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The Clinical Spectrum of Alzheimer's Disease

The Charge Toward Comprehensive Diagnostic and Therapeutic Strategies

Edited by Suzanne De La Monte

THE CLINICAL SPECTRUM OF ALZHEIMER'S DISEASE – THE CHARGE TOWARD COMPREHENSIVE DIAGNOSTIC AND THERAPEUTIC STRATEGIES

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Meet the editor

Dr. Suzanne M. de la Monte, M.D., M.P.H. is Professor of Neuropathology, Neurology, and Neurosurgery at the Rhode Island Hospital and the Alpert Medical School of Brown University, Providence, RI. She received her undergraduate degree from Cornell University, Ithaca, N.Y., M.D. from the Weill College of Medicine at Cornell University, and masters in public health (M.P.H.) from the Blumberg School of Public Health at Johns Hopkins University, Baltimore, MD. Dr. de la Monte completed her residency in Anatomical Pathology and fellowship in Pediatric Pathology at The Johns Hopkins Hospital, and a fellowship in Neuropathology at the Massachusetts General Hospital. She received postdoctoral research training at the Massachusetts General Hospital. Currently, Dr. de la Monte directs research on the role of insulin resistance as a mediator of neurodegeneration in relation to Alzheimer's disease, alcoholism, obesity, diabetes, and development. Her research led to the provocative concept that Alzheimer's is "Type 3 Diabetes".

Contents

Preface

The Clinical Spectrum of Alzheimer's Disease: The Charge Toward Comprehensive Diagnostic and Therapeutic Strategies

Alzheimer's disease, the most common cause of dementia, is a degenerative disease associated with progressive destruction of the brain, resulting in behavioral/psychiatric symptoms, memory and cognitive impairments, and eventually inability to carry out normal daily activities. For over a century, Alzheimer's disease, sometimes mispronounced "old timers' disease" has been studied by clinicians, basic scientists, and translational investigators who work to link concepts developed by each of the other two groups. The term, 'old timers' is apt, because aging is by far the most dominant risk factor for the disease. Alzheimer's disease is studied all over the world because as populations age, the prevalence rates of Alzheimer's increase, and the personal, social, societal, economic, and emotional hardships endured over its 4 to 20 year span are staggering. Given the almost crusade-like drive and enormous sums of money poured into just one field, and the thousands of publications resulting from decades of dedicated struggle, one cannot help but wonder, "what's the problem?" Why are we still so deficient in our understanding of this disease? How much more time and effort are needed to finally have ways to make early, rapid, and accurate diagnoses? When will we finally have the cure, or at least some kind of treatment that can slow down the process and provide a bit more time to enjoy life in a *compos mentis* state?

The Overview chapters in, "The Clinical Spectrum of Alzheimer's Disease: The Charge Toward Comprehensive Diagnostic and Therapeutic Strategies", summarize the basics and provide up-to-date summaries of the salient clinical, epidemiological, and genetic features of Alzheimer's. The Chapter by Dr. Lee Tih-Shih, in addition to reviewing genetic factors mediating Alzheimer's, covers the use of genomics and chip arrays, approaches that will certainly be utilized in the future to identify individuals at increased risk for developing Alzheimer's, so that preventative measures, once determined, could be implemented. The final chapter in the Overview section is unique because it highlights the shifting demographics of Alzheimer's. Previously, Alzheimer's was not prevalent among African American, but now is. The author links the increased rates of Alzheimer's among African Americans to the increased rates of diabetes mellitus. Type 2 diabetes mellitus is now a very well recognized risk factor for

sporadic AD, and its precursor, mild cognitive impairment. The author suggests practical measures to combat this emerging epidemic; the concepts expressed may have broader implications for the management and possibly prevention of sporadic Alzheimer's, which accounts for at least 90 percent of all cases.

The next section covers the non-standard features of Alzheimer's. All too often, physicians and caretakers look for only the classical features of Alzheimer's. The four chapters included in this section discuss problems related to focal cortical degenerative effects and disorders of spatial navigation and spatial memory. Such deficits quite likely account for the increased propensity of individuals with early Alzheimer's to get lost and become confused in new environments. The chapter by Dr. Ally Brandon discusses impairments in visual memory and cognition, which dovetails with the chapter on visual-spatial memory impairments in Alzheimer's. The last chapter summarizes olfactory sensory deficits in Alzheimer's. These concepts are important because, in addition to problems with perception and memory, the primary sensory organs, eyes and nose, can and often do undergo degenerative changes, some due to aging, and others possibly as components of Alzheimer's. The bottom line is that "non-standard" does not mean exceptional; instead it refers to the broader spectrum of abnormalities that exist in Alzheimer's, and that could be tapped to better understand the disease as well as improve diagnosis using non-invasive methods.

The ability to detect and monitor the progression and regional distributions of brain atrophy through neuro-imaging approaches provides excellent tools for supporting a clinical diagnosis of Alzheimer's, and can help distinguish the different causes of dementia. In addition, there is a growing realization that neuro-imaging, when combined with function, such as in vivo measures of blood flow, biochemistry, and metabolism, can be powerful for improving the accuracy of early diagnosis, and potentially monitoring responses to treatment. The section, 'Neuroimaging in the Spotlight" decodes the different approaches to neuro-imaging currently used to evaluate people with mild cognitive impairment, Alzheimer's disease, and other dementias. It is worthwhile knowing that as neuro-imaging approaches become more sophisticated and refined, functional assays will become incorporated more routinely. The limitations mainly pertain to the ability to identify pathological, biochemical, and molecular markers of neurodegeneration that correlate with structural and functional neuroimaging abnormalities, and the severity of dementia. This segment of the book is particularly useful for non-specialists and early-stage career specialists.

As mentioned, the growth and sophistication of neuroimaging are partly dependent upon understanding which molecular, biochemical, and structural abnormalities are significantly correlated with progressive neurodegeneration, and specifically, Alzheimer's. Research in the field of Alzheimer biomarkers is robust, and the combined effects of shifting targets, paradigms, and approaches, together with the difficulties in achieving high levels of inter-study concordance rates, make this area of investigation difficult to follow. The field is at the stage where clinicians, educators, and researchers must be knowledgeable about the state-of-the-art approaches to

biomarker assays for Alzheimer's, particularly since they are almost exclusively based on cerebrospinal fluid tests. The first two chapters in the section, "Biomarkers: Steps Toward Rapid Non-Invasive Tests", cover these topics in complementary fashions and with sufficient detail for even newcomers to the field to grasp what are rapidly becoming accepted tools to aid in the diagnosis of AD. However, there is still appreciable resistance in the application of cerebrospinal fluid tests, particularly if samples have to be obtained repeatedly. The chapter by Dr. Miscia highlights one of the alternative approaches, i.e. the use of peripheral blood T cells to detect molecular abnormalities related to Alzheimer's neurodegeneration. Then, taking advantage of non-standard approaches to evaluating Alzheimer's, the chapters by Drs. Marco and Panitha illustrate how such sensory deficits can be detected and used to monitor progression of Alzheimer's.. To reiterate, the term, 'non-standard features' is used to highlight the fact that most clinicians and investigators either ignore or are unaware of the primary sensory deficits in olfaction and vision that accompany Alzheimer's.

Understanding the mechanisms of Alzheimer's neurodegeneration will absolutely aid in diagnosis, treatment and prevention of disease. The inclusion of experimental animal model studies in this book may seem off-target. On the contrary, open discussion of this type of research is critical because unlike humans, the specific conditions implicated in the pathogenesis and progression of neurodegeneration can be manipulated, and the outcomes determined in a short period of time. Experimental small animal (mainly rodent) approaches have led to many translational diagnostic and therapeutic approaches in humans. As mentioned at the outset, the most potent risk factor for Alzheimer's is aging. The first chapter in the section, 'Potential Mechanisms of Neurodegeneration' utilizes an experimental mouse model to show how accelerated senescence contributes to abnormalities in intracellular signaling that are associated with Alzheimer's disease. The second chapter by Dr. Kimonis, covers the role of a novel protein that inter-relates several degenerative diseases, including their molecular and biochemical natures. This particular chapter opens readers' minds to the concept that various degenerative diseases are actually interrelated on molecular and biochemical bases, but differ with respect to brain regions and therefore clinical and pathological features. Conceptually, this chapter paves the way toward improving our understanding of Alzheimer's by indirect, backdoor means, i.e. making use of advances in other related as well as seemingly unrelated diseases. Finally, the chapter by Dr. Martinez-Marcos illustrates how the primary sensory abnormalities of olfaction in Alzheimer's are likely mediated by the same pathophysiological mechanisms already identified in the brain. This chapter champions the concept that more consistent evaluation and monitoring of "non-standard" abnormalities in Alzheimer's, even by primary care physicians, could aid in the early detection of neurodegeneration

In summary, the collection of chapters in "The Clinical Spectrum of Alzheimer's Disease: The Charge Toward Comprehensive Diagnostic and Therapeutic Strategies" provides a balanced review of the problems and state-of-the-art approaches to diagnosing and monitoring Alzheimer's disease. The chapters are very readable and

well-referenced. The works are mainly focused on human disease, which is the primary concern. However, a few chapters are devoted to experimental models because they are needed to demonstrate how our concepts evolve and the types of analyses that will likely be done in the future to improve our understanding of the pathogenesis of Alzheimer's. Data stemming from both clinical and experimental research will be need to develop objective non-invasive biomarkers for diagnosis, monitoring responses to treatment, and developing new therapeutic targets.

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Part 1

Overview: Clinical, Epidemiological, and Genetic Factors

1

Risk Factors for Disease Progression in Alzheimer's Disease

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1. Introduction

The most common form of dementia is Alzheimer's disease (AD) (Blennow et al., 2006). Due to worldwide demographic aging, its incidence and socioeconomic impact is going to be growing noticeably within the next fifty years (Sloane et al., 2002). Typically the disease progresses slowly with a mean decline of about 3 MMSE (Mini Mental Status Examination) pts/yr (Morris et al., 1993). On average, patients survive 8 years after the diagnosis has been established (Goldberg, 2007). But sometimes fast progressive AD forms with distinct clinical features are observed (Caselli et al., 1998; Josephs et al., 2009; Mann et al., 1989; Schmidt et al., 2010; van Everbroeck et al., 2004).

During the past few years AD has increasingly being understood as a disease that appears in rather heterogeneous variants (Blennow et al., 2006; Wilkosz et al., 2010; van der Vlies et al., 2009a; Iqbal et al., 2005; Querfurth & LaFerla, 2010). This accounts for its clinical profile, biomarker patterns or neuropathological features. Still, studies sufficiently interrelating symptomatology to neuropathology, pathophysiology and biopathochemistry are lacking. Factors, which might cause heterogeneity, appear to be diverse. For instance, different deterioration speeds may occur in different disease stages (Wilkosz et al., 2010; Brooks et al., 1993; Storandt et al., 2002). Also differences in the so-called cognitive reserve (Stern, 2006; Mortimer et al., 2005; Paradise et al., 2009) could account for phenotypical disparities. But furthermore, different biological causes or processes that converge on a common final pathophysiological pathway might evoke heterogeneity (Ritchie & Touchon, 1992). With ever growing evidence of AD heterogeneity, rapidly progressive AD forms (rpAD) might very well be one representative of such AD subentities.

In this book chapter, we review clinical evidence regarding AD heterogeneity in general and rapidly progressive AD (rpAD) in particular. Questions arising regard the epidemiological evidence for rpAD, its predictability, the biological / pathophysiological basis and the impact on therapeutic decision-making (subtype adapted therapy).

2. Excursus: evidence of AD heterogeneity

Different disease courses, regarding speed and slope, as well as different phenotypes might represent distinct subtypes of AD (Davidson et al., 2010; Geldmacher et al., 2000; Mangone, 2004). Several attempts have been made to characterize those subtypes, by definition of cognitive subgroup patterns, biomarker profiles in the CSF and recently using

neuroimaging (Wilkosz et al., 2010; Davidson et al., 2010; Boxer et al., 2003; Cummings, 2000).

Study	Mean survival	Age	n (patients with rpAD), gender	n, in parenthesis: n (subjects with prion disease)
Aksamit et al., 2001	n.a.	n.a.	13 (not all neuropathologically confirmed)	152 (31)
van Everbroeck et al., 2004	22mn	71	clinically diagnosed: 45 (19m, 26f); thereof 30 confirmed by post mortem	201 (52)
Collins et al., 2000	n.a.	n.a.	3	119 (14)
Gelpi et al., 2008	n.a.	n.a.	6	>900 (206)
Haïk et al., 2000	n.a.	n.a.	n.a.	465
Huang et al., 2003	n.a.	n.a.	1, m	46 (17)
Jansen et al., 2009	n.a.	n.a.	54	280 (146)
Jayaratnam et al., 2008	4.5mn	74	1, m	1
Josephs et al., 2009	3yrs	72	1, m	22 (8)
	1.2yrs	74	1, m	
Mahmoudi et al., 2010	21mn	74	1, m	1
Reinwald et al., 2004	40d	69	1, m	1
Schmidt et al., 2010	26.4mn	73	32 (15m,17f)	32
Tschampa et al., 2001	24mn	76	19 (4m, 15f)	56 (25)

Table 1. Neuropathologically confirmed rpAD cases imitating features of prion disease in different studies of rapid dementias. (Abbreviations: d=days, f=female, m=male, mn=months, n.a.=not available, yrs=years). Table modified from Schmidt et al., 2011.

2.1 Heterogeneity in AD neuropsychology and imaging

In a comprehensive overview Cummings presents the knowledge about different phenotypes of AD, which also correlate with marked differences in the focal metabolism or distinct types of focal atrophy (Cummings, 2000). Firstly, he mentions cognitive heterogeneity. Different AD phenotypes may reflect subtypes characterized by marked aphasia (Gorno-Tempini et al., 2008; Price et al., 1993), pronounced visoconstructive disturbances (Furey-Kurkjian et al., 1996), the variant denominated as "posteriortcortical atrophy" (Benson et al, 1988; Tom et al., 1998) and a frontal variant (Foster et al., 1983). For all these speculative variants, different metabolism patterns have been demonstrated e.g. by means of FDG PET imaging (Foster et al., 1983; Grady et al., 1988; Haxby et al., 1988; Pietrini et al., 1996) - as a possible reflection of neurobiological heterogeneity. Boxer and colleagues for instance examined AD patients with similar cognitive profiles but marked differences in visuoconstructive abilities. More right than left cortical gray matter loss was seen in MRI imaging in the visuoconstructively impaired group (esp. right inferior temporal gyrus in contrast to the less spatially impaired group). Right inferotemporal atrophy might therefore be able to serve as an imaging surrogate marker for visuoconstructive disabilities. Another subtype might be AD with salient extrapyramidal signs. Those patients exhibit parkinsonoid

features, more severe cognitive decline (Clark et al., 1997) and an increased number of neurofibrillary tangles in neuropathology (Liu et al., 1997). Lewy body (LB) pathology is common (McKeith et al., 1996) in AD, but the group mentioned here was free from such LB features. Behavioral symptoms such as delusion, aggression, depression etc. seem as well to be heterogeneous and also show differences especially regarding metabolism (Cummings, 2000).

2.2 CSF biomarker evidence of heterogeneity

Iqbal and colleagues defined disease subtypes based on CSF marker profiles, age at onset, clinical profile and disease course (Iqbal et al., 2005). Van der Vlies et al. could also identify three AD subtypes using CSF marker profiles (based on Tau, phosphorylated Tau (pTau), and Aβ1-42) - corrected for *Apoε* type, age, gender - showing distinct cognitive profiles on neuropsychologic testing (van der Vlies et al., 2009a, 2009b). Especially patients with very low Aβ1-42 and high Tau and pTau performed worse on Visual association testing (VAT), Trail Making Tests (TMT) and Word Fluency (WF).

The differences in CSF marker profiles might imply the underlying pathophysiology to differ between subtypes. Although this is not proven to date, some findings support this hypothesis: Cerebrospinal fluid (CSF) contains a dynamic and complex mixture of proteins, which reflects physiological and pathological state of the CNS (Gawinecka & Zerr, 2010; Weller, 2001). In AD, levels of both major key players in the disease pathogenesis, namely Tau protein and Aβ, are altered in the CSF. These CSF changes are assumed to mirror the pathophysiological process in the brain, however, direct comparisons are lacking due to a long period between lumbar puncture and CSF tests on the one side and potential autopsy and neuropathological workup on the other side.

2.3 AD heterogeneity in neuropathology

Also from a pathology point of view evidence has been found to support hypotheses of Alzheimer heterogeneity. The basis of neuropathological classification are: Braak's staging, describing the distribution of neurofibrillary tangles (NFT), CERAD staging, describing the densitiy of neuritic plaques and NIA-RIA criteria, being a synthesis of CERAD and Braak's criteria (Murayama & Saito, 2004). Regarding those criteria, neuropathological heterogeneity is observed. Ritchie et al. suggest three hypotheses to explain neuropathological heterogeneity in AD: 1) subtypes 2) disease stage effects 3) "compensation" (differences in cause / origin and progression of AD) (Ritchie & Touchon, 1992).

Especially heterogeneous cortical atrophy, of which right inferotemporal atrophy correlates with visuoconstructive impairment, can be found (Boxer et al., 2003). Recent papers reported heterogeneous Aβ deposition patterns in the end stages of the disease with variations throughout the neocortex, which cannot be completely explained by a regular built up of the pathologic protein during the course of the disease. This implies that other biological factors might be involved to build certain phenotypes (Cupidi et al., 2010). The morphology of Aß deposits is influenced by the cyto- and fibroarchitectonics of the brain region in which they are found and by the amount of amyloid present (Wisniewski et al., 1989). Factors having an impact thereupon are not fully understood (Walker et al., 2008).

Studies, which focused on neurofibrillar tangles (NFT) in AD revealed significantly different NFT densities in various areas of the cerebral cortex without significant differences in the

duration of illness, suggesting a possible existence of subgroups. Two distinct subentities in AD with different densities of neurofibrillary tangles - but apparently without distinct clinical courses could be differentiated (Mizuno et al., 2003). Even in patients with presenelin (*PSEN*) mutations, the neuropathological distribution of different types of plaques, intensity of cerebrovascular amyloid and the number of NFT substantially differed among individuals, implying that missense mutations in PSEN genes can alter a range of key gamma-secretase activities to produce an array of subtly different biochemical, neuropathological and clinical manifestations (Maarouf et al., 2008).

Although the pathological and clinical heterogeneity of AD has been recognized and addressed to some extent in the literature, direct studies on clinico-pathological phenotypes are sparse. Some authors are arguing against the hypotheses of neuropathological heterogeneity. Armstrong et al. for instance examined eighty cases (Armstrong et al., 2000). They found that neuropathological differences were rather continuously distributed in contrast to the subtype hypotheses. Heterogeneity in plaque and tangle distribution correlated more with disease stage (stage hypothesis) rather than being explained by the presence of AD subentities. Nonetheless plaque load and distribution was significantly influenced by the presence of *Apoε* type 4 allele.

3. Definition and epidemiology of rapidly progressive AD

AD has been a clinical diagnosis since the McKhann Criteria were established in 1984 (McKhann et al., 1984). Neuroimaging and CSF parameters increasingly came into use especially in the first decade of the new millennium leading to newly proposed research criteria finally being accepted as a validated instrument to support the diagnostic concept (de Meyer et al., 2010; Dubois et al., 2010; Dubois et al., 2007; Gauthier et al., 2008).

Alois Alzheimer first described the hallmarks of AD with plaques and neurofibrillary tangles (NFT) more than a hundred years ago. In synopsis with the clinical presentation, neuropathological work-up allows a definite diagnosis. But it has become obvious that AD pathology can also exist without significant simultaneous cognitive impairment (Price et al., 2009). In cases when AD was diagnosed clinically and by post mortem work-up, heterogeneity has also been found to exist e.g. in terms of tangle distribution (Mizuno et al., 2003). Until today it remains subject to controversy how to relate clinical signs and symptoms to specific neuropathological lesion patterns or profiles.

Hypothetically clinically differing disease course could represent distinct subentities of AD in terms of heterogeneity. This accounts especially for speed of decline and distinct trajectories of that deterioration speed (Davidson et al., 2010; Mangone, 2004). Some attempts have been made to characterize these subentities by defining cognitive subgroup profiles, CSF biomarker patterns and neuroimaging characteristics (Wilkosz et al., 2010; Davidson et al., 2010; Boxer et al., 2003; Cummings, 2000). (→ see section 2)

Disease progression rates have also been used to distinguish AD subtypes. But at the moment there is no consensus about the definition of the term "rapidly progressive AD". Moreover the term «rapid» has been used rather arbitrarily. It has been doubtful whether "rapid" should be applied to characterize either the rate of cognitive deterioration - and if so, on which scales - or the disease duration time (survival time). In addition, the trajectories of decline have not been and even are currently not clearly known. They might differ among subentities, making a clear definition very difficult. The majority of AD researchers assume

a linear slope, but some investigators also suggest trilinear models of decline or even more trajectories (Wilkosz et al., 2010; Brooks et al., 1993).

A variety of definitions has been used in previous studies rather at will. The term "rapid" has been applied to describe a survival time below 4 years (Josephs et al., 2009), MMSE declines of >5 pts/yr (Doody et al., 2001), >3 pts/yr (Carcaillon et al., 2007), >4pts/0.5yrs (Dumont et al., 2005) or >2,56 pts/yr (Buccione et al, 2007) as well as CDR (Clinical Dementia Rating Scale) score progression from 1 to 2 or 3 within max. 3 yrs (Bhargava et al., 2006). Ito et al. observed an average MMSE loss of 5.5 pts/yr in mild to moderate AD in a metaanalysis (Ito et al., 2010). Encouraging a discussion and attempt to reach a consensus on the term "rapid cognitive decline", a threshold of 3 or more MMSE pt loss per six months has been proposed (Schmidt et al., 2011; Soto et al., 2008).

Owed to different definitions of "rapid", rpAD seems to constitute approximately 10-30% of the AD population. In a longitudinal study with more than 600 AD patients over a two years period, Cortes et al. discovered that almost one third of the patients declined faster than 3 MMSE pts. per year. A tenth deteriorated twice as fast as the whole groups average decline of approx. 4.5 pts per year on the MMSE scale (Cortes et al, 2008). Dumont and colleagues, in another prospective study, saw one quarter of the cohort decline faster than 4 MMSE points within half a year (Dumont et al., 2005). Recently Åsa Wallin and her research group were able to show that approximately 8% of their AD study population were characterized by a significantly higher mortality and a mean speed of cognitive deterioration of almost 5 MMSE pts/yr (Wallin et al., 2010). Table 2 gives overview of different studies describing rapid progression and its frequency.

study	definition of "rapid" [MMSE decline]	proportion of study population, (n (total))
Carcaillon et al., 2007	>3pt/yr	34% (254)
Ballard et al., 2001	>4pt/yr *	60% (101)
Cortes et al., 2008	>4.5/yr	11% (686)
Wallin et al., 2010	>5pt/yr**	8% (151)
Ballard et al., 2001	>7pt/yr *	32% (101)
Dumont et al., 2005	>8pt/yr	25% (312)
Soto et al., 2008a	multiple (>3pts/6months)	10%-54%
Soto et al., 2008b	>4pts/first 6 months	14% (565)

*(«Rapid» is not explicitly defined in this study. The numbers given are mere observations.)
** Special CSF biomarker cluster

Table 2. Frequency of rpAD in several clinical studies (longitudinal, cross-sectional, retrospective). «Rapid» has been defined by the authors in terms of MMSE decline (column 1) to specify a «rapid group» out of the AD continuum. (Abbreviations: MMSE=Minimental Status Examination, n=number, pts=points, yr=year). Table modified from Schmidt et al., 2011.

4. Factors associated with rapid progression

Much is known about clinical, pathobiochemical and hereditary factors altering the *risk* of developing Alzheimer's disease, as well as how the risk to advance from Mild Cognitive Impairment (MCI) to manifest dementia is modulated by these. But there is a relative lack of

knowledge about which signs and symptoms, blood and CSF marker values as well as genetic factors actually predict the speed of deterioration in AD.

4.1 Clinical signs, symptoms and comorbidity as predictors of fast progression

Several factors such as genetic properties, environmental circumstances, cerebral atherosclerosis, cognitive reserve, medical and social support contribute to disease progression (Etiene et al., 1998).

sign / comorbidity	predictor of		
	slow progression	no influence or unclear	fast progression
apathy			Starkstein et al., 2006 (354)
apraxia (constructional)			Smith et al., 2001 (60)
atherosclerosis, atrial fibrillation, hypercholesterinemia, hypertension, microvascular disease, myocardial infarction		Abellan et al., 2009 (686)	Laukka et al., 2010 (138) Mielke et al., 2007 (135) Roselli et al., 2009 (162) Silvestrini et al., 2006 (53)
chronic systemic inflammation			Holmes et al., 2010 (300)
diabetes mellitus	Sanz et al., 2009 (608)		Roselli et al., 2009 (162)
psychotic symptoms			Mangone, 2004 (1000) Wilkosz et al., 2009 (201)
multitude of focal neurological signs			Josephs et al., 2009 (1) Schmidt et al., 2010 (32) Tschampa et al., 2001 (19) van Everbroeck et al., 2004 (45)
high educational level	Pavlik et al., 2009 (rate of decline) (478)	Pavlik et al., 2009 (survival) (478)	Roselli et al., 2009 (162)
low educational level			Mangone, 2004 (1000)
motor signs			Mangone, 2004 (1000) Portet et al., 2009 (388) Scarmeas et al., 2005 (533)
early fast decline			Soto et al., 2008b (565)
seizures			Volicer et al., 1995 (language function) (27)
severe cognitive impairment at disease onset		Hui et al., 2003 (mortality) (354)	Atchison et al., 2007 (150) Ito et al., 2010 (576) Marra et al., 2000 (45)
sex (male)			Roselli et al., 2009 (162)

Table 3. Clinical signs, symptoms and comorbidity as predictors of disease progression. Total number of subjects (AD) in the studies are given in parentheses. Table modified from Schmidt et al., 2011.

The role of comorbidity is subject to controversy. Diseases of the cardiovascular system and diabetes mellitus are commonly accepted as AD disease risk modulators. However, findings regarding their impact on disease progression are sometimes contradictory (Table 3) (Abellan van Kan et al., 2009; Mielke et al., 2007).
Fast deterioration also appears to be associated with the occurrence of certain signs and symptoms. Among those are especially early signs of the motor system. They are predictors of fast decline as well as poor outcome (Mangone, 2004; Portet et al., 2009; Scarmeas et al., 2005). Another potential indicator / predictor of a rapid disease course might be the presence of psychotic symptoms (Wilkosz et al. 2010). Table 3 provides an overview of the associations of comorbidity and symptoms with progression of AD.
Baseline cognitive status and preprogression rates in MMSE decline (estimated MMSE loss per time period from onset until diagnosis [pt/yr]) were used as predictive clinical markers as well. Another concept of predictive clinical markers has been demonstrated to be useful e.g. by Doody et al. in 2001. The baseline cognitive status as well as preprogression rates of MMSE decline were able to predict further speed of deterioration. Preprogression rates resemble the estimated MMSE loss per time period between the clinical onset to formal diagnosis (pts/yr).
It has been shown by Soto et al., that especially the early loss of 4 MMSE pts within half a year was predicting a poorer outcome (Soto et al., 2008b). Additionally, the baseline cognitive status is all the more capable of predicting the speed of decline regarding functional basic care abilities in AD (Atchison et al., 2007). The baseline level of cognition does not necessarily correlate with mortality, nonetheless, the cognitive decline rate features a considerable variability in some longitudinal studies (Hui et al., 2003). Recently a metaanalysis showed baseline ADAS-Cog values to be covariates of speed of decline (Ito et al., 2010). Santillan and coworkers proposed the use of a scale, consisting of the educational level, insight assessment, the presence of psychosis, the activities of daily living as well as MMSE. Measured at baseline this scale might be capable of estimating the risk of future deterioration (Santillan et al., 2003).

4.2 Imaging and prediction

An abundance of scientific work has been published regarding imaging in AD. The majority deals with either the early diagnosis of AD and differentiation MCI, AD and healthy subject, or makes statements about imaging and the risk of developing Alzheimer's disease, or it correlates atrophy rates to stages of AD. Literature about baseline imaging characteristics that actually predict the future speed of decline of AD patients (and not the risk of progression from MCI to AD) is scarce. Table 4 gives an overview.

4.3 Predictive biomarkers

4.3.1 CSF

CSF markers have become an important part of AD diagnostics. But also as predictors of fast decline, they might harbor a certain potential. For instance, rapid cognitive deterioration has been demonstrated to be indicated by high total Tau (Tau) protein or hyperphosphorylated Tau (pTau) as well as low Aβ1-42 (411pg/ml or less) or a high Tau/Aβ1-42 ratio (0.81 or higher) in the cerebrospinal fluid (CSF) respectively (Mungas et al., 2002). Therefore attempts have been made to suggest and validate Tau as well as its phosphorylated isoforms in particular as prognostic markers. Kester et al. discovered that especially elevated Tau

protein without proportionally elevated hyperphosphorylated Tau (pTau) might predict fast decline (Kester et al., 2009). Wallin and coworkers recently showed that subjects with very high levels of Tau (>1501 (±292) pg/ml) and pTau (>139 (±39) pg/ml) and at the same time low levels of Aβ1-42 (< 362 (± 66) pg/ml) deteriorate more rapidly and feature high mortality rates (Wallin et al., 2010).

study	slower progression or no influence	faster progression
Adak et al., 2004 (n=225, MRI)		higher ventricular volume
Kinkingnehun et al., 2008 (n=41, MRI, voxel based morphometry)		extensive cortical atrophy
Mungas et al., 2002 (n=120, MRI)		hippocampal atrophy, cortical atrophy
Ridha et al., 2008 (n=52, MRI)	hippocampal atrophy	
Sluimer et al., 2008 (n=65, MRI)	focal hippocampal shrinkage	generalized global atrophy and early onset and *Apo*ε4 negative
Swann et al., 1997 (n[AD]=24, MRI)	hippocampal atrophy	

Table 4. Imaging and the prediction of AD disease progression.

It has to be kept in mind that some studies the disease stage might be a confounder: Certain CSF marker levels or patterns could as well reflect the disease stage instead of being indicative or predictive for the deterioration rate. Data from serial, repeatedly performed lumbar punctures and CSF analyses are necessary to control this potential confounding factor. Only a small number of studies on this subject have been performed so far. The follow up intervals were short. Over a period of 24 months CSF Tau, pTau and Aβ1-42 appear to be quite constant (Sunderland et al., 1999; Blennow et al., 2007). This hypothesis has largely been undergirded by Buchave et al. However, they reported slightly increasing Tau values over two years (Buchhave et al., 2009). Contradicting these findings of constancy, Stomrud and colleagues demonstrated pTau to increase in a 4 years observation period. Furthermore this increment seemed to be associated with cognitive decline (Stomrud et al., 2010). Regarding Aβ1-42 levels, Huey and colleagues found these to slightly decrease while Tau staying stable observed over a period of 4 years (Huey et al., 2006).

4.3.2 Genetics

Efforts to investigate genetic predictors in AD have been significantly increased over the past years. A number of polymorphisms found seem to have predictive capability in regards of speed of decline. Nonetheless, several remain subject to discussion and controversy: Among those especially the Apoε gene. This polymorphism is a well established modulator of AD *disease risk*. But its significance as a *predictor* of progression is not yet as well examined. Some researchers claim, that the presence of the ε4 allele predicts fast

deterioration especially in mild AD (Cosentino et al., 2008). But in opposition, according to van der Vlies, early onset AD is especially rapid, if the subjects are negative for Apoε4 (van der Vlies et al., 2009b). A recent study of our research group came to the same result: the ε4 allele was exceptionally infrequent among rpAD cases (Schmidt et al., 2010). Clues mount up that lacking Apoε4 in AD is not only associated with a faster decline but also a more atypical course (van der Flier et al., 2011).
Nevertheless, the research group of Kester and colleagues found no predictive capability of Apoε whatsoever (Kester et al., 2009). An overview of different genetic markers associated with speed of decline is provided in Table 5.

gene/polymorphism	**decline**		
	slow	**no influence**	**fast**
Apoε4		Kester et al., 2009 (151)	Cosentino et al., 2008 (570)
no Apoε4			van der Vlies et al., 2009b (291) Schmidt et al., 2010 (32) van der Flier et al., 2011
BuChE (K allele)	Holmes et al., 2005 (339)		
G51S PNP (AA genotype)			Tumini et al., 2007 (321)
HMGCR (A allele)			Porcellini et al., 2007 (190+586, 97, 296)
PSEN1 rs3025780 (TG genotype)			Belbin et al., 2009 (714, 169)
PSEN1 rs3025787 (CG genotype)			Belbin et al., 2009 (714, 169)
PSEN1 rs7152131 (CA genotype)	Belbin et al., 2009 (714, 169)		
ACT7 (GC + CC genotype)			Belbin et al., 2008 (688+419)
ACT-17 (AA genotype)			Kamboh et al., 2006 (909)
ACT promoter polymorphism (TT genotype) + Apoε4			Licastro et al., 2005 (422)
*IL-1α -889 (*1/*1 genotype)*			Murphy et al., 2001 (114)
IL-18 -137 (CC genotype)			Bossu et al., 2007 (339)
FAS -1377 (AG + GG genotype)			Chiappelli et al., 2006 (137+144)
RAGE G82S (GS + SS genotype)			Li et al., 2010 (276+254)

Table 5. Genetic predictors of cognitive deterioration speed in AD Total number of subjects (AD) in the studies are given in parentheses.

5. Conclusion

Until recently, Alzheimer's disease has been seen as a clinically rather homogeneous disease. But during the last decade several studies have differentiated early onset or late onset entities as well as fast declining forms. Classification and characterization of these disease subentities by means CSF biomarkers and search for indicative patterns as well as neuropsychological test batteries has been attempted. However, comprehensive approaches to characterize AD subtypes relating clinical characteristics to a neuropathological molecular level are lacking (Wilkosz et al., 2010; Doody et al., 2001). Latest pharmacological trials implicated that there may be different subtypes within Alzheimer's disease exhibiting different susceptibilities to specific pharmacotherapies (Wallin et al., 2009). Hence, a superior characterization of the clinico-pathological heterogeneity and identification of predictive factors of disease progression should be able to improve our understanding of disease pathogenesis and allow better monitoring in therapeutic settings.

	rpAD	**classical AD**
survival	few years (2-3)	8-10 years
onset	still unclear, around the age of 73yrs in the study of Schmidt et al., 2010	around age 65yrs (below = early onset, above = late onset)
cognitive decline	>6 MMSE pts/yr → fast	approx. 3-6 MMSE pts/yr → slow
focal neurological signs	occurring in early stages, multiple (esp. extrapyramidal signs)	occurring in late stages
CSF biomarkers	very high Tau, very high pTau, very low A beta 1-42, proteins	high Tau, high pTau, low A beta 1-42, proteins 14-3-3
ApoE4	controversial: its influence on decline see Table 4, sometimes seen negative in very rapid cases (Mann et al., 1989)	established as a risk factor

Table 6. Classic AD and rpAD in comparison. Table modified from Schmidt et al., 2011.

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Alzheimer's Disease Genomics and Clinical Applications

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1. Introduction

The prevalence of dementia is expected to increase exponentially worldwide. Global estimates of Alzheimer's disease (AD) - generally considered to be the most common subtype of dementia - are expected to increase from the current estimated 25 million to 63 million in 2030, and by 2050, a staggering 114 million (Wimo et al., 2003).

Traditional approaches in AD diagnosis (Diagnostic and Statistical Manual- IV (DSM-IV) – Text Revision (American Psychiatric Association [APA], 2000) and National Institute of Neurological Disorders and Stroke- Alzheimer Disease and Related Disorders (NINCDS-ADRDA) (McKhann et al., 1984) criteria have varying diagnostic accuracy of 65-96% and specificity of 23-88% compared to the neuropathologic gold standard (Kazee et al., 1993; Lim et al., 1999; Petrovich et al., 2001; Varma et al., 1999). Studies have shown that the hallmark histopathological changes of AD (β-amyloid plaques and neurofibrillary tangles) precede the clinical onset of disease by as long as 20-30 years (Price & Morris, 1999). This translates clinically to functional and structural brain damage where these pathologic changes may occur prior to apparent clinical manifestations of cognitive decline by way of standard clinical assessments. This has fuelled an increasing shift of diagnostic focus to the pre-dementia transitional state between normal aging and early AD, which represents a window of opportunity for identifying subjects at a phase when pathogenesis has already begun but clinical diagnosis of established dementia is still not achievable. This would logically be the stage most amenable to disease-modifying interventions (such as β- and gamma-secretase inhibitors, anti-amyloid and anti-neurofibrillary tangle therapies). Diagnostic focus thus has shifted towards prodromal stages of Alzheimer's Disease (AD), such as mild cognitive impairment (MCI) (Morris et al., 2001; Peterson, 2004). Clinical criteria alone, which by their very nature subjective and entail judgment, are thus inadequate to identify the pre-clinical stages of AD and may have contributed to the disappointing results of therapeutic trials in MCI (a heterogeneous entity) to date. This has prompted revisions in the upcoming DSM-V criteria due in 2013 (Kupfer & Regier, 2010) which include major and minor neurocognitive disorder classification, as well as the proposed revision of NINCDS-ADRDA criteria for AD to include prodromal AD and preclinical AD, which characterises earliest stage of AD that predate crossing of the dementia threshold of functional disability. In the proposed criterion by Dubois et al (Dubois et al., 2007), other than clinical criterion of episodic memory deficit,

they have also included in criterion E, dominant genetic mutation within the immediate family of amyloid precursor protein (Chromosome 21), presenilin 1 (chromosome 14) and presenilin 2 (chromosome 1). In consideration of important genetic factors, the presence of a proven autosomal dominant mutation has been taken as evidence to support AD diagnosis even when clinical features fall outside typical AD criteria. In the working draft for the revised NINCDS-ADRDA research criteria for MCI-AD and preclinical AD, there are also considerations given to genetics and its influence on disease progression in these pre-dementia states.

The rate and presence of clinical manifestation of AD is postulated to be influenced by the complex relationship of age, genetic factors, cognitive reserve, cerebrovascular disease, which might affect the neuropathogenic progress of amyloid toxicity and subsequent clinical presentation of AD (Jack et al., 2010). Hence in recent years, there has been increasing interest in the role of genomics in understanding AD and disease progression.

In this chapter, we will review the application of genomic, transcriptomic and other 'omic' platforms and their role in the development of novel diagnostic strategies for AD diagnosis, prediction of disease progression and therapeutic drug responses. We will discuss the potential clinical applications, the current limitations, ethical dilemmas and the future direction of genomics in AD.

2. Genetics of AD

The genetic underpinning of AD is heterogeneous and complex, without a straightforward mode of inheritance for the vast majority of cases. The heritability of AD in general is estimated to be around 60% (Bergem et al., 1997a, 1997b).

In general, AD can be divided into 2 forms: early onset AD (EOAD) usually those below 65 years of age, and patients with the late onset AD (LOAD), above 65 years. EOAD largely follows a Mendelian autosomal dominant inheritance but they account for less than 5% of all AD. Linkage studies have identified three genes thus far for which multiple mutations can lead to the pathology. These genes are the amyloid precursor protein (APP) gene on chromosome 21q, the presenilin 1 (PSEN1) gene on 14q, and presenilin 2 (PSEN2) gene on 1q. These mutations all affect Amyloid Precursor Protein (APP) processing and lead to the increased synthesis of Aβ40 and Aβ42 (See Figure 1). These peptides aggregate to form amyloid plaques. Given their rarity, these three gene mutations contribute minimally to the estimated 60% heritability of AD. The importance of these rare mutations lies in the identification of pathogenic pathways, specifically those involving the catabolism of APP. Hence accumulation of Aβ40 and Aβ42 is attributed to increased activity of the β and γ secretases in familial cases of AD with APP, PSEN1 and PSEN2 gene mutations. However environmental or other non-genetic or epigenetic factors may also affect the activities of the secretases. This may account for why some cases of PSEN1 and PSEN2 mutations show incomplete penetrance and variable onset of illness (Tanzi et al., 1996, 1999).

Normal individuals with first-degree relatives affected by AD, especially one parent, are at 4 to10-fold higher risk of developing LOAD compared to those with no family history. However no clear Mendelian pattern of transmission has been identified as yet for LOAD. Those subjects with a maternal history of dementia showed reduced cerebral metabolic rate of glucose in the same regions as clinically affected AD subjects (posterior cingulate cortex, precuneus, parietotemporal and frontal cortices, medial temporal lobes) and these effects remained after age, gender and education adjustments were made. This may be suggestive

of either chromosome X transmission or inheritance of mitochondrial DNA (mtDNA). This is especially pertinent as mtDNA deficits are proposed to be involved in AD (Lin & Beal, 2006; Mosconi et al.,2007) with further recent evidence for sub-haplotype H5 of mtDNA, especially in females, to be a risk factor for late onset AD, independent of APOE status (Santoro et al., 2010).

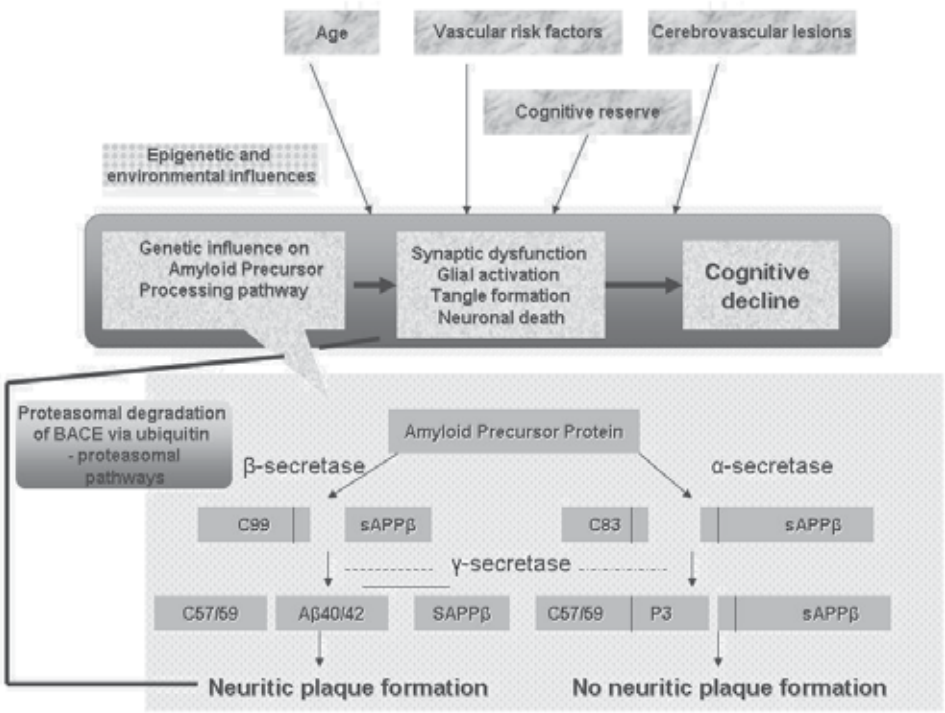

Fig. 1. Hypothetical pathophysiological cascade of AD and genetic influences on amyloid precursor pathways

Multiple association studies have showed apolipoprotein e4 (APOE e4) to be a genetic susceptibility factor, and another allele e2 to be likely protective. Apolipoprotein has three alleles e2, e3, e4 located on chromosome 19. They encode cholesterol transport protein APOE which is the primary cholesterol transporter in the brain. APOE proteins play a central role in the regulation of cholesterol and triglyceride metabolism. They are also present in amyloid plaques. In 25% of LOAD patients, there is at least one affected relative in the family (Ritchie & Lovestone., 2002). In Caucasian populations, 3-fold increased risk of developing AD has been reported for heterozygous APOE e4 and 8-fold risk in homozygous APOE e4 (Roses, 1996). Regional, racial and ethnic differences have been observed in APOE e4 genotype frequency, with lower carrier status estimates in Asian, southern European/Mediterranean communities compared to North American or North European counterparts (Crean et al., 2011). The influence of APOE e4 on AD risk, while applicable between ages 40 and 90 years, diminishes after the age of 70, and varies across ethnic groups (Farrer et al., 1997).

The mechanism for the effects of APOE isoforms on brain damage is unclear although a recent study demonstrated APOEe4 to cause mitochondrial respiratory dysfunction in neuronal cells

through APOEe4 domain interaction. APOEe4, not APOEe3, causes reduced expression of mitochondrial respiratory complexes and perturbed mitochondrial respiratory function in neuronal cells; thus suggesting that the structure of APOEe4 could be a potential therapeutic target for APOEe4-related neurodegeneration (Chen et al., 2010). Other studies have suggested that APOEe4 is a disease modifier exerting its effect on disease risk by influencing age of onset rather than disease risk per se (Serretti et al., 2007). In this hypothesis, APOEe4 genotype modulates disease risk likely by its effect on earlier amyloid β accumulation.

While APOEe4 status exerts a modulatory effect on disease trajectory and clinical expression of disease, it has not been consistently shown to predict MCI-converters (Jack et al., 1999; Killiany et al.,2002; Korf et al.,2004 ; Martins et al, 2006; Okonkwo et al., 2010; Petersen et al., 2005; Wang et al., 2011). A recent study showed APOE subjects to have 6 times increased risk of MCI conversion to AD (Barabash et al., 2009). The role of APOEe4 genotype in cholesterol metabolism and Aβ clearance and interactions in vascular risk factors is becoming increasingly recognized (Martins et al., 2006). Midlife high systolic blood pressure has a stronger adverse effect on cognitive function in the presence of APOEe4 genotype (Peila et al., 2001). Histopathologic data suggest an association between APOEe4 and small vessel arteriolosclerosis and microinfarcts of the deep nuclei (Yip et al., 2005).

In cognitively normal individuals, APOEe2 carriers have slower rate of hippocampal atrophy over 2 years than individuals with e3/3. The e2 carriers also have higher CSF β-amyloid (Chiang et al., 2010; Morris et al., 2010) and lower phosphorylated-tau (p-tau) (Chiang et al., 2010) suggesting less AD pathology. Morris also showed a gene dose effect for the APOE genotype, with greater mean cortical binding potential for Pittsburgh Compound-B binding increases and greater reductions in CSF $A\beta_{42}$ with increased numbers of APOE alleles; with no effect on CSF tau or p-tau_{181} (Morris et al., 2010). These findings are also supported by the Alzheimer Disease Neuroimaging Initiative study (Vemuri et al., 2010). Mosconi et al has also shown that normal APOEe4 carriers with subjective memory complaints have decreased cerebral metabolic rates for glucose (CMRglc) on 2-[^{18}F] fluoro-2-deoxy-D-glucose (FDG)-positron emission tomography (PET) (Mosconi L et al., 2008).

Apart from its effects on clinical progression in at-risk individuals, there have also been studies on APOE polymorphism in Alzheimer's disease patients and neuropsychiatric symptoms. APOEe4 AD subjects have been found to be associated with more depressive symptoms and apathy (D'Onofrio et al., 2010; Fritze et al., 2010). However this association has been inconsistent (Slifer et al., 2009).

The relationship of APOE genotype with brain function is complex. The APOEe4 carrier state is likely to increase the brain's vulnerability to late-life pathology or cognitive decline (Filippinin et al., 2009). APOE also has been postulated to interact with other factors, such as homocysteine (Minagawa et al., 2010), smoking (Rusanen et al., 2010), testosterone (Panizzon et al., 2010), and other genetic factors like GAB2 haplotype (Liang et al., 2011) which is possibly protective, These allude to potential gene-gene interactions between APOE and other factors for clinical AD manifestations. APOE as a genetic risk factor is not fully penetrant, and neither necessary nor sufficient for AD development (Ertekin-Taner et al., 2010).

Genetic risk factors are traditionally studied using linkage analysis followed by positional cloning, and association studies. A major drawback is that these hypothesis-driven studies depend on pre-existing knowledge limiting their potential to uncover novel genes and pathways. Over the past decade, a high-throughput hypothesis-free approach - genome-wide association study (GWAS) has taken off. This approach examines genetic variation across an entire genome and is designed to identify whether certain genes or their variants

are skewed to a particular population of individuals affected with disease when compared with a control population. This follows recent advancements in developing microarray platforms that allow researchers to survey the human genome for single base pair differences - single-nucleotide polymorphisms (SNPs) - across many disease cases and unaffected controls. For example, Illumina's Infinium HD Beadchip Human Omni1-Quad® and Human 1MDuo® now have excess of one million markers (www.illumina.com). Affymetrix Genome-Wide Human SNP Array 6.0® is a single array that features more than 1.8 million markers for genetic variation, including more than 900,000 single nucleotide polymorphisms (SNPs) (www.affymetrix.com).

By different gene discovery methods, hundreds of genes have been associated with LOAD but most have not been consistently replicated except for APOEe4 (Betram et al., 2008). Some of the other susceptibility genes reported include ubiquilin 1 (UBQLN1), a presenilin interactor that promotes the accumulation of presenilin 1 protein and regulates its endoproteolysis (Betram et al., 2005); insulin degrading enzyme (IDE), which regulates Aβ42 levels in brain neurons and microglial cells (Farris et al., 2003; Prince et al., 2003); sortilin-related sorting receptor (SORL1), which appears to play a key role in the differential sorting of APP. Under-expression of SORL1 leads to APP release into late endosomal pathways and processed by beta-secretase cleavage and yielding Aβ (Andersen et al., 2007; Rogaeva et al., 2007). A recent study has also shown MTHFD1L association with AD, which might influence homocysteine-related pathways, thus supporting biological evidence of folate-pathway abnormalities as homocysteine has been implicated in AD (Naj et al., 2010). There is also some recent evidence supporting the role of intermediate genotypes in influencing age-related cognitive decline and neuropathologically-proven AD pathology, suggesting divergent pathways to AD (Shulman et al., 2010).

The association studies results can be accessed openly on the AlzGene database - (http://www.alzforum.org/res/com/gen/alzgene) for the most up-to-date information. This is a huge and rapidly growing database of genes and proteins that researchers have found and made available in an open access platform which summarizes results of case-control AD studies across different racial populations. In the recent 2 years, results from large population GWAS studies showed association with the established APOE locus (most significant SNP,rs2075650, P = 1.8 x 10(-157)) as well as observed genome-wide significant association with SNPs at two loci not previously associated with the disease: at the CLU (also known as APOJ) gene on chromosome 8(rs11136000, P = 1.4 x 10(-9)) and 5' to the PICALM gene (rs3851179, P = 1.9 x 10(-8)) (Harold D et al., 2009; Seshadri et al., 2010); rs744373 near BIN1 (odds ratio [OR],1.13; 95% confidence interval [CI],1.06-1.21 per copy of the minor allele; P = 1.59x10(-11)) and rs597668 near EXOC3L2/BLOC1S3/MARK4 (OR, 1.18; 95% CI, 1.07-1.29; P = 6.45x10(-9)) in a separate Spanish sample (Seshadri et al., 2010). Similar results were replicated in a large European study, which showed CLU, (OR = 0.86, 95% CI 0.81-0.90, P = 7.5 x 10(-9) for combined data) and the other within CR1, encoding the complement component (3b/4b) receptor 1, on chromosome 1 (rs6656401, OR = 1.21, 95% CI 1.14-1.29, P = 3.7 x 10(-9) for combined data). Previous biological studies have supported CLU and CR1's role in Abeta peptide clearance (Lambert et al., 2009) and their interactions with APOE genotype (Gyungah et al., 2010). Another gene locus of interest is TOMM40, gene in LD with APOE, which may contribute to APOE correlations with AD risk and age of onset (Roses et al., 2009) (See Table 1). Although genetic associations have been demonstrated, such as CLU and PICALM, in a study by Seshadri, these loci did not improve AD risk prediction (Seshadri et al., 2010).

Year published	Authorship	Source	Initial Number of cases/ controls	Reported Genes
2011	Lee (Lee et al., 2011)	US	549/544	CUGBP2 (APOE4 homozygous), HPCAL1, PCDH21, LRITI, RGR, CLU, PICALM, BIN1
2010	Carrasquillo (Carrasquillo et al., 2010)	US	1829/2576	CLU, CRI, PICALM
2010	Seshadri (Seshadri et al., 2010)	US	10968/14642	APOE
2010	Naj (Naj et al., 2010)	US	931/1104	APOE,TOMM40, MTHFD1L, PVRL2
2009	Heinzen (Heinzen et al., 2010)	US	331/368	TOMM40, APOE, RFC3, TTLL7, PAX2, SASHI
2009	Harold (Harold et al., 2009)	US/ Europe	3941/7848	APOE, TOMM40, CLU, PICALM
2009	Lambert (Lambert et al., 2009)	Europe	2032/528	APOE, CR1, CLU
2009	Carrasquillo (Carrasquillo et al., 2009)	US	844/1255	PCDH11X
2009	Poduslo (Poduslo et al., 2009)*	US	140(family) & 199 (unrelated)/85 (unrelated controls)	TRPC4AP
2009	Feulner (Feulner et al., 2009)	Germany	491/479	TOMM40, APOE
2009	Beecham (Beecham et al., 2009)	US	492/496	FAM113B, ZNF224
2008	Bertram (Bertram et al., 2005) #	US	941/404	CD33
2008	Abraham (Abraham et al., 2008)	UK	1082/1239	PVRL2, TOMM40, APOE

Year published	Authorship	Source	Initial Number of cases/ controls	Reported Genes
2008	Li (Li et al., 2008)	Canada/ UK	753/736	APOE, APOC1
2008	Webster (Webster et al., 2008)	US	664/422	APOE
2007	Reiman (Reiman et al., 2007)	US/ Netherlands	415/260	GAB2
2007	Coon (Coon et al., 2007)	US/Netherlands	664/422	APOE
2007	Grupe (Grupe et al., 2007)	UK/US	1428/1666	GALP, THNK1, chr14q32.13, PCK1, LMNA, PGBD1, LOC651924, chr7p152, THEMS, MYH13, CTSS, UBD, BCR, AGC1, TRAK2, EBF3

All case control studies except # (family-based) and * (family-based and case control)

Table 1. Selected recent GWAS studies and genes reported Adapted from the Alzheimer Disease database of the National Human Genome Research Institute, NIH. (www.genome.gov)

Recent findings in an adequately powered study failed to detect evidence for association between common variants in BIN1, CLU, CR1 and PICALM with CSF Aβ42 and ptau-42; This original hypothesis that gene variants might affect risk via an additive effect has yet to be substantiated. The authors concluded that the negative findings might suggest that the rare, rather than common, variations influence the CSF biomarkers and that other complex, non-additive genetic mechanisms, such as gene-gene and gene-environment interactions play major roles (Kauwe et al., 2011).

Copy number variations (CNV) which include segments of DNA ranging from 1kb – several Mb have been postulated to show differences in regional copy number when comparing 2 or more genomes although a recent study did not show any new SNPs of genome-wide significance (Heinzen et al., 2010).

3. From genomes to transcriptomes

Beyond DNA, other sources of variability are related to transcriptional, translational and post-translational modifications all the way to environmental factors. Hence from the study of the collective genotypes of an individual, one logically moves to the study of the genome-wide gene expression products, also called 'transcriptomics'. Here we are not looking only at which genes are present in an individual but which genes are actually transcribed into

messenger RNA (mRNA). While the genome is fixed for an individual, the transcriptome, i.e. the set of mRNAs produced in an individual corresponding to its genome, varies between different tissue types, across the life cycle of the cells, the life cycle of the organism, changes in the internal or external milieu, epigenetic and other factors. Hence the transcriptome reflects the genes that are being actively expressed in the tissue or cell type under study at one point in time. Transcriptomics usually employs high-throughput techniques based on oligonucleotide microarray technology platforms. For example Illumina's Whole-genome expression HT-12 v4.0 beadchip® targets more than 28,000 coding transcripts with well-established annotations derived from the RefSeq and the UniGene databases (www.illumina.com). Another one commonly used is the Affymetrix GeneChip Human Genome U133 Plus 2.0 Array® (www.affymetrix.com).

An example of a transcriptomic study would be comparing the mRNA levels between samples obtained from AD patients versus healthy controls. Significant differences in mRNA levels would imply that the genes are expressed to different degrees and this may lead downstream to different levels of protein translation and several steps further into disease phenotypes.

The first area of interest is whether the findings from transcriptomics correspond to those obtained from classical genetics or GWAS, for example, with reference to APOEe4, APP, PSEN1, PSEN2. A seminal study by Liang and coworkers demonstrated how different regions of the brain have different expression profiles by analyzing six anatomically discrete postmortem brain regions – entorhinal cortex, hippocampus, middle temporal, posterior cingulate, superior frontal gyri and primary visual cortex, comparing AD patients with normal controls (Liang et al., 2008). They showed a correlation between their findings with those obtained by other investigators using genotyping and GWAS. They found altered expression of factors previously implicated in AD pathogenesis, including APP, PSEN1, SORL1, and BACE1. APP gene expression was markedly increased in the hippocampus, medial temporal, posterior cingulated gyri and visual cortex in AD subjects whereas BACE1 displayed decreased expression in entorhinal cortex, medial temporal gyrus and hippocampus. They also found decreased expression for microtubule associated protein tau (MAPT) and decreased expression for alpha and beta tubulin proteins (which form the building blocks of microtubules) in entorhinal cortex, hippocampus, medial temporal and posterior cingulated gyri. These observations may help in explaining the relationship between NFTs and amyloid plaques.

Other investigators have focused on specific regions of the brain. Studying specifically the neocortex, Tan and co-workers found evidence of synaptic dysfunction, disturbed neurotransmission and activation of neuroinflammation in AD subjects when compared to normal controls (Tan et al., 2010). In a case control study of 22 AD subjects and 9 normal controls, Blalock examined gene expression patterns in the hippocampus and found up-regulation of many transcription factor/signaling genes regulating proliferation and differentiation, including tumor suppressors, oligodendrocyte growth factors and protein kinase A modulators. In addition, there was up-regulation of adhesion, apopotosis, lipid metabolism and initial inflammation processes. Protein folding/metabolism and signaling pathways were conversely down-regulated (Blalock et al., 2004).

To overcome the limitations of gene expression profiling due to the substantial intra-individual regional brain differences and inter-individual heterogeneity, Dunckley compared the gene expression profiles of three groups: (1) neurofibrillary tangle-bearing entorhinal cortex neurons from 19 AD patients with (2) adjacent non-tangle bearing neurons from the same patients and (3) histologically normal non-AD controls. 225 genes showed

progressively increased and decreased expression in the 3 groups. Not unexpectedly many of the genes coded for proteins implicated in NFT formation, especially in the early stages of formation (Dunckley et al., 2006).

Another approach employed was to correlate gene expression profiles according to histological staging of AD. Bossers and co-workers correlated changes in gene expression to the progression of AD in prefrontal cortex brain samples from 49 patients using the standard Braak histopathological system of staging post mortem brain tissue samples for neurofibrillary changes as an objective indicator of AD progression et al (Bossers et al., 2010). The Braak stages range from I to VI, with an additional Stage 0 referring to the absence of neurofibrillary change. They found 2 distinct patterns of tightly co-regulated groups of genes. Firstly, there was an increase in the expression of genes involved in synaptic activity and changes in the plasticity during the early Braak stages. However, the expression of this same group of genes was reduced in the later Braak stages. There was also an increase in intracellular amyloid beta staining from Braak Stages I to II but a decrease in Braak stages IV to VI. For the genes up-regulated in the early Braak stages, there were several genes involved in amyloid precursor protein processing and beta amyloid clearance. The authors thus suggested that the temporally correlated upregulation of synaptic genes activity represents a compensatory mechanism against increased soluble amyloid beta (Aβ) levels, and that this could possibly be a good place to identify new targets of anti-dementia drug development.

The most important factor in transcriptomics and also the downstream 'omic's is the tissue of study. Human brain tissue for analysis can only be obtained post-mortem and sampled only once. This tissue usually reflects a late-stage disease unless an AD subject had died of another cause early in the disease. Moreover RNA quality and quantity are highly dependent on the pre and post-mortem conditions and around tissue harvesting. The other major concern is the region of sampling as the brain is anatomically and histologically extremely heterogeneous. The choice of which anatomical brain region to study, gray versus white matter, neocortex versus archicortex greatly influences the results. Therefore much transcriptomic research endeavor has been directed towards blood. Blood is readily available and can be re-sampled repeatedly, allowing for longitudinal assessment of gene dysregulation at different disease stages. This could assist in making diagnoses, tracking the disease course and evaluation of disease altering interventions. Blood derived nuclear material is by no means a direct proxy for neuronal tissue, but it may reflect certain aspects of the CNS milieu, or systemic manifestations of the underlying disease. More importantly they can serve as peripheral biomarkers of disease. Gene expression profiling of peripheral blood has been shown to provide distinctive profiles for a few neurological conditions (Tang Y et al., 2001, 2003). Similarly psychiatric disorders were also found to have unique gene expression signatures (Tsuang et al. , 2005). Nevertheless extrapolating blood-derived data to the brain tissue still poses major challenges. In transcriptomic studies, due to the large numbers of genes in the genome that are differentially expressed, the up or down-regulated genes may be implicated as potentially casually related to AD pathology, downstream consequences of the disorder, reflect compensatory mechanisms, or found simply by chance alone. This type-one error is likely by virtue of the large number of genes sampled.

Probably the first major publication on the blood-derived gene expression for AD was by Maes and his co-workers who studied the expression profiling of blood mononuclear cells of AD subjects versus normal controls (Maes et al., 2008). They reported that 28% of the upregulated genes and 16% of the downregulated genes have been previously reported to

exhibit similar expression patterns in AD brain, whereas only 4% of affected genes were divergent in terms of expression between blood and brain. This comparison is important as it suggested the systemic nature of altered gene expression in AD and demonstrated the usefulness of blood as putative probes of CNS dysfunction. After comparing over 6000 genes, they found a significant decline in gene expression in the pathways of cytoskeletal maintenance, cellular trafficking, cellular stress response, redox homeostasis, transcription and DNA repair (Maes et al., 2007). Moreover they reported that the majority of upregulated transcripts function in apoptotic and inflammatory pathways, including those involved in TNF alpha signaling and caspase pathways. Using whole blood samples of AD patients versus normal controls, Grunblatt et al found that five out of 33 genes were differentially expressed and showed significant correlation to the severity of AD (Grunblatt et al., 2009).

More recently, Booij et al developed a 96-gene microarray using blood samples from a large clinical cohort of 203 probable AD patients and 209 cognitively healthy age-matched controls and has patented it for commercial use, specifically for detection of early AD - ADtect® by DiaGenic ASA based in Oslo, Norway (www.diagenic.com). A disease classification algorithm was developed on samples from 208 individuals (AD = 103; controls = 105) and was validated in two steps using an independent initial test set (n = 74; AD= 32; controls = 42) and another second test set (n = 130; AD= 68; controls = 62). In the initial analysis, diagnostic accuracy was 71.6±10.3%, with sensitivity 71.9±15.6% and specificity 71.4±13.7%. (Booij et al., 2010; Rye et al., 2010).

In summary, the transcriptomic studies regardless of tissue of origin, has yielded a large number of genes and putative mechanisms and pathways, including the 'usual suspects' as outlined previously. However no simple conclusions can be arrived at from this approach at this time. The fragmented and sometime incongruous lists of pathways and mechanisms attest to the heterogeneous nature of AD and await further replication and confirmation. Furthermore, most of the transcriptomic studies have focused on neuroanatomical and histological stages of AD. The current lack of longitudinal data and translation of genetic correlations to neuroimaging changes limits its role to a primarily investigative exploration of AD pathogenesis.

4. The other 'Omics'

Looking further downstream, although microarray studies can reveal the relative amounts of different mRNAs in the cell, levels of mRNA per se are not directly proportional to the level of the proteins they code for nor the eventual protein configuration. Each protein first arises as an unfolded polypeptide when translated from mRNA to a linear chain of amino acids. The amino acids interact with each other to produce complex three dimensional structures, and their folding configuration is essential to protein function. The complete protein complement of a cell, including protein structures is now referred to as the proteome. Proteomics refer to the study of the plethora of proteins across the whole organism and is one step closer to the phenotype (Simonsen et al., 2007).

Like transcriptomics, proteomics is usually applied to compare the differences in abundances of proteins between diseased and normal subjects. The technologies involved in proteomics are very complex and beyond the scope of this review. Proteomic discoveries are limited thus far but more would be expected in the next few years. The proteomic techniques have been applied to discover biomarkers for AD in CSF but the results thus far are inconclusive. Simonsen et al found a 17-protein biomarker using cerebrospinal fluid

samples to predict the progression from mild cognitive impairment to AD in a sample of 113 patients (Papassotiropoulos et al., 2006). German et al. reported finding three discriminating peaks in common but these peaks still await identification of the proteins and peptides (German et al. , 2007). Portelius proposed that targeted proteomics on Aβ may provide novel assays for biomarkers for AD (Portelius et al., 2008). Investigating the proteome of the hippocampus, Sultana and her co-workers employed 2-dimensional gel electrophoresis and mass spectrometry to determine the changes in protein levels in AD and controls (Sultana et al. , 2007). They identified 18 proteins with altered protein levels and which were involved in regulating different cellular functions. This study gives preliminary data on the levels of key proteins in the AD brain. A comprehensive review of the state of the art of proteomics in AD has been published by Zellner (Zellner et al., 2009).

As a corollary to proteomics, metabolomics is the global approach to understanding regulation of metabolic pathways and metabolic networks (Kaddurah-Daouk et al., 2009). This involves the study of the complete set of small-molecule metabolite, such as metabolic intermediates, hormones, other signaling molecules, and secondary metabolites. The metabolome is dynamic, changing from minute to minute and provides a snapshot of the physiology of the cell. It is not currently possible to analyze the entire range of metabolites by a single analytical method. Kaddurah-Daouk compared samples from post-mortem ventricular cerebrospinal fluid of 15 AD with 15 non-demented subjects and identified alterations in tyrosine, tryptophan, purine and tocopherol pathways in patients in AD (Kaddurah-Daouk et al., 2010). She also noted a reduction in norepinephrine and its related metabolites. Barba and co-authors published a useful review of the rationale and methodology of metabolomics in AD (Barba et al., 2008). The applications for metabolomic analysis in AD is still in infancy but will emerge as a powerful tool in CNS research, as it complements data derived from the other 'omics' to assist in providing a systems approach to the study of human health and disease.

Epigenetics refer to molecular and cellular effects on gene expression without a change in the DNA sequence. These effects include DNA methylation, histone modification and RNA mediated gene silencing. Epigenomics is the genomic-wide study of epigenetic effects. While all nucleated cells in an individual contain the same genome, they contain very different epigenomes depending on tissue type, developmental stage, environmental influences and other parameters (Murrell et al., 2005; Stamatoyannopoulos et al., 2008). These affect the individual presently and can be transmitted into the next generation without a change in the underlying DNA code. Epigenetic contribution is vital to achieve either stable expression or repression of genes at various stages of development (Chouliaras et al., 2010; Wu et al., 2008).

DNA methylation is currently one of the most studied modification and is accomplished through DNA methyltransferases, which transfer a methyl group to the cystosine of CpG dinucleotides. The cystosine DNA-methyltransferase genes play a critical role in the establishment of transcriptionally repressive complexes. It plays an important role in gene silencing and regulating gene expression. Epigenetic mechanisms are dynamic and changeable even in fully differentiated brain cells. Among the various epigenetic mechanisms, histone acetylation and phosphorylation can open up the chromatin structure and may favor gene transcription. DNA methylation is more often associated with increased condensation of chromatin and gene silencing. However the effects of such epigenetic mechanisms may be gene dependent (Gräff et al., 2009).

In investigating LOAD, Wang and his co-workers proposed that epigenetic contribution in the development of the disease could be inferred from several observations (Wang et al., 2008). These include the fact that sporadic cases of AD dominate over familial ones; a concordance rate of monozygotic twins well below 100%; differential susceptibility and course of illness in males and females; parent-of-origin effect; and relatively late age of disease onset. In AD brain they found aberrant histone modifications and abnormal folate (Coppede et al., 2010) and homocysteine levels, which were indicative of abnormal methylation homeostasis and epigenetic dysregulation. Even for EOAD, the difference in penetrance and expressivity can be attributed in part to epigenetic phenomenon. They argued that the epigenome is particularly susceptible to dysregulation during embryonic and neonatal development, puberty and old age. When studying DNA methylation patterns on a series of candidate genes in postmortem brains and lymphocytes from LOAD patients versus healthy controls, they further found that the largest inter-individual variance in DNA was observed in PSEN1 and APOE promoters and postulated that hypomethylation of PSEN1 promoter could induce an overexpression of PSEN1. They also found that there were substantial differences in the epigenetic profiles between old monozygotic twins that can be attributed to one's environmental exposure, lifestyle, diet, or merely stochastic fluctuations. They reported that the strongest age-effects were detected in NCSTN gene that codes for nicastrin, which participates in the regulation of gamma-secretase cleavage of the APP. Hence they hypothesized that neuronal tissue in the AD brain may be prone to collecting epimutations with time due to their post-mitotic state, as opposed to cells which are constantly being renewed.

Mastroeni and co-workers studied the immunoreactivity for two markers of DNA methylation and eight methylation maintenance factors in the entorhinal cortex layer II, a region that has been implicated in the histopathology of AD (Mastroeni et al., 2010). They demonstrated neuronal immunoreactivity for all 10 of the epigenetic markers and factors, with significant decrements in AD cases. These decrements were particularly marked in certain neurofibrillary tangle-bearing neurons. In addition, two of the DNA methylation maintenance factors were decreased in AD subjects. Hence they concluded that epigenetic dysfunction occurred in AD-vulnerable neurons.

5. Clinical applications of the 'Omics'

The involvement of genetic approaches in the diagnosis, risk prediction of AD in the established and at-risk (pre-dementia) states includes its use in combination with clinical parameters and biomarker supplementation (Crunchaga et al., 2010). Another potential application is based on quantitative endophenotype data to provide greater statistical power for subject inclusion in clinical therapeutic trials via genetics-imaging approaches. This later approach has been adopted by the US NIH Alzheimer's Disease Neuroimaging Initiative (ADNI) and European Union FP6-funded AddNeuroMed study (Mueller SG, 2005) (Lovestone et al., 2007; Shen et al., 2010). Specific loci such as PICALM has been shown to be the most significant gene associated with entorhinal cortical thickness (Biffi et al., 2010). Over-representation of rs10845840, located in the GRIN2B gene, which encodes the N-methyl-d-aspartate (NMDA) glutamate receptor NR2B subunit has been found to be associated with lower temporal lobe volume (Stein et al., 2010). Distinct variants of SORL1 has been demonstrated to be associated with cerebrovascular and neurodegenerative

changes related to AD (Cuenco et al., 2008); and APOE and TOMM40 with multiple brain regions (Shen et al., 2010).

APOE genotype has also been shown to be associated with increased blood oxygen level-dependent (BOLD) signal on functional magnetic resonance imaging (fMRI) in the occipital and perisylvian cortices bilaterally. More work needs to be done looking at the paradigm, family history and age to further interpret the BOLD differences between e4 carriers and non-carrier states, which might provide information on default modal networks involved in AD (Ringman et al., 1993; Trachtenberg et al., 2010).

The effects of genetics on therapeutic response to AD are potentially via the use of genomic information for the selection of at-risk groups as well as genetic influences on treatment efficacy and complications in the burgeoning field of pharmacogenomics. In particular, it has been shown that those with APOEe4 genotype do better on donepezil during the initial 1-year period (Petersen et al., 2005); but fair less well during the phase II trial of rosiglitazone (Risner et al., 2006) (peroxisome proliferator-activated receptor γ agonist), and the higher incidence of reversible vasogenic edema when treated with bapineuzumab (Kaufer et al., 2009). There is also some epidemiological rationale for the use of curcuminoids in AD which may be explained by the enhanced phagocytosis of Aβ via upregulation of transcription of specific genes (MGAT3) and translation of TLR2-4 (Fiala et al., 2007).

The main utility of GWAS currently lies in its potential in their hypothesis-generating nature to understand the pathophysiological pathways and ability to correct for population stratifications using principal component adjustments in homogenous populations, especially when low levels of increased risks are involved. Although there are current studies proposing the use of a 96-gene expression array (using blood RNA) for early AD diagnosis (Booij et al., 2010; Rye et al., 2010), the clinical accuracy is modest, estimated at 80%, and comparable to current clinical assessment methods.

While the idea of a diagnostic gene test kit to diagnose early AD is appealing, we have to be mindful of the diagnosis in asymptomatic at-risk individuals, given the current lack of concrete benefit of disease-modifying treatments and the modest benefits conferred by cognitive enhancers (Caselli et al., 2010). There are also concerns about the amount of age-related AD-like pathology one would accept as normal before offering potentially useful but yet unproven treatment strategies, which might promote 'cognitive hypochondriasis'. An example would be the controversial "Alzheimer's Mirror" genetic test developed by Smart Genetics, a Philadelphia company, which developed test kit for APOE variants and ceased operations 8 months after initial launch (Erika et al., 2008).

6. Limitations of methods and works in progress

Foremost is the absence of a time axis as most studies are based on a snapshot. While this is not vital in DNA studies, it is very relevant for transcriptomic, proteomic, metabolomics, epigenomic and most imaging studies. As mentioned previously, human tissue derived from postmortem specimens largely reflects advanced or terminal stage of disease and the quality of the tissue is dependent on the agonal and postmortem conditions. CSF and blood can only be a proxy of the intracerebral condition. However sampling CSF and blood allows longitudinal study and repeated sampling. Another major issue is 'spatial'. The brain is not a homogenous tissue and regional differences are significant. Hence whole brain sampling will not reflect regional variation and significant differences may be 'lost in translation'. This

has been somewhat mitigated by microdissection and single-cell sampling. However looking at individual cells or cell types ignores the complex and interconnected nature of brain tissue and function. Other researchers have focused on studying transgenic mice or cell lines as informative proxies. Therefore there is no single best approach, and a combination of approaches, analyzed collectively and stratified according to ethnic variability will likely yield the most meaningful results.

There is currently strong interests in genomics and the various groups, for example the Dominantly Inherited Alzheimer Network (DIAN) (http://www.dian-info.org), which is presently enrolling study participants who are biological adult children of a parent with a mutated gene known to cause dominantly inherited Alzheimer's disease. Others include the ADNI Genetics Core and OPAL (Opportunity for Prevention of Alzheimer's) (http://www.opalstudy.org) which are recruiting normal subjects, defined by neuropsychological testing, between 60 and 87 years and genotyped for APOE status and TOMM40 '523 polymorphisms. Then, based on their age and TOMM40 status, they will either fall into the high-risk or low-risk category for developing Alzheimer's in the next five years with potential therapeutic drug administered.

7. Conclusions

In recent years, there has been much progress made in the recent high throughput technologies that has led to a better understanding of AD. Mutations in the genes APP, PSEN1 and PSEN2 in autosomal dominant inheritance patterns in EOAD are fairly well established. For LOAD, with an estimated heritability of around 60%, the principal susceptibility gene remains APOEe4 with several others, which play much lesser roles. There is evidence for APOE gene profiling for prospective epidemiological study research and pharmacogenetics in identifying high-risk individuals as well as the potential response to current therapeutic agent with calls for APOEe4 genotype to be a covariate for AD clinical trials in view of its modulatory effect on disease progression (Farlow et al., 2010). Broader genetic profiling via GWAS approach very recently has identified susceptibility at-risk genes. However, the individual effects of these identified gene loci are individually small but may identify genes for further functional investigation.

The endeavors in gene expression, proteomics and metabolomics, all downstream from genomics, are relatively early in their development and the results are very preliminary. This is in part due to the limitations of the technology and the tremendous challenges posed by analyzing huge datasets with current bioinformatic and computational biology capabilities. Epigenomics is another burgeoning area of tremendous promise. It runs parallel to genomics for it affects gene transcription and translation in ways beyond the actual DNA code. Extraneous and environmental factors can affect the epigenetic modifications. While DNA methylation and histone modifications are better understood, a far larger scope of epigenomics lies in the near horizon. Integrative approaches to complex multi-gene interaction and epigenetic effects using sophisticated algorithms have begun and would likely yield more robust results. However, the current state of genomics in diagnostics, risk prediction and potential inclusion in therapeutic trials still favor the established APOE given the demonstration of modulatory effect even in the preclinical stages of disease. The unfulfilled potential of these other high throughput platforms lie mainly in the lack of longitudinal data reflecting change as well as correlations with a more 'measurable' structural and functional brain measure, limiting its current role to a purely investigative one.

Moving forward, large scale collaborative efforts across high throughput technologies are required to understand the role of amyloid, lipid homeostasis and chronic inflammation in AD pathogenesis as well as therapeutic interventions directed at these various proposed pathways.

There is a need for diagnostic-therapeutic co-development approach especially in complex disorders such as AD to create diagnostic and prognostic algorithms via a multi-modal approach together with clinical and biomarker supplementation. This will lead to eventually fulfill the role of genomics in predictive, preventive and personalized medicine. In anticipation of this, a recent GRIPS (Genetic Risk Prediction Studies) Statement recommendation has also been published to enhance transparency of study reporting, and thereby improve the synthesis and application of multiple studies which may differ in terms of study design, protocol and analytical methods (Janssens et al., 2011).

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3

Addressing Risk Factors for Neurocognitive Decline and Alzheimer's Disease Among African Americans in the Era of Health Disparities

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1. Introduction

More than any other time in the past, the world is now paying attention to the public health implications of neurological health, ranging from cognitive aging, and mild cognitive impairment to neurodegenerative conditions such Alzheimer's disease. It is estimated that every 69 seconds someone develops AD related symptoms, and by 2050, the rate of developing this condition will be about every 33 seconds (Alzheimer's Association 2011). As a leading public interest organization, the national Alzheimer's Association 2011 report also mentions that in 2010, 14.9 million family members and friends provided 17 billion hours of unpaid care to those with Alzheimer's and other types of dementia, a care valued at $202.6 billion. Adverse neurocognitive functioning is now front and center of public health concerns as more than 5.4. million people are living with Alzheimer's disease (AD).

Within the continuum of health disparities, we are now learning that risk for Alzheimer's disease (AD) is 14-100% more likely for African Americans when compared to Caucasian Americans (Taylor, Sloan et al. 2004; Manly 2008; Weiner 2008). For all too many African Americans, the biopsychosocial threats to brain health and neurocognitive functioning are profoundly influenced by seen and unseen factors, ranging from inequalities in education, health, neighborhoods and communities, vocational opportunities, and access to wellness promoting resources. The cumulative effect of acute and chronic inequalities may be expressed through a differentially higher burden for brain-behavior vulnerability. Hence, identifying factors that are leading to an increased risk for adverse neurocognitive health and wellbeing is of highest priority in the black community.

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2. Public health implications of dementia related health

Dementia is a general term used when referring to symptoms caused by disorders that affect the brain. Persons with dementia have an assortment of neurobehavioral symptoms affecting and interfering with cognitive performance, memory, personality, and activities of daily living. Alzheimer's disease (AD) is a specific type of disease process, and in the early 1990s this condition was estimated to affect 1.5 to 2 million people (Brinton 1993).

Although AD, closely followed by vascular dementia, is the more common form of dementia, African Americans are more than twice as likely to develop dementia symptoms when compared to Caucasian Americans (Alzheimer's Association 2002; Demirovic, Prineas et al. 2003; Fitzpatrick, Kuller et al. 2004; Bennett 2007; Plassman, Langa et al. 2007; Fitzpatrick, Kuller et al. 2009). For over a decade, the prevalence of dementia in the African American community has been characterized as a "silent epidemic". Issues of accessibility to medical care have been linked to the prevalence of dementia in the black community (Alzheimer's Association 2002). Chances are, African Americans may receive critical neurological attention too late and miss the window for therapeutic interventions. This is particularly concerning given the growing discussion about reversal of adverse neurocognitive functioning, including dementia (Arendash, Mori et al. 2009; Etgen, Sander et al. 2010; Mangialasche, Solomon et al. 2010). Moreover, African-Americans have a higher rate of vascular disease such as T2DM, a condition thought to increase brain dysfunction in higher cortical abilities. Despite the significance of an early diagnosis of Alzheimer's disease (AD), African Americans are diagnosed in later stages of the disease, demonstrate greater cognitive impairment at the time of diagnosis, and receive less adequate treatment following diagnosis (Hughes, Tyler et al. 2009; Chin, Negash et al. 2011). As the major public health emphasis for African Americans remains focused around physical disease (e.g., heart disease, lower limb amputations, and kidney disease), there is growing concern that health care professional and the patients for whom they provide care may be missing opportunities for connecting integrative mind-body health.

Although the exact cause of Alzheimer's is not completely understood, experts have recently identified a genetic and physiological mechanism involving the insufficient breakdown and recycling of amyloid protein in the brain. This action process leads to cell death and weakened neuronal communication. Guidelines identifying and classifying individuals with possible, probable, and definite AD were established through a joint effort supported by the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) and the Alzheimer's Disease and Related Disorders Association (ADRDA) (McKhann, Drachman et al. 1984). Multi-center studies have shown these guidelines to have adequate sensitivity (83%) and specificity (84%) (Blacker, Albert et al. 1994). Additional diagnostic criteria for probable AD include medical examination, deficits in two or more neurocognitive domains, progressive worsening of memory and other neurocognitive domains, no disturbance of consciousness, and absence of systemic disorders or other brain diseases that could be the cause of decline.

3. Type 2 diabetes immediate and intermediate crises

T2DM is a blood sugar dysregulation disorder associated with insulin resistance, insulin deficiency, or both. Individuals can remain undiagnosed for as long as seven years (Harris, Klein et al. 1992). T2DM is a costly, life-threatening chronic disease that is associated with a

high degree of medical complications and an estimated price tag of $100 billion a year (O'Connor 2006). This metabolically driven chronic illness is at an all time high in black America, and tied primarily to health behaviors that include poor dietary habits and sedentary lifestyles (Bell, Bertoni et al. 2010). In addition to physical health markers, persons living with diabetes are known to live with an elevated risk of developing adverse neurocognitive problems, ranging from mild cognitive impairment to more severe brain health problems including AD related dementia. Memory related problems were initially reported, particularly among persons with complications of peripheral neuropathy and/or elevated A1c levels (Perlmuter et al., 1984). These findings have been substantiated through meta-analysis research methodology (Strachan, Deary et al. 1997). At this, based upon research published between 1980 and 1995, Strachan and colleagues found that there was a connection between history of T2DM and verbal memory dysfunction. Supplementing these findings, multi-site longitudinal studies including, the Rotterdam Study (Ott, Stolk et al. 1996), the Honolulu-Asia Aging Study (Peila, Rodriguez et al. 2002), and the Epidemiology of Vascular Aging Study (Fontbonne, Berr et al. 2001) have also found evidence of a diabetes-neurocognitive functioning compromise. When studying younger Type 2 diabetes cohorts, non-age related changes in information processing speed are observed (Ryan and Geckle 2000). As noted in their published findings from the Atherosclerosis Risk in Communities cohort, a large biracial, multisite, longitudinal investigation of initially middle-aged individuals, (Knopman, Boland et al. 2001) observed that persons with Type 2 diabetes demonstrated problems with language skills tasks.

The stated inconsistencies in the area of diabetes and brain may reflect differences in how well researchers have characterized the population. Toward achieving a more standardized covariate modeling of the data, some relevant markers need mentioning. Because the community of persons living with T2DM is not homogenous, it is important to explore the range of biopsychosocial candidate markers implicated in a loosely defined Diabetes-neurocognitive risk paradigm. Duration of diabetes is identified as a risk marker. For instance, when persons with well controlled diabetes are compared with relatively healthy individuals without diabetes, there are relatively no discernable differences on behavioral tasks measuring verbal memory, except when duration of diabetes is considered (Cosway, Strachan et al. 2001). Duration of diabetes as a brain-cognition threshold marker has been previously reported in the literature (Stewart and Liolitsa 1999; Logroscino, Kang et al. 2004).

Insulin resistance is a physiological condition where the natural hormone, insulin, becomes less effective at lowering blood sugars. Insulin resistance as also been addressed has an inflammatory process (Shoelson, Lee et al. 2006). The poor supply of glucose to the brain can also cause neurological symptoms such as: drowsiness, confusion, loss of consciousness, seizures, and permanent brain damage. Elevated blood glucose levels can damage small blood vessels (miscrovascular disease) that in turn affect the eyes (diabetic retinopathy), kidneys (nephropathy), and nerve damage (i.e.,neuropathy). Elevated blood sugar or hyperglycemia is a significant complication experienced in Type 2 diabetes. Elevated blood sugar is shown to decrease acetylcholine synthesis in rat brain (Squadrito, Trimarchi et al. 1986; Welsh and Wecker 1991). Acetylcholine is a neurotransmitter that appears to be involved in learning and memory, and is severely diminished in the brains of persons with Alzheimer's disease. Insulin resistance, as inflammatory response, may lead to a situation of brain cells losing their ability to convert glucose to energy, and begin to starve.

There is reason to believe that dementia, in the context of T2DM, is driven through a stress pathway and inflammatory sensitive process. Stressful situations repeatedly activate

Allostatic load responses leading to a wear and tear of the body (McEwen and Stellar 1993). This cumulative wear and tear or Allostatic load represents a model of how psychosocial factors impact the body and lead to disease (Nielsen, Seeman et al. 2007). There seems to be three stages of allostasis. During the primary stage the body releases stress hormone molecules and their antagonists as well as anti-inflammatory cytokines which are the Allostatic load biomarkers called primary mediators (Juster, McEwen et al. 2010). These molecules' synergistic effects impact cellular levels activities which compromise the integrity of Allostatic mechanisms (Juster, McEwen et al. 2010). In order to compensate for unbalanced production of these primary mediators, subsidiary biological systems change their operating ranges to maintain functions at chemical, tissue and organ levels (Juster, McEwen et al. 2010). At a secondary stage, also called prodromal stage, metabolic, cardiovascular and immune parameters arrive at sub-clinical levels (Juster, McEwen et al. 2010). The third and final stage or the Allostatic overload is when physiological dysregulation leads to tertiary outcomes or disease (Juster, McEwen et al. 2010). Even more dangerous are brain changes that are associated with chronic stress and allostasis since it further reduces our abilities to cognitively process and physiologically respond to stressors (Juster, McEwen et al. 2010). Therefore, this model explains how the dysregulation of systems that are supposed to balance our bodies through environmental demands serves as a key mediator for increased disease risk (Nielsen, Seeman et al. 2007).

Several, serum markers of inflammation have been speculated to burn neurocognitive abilities. Findings from the Health, Aging, and Body Composition Study demonstrated that serum markers of inflammation, especially IL-6 and CRP, were prospectively associated with cognitive decline in well-functioning African-American and white elders, as measured by the Modified Mini-Mental State Examination (3MS)(Yaffe, Lindquist et al. 2003). C-reactive protein (CRP), a marker of inflammation, is generated at a higher level in obese people, and mild elevation in CRP increases risk of heart attacks, strokes, high blood pressure, muscle weakness and fragility. In a more recent cognitive aging related study (Noble, Manly et al. 2010) , findings showed that participants in the highest high-sensitivity (hs) CRP tertile had higher adjusted odds of impaired memory (odds ratio [OR], 1.5; 95% confidence interval [CI], 1.0-2.1; $P = .03$) than participants in the lowest tertile. Subjects in the highest hs CRP tertile also had greater odds of visuospatial impairment (OR, 1.6; 95% CI, 1.0-2.3; $P = .03$). Higher hsCRP was not associated with executive or language impairment. Persons with at least 1 *APOE* ε4 allele and hsCRP in the highest tertile had the greatest odds of impaired memory (OR, 2.7; 95% CI, 1.6-4.4). While CRP is associated with body mass index, the evidence on the association between BMI and cognitive functioning in aging research is mixed. For instance, in one biracial community population aged 65 and older, BMI was not predictive of cognitive decline in a cognitively unimpaired community population (Sturman, de Leon et al. 2008); however, results from a Swedish community population study, published in the Journal of Gerontology (Dahl, Hassing et al. 2010), found that higher midlife BMI scores precede lower general cognitive ability and steeper cognitive decline in both men and women. Yet, data from the Cardiovascular Health Study (Fitzpatrick, Kuller et al. 2009) seems to have indicated no association indicated no association of BMI with greater dementia risk.

As diabetes is a constellation of signs and symptoms, a salient feature of poor glycemic control is increased risk for atherosclerosis. Atherosclerosis, a disease of vessel wall thickness and plaques of the carotid arteries, is a risk factor for non-age related changes in brain functioning (Hofman, Ott et al. 1997) and a consequence of several lifestyle factors

(e.g., cigarette smoking, sedentary lifestyle, obesity). Reporting on their findings from the Rotterdam Study, Hofman and associates (year) found that atherosclerosis is associated with dementia and the odds ratio for Alzheimer's disease in those with severe atherosclerosis compared with those without atherosclerosis was 3.0 (95%, CI 1.5-6.0; p = 0.001).

Similar to atherosclerosis, hypertension is shown to alter brain functions (Kilander, Nyman et al. 1998; den Heijer, Launer et al. 2005). Variables that may alter (i.e., moderate) the relationship of hypertension to brain functioning include age, education, several characteristics of elevated blood pressure or hypertension, and the presence of concurrent diseases (Waldstein 2003). The data suggests that while hypertension is a risk factor for cerebrovascular health independent of Type 2 diabetes, the joint contributions of both diseases elevate stroke mortality (Hu, Sarti et al. 2005). Pathologies of the peripheral vasculature are responsible for a wide variety of clinical conditions, premature disability, and early death. With these and other factors in mind, T2DM increases risk for peripheral arterial disease (PAD), a common disease affecting 8 to 12 million Americans (Selvin and Erlinger 2004) Occurring in about 8% of African Americans, 5% of Mexican Americans, and about 3% of Caucasian Americans(Rucker-Whitaker, Greenland et al. 2004; Nelson, Reiber et al. 2007; Meadows, Bhatt et al. 2009). PAD occurs when a fatty material called plaque builds up on the inside walls of the arteries that carry blood from the heart to the head, internal organs, and limbs. Peripheral arterial disease (PAD) is a manifestation of systemic atherosclerosis, is associated with significant morbidity and mortality, and is an important marker of subclinical coronary heart disease, and stroke (Selvin and Erlinger 2004; Khattab, Ali et al. 2005). PAD prevalence increases dramatically with age and disproportionately affects persons of color (Collins, Petersen et al. 2003; Selvin and Erlinger 2004), and the comorbidity of PAD and Type 2 diabetes greatly increases mortality (Leibson, Ransom et al. 2004). Three types of studies on the investigation of cognitive functioning have emerged including PAD severity compared to healthy controls (Waldstein, Tankard et al. 2003), asymptomatic PAD and inflammatory process (Mangiafico, Sarnataro et al. 2006), and PAD and coronary heart disease with stroke (Phillips and Mate-Kole 1997; Rao, Jackson et al. 1999); (Mukherjee, Eagle et al. 2007). PAD can impair physical health and diminish the ability to walk since physical activity is associated with oxygenated blood flow to brain and cognitive agility (Yaffe, Barnes et al. 2001; Allmer 2005; Singh-Manoux, Hillsdon et al. 2005). Jointly, poor circulation in the legs can be associated with poor circulation in the heart and in the brain. Such questions would concern how and to what extent persons with T2DM develop their continuum of risk for adverse brain health and neurocognitive dysfunction, including neurodegenerative disease, such as dementia of the AD type. Recent clinical data show that rates of diabetes and other cardiovascular disease health problems are increasing among African Americans in their middle-ages and younger (Krishnan, Cozier et al. 2010; Cali and Caprio 2008; Steinberger, Daniels et al. 2009). An earlier onset of physical health problems correlated with accelerated brain aging will likely translate into a greater burden of neurocognitive problems in younger cohorts. To that extent, blood circulation components, including volume, flow, access, and nutrition quality may prove beneficial in elucidating the upward-bound body-brain functionality and dynamics central to critical psychoendoneuroimmunology questions.

For the most part, T2DM does not appear to have immediate effects on brain health and cognitive functioning. All things being considered, it is equally plausible that adverse neurocognitive risk and event is the summed expression of an inverse neurocognitive resilience-inflammatory response process. That is, as one's resilience is diminished there

might be an appreciable spike in inflammatory activity. Risk for brain health complications along the diabetes-brain connectivity spectrum is more characteristic of intermediate effects, especially when there is evidence of diabetes being poorly controlled. This said, a general saying in clinical neuropsychology is "its not just the brain it happens to; its whose brain it happens to". Such a statement warrants attention to acknowledging the absence of a social-ecological perspective in addressing prevention and treatment, as well as the formulation of intervention efforts in taking serious action to eliminate the burden of dementia related disparities faced by African Americans, particularly those suffering from vascular diseases.

4. Built environment and social determinants of AD risk

The built environment in the context of health can provide insight into how African Americans may experience a differentially high risk for dementia. The term built environment is used to signal human-made structures and how those environmental components come to shape our health, whether we are talking about neighborhood-level factors or more broad ranging elements that constitute community and its accessible and inaccessible resources. Within the built environment lies a host of factors operating in concert, but are there clues and solutions in the built environment that could be used to reposition translational research on brain and neurocognitive impairment research? As some brain functions are more sensitive to aging effects than others, neurobiological and contextual factors such as environmental demands and cultural experiences independently and simultaneously influence underlying neural circuits that activate and deactivate cognitive processes (Salthouse 1996; Baltes, Staudinger et al. 1999; Von Dras and Blumenthal 2000; Park and Gutchess 2006). Environmental factors shown to affect health include housing and indoor air quality (McEwen 2008; Lan and Lian 2009; van Hoof, Kort et al. 2010), neighborhood location, noise, crowding, socioeconomic status (Glass, Bandeen-Roche et al. 2009; Hackman and Farah 2009; Van Gerven, Vos et al. 2009; Yen, Michael et al. 2009), access to grocery stores, nutritious foods (Gomez-Pinilla 2008; Rose 2010), and space and safety forphysical activity (Kramer and Erickson 2007; Hillman, Erickson et al. 2008; Lautenschlager, Cox et al. 2008; Scarmeas, Luchsinger et al. 2009).

It is generally accepted that chronic stress and chronic physical diseases accelerate physiological changes in multiple organ systems independent of age (Sieck 2003; Roth, Mattison et al. 2004). As psychosocial markers, negative emotional functioning is problematic for achieving optimal brain health. That is, *emotional instability stimulates the release of various hormones, alters metabolic activity, and is linked to risk for elevated blood glucose levels.* Hostility and impatience influence body physiological in the direction of blood pressure elevations (Yan, Liu et al. 2003). Negative emotional states and both acute and chronic social stressors increase cardiovascular symptoms (Ferketich, Schwartzbaum et al. 2000; Everson-Rose and Lewis 2005). In postmenopausal women, the evidence suggests that perceived chronic stress was associated with decreased grey matter volume in the right orbitofrontal cortex and right hippocampus (Gianaros, Jennings et al. 2007). Studies have shown that chronic stressful life events (e.g., perceived discrimination or chronic burden) may impair the sympathetic nervous system, as well as neuroendocrine stability, cognitive and emotional functions that the hippocampus supports: spatial information, regulating the HPA axis and processing the contextual aspects of emotional events.(Krieger and Sidney 1996; Sapolsky 2003; Conrad 2006; Aldo Ferrara, Guida et al. 2007; Cohen, Janicki-Deverts et al. 2007; Albert, Ravenell et al. 2008).

For sometime now, researchers have found that chronic stress derails the body's physiology, including the brain's corticotropin-releasing factor, interleukin-6, brain-derived neurotrophic factor, and insulin-like growth factor-1(Bonne, Gill et al. 2010), and has a consequential impact on AD risk (Nakajima, Ohsawa et al. 2010; Gasparini and Xu 2003; Araki, Kume et al. 2009). To the point, chronic stress can diffuse neural signaling, diminish the cells ability to cope with adversity and potentially trigger accelerated age-related damage to the hippocampus. The adrenal glucocorticoids are thought to be responsible for this damage given its ability to compromise energy metabolism and make neurons more vulnerable to glutamate excitotoxicity (Smith 1996; Mattson, Maudsley et al. 2004). Hence, stress has been found to decrease brain-derived neurotrophic activity in the hippocampus and other possible brain areas. The term disrupted energy in the context of metabolism is being used more frequently in brain and neurocognition research. The pathogenesis of neuronal circuit dysfunction connects to overwhelmed neurocognitive compensatory mechanisms and is speculated to result from perturbation in cellular energy metabolism, level excitation or inhibition and neurotrophic factor release (Kapogiannis and Mattson 2011).

As a chronic stress psychosocial marker, socioeconomic status (SES) has received increasing attention as an explanatory mechanism for health disparities. SES and its associated proxy indicators (i.e. income, employment, education, housing, social cohesion, life expectancy, etc.) are thought to negatively affect healthcare utilization and health outcomes, including premature death (Gostin et al., 2004; McGennis, Williams-Russo, & Knickman, 2002). A number of studies have indicated that African Americans are at greater risk for suffering from chronic disease and disease morbidity often operating through injustice and discrimination (Clark, 2000; Kessler et al., 1999, Williams et al., 1997). Chronic stress reduces an individual's functional capacity, and has an untoward effect on sustainable healthy functioning. The "isms" that impact health outcomes (i.e. racism, institutionalism, ageism, sexism, etc.) increase mortality rates, as discrimination is inversely related to medical well-being, e.g. cardiovascular disease, (Krieger & Sidney, 1996), as well as emotional health (Branscombe, Schmitt, & Harvey, 1999; Fischer & Shaw, 1999).

A last and central component of the built-environment is access to and quality of caregiving on neurocognitive wellbeing. The role of African American caregiver in the case of caring for someone with adverse mental functioning is loosely addressed in the dementia literature. In the Black community caregiving occurs at several levels. Within the context of social services, caregiving is defined as providing assistance or care to someone who is unable to perform some or all Activities of Daily Living (ADLs) and/or Instrumental Activities of Daily Living (IADLs). Examples of ADLs are bathing, eating (unable to feed oneself), and dressing. IADLs are complex activities such as driving, planning, and cooking. There is some evidence suggesting African American couples have others to assist them with ADLs compared to Caucasian couples who only have themselves to rely on (Feld, Dunkle, and Schroepfer ,2005; Yarry, Stevens, McCallum, 2007).

In 2009, approximately 10.9 million family caregivers in the United States did not receive payment for the care they provided to a family member with Alzheimer's disease (2010 Alzheimer's Association Report). An *informal caregiver* is someone who is not being compensated monetarily for the care they provide. An informal caregiver can be a family member (e.g. husband, cousin, extended family member), a neighbor, or a friend. Burnout is potentially a caregiving risk factor as African American caregivers are less likely to utilize formal care such as respite care or nursing homes services (Fiscella, Franks, Gold, & Clancy, 2000; Cagney & Agree, 1999; Murtaugh, Kemper, Spillman, & Carlson, 1997; Belgrave &

Bradsher, 1994. Despite of the declining health of the elderly caregiver, they are more likely to be the caregiver and care recipient ((Yarry, Stevens, McCallum, 2007; Sorensen and Pinquart, 2005; Hooyman and Kiyak,1996). In North Carolina, the number of Alzheimer's/dementia caregivers was 311,578 in 2007 but rose to 356,851 in 2009. The 2007 group provided 268,953,971 hours of unpaid care that year at an economic value of $2,845,533,016. The 2009 group provided 406,381,406 hours of unpaid care that year at an economic value of $4,673,386,174. For South Carolina, the number of Alzheimer's/dementia caregivers was 159,221. They provided 137,439,556 hours of unpaid care that year, which had an economic value of $1,454,110,507 (Alzheimer's Association, 2008).

Although not conclusive, there is strong evidence that African Americans experience less caregiving depression compared with Caucasian Americans. (McCallum, Longmire, Knight, 2007; Sorensen & Pinquart, 2005; Plant & Sach-Ericsson, 2004; Dilworth-Anderson, Williams, Gibson, 2002; Knight, Silverstein, McCallum, & Fox, 2000; Cox, 1995; Allen, 1993). The church environment has been shown to serve as a potential emotional buffer among this population(Hines-Martin, 1998). Religion has been found to be a significant coping mechanism for African American informal caregivers. Caregivers who attend or are active members in a church report less experience of depression and stress (Sorensen & Pinquart, 2005; Coon et al., 2004; Haley et al, 2004; Stuckey, 2001; Hines-Martin, 1998; Wood & Parham, 1990). More than likely, the church buffering effect may be acting in concert with what has been termed the mutual aid system (Dilworth-Anderson, 1992; Wood & Parham, 1990). The mutual aid system is the extended family which included the parents, children, uncles and aunts, grandparents, close friends, and the community. Specifically, one study investigating the kin support and use of formal caregivers (i.e. licensed or certified professionals such as nurses, home aids, etc.) found that African American caregivers who are caring for an impaired elderly family member had a larger number of "nuclear kin" available to provide assistance compared to Caucasian caregivers (Sterritt, Pokorny, 1998). It has been further observed that African Americans caregivers are less likely to be spouses, and more likely to be extended family members (Dilworth-Anderson et al., 2002; Knight & McCallum, 1998).

5. Opportunities for promoting and restoring neurocognitive health

Restoration is generally thought of as involving an integrative renovator-rejuvenating dynamic embodying a holistic reserve necessary to achieving optimal physical, mental, and emotional health functioning. However, the pathways of restoration and reversal of adverse neurocognitive health remain poorly understood. There is growing speculation that increasing cognitive stimulation, consuming more fruit and vegetables, increasing physical activity, maintaining emotional balance, and reducing diabetes and its health related complications would have a noteworthy public health impact on reducing the incidence of dementia, outweighing even the effects of removing the principal known genetic risk factor (Ritchie, Carriere et al. 2010). As a component of the built environment, social network engagement has been described in terms of its brain health benefits. For instance, social network research suggests that social engagement surrounding mental, social, productive, and leisure activities appears to have an inverse relationship to dementia incidence (Amieva, Stoykova et al. 2010; Wang, Karp et al. 2002; Akbaraly, Portet et al. 2009; Hughes, Chang et al. 2010).

Caffeine's role in cognitive impairment research provides some preliminary insights. For instance, in the basic sciences, AD transgenic mice given a moderate level of caffeine intake

have been shown to experience a memory restoration and reversal of AD pathology, suggesting the treatment potential of caffeine in cases of established AD (Arendash, Mori et al. 2009). From human-level research, there are clinical findings demonstrating the association between caffeine consumption and lower cognitive change over time to be statistically significant for women only and dose-dependent (Ritchie, Artero et al. 2010).

The impact of physical activity, nutrition and dietary quality on the aging central nervous system has multiple implications for African Americans neurocognitive health.

There is increasing evidence that physical activity and diet are equally beneficial in reducing risk for AD (Scarmeas, Luchsinger et al. 2009). Results from the Cardiovascular Health Cognition study (N=299; mean age 78 years) show that greater amounts of walking related physical activity was related with greater gray matter volume, which is associated with protection from neurocognitive impairment(Erickson, Raji et al. 2010). Although typically treated as a separate component of health and wellbeing, diet and, nutrition are as relevant to deconstructing the neurological consequences of the built-environment as SES, chronic stress, and other psychosocial markers. As one of the Omega-3 fatty acids, DHA is considered one of the primary structural fatty acids in brain gray matter, influencing and promoting communication between brain cells.. Specifically, dietary intake of omega-3 fatty acids and weekly consumption of fish may improve visual, mental, cardiovascular health and reduce the risk of incident Alzheimer disease (Morris et al., 2003.; Mattson, 2004; Arterburn, 2006). In persons characterized with mild to moderate AD from the randomized double blind OmegAD trial, researchers found that administration of ω-3 fatty acid did not delay the rate of cognitive decline according to the MMSE or the cognitive portion of the Alzheimer Disease Assessment Scale. However, positive effects were observed in a small group of patients with very mild AD (MMSE >27 points) (Freund-Levi et al., 2006).

Data from the National Health and Examination Survey III examining the association of race, education, and dietary intake, showed that while education attainment was positively related to intake of calcium, magnesium, and potassium among whites, African Americans consumed less calcium, magnesium, and potassium regardless of educational achievement (Ford, 1998). As a function of diet, potassium supplementation has been studied on its effects to the blood pressure of African Americans, showing positive results in substantially reducing blood pressure in persons with low potassium consumption (Brancati et al., 1996; Appel et al., 2006). In a more recent study by Individuals and the National Health and Nutrition Examination Survey, utilizing the Continuing Survey of Food Intakes questionnaire, investigations revealed similar findings that African Americans in all age groups consume fewer mean servings of total dairy, milk, cheese, and yogurt per day than non-African Americans, and have lower mean intakes of calcium, magnesium, and phosphorus (Fulgoni et al., 2007). Among African Americans, the intake of fruits and vegetables, as well as food store characteristics, store location and perceptions of the selection/quality and affordability of fresh produce mediate perceived nutritional options, at the community level (Zenk et al., 2005;). Elsewhere, African Americans' higher frequency of eating at fast-food restaurants was positively associated with fair/poor self-rated health, weak belief in a diet–cancer relationship, low self-efficacy for healthy eating, weight dissatisfaction, and perceived difficulties of preparing healthy meals and order healthy foods in restaurants (Satia et al., 2004). At last, the issues of nutrition and dietary self-management represent a major piece of neurocognition health and the brain protection campaign. This is largely speculated from basic science studies, clinic-base trials, and cognition related epidemiological studies observing that higher dietary intake of

antioxidants, vitamins B6, B12,folate, unsaturated fatty acids and fish are related to greater likelihood of neurocognitive protection reducing risk for untoward neurocognitive functioning, including AD (Burgener, Buettner et al. 2008; van der Beek and Kamphuis 2008; Carrie 2009; Dangour, Whitehouse et al. 2010).

T2DM is almost always preceded by obesity. Obesity, eating behavior, and physical activity are also associated with multiple behavior changes including brain-behavior functioning. As the body of evidence linking nutrition and dietary behavior to incidence of neurocognitive illnesses increases, community-based researchers and practitioners will need to translate this knowledge into procedures and strategies to advance African American neurocognition risk prevention research. Such community-level interventions will want to be mindful of the need to better understand the barriers to consuming specific dairy products at all levels of African American communities (e.g., SES, education attainment, gender, urban vs. rural locations). While diet and nutrition may be important modifiable risk factors in preventing adverse neurocognitive disease, conclusive evidence has yet to emerge. (Coley, Andrieu et al. 2008).

In short, whether brain and its supporting abilities can be restored is stimulating debate across scientific fields. At the heart of this debate is a gene-environment that may actually represent the subcomponents of where you live, what you experience, how you feel, and how you relax. Stated slightly different, neurocognitive aging may be a function of built-environment-brain energy balancing operating on some biopsychosocial continuum across time.

6. Conclusion

6.1 Challenges, opportunities, and implications

Pathologies of the central nervous system and peripheral vasculature are responsible for a wide variety of clinical conditions, premature disability, and early death. T2DM is a diet, energy, nutrition, health behavior, and lifestyle driven condition. Brain health protection and promotion are equally responsive to diet, energy, nutrition, health behavior, and lifestyle components. Several nutritional factors can influence mental health, including: overall energy intake, intake of the energy-containing nutrients (proteins, carbohydrates, and fats), alcohol intake, and intake of vitamins and minerals. The downstream occurrence of elevated risk for acquired brain changes in Americans African is inextricably linked to the upstream chronic diseases strongly tied to the social determinants of health. The social determinants of neurocognitive impairment link to physical health conditions such as high blood pressure, diabetes, heart disease, stroke, and psychiatric health conditions such as chronic depression and excessive psychological distress, as well as social resource deprivation, all known risk factors for neurocognitive illness. The built environment in the context of health can provide insights about how African Americans may experience a differentially high risk for dementia. In turn, the socio-environment plays a major role from brain development, brain stimulation to brain nutrition, brain trauma and brain aging.

The bourgeoning research underscores that there exists multiple explanations for why Africans Americans are at higher risk for preventable neurocognitive illness. There appears to be consistent findings that social factors (e.g., discrimination, biased access to resources, environmental social injustice) are a major deterrent to sustainable neurocognitive quality of life. For T2DM, untoward neurobehavioral changes will negatively impact quality-of-life and self-care management, which could place African Americans living with chronic health

complications at additional disadvantage for underperforming in their daily physical disease management regimen, including adhering to medical regimen, scheduling appointments, health knowledge, health service utilization behavior, and prevention of secondary diseases. The factors contributing to psychosocial wellbeing may be important predictors of decreasing brain-behavioral status and might partly be related to experiences with racial discrimination. Understanding neurocognitive variability among persons living with diabetes, their activities of daily living, as well as their perceived quality of life requires a commitment to work across conventional disciplinary boundaries. Showing the relationship of psychosocial factors to differential risk for brain-behavior connectivity in a well characterized population will serve to help clinicians identify adults at high-risk for non-age related brain and cognitive changes and those who may be at additional high risk for self-management difficulties that could lead to stroke and/or dementia. Figure I, below, can be utilized to inform other researchers about these translational research efforts.

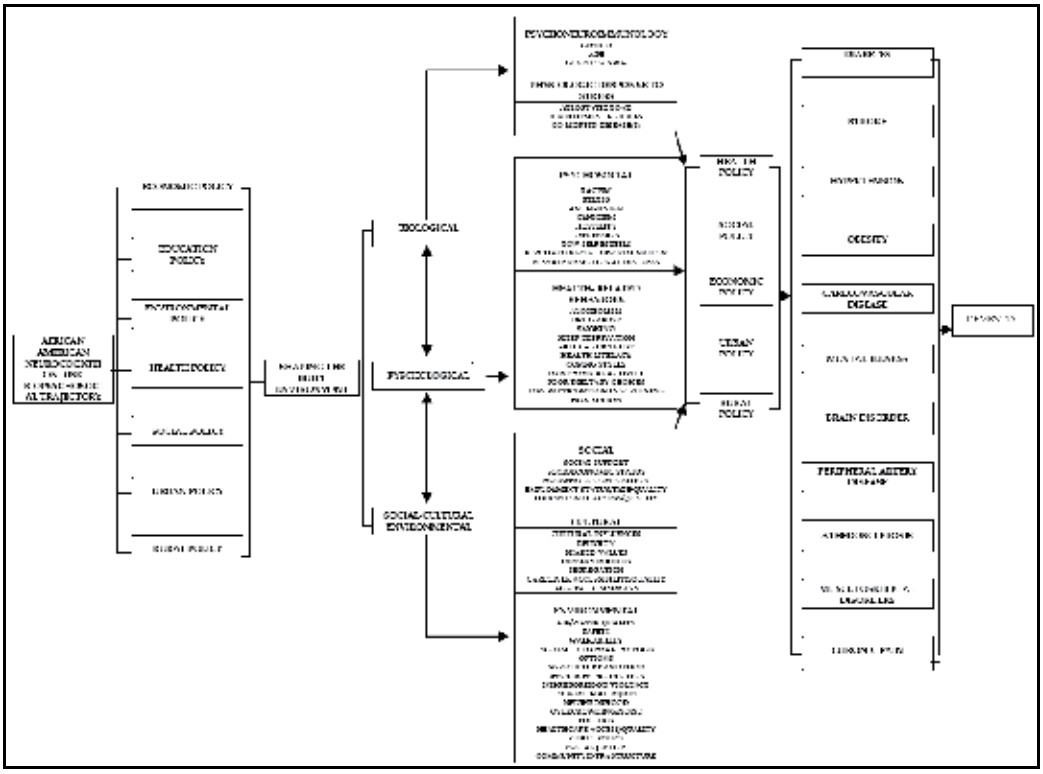

Fig. 1. Alzheimer's disease risk as a biopsychosocial determinant of health

One of the most challenging health care issues facing African Americans and the United States as a whole is the rising rates of dementia. Dementia prevention is one of the most critical health disparity agenda items of the 21st century. Dementia not only compromises the patient, it affects the family, the church, the neighborhood, the community, the workforce, the healthcare system, as well as the public policy programs. From this viewpoint, no one can afford to ignore the need to develop programs and interventions that prevent, delay, and/or eliminate the public health burden of dementia. Our research team has planned, developed, and are working toward implementing several programs.

6.2 Neurocognitive health screening and monitoring in primary care

While African Americans may have low access to specialty care, developing state-of-the-art primary care screening, evaluation, and monitoring will prove necessary in order to address dementia risk in the black community. This said, important gaps remain in our knowledge about screening for and early treatment of dementia. There are several benefits to early detection of dementia (e.g., individual and family planning, care coordination); however, there is a need for determining and examining the evidence that screening serves as an added value in the context of patient care. More attention is needed centered around identifying promising brief screening tools that can be implemented in primary care settings.

6.3 Participatory community-based AD awareness promotion

Developing drug therapies are essential, but drugs will not achieve therapeutic intent if patients are not willing to take them as prescribed. Our research team is focused on developing, testing, and implementing community outreach and health promotion. This work addresses the underrepresentation of African Americans in dementia related clinical research in urban and rural communities. Some of our objectives include identifying barriers to recruitment and addressing factors associated with lower participation rates in clinical research. The use of community-based participatory engagement as a strategy for addressing barriers to recruitment and retention is at the heart of our work.

In conclusion, because of a possible dementia epidemic, focusing narrowly on susceptibility genes and identifying genetic, clinical, and neuropathological subtypes of AD may miss the larger story in the context of upstream factors and cognitive health in black America. The critical question seems to be what factors are setting African Americans up on this downward neurocognition slope? With an eye toward protection, prevention, and neurocognitive promotion, we must start to unpack the biopsychosocial dimension of health that influences risk for adverse neurocognitive wellbeing and that provides clues to potential risk factor modification strategies among African Americans.

At this point in the field of neurocognitive health research and public health protection and promotion, researchers have not been able to pin down or know exactly what causes Alzheimer's disease. But, the accumulating body of research in the chronic physical disease literature is motivating brain researchers to consider mind-body explanations in understanding risk for dementia related conditions. Threats to brain health appear to be elevated in the presence of Type 2 diabetes and its associated health complications, including high blood pressure, high cholesterol, lack of exercise, malnutrition and psychological distress. This chapter has identified and characterized potential modifiable risk factors for adverse neurocognitive health in the context of African Americans diagnosed with T2DM.

This chapter should have advanced insights about the strategies that could promote awareness of AD risk and health care outcomes among African Americans with T2DM. Compared to the amount of public health attention focusing on African Americans and T2DM, little attention has focused on treating T2DM among African Americans as a neurodegenerative primary prevention public health concern. Moving forward, it will be critical to ensure that we clearly understand the lay health perspectives as it relates to meaning, health beliefs, and socio-ecological determinants attached to risk for neurocognitive impairment and AD within and across the black community. If African Americans continue to receive little education on neurocognitive risk factors and health

promotion, continue to develop chronic diseases that threaten brain health at earlier ages, continue to experience psychosocial trauma, and continue to experience problems obtaining early intervention for changes in neurocognitive functioning, the possibility that neurocognitive illness will disrupt this community remains very high. The information regarding functions protecting, promoting, and interfering with neurocognitive health lag further behind information on cancer, heart disease, and diabetes. Health conditions that were once considered age-related are now being diagnosed in young and middle adulthood. This heightens the public health urgency for why funding supporting research, treatment, education, and community outreach addressing African American neurocognition health is the new civil rights issue of the 21st century.

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Part 2

Non-Standard Features of Alzheimer's

4

Focal Cortical Presentations Genetically Proven Alzheimer Disease

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1. Introduction

The term Alzheimer Disease (AD) was first proposed by the famous German psychiatrist, Emil Kraepelin, over a century ago, in the eighth edition of his textbook of psychiatry. AD was, at this time, included in the differential diagnosis of presenile dementia. Kraeppelin chose this name to honor one of his pupils Aloïs Alzheimer who was one of the first to describe the pathological pattern that hallmarks the disease (1,2): miliary foci of extracellular structures (senile plaque) and the intracellular flame-shaped fiberlike bundles (tangles) observed in the brain of a fifty years old woman who presented with dementia and delusions he had first clinically followed (figure 1, copyright-free).
For decades, the term AD was used for patients whose onset of dementia was before 65 years old. In 1977, it was admitted that physiopathological changes were the same whatever was the age of clinical illness begining (3). The classic clinical pattern consists in early episodic memory loss followed by a various combination of higher function modalities alterations (executive, language, visuo-spatial impairment…) reflecting the spread of the pathology from the medio-temporal lobe to other neo-cortical areas (4,5). Thus, actual diagnostic criteria for AD (NINCDS-ADRDA and DSM IV) claim that the disease is probable if there are cognitive impairments in two or more areas of cognition, whether memory and one other (aphasia, apraxia, agnosia or executive dysfunctions for DSM-IV) or two from the following eight: memory, language, perceptual skills, attention, constructive abilities, orientation, problem solving and functional abilities for NINCDS-ADRDA. Assuming that the deficits were progressive for both, the onset between 40 and 90 years old for NINCD-ADRDA and that disease masquerading AD were excluded. Against neuropathological gold

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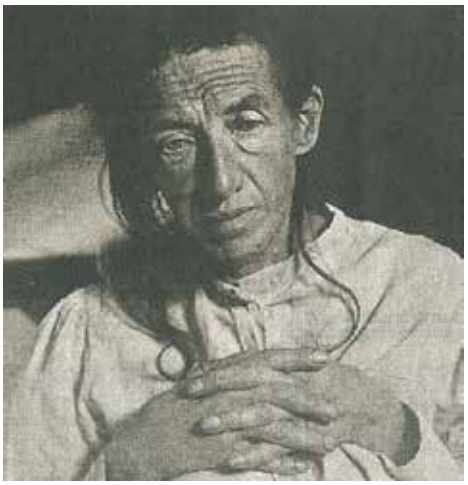
standards, the accuracy of those criteria is low: 65-96% and their specificity against other dementias even lower 23-88% (6,7,8). Furthermore, these criteria impose the necessity of presenting dementia to make a diagnosis of AD. Howhever, it is very clear today that such disease begins to express itself well before the presence of dementia. Thus, focal cortical presentation of AD escapes diagnostic by those criteria whether because memory loss remains isolated like in isolated progressive amnesia (IPA), amnesic mild cognitive impairment (MCI) or because the presenting feature that involves an other area of cognition than memory like in posterior cortical atrophy, semantic dementia, primary progressive aphasia, etc…



The senile plaque found in the hippocampus, temporal cortex and the nucleus basalis of Meynert of patient suffering from AD are made of a core of Aβ-amyloid, proteoglycans, ApoE, antichimotrypsin and other proteins. Aβ-amyloid is a protein derived proteolytically from a larger transmembrane protein: amyloid precursor protein (APP). The cleavage of APP is processed either in a non amyloidogenic way in a two step manner by the action of an α- followed by the action of γ-secretase and lead to the production of the non toxic P3 peptide. The amyloidogenic way is due to the action of a β-secretase in toxic Aβ42 and less-toxic Aβ40. Three genes have been associated with autosomal dominant AD, the APP gene, the Presenilin 1 (PSEN1) gene that codes for a protease that is part of the γ-secretase complex and Presenilin2 (PSEN2) that is also part of the γ-secretase complex (9).

Actually, those three genes can be screened for AD and are estimated to be responsible for 0.5% of all AD and 13% of early onset AD.

According to the Alzheimer Disease & Frontotemporal Dementia Mutation Database (http://www.molgen.ua.ac.be/ADMutations), of the EOFAD mutations identified, *PSEN1* mutations account for the majority (81%), followed by *APP* (14%) and with *PSEN2* mutations identified only in a handful of families (6%). An earlier age of onset is linked to the presence of an APOE ε4 allele and is also sought in suspected genetic AD (10,11).

The second pathological finding in AD are neurofibrillary tangles. These tangles are filamentous inclusions in neurons that are mostly made of an hyperphosphorylated and aggregated form of τau. The normal τau is soluble and favors vesical transport in axons by promoting assembly and stability of microtubules. Abnormal accumulation of the hyperphosphorylated and insoluble form of τau is toxic and can be found in several

neurodegenerative disorders including AD. In AD, The number of tangles is a marker of the disease's severity.
Recently, cerebro-spinal fluid (CSF) biomarkers (12), iconographic (13,14) and metabolic markers (15,16,17) have been validated as supportive criteria for the diagnostic of AD and cases of pathogenic mutations have been described in the preseniline genes in patients presenting with focal cortical presentation of AD. New approaches challenge NINCDS-ARDA and DSM IV criteria, notably the criteria proposed by Dubois et al in 2007 (18,19) that include brain imaging, CSF biomarkers and genetic considerations.
In this chapter, we will present two patients with new mutations in the PSEN 1 gene for one, APP gene for the other and isolated cognitive loss in both cases. We will discuss the various presentation of focal cortical presentation of AD and try to broaden the new criteria of Dubois et al to fit those presentations.

2. Cases- reports

2.1 Isolated progressive amnesia (IPA) associated with the London mutation of the APP (20)

A fifty year old, right handed salesman consulted for memory deficits, he first noticed five years before. The trouble began by subjective blunders in his professional activities as forgetting to collect information from his customers that increased and eventually cost him his job. His medical history was unremarkable except for a road accident 43 years before without loss of consciousness. Interestingly, his family history was significant for personality and memory disorders in his mother, grandmother and maternal aunt who required institutionalization in their early sixties. Neurological examination was within normal limits and his Minimal Mental State Examination (MMSE) score was 28/30. Blood analysis and electroencephalogram didn't reveal any abnormalities. Brain MRI was normal. Extensive neuropsychological examination highlighted an isolated deficit in verbal learning. Visual analysis of his cerebral 2-fluoro-2-deoxy-D-glucose (FDG)-PET obtained at this stage showed slight cortical hypometabolism on temporal tips. Those findings coupled with positive family history and early age of onset prompted us to perform direct sequencing on AD genes, namely of exons 16 and 17 of APP, exons 3 to 12 of PSEN1 and exons 9 to 13 of microtubule associated protein Tau. The analyses lead to the discovery of the London mutation (Val717Ile) in the APP gene that was reported in several autosomal dominant AD families. The clinical evolution was remarkable for the initial striking stability of the isolated progressive amnesia (IPA). Such long lasting IPA is not unheard, a case of 13 years duration IPA was found on autopsy to have pathological AD anomalies and there are reports of atypical AD preceded by years of isolated amnesia. This case shows that IPA can be a variant of genetically proven AD. Yet this case, even with the genetic analysis would not have fulfilled the NINCDS-ADRDA or DSM IV criteria (20).

2.2 Progressive non fluent aphasia and executive disturbances associated with a Gly266Cys mutation in PSEN1 (unpublished data)

A 56 years old physiotherapist consulted with her daughter for gradual-onset speech difficulties over a year. She never presented space or time disorientation nor had important behavioural changes. The core complaint was trouble to find words and effortful speech output due to loss of proper and common nouns. She was still working but felt increasing psychological tension due to her communication problems. Her familial history was

unremarkable, her mother died of cancer without cognitive impairment at 75 and her father is still alive and alert at 83 years old. Neurological examination was normal. She presented dyscalculia and her MMSE was 28/30, losing one point for sentence rehearsal and one for making a mistake in "world" spelling. Complete neuropsychological examination showed a mixed pattern dominated by dysexecutive and loss of speech production features and saliently a complete lack of episodic memory deficit. The initial diagnostic hypothesis was a predominantly logopenic form of primary progressive aphasia (PPA). Biological work-up was unremarkable. Brain MRI showed a discrete cortical atrophy and FDG-Pet highlighted slight biparietal cortex hypometabolism. Genetic testing was undertaken and positive for a heterozygous mutation in PSEN1 gene never reported before. The mutation in exon 8 implicated an amino acid substitution of Glycine to Cysteine at codon 266. The mutation was absent in 500 belgian controls and mutation in the codon 267 are associated with Early onset AD in polish and English families which make us think that the mutation is pathogenic in our patient. Furthermore our patient improved with treatment by memantine chlorhydrate. As in our previous case of IPA, this patient would not have fulfilled the actual criteria for AD.

3. Other focal cortical forms of AD

Classical AD is diagnosed in vivo thanks to criteria centred on memory loss as the first and predominant complaint. Patients presenting with features masquerading other forms of neurodegenerative diseases like PPA, fronto-temporal dementia (FTD), Cortico-Basal Dementia (CBD) can be overlooked. An important study by Alladi et al sought to confront pathological examination with focal cortical syndromes and found that AD was the primarily pathological diagnosis in 34% of the cases (21). Interestingly, the proportion of focal cortical syndromes associated to AD pathology was different according to their clinical presentations. One hundred percent of posterior cerebral atrophy (PCA) was found to be due to AD whereas 50% of CBD, 36% of PPA and 7 % of FTD were associated with AD pathologies.

We will develop in the following paragraphs the clinical features of those focal cortical presentations.

3.1 Behavioural variant

The initial case reported by Aloïs Alzheimer, Auguste D. (figure 1, copyright-free), presented with behavioural disorders, psychosis and delusions in combination with dementia… and had the pathologic anomalies that now hallmark AD. Yet behavioural presentation are not considered in NINCDS-ADRDA and DSM IV criteria and are even an exclusion feature in recently proposed Dubois et al criteria. The behavioural variant of FTD (bv-FTD) is the most common differential diagnosis of AD presenting with behavioural pattern, especially in presenile dementia. bv-FTD is the most common clinical manifestation of FTD that represents one of the seconds causes of cortical dementia after Alzheimer's disease (22). Initial symptoms in bv-FTD patients usually include progressive personality and social conduct changes coupled with executive functions deficits (23,24). Changes in personality, loss of social abilities, apathy, reduced empathy, stereotypic behaviour, disinhibition are in correlation with predominantly orbito-basal and dorsolateral frontal atrophy found on MRI (22). Many studies tried to find distinctive neuropsychological

patterns or CSF biomarkers that could reliably make the difference between AD and bv-FTD. However, pathological studies continue to demonstrate an important proportion of mistakes.

3.2 Progressive aphasia

Losing words, especially proper names, may be the first complaint of patients and may thus bring them to consultations. Later, difficulties classically involve common nouns and progress to points where fluency of speech could be seriously impaired. Every sentence can be broken by pauses and search for wanted words. If not found, circumlocutions can be substituted or sentences left unfinished. Depending on which modality of aphasia is mostly affected, PPA is separated in progressive non-fluent aphasia (PNFA) when effortful speech and phonological and/or syntaxic errors is prominent, semantic dementia (SD) (26) and more recently by logopenic aphasia. In SD, speech fluency is preserved but there is a striking anomia, impaired word comprehension and deficits in non-verbal semantic association tasks such as sorting and grouping objects on basis of functional characteristics (26). In logopenic aphasia, repetition is more affected. The localization of semantic memories is usually lateralized. Left parietal and temporal lobes store verbal-language semantic memories whereas the right parietal and temporal and parietal lobes store predominantly visual-spatial semantic memories. These modalities seem to be electively affected in semantic dementia (26,27).

3.3 Corticobasal syndromes (CBS)

The term corticobasal dementia was coined to describe a specific nosologic form of cortical-subcortical degenerative disorders. Patients classically present with an asymmetric apraxia and extrapyramidal syndromes with rigidity, bradykinesia and tremor indicating basal-ganglionic impairment (28). This is usually combined with an "alien hand" phenomena suggesting cortical involvement: the patient, though able to exert normal muscle strength, fail to direct voluntary action, the affected limb is unable to produce purposeful action and attempts result in inappropriate movement. The limb may remain in an odd posture without the patient's awareness; there is some kind of hemisensory neglect and visuo-spatial impairment. Myoclonus may also be present and may acquire a sensitive stimulus myoclonus pattern as the disease progresses. Initially the disease begins with one limb and then progresses to the other side and to cranial nerves. Apraxia of gaze and eyelids opening are frequent. CBS are considered distinct of AD for localized thalamoparietal metabolic asymmetries and lack of histopathologic hallmarks on autopsies. Yet, as time passes, most pathological changes associated with dementia have been described: usually tangles, amyloid deposits and lewy bodies. In the Alladi et al study, half of CBS had pathological hallmarks of AD (21) and several mutations of PSEN-1 have been described with clinical extrapyramidal syndromes and myoclonies (29,30).

3.4 Posterior cortical atrophy (PCA)

Initial manifestations are characterised by progressive visual impairment. Patients even usually consult an ophtalmologist for reading or driving difficulties. Features of Balint's syndrome can be found such as simultagnosia (patients see the tree instead of the forest), ocular apraxia (inability to direct gaze accurately) and optic ataxia (an object cannot be

reached by visual guidance). Both simultagnosia and ocular apraxia lead to obvious reading difficulties (31). Visuo-spatial orientation becomes defective. Patients are thus unable to park a car. Their arms do not find correct sleeves of the dressing gown. The route from one place to another cannot be described nor can been understood. PCA can be further subsided in bi-parietal syndrome, where object recognition and reading are preserved but with marked apraxia, agraphia and visuo-spatial difficulties. Occipito-temporal syndrome presented with alexia, aperceptive agnosia and some degree of prosopagnosia. In visual variants, there is a primary visual failure and failure of perceptual abilities (32). The immense majority of the patients with PCA do have AD with lewy body disease, prion disease, and ταupathies as major differential diagnosis (21). The patient in the seminal description of Aloïs Alzheimer, Auguste D, could maybe presented initially with PCA explaining partially precocity of delusions described.

3.5 Others (myoclonia, cerebellar…)

Studies of the clinical characteristics of families with mutations in PSEN-1 show wide phenotypic features variety. Ranging from families in which myoclonus and seizures were prominent to ataxia attributed to some cerebellar pathology. Interestingly, extrapyramidal signs were reported in some series in as much as 50% of patients with PSEN-1 mutations (29) and seizures in over 20 % PSEN-2 mutations (33).

4. Recents Dubois et al criteria and our proposed amendements

In some way, the situation of focal cortical presentation of AD can be compared to the diagnosis of clinically isolated syndrome in multiple sclerosis (MS). To speak of MS, amnestic, clinical and paraclinical examination must reveal objective anormalities that implicate two or more areas of the central nervous system. If the anomaly is isolated, waiting for another anomaly is mandatory to confirm the diagnostic. This pitfall is responsible for time loss and thus of psychological and physical burden for the patient and in some cases, the worsening of his prognosis. Therefore, in MS, there has been considerable efforts to include paraclinical features in diagnostic criteria to reduce time to diagnosis. Since several years, Central Nervous System MRI, CSF markers and evoked potentials are important features to increase the suspicion of the diagnosis. The same situation happens with actual criteria proposed for AD diagnosis. In cases where there is only an isolated cortical dysfunction without memory impairment or isolated memory impairment without other cortical areas involvement, a diagnosis would be postponed until some new features are added to the pattern. Contrary to MS, where evidenced of diagnosis can be gathered from many clinical or paraclinical sources, actual criteria of AD fail to consider the knowledge that has been accumulating for years in the field of brain imaging and discoveries of relatively reliable CSF biomarkers. Recently, Dubois et al concerned by the situation proposed their new criteria (Table 1) to replace those previous (from 1984) of the NINCDS-ADRDA (18). The argumentation behind the new criteria is that the previous NINCDS-ADRDA/DSM IV-TR ones were not including recent progresses of imaging and biomarkers for AD. This would be responsible for a low sensibility and sensitivity that could be much improved with the inclusion of CSF analysis, structural MRI and neuroilmaging with PET features. Those additions would lead to more accurate detection of the earliest stages of the disease and of its full spectrum.

Probable AD:
A plus one or more supportive features B,C,D or E

A. Presence of an early and significant episodic memory impairment that includes the following features:

1. **Gradual and progressive change in memory function reported by patients or informants over more than 6 months**
2. **Objective evidence of significantly impaired episodic memory on testing: this generally consists of recall deficit that does not improve significantly or does not normalise with cueing or recognition testing and after effective encoding of information has been previously controlled**
3. **The episodic memory impairment can be isolated or associated with other cognitive changes at the onset of AD or as AD advances**

Supportive features

B. Presence of medial temporal lobe atrophy

- **Volume loss of hippocampi, entorhinal cortex, amygdala evidenced on MRI with qualitative ratings using visual scoring (referenced to well characterised population with age norms) or quantitative volumetry of regions of interest (referenced to well characterised population with age norms)**

C. Abnormal cerebrospinal fluid biomarker

- **Low amyloid $A\beta_{1-42}$ concentrations, increased total τau concentrations, or increased phospho-τau concentrations, or combinations of the three**
- **Other well validated markers to be discovered in the future**

D. Specific pattern on functional neuroimaging with PET

- **Reduced glucose metabolism in bilateral temporal parietal regions**
- **Other well validated ligands, including those that foreseeably will emerge such as Pittsburg compound B or FDDNP**

E. Proven AD autosomal dominant mutation within the immediate family

Exclusion criteria

History

- **Sudden onset**
- **Early occurrence of the following symptoms: gait disturbances, seizures, behavioural changes**

Clinical features

- **Focal neurological features including hemiparesis, sensory loss, visual field deficits**
- **Early extrapyramidal signs**

Other medical disorders severe enough to account for memory and related symptoms

- **Non-AD dementia**
- **Major depression**
- **Cerebrovascular disease**
- **Toxic and metabolic abnormalities, all of which may require specific investigations**
- **MRI FLAIR or T2 signal abnormalities in the medial temporal lobe that are**

consistent with infectious or vascular insults

Criteria for definite AD

AD is considered definite if the following are present:

- **Both clinical and histopathological (brain biopsy or autopsy) evidence of the disease, as required by the NIA-Reagan criteria for the post-mortem diagnosis of AD; criteria must both be present**
- **Both clinical and genetic evidence (mutation on chromosome 1, 14, or 21) of AD; criteria must both be present.**

Dubois et al remain focused on early episodic memory impairment as core diagnostic criteron. The degradation of memory function must have started progressively and for over six month. Memory complaints can come from the patient itself or a close relative as both situations are associeted with high risk of developping AD (34,35). When the complaint is reported, objective memory testing must confirm an impaired delayed recall that is not normalised with cuing or recognition testing. Some studies found that delayed recall was a reliable predictor for AD in patients with MCI. Memory impairment can be isolated or associated with other cognitive changes.

The inclusion of objective paraclinic tests as supporive features is, we believe, the important inovation of proposions of Dubois et al. They include findings from structural MRI, abnormal CSF markers, specific pattern on functionnal imaging and genetic that are questionnably absents from the previous NINCDS-ADRDA/DSM IV-TR criteria. Unfortunately, their exclusion criteria are actually quite strict. We guess that for a high specifity of their criteria, they choose not to consider any atypical pattern, excluding patients presenting with predominantly behavioural changes, extrapyramidal signs, sensory loss, visual fields deficits. As a consequence, all the AD focal cortical presentations exposed before would fail to qualify for probable AD except for IPA. The 2 cases we here report and other studies examining post-mortem brain pathologies from patient with alleged CBS or focal cortical presentations clearly argue against such exclusion criteria. Yet, it is our belief that with slight modifications, the score proposed by Dubois et al could encompass "atypical-AD". The major modification we thus propose (table 2) is to get free of the memory impairment dogma in AD.

We do not question the fact that most AD begin with episodic impairment and that accumulation of pathologenic almyloid plaques and tangle preferentially starts from the medio-temporal lobe to spread towards other neo-cortical areas. Yet, a significant proportion of patients presents initially with alterations of other cortical skills, even if they represent a small part of all AD. As the prevalence of AD is high and the population is getting old, this small part of a high population will make many thousands of patients to fail to be diagnosed. Changing episodic memory impairment as core criteria to any gradual cortical impairment with the supportive feature listed by Dubois et al would support a diagnosis of probable AD in most of these cases. We also think that early occurence of gait disturbances, behavioural changes, sensory loss, visual field deficits and extrapyramidal signs should not be exclusion criterias. There is a risk to diminish our specificty to diagnosis by using those amended criteria, but the active searching for supportive features wether genetic, CSF biomarkers or iconographic will help to diagnose early many patients and perhaps help to broaden visions more and more many people have of the AD clinical spectrum.

Probable AD:
A plus one or more supportive features B,C,D or E

A. Presence of an early and significant episodic memory impairment that includes the following features:

1. **Gradual and progressive change in any cognitive function reported by patients or informants over more than 6 months**
2. **Objective evidence of significantly impaired at least this higher function on testing: in case of memory, this generally consists of recall deficit that does not improve significantly or does not normalise with cueing or recognition testing and after effective encoding of information has been previously controlled**
3. **The cognitive function impairment can be isolated or associated with other cognitive changes at the onset of AD or as AD advances**

Supportive features

B. Presence of predominantly medial temporal lobe atrophy

- **Volume loss of hippocampi, entorhinal cortex, amygdala evidenced on MRI with qualitative ratings using visual scoring (referenced to well characterised population with age norms) or quantitative volumetry of regions of interest (referenced to well characterised population with age norms)**

C. Abnormal cerebrospinal fluid biomarker

- **Low amyloid $A\beta_{1-42}$ concentrations, increased total τau concentrations, or increased phospho-τau concentrations, or combinations of the three**
- **Other well validated markers to be discovered in the future**

D. Specific pattern on functional neuroimaging with PET

- **Reduced glucose metabolism in bilateral temporal parietal regions**
- **Other well validated ligands, including those that foreseeably will emerge such as Pittsburg compound B or FDDNP**

E. Proven AD autosomal dominant mutation within the immediate family

Exclusion criteria

History

- **Sudden onset**
- **Early occurrence of the following symptoms: gait disturbances, seizures, behavioural changes**

Clinical features

- **Focal neurological features including hemiparesis, sensory loss, visual field deficits**
- **• Early extrapyramidal signs**

Other medical disorders severe enough to account for the cognitive decline and related symptoms

- **Non-AD dementia**
- **Major depression**
- **Cerebrovascular disease**
- **Toxic and metabolic abnormalities, all of which may require specific investigations**
- **MRI FLAIR or T2 signal abnormalities in the medial temporal lobe that are**

consistent with infectious or vascular insults

Criteria for definite AD

AD is considered definite if the following are present:

- **Both clinical and histopathological (brain biopsy or autopsy) evidence of the disease, as required by the NIA-Reagan criteria for the post-mortem diagnosis of AD; criteria must both be present**
- **Both clinical and genetic evidence (mutation on chromosome 1, 14, or 21) of AD; criteria must both be present.**

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5

Spatial Navigation Impairment in Healthy Aging and Alzheimer's Disease

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1. Introduction

The prevailing number of studies dealing with spatial navigation in animals and also in human have been connecting it to the function of the medial-temporal lobe (MTL), hypothesized to serve as the neuronal basis for the spatial cognitive map. Changes in the MTL are characteristic for both healthy aging and Alzheimer's disease (AD), with markedly more severe changes in AD. The level of hippocampal dysfunction therefore counts among the boundaries between healthy aging and development of AD. Furthermore, the decline in the spatial navigation abilities has been described for healthy aging and is among the diagnostics marks of early AD. While the impact of this decline on the life of elderly seems to be minor, subjects suffering AD become lost in new environments and later in the course of the disease even inside their homes. The spatial navigation is however a complex process using a number of brain regions besides MTL. The regions discussed in this review also include prefrontal cortex (PFC), whose changes in healthy aging are often considered more typical than changes in MTL, and parietal cortex documented to deteriorate from the very early stages of AD. Due to its complexity, spatial navigation is a promising cognitive ability to be investigated in the phases of development from healthy aged to AD.
The aim of this review is to investigate the complexity of the spatial navigation and its neural basis in healthy aging and in AD, including several available studies concerning the boundary between these two. We will focus on the differences in the spatial navigation abilities between healthy aging and AD and on the potential of spatial navigation to differentiate between them. The current review follows up two papers on similar topic (Iachini et al., 2009; Moffat, 2009) and in contrast to them focuses just on wayfinding and navigation, its underlying neural structures and the distinction between healthy aging and Alzheimer's disease.

2. Neural basis of spatial navigation

Functional neuroimaging and lesion studies have identified a complex network of structures that are involved in spatial navigation. The proposed network includes the hippocampus, parahippocampal gyrus, medial and right inferior parietal cortex, regions within prefrontal cortex, cerebellum, parts of the basal ganglia, posterior cingulate cortex and retrosplenial cortex (Aguirre et al., 1996; Barrash, 1998; Ekstrom et al., 2003; Hartley et al., 2003; Maguire

et al., 1998a). Here we will shortly review the role of some of those areas whose changes in elderly and Alzheimer's subjects have been documented in relation to spatial navigation.
The concept of hippocampus as a neural basis for cognitive map (O'Keefe & Nadel, 1978) stems from the discovery of place cells responding when an animal is in a particular location as defined by the spatial configuration of objects in the environment (O'Keefe & Dostrovsky, 1971) and from the effect of hippocampal lesions impairing navigation to an invisible goal in a water maze from variable start positions (Morris et al., 1982). The cognitive map theory dissociated the navigation based on a configuration of distal landmarks from the navigation to and from landmarks. This concept has evolved into the dissociation between allocentric and egocentric navigation. The allocentric navigation uses flexible representation (Eichenbaum et al., 1999) of an ensemble of distal landmarks and independent of actual subject positions. On contrary, the egocentric navigation uses distances and angles to or from individual landmarks. In humans, the allocentric mode of navigation was shown to be connected with the hippocampal function in analogues of the Morris water maze (Astur et al., 2002; Feigenbaum & Morris, 2004), in place navigation inside a virtual town (Maguire et al., 1998a), and also in remembering the location of objects on a table (Abrahams et al., 1997). The hippocampal function have often been confused with the function of parahippocampal gyrus, but there is evidence that parahippocampal cortical areas are required for iconic representations of scenes, with hippocampus being required in addition when memory for locations in three-dimensional space is required. Functional neuroimaging of recognition memory for object location within a two-dimensional array (Johnsrude et al., 1999) and the perception of spatial scenes (Epstein & Kanwisher, 1998) and buildings (Aguirre et al., 1998) consistently activates the posterior right parahippocampal gyrus, but not the hippocampus.
While allocentric representation of space has been connected with the MTL, the egocentric representations were documented within the parietal cortex (Hyvarinen & Poranen, 1974; Mountcastle et al., 1975). Posterior parietal areas appear to support the necessary translation of spatial information between allocentric and body-centred reference frames (Snyder et al., 1998) and between the various egocentric reference frames (Andersen et al., 1985). Lesions of the right posterior parietal cortex are characteristic by an egocentric orientation deficit presenting as the inability to represent the spatial relationship between the subject and other objects (Kase et al., 1977; Levine et al., 1985; Stark et al., 1996).
Evidence of PFC involvement in spatial navigation is based mainly on animal research, however its functional activation associated with navigation in human was described several times including activation during successful relative to unsuccessful navigation (Maguire et al., 1998a) or activation associated with navigational goals and conjunction of goals and places (Ekstrom et al., 2003). Ablating or inactivating part of the rat orbitofrontal cortex impairs performance of an allocentric foraging task (Corwin et al., 1994), escape in the Morris water maze and conditioned place avoidance (Vafaei & Rashidy-Pour, 2004). Several studies have reported double dissociation with hippocampal and/or fimbria fornix lesions impairing place learning in the place version of the Morris water maze and PFC lesions impairing response learning (de Bruin et al., 1997; de Bruin et al., 2001).

3. Healthy aging

The decline of cognitive function in elderly people have been thoroughly studied, the role of spatial navigation impairments in the difficulties experienced in daily life is however probably underestimated. During driving elderly individuals have self-perceived deficits in

navigation and develop behavioural patterns to avoid unfamiliar routes and places (Burns, 1999). These deficits can have negative implications for peoples' well-being and independence. In addition, several studies in elderly describe objective route learning and navigational deficits that can affect daily life not only during driving.

3.1 Cognition and neural changes

Before reviewing literature concerning changing spatial abilities during aging we will review shortly general cognitive changes. The prevailing concept explains most of the cognitive effects of aging by changes in prefrontal cortical function. This viewpoint is evidenced by both neuropsychological and functional brain imaging data and related decrements are typically found in tasks as working memory, episodic free recall or recollection of the source of the learned information (Johnson et al., 1993). Working memory tasks require to manipulate in some way the material held in memory, in contrast to short-term memory tasks, requiring only holding the presented material in memory for several seconds. One example is repeating a sequence of presented digits in a reversed order. Working memory is dependent on the prefrontal regions (Courtney et al., 1998; D'Esposito et al., 2000; Smith & Jonides, 1999) and deteriorates with aging (Salthouse & Meinz, 1995). Also free recall of episodic information without contextual or any other cues activates prefrontal brain areas (Petrides, 2002) and there is considerable evidence that age differences in memory performance diminish when retrieval is facilitated by providing additional cues at the time of memory test (Craik & Mcdowd, 1987; Rabinowitz, 1984).

Another source of evidence concerning age dependent changes in prefrontal function comes from functional imaging experiments. Aging is generally connected with reduced activation of the left PFC during encoding (Cabeza et al., 1997; Grady & Craik, 2000) and a pattern of reduced activation in those prefrontal regions that are most activated in younger subjects together with an increased activation in the contra-lateral regions. This pattern, referred to as reduced hemispheric asymmetry (Cabeza et al., 2002), is often interpreted as a sign of compensatory function, as those older individuals who have the most successful task performance tend to display the largest bilateral PFC activations (Reuter-Lorenz & Lustig, 2005). Several studies document another pattern of age dependent changes in brain activation with decreased activity of hippocampal region and simultaneously increased prefrontal activation during encoding (Gutchess et al., 2005; Park et al., 2003), interpreted as compensatory mechanism for the decrease of hippocampal function. Structures of the PFC also exhibit age-related volumetric changes that are larger than in any other cortical region (Raz et al., 2004; Raz et al., 2005; Resnick et al., 2003; West, 1996).

The age dependent changes in the MTL are less clear. Results of the volumetric studies are somewhat ambiguous: while numerous both cross-sectional and longitudinal studies show an effect of age on the hippocampal volume (Golomb et al., 1993; Jernigan et al., 1991; Persson et al., 2006) with the rate of decline in the range of 0.79–1.5% per year (Jack, Jr. et al., 1998; Pruessner et al., 2001; Raz et al., 2004; Raz et al., 2005), some studies did not find any age dependent hippocampal volume decline (Sullivan et al., 1995; Van et al., 2004). The finding of decreased medial-temporal involvement for older adults compared to young adults during encoding is robust however and has been reported in a number of studies, including encoding of faces (Grady et al., 1995), natural scenes (Park et al., 2003), verbal materials (Daselaar et al., 2003; Grady et al., 1999) and line drawings (Grady et al., 1999). The neuropsychological evidence of age related changes in MTL is scarce and confined mainly to the domain of spatial navigation.

3.2 Spatial memory and navigation

Results from various types of spatial memory tasks have been explained by limits in cognitive capacity connected to the PFC. Age dependent deficit in the memory for location of several object was shown in a number of studies (Light & Zelinski, 1983; Naveh-Benjamin, 1987; Naveh-Benjamin, 1988; Park et al., 1983; Sharps, 1991), but not when using specific experimental conditions (Sharps & Gollin, 1987; Waddell & Rogoff, 1981; Waddell & Rogoff, 1987). The factors influencing similar performance of elderly to young subjects seem to include intentional versus incidental learning and distinctiveness of the object locations (Uttl & Graf, 1993). In experiments focusing on this distinctiveness theory, Sharp and Gollin (1987) showed that the age dependent deficit is absent when the objects are presented in a real space or are in different colors or are three-dimensional blocks of wood. Similar effect of the form of the objects these authors described even for the free recall of identity of objects located on a table-top map (Sharps & Gollin, 1988). They suggested that visually distinctive context facilitates object memory because it provides a rich associative base for elaboration of memory traces. It seems that elderly participants spontaneously engage in less elaboration than do younger respondents. This interpretation is in agreement with reports showing less efficient encoding in elderly adults (Grady et al., 1995; Stebbins et al., 2002), was however not confirmed in a control study on a different group of subjects (Park et al., 1990). Interestingly, object location memory was shown to be dependent on the MTL region (Nunn et al., 1999; Pillon et al., 1999; Stepankova et al., 2004) or more specifically on the parahippocampal gyrus (Maguire et al., 1998b; Owen et al., 1996). Thus another interpretation of this age dependent object location memory deficit may attribute it to the decline in MTL activity. The incidental learning and distinctiveness may then facilitate compensational strategies supported by the PFC.

3.2.1 Large-scale real spaces

Other authors studying spatial memory in large-scale environments proposed explanation of age related deficits by working memory demands. In one of these studies, subjects should remember spatial position of building pictures standing in a large room either in a spatial configuration corresponding to the real position in the subject's hometown or in a novel spatial configuration. Most of the pictures were hidden and the subjects should learn their position by replacing them several times until they could do all items accurately. Then, at several points in the room, they should estimate direction and distance to the current position from these hidden pictures. They were instructed to do so either by mental rotation of their current position or by taking perspective from the picture. Mental rotation and perspective taking was more difficult for elderly subjects in the novel but not in the known environment (Kirasic, 1989). Because all subjects learned the object positions before testing, the understanding of the experiment was based on working memory demands larger in novel configuration than in known configuration. Similar effect of increased deficit associated with aging in a novel relative to a familiar environment was documented in a navigational task in supermarket (Kirasic, 1991) and explained also by working memory demands.

The limits in cognitive capacity in temporospatial processing were assessed by another study presenting to the subjects a set of colour slides of two routes through unfamiliar neighbourhood. The two routes shared a common section. In a series of tests following the presentation, older adults were less likely to recall the landmarks sequentially, more likely to recall non-spatial associations to the routes and more likely to regard salient landmarks,

rather than turns, as critical route-maintaining events (Lipman, 1991). The older adult's landmark memory was organized according to the distinctiveness of landmarks rather than their spatial order. The authors hypothesized that with the advancing age sequential processing of route information is curtailed or precluded by the cognitive capacity limits. Preferred mode of spatial processing used by older people, which captures distinctive but not critical route events, may require less cognitive effort.

Similar age related deficits were found in an experiment by Wilkniss et al. (1997), where subjects were guided along a complicated route through a university building. After the tour they were asked to lead the experimenter along the same route and their errors were recorded. Then they completed landmark recognition and ordering tasks, followed by another test on navigation in the same building using a just learned map of a new route. The elderly adults recognized well the landmarks, but were impaired in their temporal ordering, in recalling the route, and in using the learned map to navigate successfully. Although the older adults encoded visual information and recognized landmarks, they were less likely than younger adults to select or effectively use critical cues. This was interpreted as consistent with the studies by Kirasic (1991) and Lipman (1991) (see above) by an age-related deficit in selection of information most useful in route maintenance. The impairment of navigation using a learned map in the second part of the test offers additional explanation, mentioned by the authors, by deficit in using a configural spatial representation to navigate a route.

Real space route learning ability in a hospital lobby was the subject of another study (Barrash, 1994). The participants in the age of 18 to 78 were lead by the experimenter through a long route containing 28 intersections and 16 turns and at the end they were asked to lead the experimenter back to the route origin. The complete test consisted of three trials. Although all groups showed significant learning between the trials, the participants 60 and older made significantly more errors than the youngest group and the oldest participant in their 8^{th} decade committed almost 4 times more errors than the youngest group. There was a tendency even for the participants in their 6^{th} decade to be impaired. The impairment seems to be explicable by cognitive capacity limits in the elderly subjects, because the retracing test was performed in an opposite direction than the learning and there were only three trials to learn the route.

3.2.2 Virtual maze environments

Other studies employed virtual reality design to assess navigation in highly controlled environment. In a simple virtual maze composed of richly textured alleys connected by four intersections, participants were required to find a trophy at the goal point as fast as possible and to learn the correct route in five learning trials (Moffat et al., 2001). The time, distance and number of errors to reach the goal increased with age, with the groups of 45-65 and 66-91 years old participants impaired relative to younger subjects. The impairment resulted from incorrect entrances to dead ends or already visited alleys. The authors found significant correlations between the performance in the maze and measures of working, non-verbal and verbal memory and mental rotation.

Spatial relational learning was investigated in a virtual reality study using a treadmill to simulate locomotion (Lovden et al., 2005). The subjects were assessed in a virtual museum using combination of two conditions: city-block topography with straight corridors vs. variable topography with winding corridors and with vs. without walking support. Variable topography was expected to be more difficult than city-block topography when using

spatial strategy, but not when using non-spatial cued navigation strategy. Elderly subjects were impaired under all conditions, and their performance was slightly better using walking support and not influenced by variable topography. This contrasted with the increase in travelled distance with variable topography in younger subjects and no effect of walking support. The elderly subjects were also less successful in locating landmarks from the museum on its map. Findings of this study suggest that elderly subjects do not use spatial relations between parts of the maze. In analogy, animal research showed that aged organisms more often adopt stimulus-response learning and cue guidance strategies to locate places in space, whereas younger organisms rely more on spatial relational learning in tasks where both activities could be used (Rapp et al., 1987; Tanila et al., 1997). In addition, the finding of walking support helping elderly subjects in navigation indicates that walking per se is an attention demanding activity for elderly subjects.

Results distinct from the real space study by Wilkniss et al. (1997, see above) were described in an experiment using immersive virtual reality with head mounted display in simple city model (Zakzanis et al., 2009). The elderly subjects were impaired in retracing a short route with four intersections after passively viewing it, but made also more false alarm errors in the landmark recognition test, contradicting the above mentioned study. It is questionable whether the landmark recognition error could result from small angle of view (40°) or the passive way of learning the route.

In an interesting recent study using virtual reality, subjects were trained thoroughly to acquire the spatial relationships of the environment and tested in this knowledge by locating six landmarks on a schematic map (Iaria et al., 2009). Elderly subjects not only spent longer time learning the environment, but even after meeting the criterion of knowing it, they were slower and made more errors in the navigational tasks. The authors interpret this finding as impairment both in the formation of a cognitive map and in using the acquired cognitive map for navigation, similarly to the above mentioned study by Wilkniss et al. (1997). It may be questionable however if during the training the elderly subjects really learned a map of the environment containing spatial relations or it they learned rather a visual view of the schematic map with landmarks. This visual view would then be naturally more difficult to use for navigation. Anyway, this approach should be a fruitful one and deserves further elaboration.

3.2.3 Analogies of the Morris water maze

The theory explaining spatial navigation deficit in elderly by changes in using of the cognitive map was directly tested using human analogues of the Morris water maze (MWM) (Morris, 1981). The cognitive map, as introduced by Tolman (1948) and later by O'Keefe and Nadel (1978), contains flexible representation of the environment, which can be used in novel circumstances independently on actual subject's position. Navigation using this flexible representation should not be affected by rotation or deletion of individual landmarks (Markus et al., 1994; O'Keefe & Speakman, 1987). In the experiment using a real space analogue of the Morris water maze (Newman & Kaszniak, 2000), subject were required to learn to find an invisible target position in an octagonal arena 7.3 m in diameter. Then they were assessed in three testing conditions: after arena rotation, cues deletion and delay. The elderly subjects were impaired under all of these conditions but not in the control trials allowing non-spatial strategy, confirming the assumption of impaired acquisition or use of their cognitive map of the maze.

In navigational experiments using virtual analogue of the MWM with variable start position, strong association was found between age and time to find the invisible platform and also between age and time spent in the correct quadrant during the probe trial with the platform removed (Driscoll et al., 2005; Driscoll et al., 2003; Moffat & Resnick, 2002). In the latter of these experiments (Moffat & Resnick, 2002), older participants were able to use proximal objects but not room-geometry cues to locate the goal on overhead diagrams of the environment. This difference in strategy in aged subjects is similar to the effect of hippocampal lesions in rats, which results in impaired use of distal but not proximal landmarks (Save & Poucet, 2000). This similarity suggests that the age dependent atrophy in the human hippocampus (Jack, Jr. et al., 1998) underlies the age related shift to cue-use strategies observed in the present study. In a strong relation to these views are the findings from another report using virtual MWM analogue in elderly subjects (Laurance et al., 2002). The elderly participants were impaired in learning the goal position and also in locating it on a diagram of the maze, but on the same diagram they were able to arrange correctly the patterned walls and objects on the walls. It seems therefore that they acquired cognitive map of the maze structure but not of the goal position. Two dissociable cognitive processes could be responsible for these two abilities, according to authors' suggestion. The possibility should be excluded however, that the elderly subjects used pure visual memory to reconstruct the maze, but could not use it to estimate the goal location that was never shown.

Important observation from the Moffat and Resnick (2002) study was that elderly subjects were impaired even in the first trial of the test, following only practice trials with different goal location. Elderly individuals probably continued to search in locations that have been already adequately explored, suggesting a preservative component to their behaviour, the working memory deficit, or more generally, the selection of inefficient spatial search strategy. Thus, besides relational spatial memory, executive and strategic functions probably play a significant role in spatial skill acquisition in the MWM.

3.2.4 Brain imaging

From the above results we can hypothesize about the underlying neural structures, primarily the PFC and MTL. More direct evidence about the connection between neural function changes during aging and navigational performance was provided however by several brain imaging studies. Two approaches used in the aging research include correlating brain volumes with spatial navigation performance and functional imaging. The first approach was used in three studies with surprisingly contrasting results. The hippocampal volume was significantly correlated with navigational performance in the virtual MWM in the first study (Driscoll et al., 2003); more specifically the positive correlation was found with the time in the target quadrant in the probe trial in all subjects together, younger and older. In contrast, another study using also virtual MWM (Moffat et al., 2006b) described correlation of the hippocampal volume with the distance travelled only in the first learning trial (with not yet known goal position) and only in the younger subjects group. Finally, in a report describing navigation in a maze consisting of several alleys and seven intersections (Head & Isom, 2010) significant correlation was found between the wayfinding performance and hippocampal volume in only elderly subjects. Spatial strategy selection could account for these discrepancies. The brain areas correlating with the navigational performance was shown to depend on the strategy selection (Bohbot et al.,

2007). In addition, the virtual MWM performance depends on extrahippocampal brain areas, namely PFC and caudate nucleus (Moffat et al., 2006b). The link between hippocampal volume and navigational performance probably depends on the specifics of the virtual maze and successfulness of individual spatial strategies.

The second approach to assess changes in brain function in spatial navigation accompanying aging is to use functional imaging. In the first route encoding and recognition study in a virtual reality building (Meulenbroek et al., 2004) subjects should remember a set of turns marked by arrows on the walls and then, during the recognition phase, on the same places choose the correct one of two arrows to follow the route. The elderly subjects reached slightly lower scores than young subjects, and compared to the young they showed diminished posterior fusiform-parahippocampal and parietal activity during route encoding, corresponding to the role of these brain areas in navigation. In addition, the elderly subjects exhibited increased anterior parahippocampal activity relative to the young subjects during the route recognition, which might indicate decreased familiarity with the route. Finally, the elderly subject activated more the anterior cingulate and perisylvian cortices during encoding, interpreted as a failure to suppress irrelevant information. In a related paper by Moffat et al. (2006a) subjects learned a virtual environment consisting of several rooms and hallways by navigating between various objects. Compared to their younger counterparts, elderly adults showed reduced activation in several regions of the MTL and of the parietal lobe. They also showed greater anterior cingulate gyrus and medial frontal lobe activation during encoding than younger subjects. The findings were interpreted as aging dependent compensatory shift from more posterior and medial temporal systems supporting navigation to more anterior frontal systems. Contrasting activation pattern was found in the only fMRI study in virtual MWM in elderly subjects (Antonova et al., 2009). Differently from younger group, the elderly adults did not activate hippocampal-parahippocampal region either during encoding or retrieval and also lacked activation of the frontal pole and dorsolateral prefrontal cortex. The compensatory shift to frontal system seems therefore to be limited to several structures, some of which may be related to inhibitory processes.

In summary, many descriptions of spatial navigation impairment in aging are compatible with the lower cognitive resources theory of aging and weaker prefrontal function. Evidence for mediotemporal function decline in aging comes mainly from studies using analogies of Morris water maze and brain imaging.

4. Alzheimer's disease

4.1 Progression of Alzheimer's disease

AD is a neurodegenerative disorder, which predominantly and initially affects mediotemporal structures, especially hippocampus and parahippocampal gyrus. Its early symptom is the impairment in episodic memory. Patients have difficulty with learning of new information and retaining it for more than a few minutes. As the disease advances, the ability to learn is increasingly impaired and the access to older, more distant memories is lost. With the development of the disease also other cognitive domains than memory are impaired, like judgment and executive functioning, together with aphasia, apraxia, disorientation and visuospatial functioning. Important diagnostic criterion for dementia is that these cognitive deficits affect daily life of the patients.

Due to its neurodegenerative nature, the development of AD from normal ageing is gradual and long. Increasing attention is now being paid to the mild end of the cognitive spectrum from normal ageing to AD. There is probably a transitional period between normal ageing and the clinical diagnosis of probable very early AD and this transitional zone is now mostly described using term mild cognitive impairment (MCI) (Petersen et al., 2001). Originally, the concept of MCI was limited to memory deficits with relative preservation of other cognitive domains (Petersen et al., 1999). As the research on MCI has advanced, it has become apparent that several clinical subtypes of MCI exist, covering all cognitive domains (Petersen et al., 2001; for review see Petersen, 2004). Besides memory, the impaired cognitive domains can be language, executive function or visuospatial skills. Based on this pattern of impairment, the MCI subtypes comprise amnestic and non-amnestic forms, both possibly single or multi domain. This distinction is particularly relevant when considering the outcomes of subjects with MCI, which can be also non-AD forms of dementia such as vascular dementia or dementia with Lewi bodies. All of these clinical subtypes of MCI have minimal impairments in functional activities and do not meet criteria for dementia. Although amnestic MCI presents a high risk of developing AD, this category includes patients who will develop AD together with others who will never convert. The conversion rates from MCI to dementia reported by different authors range from 7.2% per year (Morris et al., 2001; Rubin et al., 1998) to 12% (Bowen et al., 1997; Petersen et al., 1999) or 13.5 % (Tierney et al., 1996). This is in contrast to conversion rate from healthy elderly subjects to dementia which is between one and two percent per year (Petersen et al., 1999).

4.2 Cognition and neural changes

The MTL atrophy is the hallmark of AD. In individuals at risk of autosomal dominant familial AD it can even precede the onset of cognitive changes (Fox et al., 1996). Neuropathological investigations (Braak & Braak, 1991; Hyman et al., 1984) suggest that AD-related changes may begin in the entorhinal cortex and subsequently spread to the hippocampus. Entorhinal volume was found to be the most useful metric for discriminating healthy from MCI individuals, whereas hippocampal volume was best for classifying MCI and Alzheimer's disease patients (Pennanen et al., 2004). Volumetric studies in AD subjects consistently reveal both volume reductions in the hippocampus relative to age-matched controls (e.g. Jack, Jr. et al., 1992; Jack, Jr. et al., 1997; Killiany et al., 1993) and higher rate of hippocampal decline (Fox et al., 1996; Jack, Jr. et al., 1998; Jobst et al., 1994). The brain pathology in AD shows also in functional brain imaging studies. A pattern of bilateral temporoparietal hypoperfusion or hypometabolism well discriminates AD patients not only from age-matched healthy controls but also from patients with vascular or frontotemporal dementia (Silverman, 2004).

This MTL atrophy is closely related to the episodic delayed recall impairment from the onset of the disease (Greene et al., 1996; Small et al., 2003). Memory for both verbal and visual material is affected in the majority of cases (Greene et al., 1996; Hodges & Patterson, 1995; Small et al., 2003). AD patients seem incapable of learning due to deficient encoding rather than due to impaired retrieval since their free recall performance is as poor as their recognition performance (Greene et al., 1996).

In addition to the MTL, other brain regions degenerate as the disease spreads beyond the hippocampus. Parietal lobe atrophy is well documented from moderate stages (Braak & Braak, 1991; Brun & Gustafson, 1976; Foundas et al., 1996), but medial parietal atrophy

could be detectable even from presymptomatic AD (Scahill et al., 2002) and even in MCI patients converting to AD (Chetelat et al., 2005). Also frontal lobe atrophy was shown from moderate stages (Haxby et al., 1988; Scahill et al., 2002). This extrahippocampal brain affection is related to deficits of other non-memory domains, manifesting as impairment of executive functions with later involvement in constructional praxis, language and sustained attention (Baudic et al., 2006).

Distinguishing evolving from stable amnestic MCI in order to prescribe appropriate treatment as quickly as possible in the evolution of AD is a primary objective of majority of the MCI research. The available biological markers predicting the disease development are not reliable, a combination of several markers increases however the discrimination power. Markers in use include biochemical measures, like amyloid β-protein and tau levels in the cerebrospinal fluid (Hulstaert et al., 1999), brain imaging of hippocampal (Jack, Jr. et al., 1999) or parahippocampal (Visser et al., 1999) atrophy and hippocampal hypoactivation (Johnson et al., 1998; Small et al., 1999), and cognitive neuropsychological evaluation. Episodic memory deficits occur very early in the course of the disease and decreased performance in delayed recall of words, sentences, or series of objects is the most reliable predictor of AD in preclinical individuals (Masur et al., 1994; Tierney et al., 1996). Memory deficit may be a more efficient indicator than MTL atrophy on MRI (Laakso et al., 2000; Visser et al., 1999).

4.3 Spatial navigation impairment in AD

Deficit in the spatial domain is well documented in Alzheimer's disease. Clinically-relevant impairments in navigational skills are often apparent in the early stages and reports of impaired spatial behaviour, like getting lost in familiar places, can in many cases lead to the recognition of cognitive impairment and diagnosis of dementia (Klein et al., 1999). Disorientation and episodes of getting lost were documented both in outpatients (McShane et al., 1998) and patients residing in a community (Pai & Jacobs, 2004). According to global deterioration scale of Reisberg (Reisberg et al., 1982) the first orientation difficulties at the early stage of the disease (stage three) may affect journeys in unfamiliar and macro-scale environments, while at stage six patients only can move in very familiar home settings. Patients with AD have a tendency to become lost and then wander even in familiar surroundings. This "wandering" seems to be associated with an increased tendency to walk (Hope et al., 2001), parietal dysfunction (de Leon et al., 1984) and general confusion.

Many Alzheimer's disease patients suffer from higher visual motion processing deficits (Rizzo & Nawrot, 1998), including selective impairments in perceiving the optic flow (Tetewsky & Duffy, 1999). Optic flow is the patterned visual motion seen by a moving observer (Gibson, 1950). It provides cues about heading direction and the three-dimensional structure of the visual environment (Royden et al., 1992; Warren & Hannon, 1988) and its perception activates right posterior parietal cortex (Morrone et al., 2000). An atypical form of AD with higher visuospatial impairment was described (Kiyosawa et al., 1989), based also on deficits in judging spatial relations (Cogan, 1985) and atrophy (Benson et al., 1988), hypometabolism (Pietrini et al., 1996) and neuropathology (Hof et al., 1989) of the posterior cortical regions.

4.3.1 Perceptual deficits

Most studies on spatial disorientation in AD focus on its connection with optic flow discrimination deficit. This theory was documented several times by a significant correlation

of optic flow discrimination thresholds with several measures of spatial navigation. In a test of navigation in a hospital lobby (Tetewsky & Duffy, 1999) poor performance was associated with an elevated optic flow threshold. In contrast, there was no significant correlation between the MMSE score and spatial navigation score and adding MMSE scores to optic flow threshold in a regression model did not explain more variance in the spatial navigation scores. Another study found significant correlation of the optic flow thresholds also with a score in the table-top left-right orientation Money Road Map test and the ability to respect lane boundaries during sustained driving in On-the-Road Driving test (O'Brien et al., 2001). Rather convincing evidence of the importance of visual perception deficit in spatial disorientation in AD was recently provided in a study by Kavcic et al. (2006). The authors required elderly and AD subjects to attend to a 300 m path through a hospital lobby being pushed on a wheelchair, retrace it and complete a set of tests. Then they correlated navigational to neuropsychological, perceptual and neurophysiological measures. The AD subjects were impaired in all navigational tests, with best results in route and location knowledge and worst results in identifying photo and video location along the route. In a multiple linear regression model, the total score of navigation in AD subjects correlated significantly with optic flow discrimination thresholds, amplitude of the evoked occipital EEG responses N200 and with contrast sensitivity, but with none of the memory tests. The authors concluded that navigational impairment in Alzheimer's disease is linked to a disorder of visual cortical motion processing reflected in specific perceptual and neurophysiological measures.

Consistent with this perceptual deficit theory of spatial disorientation in AD are also two other experiments using route learning in a hospital lobby. The perceptual nature of disorientation was inferred either from correct recognition of landmarks mentioned during the walk in contrast to impaired recognition of incidental not-mentioned landmarks in AD patients (Cherrier et al., 2001) or a lack of relationship between disorientation and memory tests and a failure to use spatial architectural information (Monacelli et al., 2003). The discrepancy between memory impairment, being the defining characteristics of AD, and perceptual nature of disorientation in these patients have been explained by the memory deficits limiting the usage of spatial navigation strategies to these based on visual perceptual analyses (Monacelli et al., 2003), unfortunately without specifying these strategies.

4.3.2 Complex cognitive deficits

While the series of just reviewed studies consistently propose that the factor defining mostly the spatial disorientation in the AD is the perceptual deficit, other articles describe also the role of other cognitive processes. In a study using Memory and Behavior Problems Checklist (Zarit et al., 1985), which contains four items dealing specifically with spatial disorientation (wandering, getting lost indoors, getting lost on familiar streets, being unable to recognize familiar surroundings), Henderson et al. (1989) compared the sum score of these four items with a set of neuropsychological measures. Stepwise regression analysis showed scores from delayed recall memory test and clock drawing and house copying visuoconstructive test as the significant predictors of the disorientation score, but not disease severity, attention or language impairment. As wandering or getting lost is unusual among focal-lesion patients with constructional deficits and is also not reported in association with discrete disturbance in long-term memory, the authors propose that it is the combination of the visuoconstructive and memory deficits that is crucial.

The importance of perceptual and higher cognitive spatial skills for navigation in AD was examined in another study comparing these skills in AD and healthy control subjects with

the functional spatial skills in the subject's own home and in an unknown building (Liu et al., 1991). The AD group was worse than elderly healthy subjects in navigation inside the unknown building but not in their homes. The AD group was also impaired in all cognitive spatial orientation tests and a subset of perceptual spatial orientation tests requiring to mentally represent shapes. Some of the basic orientation skills were intact: visual object recognition of shape, visual and tactual discrimination of size and left-right discrimination. The visuospatial deficit seen in early AD, such as getting lost or misplacing objects, are probably due to the impairment of the mental shape representation or other higher order processes, rather than to visual-perceptual skills.

Another approach was chosen in an experiment by Passini et al. (1995). Spatial navigation was considered as a problem solving task demanding development of a decision plan containing a hierarchy of sub-problems. Subjects diagnosed with AD were told to take the experimenter to the dental clinic in an unknown hospital and were asked to express verbally everything that went through their mind. By interventions from the experimenter the subject were reminded about the task to minimize the effect of memory and attentional deficit. It was observed that irrelevant stimuli affected behavior of the subjects. Some patients acted impulsively without analyzing the constituent elements, e.g. they tended to read uncritically almost all of the written information that they encountered on the trip. The behaviour of many DAT patients was seemingly more driven by external stimuli than by the goal of the wayfinding task. The major difficulty for DAT patients was probably to distinguish relevant from irrelevant information and to structure their decision plan.

Virtual analogue of hospital lobby can probably substitute the real space environment in estimating the navigational deficits of MCI and AD patients, as implied by similar group differences and a strong correlation across all subjects in a recent study (Cushman et al., 2008). The subjects were taken passively (in the real space they were pushed on a wheelchair) along a 300 m path through a hospital lobby, and then should retrace it and complete a set of tests. Stepwise discriminant analysis was then used to find which scores of the follow-up tests distinguished best between the groups. The MCI and AD subjects were best differentiated by tests assessing the ability to locate of scenes from the lobby on its map and the free recall of the landmarks along the route. The cognitive deficit of MCI and AD was interpreted as a dual one consisting of visuospatial and verbal memory deficits. Slight gender differences in performance in a similar task in a hospital lobby were described in AD and MCI patients, with only women performing better in recognition of photographs from the lobby and in free landmark recall (Cushman & Duffy, 2007). Using multiple regression, their general navigational performance was however explained by scores in different neuropsychological tests: mostly radial optic flow perceptual thresholds in men and category naming and figural memory in women. The impairment found in AD and MCI patients can therefore results from different grounds in men and women.

An interesting study used positions of home locations and two familiar landmarks individual for each subject to compare the knowledge of their spatial relations with learning a new environment (Jheng & Pai, 2009). Early AD patients were similarly successful to healthy elderly subjects in drawing simple diagrams of the home and two landmarks positions. The two groups were also similar in learning new goal position in an virtual reality water maze analogue; the elderly subjects were only more successful in learning to navigate to home location in the same water maze analogue using positions of the two familiar landmarks. It is difficult to estimate however if the similarity between the two groups could results from difficulty of the tests even for the healthy subjects.

4.4 Spatial navigation in MCI

The question not sufficiently answered yet concerns with the spatial navigation impairment in a MCI, a diagnosis with a high progression rate to AD. Optic flow perception, the visuoperceptual ability compromised in AD, can be impaired already in amnestic MCI subjects, as documented by Mapstone et al. (2003). In this study, approximately half of the amnestic MCI patients were impaired in radial motion perception, suggesting a visuospatial subtype of MCI based on spatial perception. The motion perception thresholds correlated significantly with the results of the Money Road Map test, requiring subjects to follow a path through a city on a map and indicate left and right turns, but not with figural and verbal memory. However, the MCI subjects were not impaired in the Money Road Map test. The study, therefore, does not document any spatial navigation deficit.

Results of another study using two types of virtual environments might also be interpreted in the context of deficits in parietal lobe function (Weniger et al., 2010). The MCI patients and elderly control subjects should learn in five trials a route through a maze, without any landmarks and containing six intersections, and through a virtual park, a model of a village with many landmarks, both near and distant. The MCI patients performed worse than the control group in both environments, more impaired were however in the maze environment without any learning evident during the five trials. In addition, the performance in this environment was correlated with right precuneus volume. The MCI seem therefore to be connected to both egocentric navigation deficits in the maze and allocentric navigation deficits in the park, with more evident egocentric deficits. This is consistent with the following studies documenting general spatial navigation impairment in multi-domain MCI subjects.

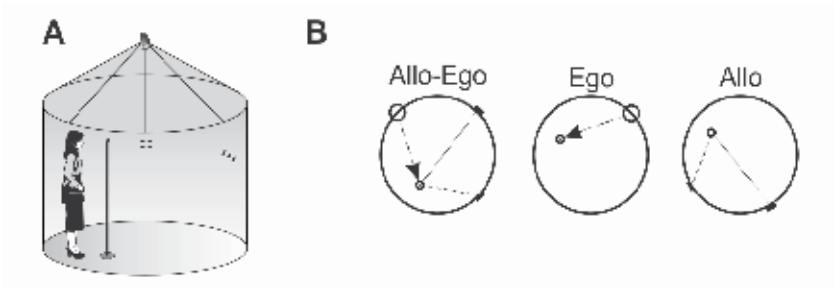

Fig. 1. Experimental environment and the scheme of the test from Hort et al. (2007)

That spatial navigation deficits could be detected even in amnestic single-domain MCI patients and that the memory deficit is the determining factor in this deficit was suggested by our recent experiment (Hort et al., 2007). In a real space analogue of the Morris water maze, we found navigational impairment in single-domain amnestic MCI subjects only in allocentric but not in egocentric configuration. To locate an invisible goal, the subjects were required to use two landmarks in the allocentric configuration or their own position in the egocentric configuration. The specificity of their impairment only to the allocentric part suggests that spatial configuration memory was the critical factor affecting the results of the amnestic MCI group in our experiment, in contrast to visuospatial perceptual functions which were required probably throughout the whole test. This interpretation is consistent with the MTL atrophy found in MCI (Du et al., 2001) and with the progression rate to AD from amnestic MCI higher than from non-amnestic subtypes of MCI (Yaffe et al., 2006). In

contrast to the results in single-domain amnestic MCI subjects, the group of multi-domain amnestic MCI patients was impaired in all subtests including both egocentric and allocentric configuration. This suggests that together with the decline in other domains, but before progression to AD, the impairment includes even simpler forms of navigation, presumably not dependent on the MTL. Similar pattern of impairment mainly in allocentric navigation was described in our previous studies in the AD group, which was broadly defined mainly by severe memory problems (Kalova et al., 2005; Laczo et al., 2010).

The view of multiple domain based disorientation in MCI patients was supported by several other reports. MCI patients reporting problems with navigation in a structured interview had lower volumes in temporo-parietal brain regions, including hippocampus and parahippocampal gyrus, than patients without navigation problems (Lim et al., 2010). Another study correlated navigational impairment in AD and MCI patients to standard neuropsychology tests and regional neural atrophy (deIpolyi et al., 2007). The key finding was that the patients who made at least one error in retracing a route through a hospital lobby did not differ from other patients in any memory or executive neuropsychological tests but had lower right posterior hippocampus and right posterior parietal cortex volumes. The fact that both medial temporal and parietal volumes corresponded to errors in navigation supports the view of visuoconstructive and memory deficits are in the basis of spatial navigation impairment in AD and MCI.

In the last published study focusing on spatial navigation in MCI, a small virtual city was used (Tippett et al., 2009). The subjects passively watched movement along a path though the virtual city comprising four intersections and should reproduce it in five trials. The group of MCI subjects differed only in the speed of movement from the control subjects, but was similar in the number of errors. This similarity was probably due to the ceiling effect in the very simple maze.

In summary, both visual perception changes and deficits in spatial memory seem to predict navigational performance of Alzheimer's disease patients. While the severe memory deficits in AD may emphasize the role of visual perception efficiency, both cognitive abilities apparently contribute to navigational capacity of MCI patients.

	Brain changes	**Cognitive abilities linked to spatial navigation impairment**
Healthy Aging	Prefrontal areas Medial temporal lobe	Working memory Cognitive capacity limits Temporal ordering Relevant information selection Formation and usage of cognitive map Place recognition by room geometry
Mild cognitive impairment and Alzheimer's Disease	Medial temporal lobe Parietal lobe	Optic flow perception Long-term memory Relevant information selection Egocentric navigation

Table 1. Summary of the brain and cognitive changes linked to spatial navigation impairment in healthy aging and Alzheimer's disease

5. Conclusion

From the reviewed literature some important points can be made. During aging, the most affected brain regions are in the PFC and most neuropsychological studies on episodic memory and also spatial memory explain the deficit in elderly subject by this prefrontal decline. Some navigational experiments in a real space or in a virtual reality points however to the hippocampal and MTL affection in aging and several object location memory experiments allow similar interpretation. More experiments are needed to compare these explanations, but in any case the spatial navigation is a promising choice of hippocampal function assessment in elderly.

Spatial navigation impairment in AD is probably closely related to its multi-domain nature. The necessary condition for navigation in a novel environment is a contribution of higher visual perceptual functions which are impaired in AD. Due to this impairment, the perception seems to be the more important factor in the documented navigational impairment than memory. In a known environment however, like in a familiar building or in familiar streets, the perceptual requirements may become smaller and the spatial memory seems to become another important factor of navigational impairment in AD.

The boundary between the healthy aging and AD in the domain of spatial navigation presumably lies in the cognitive changes connected to parietal lobe atrophy during the dementia development. Optic flow perception deficits and egocentric navigation impairment appear to be detectable in some MCI patients and are profound in AD patients. This hypothesis, implied by the current review, deserves however further examination. Future studies should focus on connection between these abilities and spatial disorientation also in other types of environments, as in water maze analogues and virtual mazes, and on the possibilities of outcome prediction of MCI patients. MCI subtypes differentiation based on individual cognitive domains could be essential for these predictions. Another area worth more experimentation is the navigation of AD patients in familiar environments. The knowledge of the strategies and orientation abilities these patients have in their homes should help with their well-being.

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6

Visual Cognition in Alzheimer's Disease and Its Functional Implications

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1. Introduction

Alzheimer's disease (AD) is a common form of neurodegeneration that entails a progressive breakdown of cognitive functions. Cognitive impairments commonly begin with noticeable difficulties in remembering recent events (Gold & Budson, 2008; Ally, Gold & Budson, 2009), as well as spatial disorientation (Duffy, Cushman & Kavcic, 2004) and semantic memory impairments (Milberg, McGlinchey-Berroth, Duncan & Higgins, 1999). As the disease progresses, AD also entails impairments to visual cognition, which encompasses processes that help us to understand what we see (Cronin-Golomb, 1995). The purpose of this chapter is to review recent progress towards understanding aspects of visual cognition in AD, including visual memory and visual attention, and then explore how this research can be applied to assist in the daily living of AD patients.

Humans normally rely tremendously on vision for interacting with the world. Therefore, there is a profound impact on everyday activities when visual impairments develop. For example, Perry and Hodges (2000) showed that measures of visuospatial functions, semantic memory, and attention correlated significantly with measures of daily living in patients with AD, but measures of episodic and verbal short-term memory did not. Further, impairments to visual attention and memory in patients with mild-to-moderate AD lead to poor performance in complex situations like driving (Rizzo, Sparks, McEvoy, Viamonte, Kellison & Vecera, 2009) and measures of financial responsibility (Sherod, Griffith, Copeland, Belue, Krzywanski et al., 2009). Finally, certain visual abilities, like assembling an image from its parts, deteriorate as AD progresses, providing a reliable means of tracking the progress of AD in a given patient (Paxton, Peavy, Jenkins, Rice, Heindel & Salmon, 2007). Changes in everyday visual abilities can be observed very early in the disease, even in patients with mild cognitive impairment (MCI), which is thought to be a precursor to AD (Farias, Mungas, Reed, Harvey et al., 2006). Together, these studies suggest that measurable visual impairments in AD are strongly related to deterioration in everyday activities.

Since the decline of visual abilities can have a very profound effect on daily living, a better understanding of AD-related changes to visual cognition, and the development of subsequent interventional strategies, promise to lead to improvements in patients' quality of life. This chapter will place an emphasis on visual short-term memory (VSTM) in patients with AD, which has only recently been investigated. We will first describe the basic process of VSTM and then review the changes known to occur in AD. Then, we will review the interaction between visual attention and VSTM in AD patients and indicate how these two

processes have sometimes been confounded with each other in the literature regarding AD patients. Finally, we will discuss possible functional interventions to improve daily living in AD patients based on findings in visual cognition.

2. Visual short-term memory

Visual memory is extremely important in normal daily functioning. Consider how one navigates a room during a sudden blackout or how one recalls the color of a pill he or she just ingested. More fundamentally, visual memory is crucial for one's basic understanding of the visual environment. Objects often become occluded, like when a person walks behind a tall bookshelf. Visual processing is also suppressed during saccadic eye movements, when one's eyes suddenly move from one position to another. The situations described above are discontinuous visual events: the bookshelf disrupts the image of a person in the former situation, and the saccadic suppression disrupts the image of the entire world in the latter. Such discontinuities introduce a problem of correspondence to the visual system. In the first situation, how do we know that the person walking behind the bookshelf is the same person that emerges from behind that bookshelf? In the second situation, the image of an object will project to different regions of the retina before and after the saccadic eye movement. Given that visual information is unavailable during saccadic suppression, how are the images matched across saccades? Both solutions require visual memory.

Several forms of visual memory are available to the visual system following the offset of a visual stimulus, including visible persistence, iconic memory and visual short-term memory (VSTM). Visible persistence refers to the subjective impression of an image remaining visible after it has offset. For example, lightning is only physically present for less than 100 milliseconds (ms), but we perceive it to last much longer. Iconic memory is a highly detailed representation of an image, but only lasts for 300 – 500 ms (Sperling, 1960). Unlike visible persistence, iconic memory does not necessarily involve an impression that the stimulus is still present (Coltheart, 1980; Irwin & Yeomans, 1991), and the information extracted from iconic memory has undergone some higher-level processing, such as rapid matching to long-term memory representations (Chun & Potter, 1995). Another form of memory is visual short-term memory. VSTM involves a less detailed representation of an image compared to iconic memory, but it can endure for up to nine seconds or longer (Phillips, 1974). In relation to models of cognition, VSTM is similar to the visuospatial sketchpad of Baddeley's multi-component model of working memory (Baddeley & Hitch, 1974).

Phillips (1974) first distinguished iconic memory from VSTM with a change detection task. A typical trial in this task entails presenting observers with a brief visual pattern, followed by a blank interval of a specified duration, and then another visual pattern. The observer's task is to report whether the two patterns are the same. It is assumed that the ability to detect whether a change has occurred is mediated by comparing the memory of the first pattern to the perception of the second pattern (Figure 1). Using this task, Phillips (1974) discovered that visual memory exhibited different properties depending on how much time intervened between the visual patterns. The early memory was related to iconic memory, and the late memory eventually became known as VSTM. These memories differed in two important ways. First, unlike iconic memory, VSTM is not tied to the retinotopic region upon which the image was projected. Second, VSTM is resistant to backward masking, which is erasure that could occur due to visual stimulation occurring after a stimulus has

been encoded in memory. In contrast, iconic memory is not resistant to backward masking, so it can be erased by subsequent visual stimulation.

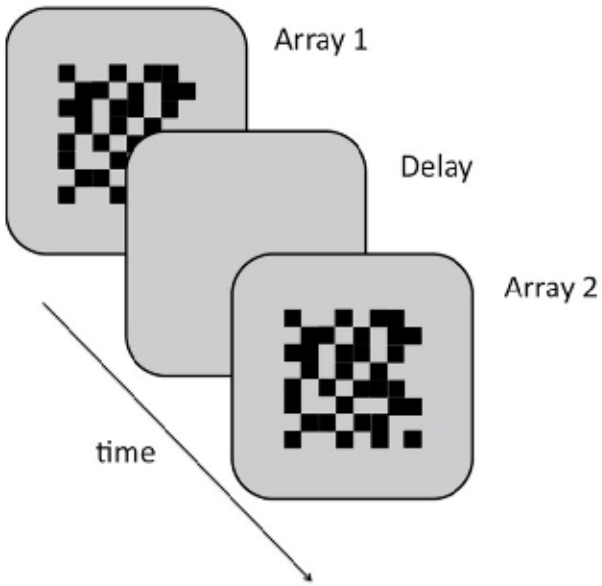

Fig. 1. One trial of a change detection task, adapted from Phillips (1974)
Two visual patterns appear sequentially and separated by a blank screen enduring for a certain amount of time. The ability to detect any difference between the patterns is assumed to depend on a form of memory that must span the delay interval. This delay could be between 100 – 500 ms to capture iconic memory. Delay intervals of longer than 500 ms could capture VSTM.

The properties of VSTM discovered by Phillips (1974) make it an ideal component in performing everyday activities. The finding that VSTM is not retinotopic suggests that it plays a role in resolving the correspondence of images across saccadic eye movements. Irwin (1991) showed that subjects were able to detect changes between the visual patterns, even when they made eye movements during the inter-pattern interval. Irwin's (1991) results closely resembled those of Phillips' (1974) study, suggesting that VSTM mediated the retention of visual information across saccadic eye movements. VSTM is also vital in correcting eye movement errors (Hollingworth, Richard & Luck, 2008; Hollingworth & Luck, 2009). Eye movements often land inaccurately and miss their targets, especially in crowded situations where one must perform a visual search and move the eyes frequently. Hollingworth and colleagues (2008) showed that the ability to correct saccadic eye movement errors was significantly slowed when subjects concurrently maintained information in VSTM, but not when they held information in verbal short-term memory. The use of VSTM in eye movements is also suggested by the finding that it is not susceptible to backward masking. Some theories propose that finding an object during visual search is facilitated by keeping the target in VSTM (Desimone & Duncan, 1995). It would then be important that contents of VSTM are not easily erased as the eyes land on different objects in the scene during the search.

The differing properties of iconic memory and VSTM suggest a possible transformation of information between the two systems. This transformation of information is referred to as consolidation (Chun, 1997; Jolicoeur & Dell'Acqua, 1998). Consolidation of an item into short-term memory is cognitively demanding, so that the performance of other tasks is delayed until it concludes (Jolicoeur & Dell'Acqua, 1998). The time that is required for consolidation to conclude depends on the amount of information being transitioned between iconic memory and VSTM (Vogel, Woodman & Luck, 2006), as well as difficulties in target identification caused by low-level factors, such as backward masking (Seiffert & Di Lollo, 1997).

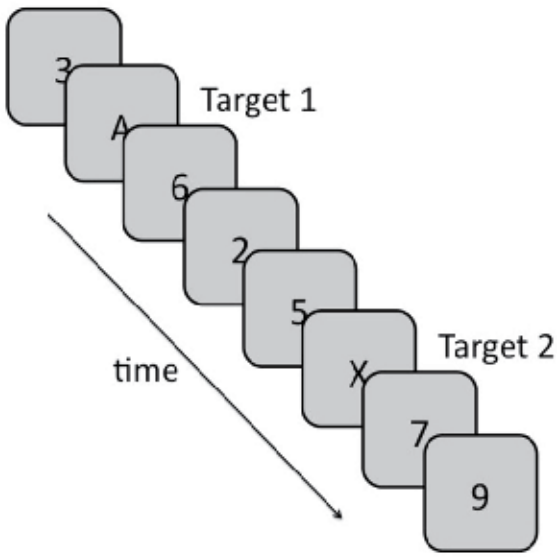

Fig. 2. One trial of a target discrimination task using RSVP.
A schematic of a typical multiple target discrimination task using rapid serial visual presentation (RSVP). The stimuli are typically presented at a rate of 100 ms per item, although this varies across studies. The task is to report the identities of items belonging to a target category, in this case letters. The attentional blink refers to the difficulty of reporting the second target if it appears too closely to the first target in the sequence. However, accuracy of reporting the second target is often high when it immediately follows the first, a phenomenon called "Lag-1 sparing" (Chun & Potter, 1995).

One paradigm that nicely illustrates the consolidation process is multiple target discrimination during a rapid serial visual presentation (RSVP; see Figure 2). In this task, participants view several items in rapid succession, and are required to identify two items belonging to a specific target category, such as letters appearing among digits. One well-known phenomenon that occurs in this paradigm is the attentional blink (Raymond, Shapiro & Arnell, 1992). When participants correctly identify the first target, they are often impaired at identifying the second one if it follows the first target too closely in the sequence. It is thought that the processing of the first target delays the processing of other incoming

information. If the second target falls within this window of time, it cannot be reported. The processing during the attentional blink is thought to include the consolidation of the first target from iconic memory to VSTM (Chun & Potter, 1995). The actual processing of the first target may entail binding its features together as it is encoded in VSTM (Chun, 1997). Together, visible persistence, iconic memory, and consolidation can be conceptualized as progressive stages of encoding information into VSTM. A rough schematic of this process is shown in Figure 3.

What determines the limit of VSTM storage capacity? Using change detection tasks, Luck and Vogel (1997) found similar VSTM capacity when memorized items differed from each other by a single feature, as well as when the items differed from each other by a specific conjunction of color and orientation. In other words, they found that change detection of conjunctions was just as good as that of basic features. This result showed that VSTM capacity was not defined by the total amount of differing information available to the observer. Instead, the basic unit of VSTM is not individual features, but rather integrated objects. This may have occurred because each item underwent processing that integrated their component features. Another important result was that VSTM capacity had an upper limit of about four objects, which resembled the capacity of several short-term memory studies (Cowan, 2001).

To illustrate this finding, imagine that a person with a VSTM capacity of 2 objects must remember to take two pills. This person would have the same capability to remember to take two red and blue circular pills, and to take a red circular pill and blue cylindrical pill. In the first case, there are only two pieces of differing information, being the colors. In the second case, there are four pieces of differing information, being the colors and the shapes. This person would have adequate memory in both cases since they both involve only two objects, which fits the person's VSTM capacity. With some modification, Luck and Vogel's (1997) results have been replicated many times (Vogel, Woodman & Luck, 2001; Olson & Jiang, 2002; Xu, 2002; Alvarez & Cavanagh, 2004; although see Wilken & Ma, 2004).

However, an important debate in the VSTM literature has regarded exactly how objects are represented in VSTM. Luck and Vogel (1997) proposed that the objects of VSTM were fully integrated, such that no other process but VSTM storage was necessary to maintain them. Alternatively, Wheeler and Treisman (2002) proposed that sustained visual attention was necessary to keep the features of each object bound together. Treisman had shown years earlier that visual attention plays a similar role in visual perception (Treisman & Gelade, 1980), thus providing a parsimonious theory of how objects are represented in visual cognition: in both perception and memory, objects are comprised of separate features bound together by visual attention. Studies have examined Wheeler and Treisman's (2002) binding account of VSTM by having participants perform a visual attention task during VSTM retention. The binding account predicts that, in such conditions, VSTM for features would be unaffected by the intervening task, but VSTM for conjunctions would be profoundly impaired since visual attention would be occupied with the intervening task. Many studies failed to show any differences in VSTM for features and conjunctions (Yeh, Yang & Chun, 2005; Allen, Baddeley & Hitch, 2006; Johnson, Hollingworth & Luck, 2008). However, when Fougnie and Marois (2008) used a multiple-object tracking task during VSTM maintenance, which places heavier demand on visual attention than the previous studies, they showed significant impairments in VSTM for conjunctions, but not for features. In further support for a bound feature-based representation in VSTM, it has been shown that participants can selectively encode specific features of an object in memory (Woodman & Vogel, 2008).

Furthermore, participants can selectively update a specific feature of an object in VSTM, while leaving the other features of that object unchanged (Ko & Seiffert, 2009).

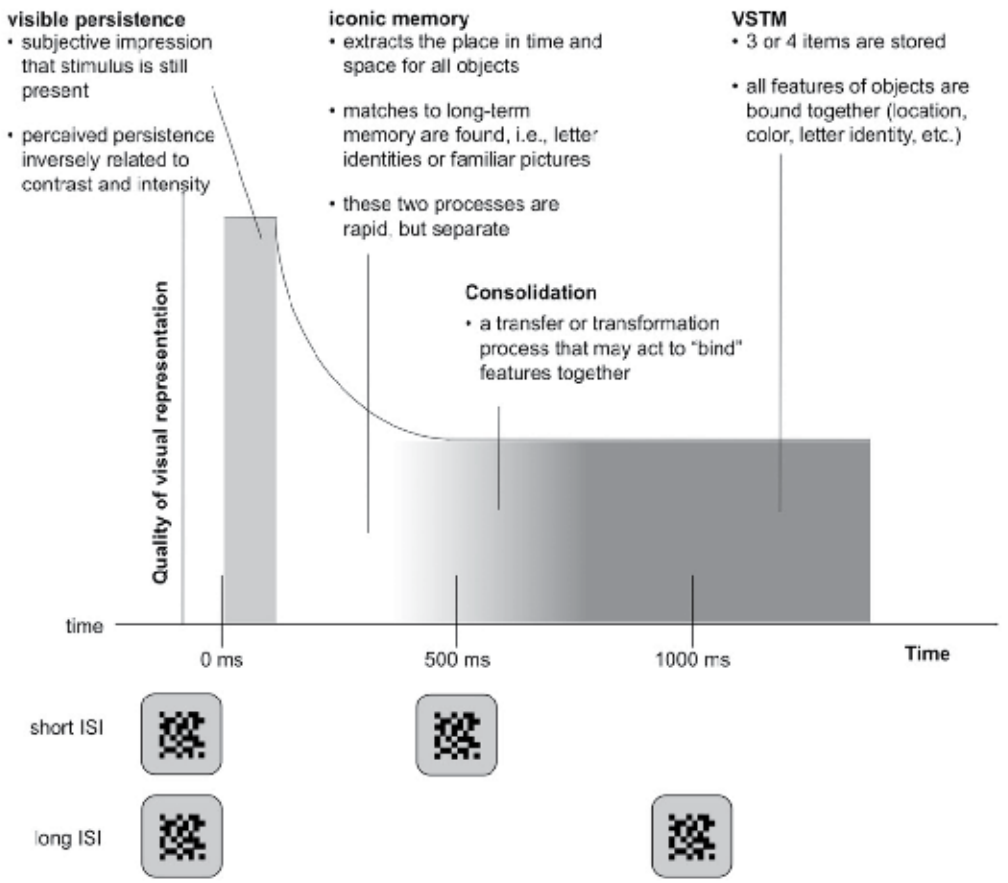

Fig. 3. A schematic of visual memory stages.
The y-axis depicts a vague concept of "quality", indicating how similar the memory representation is to the original perceptual representation. This visual quality is plotted as a function of time on the x-axis. In visible persistence, quality is high because the subjective percept is identical to the stimulus. Iconic memory marks the exponential decay of visual quality over time. The gradient between the iconic memory stage (white) and VSTM stage (dark gray) represents the time of consolidation. The decay in quality plateaus as the information is being consolidated into VSTM and then there is no further loss of information. This decay in visual quality trades off with the level of abstraction from the original stimulus, so by the time information is consolidated into VSTM, it is processed at a high-level. Below the x-axis, the stimuli for change detection are depicted as they would appear in time. This illustrates how manipulating the time interval between the two arrays captures different kinds of visual memory. It should be noted that this summary of findings is specific to change detection and RSVP methods. Other psychophysical methods, like method of adjustment, capture important details of VSTM not usually found by change detection (Zhang & Luck, 2008; Bays & Husain, 2009).

What are the neural correlates of VSTM? Studies using functional magnetic resonance imaging (fMRI) have related activity in the posterior parietal cortex to VSTM capacity (Todd & Marois, 2004). However, there may be multiple sites of neural representation related to VSTM. For example, different regions of the parietal cortex have been related to the number of items stored in VSTM and the visual complexity of the items (Xu & Chun, 2006). Recent studies using sensitive multivoxel pattern analyses have shown evidence that primary visual cortex and extrastriate areas are also involved in VSTM representation (Harrison & Tong, 2009; Serences, Ester, Vogel & Awh, 2009). Studies using electroencephalograms (EEG) have identified an event-related potential (ERP), recorded from posterior electrodes in the hemisphere contralateral to the stimulus, that modulates with VSTM capacity (Vogel & Machizawa, 2004). This ERP, called the contralateral delay activity (CDA), originates from activity recorded by posterior electrodes contralateral to the stimulus location. It emerges 200 ms after stimulus onset, and remains active during VSTM maintenance.

In summary, VSTM emerges from earlier stages of visual memory. Visual information is initially available in high resolution visual memories, including visible persistence and iconic memory. Visual information then must undergo a time-consuming, cognitively demanding process of transfer or transformation into VSTM, during a stage called consolidation. These early stages of visible persistence, iconic memory, and consolidation can be conceived of as progressive stages of VSTM encoding. Information is properly stored in VSTM, which exhibits three important properties:

1. It is not tied to the original retinotopic location of the perceptual image being stored.
2. It is resistant to incoming visual stimulation, so one can hold information in VSTM while looking at other things.
3. VSTM stores relatively complex objects; the mechanism by which this is possible may be sustained visual attention that binds the features of each object together.

These properties indicate that VSTM is critical in common tasks that involve frequent eye movements around a scene, such as visual search. VSTM enables people to match images across saccades, correct saccadic eye movement errors, and keep a target item in memory during a search process. Understanding this system in AD patients is important when considering changes to visual cognition associated with the disease. In the next section, we will examine what is known about visual memory and VSTM in AD patients.

3. VSTM in Alzheimer's disease

There has been relatively little direct investigation of VSTM in patients with AD. However, examining VSTM in AD patients is potentially important for several reasons. First, methods that tap into VSTM processing are relatively simple, sensitive, and inexpensive, providing a means to detect early signs of AD-related changes to VSTM. Second, AD may also involve specific changes to VSTM that can be measured to distinguish AD from other neurodegenerative diseases and memory disorders. Finally, understanding changes in VSTM in patients with AD can help lay the groundwork for developing meaningful behavioral strategies and interventions that can be easily implemented in the clinic or home. In this next section of the chapter, we will first review early research on visual memory in AD and its relationship with visual perception and long-term memory. Then, we will review more recent research on VSTM in AD, and emphasize AD-related changes to VSTM encoding and storage.

Various techniques in neuroscience have revealed deficits in functions that may overlap with VSTM in AD patients. fMRI has shown that when encoding visual information, medial

temporal activity that is typically observed in normal adults is absent in patients with AD (Kato, Knopman & Liu, 2001). However, this finding may be specific to encoding information into long-term memory. AD-related differences in neural activity during working memory may suggest that AD patients use different cognitive strategies than controls to accomplish such tasks (Yetkin, Rosenberg, Weiner, Purdy & Cullum, 2006). Animal models of AD using non-human primates have shown that lesions to the cholinergic and noradrenergic systems impair VSTM (Dudkin, Chueva, Makarov, Bich & Roer, 2005), suggesting that deficits in visual memory can be related to pathology in areas such as the nucleus basalis of Meynert and the locus coeruleus.

Early studies suggested that AD-related impairments in visual memory were independent of problems with visual perception. Grossi, Becker, Smith and Trojano (1993) showed that AD patients had impaired short-term memory for movement patterns and visual patterns, without showing impairments of visual perception. Similarly, Trojano, Chiacchio, De Luca and Grossi (1994) demonstrated that AD patients had impaired short-term memory for abstract visual patterns, while exhibiting no deficits in visual perception. More recently, Rizzo and colleagues (2000) showed that while AD patients exhibited intact performance on tasks measuring low-level perceptual functions like motion discrimination and visual acuity, they were impaired on higher-level visual processes, such as divided attention, selective attention, and visual memory. Together, these early findings suggested that patients with AD have impairments in VSTM, while their visual perception remains intact.

However, not all low-level perceptual functions are left intact by AD. Rizzo et al. (2000) showed that contrast sensitivity was impaired in AD patients. This perceptual impairment in AD has profound effects on higher-level visual tasks. Supporting this idea, Cronin-Golomb and colleagues (2007) showed that enhancing the contrast of visual stimuli facilitated the performance of AD patients to match that of healthy older adults on several tasks of high-level visual cognition. This finding is remarkable, because it indicates a simple solution to significant visual problems in AD patients. Interestingly, although the enhanced stimuli aided AD patients on several visual-based tasks, it did not help them on the Raven's Progressive Matrices task. This task requires viewing a patterned sequence of abstract images and choosing another abstract image to complete the sequence. Their impairment on the matrix task was remarkable, because enhanced contrast did benefit them on a face-matching task that was arguably equal in task demands – both required multiple fixations, and both entailed a form of matching a target to a sample. In other words, both had similar oculomotor demands, and both likely required visual memory. The crucial difference between the tasks could have been that the abstract visual patterns contained no semantic information, in contrast to the stimuli in the face-matching task. This suggests that compensating for low-level impairments in contrast sensitivity effectively enhances retention of images containing familiar semantic information but not images devoid of semantic content.

This idea is supported by results from Ally, Gold and Budson (2009), who showed that mild AD patients have a relatively preserved long-term memory for real-world pictures compared to memory for the verbal referents of those pictures. Pictures of real objects produce a great deal of semantic or conceptual activation, in addition to the rich amount of perceptual information that they contain. To follow-up on this finding, Ally, McKeever, Waring and Budson (2009) found that ERP components related to memorial familiarity were preserved in amnestic MCI patients when they memorized pictures, but were diminished when they memorized words. Familiarity can be rooted in the ease at which both perceptual

and conceptual information related to the test item is processed. Since MCI patients demonstrated intact familiarity for visual stimuli compared to verbal stimuli, Ally, McKeever, et al. (2009) suggested that implicit or stored visual representations were responsible for the increased familiarity and discrimination for pictures. Further, O'Connor and Ally (2010) found that recognition memory in amnestic MCI and mild AD patients was superior when the perceptual and conceptual aspects of studied items were preserved from study to test, compared to when only the conceptual aspects were preserved from study to test. Importantly, this advantage was much greater when the stimuli were pictures rather than words.

Together, these findings show that storing information as pictures leads to greater success of long-term memory retrieval in AD patients. This might stem from the rich retrieval cue provided by the combination of visual and semantic information in pictures compared to words alone. Another likely hypothesis is that neural regions dedicated to VSTM remain intact, allowing for storage of visual representations that can be used for subsequent retrieval. In support of this idea is evidence that similar neural regions are activated during retrieval from VSTM and visual long-term memory (Ranganath, Cohen, Dam & D'Esposito, 2004).

Direct investigation of AD-related changes in properties of VSTM has become more popular in the last decade. In an initial study, Vecera and Rizzo (2004) used change detection tasks to show that AD patients exhibit a decreased VSTM capacity. What is the root of this decreased VSTM capacity? Subsequent research has shown AD-related deficits in at least three important stages of the VSTM process. First, iconic memory decays much faster in patients with MCI compared to healthy controls. Second, the consolidation of information in VSTM may be impaired in AD patients. These first two findings suggest that the encoding of information into VSTM from earlier memory representations may be corrupted. Third, VSTM storage itself is changed in patients with AD. Specifically, AD patients appear to have a specific deficit in how features of objects in VSTM are bound together.

Lu, Nuese, Madigan and Dosher (2005) showed that patients with MCI exhibited much faster decay of iconic memory than healthy younger and older adult controls. In this particular visual task, the duration of iconic memory was calculated to be approximately 70 ms in patients with MCI, while younger and older adults showed durations of nearly 340 ms and 300 ms, respectively. This accelerated decay of iconic memory could potentially lead to fewer items being transferred to VSTM, especially as MCI patients develop AD.

In addition to iconic memory being shorter in duration for patients with very mild AD, the consolidation of information from iconic memory into VSTM appears to be somewhat impaired. Kavcic and Duffy (2003) showed that the attentional blink window was much more severe and longer enduring for AD patients than for healthy older adults. Furthermore, the authors noticed that AD patients tended to report the second target correctly while failing to identify the first target. This impairment was attenuated when 5 or more intervening distractors appeared between the first and second target. In other words, the identification of the second target seemed to retroactively mask identification of the first target, analogous to perceptual backward-masking effects. However, this effect was attentional in nature because it occurred between items belonging to the target category. This effect may stem from the same mechanism responsible for object substitution masking, a phenomenon thought to reflect the overwriting of one visual object by another (Enns & Di Lollo, 1997). An object may be susceptible to this overwriting if there is a failure to encode it into VSTM (Prime, Pluchino, Eimer, Dell'Acqua & Jolicoeur, 2010). Interestingly, this

alteration to the attentional blink may explain some results in a study directly examining VSTM in AD patients. Alescio-Lautier and colleagues (2007) had AD patients report any changes between a visual image in memory and three sequentially presented probe images. AD patients failed to detect changes in the first of the three probe images but successfully detected changes in the second and third image. This temporary failure to detect changes between the image in memory and a subsequent probe image is consistent with Kavcic and Duffy's (2003) finding of a prolonged attentional blink in AD.

The finding of AD-related changes to the attentional blink (Kavcic & Duffy, 2003) differs from the results of two studies with MCI patients. Perry and Hodges (2003) found that patients with MCI did not show alterations to the time-course of the attentional blink. Also, Lu et al. (2005) found that, although MCI patients have shortened duration of iconic memory, they have normal transfer of items from iconic memory into VSTM. Together, these results could reflect the natural course of AD, such that the consolidation process from iconic memory to VSTM is still intact in MCI patients but becomes corrupted once MCI patients develop into AD patients. Alternatively, they could reflect the methodological differences between the studies. For example, the stimuli used by Perry and Hodges (2003) and Lu et al. (2005) were spatially distributed, but the stimuli used by Kavcic and Duffy (2003) were all centrally presented. Subjects could therefore use the spatial pattern formed by multiple items as a memory cue (Jiang, Olson & Chun, 2000).

AD patients have also been shown to exhibit changes in VSTM storage. These studies suggest that AD patients do not have impaired VSTM storage per se, but rather have a specific problem in maintaining how features of objects are bound together. For example, Parra and colleagues (2009a) had AD patients and healthy older adults view visual arrays of multiple items. Each item was either distinguishable by a single feature, like color or shape, or by a specific conjunction of color and shape. Participants then provided a free recall of the items after the array was removed. AD patients did not differ from controls in their memory accuracy for features, but showed significantly worse accuracy for conjunctions. This suggested that short-term memory deficits in AD might not involve a decreased capacity per se, but a specific impairment in maintaining the relationship between the component features of each object. However, since the participants provided verbal reports, the authors did not claim that these short-term memory deficits were specifically visual.

Further studies from the same group have supported the idea that patients with AD have specific problems keeping information bound in VSTM. These results have practical applications, such as early detection of AD onset and distinguishing AD from other memory disorders. Parra et al. (2009b) measured VSTM for features and conjunctions in patients with familial AD caused by mutation of the presenilin-1 gene. Binding deficits were detected in patients with early onset AD, as well as asymptomatic carriers of the gene, when compared to non-carriers of the same families. This suggests that measuring a specific impairment in VSTM can be used as a means for detecting AD early in the course of the disease, perhaps prior to the onset of noticeable symptoms. Parra and colleagues (2010) also showed VSTM binding deficits to be specific to patients with AD compared to depressed patients, who also suffer from poor memory. The detection of VSTM binding deficits is more accurate than other binding problems in distinguishing AD patients from healthy older adults. Healthy older adults show deficits in binding features in long-term memory (Chalfonte & Johnson, 1996), but AD patients show this deficit as well (Dierckx, Engelborghs, De, et al., 2007; Swainson, Hodges, Galton et al., 2001). However, healthy older adults do not show deficits in VSTM bindings (Brockmole, Parra, Della Sala, & Logie, 2008; Parra et al., 2009c).

Together, these studies show that deficits in VSTM binding could better distinguish AD from healthy aging compared to other impairments in memory.

In summary, important findings have begun to reveal AD-related changes in certain VSTM processes. Changes to visual encoding can be detected early in the course of AD, as suggested by shortened iconic memory in MCI patients. While consolidation may remain intact in MCI, it becomes corrupted as MCI patients convert into patients with AD. As information is consolidated from iconic memory to VSTM, AD patients have trouble preventing incoming information from interfering with representations already in VSTM, sometimes resulting in the overwriting of information in VSTM. This impairment would subsequently interfere with normal cognitive processes, such as making correspondence between images across eye movements. Imagine that, in such a situation, rather than being able to compare the images across an eye movement, the new information acquired once the eye has landed subsequently erases the memory of information acquired before the saccade. This would greatly impair common activities, like trying to find one's wallet on a crowded countertop.

Finally, the storage of information in VSTM is changed in AD. AD patients have a specific difficulty retaining the relationship between features of objects in VSTM. For example, if a patient kept the image of two parking lot symbols in VSTM, such as a red square and blue oval, a potential problem might be that he or she could falsely recognize a blue square or red oval. It is important to note that this finding indicates information in VSTM is still preserved in AD patients. The key to helping patients could be to adapt their environment according to this preservation.

4. Visual attention and memory in Alzheimer's disease

Attention is considered to be involved in the high-level processing of information in a manner that is limited in capacity (Pashler, 1998). This capacity limitation has many consequences, including the processing of some information at the cost of neglecting other information (James, 1890) and the inability to adequately perform two tasks simultaneously (Pashler, 1994). Visual attention refers to when an aspect of vision is the basis of this capacity limitation. For example, visual attention may select and process a small region of visual space at the cost of processing surrounding regions. In this section, we will examine the relationship between visual attention and VSTM in AD. Although some researchers consider visual attention and VSTM to be synonymous (Cowan, 2001), some research suggests that there is only a partial overlap in these processes and at least some important distinction exists between them (Fougnie & Marois, 2006; Ko & Seiffert, 2006). It is important to understand this relationship in the context of AD, because AD-related impairments in visual attention could actually be rooted in changes to VSTM (Vecera & Rizzo, 2004). For example, one early study by Simone and Baylis (1997) suggested that AD-related problems with selective attention could be significantly alleviated once memory demands had been removed. However, removing such demands did not relieve all attention-related impairments.

AD patients have long been known to have disturbances in visual attention. Extensive reviews on this topic have been recently published (Vecera & Rizzo, 2004; Iachini, Iavarone, Senese Ruotolo & Ruggiero, 2009; Tales & Porter, 2009), so this section will not provide an exhaustive review. Instead, we will examine whether AD-related problems in visual attention could be re-interpreted as problems with VSTM, as first proposed by Vecera and

Rizzo (2004), prior to much of the research conducted on VSTM in AD patients. We will show that, although there is some evidence to support Vecera and Rizzo's (2004) theory in regards to selective attention, visual search, and eye movements, there is also evidence to support the opposing theory – problems with VSTM could be rooted in deficits of visual attention.

Selective attention processes must enhance or facilitate relevant information while also filtering or inhibiting irrelevant information (Neill, 1977). Priming measures have been used to examine selective attention in AD patients. In these studies, participants attend to one stimulus while concurrently ignoring another stimulus. Effects of this selection can be observed by subsequently measuring responses to a quality of the attended or ignored stimulus, such as a color, a location, or a name. Faster responses, or positive priming, are predicted to occur for a feature of the attended stimulus, indicating facilitated processing of that stimulus. Slower responses, or negative priming (Tipper, 1985), are predicted to occur for features of the ignored stimulus, indicating inhibited processing of that stimulus. Research using priming has suggested that AD patients have trouble inhibiting irrelevant information (for a review, see Amieva Phillips, Della Sala & Henry, 2004). For example, Grande, McGlinchey-Berroth, Milberg and D'Esposito (1996) demonstrated that AD patients not only failed to suppress information to be ignored, but that such information was actually enhanced, as if it were attended. Similar studies have shown that AD patients fail to suppress the visual image of an object that should have been ignored (Sullivan, Faust & Balota, 1995; Amieva, Lafont, Auriacombe, Le Carret, Dartigues et al., 2002). Together, these studies suggest that AD patients are impaired at inhibiting irrelevant information. Impaired filtering of irrelevant information becomes particularly problematic for AD patients because once they attend to something like a spatial location, they have trouble disengaging from that location (Parasuraman, Greenwood, Haxby & Grady, 1992), especially if their attention has been captured in an exogenous manner (Tales, Muir, Bayer & Snowden, 2002).

Visual selective attention to spatial locations in AD patients has also been investigated with priming. Vaughan, Hughes, Jones, Woods and Tipper (2006) presented participants with two ovals of different sizes that could each appear in one of four possible locations in space and instructed them to attend to the location occupied by the larger oval. AD patients did not behave differently for ignored locations and baseline conditions, suggesting that they failed to inhibit ignored locations. In contrast, Ko, Higgins, Kilduff, Milberg and McGlinchey (2005) used a similar task and showed that both the facilitative and inhibitive components of spatial attention were intact in AD patients. One reason for this discrepancy may stem from task-related differences across the studies. Vaughan et al.'s (2006) targets and distractors were defined by a rule: the larger stimulus was the target, so that the appearance of the target could have changed across trials. In contrast, Ko et al. (2005) used the same visual identities for targets and distractors throughout the experiment, thereby allowing participants to habituate to their appearance. Could this difference account for intact selective attention in AD patients? Recently, Fernandez-Duque and Black (2008) also demonstrated intact selective attention in AD patients, but they found severe performance costs in conditions when the targets and distractors could switch identities across trials. In support of this, Langley, Overmier, Knopman and Prod'Homme (1998) also found intact selective attention when the identities and locations of targets and distractors were kept constant. Together, these results suggest that habituation may be important in preserving visual selective attention in AD. Dynamically changing the appearance of what is to be attended or ignored, and therefore requiring a memory system like VSTM, could severely impair AD patients in ways that obscure the benefits of selective attention.

The shifting of visual attention, as assessed by visual search, could also be interpreted as a VSTM deficit in AD patients. Many studies of visual search in AD have been conducted under the framework of Treisman and Gelade's (1980) feature integration theory, whereby search for targets differing from distractors by a single feature requires little attention because they can be found primarily by bottom-up processes. However, search for targets differing by distractors by a conjunction of features requires attention to bind the features of a single object in order to discriminate targets from distractors. Foster, Behrmann and Stuss (1999) showed that AD patients were impaired at finding targets defined by conjunctions compared to those defined by single features, especially as set-size increased, indicating an attentional binding problem during search. Tales, Butler, Fossey, Gilchrist, Jones and Troscianko (2002) also showed that AD patients were selectively impaired at conjunction search, even though feature search was equated for attentional demands. More recently, AD patients were shown to have increased pupil dilation during visual search for conjunctions rather than features, suggesting increased effort during such conditions (Porter, Leonards, Wilcock, Haworth, Troscianko & Tales, 2010). Together, these results show that AD patients have specific impairments search for objects defined by conjunctions of features rather than single features.

Some neural models of visual search require VSTM for the control of attention, such as biased competition theory (Desimone & Duncan, 1995). This model proposes that visual search for a target requires a "template" of that target be stored in VSTM during the search. This target-to-template theory of visual search is supported by behavioral evidence (Soto, Heinke, Humphreys & Blanco, 2005; Soto, Hodsoll, Rothshtein & Humphreys, 2008; Olivers, Meijer & Theeuwes, 2006) and electrophysiological evidence (Woodman & Arita, 2011). This may help to explain AD patients' specific impairment at conjunction searches. It is possible that conjunction search is supported by attentional binding of perceptual information (Treisman & Gelade, 1980), as well as a memory template for the conjunction target. AD patients may be missing the search template, since they have a specific impairment at maintaining feature bindings in memory (Parra et al., 2009a, 2009b). This would leave search for conjunctions possible for AD patients, but severely impaired compared to the healthy older adults.

Like visual search, eye movements provide a measure of spatial shifting in attention, albeit in an overt manner. Early research suggested that AD patients have abnormal smooth pursuit eye movements (Hutton, 1985). Bylsma and colleagues (1995) found that AD patients also made saccadic eye movements when they were supposed to fixate, and the increase in these intrusive saccades correlated with lower scores on the Mini-Mental State Examination (MMSE; Folstein, Folstein & McHugh, 1975). AD patients also exhibit neural changes with abnormal eye movement behavior. Using fMRI, Thulborn, Martin and Voyvodic (2000) showed that right-parietal dominance during a saccadic eye movement task was reversed to left-parietal dominance in AD patients.

Some research has shown that AD patients show relatively intact performance when asked to make saccadic eye movements towards a visual target, or pro-saccades, but they are error-prone when instructed to make a saccade away from a target, or an anti-saccades (Crawford, Higham, Renvoize, Patel, Dale et al., 2005; Boxer, Garbutt, Rankin, Hellmuth, Neuhaus et al., 2006; Kaufman, Pratt, Levine & Black, 2010). AD patients make fewer spontaneous self-corrections after anti-saccade errors, while other types of dementia patients readily make corrections as well as normals (Garbutt, Matlin, Hellmuth, Schenk, Johnson et al., 2007). This is consistent with the finding that AD patients have trouble disengaging after

being exogenously drawn towards a location (Tales, Muir et al., 2002). Together, these results may reflect impairments in inhibiting overt attention.

Another possibility regarding antisaccades in AD is that their high error rate reflects a working memory deficit. For example, AD patients could have simply forgotten the task instruction to saccade away from the target rather than saccade to it. Performance on antisaccade tasks have been linked to working memory (Mitchell, Macrae & Gilchrist, 2002) and measures of working memory have correlated with antisaccade performance in AD (Boxer et al., 2006). However, this theory remains controversial (Crawford, Parker, Solis-Trapela & Mayes, 2011). Oddly, there is no research on memory-guided saccadic eye movements in AD to our knowledge. In this paradigm, a participant must hold target location in VSTM prior to making a saccade toward that location. VSTM is not only important in guiding such saccades (Desimone & Duncan, 1995), but also in correcting them in case of errors (Hollingworth et al., 2008). The memory-guided saccade task has been shown to be a useful biomarker for Huntington's disease (Blehker, Weaver, Cai, Hui, Marshall et al., 2009). Exploring memory-guided saccades in AD would provide a better understanding of how VSTM could guide overt shifts of attention.

In contrast to Vecera and Rizzo's (2004) theory that AD-related attentional impairments actually stem from deficits in VSTM, a more recent study has suggested the opposite: deficits in VSTM could be traced to an underlying deficit in visual attention. Alescio-Lautier, Michel, Herrera, Elahmadi, Chambon et al. (2007) examined VSTM in patients with probable AD and MCI. In their task, participants memorized multiple real-world objects presented in one image, followed by a variable delay, and then three probe images that could be either the same or different than the sample image. AD patients showed impaired task performance at 30-second delay intervals, but strangely, these impairments were restricted to the first probe image, and not the second or third probe. High performance on the later images suggested that VSTM is intact in AD. The authors speculated that the AD patients' performance suffered during the first probe due to an attentional blink (as discussed above; see Kavcic & Duffy, 2003). In contrast, AD patients' performance on a task requiring only memory for spatial location decreased as the time interval increased. Together, these results suggest that VSTM for appearance may stem from an attentional deficit, while VSTM for spatial locations stem from a memory deficit. The fact that the stimuli were real-world images may also be important; as discussed above, memory tests using real-world images have shown high rates of successful retrieval in AD patients (Ally et al., 2009a, 2009b; O'Connor & Ally, 2010).

Additionally, the finding that AD patients have a specific deficit in maintaining feature bindings in VSTM (Parra et al., 2009a; 2009b) could be interpreted as a deficit of attention at its core. One prominent theory proposes that maintaining conjunctions in VSTM relies on sustained visual attention to keep the separate features of the conjunctions bound together (Wheeler & Treisman, 2002; Fougnie & Marois, 2008). AD patients in the studies by Parra and colleagues may not have suffered from a memory failure per se, since they showed normal VSTM for features. Instead, they showed VSTM binding deficits because they lacked the attention to sustain those bindings.

Do these results imply a dissociation between AD patients with visual attention deficits and those with VSTM deficits? Research examining neurotransmitter systems suggests that this is a possibility. One hallmark of AD is the breakdown of the cholinergic system, which has profound effects on attention (Contestabile, 2010) and inhibiting the processing of irrelevant sensory stimuli (Ally, Jones, Cole & Budson, 2007). Studies have also shown acetylcholine to

play a role in working memory (Furey, Peitrini & Haxby, 2000). However, acetylcholine may only have similar effects on stages of memory that are shared by attention. Consistent with this, Bentley, Husain and Dolan (2004) found that pharmaceutically induced cholinergic enhancement increased activity in extrastriate regions while suppressing parietal activity during spatial attention and spatial memory encoding. However, this effect was absent during the spatial memory delay. This showed that acetylcholine has distinct effects on attention and memory. Importantly, Voytko and colleagues (1994) found deficits in visual attention, but not VSTM, after lesioning cholinergic neurons in crab-eating macaques. Together, these studies suggest that acetylcholine may play a specific role in visual attention, but not VSTM.

In contrast to results by Voytko et al. (1994), Dudkin and colleagues (2005) found that by lesioning both cholinergic and noradrenergic neurons in rhesus macaques, VSTM was impaired. Related to this finding, one theory proposes that dynamics of norepinephrine activity in the forebrain is the primary mechanism behind the attentional blink (Warren, Breuer, Kantner, Fiset, Blais & Masson, 2009), supporting the role of the noradrenergic system in VSTM encoding. Together, these results highlight the importance of understanding, not only cholinergic systems in AD, but also noradrenergic systems. AD has long been known to affect the noradrenergic system (Bondareff, Mountjoy, Roth, Rossor, Iversen et al., 1987). Besides the cognitive consequences, norepinephrine plays a mechanistic role in the pathology of AD. Recent work has shown that norepinephrine activity from the locus coeruleus is critical in clearing beta-amyloid plaques in the brain (Heneke et al., 2010).

Together, these studies suggest that AD patients could be split into cognitive subtypes, some with more severe impairments in visual attention, and others with more severe impairments in VSTM, depending on how the pathology has affected specific neurotransmitter systems. However, this possibility has not been investigated to our knowledge. Possible research could include the use of pharmacological agents, such as donepizil (Rokem, Landau, Garg, Prinzmetal & Silver, 2010; Dickerson, 2010) and clonidine (Coull, Nobre & Frith, 2001) to manipulate levels of acetylcholine and norepinephrine, respectively, and observe the effects on visual attention and VSTM.

In summary, the literature indicates several areas of visual attention that could be recast as impairments with VSTM, including selective attention, visual search, and eye movement behavior. However, some findings in the VSTM literature could also be re-interpreted as impairments of visual attention. This possibly reflects the difficulty in teasing apart the effects of visual attention and memory. Since these processes are closely interrelated, it may be better to always consider both visual attention and VSTM in investigations of visual cognition in AD. Findings that could not link visual attention to problems of daily living may have ignored memory systems like VSTM (Liu, McDowd & Lin, 2004). In contrast, research that has integrated measures of visual attention and VSTM in formal computational models, such as Bundesen's Theory of Visual Attention (Bundesen, 1990) have had success in creating a more full picture of changes to visual cognition in AD (Bublak, Redel, Sorg, Kurz, Forstl et al., 2009; Redel, Bublak, Sorg, Kurz et al., 2010). Alternatively, the discrepant findings in visual attention and VSTM of AD patients may be rooted in distinct subtypes of AD. One possible reason for differences in patients could be how AD pathology affects different neurotransmitter systems, including those mediating acetylcholine and norepinephrine activity. This could possibly lead to differences in pharmacological treatments of AD, depending on the specific nature of changes to visual cognition.

5. Functional implications on daily living

A better understanding of visual cognition in AD patients potentially leads to inexpensive alterations of their visual environment that could prolong a high quality of patients' lives. In this section, we speculate on such alterations based on the known research. Understanding changes in visual processing in AD has been shown to be clearly effective. For example, Dunne, Neargardner, Cipolloni and Cronin-Golomb (2004) applied the finding of low contrast sensitivity in AD patients (Cronin-Golomb et al., 2007) to known problems with food intake in AD patients. By using high contrast plates, cups and utensils, they were able to increase food intake by 25% and liquid intake by 84%. We believe that similarly effective applications can be inspired by basic research in visual cognition to improve the lives of AD patients. In this section, we focus on how the following findings can lead to improved daily living for AD patients:

1. Visual perception and VSTM for feature bindings is impaired in AD patients.
2. AD patients have impaired mechanisms of encoding visual information into VSTM.
3. "Enhancing" visual stimuli for AD patients facilitates performance in higher-level visual cognition, but only for real-world pictures.
4. Visual selective attention in AD may be intact, but only for overlearned, habituated stimuli.

It must be noted that each of these findings requires further verification with basic research involving AD patients. Further basic research will also provide psychological and physiological explanations in understanding why the proposed applications may work. However, there is no reason to refrain from testing the effectiveness of these proposed applications until the theoretical verification is exhausted. Testing the impact of inexpensive ideas now could lead to tremendous savings in healthcare costs in the near future. One study by the Department of Veteran's Affairs found that delaying nursing home placement by only one month could realize a $4 billion annual savings to the healthcare system (Clipp, 2005). Below, we will discuss two potential applications to the benefit of AD patients: First, we provide suggestions on organizing patients' visual environment and how they interact with it. Second, we suggest that a visual mnemonic, in the form of a cheap booklet of pictures, would facilitate several everyday abilities.

AD patients are impaired at perceiving and remembering feature bindings. Everyday objects are generally composed of several different features, such as color and shape, and often the objects in a given scene share many visual features. For example, many objects on your desk, like a computer mouse, cell phone, and pen, could be colored black or white, and shaped as a rectangle or oval. Binding is necessary when you must find the black-oval object, like the computer mouse. This attentional process is impaired in AD (Foster et al., 1999; Porter et al., 2010), and even when they successfully find such items, they cannot remember them well (Parra et al., 2009). It would therefore benefit AD patients if the objects in their surroundings were distinguishable by a single, salient feature, such as color. If the objects all appeared in different colors, the attentional cost of finding them in a cluttered scene would be significantly reduced. Since visual search is likely to be facilitated by VSTM contents (Woodman & Arita, 2011), patients with AD would be better able to maintain their search target in memory, and therefore find the target sooner. Instead of having to find the "black-oval" object, they just have to find the "black" object.

Additionally, with regards to AD patients' environments, it is important to consider impairments to their visual selective attention. It has been shown that selective attention in

AD may be intact when aspects of targets and distractors are fixed (Ko et al., 2005), rather than dynamic and changing (Vaughan et al., 2006; Fernandez-Duque & Black, 2008). It may be damaging for a patient to perform too many attentive tasks in succession. For example, if a patient must quickly find her keys and then her cell phone, the search for these items may be impaired due to the rapid switching of the search target. Also, if too many people are addressing them at a time, the focus of their attention will rapidly switch, introducing confusion that could obscure otherwise intact selective attention. Related to this notion is that AD patients have trouble disengaging from an attended object or location (Parasuraman et al., 1992). This is most problematic when their attention has been captured in a bottom-up manner (Tales, Muir, et al., 2002). Therefore, it is important to reduce sudden loud noises or visual transients in their environment. For example, if a telephone rings or the television suddenly turns on while a patient with AD patient is being addressed by a family member, the patient could suddenly attend to the phone or television and subsequently fail to return attention to the family member. The flow of the conversation could easily be lost by these sudden interruptions.

Keeping in mind that VSTM helps to guide visual search and that VSTM is impaired in AD, it may be useful for AD patients to keep a booklet containing pictures of common targets of their visual search. Such objects could be a patient's wallet, cell phone, or keychain. While a patient searches for a specific object, like her keys, she could visually consult this booklet repeatedly during the search. The booklet image would effectively serve as the search template, alleviating the need to use an impaired VSTM system, and potentially facilitating the search. This booklet might also be useful because AD patients have trouble encoding information into VSTM. This impaired encoding not only prevents a normal range of information to be stored in VSTM, but information already in storage will be vulnerable to incoming perceptual information. This scenario is relevant to eye movements made in perceptual comparisons – information stored prior to a saccade could be fragile and may be prone to erasure once the saccade is complete, since the observer is looking at a new visual scene after the eye movement. A booklet of objects would also be useful in this situation. It can be held next to objects of the comparison to reduce eye movements. For example, if a patient is looking for his wallet, he could hold up a picture of his wallet from the booklet next to each object that could potentially be the wallet. The spatial proximity of the picture to each candidate object would minimize eye movements and the need for VSTM.

The use of real-world pictures, collected into a handy booklet, may also be useful in recognizing other important information, like the faces of family members. Writing family members' names underneath each picture would maximize the perceptual and conceptual information that could trigger familiarity processes. AD patients could use these pictures to rehearse the information and potentially reduce the amount of retrieval failures when encountering those faces in person.

In summary, changing the visual environment of AD patients can potentially facilitate their performance on everyday visual tasks. Our suggestion is to essentially simplify the objects in their environment, so that each object is defined by a single, salient feature. Keeping the appearance of their objects as stable as possible may also help their selective attention. Finally, the use of a visual mnemonic, like a booklet of pictures, is potentially useful in activities involving visual attention and VSTM, including search and perceptual comparisons. It could also help AD patients rehearse the names and faces of friends and family who interact with them regularly. It is important to note two factors in the use of such a booklet: (1) AD patients have low-level perceptual impairments, so the pictures must

be high contrast and devoid of visual clutter; (2) Enhancing the quality of visual aids may only help when the images carry semantic information. So, real-world pictures should be used as opposed abstracted icons.

6. Conclusions

AD patients experience changes to processes that enable them to understand what they see. In this chapter, we have referred to such processes as visual cognition, which includes visual attention and VSTM. Although much research has been conducted in understanding visual attention in AD, less research has examined changes in other components, like VSTM. Further examination of this component of visual cognition could reveal a greater understanding of everyday visual abilities in AD patients. The relationship between visual attention and VSTM remains unclear, so future research may benefit from always considering both components. It is also important to understand how these short-term processes relate to long-term memory and visual perception.

Understanding visual cognition in AD patients leads to ideas of how their visual environment could be optimized to suit their psychological changes. Such inexpensive visual alterations could enhance patients' quality of life and save the healthcare industry billions of dollars. Here, we provide some suggestions on changing AD patients' visual environment, as well as a possible mnemonic that could be used to aid patients in everyday tasks.

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7

Olfactory Dysfunctions in Alzheimer's Disease

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1. Introduction

Alzheimer's disease is not only the most frequent cause of dementia that takes its toll on the elder population, but it is also the disorder for which, despite the complex research performed, a certain diagnosis may only be performed through anatomic-pathologic examination.

Alzheimer's disease is a neurodegenerative disease whose defining anatomic-pathologic trait is the deposit of amyloid plaques at the level of certain areas of the brain, the presence of fibrillary degeneration in the neurons and vascular changes with an amyloid deposit, all of which result in the impressive reduction of the cerebral mass noticed in the final stages of the condition.

Studies of over 30 years have established that olfaction is impaired in AD, however not invariably.

As early as 1987, Rezek described olfactory deficit as a neurological sign in Alzheimer dementia. Recent research has started to clear up potential mechanisms of olfactory loss in Alzheimer's disease.

Alzheimer disease has a selective vulnerability of the cerebral structures to the pathologic process, inducing a distinctive lesion pattern with a slow evolution in time and that is constantly invariable in the study cases. It begins in the transtentorial region and it then expands to the cortical and subcortical components of the limbic system and it may include the association areas of the neocortex. The pathologic process may be progressive, containing 6 stages. In stages 1-2, the pathologic process takes place at the level of the anteromedial temporal lobe mesocortex, the entorhinal allocortex and the horn of Ammon. Such structures are not only related to memory and learning, but also to the olfactory system. Therefore, stage 1-2, considered "clinically silent", includes a series of non-cognitive clinical changes that are essential for the timely diagnosis of the disease, including olfactory changes.

The deficit of the olfactory system in Alzheimer's disease, as early as its incipient stages, is currently a generally recognized fact. The correlation between olfaction and Alzheimer's disease is particularly exciting, with important diagnosis practical implications.

The olfactory system is a unique system of the human brain; it belongs to archaic structures, its anatomic route is distinguished by the fact that it is the only means of sense that approaches the brain directly, it is the only sensatory system with direct cortical projections, without a thalamic relay, it is the only part of the brain where neurogenesis persists, it has a special reaction to the aging process, it plays an essential role in behavior (food behavior, orientation and sexual behavior) and it influences memory, as there is also a smell memory.

2. Olfaction and memory - investigating the olfactory system

The olfactory primary cortex is the place processing the olfactory information at the highest level and it is directly related to the amygdale and the hippocampus. The amygdale plays an important role in the formation and modulation of emotions, as well as in emotional memory. The hippocampus is involved in memory processes, especially in working memory and in short term memory. Olfaction is the sensory manner that is closest to the limbic system, including both the amygdale and the hippocampus, and that is responsible for emotions and memory. Olfaction and memory are so infused that they allow us to make connections with certain experiences we have been subject to (the capacity of a smell to evoke memories).

This is probably why memories evoked by smells are particularly strong at an emotional level.

An individual olfactory stimulus may trigger different perceptions that depend on previous experiences and olfactory learning. Recent studies of cerebral imagery have indicated changes based on experience of the piriform cortex. (Li et al 2008) The anatomic bases of olfactory learning in such cerebral regions may involve a complex system of association fibers connecting neurons from the same areas or different cortical areas and whose synapse power may be changed by olfactory experience. (Wilson et al 2004) Olfactory learning may amplify the discrimination of smells and it may be important in survival. In humans, changes in the neuronal cortical activity determined by olfactory learning are correlated with the improvement in the discrimination of similar smells. (Li et al 2008)

Olfaction has often been involved in learning processes.

Since the olfaction system is connected to the limbic system, which explained earlier is responsible for the storing and creation of memories, this leads us to the conclusion that each receptor will recognize a specific scent if it had previously been exposed to it, basically creating memories.

It can also be significant that demyelinized olfactory fibers make olfaction the sense with the slowest driving. Not only does it require a longer time for the brain to perform the olfactory perception, but the sensation of a smell also persists more than, for example, visual or auditory sensation. Moreover, the fact that olfactory receptors are the only sensory receptors that are directly exposed to the environment may explain the relation between olfaction and memory.

Despite the fact that initial studies have not recognized it, there is also a short term olfactory memory.

White and Treisman have suggested that olfactory memory occurs due to the fact that humans assign verbal meanings to olfactory stimuli. The memory of smells is improved by familiarity and the capacity of identification.

Episodic information is an essential constituent of olfactory memory, comparative with the form and structure of the visual and auditory memory systems. All smells are encoded as "items" in the piriform cortex. The perception of smells is totally dependant on the integrity of this memory system and the loss thereof leads to the dysfunction of perception.

Neuro-transmitters in the olfactory system are responsible for the neuronal plasticity and behavioral changes. Noradrenaline and acecoline influence both implicit and explicit memory.

Implicit memory

It does not imply the conscious memory of the initial exposure to smell. The proof of the implicit memory formation of smells is given by habit, sensitization, perceptual learning and classic conditioning tests. Olfaction includes a strong tendency of habit.

Habit

It involves the decrease of the level of attention and response to a stimulus that is no longer perceived as new. It refers to decreasing the levels of response to a smell due to extended exposure. It involves the adaptation of cells in the olfactory system. It concerns both receptors, mitral cells of the BO, and cells in the piriform cortex with a rapid adaptation rate. Noradrenaline is deemed to have an effect in the operation of mitral cells and in increasing the response level thereof.

Explicit memory

Is a phenomenon encountered exclusively in humans. It concerns the assigning of associative meanings to different smells. The testing thereof uses the identification and recognition of smells.

In humans it was noticed that the Korsakoff syndrome, with an important memory dysfunction, the memory of smells is less impaired than other types of memory, thus suggesting a separate mechanism than other types of memory.

The testing of the olfactory sense is relatively complex and difficult at the same time. An attempt is made to asses the capacity to detect, identify, recognize and differentiate smells.

- The odor detection threshold is the lowest concentration of a certain odor compound that is perceivable by the human sense of smell. The threshold of a chemical compound is determined in part by its shape, polarity, partial charges and molecular mass
- The identification of smells is a semantic task, referring to general individual knowledge or to experience with a specific smell. (Schab 1999, Tulving 1993) The identification of smells is related to semantic memory, as there is a relation between smells and the name thereof. No significant relation was ascertained with intelligence, short term memory and episodic memory.

The episodic memory of smells is mediated by semantic factors (familiarity and identification of smells); the difficulty in the identification of common smells is based on deficits of the smell memory.

It is worth noting that it is the strong influence of the perception of intensity on the identification of smells. Individual variations in the perception of intensity are a major element for successful identification and it must be considered in the study assessing identification.

Odor identification ability is sensitive to prefrontal lobe dysfunction.

The objectification of smells by the identification of the name makes the subtle connection between memory and the environment.

Olsson (1999) suggested that the identification of smells interferes with memory. The identification of smells has a positive effect on the recognition of smells and it deems the name of smells to be a high level of cognition.

There is also a peri-semantic implicit episodic memory of smells and this spontaneous smell memory remembers especially connections with experienced situations.

Identification provides the individual with a secondary memory channel, however, the question remains if this is for smell itself.

Once identified, the name is remembered and the memory of this name is reactivated when the smell is identified again.

In order to test whether smell can be identified without verbal mediation, Moller carried out an experiment of incidental versus intentional learning recognition with unusual smells,

using young and old subjects. In incidental learning, the elderly proved more successful; in intentional learning, the young proved better, as they had fewer false alarms and because they did not use verbal mediation and not because of the deterioration of their working memory.

If smell memory operates independently from its name, it is mainly tuned at detecting changes.

- Smell recognition is a simple method of checking olfactory memory that does not involve language. It only requires that the subject establish, after a period of latency, if the substance it smells is the same as that which was provided for it to smell before.

3. Examination of olfaction

Ideal examination is carried out for each nostril separately. Starting with the asymmetry of neuropathologic changes, there is a functional asymmetry in olfactory performances. Apparently, in order to diagnose the difference of the olfactory identification capacity and of olfactory memory between subjects with MCI and AD, it is useful to test unirinal smell. The testing of performances it carried out by considering the affected nostril; a difference may be noticed between patients with MCI and AD. If the assessment is made by considering the healthy nostril (the olfaction of the two nostrils), the differences between MCI and Alzheimer's disease are no longer present. (Bacher-Fuchs, Moss, 2010).

Olfactory tests:

It is necessary for the battery of tested smells to reduce errors and to be adapted at a multicultural and ethnic level. It should consider all causes that may lead to the dysfunction of the olfactory sense, paying great care to pathological history, associated disorders, the therapy the patient is subject to, the pollution it was exposed to (job), etc.

They must be supplemented and correlated with other factors involved in AD, as well as with other situations that may prevent the cognitive function.

The used tests are:

1. UPSIT - The University of Pennsylvania Smell Identification Test, "a scratch and sniff odor identification test", including:
   - tests for odor threshold (n butanol testing by means of a single staircase)
   - odor discrimination (16 pairs of odorants, triple forced choice)
   - odor identification (16 common odorants, multiple forced choice from four verbal items per test)
2. "Sniffin' sticks" - pen like odor dispersing devices which include tests for odor identification, discrimination and thresholds
3. CCCRC (Connecticut Chemosensory Clinical Research Center Test) - a combined odor identification and odor threshold test.

Specialized testing may include EEG derived measured such as the recording of olfactory event-related potentials.

4. Olfaction and aging

Aging also leads to the decrease of all senses: sight, hearing, tactile, including taste and smell. There is a reverse proportionality between age and olfactory sensibility.

Olfactory senescence begins at 36 for both genders, and it accelerates with aging, impairing pleasant smells. Despite the fact that it is of lower interest for the patient compared to, for example, the loss of sight or of hearing, the loss of smell is frequent and often unacknowledged. Patients with congenital anosmia are diagnosed with it at about 10 years old.

It was reported that over 75% of the population over 80 years old has a tendency towards major olfactory dysfunction, as olfaction decreases considerably after the 7th decade. A more recent study has indicated that 62.5% of people between the ages of 80 and 97 are subject to olfactory dysfunction.

Aging is also accompanied by the decrease in the capacity to identify smells and of discrimination.

The loss of olfactory acuteness in elders may be caused by changes in the anatomy of the involved structures, the action of environmental factors, medication. Apparently, the main causes are: chronic illness, medication, dental and sinus problems.

Medical causes affecting smell:

i. Neurological: - Bell's paralysis
   - chorda tympani dysfunction
   - epilepsy
   - cranial trauma
   - Korsakoff's syndrome
   - multiple sclerosis
   - Parkinson's disease
   - tumors

ii. Nutritional:
   - cancer
   - renal chronic illness
   - liver disease
   - vitamin PP deficit
   - vitamin B12 deficit

iii. Endocrinous:
   - corticoadrenal insufficiency
   - congenital adrenal hypoplasia
   - panhypopituitarism (Simon's disease)
   - diabetes mellitus
   - hypothyroidism
   - Kallman's syndrome
   - McCune–Albright syndrome
   - Turner syndrome

iv. Local:
   - sinusitis, rhinitis, polyposis
   - asthma
   - xerostomic conditions, including the Sjogren's syndrome

v. Viral:
   - acute viral hepatitis
   - flu-like infections

However, the studies have indicated that a series of disorders in elders do not impair olfaction, such as arterial hypertension and heart diseases.
The decrease of the olfactory sense in elders may occur by the alternation in the distribution, density, functionality of specific receptor proteins, of ionic channels or of signaling molecules affecting the ability of the neurons in the olfactory path to signal and process the odoriferous information.
The mechanisms leading to the decrease of the olfactory sense in high age are: the decrease of neurogenesis, the decrease in the number of synapses, a decrease of the total synaptic density in the glomerular layer, (STABLE TUBULE ONLY POLYPEPTIDE could be responsible for this phenomenon), the change of growth factors (TGF – alpha, FGF2, BMPs, TGF-beta, EGF, BDNF) ,the decrease in the number of nerve terminations at the level of the nasal mucous, the decrease in the quantity of mucus at the level of the nasal mucous, respectively of the olfactory epithelium, the decrease of neurotransmitters.
Anatomic changes consist of decreasing the number of olfactory receptors and fibers in the olfactory bulb. Losses in the olfactory bulb may be secondary to the reduction of neurons in the nasal mucous. Olfactory receptors die through an apoptosis process. The decrease in regeneration with age leads to reducing the surface of the olfactory epithelium. This is also accompanied by an increase in the death rate of receptors.
In some cases, olfactory loss occurs due to bone growth and the consequent reduction of the cribriform lamella orifices of the ethmoid bone with microlesions of the nerve fillets when crossing this structure.
Functional imagery studies have proved that the activity of the piriform/amygdaliane region and of the orbitofrontal cortex is reduced in elder patients exposed to olfactory stimuli.
Moreover, it was noticed that areas of the cerebellum are activated by olfactory stimuli: the upper and inferior semilunar lobe, the posterior quadrangular lobe. In elders, olfactory stimulation leads to a higher activation of the cerebellum, suggesting a high response to attention requests or a compensating mechanism.
Age-related deficits concern both the recognition of smells and the identification of smells, which may be attributed to cognitive limitation.
The reduction of the olfactory sense has major consequences of the state of health and on the security of the respective patient: hyposmia is inseparably related to the altering of taste, leading to inappetence, the decrease of food intake, weight loss, malnourishment, reduction of immunity, the deterioration of the state of health. The altering of taste leads to the loss of the please of eating, as the patient is deprived of the ability to savor a meal, which may lead to depression. As taste is altered, the patient has the tendency to have a high intake of salt and sugar which may lead to the aggravation of cardio-vascular diseases or, respectively, to diabetes.
The reduction of the olfactory sense may lead to anxiety, the tendency of isolation. The altering of the perception of smell related to one's own body may lead to the decrease in the degree of personal hygiene, with consequent social implications.
The security of the life of patients with hyposmia is affected by the possibility of ingesting altered food, gas intoxication, etc.
Physical health, financial security, profession, partnership, friendship, emotional stability, free time are severely affected by the loss of olfaction.
The treatment of hyposmia generally has relatively modest results. Treatment may include the intake of zinc, vasodilatation substances such as pentoxifylline, as well as of vitamin A,

alpha-lipoic acid and NMDA receptor antagonists such as caroverine. Aside from such substances, the use of flavoring agents improves taste changes. Studies have indicated that the use of food supplements such as flavors leads to the increase of salivary Ig A, the increase in the number of T and B cells.

5. MCI and olfaction

The assessment of the olfactory function may be a method that is worth trying in order to identify patients with memory deficits. Identification performances were studied by using MCI. Comparing normal elder subjects with an MCI group, where the identification and the remembering of smells were studied, noticed that MCI subjects had significantly lower results in their tests, however, the performance in the assessment of smells was less affected than the cognitive assessment.

Moreover, after monitoring elder patients in time (5 years), who did not initially have cognitive dysfunctions, where the capacity of olfactory identification was also determined, it was ascertained that for those who have developed MCI, the olfactory identification score had a predictive value. Respectively, an olfactory score increased the risk of MCI by 50%. The results were not changed by the cognitive level in the presence of smoking. The reduction of olfaction was associated with a lower basic cognitive level and with a faster decline of episodic, semantic memory and of the perceptual speed. Therefore, in elders with no manifest cognitive disorder, the difficulty in identifying smells plays the role of prediction in the MCI development.

An important study has monitored 471 elder patients that were not subject to dementia or to cognitive disorder, for 5 years (Wilson-Olfactory impairment in presymptomatic Alzheimer's disease), for whom the capacity to identify familiar smells was initially assessed, by using the Brief Smell Identification Test, and they well clinically assessed annually and they were subject to cerebral anatomo-pathologic examination after their death. Moreover, the presence of the APOE epsilon 4 allele was determined. Low BSIT scores were associated with a faster decline of episodic memory, with a high risk of developing MCI. People who have deceased without a cognitive deficit and with lower BSIT scores were associated with a high level of the Alzheimer disease pathology, particularly with fibrillary degenerations in the central olfactory regions, especially in the entorhinal cortex and the horn of Ammon.

Such analyses suggest that in an elder population without clinical manifestations of Alzheimer's disease or MCI, the olfactory dysfunction is correlated both with the level of the Alzheimer's disease pathology in the brain and with the risk of the subsequent development of prodromal signs of the Alzheimer's disease symptoms as MCI and of the episodic memory decline. Therefore, olfactory manifestations may precede cognitive disorders in Alzheimer's disease with a substantial amount of time.

Furthermore, decline also occurs with age in other sensory systems in association with cognitive decline. As the entorhinal cortex processes multiple sensory impulses, it is possible that olfaction may also be accompanied by subtle changes in other sensory functions.

Therefore, the olfactory deficit occurs both in patients with symptoms of Alzheimer's disease, which was long proven, and in MCI patients or in carriers of the epsilon 4 allele, a well established risk factor for Alzheimer's disease. The respective analysis indicates that

the association between the olfactory dysfunction and the pathological changes specific to Alzheimer's disease may also occur in asymptomatic elders.
Another study has proven that in elders, the presence of APOE is associated with a high risk of MCI and a fast cognitive decline. It was also indicated that hyposmia associated with APOE has a 5 times higher risk of developing Alzheimer dementia than the general population.
The preliminary data of a study indicates the fact that MCI patients not only have an olfactory dysfunction compared to healthy persons, that progresses in time, but that those who are not aware of the decline of their olfactory sensibility, suffering from amnestic MCI, will develop the criteria for the diagnosis of Alzheimer's disease in the near future.

6. Alzheimer's disease and olfaction

Despite all the research carried out so far, it is still not known why the loss of olfaction occurs in victims of Alzheimer's disease. What is known is that the loss of olfaction is current. Ansomia and Alzheimer's disease go hand in hand. Anosmia was currently studied as a potential diagnosis instrument for Alzheimer's disease.
In 1994, Solomon examined the first cranial nerve in patients with Alzheimer's disease. He noticed that 90% of the patients had different degrees of anosmia.
Another experiment compared the olfactory capacity of 80 normal elders with that of 80 elders suffering from Alzheimer's disease, and the latter had a significantly lower olfactory capacity.
An attempt was even made to quantify the difference of olfactory capacity. Respectively, the study performed by Nordin in 1995 proved that 74% patients with Alzheimer's disease had a satisfactory olfaction only after smelling a sample with a concentration that was 9 times higher than the initial one, which 77% of the normal elders managed to identify. The same study also indicated that patients with Alzheimer's disease were not aware of the debut of their anosmia, nor of the severity of their impairment which is why they did not acknowledge the loss of their olfactory sense.
The amyloid deposit-olfactory impairment relation was proved by Zucco in 1994 in his study on patients suffering from Down's syndrome. It is known that due to the trisomy of the chromosome 21 they carry, they have an overexpression of the genes of this chromosome, including that of the amyloid precursor protein. Starting with the age of 40, they indicate deposits of amyloid plaques at a cerebral level, without however necessarily developing it. The risk of developing dementia in a patient suffering from Down syndrome is higher in persons with a family history of Alzheimer's disease, while others have the same risk as the general population. With age, patients with Down syndrome are also subject to an increase in the rate of Alzheimer's disease, so that, at 60 years old, 50-70% have Alzheimer's disease. Moreover, Alzheimer's disease has an earlier debut in patients with Down syndrome.
The decrease of the olfactory capacity was also noticed in adults with Down syndrome, many of which have a cerebral pathology that is analogue with Alzheimer's disease.
The question that was raised next was if adolescents suffering from Down syndrome and that were not subject to clear neuropathologic changes similar to Alzheimer's disease will develop an olfactory dysfunction.
The olfactory sense (the capacity of identification and discrimination) was tested for 20 teenagers suffering from Down syndrome (13.8 years average age), and the results were

compared with 20 patients with Down syndrome who were mentally retarded and 20 patients with Down syndrome who were not retarded. There were no differences between the three groups.
Another study tested the olfactory sense for 14 young people (20 - 31 years) and 14 adults (32 - 54 years) with Down syndrome. The scores were definitely lower in the older group. (Amyloid plaques were emphasized together with neurofibrillary degeneration in the olfactory mucous).
Such corroborated studies suggest that the olfactory impairments correlated with Down syndrome only occur in elders, when the pathology similar to Alzheimer's disease is present.
In MCI, the decrease in olfactory identification may be a marker for early Alzheimer's disease, and the Apo E genotype may be a part of the olfactory decline base.
In his study, Devanand proved that in patients with MCI, low base olfactory identification scores predict the diagnosis of Alzheimer's disease during monitoring. However, the MMSE should be considered, together with whether the patient is aware of the olfactory deficit or not. Devanand has indicated an association of olfactory and neuropsychological tests with an MRI examination for the cerebral volume (entorhinal cortex and horn of Ammon), the sensibility of predicting the MCI conversion to AD increases. Such studies have suggested that olfactory dysfunction may be a potential useful biomarker in estimating the debut and the progression of the disease.
The power of prediction increases if it is associated with the lack of awareness of the olfactory deficit. Low olfactory scores associated with the subjective reporting of the lack of olfactory problems are a stronger prediction factor. The correlation between being non-critical towards olfactory issues and the development of Alzheimer's disease is important as the awareness of the loss of olfactory sense may also be located in the medial temporal lobe structures, known to be impaired in the preclinical stages of the disease and associated with attention deficits.
Olfaction was studied (detection, quality of dissemination and identification) together with cognition (attention, rationale, memory, name, fluency) in patients with Alzheimer's disease, MCI and normal elders.
Patients with MCI had their olfactory sensibility and identification diminished, while discrimination was considered to be under normal limits.
Alzheimer's disease impairs all three areas, more than in MCI. It was noticed that the performances in the identification and discrimination of smells is correlated better than the detection with neuropsychological tests. Therefore, the deficits in detection and identification occur early in Alzheimer's disease, prior to the development of the clinical symptoms, and it progresses with the evolution of the disease. High detection thresholds together with the impairment of identification may be an early indicator of Alzheimer's disease.
Electrophysiological - olfactory evoked potential studies have confirmed the olfactory dysfunction both in patients suffering from Alzheimer's disease and in the preclinical MCI stage.
In Alzheimer's disease, the olfactory dysfunction progresses together with the disease and it is correlated with its severity. More advanced stages also register the impairment of olfactory discrimination.

1996 meta-analyses were performed on 43 olfactory perception and AD studies, and significant deficits were noticed in the olfactory detection threshold, the olfactory identification and olfactory recognition in confirmed or potential AD cases compared to the case-controls of the same age. Such deficits were correlated with genetic factors associated with a high risk for AD.
Gilbert and Murphy have proved that even one or two copies of the APOE allele e4 have a significant deficit in smell recognition compared to patients who do not have this allele. The authors have indicated that the deficit only applies to olfaction and not to visual stimuli.

7. The theoryes of olfactory impairment in Alzheimer's disease

In terms of olfactory impairment in Alzheimer's disease, two theories were raised: the olfactory vector theory and the degenerative theory.
The olfactory vector theory
In 1985, Pearson et al suggested that in terms of the impairment of the olfactory system in striking contrast to the minimal changes of other cerebral areas, it is possible that the olfactory system is the gateway for the agents triggering the disease. Therefore, the loss of smell may be a consequence of virus access or the access of toxins from the nose to the brain via the olfactory path.
This theory is supported by the following:

1. In the intranasal instillation of virus or toxins, they can enter the brain by the active transport of olfactory cells and it induces the alteration of olfactory structures (Stroop, 1995)
2. Studies suggesting histopathologic changes occur in the olfactory epithelium in patients with Alzheimer's disease (Jafek et al, 1992)

Amyloidal plaques and degeneracy are preferentially located in the limbic system receiving fibers directly from the olfactory bulb, including the anterior olfactory nucleus, uncus and the medial amygdaline nucleus. (Pearson et al, 1985)
Secondary degeneration theory
It postulates that the loss of olfaction is determined by retrograde secondary degeneration. A version postulates that limbic structures are particularly susceptible to alteration in the processes of Alzheimer's disease.
Potential arguments are:

1. Patients with Alzheimer's disease have a reverse correlation between the UPSIT scores and the metabolic activity in the anterior portion of the medial temporal cortex measured by PET (Buchsbaum et al, 1989)
2. The association between low olfactory scores and the number of hippocampal lesions in patients with Alzheimer's disease (Serby et al, 1992)
3. Mice which have recently become anosmic do not have a learning deficit, unlike those in which anosmia was induced earlier (Kurtz et al, 1989)

8. The structural bases of the olfactory impairment in Alzheimer disease

Multiple areas of the brain that are crucial for the normal olfactory function are severely impacted by the pathology of Alzheimer's disease. The olfactory dysfunction of Alzheimer's disease is associated with anatomical-pathological changes specific to the disease both at the

level of the olfactory mucous and at the level of the olfactory bulb, of the olfactory tracts and of the central region for the projection of olfactory tracts, including regions involved in the recognition of smells and memory (entorhinal cortex and the horn of Ammon).
Deposits of amyloid and neurofibrillary tangles, the two pathological hollmarkes of AD, are located differentially by crossing these regions. Few studies have approached the connection between the prevalence of these pathologies with the olfactory function.
A study has suggested that for elders (87 ± 6 years), olfactory loss is determined by the loading of neurofibrillary tangles in the central areas processing olfactory information; another study has indicated that the severity of the pathology in the olfactory system is correlated with the neuropathologic progression of the disease starting from the MCI stage.
Olfactory identification is impaired early on in Alzheimer's disease and it may be influenced by the cognitive status more than the olfactory acuity or detection that is not alerted until late stages. The hyposmia pattern in Alzheimer's disease suggests that the disease does not begin "in the nose", as was theorized so far.
Talamo examined the changes in the olfactory neurons in Alzheimer's disease and he indicated that there are histopathologic changes at the level of the olfactory epithelium.
In 1991, Hyman used neuroanatomic and neurochemical studies to describe the changes at the level of the olfactory bulb with deposit of amyloid plaques and neurofibrillary tangles with degenerations of other areas: the anterior olfactory nucleus, the olfactory tubercle, uncus and subiculum.
A 1993 study indicated a less severe impairment in Alzheimer's disease of the olfactory tract and bulb in Alzheimer's disease than in the central part of the olfactory system.
Immunohistochemical determinations in the olfactory epithelium for the polyclonal protein, for amyloid plaques and for ubiquitin have proved the presence thereof in Alzheimer's disease, so that nasal mucous biopsy may be useful.
It was also noticed that neuroblasts from donors with Alzheimer's disease have high levels of precursor amyloid.
Imagistic and immunohistochemical studies have proven the correlation between the deposit of amyloid plaques and neurofibrillary tangles at the level of the olfactory bulb with those at the level of the cortex.
The study of Wilson and his collaborators, performed in 2007, indicated that the difficulty in identifying familiar smells at a high age is partially due to the neurofibrillary pathological accumulation in the central olfactory regions, respectively in the entorhinal cortex and the CA1/subiculum area in the horn of Ammon, as the density of degeneracy is inversely proportional with the olfactory identification.
In 2009, Thomann carried out MRI studies to indicate the reduction in the sizes of the olfactory bulb and tract in the early stage of Alzheimer's disease.
An MRI study also described the decrease in the volume of the left horn of Ammon in Alzheimer's disease, reflected in the reduction of olfactory identification performances.
Experiments carried out on rodents with AD have provided evidence that both the neurofibrillary tangles and the beta-amyloid lead to olfactory loss. For example, a recent study carried out on a transgenic mouse with the overexpression of the human amyloid beta protein precursor indicated that such mice have an age-dependent olfactory dysfunction compared to the control group of the same age. Such olfactory deficits include the abnormal investigation of smells, olfactory habit (short term memory) and olfactory discrimination. Furthermore, such behaviors are correlated with the spatial-temporal deposit of the fibrillary and/or non-

fibrillary beta-amyloid. It is worth mentioning that the amyloid deposit first occurs in the olfactory bulb, followed by the deposit in the olfactory cortex and horn of Ammon.Such data suggest that the beta-amyloid deposit in the olfactory bulb and in the olfactory cortical areas may contribute to olfactory loss in early and, respectively, late stages.

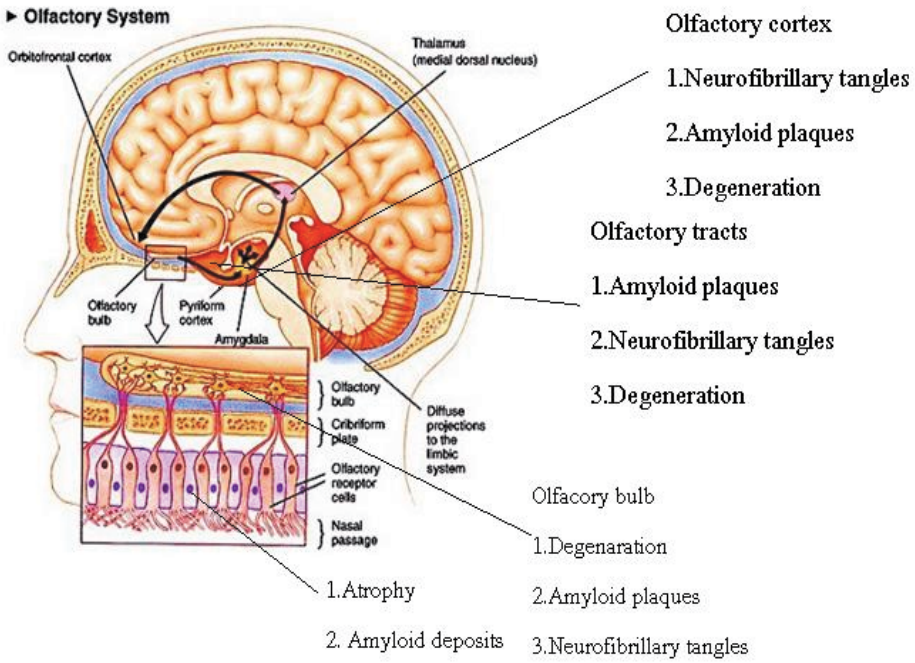

Fig. 1. Anatomical changes of the olfactory pathways in AD

9. Olfactory sense and other neurodegenerative disease

The decrease of the olfactory sense is also associated with other neurodegenerative diseases: Parkinson's disease and Lewy corpuscles dementia,

Olfactory impairments in Alzheimer's disease and Parkinson's disease are initially similar, and they are later on set apart in terms of quality. In Parkison's disease, cerebral lesions are rarer and the olfactory impairment is more stable. However, it is worth noting that in initial studies, the extent of the olfactory changes in Parkinson's disease is the same as that in Alzheimer's disease.

During the post-mortem examination, patients suffering from anosmia dementia seem more likely to have Lewy body dementia.

Progressive supranuclear palsy -In this disease, the olfactory function is relatively intact, with no significant differences between the olfactory scores of such patients compared to the case-controls.

Multiple system atrophy- They have lower olfactory scores than the case-controls, however they are better than patients with Parkinson's disease (Wenning et al, 1993). The olfactory impairment has no correlation with the disability scores, and the pathological changes are more expanded than in Alzheimer's disease.

In Huntington's chorea there is impairment in the discrimination and identification of smells, however not in the detection thereof.

10. Olfaction-marker of Alzheimer disease

A major effort in AD research is directed towards the identification of the markers of the disease. Such biomarkers should ideally predict the AD prognosis before the significant development of the neuropathology and the consequent loss of the cognitive function. Early indicators of the disease are especially important for the implementation of interventions as long as the brain is still operating normally. Therefore, the finding of a robust and accurate biomarker may be a pivot in the reduction of the AD global impact.
An overview of the biomarkers' progression has indicated that the first elements that are detected are beta-amyloid, followed by the neurodegeneration and the cognitive markers.
Despite the fact that they are not normally used, perception impairments may serve as AD biomarkers. Perception impairments are common in AD, including the loss of olfaction, of visual and hearing abilities.
The answer to the question on whether olfaction can be used as an AD marker is not simple. There are numerous causes of hyposmia. The high prevalence of anosmia in other neurodegenerative diseases - PD, DLB, D fronto-temporal indicates that olfaction alone is not specific enough as an AD biomarker. Together with other biomarkers, the olfactory perception screening may be useful in consolidating the sensitivity and specificity of an AD diagnosis, especially due to the fact that it is non-invasive, easy to repeat, it reflects the operation of the neuronal circuits impaired in the initial stages of AD, it is not costly and it does not require technological equipment.
Considering the fact that the olfactory deficit is developed with a high frequency in intact cognitive subjects, carriers of the e4 allele, olfaction can be a particularly useful and non-invasive measure in the intervention or prevention of risk trials.
The testing of the olfactory sense may be useful in differentiating Alzheimer's disease from major depression.
Anosmia may be a respectable method of diagnosis in Alzheimer's disease. The careful monitoring of the decline of the olfactory sense may indicate the debut of Alzheimer's disease.
Anosmia may be used as a probable indicator in the diagnosis of Alzheimer's disease, however it cannot be a decisive factor by itself.
Longitudinal studies during the progression of the disease, correlated with independent measurements of the structural and functional deficits in relevant areas of the brain will establish the usefulness of olfactory tests.
The smell identification function may be useful as a clinical measure for the assessment of the clinical response to donepezil, as the entorhinal cortex, the olfactory bulb, critical areas for smell, are high in acecoline, a neurotransmitter involved in the pathology of Alzheimer's disease and treatment.

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Part 3

Neuroimaging in the Spotlight

8

Currently Available Neuroimaging Approaches in Alzheimer Disease (AD) Early Diagnosis

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1. Introduction

Alzheimer´s disease (AD) is a condition mainly diagnosed through clinical interpretation and neuropsychological testing. Unfortunately this leads to problems due to variations in expertise of the clinicians involved. Moreover, its diagnosis is usually achieved in later phases of the disease, which reduces the scope of useful interventions. In this chapter we will review the different brain imaging approaches used for both AD and mild cognitive impairment (MCI), a condition which evolves into AD in an important proportion of cases (between 10-15 % yearly) (Petersen, 2008).

It has been hypothesized that the accumulation of β-amyloid (Aβ) in the AD brain triggers a cascade of neurodegenerative events, including inflammatory processes, neurofibrillary tangles, oxidative stress, and neuronal network dysfunction with synaptic loss and neurotransmitter deficits. Such events are manifested by progressive impairment of cognitive functions (Kadir et al, 2011). Years ago, an accurate diagnosis of AD was considered only possible using a demonstration of Aβ plaques and neurofibrillary tangles at post-mortem histopathological analysis of the brain (Wiley et al, 2009).

At present there are promising methods for the *in vivo* assessment of the extent of AD. The typical findings of these brain imaging techniques are different and they are summarized in Table 1. These neuroimaging methods may help in AD early diagnosis as well as helping in differentiating AD from other neurodegenerative conditions. Although some pioneering work started in the 1970s, the explosion of knowledge regarding brain imaging methods for AD diagnosis is a matter of the last two decades.

1.1 Computerized Tomography (CT)

Nowadays CT is not considered a standard technique for diagnosing AD, least of all in early stages. Its main usefulness is in differential diagnosis since it is less expensive, faster and more widely available than MRI. CT allows ruling out some clinical situations which lead to

behavioural alterations but are not AD, e.g., normotensive hydrocephalus, intra and extra-axial bleeding, etc.

Neuroimaging technique	Finding
CT	Tissue atrophy.
MRI	Tissue atrophy. It is more specific about grey matter.
fMRI	Changes in blood oxygenation level (BOLD signal) representing synaptic activity.
DTI	Connectivity and organization in white matter tracts.
Spectroscopy	Chemical content of the brain, such as NAA/Cr ratio or others.
SPECT	Changes in cerebral perfusion.
PET	Changes in glucose metabolism.
PET-amyloid	Measures accumulation of β-amyloid, an AD-specific protein.
MEG	Measures magnetic fields and, indirectly, yields rather precise information about brain electrical activity

CT = computerized tomography; MRI = magnetic resonance imaging; fMRI = functional magnetic resonance imaging; DTI = diffusion tensor imaging; SPECT = single-photon emission computed tomography; PET = positron emission tomography; MEG = magnetoencephalography; BOLD = blood oxygen level-dependent; NAA = N-acetyl aspartate; Cr = Creatinine; AD = Alzheimer's disease

Table 1. Typical findings of different brain imaging methods used in AD and MCI diagnosis

1.2 Magnetic Resonance Imaging (MRI)

1.2.1 Structural MRI

This technique provides the best possible spatial resolution. Given the fact the most robust neuroimaging finding in AD is the atrophy of mesial temporal structures, structural MRI is the most widely used approach. Hippocampal atrophy is another well known finding, but atrophy within other brain areas has also been described in entorrhinal cortex, amygdala, basal ganglia (nucleus basalis of Meynert), thalamus and bilateral parietal cortex. It is relevant to mention here that in terms of early diagnosis some consider the entorrhinal cortex as the earliest area affected by AD. Other researchers have stated that the nucleus basalis of Meynert is a key structure in AD early diagnosis (Herholz et al, 2004; Grothe et al, 2010).

1.2.2 Functional MRI (fMRI)

fMRI provides signal intensities images associated with a relative cerebral blood flow during cognitive tasks. Resting and activation functional MRI studies have showed a lesser coordinated activity in the hippocampus, inferior parietal lobes –both bilaterally- and cingulate cortex in patients with AD. This neuroimaging technique is nowadays used to monitor AD patients' treatment.

Combining genetic risk with functional MRI memory task paradigms has shown to be a very accurate pre-symptomatic predictor of cognitive decline.

1.3 Diffusion Tensor Imaging (DTI)

DTI allows visualizing neuronal connectivity or, more precisely, local fiber orientation and white matter tracts integrity. Patients with AD show decreased fiber density in temporal white matter, probably related to the medial temporal grey matter atrophy, as well as in the splenium of the corpus callosum.

In mild cognitive impairment (MCI), however, this decreased fiber density has been located in the anterior part of the corpus callosum.

1.4 Spectroscopy

It provides information on tissue substrate or metabolite concentrations. Specifically the N-acetyl aspartate (NAA) has been used as a biomarker of neuronal death, and it has been demonstrated that in patients with MCI and AD is significantly reduced compared to healthy controls.

1.5 Nuclear medicine brain imaging techniques

1.5.1 Single-photon emission computed tomography (SPECT)

It measures cerebral flow by detecting a single-photon emitting tracer after its intravenous injection and brain uptake. It has a very low spatial resolution and its diagnostic accuracy is lower than PET. Nevertheless, in clinical practice, it can be useful for differentiating AD from other dementias such as frontotemporal dementia.

1.5.2 Positron emission tomography (PET)

Recently a new technique using PET with Pittsburg compound B (PIB; PIB-PET) has emerged. PIB is a tracer which allows marking β-amyloid plaques. This technique has been highly efficacious to detect β-amyloid *in vivo* and, therefore, could be rather useful to detect AD in its early phases.

1.6 Magnetoencephalography (MEG)

MEG is a technique which measures brain magnetic fields both during basal state and during cognitive activation. Its time resolution is measured in milliseconds. It is precisely this very high temporal resolution which allows evaluating small changes in brain processing during cognitive stimulation. These changes may help to differentiate between AD, MCI and normality as well as providing some clues about differences in information processing in these same conditions

Different studies carried out with MEG show a decrease in magnetic fields in people with AD in temporal mesial areas during memory tasks. People suffering from AD exhibit increased slow frequencies in temporoparietal areas in basal evaluation.

2. Computed Tomography (CT)

Since the 1970s, there have been many CT studies searching for evidence of focal atrophy in the brains of subjects with AD (DeCarli et al, 1990). The search was complicated by the overlap between changes common to AD and normal ageing and because AD cases were only diagnosed clinically (Smith and Jobst, 1996). There are both generalized and focal changes, both in cortical areas as in the ventricular system, in brain atrophy. Unfortunately focal atrophy is not consistently found in standard axial CT studies though subjects with AD may show a greater degree of generalized atrophy revealed by sulci enlargement and ventricular dilatation.

DeCarli et al (1990) concluded as follows: "Unfortunately, at present there is little definite evidence for clear anatomic brain changes that accurately predict the cognitive dysfunction within a group of patients suffering Alzheimer's disease". The dictum still remains valid.

The standard axial CT system used internationally does not show the medial temporal lobe well, mainly because of the scan angle used. Changes in the medial temporal lobe itself have to be inferred from dilatation of the temporal horns of the lateral ventricles and enlargement of the suprasellar cisterns. Indeed, these changes have been observed in AD (Kido et al, 1989). By 1988, an alternative camera angle was used for hippocampus evaluation in patients with temporal lobe epilepsy and this system was also used by de Leon et al (1989) to study AD patients. The angle is parallel to the long axis of the temporal lobe and scanning is carried out from below and upwards until the inferior margin of the orbit is reached; in this way a complete series of images through the temporal lobe is obtained without exposing the eyes. Using this "temporal-lobe-oriented" CT procedure, de Leon et al (1989) found that 87% of patients with moderate to severe AD had dilatation of the hippocampal fissure compared with age-matched controls.

Hippocampal atrophy detected with CT was also more common in a group that did not reach the clinical research criteria for AD but showed minimal memory impairments. This led them to suggest that hippocampal atrophy might be an AD marker which occurs early in the course of the illness, before the diagnosis is established, and predicts deterioration (De Leon et al, 1989). Hippocampal atrophy is not the only neuroimaging sign in AD, but it is rather characteristic.

The axial temporal lobe oriented CT scan can be used to look for other possible causes of dementia, such as multiple infarcts, tumours or hydrocephalus, and the temporal lobe-oriented scan (in 1.5-2 mm slices) to reveal atrophy of the medial temporal lobe (Smith et al, 1996) (Fig 1).

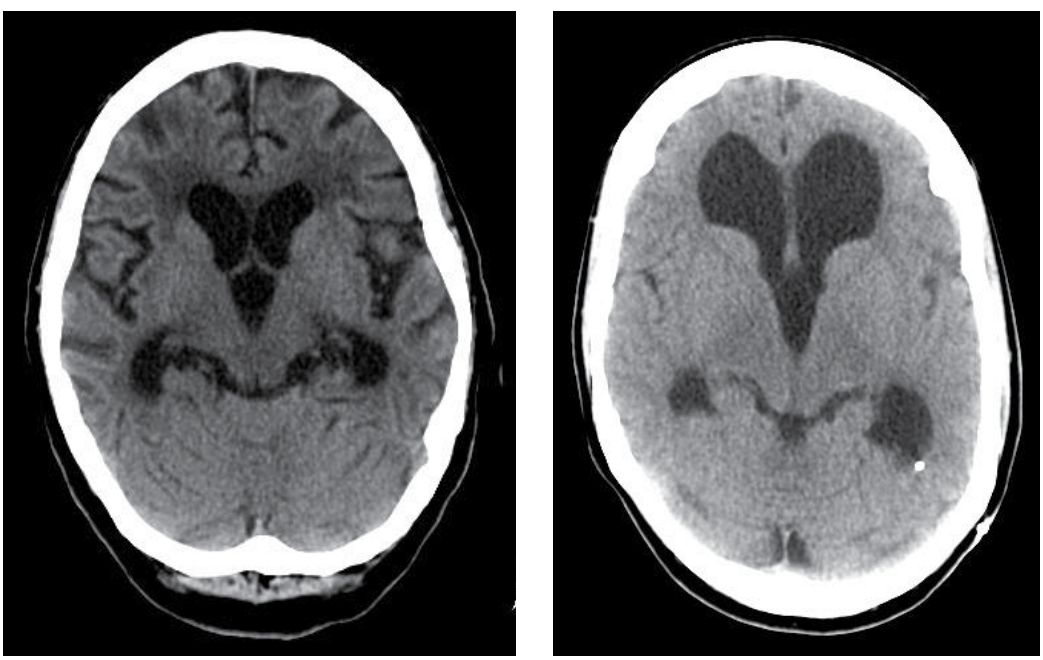

Fig. 1. CT in AD on the left and a patient with normotensive hydrocephalus on the right, where a catheter can be seen. CT is helpful to differentiate between AD and other brain conditions.

Unfortunately CT usefulness in AD diagnosis is very limited (DeCarli et al, 1990). In the last 20 years MRI became increasingly available and the vast majority of structural neuroimaging used since then has been MRI. This is the reason why there are no relevant papers written since then where CT has been used for AD.
Atrophy is evident, at hippocampal level too. Dilatation of the lateral ventricles can also be seen.

3. Structural magnetic resonance imaging (MRI)

MRI volumetry was one of the earliest brain imaging techniques used to identify AD. Given the temporal lobe atrophy found in AD patients, it seemed that this finding per se could diagnose AD. Further research revealed this simplistic approach was flawed.
The first technique was manual volumetry. This required an excellent working knowledge of neuroanatomy as well as good dexterity in delineating the ROIs (regions of interest). This method is highly operator-dependent and multiple subjective issues can arise. This is overcome by double or triple-blind studies. Manual volumetry is also very time-consuming. On the other hand you have to have a prior hypothesis, such as a ROI to analyze. There have been many hypotheses in AD. Hippocampal analysis stands out (Besga et al, 2010) amongst them, though more recently other structures, such as the caudate nucleus or thalamus (Skup et al, 2011), or even corpus callosum, are being considered.
A newer method is the so-called Voxed-based morphometry (VBM). This is an authomatized approach which allows distinguishing between grey and white matter. It also allows performing statistical studies amongst different populations (Kinkingnéhun et al, 2008).
Multiple computer techniques have evolved since then. They allow analysing the brain, either globally or in any 3D ROI. For instance, in hippocampus, a key structure in AD, there are "radial atrophy mapping approach" or "large-deformation high dimensional brain mapping". The former computes the 3D distance from the hippocampal centroid in each coronal section (also called a medial curve or core) to each hippocampal surface point. This provides an intuitive 3D measure of hippocampal thickness, as well as metric estimates of hippocampal atrophy that can be compared point-by-point across individuals and groups (Thompson et al, 2004). The latter is also used to study the changes in hippocampal morphology in patients with AD (Apostolova et al, 2007). Rather than relying on hippocampal manual tracings, a template hippocampus is traced on a single subject and fluidly warped to match the anatomy of new subjects. The transformation involved is high-dimensional, i.e. involves millions of degrees of freedom to produce a deformation that captures shape differences in detail. It is *de facto* a 'large-deformation' approach, that is to say the deformation model follows a continuum-mechanical law that prevents folding or tearing of the deforming template (this is known mathematically as a *diffeomorphism*).
Similarly to VBM, many cortical computational anatomy techniques use the segmented grey matter maps and the grey matter density approach (Thompson et al, 2004) or a more advanced grey matter thickness approach (Lerch et al, 2005; Singh et al, 2006; Apostolova et al, 2008).
Using the hippocampal radial atrophy mapping approach, Thompson et al (2004) showed profound hippocampal differences between normal controls and AD patients. These differences were correlated with MMSE scores. Two cross-sectional large-deformation high-dimensional brain mapping hippocampal studies have suggested the CA1 subfield may

discriminate between normal aging and questionable AD (Clinical Dementia Rating Scale (CDR) = 0.5) (Csernansky et al, 2000; Wang et al, 2006).

Along the last 10 years all sorts of volumetry studies aiming at early diagnosis of AD have been carried out. They mainly focus on either comparing AD versus MCI or healthy controls category or finding AD progression biomarkers progression.

One can reliably classify AD patients and controls in the first group. Most studies revolve around temporal mesial lobe (MTL) structures. Many volumetric MCI studies measure hand-traced ROIs of specific MTL structures and most of them focus on the hippocampus. The majority of published studies indicate MCI patients have less hippocampal volume than cognitively intact controls (Becker et al, 2006; Kantarci et al, 2002), and AD patients have less volume than those with MCI (Wolf et al, 2004).

Other studies show average entorhinal cortex volume in MCI patients is decreased compared with cognitively intact controls. Cross-sectional ROI studies have shown that the hippocampal and entorhinal volumes (Juottonen et al, 1999; Xu et al, 2000) can reliably differentiate AD subjects from normal older adults. Although some researchers claim that the precision of differentiating MCI from normal controls and MCI from AD is substantially less accurate when using hippocampal volume (MCI vs controls: sensitivity = 52– 80 % and specificity = 79–80% ; MCI vs AD: sensitivity = 45–60 % and specificity = 80%) than when using entorhinal cortex volumetry (Xu et al, 2000; Du et al, 2001; Kantarci et al, 2002), they concluded that, given the ambiguity imposed by the boundaries of the entorhinal cortex, it is easier to make these measurements in the hippocampus. Which structure is the more powerful discriminator to distinguish MCI patients from normal controls is currently being debated. Some studies side with the hippocampus and others with the entorhinal cortex (Pennanen et al, 2004).

The second group of studies is made up of longitudinal research based on disease progression. Two hypotheses have emerged: the degree of atrophy progression and the basal atrophy found in AD patients.

Several longitudinal studies have examined the relationship between the brain atrophy rate and its progression from MCI to AD. They have found significant group differences between MCI patients who progress to AD versus those who do not rapidly progress. They show that the hippocampus shows higher annual rates of atrophy in patients with MCI that progress to AD than in those who remain stable (Chetelat et al, 2005; Apostolova et al, 2006; Jack et al, 2004). Others have found that entorhinal cortex atrophy rate, but not hippocampus, is faster for individuals progressing to AD (Stoub et al, 2005). Moreover, other studies have shown that not only mesial temporal lobe regions show greater atrophy linked with progression towards AD, but also some cortical structures, such as posterior cingulum, precunneus or temporal medial lobe (Jack et al, 2004; Jack et al, 2005; Chetelat et al, 2005).

Several studies have examined the accuracy of a single baseline brain MRI in predicting the progression towards AD, or stability, using a longitudinal clinical status follow-up. Studies using VBM for whole-brain analysis also find that a lower hippocampal baseline predicts progression towards MCI (Whitwell et al, 2007; Chetelat et al, 2005; Bozzali et al, 2006). Others studies using manual tracing methods have failed to replicate this association and report the entorhinal cortex as a more sensitive predictor of progression (de Toledo-Morrell et al, 2004; Stoub et al, 2005).

Investigations using VBM have identified areas outside the mesial temporal lobe in which lower baseline volume in certain areas is related to later progression to MCI. These areas are

fusiform gyrus (Whitwell et al, 2007; Chetelat et al, 2005), medial and inferior frontal regions (Whitwell et al, 2007; Bozzali et al, 2006) and the posterior cingulate and precuneus ones (Whitwell et al, 2007; Hamalainen et al, 2007).

To summarize, it is in the last decade when we have been able to have an accurate idea about the structural brain changes in MCI as well and its progression towards AD. Those structural changes are multiple. Hippocampal atrophy is important, but there are also other important cortical biomarkers. Atrophy is more diffuse than one would expect, but pinpointing the specific regional areas of atrophy can be useful in the diagnosis of AD and differential diagnosis with other types of dementia. VBM is a technique which can help in identifying the regions of atrophy.

Nevertheless it is important to take into account that all these studies are population ones, so none of them serves as an individual maker. Though reliable whole brain measurements can be accomplished with authomatized methods there is no way yet to know which MCI patients will evolve into AD. However these structural ancillary tests are clinically useful in combination with neuropsychological testing, augmenting significantly the prognosis accuracy.

4. Functional magnetic resonance imaging (fMRI)

It can be described as a non-invasive form (it does not require the injection of a paramagnetic contrast) of MRI. It measures the oxygen consumed by certain brain areas when they are challenged with specific stimuli or tasks. This technique has got a high spatial resolution and a rather acceptable temporal resolution too. Its major inconvenience is its high sensitivity to small head movements. It is carried out taking brain images during a specific activity and in basal state. Basal state activity is subtracted from the specific task activity to yield the specific brain areas where blood flow has increased due to the specific activity tested.

fMRI physical foundation relies on the inherent magnetic properties of blood. Red blood cells (RBC) have a haemoglobin molecule which has an iron (Fe) atom in its centre. Oxygen binds to haemoglobin yielding oxihaemoglobin and haemoglobin configuration changes when oxygen is delivered to tissues (deoxihaemoglobin). Both oxihaemoglobin and deoxihaemoglobin have different paramagnetic properties. Deoxihaemoglobin will create an in-homogeneous magnetic field around itself which will lead to a decreased signal. In other words, when there is less deoxihaemoglobin (and more oxihaemoglobin) due to increased activity and subsequent increased blood flow and associated oxygen concentration within a specific area, there will be a greater signal. Blood oxygenation dependent level (BOLD) gives a variable signal intensity between stimulus and resting state. BOLD signal relies upon the different magnetic susceptibility of oxyhaemoglobin and deoxyhaemoglobin (Westbrook, 2000).

Task testing is prepared according to paradigms. There is a huge variety of paradigms depending on the task. Broadly speaking these paradigms are classified into two main groups: “block design” or “event-related”. The former are characterized for stimulus presentation for a longer time while in the latter group they are presented for a shorter time and in an alternating fashion.

Numerous studies have shown activation during memory tasks in the anterior hippocampal region in both healthy older adults and young people. This activation has a lesser extension in healthy older adults. On the other hand healthy young adults exhibit a greater activation of the prefrontal cortex and a lesser parietal cortex activation in comparison with older adults (Sperling, 2003b).

AD patients show a consistent decreased activation in hippocampal and parahippocampal areas in comparison with healthy controls (Pariente, 2005). Dickerson et al (2004) suggested the cognitive deterioration degree could be inferred from the hippocampal activation at the time of fMRI basal scanning; the more hippocampal activation, the greater the deterioration. Interestingly, an increased activity in some neocortical regions has also been described (Sperling, 2003). This may be understood as a hippocampal failure compensatory mechanism. In fact these frontal regions have been labelled "default mode network", implying that AD patients would have difficulty in deactivating them (Buckner, 2005).

There are few fMRI studies addressing the differences between MCI and healthy adults. Moreover, their results show disparity, from mesial temporal lobe hyperactivation (Dickerson, 2004) to hypoactivation (Johnson, 2005).

Dickerson et al (2004) have tried to clarify this issue and they state that whenever there is MCI, there is a compensatory hyperactivation, and both the extension and localization of this compensatory activity depends on memory tasks demands and memory performance. They also affirm that memory starts to fail in advanced MCI stages, which is shown by hippocampal hypoactivity. In this way they describe an fMRI activity non-linear trajectory in a patient which evolves from MCI to AD, presenting first a hyperactivity phase in pre-clinical stages followed by an activation decrease. This is what they called "inverse-U shape curve" (Sperling, 2007; Dickerson, 2008). This curve poses a problem if it is used as an AD biomarker, namely the brain activity pseudo-normalization when people with MCI start deteriorating more and at that moment they show a loss of the MCI characteristic hyperactivation. They conclude that if there is minimal clinical symptomatology and little atrophy of the mesial temporal lobe for a given degree of hyperactivation and more clinical symptomatology with greater mesial temporal atrophy for the same degree of activation, we are in the ascending and descending parts of the inverse-U shape curve.

Johnson et al (2004) used a paradigm with repetitive presentation of faces to prove MCI patients do not show the same slope of decreasing hippocampal activity than in healthy older controls. This suggests a certain disruption of this adaptive response in the medial temporal lobe.

Dickerson (2008) stated the direct linear relation between the clinical deterioration and the extension of activity (measured as a function of the number of activated voxels) detected by fMRI both in hippocampus as in parahippocampus. Thus, a greater clinical deterioration correlates with a greater activity extension in the mesial temporal lobe which, in turn, correlates negatively with the degree of hippocampal atrophy.

Amongst those studies where different patient groups have been compared (healthy ones, very mild MCI, MCI and AD), Celone et al (2006) stands out because of the strong correlation they found between the degree of preserved memory and the activity of mesial temporal areas and deactivation of precunneus and parietal cortex, using always a "default-mode network" approach.

5. Diffusion tensor imaging (DTI)

This technique is also known as diffusion MRI. Its underlying physical basis relies upon detection and quantification of the random movement of water known as the Brownian movement. Molecules undergoing the Brownian movement follow a chaotic route due to continuous impacts against other particles in their environment and their speed is directly proportional to the system temperature.

Even though the system displacement induced by a single hydrogen molecule cannot be appreciated, the impacts of a great number of them generate a significant and quantifiable displacement of a chaotic nature. However it is possible to calculate the distance run by a particle in a given time through the formula: $R=\sqrt{6}\ D\ \tau$. D is the molecule diffusion coefficient, which is dependent on the temperature, while τ is the time interval. In biological tissues D is not the only cause of molecular movement since microcirculation, amongst other variables, throughout the capillary net has a diffusion increase net effect. This is the reason the term apparent diffusion coefficient (ADC) is used.

When the diffusion in a structured system, such as the brain, is quantified, molecules displacement is limited by physical barriers. The latter make the displacement dependent upon direction (anisotropy). In a system without barriers a particle undergoing the Brownian movement can displace freely in any direction, with an isotropic diffusion. Whenever there are physical barriers, such as a membrane or axonal fibres, the particle loses movement freedom and the diffusion is therefore restricted (anisotropic).

A set of DTI images permits the identification of a predominant direction of diffusion (anisotropy). The processing of diffusion values is carried out through a structure called tensor. A tensor can be defined as a set of co-existing magnitudes dependent upon direction and coordinates.

Tractography permits the reconstruction and 3D visualization of the neuronal column structures. If is applied to white matter axon fibres, it provides information about connectivity and fibre deviations caused by tumours and infarcts (Martí-Bonmatí, 2008; Le Bihan et al, 2001; Melhem et al, 2002).

In short, MRI water diffusion measurements include those images weighted in diffusion (DWI) and tensor diffusion (DTI). DWI provides a mean without direction (isotropic) of tissue water diffusivity. DWI is described in terms of ADC. ADC increases reflect neuronal loss and increased extracellular space, where water diffusion is faster, and it is an indirect indicator of grey or white matter integrity. DTI can be understood as an index of tissue permeability difference in different directions (anisotropic) and it is measured in terms of mean diffusivity (MD). Another important measurement to take into account is the anisotropic fraction (AF). AF is very sensitive in the evaluation of the microstructure integrity of the white matter. AF is obtained measuring water diffusivity along white matter long tracts (Ries et al, 2008).

The use of diffusion techniques is justified because of the AD ethiopathogenic mechanism, e.g., β-amyloid depositions and neurofibrillary tangles. They seem to interfere with neuronal function at early stages producing a cascade of ultra-structural changes in axons. The resulting damage affects axonal transport, microtubules structure, neurofilaments and the integrity of the myelin sheath (Englund, 1981). All of these factors produce microstructural changes in the white matter which, in turn, affect the water protons diffusivity, a parameter measurable by DTI. This technique has the advantage of being very sensitive in detecting microstructural abnormalities, something not revealed by volumetric measurements.

The loss of tissue organization causes a decrease in anisotrophy (or its correlate, AF). It is assumed that reduced water diffusion parallel to axonal tracts is indicative of axonal degeneration, disruption and partial breakdown of cytoarchitecture (Beaulieu, 2002) or demyelinating processes (Song et al, 2002), which modifies AF.

We should focus on the corpus callosum (CC), the largest white matter structure in the brain. The CC provides inter-hemispheric communication and anterior-posterior

connections which link cortical association areas. CC anisotropy decreases with age. Progressively reduced AF has been reported as part of the normal aging process (Sullivan et al, 2010). This AF decrease seems to follow an anterior-posterior gradient. The genu has a lower AF compared to the splenium. Several major white matter fibre bundles, including those connecting to the hippocampal structures, pass through the genu of the CC (Serra et al, 2010).

Anatomical localization of AF reductions in amnestic MCI (aMCI) seems to be a robust finding across a variety of approaches. Thomann et al (2006) found an AF reduction in the rostral subregions and anterior body of the CC in MCI patients compared with healthy controls using region of interest (ROI) methods. Similarly Wang et al (2009) and DiPaola et al (2010), using voxel based morphometry (VBM), identified a density reduction in the genu in aMCI patients compared with healthy controls. Other researchers like Ukmar et al (2008), Cho et al (2008) or Parente et al (2008) found an AF reduction in MCI patients in the CC splenium. The splenium of the corpus callosum, crux of the fornix (Zhuang et al, 2010) and the cingulum were sensitive discriminators to differentiante between aMCI and normal subjects (Chua et al, 2009). Ortiz Alonso et al (2000) found a volume reduction of the anterior body, mid body and isthmus of the corpus callosum in a homogenous group of moderately advanced AD compared with healthy controls.

Several theories have been proposed to explain the early involvement of frontal white matter in MCI. The pattern of white matter integrity disruption tends to follow an anterior-posterior gradient with greater damage noticeable in posterior regions in AD and MCI. The aMCI group showed significant macrostructural atrophy only in the anterior CC section. In the mild AD group this atrophy extended to posterior CC subsections and was accompanied by anterior and posterior microstructural modifications (Di Paola et al, 2010).

It has been recently suggested that myelin breakdown is an important component of the illness process in AD (Bartzokis, 2004; DiPaola et al, 2010). According to this hypothesis late myelinating fibers should be more susceptible to myelin breakdown. This may constitute an alternative mechanism which may explain AD cortical progression in the direction opposite to myelination (Braak and Braak, 1997). Wallerian degeneration affects the posterior CC sub-region that gets afferent axons directly from those brain areas primarily affected in AD (temporoparietal regions). However the myelin breakdown process might affect the late myelination of the CC subregion, causing changes in the CC genu at early stages of the disease (DiPaola et al, 2010).

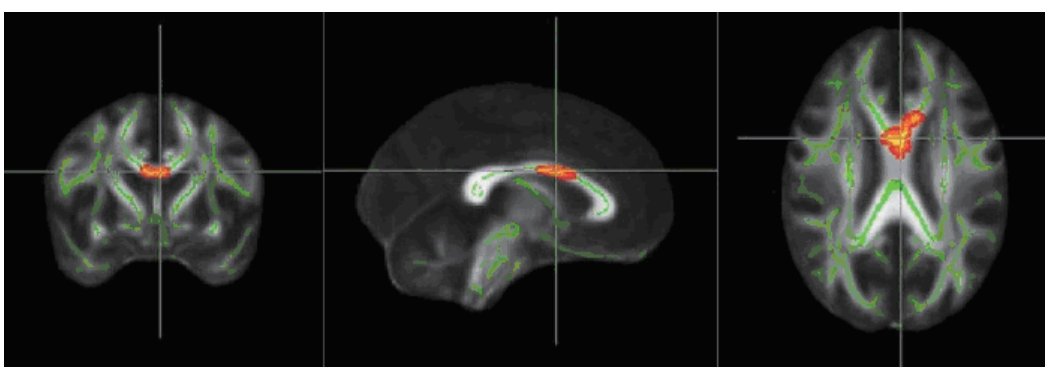

Fig. 2. Coronal, sagital and axial planes DTIs showing AF differences between patients with MCI and controls.

The controversial results concerning CC regional atrophy are most likely due to the methods adopted in various studies, such as different criteria used to select patients, different stages of the illness or variation in the number of participants. Those investigators who found that the genu was more adversely affected thought that earlier affectation of late maturing regions might be responsible, whereas others who found that the splenium was more affected said it might be due to the overall degenerative pattern occurring in the posterior circuitry of the temporal parietal areas.
A positive correlation between AF values and MMSE scores has been demonstrated in several studies, arguing in favour that white matter degeneration has an impact in cognitive performance (Bozzali, 2002; Ukmar et al, 2008; Ortiz-Terán et al, submitted) Other studies (e.g., Sullivan et al, 2010) found a correlation between AF decline in the CC with performance on the alternate finger tapping test, which is a test of inter-hemispheric communication transference.
DTI is more used nowadays for correct identification of dementia subtypes.

6. Magnetic Resonance Spectroscopy (MRS)

MRS is used to measure the levels of different metabolites in body tissues. It detects and quantifies resonance signals of certain molecules present at much lower concentrations (approximately 10^{-3} mol/g_{tissue}) than water ones (circa 55 mol/g_{tissue}). The metabolic profile of a concrete region within a specific organ can be obtained through a single voxel MRS (SVMRS) or through multivoxel MRS imaging. SVMRS provides a metabolic information average score within the selected voxel while multivoxel MRS provides, within a unique image acquisition, various molecular images which indicate the spatial distribution of different metabolites (Martí-Bonmatí, 2007).
The major drawback of SVMRS is the lack of spatial information, though the magnetic field homogenization is excellent and, consequently, so is its spectral resolution. On the contrary, multivoxel MRS yields spatial distribution information of different metabolites but its magnetic field homogenization is rather complicated since the brain areas are bigger and their magnetic susceptibility differences greater. Hence the characterization of a given specific volume is more precise with SVMRS but multivoxel MRS generates additional molecular information about surrounding areas. The latter provides more information about the heterogeneity of the lesion, analyzes peripheral infiltrations and allows comparisons with contralateral areas (Martí-Bonmatí, 2007).
The most common CNS metabolites measured with MRS are (1) N-acetyl-aspartate (NAA), a neuronal marker localized in neurons and axons, (2) choline (Cho), a marker which indicates membrane changes, (3) Creatine/phosphocreatine (Cr/PCr), an index of energetic metabolism, (4) lactate (Lac), a final product of glycolisis generated because of oxidative metabolism failure which is found both in intra and extra-cellular compartments, but never found in healthy tissues, (5) myoinositol (mI), mainly found within astrocytes where it regulates the cell volume, (6) lipids (Lip) and polypeptides, mostly in myelin sheaths and cell membranes and (7) glutamine (Gln) and glutamate (Glu), important neurotransmitters involved in multiple synaptic processes which give complex signals because of their ubiquity, scalar coupling and multiple complexes therefore posing a real challenge for their quantification.
In short, MRS is the only available technique for obtaining fast and simultaneous metabolic information *in vivo* and different molecular images within a single image acquisition. It is a

reliable method for clinical molecular follow-up and studying therapeutic effectiveness in different diseases.

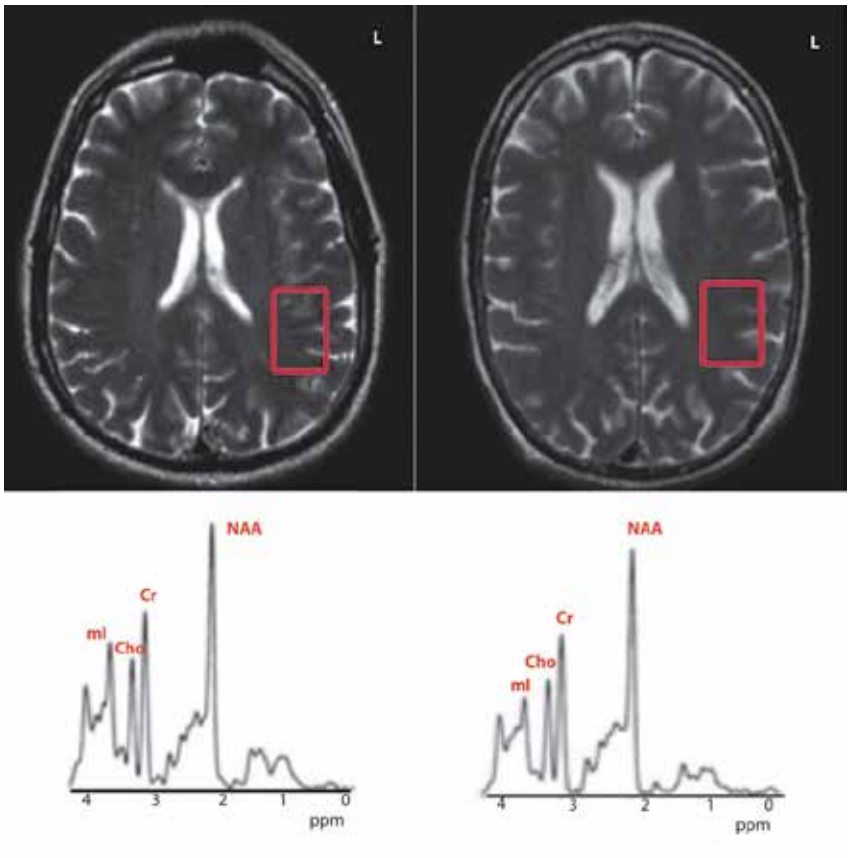

Fig. 3. MRS in Alzheimer Disease
Axial T2-weighted images from an AD patient (L, left) and a healthy control (R, right). These images show the left parietotemporooccipital region where the spectra was acquired, and the results are shown just below. Please note the differences between the two subjects in the mI.

With regards to AD, and from the early nineties, MRS has become a frequently used technique. The typical 1H-MRS includes the following peaks: NAA, Cr/PCr, Cho containing compounds, Glu and mI. Most authors have opted for following up AD progression using voxels in the medial temporal lobe. However Kantarci et al (2002) states that from a biologic standpoint there is evidence from MR imaging, fluorine 18 (18F) fluoro-deoxy- glucose PET, amyloid ligand PET, and fMRI studies that the posterior cingulate gyri (bilaterally) and the inferior precuneate gyri exhibit atrophy, decreased metabolism, amyloid deposition and deactivation early in the course of AD (Whitwell et al, 2007; Petrella et al, 2007; Chetelat et al, 2003). Moreover, the quality and reliability of 1H MR spectra from these voxel locations are superior to those from the medial temporal lobe because of the close proximity of the medial temporal lobe to the skull base, an area susceptible to magnetic artefacts.

Besides of the voxel location, NAA has been considered a prominent candidate for AD investigation and diagnosis since it is localized only in neurons and absent in glial tissue in mature brains (Simmons et al, 1991). Hence NAA is a general marker of neural integrity. The first ever described finding in relation to AD was a decrease in NAA concentration in the brain (Miller et al, 1992), a reduction positively correlated with the density of senile plaques and neurofibrillary tangles (Klunk et al, 1992). Because NAA is a neuronal metabolite, the reduction of NAA levels in patients with AD is the result of loss of neuronal components, neuronal function disruption, or both; these phenomena are strongly associated with increasing neurofibrillary degeneration. On the basis of the correlation between mitochondrial ATP production and NAA level, NAA production within the neuron is thought to be related to mitochondrial function (Bates et al, 1996).

Both Shonk et al (1995b) and Kantarci et al (2000) described bilateral temporoparietal increase of Cr and mI concentrations as well as the mI/NAA ratio in AD. These authors also found significantly elevated mI and Cr concentrations in the parietal region in early AD stages while NAA remained largely unchanged. Based on these findings they suggested mI and Cr abnormalities could be detected with MRS prior to abnormalities in neocortical NAA (Shonk et al, 1995b). Nevertheless, only the mI/NAA ratio was capable of classifying AD and healthy controls without any overlap between the groups (Panertti et al, 1998). Kantarci et al (2002) found a sensibility around 82% and a specificity ranging from 80 to 90% for MRS in classifying AD, clinically defined, and healthy controls.

The observation of an increased mI concentration not coupled with a cortical NAA decrease had already been reported in a group of AD patients with a low MMSE score and, also, in a group of Down's syndrome patients in the pre-dementia phase (Huang et al, 1999). Therefore mI, but not NAA, showed an inverse linear correlation with MMSE scores (Huang et al, 2001).

The most plausible up-to-date explanation for the increased levels of mI in the brain of AD patients is the contribution of the glial component. Glia contains much higher concentrations of mI than neurons (Glanville et al, 1989) and gliosis may cause the increase in mI found in AD patients (Huang et al, 2001). This may suggest that glial proliferation is a sensitive measure of AD before neuronal loss, the latter reflected by NAA (Fig 3).

Shonk and Ross (1995a) had previously supported the aforementioned hypothesis by showing that adults with Down's syndrome exhibited a significant elevation of mI before the onset of dementia without any concomitant reduction in NAA. On the other hand, cholinergic receptors in the CNS are believed to act in part through the phosphoinositide pathway (Honchar, 1990; Kennedy, 1990). Jolles et al (Jolles, 1992) showed a reduced activity of the inositol polyphosphate enzyme phosphatidyinositol kinase and postulated a specific defect in the inositol polyphosphate cascade in AD to such a degree that cholinergic activity could be affected. Ross et al (2005) summarized this evidence proposing that alterations in mI must have consequences for enzyme equilibrium and the metabolite concentrations in the inositol polyphosphate cascade. Accordingly, altered cholinergic sensitivity could be expected.

During the last decade 1H MRS *in vivo* studies have consistently indicated NAA decreases and mI increases in patients with AD and aMCI (Valenzuela and Sachdev, 2001). The strongest association between MRS and anatomopathologic findings was observed when two metabolite ratios were combined to yield the NAA/mI ratio; this result suggests that NAA and mI have complementary roles in predicting AD pathology. As previously said, NAA is located primarily in neuron bodies, axons, and dendrites. Therefore, it is a sensitive

marker for neuronal density or viability. In patients with amnestic MCI mI/Cr ratios are elevated but NAA/Cr ratios are only mildly decreased. These findings suggest that the mI/Cr ratio increase happens earlier than does the decrease in NAA/Cr ratio decrease in AD (Parnetti et al, 1997).

In a recent 1H MRS study, Cho/Cr ratios longitudinally increased in patients with amnestic MCI who progressed to AD (Kantarci et al, 2007). In contrast, Cho/Cr ratios decreased in patients with amnestic MCI who remained stable.

Wang et al (2009) described: a) Hippocampal mI/NAA increases were significantly larger than those in the posterior cingulate area (an area with a recognized relationship in the pathological progression of AD); b) Hippocampal and posterior cingulated mI/NAA together provided valuable discrimination to differentiate AD, MCI and control groups and c) there were significant correlations between mI/NAA in the hippocampus and the posterior cingulate area and MMSE scores. The posterior cingulate area suffered later neuropathological and spectroscopic changes.

A reduced NAA was found in the right hippocampus ($p = 0.01$) in MCI patients while increased mI was reported in the left hippocampus ($p = 0.02$). mI/NAA ratios were higher in both right ($p = 0.03$) and left ($p = 0.01$) hippocampi of MCI subjects (Franczak et al, 2007). Greater mI concentrations have also been found in patients with MCI (Godbolt et al, 2006; Kantarci et al, 2000). Neuropathological studies of MCI have shown the existence of hippocampal damage. This goes along with the hypothesis that MCI is a transitional state between normal aging and AD. A recent study found reductions in NAA/mI ratios in presenilin 1 and amyloid precursor protein mutation carriers who have a nearly 100% risk of developing AD (Godbolt et al, 2006). Metastasio et al (2006) observed a significant a NAA/Cr decrease in the parietal white matter in a group of five patients who developed AD after being diagnosed with MCI. This was replicated by Pilatus et al (2009). Modrego et al (2005) identified the occipital cortex as the most sensitive area for NAA/Cr ratios. To summarize, MI/NAA ratio may be a useful biomarker for diagnosing MCI and it may help to commence early treatment in affected individuals (Franczak et al, 2007).

In AD patients NAA levels may also return to normal within the first 6 weeks of treatment with donepezil (Krishnan et al, 2003). NAA is not just a marker for neuronal function and, possibly, neuronal mitochondrial function, but can also be a surrogate marker to evaluate progression and decline in neuronal integrity during the transition period from MCI to dementia.

Other attempts to evaluate alternative metabolites have been done. Glu and Gln are decreased in the cingulate gyrus in AD patients (Antuono et al, 2001). However, the precise role of glutamate excitotoxicity in the development of MCI and AD remains largely unknown.

In short, and in spite of the difficulties posed by the voxels localization in the mesial temporal lobe, this seems to be the best place to accurately discriminate between MCI and AD as well as measuring MCI progression. The most relevant metabolites in MCI and AD are mI and NAA. They also allow to correctly classify the patients between those two groups and to evaluate their response and treatment and disease progression.

7. Single-photon emission computed tomography (SPECT)

Brain SPECT allows the study of regional cerebral blood flow (rCBF), which correlates at rest with the regional consumption of glucose and reflects neuronal activity. Thus, the rCBF

indirectly reflects neural activity in each brain region, and allows the earlier detection of functional abnormalities, preceding the stage of symptomatic disease.

The tracers currently used in the clinical practice are ^{99m}Tc-Hexamethyl-propylene-amine-oxime (^{99m}Tc-HMPAO), which is considered to be the most reliable SPECT method, and ^{99m}Tc-ethyl cysteinate dimer (^{99}mTc-ECD). They enter cells due to their lipophilic character and remain trapped because of their conversion to hydrophilic compounds. Their incorporation is proportional to rCBF in the first few minutes after injection. Modifications in rCBF after injection do not change the initial distribution of the tracer because of its intracellular trapping (Farid et al, 2011).

Both radiotracers have a similar behaviour, being captured in the brain, with a distribution pattern corresponding to the rCBF (Koyama et al, 1997). Brain SPECT reveals a specific pattern of abnormalities in AD by demonstrating reduced rCBF in the medial temporal, superior temporal, parietal, posterior cingulate cortex, and precuneus before becoming diffuse and affecting the frontal cortex in advanced stages (Farid et al, 2011).

SPECT images represent the topography of physiopathologic changes in AD (Fig 4) and it is a promising diagnostic tool in the early diagnosis and its differentiation from other potentially treated causes of dementia such as normal pressure hydrocephalus or depression (Bartenstein et al, 1997).

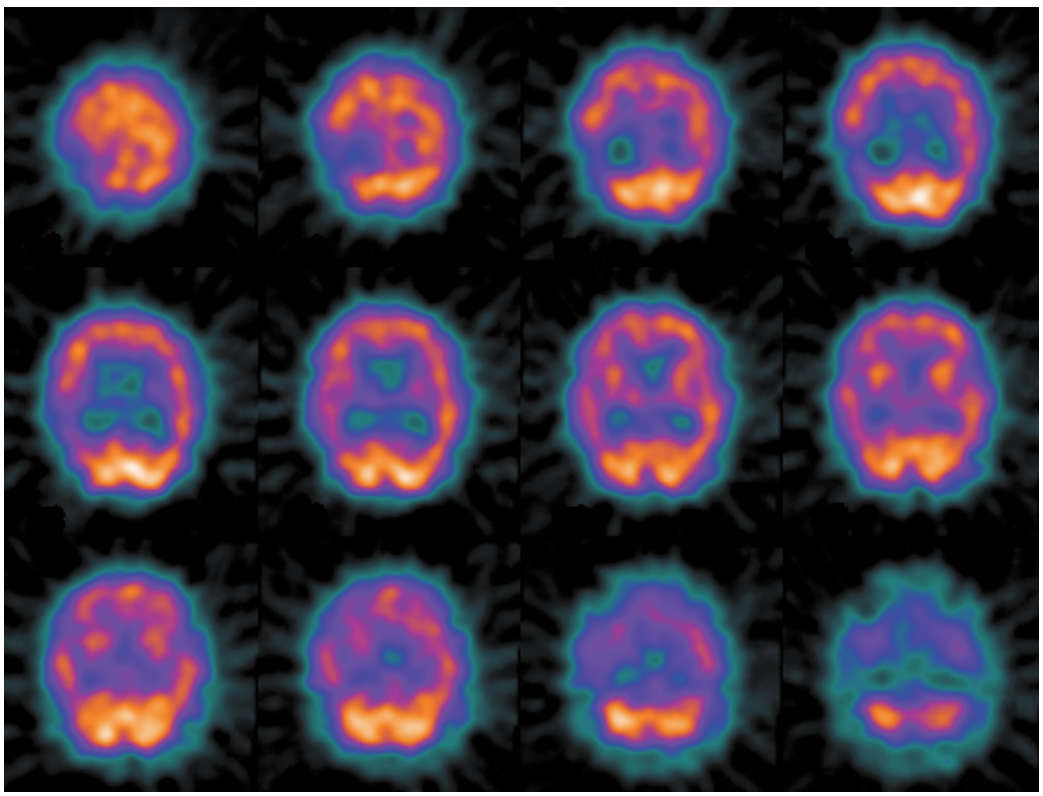

Fig. 4. SPECT in AD. Transversal slices of a 99mTc-ECD SPECT study in a patient with early AD. Bilateral temporal hipoperfusion and assymmetrical parietal hipoperfusion, more evident on the right cortex, can be seen

Haxby et al (1985) were able to link metabolic function, measured with SPECT, with a variety of cognitive and behavioural domains, including memory.

8. Positron emission tomography (PET)

In the last decades there has been a progressive advance in the development of techniques able to explore neurophysiological and neurochemical processes in humans. Positron emission tomography (PET) is a powerful technique allowing the visualization of physiological and biochemical changes both in healthy and ill subjects. This type of unique information may not be available with structural brain imaging techniques. PET has been useful to elaborate the hypothesis of the pathogenesis of AD, in correlating symptoms with their biological markers and in studying individuals at increased risk (Giovacchini et al, 2011).

Glucose is the main energy supply for the brain and its metabolism maintains ion gradients and glutamate turnover, and it is related to neuronal function at rest and during functional activation Its measurement by [^{18}F]-2-fluoro-2-deoxy-D-glucose (^{18}F-FDG) is based on phosphorylation of the tracer by hexokinase, which is the pivotal first step of that metabolic pathway. Measurement of regional cerebral glucose metabolism (rCMRglc) using ^{18}F-FDG PET has become a standard technique in dementia research (Herholz et al, 2007). FDG PET is the approach most efficiently used in AD and MCI diagnosis.

The visualization of metabolic abnormalities using ^{18}F-FDG PET, reveals similar patterns to that of SPECT, with hipoactivity in temporal and parietal neocortex in association with AD, detected either by visual inspection or by conventional quantitative analyses selecting regions of interest (ROI).

PET and SPECT studies using SPM-based image analysis methods have replicated temporoparietal neocortical hypoactivity findings in AD; moreover, these studies have been able to document activity decrements also in the medial temporal region (where the neuropathological process occurs first, with greatest severity and in the posterior cingulate gyrus and precuneus (Matsuda, 2001). That pattern of hypometabolism has been detected even in very early stages of AD (Fig 5) and in subjects at genetic risk for the disorder (Duran et al, 2007).

As AD progresses, the frontal association cortex becomes involved, while cerebellum, striatum, basal ganglia, primary visual, and sensorial and motor cortex remain unaltered (Mosconi, 2005). Hemispherical asymmetries are often observed, especially at early stages of AD and may be attributed to co-morbidity factors (e.g., vascular brain disease) or compensatory mechanisms (e.g., neuroplasticity). This *in vivo* pattern of hypometabolism is found in the majority of clinically diagnosed AD patients and in over 85% pathologically confirmed AD cases (Mosconi et al, 2010).

^{18}F-FDG PET is therefore a useful diagnostic tool to identify the cause of dementia and to have a differential diagnosis with other diseases, and to determine the neurophysiological mechanisms underlying these pathologies. Table 2 shows the characteristic locations of hypometabolism according to different types of dementia. (Montz et al, 2002).

^{18}F-FDG PET is more sensitive than clinical criteria in the detection of AD and in the differential diagnosis with other dementias, reaching values of diagnostic accuracy between 80 and 100%, with sensitivity of 90-96 % and specificity of 67-97% for AD (Gambhir et al, 2001, Knopman et al, 2001 Silverman et al, 1999).

It should be noted that this technique has been helpful in diagnosing AD, irrespective of the degree of cognitive impairment. In fact, abnormal parietotemporal uptake patterns were

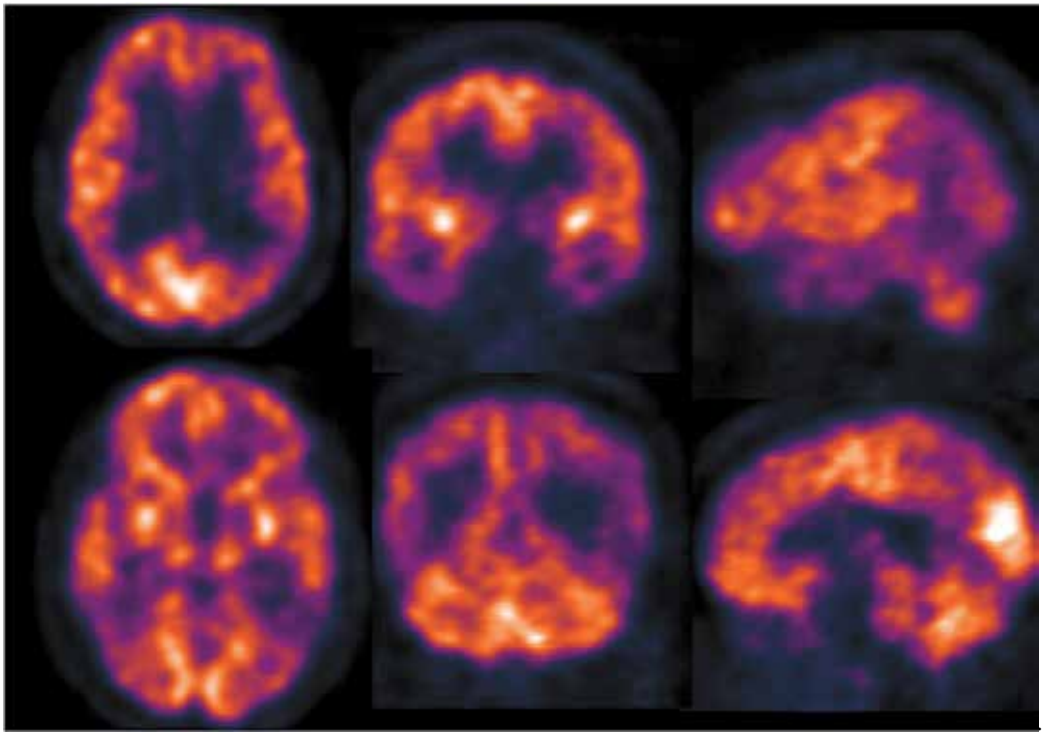

Fig. 5. PET in AD. Transversal, coronal and sagital slices of a cerebral 18F-FDG study of a patient with mild cognitive impairment. Hypometabolism of bilateral temporal and parietal cortex including part of the posterior cingulate gyrus (left dominance) was observed, corresponding to incipient AD.

TYPE OF DEMENTIA	**HYPOMETABOLIC BRAIN AREAS**
Alzheimer's disease	Parietal and superior/posterior temporal abnormalities; bilateral temporoparietal hypoperfusion (frontal lobe hypoperfusion may happen in conjuncion with temporoparietal hypoperfusion) Earliest changes in medial portion of parietal cortex, posterior cingulated or retrosplenial region
Diffuse Lewy body disease	Temporoparietal associatedwith occipital hypoperfusion
Corticobasal degeneration	Cortex and contralateral thalamus to the affected limb
Progressive supranuclear palsy	Motor/premotor frontal cortex, anterior cyngulate gyrus, hippocampus, basal ganglia and thalamus
Frontal dementia	Frontal, anterior cyngulate gyrus, anterior temporal lobe, hippocampus, basal ganglia and thalamus
Vascular dementia	Irregular pattern of uptake due to strokes
Parkinson's disease	Frontal-temporal-parietal

Table 2. ^{18}F-FDG PET pattern of uptake in different types of dementia

seen on ^{18}F-FDG PET images obtained in asymptomatic members of families in which a familial form of early AD was present. Similarly, asymptomatic subjects with the apolipoprotein ε4 allele were found to have significantly less parietotemporal ^{18}F-FDG uptake than those without this allele. ^{18}F-FDG PET has also proved useful to follow the course of AD patients. A normal PET study indicates that pathologic progression of cognitive impairment during a 3-year follow-up period is unlikely to occur. In another study, investigators were able to predict the clinical course of patients with mild cognitive impairment by using ^{18}F-FDG PET images obtained early in the course of disease (Hammoud et al, 2009).

Few studies have directly compared brain perfusion SPECT and ^{18}F-FDG PET in AD. Ishii et al. in 1999 compared ^{99m}Tc-ECD SPECT images with ^{18}F-FDG PET images in the same ten patients with Alzheimer's disease. ^{99m}Tc-ECD SPECT showed a reduction in parieto-temporal perfusion in 8 of the 10 patients, whereas ^{18}F-FDG PET showed a reduction in temporoparietal metabolism in nine of them. The contrast between the radiotracer uptake in the sensorial and motor areas and that in the parietotemporal region was not as great in the ECD images as it was in the FDG images (Ishii et al, 1999). Messa et al, in 1994, performed ^{99m}Tc-HMPAO SPECT and ^{18}F-FDG PET in patients with mild to moderate AD and in healthy control subjects. They reported that these techniques had similar abilities for visualizing decreased perfusion and metabolism in the temporoparietal cortex and similar diagnostic accuracies (Messa et al, 1994). In 2002, Herholz et al. showed good correlation between ^{18}F-FDG PET and ^{99m}Tc-HMPAO SPECT for detecting changes in the temporoparietal cortex in mild to moderate AD by using voxel-based statistical image analysis (Herholz et al, 2002), although ^{18}F-FDG PET demonstrated a more robust differentiation of patients with AD from healthy volunteers than did SPECT (Matsuda, 2007).

The meta-analysis performed by Yuan et al. in 2008 comparing results of ^{18}F-FDG PET, SPECT and structural MR imaging to predict conversion to AD in patients with mild cognitive impairment, including data from 1112 patients, showed that ^{18}F-FDG PET had moderately better concordance with follow-up results for the prediction of conversion. Approximately 88.9% of the patients with progressive mild cognitive impairment were considered affected by ^{18}F-FDG PET, whereas 84.9% of stable patients had negative ^{18}F-FDG PET (Yuan et al, 2008).

Another method for *in vivo* assessment of the extent of AD is the use of new radiotracers including the amyloid PET tracer Pittsburgh Compound B (^{11}C-PIB) for visualizing fibers Aβ (Morris et al, 2009, Kadir et al, 2011) or 2-(1-{6-[(^{18}F-fluoroethyl)(methyl)amino]-2naphthyl}ethylidene) malononitrile (^{18}F-FDDNP) that has been reported to label not only amyloid but also neurofibrillary tangles (Tolboom et al, 2009), and these methods have generated new possibilities for early diagnosis of brain impairments.

The first attempt to image brain Aβ accumulation in AD was reported by Friedland et al. in 1997 using a monoclonal antibody fragment labelled with ^{99m}Tc for single SPECT. This first attempt failed, but it was fundamental for subsequent efforts (Friedland et al, 1997). The second used PET, and the report of this work was published in 2002 (Shoghi-Jadid et al, 2002). The radiotracer used was ^{18}F-FDDNP but had the disadvantage of a limited experience worldwide (Klunk & Mathis, 2008). The third study of *in vivo* Aβ imaging with ^{11}C-PIB, was reported in 2004 (Klunk et al, 2004).

The quantity of radiotracer measured must be proportional to the amount of Aβ in that area and there must be sufficient signal-to-noise ratio to detect the tracer when levels of Aβ have become clinically significant. There are some published data for PIB demonstrating these

properties both *in vivo* and in post-mortem studies (Klunk et al, 2005, Maeda et al, 2007, Wiley et al, 2009). There is no doubt that the validation of an in-vivo Aβ tracer needs *in-vivo*–post-mortem correlation study in humans. ^{11}C-PIB has the advantage of not showing significant changes over 2 years during clinical AD (Engler et al, 2006).
Several tracers including ^{18}F-BAY94-9172 (Wang et al, 2011) (figure 3), ^{18}F-AV-45, ^{18}F-AH110690, ^{18}F-3'F-PiB, ^{11}C-AZD2995 and ^{11}C-AZD2184 have recently been or soon will be used in multi-centre phase II or phase III trials.
To understand the behaviour of a tracer in neurodegenerative disease, it is important to note that the population should include not only patients with AD or healthy controls. Studies on populations with mild cognitive impairment, early onset familial autosomal dominant AD, Parkinson's disease, Lewy body disease, fronto-temporal dementia, cerebral amyloid angiopathy, prion disease and other atypical dementias and cognitively normal aging contribute to our understanding of Aβ imaging with ^{11}C-PIB. A third principle for acceptance should be widely available of any Aβ imaging tracer.
The leading difference between these radionuclides is their decay 'half-life'. The decay half-live of carbon-11 is 20 min and that of fluorine-18 is 110 min. The main advantage of carbon-11 is that it decays so rapidly that sequential imaging studies in the same patient can be performed on the same day, thus, a ^{11}C-PIB study can be followed by a study using ^{18}F-Fluorodeoxyglucose (FDG). This would require two different days with a fluorine-18-labeled Aβ tracer. However, the 110 min half-life of fluorine-18 allows distribution within a 2–4 h travel radius. Thus, the need for a good fluorine-18 Aβ imaging tracer is mainly a matter of widening the availability. The properties of ^{11}C-PIB appear quite sufficient for imaging Aβ with a carbon-11 tracer, so the current goal is to obtain a fluorine-18 tracer with similar characteristics to those of ^{11}C-PIB (Klunk & Mathis, 2008).
To conclude about these two nuclear medicine brain imaging approaches (SPECT and PET), SPECT with ^{99m}Tc-HMPAO and ^{99m}Tc-ECD and ^{18}F-FDG PET are very useful diagnostic tools for early diagnosis of AD, even in pre-clinical stages. Posterior cingulated hypometabolism, measured with SPECT, may help to predict progression from MCI to AD. Some authors have stated that SPECT can be highly accurate in identifying the type of dementia (Read et al, 1995). The severity of temporoparietal metabolism impairment, measured by FDG PET, is helpful to distinguish those people with a progressive course of their condition from those who have a non-progressive course. This is useful for delivering MCI prognosis. The development of new radiotracers able to demonstrate the presence of Aβ plaques in AD patients, will improve the specificity of those methods. Finally, it is worth mentioning these nuclear medicine neuroimaging methods can serve as surrogate markers in clinical trials of therapeutic agents (Mueller et al, 2005; Teipel et al, 2006).

9. Magnetoencephalography (MEG)

MEG is a non-invasive technique that allows recording the magnetic fields generated by the human brain. MEG provides an excellent temporal resolution up to milliseconds, magnitude orders better than in other methods for measuring cerebral activity, such as CT, MRI, SPECT or PET (Hämäläinen et al, 1993). It generates functional maps with delimitation of cerebral structures in the range of few cm and, even, cubic millimeters. Therefore these functional maps can be organized both temporal and spatially. MEG signal is generated by synchronous oscillations of pyramidal neurons; the MEG detects slightly different features of the simultaneous electromagnetic brain activity and MEG power represents the activity of a given number of neurons discharging synchronously.

Some MEG background activity abnormalities in moderate and severe AD have been observed. AD patients show a decrease of MEG coherence values (Berendse et al, 2000). This biological marker is accompanied by a slowed MEG activity which becomes evident when analyzing the power spectral density of selected frequency bands. In this way, spontaneous MEG activity shows increased slow rhythms and reduced fast activity in AD patients compared with healthy subjects (Berendse et al, 2000; Fernández et al, 2002; Osipova et al, 2005).

It has been proposed that such slowing might be due to an increase in activation of low frequency oscillators rather than slowing of existing sources (Osipova et al, 2005). Fernández et al (2002) analyzed the presence of low frequency magnetic activity (delta and theta bands) associated with AD degeneration. Their results showed that people with AD had a significant increase of this type of frequencies in the temporoparietal area greater in the left hemisphere. Moreover, the values of low frequency were associated with the cognitive and functional state of these patients. Temporoparietal delta activity predicted the scores in mental status scales such as MMSE or CAMCOG. Delta activity in right parietal areas allowed predicting the functional status (FAST stage). Fernández et al (2006C) replicated previous studies stating that temporoparietal low frequency plays a key role in the process that leads from MCI to AD. There is a slow and practically linear low frequency activity from normal aging to dementia where MCI has an intermediate position. This study, once the parietal low frequency was defined as the most relevant feature to characterize these patients, carried a follow-up study of the people with MCI. The next step was to find out if MCI patients with more marked low frequency had more probability to evolve into AD. This group showed that people with MCI and higher parietal delta activity had 3,5 times more chance to develop AD (Fernández et al, 2006A). Broadly speaking, from the perspective of spontaneous MEG activity, one can say that a tendency towards brain activity slowing means a higher risk to develop dementia.

Fernandez et al (2006B) carried out a detailed analysis of spontaneous MEG activity spectral changes where the frequencies spectrum 2-60 Hz was subdivided "microscopically" in 2 Hz bands. The relative power of these micro-bands was then calculated. This approach served to overcome the difficulties in comparing data with previous literature, since there is some variation in establishing the limits of traditional EEG bands. A narrow temporoparietal 20-22 Hz band differentiated clearly the AD group from healthy elderly people and was correlated with the cognitive status.

MCI is an ideal target for this type of studies because the rate to progression to AD in healthy elderly people is about 1-2 % yearly, but in the MCI group this rate is 10-15 %. In fact AD and MCI share some characteristics which make difficult to establish a dividing line between both of them. Essentially anyone diagnosed with MCI will exhibit some cognitive impairments, specifically in the memory domain, that can be measured objectively but allow this patient to carry out a normal lifestyle. Therefore he cannot be diagnosed as suffering from dementia. Because of this fact many authors consider MCI as an intermediate or transitional state between normal aging and AD.

Once MCI was chosen as a target, we did a first study following-up a series of normal elderly people for two years. We realized those patients developing MCI had a MEG activity reduction in medial temporal regions during a memory task compared with those who had normal aging (Maestú et al, 2006). Along this line Poza et al (2007) evaluated the discriminative power of five measures extracted from the MEG power spectrum. The best one of them was the spectral mean frequency (sensitivity = 85%, specificity = 85.71%), thus

confirming the presence of spectral slowing in AD. Spectral changes can also be characterized in terms of ratios between powers in different frequency bands. In a later study Poza et al (2008) showed that a ratio of relative power in fast bands (alpha, beta and gamma) and slow ones (delta, theta) had a 75% sensitivity and a 95% specificity for the same purpose. A comparison of spectral median frequency with various nonlinear measures indicated median frequency was the single best discriminator between AD patients and controls (Hornero et al, 2008).

Another important issue during basal MEG, as well as during basal EEG, is brain complexity. Some authors have found a global decrease in irregularity and complexity in AD during basal (rest) MEG recordings (Poza et al, 2006; Gomez et al, 2006). However, the loss of complexity has been only proven in the high frequencies when a detailed spectral analysis in several frequency bands was performed.(van Cappellen et al, 2003).

Other approaches have focused on analyzing functional connections rather than local abnormalities in AD. A decrease of coherence values in the alpha band (Franciotti et al, 2006) and a general decrease of coherence in all frequency bands (Berendse et al, 2000) have been described. A reduced level of synchronization has also been reported in the upper alpha, beta, and gamma bands of AD patients in comparison with controls suggesting a loss of functional connectivity (Stam et al, 2002). Using resting MEG some authors have shown functional connectivity in AD is characterized by specific changes of long and short distance interactions in several frequency bands (Stam et al, 2006). Decreased levels of functional connectivity point towards the relationship of AD with an abnormal function of the large scale brain networks.

Recently Fernández et al (2010) have shown AD non-linear analysis is characterized by a reduced complexity and connectivity. They calculated Lempel-Ziv complexity (LZC) values and found that MCI patients exhibited intermediary LZC scores between AD patients and healthy controls. A combination of age and posterior LZC scores allowed AD-MCI discrimination whereas no LZC score allowed MCI-healthy controls discrimination. AD patients and controls showed a parallel tendency towards diminished LZC scores as a function of age, but MCI patients did not exhibit such "normal" tendency. Accordingly, anterior LZC scores allowed MCI-healthy controls discrimination for subjects below 75 years. People with MCI exhibited a qualitatively distinct relationship between aging and complexity reduction, with scores higher than controls in older individuals. This fact might be considered a compensatory mechanism in MCI before fully established dementia.

A third type of MEG studies is through brain synchronicity. There is increasing evidence that ubiquously distributed neural systems may communicate through synchronization of their activity. Synchronization here refers to the existence of a consistent relationship between activity patterns of two or more spatially separated neuronal groups. Synchronization can be defined at action potentials level or at oscillatory activity one. Synchronization in different frequency bands has been associated with various cognitive functions and the integration of information in the healthy brain (Stam, 2010).

Using the synchronization likelihood as a measure of functional connectivity, a loss of functional interactions between brain regions in alpha, beta and gamma bands was demonstrated in AD patients (Stam et al, 2002). In the same study coherence analysis did not show any significant differences between AD patients and controls, suggesting that nonlinear coupling might play a role. In a further analysis of the same data, van Cappellen et al (2003) showed that a neural complexity measure was increased in AD in both delta and theta bands, whereas the multichannel correlation dimension was increased in the beta

band; these results argue against a simple concept of 'loss of complexity' in AD and suggest a pattern of decreases as well as increases of functional connectivity in different frequency bands. This pattern was subsequently elucidated in another study showing a loss of mainly long distance left hemisphere functional connectivity in low alpha and beta bands, and an increase in parietal theta and occipito-parietal beta/gamma connectivity in AD patients compared to controls (Stam et al, 2006). The preferential loss of left hemisphere connectivity is interesting because some researchers (Osipova et al, 2003; Fernandez et al, 2006) have suggested a left hemisphere vulnerability rather characteristic. The increased connectivity in occipital and parietal areas is remarkable. Possibly, these changes could also reflect a kind of compensatory mechanism. Coherence analysis of the same data showed a roughly comparable pattern of changes. Furthermore, inter-hemispheric alpha band synchronization likelihood was significantly correlated with the MMSE score, suggesting the functional significance of these resting-state measurements.

A problem of functional connectivity studies based upon correlations between raw EEG or MEG signals is a single source is often picked up by many sensors, giving rise to spurious correlations. This volume conduction problem can be solved by special measures such as the phase lag index, which eliminates possible contributions from common sources, and reflects more accurately true interactions between distributed brain areas, reconstructed functional brain networks (Stam et al, 2007). This approach allows the classification of networks on a scale ranging from completely ordered to completely random. It was shown that in the alpha band brain networks in AD were more random than brain networks of healthy controls. Finally, using a modeling approach, it could be shown that the process that gives rise to abnormal brain networks in AD probably attacks critical connections between networks hubs preferentially.

Lastly, cognitive function studies through magnetic evoked potentials have been widely used both in MCI and AD. The MEG activity pattern during cognitive task is significantly altered (Maestú et al., 2001). A study showed greater MEG parietotemporal activity in the left hemisphere during a memory task in healthy elderly people compared with AD patients. The latter showed a reduction of MEG activity accompanied by an anomalous localization in frontal areas. This activation profile can predict performance in cognitive tests such as the MMSE or CAMCOG, as well as with daily life functional outcomes. Thus a lesser parieto-temporal activation during a memory task is associated o lower scores in cognitive tests, what makes MEG a functional neuroimaging technique useful for diagnosis.

In combining MEG with volumetry data obtained from MRI, we have found a direct relationship between hippocampal atrophy with both an increased magnetic activity of low frequency (Fernández et al, 2003) and a decreased magnetic dipoles during a memory task (Maestú et al, 2003).

A relevant aspect is studying those MEG normal changes which are part of normal aging. We have studied elderly people at different ages and we have found a cortical reorganization as age progresses (Maestú et al., 2004). Besga et al (2010) also found that hippocampal volume reduction allowed the discrimination between AD and MCI patients as compared with controls. The percentage of correct classification was 91.3% when AD patients versus controls were compared, and 83.3% in the case of MCI versus controls. MEG data showed that AD patients exhibit higher theta and delta activity than MCI and controls. Such higher activity was significant in parietal, temporal, and occipital areas. Left parietal theta classified correctly MCI patients (vs controls) in 78.3% of the occasions. Right occipital theta and the left parietal delta allowed correctly discrimination of AD patients (vs controls)

at a 81.8% rate. Left parietal theta discriminated between AD and MCI in 56.6% of the occasions. In addition, the combination of both techniques significantly improved the rate of correct classification, thus indicating that a multidisciplinary perspective of techniques may improve the diagnostic capabilities (Besga et al, 2010).
Osipova et al (2006) used source estimation of ongoing MEG oscillations to detect changes in subjects with MCI. In this study, the distribution of alpha sources did not differ between subjects with MCI and healthy elderly controls. Maestú et al (2006) suggested MEG can provide useful information about the risk of developing MCI. They recorded MEG during a memory task in 15 healthy subjects. Five of these subjects developed MCI after two years. These subjects were characterized by abnormal low frequency activity in the left temporal lobe in the initial recording. The MEG mean frequency power spectrum in MCI subjects was decreased compared to healthy controls, and increased compared to Alzheimer patients (Fernández et al, 2006 A). This suggests once more MCI is, in some respects, an intermediate state between health and AD. Furthermore, an average decrease of 0.17 Hz/year of the mean frequency in healthy subjects was shown. Slowing of MEG in MCI could be related to the risk of developing AD.

10. Conclusion

Along this chapter we have highlighted some issues in relation to MCI. MCI is less known from the brain imaging point of view than AD. The high chance to progress from MCI to AD indicates the need to do more studies on this domain. MRS and those neuroimaging techniques based on neurophysiological measurements, e.g. MEG, can throw light on this area.
Brain imaging techniques have not reached a point yet where they can provide the confirmatory diagnosis of AD. However the likelihood of suspicion of AD can be enhanced by these methods. Table 3 summarizes these brain imaging techniques in AD and MCI.

Neuroimaging technique	**Strengths**	**Limitations**	**Comparison AD-MCI**
CT	Low cost, high availability	Relative poor resolution Poor differentiation grey matter-white matter	Does not add
MRI	Extensive use, high availability	Poor correlation with changes in function and metabolic findings	Volumetry and ROI volumetry can be useful, even more so longitudinally
fMRI	Gives insight into cognitive performance	Conflicting results	Can be useful as well, but results interpretation may be difficult
DTI	Can assess structural connectivity	Utility not proven	AF reductions in aMCI is a robust finding

Neuroimaging technique	Strengths	Limitations	Comparison AD-MCI
Spectroscopy	Provides information about brain chemical content	Utility not proven	NAA and mI may measure progression from MCI to AD
SPECT	Low cost compared with PET	Mediocre resolution Radiation exposure	Limited utility
PET	Can detect metabolic changes in AD	Expensive Lack of availability	Most useful tool to differentiate AD from MCI, so far
PET-amyloid	Detects most specific metabolic change linked to diagnosis	Difficult interpretation as neurometabolic changes may antecede disease	Can be a risk biomarker, but results interpretation may be difficult
MEG	Gives insight into cognitive performance High temporal resolution	Very expensive	Some usefulness for this purpose has been demonstrated

CT = computerized tomography; MRI = magnetic resonance imaging; fMRI = functional magnetic resonance imaging; DTI = diffusion tensor imaging; SPECT = single-photon emission computed tomography; PET = positron emission tomography; MEG = magnetoencephalography; NAA = N-acetyl aspartate; mI = myoinositol; AD = Alzheimer's disease; MCI = mild cognitive impairment; ROI = region of interest

Table 3. Comparison of different brain imaging techniques for AD, with relative advantages and disadvantages, and usefulness for AD-MCI differentiation

Structural MRI is nowadays a standard technique for this purpose, but cheaper techniques, such as CT, may still play a role, even more so in underdeveloped countries. Structural brain imaging, such as CT or MRI, is quite useful for the differential diagnosis of AD with non-neurodegenerative diseases. PET and SPECT are not sufficiently accurate to replace clinical judgment, though they supplement the diagnosis of AD and MCI and help to improve the accuracy of diagnosis. Neuroimaging can be very useful for differentiating AD from frontotemporal dementia as well.

At this moment the rest of the neuroimaging techniques previously mentioned are more used for research than for clinical purposes. Part of the problem is that the brain imaging sensitivities and relative utility of each one of them in AD and MCI are not clearly established. The bulk of neuroimaging research in AD continues to be centered on glucose metabolism, perfusion and tissue content. In the future it is likely the functional neuroimaging techniques will continue to expand. Their refinement will bring greater clarity in the diagnosis of AD as well in the MCI status. Combination of brain images approaches, some of them at least based on molecular issues, such as amyloid imaging, may be promising.

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9

The Clinical Use of SPECT and PET Molecular Imaging in Alzheimer's Disease

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1. Introduction

Single photon emission computed tomography (SPECT) and positron emission tomography (PET) are functional nuclear medicine techniques which allow for accurate non-invasive in vivo measurements of a wide range of regional tissue functions in man. Brain functional imaging with SPECT and PET is based on the recording of the distribution of administered radionuclides in three dimensions, thus producing maps of brain biochemical and physiological processes. SPECT and PET techniques are able to image brain perfusion and metabolism, as well as various neurotransmission or other cellular processes using specific radioligands which mark in vivo receptors, transporters or enzymes.
Brain SPECT and PET imaging - or molecular imaging -, has been applied to the study of Alzheimer's disease (AD) for over two decades. These functional neuroimaging approaches have the capability of identifying subtle pathophysiologic changes in the brain before structural changes are present (Xu et al., 2000). Therefore they possess greater potential for accurate and early diagnosis, monitoring disease progression, and better treatment follow-up. Furthermore, the application of SPECT and PET techniques to the study of AD has led to increased understanding of the underlying pathology and the disease processes and improved the differential diagnosis from other neurodegenerative causes of dementia.

2. Clinical applications of SPECT and PET molecular imaging in AD

2.1 Radiopharmaceuticals for SPECT and PET brain imaging

Brain SPECT and PET imaging is performed using radiopharmaceuticals which utilize the highly selective properties of Blood Brain Barrier (BBB). The intact BBB has been a significant limitation whenever a nuclear imaging technique is employed to study the function of the living human brain since it may impede brain uptake of radiotracers (Jolliet Riant & Tillement, 1999). The main factors which regulate passage across the BBB are ionic selectivity and lipid solubility of substances (Costa, 2004). Osmotic pressure and specific and non-specific binding to plasma proteins, cell membranes and other components present in the bloodstream, may also affect the permeability of BBB and brain uptake of the administered radiopharmaceuticals (Tanaka & Mizojiri, 1999). In the absence of radiotracer

binding to these metabolic (biological) barriers, free diffusion of lipophilic small neutral compounds occurs directly through the endothelial cells of BBB (Waterhouse, 2003).

2.1.1 Properties and mechanisms of brain uptake

Radiopharmaceuticals used for brain perfusion SPECT imaging are lipophilic and neutral compounds with limited protein binding, which penetrate freely the intact BBB by simple diffusion (Costa, 2004). They distribute in proportion to regional cerebral blood flow (rCBF) and remain trapped in neuronal tissue without redistribution for a suitable amount of time to permit SPECT imaging.

Brain perfusion SPECT radiopharmaceuticals are labeled with 99mTechnecium (^{99m}Tc) which has excellent physical characteristics for imaging purposes and dosimetry and it is always available at a low cost.

^{99m}Tc-bicisate (ECD) and ^{99m}Tc-exametazime (HMPAO) are the most common radiopharmaceuticals used in routine clinical practice (Kung et al., 2003). They are both lipophilic and neutral tracer agents with suitable characteristics to pass the BBB by passive diffusion. ^{99m}Tc-bicisate (ECD) is retained in brain tissue after being hydrolysed to ionized non-diffusible metabolites by interaction with esterases in brain cells (Walovitch et al., 1994), while ^{99m}Tc- HMPAO is converted to one or more polar species by an assumed interaction with glutathione (Jacquier-Sarlin et al., 1996).

The PET radiopharmaceuticals are labeled with isotopes of elements that naturally occur in the various substrates (Newberg & Alavi, 2003). The most common radioisotopes used for labeling are ^{18}F and ^{11}C. ^{18}F labeled compounds have the advantage over ^{11}C labeled compounds of the longer half life of ^{18}F (110 min versus 20 min) which allows longer imaging protocols. The advantage of ^{11}C labeling is that, theoretically, any organic molecule could be labeled by isotopic substitution of ^{11}C for natural carbon, retaining the full properties of the parent molecule (Pimlott, 2005).

The most common application of PET in AD is the study of regional cerebral glucose metabolism (rCGM) and to a lesser extent the measurements of regional cerebral blood flow (rCBF) and oxygen metabolism, with the use of radiotracer concentrations in the picomolar range which rarely exerts any pharmacological or toxicological effect (Gee, 2003).

The radiofluorinated analogue ^{18}F-2-fluoro-2-deoxy-D-glucose (^{18}F-FDG) is used for brain glucose metabolism studies. ^{18}F-FDG is transported into the brain cells by facilitated diffusion, then phosphorylated to FDG-6-PO_4 and trapped intracellularly where it can be measured, without further metabolism (Newberg & Alavi, 2003). [^{15}O] H_2O and $^{15}O_2$ are used for the measurement of rCBF and oxygen metabolism, respectively.

SPECT and PET receptor imaging radioligands are neutral and lipophilic compounds with high plasma clearance and low plasma protein binding, and the ability to pass the intact BBB by simple diffusion, while regional cerebral distribution reflect receptor density (Pimlott, 2005). Furthermore they perform high affinity and specificity and/or selectivity for the specific receptor of interest over other receptors, limited or measurable metabolism, low toxicity and good in-vitro stability (Wong & Pomper, 2003).

Although ^{99m}Tc has so far been incorporated in most SPECT imaging studies, a general problem with ^{99m}Tc complexes is the low brain uptake due to the large molecular weight of linking moieties required to radiolabel compounds with ^{99m}Tc (Johannsen & Pietzsch, 2002). The incorporation of the much smaller radioiodine into a radiotracer can increase brain uptake and currently most of the research studies on SPECT neuroreceptor brain imaging are performed using agents labeled with ^{123}I (Pimlott, 2005).

2.2 Imaging of brain perfusion and glucose metabolism in AD

A substantial number of rCBF and rCGM studies with SPECT and PET have been performed in AD patients as well as in other neurodegenerative disorders. These studies have demonstrated characteristic patterns of perfusion and metabolism abnormalities which distinguish AD from other types of dementia and supported the use of SPECT and PET imaging as biomarkers of AD for the detection of the underlying changes of perfusion and metabolism and monitoring disease progression and response to treatment. In general, there is a concordance between brain perfusion and metabolism deficits, exhibited on SPECT and PET studies, respectively.

Brain perfusion SPECT imaging in AD patients typically shows bilateral hypoperfusion of the parietal and posterior temporal lobes (Ichimyia, 1998; Ishii et al., 1996; Lojkowska et al., 2002). The perfusion deficits are frequently symmetric but not necessarily of the same magnitude and severity. Motor and sensory cortices are usually spared. Hypoperfusion of the posterior association cortices is a finding that some authors consider specific for AD and positive evidence for its diagnosis, although other conditions may display a similar pattern (Hirao et al., 2006). Prospective studies with histological confirmation in demented patients and control cases have shown that the sensitivity and specificity of rCBF SPECT imaging for the differentiation of patients with AD from control subjects is 89% and 80%, respectively (Jobst et al., 1998). With progression of the disease, hypoperfusion spreads from the posterior to the anterior temporal and frontal lobes (Fig. 1) (Brown et al., 1996).

The pattern and degree of hypoperfusion have been correlated in many studies with the onset, the severity, the clinical features and the prognosis of the disease, although with contradictable results in several cases. Temporoparietal hypoperfusion has been shown to be more severe in early-onset than in late-onset AD (Weinstein et al., 1991). Late onset patients tend to present with the characteristic involvement of the medial temporal lobes producing marked memory loss whereas early onset patients present with predominant posterior cortical association area involvement (Kemp et al., 2003). The Mini Mental Examination scores in AD patients correlate with the rCBF in temporal and parietal regions (Rodriguez et al., 1999). Moreover, specific clinical symptoms that AD patients may present are associated with perfusion abnormalities in discrete cortical areas. The right middle medial temporal region emerged as an important neural correlate of aggression (Lanctôt et al., 2004); depressive symptoms were associated with relative hypoperfusion in the prefrontal cortex (Levy-Cooperman et al., 2008); a significant association was also found between anosognosia and decreased perfusion in the orbitofrontal cortex (Shibata et al., 2008); hypoperfusion in the inferior, medial and orbital frontal lobes as well as the anterior cingulate gyri were found to be associated with the lack of awareness in patients with early AD (Hanyu et al., 2008); apathetic AD patients performed hypoperfusion in the left anterior cingulate and right orbitofrontal cortex (Lanctôt et al., 2007); hypoperfusion in prefrontal cortex, anterior cingulate gyri, inferior to middle temporal cortices, and parietal cortex of the right hemisphere has been observed in AD patients with delusions (Nakano et al., 2006); finally, hypoperfusion in the left anterior cingulate and left orbitofrontal cortices, and relative sparing of perfusion in the right anterior cingulate, right orbitofrontal and left middle mesial temporal cortices emerged as predictors of appetite loss in AD patients (Ismail et al., 2008). Decreased blood flow in the frontal lobe of AD patients is correlated not only with reduced cognitive function at the time of the evaluation but with rapid progression in the subsequent clinical course, as well (Nishimura et al., 2007). Hypoperfusion in the left temporal region has been associated with lowering of the median

survival and higher death rates (Claus et al., 1999), though perfusion in the right parietal lobe has also found to be a significant predictor of survival in patients with AD (Jagust et al., 1998).

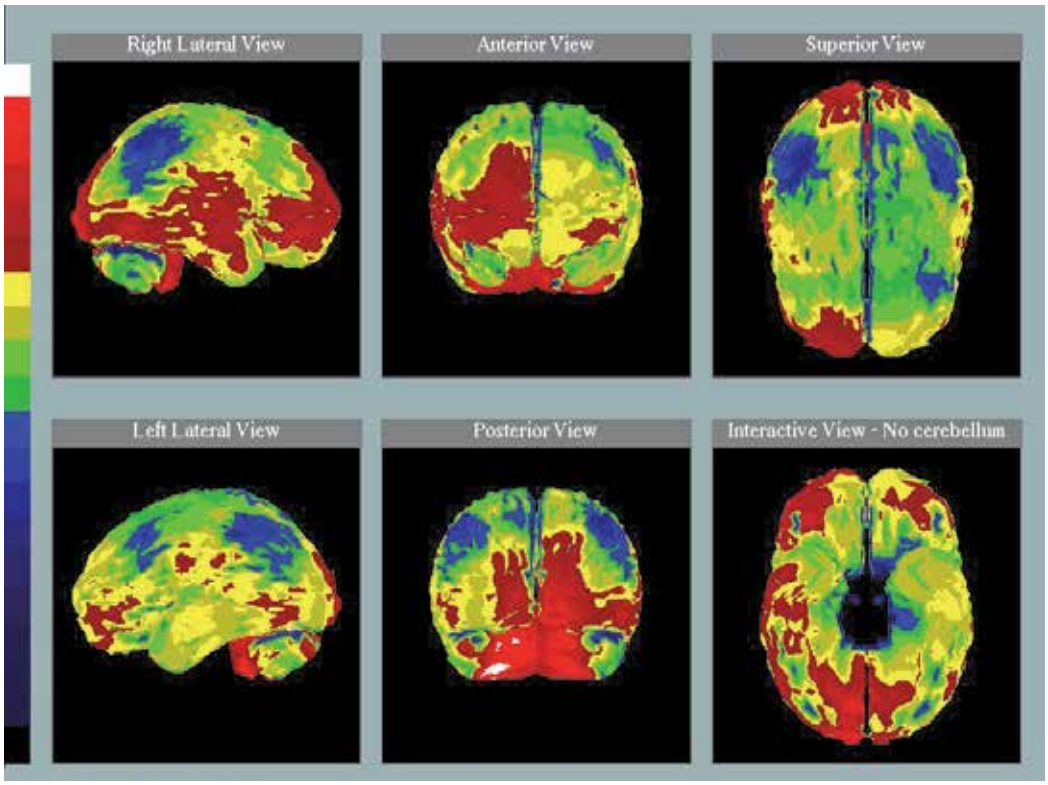

Fig. 1. Brain perfusion SPECT study in a patient with Alzheimer's disease. Reduced ^{99m}Tc-HMPAO uptake in parietal, temporal and frontal lobes. Hypoperfusion is more severe on the left hemisphere.

FDG PET studies in AD patients have demonstrated a typical pattern of reduced temporoparietal FDG uptake with sparing of the basal ganglia, thalamus and cerebellum (Coleman, 2005). Hypometabolism begins typically in the superior parietal cortex, then spreads inferiorly and anteriorly to involve the inferior parietal, superior temporal, and prefrontal cortices. The extent of hypometabolism correlates with the severity of cognitive impairment and often shows right/left hemispheric asymmetry (Haxby et al., 1990**)**. More recent studies using higher resolution PET scanning have reported marked hypometabolism of the hippocampal head and amygdala in AD (Stein et al., 1998**)**.

FDG PET demonstrated high sensitivity and specificity (94% and 73%, respectively) for detecting the presence of AD in histopathologically confirmed demented patients. In contrast, clinical evaluation without FDG PET showed lower sensitivity and specificity (83%–85% and 50%–55%, respectively), as determined by an entire series of evaluations repeated over a period of years (Silverman et al., 2002). Even early in the disease process, before the appearance of volume loss, FDG PET has been helpful in diagnosing AD, with a sensitivity and specificity of about 90%, irrespective of the degree of cognitive impairment

(Hoffman et al., 2000). It is the neuroimaging technique that has been shown to yield the highest prognostic value for providing a diagnosis of presymptomatic AD 2 years or more before the full dementia picture is manifested (Silverman et al. 2001). Thus, PET is able to measure cognitive decline at some of the earliest possible stages, providing evidence of its usefulness for early AD detection.

SPECT and FDG PET studies have also been applied in patients with mild cognitive impairment (MCI) in order to predict progression from MCI to AD. Reduced glucose metabolism in the inferior parietal cortex and hypoperfusion in the parahippocampus, lateral parietal and posterior cingulate in converters as compared with non-converters have been reported (Ishiwata et al., 2006; Mosconi et al., 2004). Longitudinal FDG PET and perfusion SPECT studies have shown that hypometabolism in the parietal association areas and hypoperfusion in the bilateral inferior parietal areas, angular gyrus and the precunei had a high predictive value and discriminative ability of converters and non-converters, while hypometabolism in the posterior cingulate gyrus had a lower predictive value (Chetelat et al., 2003; Hirao et al., 2005). Combined baseline memory deficits and rCBF SPECT images identified pre-clinical AD with a sensitivity and specificity of 77.8% (Borroni et al., 2006). These SPECT and PET findings suggest that initial functional neuroimaging studies of individuals with MCI may be useful in predicting who will convert to AD in the near future.

2.3 Beta amyloid imaging

In the last years, the detection of senile plaques (SPs) and neurofibrillary tangles (NFTs) has been a target for nuclear molecular imaging in the field of AD. The development of radiotracers able to localize SPs and NFTs could be useful not only in the diagnosis of AD but also in the investigation of the temporal relationship between amyloid deposition, neuronal loss, and cognitive decline and assessment of the effects of drugs in disease progression. Also, these radiotracers could provide treatment for AD patients early in the course of the disease when response to treatment is usually better.

The development of plaque-binding compounds started with monoclonal antibodies against beta-amyloid (Aβ) and self-associating Aβ fragments, and was followed by analogues of histopathological dyes such as Congo Red, Chrysamine G, and Thioflavin T, which are used to stain SPs and NFTs in postmortem AD brain sections (Valotassiou et al., 2010; Villemagne et al., 2005). Recently, malononitrile analogues, which share the same binding site on Aβ peptides with the nonsteroidal anti-inflammatory drugs (NSAIDs), have been developed as potential tracers for Aβ imaging (Agdeppa et al., 2001, 2003a; Shoghi-Jadid et al., 2002).

2.3.1 Radiolabeled antibodies

Several radiolabeled anti-Aβ antibodies and self- associating Aβ amyloid fragments have been developed for potential in vivo SPECT amyloid imaging in AD. ^{99m}Tc-10H3, ^{111}Indium (^{111}In) AMY33 and 10D5 (Bickel et al., 1994; L.C. Walker et al., 1994) although gave promising results in vitro, however, they didn't meet success for in vivo studies mainly due to poor BBB penetration. Despite the efforts that have been made to modify the structure of antibodies and to develop different drug delivery methods suitable for brain studies in vivo, significant problems still constrain the potential application of these probes in human subjects.

2.3.2 Radiolabeled derivatives of histopathological dyes

The first chemically modified neutral thioflavin derivatives were labelled with I-123 in an effort to develop radioiodinated tracers for SPECT imaging of Aβ plaques. Radioiodinated TZDM, TZPI, IBOX and IMPY showed good Aβ plaque binding in vitro but low brain uptake in vivo since they lack sufficient hydrophobicity for diffusion through the BBB, except IMPY which exhibited more promising binding properties (Ono et al., 2002; Zhuang et al., 2001). [^{11}C]-SB-13, a radiolabeled Congo Red derivative, (Verhoeff et al., 2004) has been recently evaluated in AD patients and healthy control subjects. [^{11}C]-SB-13 showed increased retention in the frontal and posterior temporal-inferior parietal association cortices in the AD patients, but not in the comparison subjects.

Another radiolabeled benzothiazole aniline (BTA) analogue [N-methyl-^{11}C]-2-(4'-methylaminophenyl)-6-hydroxylbenzothiazole ([^{11}C]6-OH-BTA-1), which is a neutral derivative of thioflavin T, has been studied extensively, in both preclinical and clinical studies (Klunk et al., 2001; Mathis et al., 2002). It was named "Pittsburgh Compound-B' or PIB and exhibited high affinity for aggregated amyloid but not for NFTs (Ye et al., 2005), and reasonable lipophilicities for crossing the BBB. In AD patients, the distribution pattern of ^{11}C-PIB is characterized by significantly great uptake in the frontal, temporal, parietal, and occipital cortices and the striatum but low entry into the cerebellum and subcortical white matter (Nordberg, 2008). The retention of PIB in cortical AD brain regions was found to be inversely related to the rCGM as measured by FDG PET in the same brain regions. ^{11}C-PIB uptake did not show significant correlation with the degree of cognitive impairment. Similar PIB retention was observed in both AD patients and controls in areas with low Aβ amyloid deposition (Klunk et al., 2004). Elevated ^{11}C-PIB uptake was also observed in dementia with lewy bodies and about 50% of mild cognitive impairment subjects, compared to healthy controls (Morris & Price, 2001). Interestingly, about 25% of the healthy controls demonstrated cortical binding of ^{11}C-PIB, predominantly in the prefrontal cortex, a finding which supports the in vitro observations that Aβ aggregation predominantly occurs before onset of dementia. ^{18}F-flutemetamol (or ^{18}F-GE067), a fluorolabeled structural thioflavin analogue of PIB, was developed recently. The spatial distribution of ^{18}F-flutemetamol uptake in AD resembles closely the distribution typically seen with ^{11}C-PIB binding (Nelissen et al., 2009). High ^{18}F-flutemetamol uptake was observed in striatum while the uptake in medial temporal cortex, one of the areas of predilection for neurofibrillary tangles in AD, was relatively low. Furthermore, the retention of the radioligand was similar in AD patients and healthy controls in brain regions known to be relatively unaffected by amyloid deposition.

2.3.3 Radiolabeled malononitrile analogues

Newer radioligands such as the radiofluorinated [^{18}F]FDDNP and [^{18}F]FENE, which are analogues of the 2-{1-[6-(dimethylamino)-2-naphthyl]ethylidene} malononitrile (DDNP), have been used to label not only SPs but also NFTs for the first time in the living brain of AD patients with PET (Agdeppa et al., 2003b). FDDNP and NSAIDs share a previously unrecognized common binding site on Aβ (1-40) fibrils and senile plaques and also exhibit anti-aggregation effects on Aβ peptides.

The PET imaging data showed increased retention of [^{18}F]FDDNP in the hippocampus, amygdale, entorhinal and temporal lobe regions of the brain, which are consistent with areas known to develop SPs and NFTs. The findings were associated with hypometabolism, as measured with FDG PET, and atrophy, as observed with MRI, in the same brain areas

and correlated with lower memory performance scores (Agdeppa et al., 2001; Shoghi-Jadid et al., 2002). [18F]FDDNP provides a disease-specific, in vivo imaging tool for localization and loading of AD-related lesions, which in turn, could aid in early diagnosis of AD in combination with other diagnostic tests (Agdeppa et al., 2003a). Indeed, [18F]FDDNP has greater sensitivity at early stages of AD, before clinical evidence of cognitive decline.
Moreover, [18F]FDDNP-PET may contribute in the elucidation of the relation between possible neuroprotective NSAIDs and Aβ aggregates. The shared binding site on Aβ fibrils and plaques may be a site of anti-aggregation drug action (e.g., naproxen and ibuprofen). Naproxen, ibuprofen and even FDDNP significantly inhibit aggregation of the Aβ (1-40) peptide in the micromolar range (Agdeppa et al., 2003b). Therefore, [18F]FDDNP-PET could be used in determining the occupancy rate of NSAIDs and experimental drugs in plaques in the living brain of AD patients, thus offering new opportunities for early diagnosis, prevention and treatment of AD.

2.3.4 Flavonoids

Flavonoids and their derivatives (chalcones and aurones) have been proved to have anti-oxidant effect due to matrix metalloproteinases (MMP) inhibitory activity (Calliste et al., 2001), as well as anti-inflammatory and neuroprotective properties by modulating microglia-related immune responses in the brain (Rezai-Zadeh et al., 2008). Radioiodinated flavones have also been used in experimental studies as possible amyloid imaging probes. They displayed high brain penetration, high brain uptake, fast washout from the brain and good binding affinity not only on $A\beta^{1-40}$ aggregates but on $A\beta^{1-42}$ aggregates as well. Moreover, they showed high binding affinity for NFTs, too (Ono et al., 2005, 2007).

2.4 Imaging of the acetylcholine system

PET and SPECT can evaluate noninvasively the acetylcholine system in the human brain with the use of appropriate radiotracers, in order to detect impairments even at the presymptomatic stage of AD as well as monitoring treatment outcomes of the drugs that enhance acetylcholine activity in AD.
The available radiotracers target various elements and processes involved with cholinergic neurotransmission and function. These include the study of acetylcholine receptors and acetylcholine neuronal integrity.
Radioligands have been developed to measure both nicotinic and muscarinic receptors. 11C-labeled nicotine (Nordberg et al., 1991) as well as epibatidine and azetidine derivatives labeled with 11C or 18F were used to visualize and quantify nicotinic receptors in the brain. Although epibatidine demonstrated high affinity and specificity for nicotinic receptors, unfortunately is very toxic, which may preclude its use in humans (Villemagne et al., 1997). Nevertheless, epibatidine and azetidine analogs didn't meet clinical application (Sihver et al., 1999). Early in the course of AD, PET studies revealed a reduced 11C-nicotine uptake to nicotinic receptors in frontal and temporal cortex and in the hippocampus in comparison with that of age-matched healthy control subjects (Volkow et al., 2001).
Several radiotracers have been developed for mapping muscarinic receptors. For the most part these radiotracers are limited by the lack of selectivity for the muscarinic receptor subtypes (M1–M4), except for [18F]FP-TZTP, which appears to bind predominantly to M2 receptors (Carson et al., 1998).
For the study of acetylcholine neuronal integrity, radioigands have been developed to measure both the activity of acetylcholinesterase and the acetylcholine vesicular transporter.

The activity of acetylcholinesterase can be measured with PET either using radiolabeled acetylcholine analogues that serve as substrates for acetylcholinesterase and hydrolyze to a hydrophilic product that is trapped in the cell or using radioligands that directly bind to acetylcholinesterase (Kuhl et al., 1999; Pappata et al., 1996). Radiolabeled acetylcholine analogues N-methyl-3-piperydyl-acetate [MP3A], N-methylpiperidin- 4-yl-acetate [MP4A], and N-methylpiperidin-4-yl-propionate [PMP] have been used for this purpose. PET studies in patients with AD demonstrated a widespread reduction of acetylcholinesterase activity in the cerebral cortex. In normal aging no changes were observed. Additionally, the early loss of cholinergic transmission in the cortex could be shown with these tracers, which precedes the loss of cholinergic neurons in the nucleus basalis of Meynert (Herholz et al., 2004).
Several radioligands that target the acetylcholine vesicular transporter have been labelled but only (2)-5 [^{123}I]iodobenzovesamicol (^{123}I-IBVM) has been used in SPECT studies to image the living human brain (Kuhl et al., 1994). ^{123}I-IBVM is an analogue of vesamicol that binds to the acetylcholine vesicular transporter. Cortical binding of ^{123}I-IBVM in normal subjects was found to decline only mildly with age (3.7% per decade), but it was markedly reduced in AD patients. The reductions predicted dementia severity while the binding levels were also determined by the age of disease onset (Kuhl et al., 1996). Patients with an early onset demonstrated reductions throughout the cortex and hippocampus, whereas patients with late onset had reductions only in the temporal cortex and hippocampus. This finding may reflect the greater cholinergic loss in early- rather than in late-onset AD (Rossor et al., 1984).

2.5 Imaging of neuroinflammation

The process of neurodegeneration in AD is associated with activation of resting microglial cells and local glial responses. Peripheral benzodiazepine receptors are the mediators of central nervous system inflammation. Radiolabelled isoquinoline ([^{11}C]*R*-PK11195) has been used in PET studies as an indicator of microglia activation in AD. [^{11}C](*R*)-PK11195 is a ligand for the peripheral benzodiazepine receptors which binds to the outer mitochondrial membrane of activated, but not resting, microglia (Banati, 2002). PET studies in AD patients demonstrated increased [^{11}C](*R*)-PK11195 uptake in the temporal cortex (particularly the fusiform, the parahippocampal and the inferior temporal gyri), the inferior parietal cortex, the posterior cingulate and the amygdala (Cagnin et al., 2002). These areas with high [^{11}C](*R*)-PK11195 uptake subsequently underwent the most marked atrophic changes within the following year as shown by a longitudinal serial volumetric MRI scan. This suggests that an in vivo measure of activated microglia provides an indirect index of disease activity.
^{123}I-PK11195, a SPECT ligand for the peripheral benzodiazepine receptors, has been recently studied in AD patients. Significantly increased uptake was found in the frontal and right mesotemporal regions which correlated with cognitive deficits (Versijpt et al., 2003).

2.6 Imaging of the serotonergic system

Several SPECT and PET studies have investigated the implication of serotonin (5HT) in the modulation of cognitive and behaviorial/neuropsychiatric disturbances of neurodegenerative dementias (Meltzer et al., 1998). Post-synaptic 5HT2A receptors and pre- and post-synaptic 5HT1A receptors have been studied in vivo in AD.
PET studies with ^{18}F-setoperone, a 5HT2A receptor antagonist, demonstrated reduced parietal, temporal, frontal and occipital cortical binding in untreated moderate-severe AD patients (Blin et al., 1993), while reduced ^{18}F-altanserin binding were also observed in mild-moderate AD in the anterior cingulate, prefontal, temporal, and sensorimotor cortices

(Meltzer et al., 1999). No correlation was found between cortical ^{18}F-altanserin binding and MMSE scores. Pre- and post-synaptic 5HT1A receptors studies with ^{18}F-MPPF have shown decreased binding in the hippocampus and the raphe nucleus of AD patients (Kepe et al., 2006).

2.7 Differential diagnosis

2.7.1 Differential diagnosis of AD from dementia with lewy bodies

The discrimination of AD from dementia with lewy bodies (DLB) is difficult since these disorders share common scintigraphic findings. Temporoparietal hypoperfusion and hypometabolism on SPECT and FDG PET studies is common to both AD and DLB (Minoshima et al., 2001; Pasquier et al., 2002), although subtle differences in perfusion and metabolism patterns have been reported (Colloby et al., 2002), with a relative preservation of medial temporal lobe structures and rCBF in DLB and more extended biparietal hypoperfusion in DLB compared to AD patients.

The differential diagnosis of AD from DLB is based on the greater degree of occipital hypoperfusion or hypometabolism in DLB than in AD. The reported sensitivity and specificity for the accuracy of discriminating AD from DLB on the basis of the finding of hypoperfusion and hypometabolism in the occipital cortex ranged between 65-90% and 80-87%, respectively (Lobotesis et al., 2001; Minoshima et al., 2001).

Post mortem brain studies have shown that the presynaptic dopaminergic terminals in the putamen of DLB patients show a 57% reduction compared to controls. This reduction in dopaminergic terminals leads to loss of the presynaptic dopamine transporter system (DAT) (Piggott et al., 1999). If one considers the DAT a surrogate marker of dopaminergic nigrostriatal neurons, imaging of the DAT sites with a specific marker, will be able to identify nigrostriatal dopaminergic degeneration in DLB patients during life. [^{123}I]-ioflupane is a cocaine analogue that binds specifically to the DAT in the membrane of the presynaptic dopaminergic neurones. SPECT studies with [^{123}I]-ioflupane in DLB patients demonstrated reduction of the presynaptic tracer uptake in the striata of both hemispheres, which was clearly more marked in the putamen than caudate nucleus and linked to significant loss of DAT (Z. Walker et al., 2002). This finding enabled the clear differentiation from AD that showed the striatal uptake to be within the normal range. Separation between DLB and AD based on [^{123}I]-ioflupane SPECT imaging is achieved with sensitivity, specificity, and positive predictive value of 78%, 94%, and 90%, respectively (O'Brien et al., 2004).

Recently, an alternative scintigraphic method - cardiac uptake of ^{123}I-metaiodobenzylguanidine (MIBG) - for differentiating AD from DLB was reported. Markedly decreased cardiac uptake was observed in DLB because of cardiac sympathetic denervation (Tateno et al., 2008). Thereafter, the combination of perfusion and [^{123}I]-ioflupane SPECT and MIBG scintigraphy could increase the accuracy of clinical diagnosis of DLB.

2.7.2 Differential diagnosis of AD from vascular dementia

The pattern of hypoperfusion and hypometabolism on brain perfusion SPECT and FDG PET studies in vascular dementia (VaD) varies greatly and depends on the location of the ischemic lesions (Mori et al., 1999). In the multi-infarct type of VaD, the pattern of perfusion and metabolism is characterized by small or large, single or multiple cortical defects randomly distributed. Motor and sensory cortices may also be involved (DeReuck et al.,

1998). In demented patients with white matter lesions, hypoperfusion and hypometabolism are seen mainly in frontal, posterior frontal and anterior temporoparietal cortical regions due to disruption of cortico-cortical connections. In VaD patients with subcortical lesions alone, without cortical lesions on CT, remote cortical metabolism and perfusion defects on intact cortical and subcortical structures are seen, due to disconnection or diaschisis of cortico-subcortical pathways (Kwan et al., 1999).

The great overlapping of hypoperfusion and hypometabolism patterns between AD and VaD, which some times reflect the presence of mixed dementia too, may complicate the interpretation of SPECT and PET images, making the differential diagnosis of AD and VaD difficult. In such cases the administration of acetazolamide has been proved to be a useful tool in the evaluation of vascular reserve capacity (Tikofsky & Hellman, 1991) and can contribute significantly in the differential diagnosis. Acetazolamide is a carbonic anhydrase inhibitor which increases the local pCO_2 in the brain tissue leading to arteriolar dilatation and local increase of rCBF. In AD, vascular reserve capacity is preserved and the administration of acetazolamide results in increased rCBF in the hypoperfused areas seen on SPECT perfusion study. In VaD, acetazolamide fails to increase rCBF in areas with vascular lesions where the vascular reserve capacity is impaired (Pavics et al., 1999).

2.7.3 Differential diagnosis of AD from frontotemporal lobar degeneration

In patients with frontotemporal lobar degeneration (FTLD), PET and SPECT studies revealed the preferential involvement of the frontotemporal regions (Jeong et al., 2005; McNeill et al., 2007). More specifically, these studies demonstrated an extensive decrease of glucose metabolism and perfusion in the frontal and temporal areas, cingulate gyri, uncus, and insula, and subcortical areas, including the basal ganglia and medial thalamic regions (Fig. 2).

The widespread abnormalities observed in FTLD patients may reflect the cumulative findings of the specific variants of FTLD i.e. the frontal or behavioural variant (bvFTD) and the temporal variants of semantic dementia (SD) and progressive non fluent aphasia (PNFA). FDG PET and perfusion SPECT studies in patients with PNFA and SD showed hypometabolism and hypoperfusion in the left hemisphere including the temporal, parietal and middle frontal lobe, whereas in bvFTD patients prominent frontal lobes deficits have been demonstrated (Perneczky et al., 2007; Sinnatamby et al., 1996).

PIB PET studies could potentially aid in differentiating between FTLD and AD patients. FTLD patients showed significantly lower PIB retention compared to AD in frontal, parietal, temporal, and occipital cortices as well as in putamen. The PIB uptake in these FTLD patients did not differ significantly from the healthy controls in any region (Engler et al., 2008).

2.7.4 Differential diagnosis of AD from other dementias

In Creutzfeldt-Jakob encephalopathy, brain perfusion and metabolism studies have revealed various degrees of focal or diffuse hypoperfusion and hypometabolism, which correlated with the severity of the disease, while the use of [^{11}C]-L deuterodeprenyl (DED) -a tracer to assess astrocytosis- showed parallel increases in DED uptake indicating astrocytosis (Engler et al., 2003). Use of iomazemil SPECT to bind with benzodiazepine receptors in a case of Creutzfeldt–Jakob disease has been described with reduced uptake in later stages suggesting neuronal degeneration (Itoh et al., 1998).

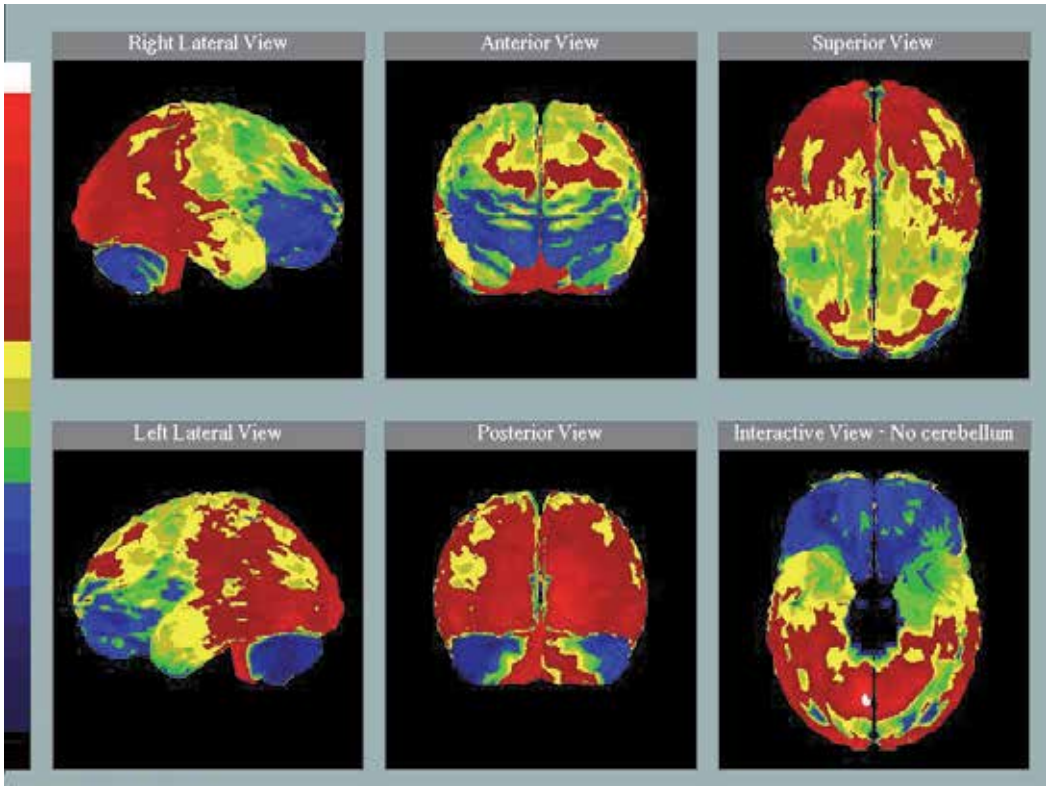

Fig. 2. Brain perfusion SPECT study in a patient with Frontotemporal lobar degeneration. Reduced ^{99m}Tc-HMPAO uptake is observed in frontotemporal cortical areas, more marked in the frontal lobes.

In acute immunodeficiency syndrome (AIDS) dementia, brain perfusion SPECT and FDG PET images demonstrated randomly distributed multiple focal cortical and subcortical deficits of perfusion and metabolism with a predilection for the basal ganglia. These perfusion and metabolism abnormalities may be present even when patients are asymptomatic and correlate better with cognitive improvement after therapy than do structural images (Kim et al., 1996; Tatsch et al., 1990).

Demented patients with Parkinson's disease or other parkinsonian syndromes such as corticobasal degeneration (CBD), progressive supranuclear palsy (PSP) and multiple system atrophy (MSA) may present overlapping perfusion and metabolism templates with AD patients. Demented patients with Parkinson's disease and AD share a common pattern of marked posterior hypoperfusion involving the parietal, temporal, and occipital lobes, as well as hypoperfusion in the dorsolateral prefrontal cortex (Eckert et al., 2005; Spampinato et al., 1991). In PSP, glucose metabolism and perfusion was decreased in the midbrain and medial frontal cortex (Eckert et al., 2005; Okuda et al., 2000). Relative hypometabolism and hypoperfusion in the basal ganglia and fronto-parietal cortex contralateral to the most affected side was a characteristic finding in CBD (Eckert et al., 2005; Hossain et al., 2003). MSA patients exhibited a pattern characterized by marked bilateral reductions of perfusion and metabolism in the lentiform nuclei, the pons and the cerebellum (Cilia et al., 2005;

Eckert et al., 2005). Recent neuroreceptor studies have found that decreased striatum uptake on the presynaptic DAT SPECT imaging in demented patients with Parkinson's disease or other parkinsonian syndromes, may be a useful marker for the discrimination from AD (Hilker et al., 2005; Pirker et al., 2000).

In symptomatic patients with Huntington's disease (HD) brain perfusion SPECT imaging shows decreased or absent tracer uptake in the caudate nucleus or basal ganglia (Nagel et al., 1991). The impairment of basal ganglia may not be permanent and tracer uptake may return to normal after therapy with olanzapine (Etchebehere et al. 1999).

2.8 Assessment of treatment

Acetylcholinesterase inhibitors have been the most widely used drugs to treat AD. Perfusion, metabolism and nicotinic receptors SPECT and PET imaging can be used to assess the efficacy of these drugs in inhibiting acetylcholinesterase, to determine the doses required to achieve optimal inhibition and identify patients in whom the concentration of acetylcholinesterase may be too low for acetylcholinesterase inhibitors to be effective (Kuhl et al., 2000).

Perfusion SPECT studies have shown that treatment with donepezil appeared to reduce the decline in rCBF, suggesting a preservation of functional brain activity (Nakano et al., 2001; Staff et al., 2000). Increases in rCBF in anterior cingulate, lateral orbitofrontal, dorsolateral prefrontal, and temporoparietal areas after short term acetylcholinesterase inhibitor therapy was significantly related to behaviors of irritability, disinhibition, and euphoria (Ceravolo et al., 2004; Nakano et al., 2001). These data suggest that cognitive or behavioral benefits after cholinesterase inhibitor therapy are related to clear increases in rCBF in crucial areas specifically involved in the attention and limbic networks.

Increases in rCBF in AD patients have also been reported after acute and fairly short periods of treatment with other cholinesterase inhibitors such as tacrine and velnacrine, and with the acetylcholine releaser linopirdine (van Dyck et al., 1997). Tacrine treatment increased cerebral blood flow, cerebral glucose metabolism, and uptake of ^{11}C-nicotine to the brain paralleled by improvement in neuropsychological performance. Though the effects of tacrine on nicotine receptors occurred early in the course of treatment (3 weeks), those in metabolism were observed only after months of treatment (Nordberg et al., 1998). Tacrine increased binding of ^{11}C-nicotine in the temporal cortex of AD patients was interpreted as reflecting a restoration of nicotinic receptors (Nordberg et al., 1997). These results are in agreement with preclinical data showing that cholinergic stimulation leads to upregulation of nicotinic receptors (Svensson & Nordberg, 1996).

Acetylcholinesterase inhibitors can be labeled with positron emitters without changing their pharmacologic properties. This would allow for the investigation of their regional distribution and pharmacokinetics in the human brain. Studies that have assess the effects of these drugs at their molecular target show the relationship between doses of a drug and percent occupancy of receptors or transporters, or percent of enzyme inhibition. This can be achieved either by using the radiolabeled drug itself, if it has a good specific-to-nonspecific binding ratio, or by using a radioligand that binds to the same site as the drug (Traykov et al., 1999). This same strategy can be applied to measure the receptor occupancies achieved by nicotinic or muscarinic drugs at doses that improve cognitive or behavioral function (Ding et al., 2000). Equivalent studies can also be done to assess the efficacy of cholinesterase inhibitors in inhibiting acetylcholinesterase (Pappata et al., 1996). Acetylcholine-enhancing

drugs that have been labelled with positron emitters include nicotine, tacrine, and physostigmine.

2.9 Genetic risk for AD and PET

Subtle changes in brain function may occur prior to overt manifestations of the disease in genetically at-risk individuals. The combination of functional brain imaging with genetic risk factors may enhance the ability to detect differences predictive of disease development prior to onset and assist in the potential for increasing the efficacy of therapeutic treatments (Reiman et al., 2001). FDG PET studies in asymptomatic apolipoprotein ε4 allele (APOE e4) carriers demonstrated a decline of metabolism in the left posterior cingulated, inferior parietal, and lateral temporal regions (Kennedy et al., 1995; Small et al., 2000). Likewise, perfusion SPECT studies in asymptomatic presenilin-1 mutation subjects demonstrated reduced perfusion in the hippocampal complex, anterior and posterior cingulate, posterior parietal lobe, and anterior frontal lobe (Johnson et al., 2001).

3. Conclusion

The application of SPECT and PET techniques to the study of AD patients has elucidated the in vivo understanding of the underlying pathology of the disease. The variety of the available radiotracers has rendered SPECT and PET objective biomarkers for monitoring of biochemical processes altered by neuronal loss. Nuclear molecular imaging of changes in brain Aβ deposition, perfusion, metabolism and neurotransmitter turnover, as well as alterations in receptor, transporter or enzyme concentrations can provide unique information not attainable by other methods. The noninvasive PET and SPECT imaging provided novel ways to improve early and differential diagnosis of AD and monitor the disease progression and the effects of symptomatic or disease modifying therapies.

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Part 4

Biomarkers: Steps Toward Rapid Non-Invasive Tests

10

Cerebrospinal Fluid Based Diagnosis in Alzheimer's Disease

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1. Introduction

The Alzheimer's disease (AD) is the most frequent form of dementia worldwide. The major neuropathological hallmarks of the disease are loss of neurons and synapse, senile plaques (extracellular aggregates primarily composed of ß-amyloid; Aß) and neurofibrillary tangles (aggregates of hyperphosphorylated forms of the microtubule-associated tau protein) throughout cortical and limbic regions of the brain. The definite diagnosis still requires histopathological conformation according to the criteria, however, in recent years substantial progress has been made in the area of early biomarker development. The use of cerebrospinal fluid as a testing platform is very promising because the CSF protein composition can reflect the pathological processes of the brain and because it is easily accessible by a lumbar puncture. Some proteins and peptides such as ß-amyloid 1-42 (Aβ1-42), ß-amyloid 1-40 (Aβ1-40), total tau (tau) and hyperphosphorylated tau (p-tau) have been reported to meet the criteria for a biomarker. Another series of publications reported transthyretin, isoprostane, BACE1 activity and other proteins and enzymes as a potential biomarkers in AD. Whereas the biomarkers mentioned first have been studied extensively and were suggested to be included into clinical AD criteria, less information is available on the others. This review will focus on the importance of CSF based biomarkers in AD, covers the data available from the literature and highlights their role in the differential diagnosis of dementia.

2. Why to use CSF as a testing platform in dementia? — Neuroanatomy driven approach

Cerebrospinal fluid (CSF) is the main component of the brain extracellular space and participates in the exchange of many biochemical products in the central nervous system (CNS). Consequently, CSF contains a dynamic and complex mixture of proteins, which reflects physiological or pathological state of CNS. Changes in CSF proteome have been described in various neurodegenerative disorders. These alterations are discussed to reflect pathological changes in the brain and thus contribute to a better understanding of the pathophysiology of the underlying disorder (Gawinecka et al. 2010).
CSF analysis is extremely important to identify autoimmune disorders and inflammatory conditions, which might lead to dementia. Although changes are non-specific, like

pleocytosis, elevated protein content, increased albumin ratio and oligoclonal IgG synthesis within the central nervous system, their presence clearly differentiate inflammatory and autoimmune disease from neurodegenerative dementia.

3. How to select a biomarker – a concept-of-pathogenesis-driven approach

Several potential biomarkers in the CSF and blood have been already suggested. Some of them like Aβ1-42 and tau (and its phosphorylated form) became important biomarker in dementia diagnosis. The advantage of these biomarkers is their clear link to the pathological process and abnormalities, which are detected in the brain of AD patients (Aß and amyloid hypothesis as well as tau pathology).

3.1 Amyloid hypothesis

The amyloid core of senile or neuritic plaques contains an amyloid-like substance formed by peptides, which originate from proteolytic cleavage of the membrane-associated precursor protein (amyloid precursor protein, APP). They are generated by a sequential cleavage of amyloid precursor protein (APP) by β- and γ-secretase. Aβs are small hydrophobic peptides existing mainly in two lengths: Aβ1-40 and Aβ1-42. It was initially assumed that the production of Aβs occurs only under pathological conditions. Later, Aβ was shown to be constitutively released from APP and secreted to blood and CSF. In AD, Aβ peptides are involved in pathological processes and accumulate in the brain as amyloid or senile plaques. There is clear correlation between Aβ levels in CSF and plaque depositions in the brain coupled with the concept of casual involvement of APP and Aβs in the pathogenesis of AD.
The concentration of Aβs is thought to reflect disease-associated changes and is widely applied as a diagnostic biomarker of AD (Gawinecka et al. 2010).
Table 1 gives an overview of publications related to this topic from past 5 years. The vast majority of publications is related to the detection of abnormal levels of Aß1-42 in AD as compared to other dementia, however, some data are also available for Aß1-40. Recently, a ratio between both peptides has been suggested as a potential biomarker in AD (Table 2). Aß1-42 level is decreased in patients with AD, but might also decrease in other dementia, too. Test sensitivity for Aß1-42 alone is given from 60 to 96%, depending on the design of the study.
In MCI, Aß42 level is lower in patients with a subsequent AD diagnosis (De Meyer et al. 2010; Diniz et al. 2008; Mattsson et al. 2009; Stefani et al. 2006), which leads to the conclusion that this parameter might also serve as an preclinical (potential predictive?) biomarker (Stefani et al. 2006) for cognitive decline.
Aß42 level is decreased in other conditions, including prion diseases, Parkinson's disease (Siderowf et al. 2010) and DLB. In PD, decreased levels correlate well with cognitive decline, in contrast to tau/p-tau ratio (see below) (Siderowf et al. 2010). According to some studies which used patients with non-AD dementia as controls, this marker is highly sensitive for detection of dementia, but it seems that it does not allow to discriminate between various dementia types because of limited specificity (Formichi et al. 2006; Gloeckner et al. 2008).
One approach to improve test sensitivity and specificity was to calculate an Aß42/40 ratio, which is significantly decreased in AD patients. It seems also to discriminate between different dementia including AD and non-AD (vascular, mixed, FTD, alcohol toxic and controls) (Lewczuk et al. 2004). However, the significance of this finding has still to be proven on higher numbers of patients in a prospective study.

	Patients (n)	Sensitivity (%)	Specificity (%)	Reference
Aß1-42	MCI -> AD (422) controls (429)	68	93	(Diniz et al. 2008)
Aß1-42	PD (109) AD (20) controls (36)	n.a.		(Alves et al. 2010)
Aß1-42	AD (131) controls (72)	92	89	(Sunderland et al. 2003)
Aß1-42	PD (45)	n.a.		(Siderowf et al. 2010)
Aß1-42	MCI	n.a.		(Okonkwo et al. 2011)
Aß1-42	AD (33) ARCD* (20) controls (50)	70-84	80-85	(Kapaki et al. 2005)
Aß1-42	MCI (750) AD (529) controls (304)	79	65	(Mattsson et al. 2009)
Aß1-42	autoptic AD (68) MCI (57)	94	n.a.	(De Meyer et al. 2010)
Aß1-42	AD	>85	>85	(Slats et al. 2010)
Aß1-42	mild AD (100) MCI (196) controls (114)	96	77	(Shaw et al. 2009)
Aß1-40	AD (82) DLB (44) controls (71)	AD vs controls 97	AD vs controls 83	(Mollenhauer et al. 2011)
Aß1-40	DLB (21) AD (23) PDD (21)	81	71	(Bibl et al. 2006a)
Aß1-40 Aß1-42	AD (23) NPH (13) DLB (23) CJD (18) DLB (23) FTD (10) controls (19)	61	78	(Gloeckner et al. 2008)

*ARCD = alcohol related cognitive disorder

Table 1. Aß40 and Aß42 in dementia diagnosis

	Diagnosis (n)	Sensitivity (%)	Specificity (%)	Reference
Aß1-42/ Aß1-40	MCI (65)	86	60	(Brys et al. 2009)
Aß1-42/ Aß1-40	AD (22)	95	88	(Lewczuk et al. 2004)
Aß1-42/ Aß1-40	AD (157)	59	88	(Shoji and Kanai 2001)
Aß1-42/ Aß1-40	AD (69)			(Spies et al. 2010)
Aß1-42/ Aß1-40	AD (18)	AD vs control: 100 AD vs DLB: 100 AD vs both groups: 100	AD vs control: 93 AD vs DLB: 68 AD vs both groups: 77	(Bibl et al. 2006b)
Aß1-42/ Aß1-40	AD (109)	AD vs control: 79 AD vs all: 70	AD vs control: 71 AD vs all: 71	(Brettschneider et al. 2006)

Table 2. Aß1-42/ Aß1-40 ratio as potential biomarkers in AD

3.2 Tau hypothesis

Intracellular neurofibrillary tangles (NFT), which are neuronal inclusions consisting of abnormal cytoskeletal elements of hyperphosphorylated tau protein are another characteristic pathological feature of AD. These tangles are found throughout the neocortex, in the nucleus basalis Meynert, thalamus, and in the mammillary bodies. Tau protein is a microtubule-associated protein (MAP), which interacts with tubulin and promotes microtubule assembly and stability; it is also involved in neurogenesis, axonal maintenance and axonal transport. There are six different tau isoforms present in the human adult brain, which are generated by an alternative mRNA splicing from a single gene (Goedert et al. 1989). Tau is a phosphoprotein, with 79 putative serine or threonine phosphorylation sites on the longest tau isoform. The hyperphosphorylated tau has a reduced affinity for microtubules and reduced ability to promote their assembly (Lindwall and Cole 1984). In AD, tau detaches from microtubules and aggregates in paired helical filaments (PHFs). Tau isolated from these aggregates is found to be about 4 times more phoshorylated than tau isolated from nondemented individuals (Alonso et al. 2001; Kopke et al. 1993, Gawinecka and Zerr 2010).

The elevated CSF level of nonphosphorylated and phosphorylated tau is one of AD hallmarks (Andreasen et al. 1999; Arai et al. 1997; Galasko et al. 1997; Ishiguro et al. 1999; Itoh et al. 2001; Mecocci et al. 1998). Since the first description of this abnormality and availability of an ELISA test, extensive research has been conducted. Data on tau level in AD and other dementia are given in Table 3. Again, elevated levels can be observed in other conditions than AD too, the test sensitivity and specificity seems to be above 80% in majority of the cases (Table 3). However, it has to be kept in mind that tau levels increase in CSF with age and this physiological finding hast to be kept in mind when cut-off level are established (Figure 1). In pathological conditions, total tau levels in MCI patients indicate increased AD risk (Hertze et al. 2010; Mattsson et al. 2009; Pauwels et al. 2009) and increased level correlates well with disease severity (Buchhave et al. 2009; Stefani et al. 2006). Some studies even demonstrated that extremely high tau levels might be indicator of poor prognosis (Snider et al. 2009).

Patients (n)	Sensitivity (%)	Specificity (%)	Reference
MCI (166)	78	83	(Hertze et al. 2010)
AD (131)	92	89	(Sunderland et al. 2003)
AD, CJD, LBD, FTD, VD	73-91	74-98	(van Harten et al. 2011)
AD (33) ARCD* (20)	88-94	95-96	(Kapaki et al. 2005)
AD NPH	91-93	78-96	(Kapaki et al. 2007)
MCI (750) AD (529)	86	56	(Mattsson et al. 2009)
AD, other dementia, psychiatric (219)	88	80	(Ibach et al. 2006)
early AD (269) mild AD (468) late AD (495)	n.a.		(Stefani et al. 2006)
MCI -> AD	82	87	(Pauwels et al. 2009)
mild AD (100) MCI (192) autoptic AD (56) controls (114)	70	92	(Shaw et al. 2009)
MCI -> AD (422) controls (420)	68	93	(Diniz et al. 2008)
DLB (34) AD (31) other dementia (4)	85	95	(Kasuga et al. 2010)

*ARCD = alcohol related cognitive disorder

Table 3. Total tau level in cerebrospinal fluid in AD and other dementia

Regarding phosphorylated tau level in CSF, a recent metaanalysis on 51 publications from the area revealed that p-tau contributed to the separation of MCI from healthy individuals with a sensitivity of 80% and specificity of 84% (Mitchell 2009). CSF p-tau is a good diagnostic biomarker of AD too, with test sensitivity mostly >80% (see Table 4). MCI patients with low Aß1-42 and high p-tau levels are at a clear AD risk (Hertze et al. 2010). AD patients with higher p-tau level have greater hippocampal atrophy, poorer neuropsychological test results and it is also indicator of disease progression (Henneman et al. 2009). Whereas p-tau levels indicate AD or development of AD with good accuracy, unfortunately, this biomarker is also less adequate in separating AD from other dementias (Mitchell 2009).

Due to these considerations, several studies tried to analyse the diagnostic potential of p-tau/total tau ratio. Some of them reported test sensitivity between 88-96% with a specificity of 60-100%. While its ratio is promising, again, it has to be analysed in a prospective setting since the numbers of analysed patients so far are too low for any definite conclusions (Buerger et al. 2006; Hu et al. 2002; Kapaki et al. 2007).

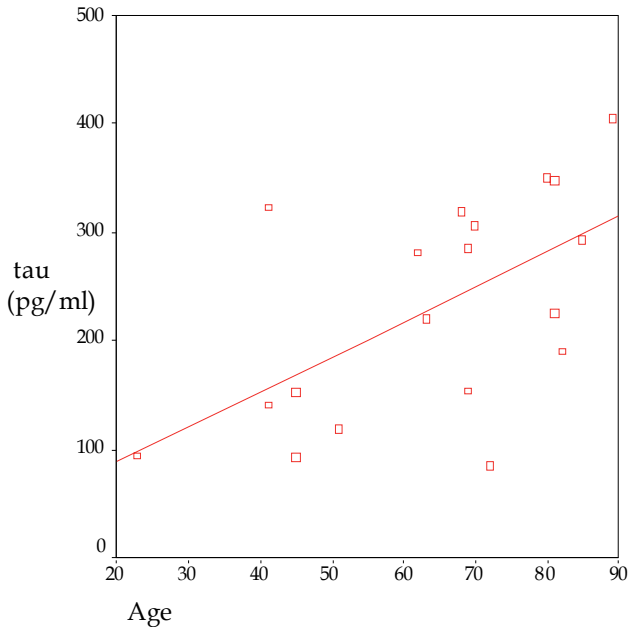

Fig. 1. Total tau level in cerebrospinal fluid stratified by age in healthy controls (Gloeckner et al. 2008)

	Patients (n)	Sensitivity (%)	Specificity (%)	Reference
p-tau	AD neurological controls (46)	>85	79-91	(Scheurich et al. 2010)
p-tau	AD (94) MCI (166) depression (29) controls (38)	>85	79-91	(Hertze et al. 2010)
p-tau	AD (49)	46	94	(Snider et al. 2009)
p-tau	AD (251) controls (122)	62	93	(Formichi et al. 2006)
p-tau	AD, CJD, LBD, FTD, vascular dementia	79-88	78-83	(van Harten et al. 2011)
p-tau	AD, NPH, controls	89	87	(Kapaki et al. 2007)
p-tau	AD, other dementia, psychiatric, controls	89	87	(Ibach et al. 2006)
p-tau	AD (31) MCI (25)	72-81	78-88	(Henneman et al. 2009)

	Patients (n)	Sensitivity (%)	Specificity (%)	Reference
p-tau	autoptic AD (68) MCI (57)	AD 94 MCI 100		(De Meyer et al. 2010)
p-tau	mild AD (100) MCI (196) autoptic AD (56) controls (114)	68	73	(Shaw et al. 2009)
p-tau	MCI (750) AD (529) Controls (304)	84	47	(Mattsson et al. 2009)
p-tau/total tau	AD (52) controls (56) non AD (37) vascular dementia (46)	96	94	(Hu et al. 2002)
p-tau/total tau	AD (67) NPH (18) controls (72)	88-93	60-100	(Kapaki et al. 2007)
p-tau/total tau	AD (37) CJD (21) controls (10)	91	97	(Buerger et al. 2006)
p-tau/total tau	AD (71) FTD (18) CJD (20) controls (43)	ratio AD: 1,27 (mean) ratio FTD: 1,13 (mean) ratio CJD: 0,05 (mean) ratio controls: 1,7 (mean)		(Riemenschneider et al. 2003)
p-tau/total tau	CJD (21) AD (49) neurol. controls (164)	86	90	(Bahl et al. 2009)

Table 4. Phosphorylated tau level in cerebrospinal fluid in AD and other dementia

In general, many studies on Aß1-42, total tau level and its phosphorylated isoform have been performed. In most studies, the number of patients per group is limited and various criteria and diagnostic techniques have been applied. Of importance, a recent multicenter study demonstrated once again that CSF Aß1-42, total tau and p-tau identify incipient AD with good sensitivity and specificity, however, the data are less reliable than reported from single-center studies (Mattsson et al. 2009). Thus, improvements are necessary, with respect to standardisation protocols between centers, but also with respect to identification of further disease- specific biomarkers in biological fluids.

3.3 The role of ApoE

The presence of the apolipoprotein E allele is a well documented risk factor for AD (Lamb et al. 1998; Saunders et al. 1993) and is associated with a decreased age of clinical onset, with a higher stage of ß-amyloid deposition and neurofibrillary change formation, severe disease course, higher brain atrophy and a more rapid disease course (Ohm et al. 1999). The ApoE polymorphism includes three common alleles (ε2, ε3, ε4) at a single gene locus resulting in

six ApoE genotypes ε2/ε2, ε3/ε3, ε4/ε4, ε2/ε3, ε2/ε4, ε3/ε4. The ApoE ε4 allele is an established risk factor for AD (Saunders et al. 1993) and there is a large body of evidence for a role of ApoE in the pathogenesis of AD (Davidson et al. 2006; Varges et al. 2011).

CSF marker	Patients (n) [diagnosis confirmed]	Influence of ApoE ε4 allele	Reference
Aß1-42	82	dose-dependent reduction	(Galasko et al. 1998)
tau		heterozygous: elevation homozygous: reduction	
Aß1-42	50	dose-dependent reduction	(Riemenschneider et al. 2000)
Aß1-42	84	dose-dependent reduction	(Hulstaert et al. 1999)
Aß1-42	73	more reduced levels in ε4 carriers	(Smach et al. 2008)
tau		no influence	
Aß1-42	60	no influence	(Ewers et al. 2008)
Aß1-42	50	no influence	(Engelborghs et al. 2007)
tau		no influence	
tau	19	dose-dependent elevation	(Golombowski et al. 1997)
Aß1-42	121 [41 NP]	more reduced levels in ε4 carriers	(Tapiola et al. 2000)
tau		more elevated levels in ε4 carriers	
Aß1-42	563	more reduced levels in ε4 carriers	(Prince et al. 2004)
Aß1-42	150	more reduced levels in ε4 carriers	(Sunderland et al. 2004)

Table 5. Influence of the ApoE ε4 allele on CSF markers in AD (modified from Varges et al. 2011)

The ApoE ε4 allele status is important to be analysed in the context of CSF biomarker. Some studies report no association between ApoE ε4 allele and tau level, whereas others show higher tau level among ApoE ε4 carriers when compared to non-carriers among AD patients. The situation seems to be clearer for ß-amyloid 1-42: several studies report correlations between Aß1-42 levels and the ApoE ε4 allele (Table 5) (modified from Varges et al. 2011). Although the pathological links are not clearly identify at the moment, it is apparent that the ApoE genotype has to be taken into consideration.

4. Clinical criteria for AD

CSF biomarkers play an important role in clinical diagnosis and differential diagnosis of neurodegenerative dementia. A lot of research in this area has been already conducted. Recently, these markers which are associated with disease pathology in the brain namely Aß1-42 as a parameter of the amyloid cascade and tau and its phosphorylated isoforms have been suggested to be included into diagnostic criteria for AD. The typical AD signature comprises low CSF Aß1-42 levels and high total tau/p-tau level and it was suggested as a parameter of one of the supportive features at the same level as structural and functional brain imaging for probable AD diagnosis (Dubois et al. 2007).

5. Conclusions

The PubMed search on 31.1.2011 using keywords cerebrospinal fluid and Alzheimer reveals 2336 hits. Although not all are dealing directly with biomarker discovery and identification of novel proteins for diagnosis, they are linked to the topic and demonstrate once again the importance of the area. Adequate biomarker, which can be easily analysed in CSF and, even better, blood, will have a great potential for clinical and also preclinical diagnosis of the disease. In neurodegenerative disorders, we have to meet the problem of early disease diagnosis, because it is likely that neuroprotective and other pharmacological strategies will allow better treatment response in earlier disease stages.

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11

Alzheimer's Diseases: Towards Biomarkers for an Early Diagnosis

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1. Introduction

Alzheimer's diseases (AD) are the most frequent dementias across the world and represent more than 60% of all neurodegenerative dementias including those with Lewy bodies (DLB) and frontotemporal lobar degeneration (FTLD). Most neurodegenerative dementias are induced by aggregation of different brain proteins; they are nosologically grouped into a large pathological entity named proteinopathy. Prevalence of dementias is more than 20% in 85 years old aged population and linearly increases with the age (Ferri, Prince et al. 2005). The international impact of degenerative dementias across the world is estimated to touch more than 24 million patients with an incidence about 4.6 million new cases by year.
Diagnosis of AD is still based on the integration of clinical examination, neuropsychological data, radiological and biological analyses. The final diagnosis is established after neuropathological examination of brain and observation of typical cerebral failures, in consequence, the clinical diagnosis is "probable AD" when typical criteria are found and "possible AD" if atypical elements are present during clinical examination. Nevertheless, the clinical diagnosis performance for "probable AD" is relatively low with a sensitivity and a specificity of 80% and 70% respectively (Knopman, DeKosky et al. 2001). Because a consequent clinical overlap does exist between etiological AD and others causes of dementia, the clinical diagnosis specificity for a possible AD is less than 50%. Paraclinical analyses such as radiological and biological examinations tend to increase accuracy of differential diagnoses as well as diagnosis precocity for these patients.

2. How can we deal with the clinical diversity of Alzheimer's phenotypes?

AD is associated with a progressive disruption of the neuronal function and subsequent gradual deterioration in cognition and behaviour leading finally to the death (Khachaturian, 1985). The typical clinical beginning of AD is characterised by a progressive cognitive alteration affecting predominantly episodic memory. Other symptoms such as language deficit, visuoperceptive alterations or even dysexecutive syndromes appear during the course of the disease in relation to the progression of cerebral failures into the associative brain areas. Decrease in episodic memory is associated with radiological finding of obvious

medial temporal lobe atrophy during the first step of the disease progression (Scheltens, Fox et al. 2002). Effectively, this cerebral structure highly participates in the memory process; even more, neuropathological findings demonstrated the strong implication of typical protein depositions met in AD that occur during this medial temporal lobe atrophy (Burton, Barber et al. 2009). Nevertheless, this kind of atrophy can be met in other pathologies such as FTLD or DLB, without any cerebral pathological hallmark of AD (O'Brien, Paling et al. 2001; van de Pol, Hensel et al. 2006). Moreover, frequent clinical overlaps (Kertesz, McMonagle et al. 2005; Josephs, Petersen et al. 2006; Hort, O'Brien et al. 2010) as well as pathological co-occurrence (Merdes, Hansen et al. 2003; Amador-Ortiz, Lin et al. 2007; Schneider, Arvanitakis et al. 2009) between these 3 dementias can occur, resulting in a misleading clinical diagnosis during lifetime.

During the AD progression, other symptoms than the alteration of the episodic memory progressively appear, but they can also be clinically predominant at the first step of the disease. For example, a dysexecutive syndrome, concordant with a frontal atrophy, and then the first hypothesis of frontotemporal dementia (FTD) diagnosis can finally be an AD. As well, an aphasic clinical presentation associated with temporal cerebral areas atrophy, suggestive of primary progressive aphasia, can reveal an etiological AD. Other clinical presentations can occur in the first symptoms of AD, such as apraxic forms suggesting corticobasal degeneration (CBD) or even visual deficits associated with posterior cortical atrophy (PCA).

These focal presentations of AD are clinically difficult to distinguish from other previously adverted neurodegenerative dementias which typically begin with these kind of clinical features (Alladi, Xuereb et al. 2007). It is why there is a need to identify inside this spectrum focal presentations underlying AD as well as other pathologic processes such as FTLD or DLB.

3. Neuropathological hallmark and physiopathological pathways of AD

The two neuropathological hallmarks of AD are the deposition of extracellular amyloid plaques and accumulation of intracellular neurofibrillary tangles (NFT). The first ones are principally constituted by amyloid protein (Aß peptide), a product of proteolytic processing by secretases of the amyloid precursor protein named APP (Haass and Selkoe 1993), and the second involves phosphorylated tau protein (Masters, Simms et al. 1985; Braak and Braak 1991). Distributions of amyloid plaques and NFTs do not overlap in all anatomical regions suggesting a complex pathogenetic scenario.

Tau protein is a microtubule associated protein expressed abundantly in the neuronal axons that promotes assembly and stability of microtubules. 6 different isoforms of tau are present in the human brain, constituted of 352 to 441 amino acids and harbouring more than 70 phosphorylation sites (Buée, Bussiere et al. 2000; Lewczuk, Esselmann et al. 2004). Its physiological function is regulated by these phosphorylations (Johnson and Hartigan 1999). The abnormal hyperphosphorylation of tau proteins in AD brain reduces their biological activity and results in disintegration of the microtubules (Uversky, Winter et al. 1998). Moreover, this phenomenon induces conformational changes of tau protein and then permits its assembly in paired helical filaments (PHF), the major constituent of NFTs (Kidd 1963; Alonso, Zaidi et al. 2001). These PHF are highly phosphorylated since more than 30 phosphorylation sites were observed in PHF deriving from NFTs (Liu, Liang et al. 2006). The stereotypic development of tau pathology with NFTs is clearly correlated with clinical

impairment of memory, starting in the transentorhinal cortex to finally involve associative areas and the whole neocortical areas (Braak and Braak 1995; Berg, McKeel et al. 1998).
In an other scheme, Aß depositions neither correlate with a given clinical presentation and seem to begin in the neocortex before involving the neuronal projections of already involved areas (Arriagada, Growdon et al. 1992). These amyloid plaques are also encountered in asymptomatic aged peoples without AD (Wolf, Gearing et al. 1999). Furthermore, metabolic imaging and particularly positron emission tomography with ^{11}C-PIB (a compound that specifically stain amyloid plaques) demonstrate that these amyloid accumulations are observed in subjects without any cognitive alteration (Villemagne, Fodero-Tavoletti et al. 2008). Although numerous proteins are associated with amyloid deposits in AD (Buée, Hof et al. 1992; Uchihara, Duyckaerts et al. 1996; Burns, Noble et al. 2003), the major proteinaceous component is the 42 amino acid Aß peptide ($Aß_{42}$) which is the most hydrophobic form of Aß (Jarrett, Berger et al. 1993; Gravina, Ho et al. 1995). Nevertheless, it was recently demonstrated that aggregation into amyloid plaques may provide truncated forms of this peptide in the N-terminal ($Aß_{4-/5-/8-/9-42}$) during the first steps of the disease (Sergeant, Bombois et al. 2003; Vanderstichele, De Meyer et al. 2005). These truncated forms could represent more than 60% of all cerebral Aß forms (Sergeant, Bombois et al. 2003).
Interestingly, the symptomatology observed in AD (or other neurodegenerative dementia) seems not due to these deposits and symptoms could appear before protein aggregations (Moechars, Dewachter et al. 1999). Several *in vitro* studies demonstrated that the toxicity against neurons is related to oligomeric forms of proteins and that the consequence is cognitive alteration in animal models (Kirkitadze, Bitan et al. 2002; Cleary, Walsh et al. 2005; Lesne, Koh et al. 2006). For AD, soluble oligomeric forms have been described, aggregating later on into amyloid plaques and it was suggested that effects of these oligomeric species could induce the first toxic events of amyloidopathy (Simmons, May et al. 1994; Kirkitadze, Condron et al. 2001; Walsh and Selkoe 2007).
Some controversy still remains on the course and distribution pattern of the Aß and tau pathologies. Some authors suggest that amyloid dysfunction is the pathogenic key leading to AD pathogenesis. This hypothesis was developed as "amyloid cascade" and the Aß metabolism dysfunction may initiate others pathologic events such as tau protein hyperphosphorylation, synaptic loss or neuroinflammation (Hardy and Higgins 1992; Hardy and Selkoe 2002). Nevertheless this hypothesis is still discussed and does not support all the AD physiopathology.
During the preclinical phase of AD, the neuronal degeneration proceeds and at a certain, yet unidentified, threshold the first symptoms appear. Otherwise, it seems that first cerebral failures begin several decades before symptoms appear (Braak and Braak 1997). Nevertheless the early and differential diagnosis remains difficult and reliable proof of AD process is difficult to obtain. An early and reliable diagnosis in order to establish appropriate drug treatment is essential to decrease symptomatology. Moreover, since research is conducted to develop treatments that could retard or stop cerebral failure progression, we need to improve AD diagnosis to identify this process before brain damages are too significant.

4. Which CSF biochemical markers might allow us to detect Alzheimer's diseases?

It is convincing that we could identify these neurodegenerative processes early during the course of AD with the help of paraclinical means such as functional imaging or use of biological markers.

An ideal biomarker for Alzheimer disease would have to meet three specific criteria:

- To be a quantitative and objective measure providing an indication of disease risk and rate of disease progression long before onset of symptoms
- To be reliable, reproducible and inexpensive to measure
- To be measurable in an easily accessible tissue of the patient.

During the past years, great efforts have been made to identify reliable biomarkers for AD in body fluids of patients that are suitable for minimal invasive early diagnosis of AD, mainly cerebrospinal fluid (CSF) and blood. Cerebrospinal fluid (CSF) closely reflects the composition of the brain extracellular space and is likely to have the highest yield in biomarkers (Wiltfang, Lewczuk et al. 2005). Furthermore, CSF biological modifications seem to appear before first anatomical modifications seen on magnetic resonance imaging (Schoonenboom, van der Flier et al. 2008). Nevertheless, lumbar puncture is relatively invasive even if complications appear in less than 2% of patients (Blennow, Wallin et al. 1993). So research strives for a less invasive biological diagnosis with development of blood markers for AD.

A high number of CSF brain-derived proteins have been investigated as potential markers for AD. The more promising are proteins resulting from cerebral failures meet in AD: tau proteins with total tau (T-tau), phosphorylated tau proteins (P-tau) and $A\beta_{42}$ which are solubles in CSF. Moreover, AD diagnosis criteria were reviewed for research in 2007 and these 3 biomarkers were included (Dubois, Feldman et al. 2007).

- Aß peptide

In AD patients the CSF concentration of soluble $A\beta_{42}$ decreases and it has been suggested that this may be due to the preferential deposition of Aß peptides into cerebral amyloid plaques reflecting parenchymental sequestration with lower levels diffusing to CSF (Strozyk, Blennow et al. 2003; Wiltfang, Lewczuk et al. 2005; Fagan, Mintun et al. 2006). Interestingly, this CSF concentration is linked to ApoE genotype which was identified as a susceptibility gene to develop AD: patients with homozygote genotype of ApoE4 have lower $A\beta_{42}$ CSF concentration (Galasko, Chang et al. 1998). Nevertheless, a CSF $A\beta_{42}$ concentration decrease is also observed in other pathologies such as DLB or even Creutzfeldt Jakob disease (CJD), pathology with sometimes cerebral Aß deposits (Andreasen, Minthon et al. 2001; Wiltfang, Esselmann et al. 2003; Mollenhauer, Bibl et al. 2005). These results are in accordance with pathological overlaps observed in these diseases (Schneider, Arvanitakis et al. 2007; Schneider, Arvanitakis et al. 2009; Kovacs, Seguin et al. 2011). Nonetheless, measurement of CSF $A\beta_{42}$ concentration could differentiate AD patients from subjects without any neurodegenerative process with sensitivity and specificity of 86% and about 90% respectively (Blennow 2004). ELISA measurement of CSF $A\beta_{42}$ reveals values comprised between 600 and 1230 pg/mL in normal subjects and 260 and 500 pg/mL in AD patients (Riemenschneider, Lautenschlager et al. 2002; Riemenschneider, Wagenpfeil et al. 2002; Lewczuk, Esselmann et al. 2004).

Several works postulate that Aß aggregates will be more reliable biomarkers than soluble Aß forms, as the aggregates are directly involved in the pathologic events of AD. Monoclonal and polyclonal antibodies specific to soluble oligomeric forms of Aß were developed (Kayed et al, 2010, Ying Z et al, 2009) and used to demonstrate that these oligomers are significantly more abundant in the soluble brain extracts of AD patients (Meli et al., 2009) suggesting that diffusible oligomers would also appear in the CSF. Sensitive techniques were applied to detect CSF Aß oligomers in AD patients (Pitschke, Prior et al. 1998). Recently, a study showed that levels of CSF aggregated Aß forms are higher in AD

patients than in patients with other neurological disorders without neurodegenerative processes (Fukumoto, Tokuda et al. 2010). Moreover, the CSF oligomer concentrations correlate with cognitive alteration in AD. While detection of these CSF oligomeric forms appears to have a considerable diagnosis interest, low concentrations of these pathologic forms do not allow considering this assay routinely?.

In parallel to $A\beta_{42}$, numerous N- and C- terminal truncated forms have been identified in clinical samples from AD patients (Gabelle, Roche et al. 2010). Most abundant Aß peptides in CSF are, in increasing order concentrations, $A\beta_{40}$, $A\beta_{38}$ and $A\beta_{42}$ (Wiltfang, Esselmann et al. 2002). Concentration measurements of the major form $A\beta_{40}$ in CSF are greatly instructive because they well reflect total amount of Aß peptide release in this biological fluid (Wiltfang, Esselmann et al. 2007). By the APP metabolism and the amyloidogenic way, strong cerebral producers of Aß peptides have higher Aß CSF levels (and thus also $A\beta_{42}$) than weak producers. Instauration of $A\beta_{40}$ CSF measurements associated with $A\beta_{42}$ could permit to rectify mistakes in interpretation of biological results with CSF $A\beta_{42}$ levels alone.

In addition to CSF $A\beta_{40}$, $A\beta_{38}$ and $A\beta_{42}$, other forms of this peptide are produced by physiological regulation of γ-secretase (Gabelle, Roche et al. 2010). Numerous both cerebral and CSF truncated forms of Aß peptide were identified in AD and MCI patients using immunobloting or mass spectrometry analyses (such as SELDI-TOF) (Lewczuk, Esselmann et al. 2003; Sergeant, Bombois et al. 2003; Vanderstichele, De Meyer et al. 2005). Part of research concentrates on identification of novel Aß truncated forms that could allow better predictive power and specificity of biological diagnosis (Bibl, Mollenhauer et al. 2007). Indeed, new $A\beta_{13}$, $A\beta_{14}$ or else $A\beta_{16}$ peptides were described within CSF of AD patients and surprisingly $A\beta_{16}$ CSF levels increase in AD patients (Portelius, Zetterberg et al. 2006). Identification of these Aß fragments in the CSF could lead to a more specific differential diagnosis since it may exist distinct CSF Aß fragment profiles between AD, DLB or FTD, these profiles could reflect distinct physiopathological events between these pathologies (Bibl, Mollenhauer et al. 2007). So, the use of these new biomarkers could lead to novel diagnostics and therapeutics approaches for Alzheimer's diseases.

- Tau proteins

Increase of T-tau CSF levels was found in AD patients as well as MCI patients who will develop later an AD (Riemenschneider, Buch et al. 1996; Andreasen, Minthon et al. 2001; Riemenschneider, Lautenschlager et al. 2002; Hansson, Zetterberg et al. 2006). Measurement of T-tau levels with ELISA test (Innotest hTau Ag, Innogenetics) reveal concentrations between 150 pg/mL and 450 pg/mL in control subjects and between 300 and 1100 pg/mL in AD patients (Riemenschneider, Lautenschlager et al. 2002; Lewczuk, Esselmann et al. 2004; Grossman, Farmer et al. 2005). Nevertheless, increase of T-tau CSF levels is not AD specific since it is also observed in different concentrations in CJD or even an acute stroke (Otto, Wiltfang et al. 1997; Hesse, Rosengren et al. 2000). T-tau CSF levels in patients with sporadic CJD are higher than those observed in patients with other neurodegenerative dementias including AD (Otto, Wiltfang et al. 1997; Otto, Wiltfang et al. 2002). Threshold for CSF T-tau concentration in sporadic CJD was determined at 1300pg/mL in 2002 in a study conducted on 300 patients with CJD with 109 neuropathological confirmations (Otto, Wiltfang et al. 2002). In other forms of CJD as new variant, CSF T-tau levels are lower than those observed in sporadic disease (Sanchez-Juan, Green et al. 2006).Thus, T-tau is more a neuronal death marker than a specific AD biomarker. Several diseases are neuropathologically linked to tau dysfunction such as in some FTD, progressive supranuclear palsy (PSP) and CBD. Numerous studies have investigated measurement of CSF T-tau levels in FTD but results are

in accordance with pathological heterogeneity of FTD (neuropathological tau inclusions or without tau inclusions) since existence of discordance between studies with increase of CSF T-tau levels or not (Riemenschneider, Wagenpfeil et al. 2002; Pijnenburg, Schoonenboom et al. 2004; Grossman, Farmer et al. 2005). Any biological argument does exist with T-tau CSF levels in pathologies like FTD or other tauopathies as PSP and CBD (Urakami, Wada et al. 2001). Interestingly, a distinct profile of T-tau in electrophoretic separation was observed in CSF of sporadic CJD patients in a very recent study (Chen, Shi et al. 2010).

More interestingly, some modifications as truncated forms can be identified on tau proteins. A recent study permitted to demonstrate differences in CSF levels of truncated forms of tau in PSP patients compared with other neurodegenerative diseases (FTD, CBD, AD, DLB and Parkinson disease) or control subjects (Borroni, Gardoni et al. 2009). Other proteolytic processes on tau protein can occur in neurodegenerative disorders and could be identified.

CSF P-tau level specifically increase in AD patients compared with those with FTD, vascular dementia or control subjects (Hampel, Buerger et al. 2004). Regarding DLB, results are more conflicting with studies describing increase of P-tau CSF levels and others reporting normal concentrations (Vanmechelen, Vanderstichele et al. 2000; Parnetti, Lanari et al. 2001). So, an increase in P-tau CSF levels is observed in AD and MCI patients (Itoh, Arai et al. 2001; Andreasen, Vanmechelen et al. 2003; Herukka, Hallikainen et al. 2005). Moreover, cognitive decline is correlated with CSF increasing levels of P-tau, these CSF tau species might specifically mark a cerebral degenerative process (Maccioni, Lavados et al. 2006; Wallin, Blennow et al. 2006). While other P-tau species have been measured, only CSF levels of tau proteins phosphorylated at serine 181 ($P\text{-}tau_{181}$) or threonine 231 ($P\text{-}tau_{231}$) seem to clearly improve the accuracy of the diagnostic of AD (Buerger, Zinkowski et al. 2002; Hampel and Teipel 2004; Mitchell 2009). Some phosphorylations are more specific for AD, CSF $P\text{-}tau_{181}$ significantly increases in AD patients compared to patients without neurodegenerative processes as well as patients with other neurodegenerative dementias (Vanmechelen, Vanderstichele et al. 2000; Vanderstichele, De Vreese et al. 2006). The P-tau181 assay in CSF is now routinely used for biological differential diagnosis between AD and other forms of dementias. ELISA measurements of this protein (Innotest phospho-$tau_{(181P)}$, Innogenetics) reveal CSF levels between 30 pg/mL and 50 pg/mL in control subjects and between 75 pg/mL and 100 pg/mL in AD patients (Lewczuk, Esselmann et al. 2004). This phosphorylated form of tau protein permits to distinguish an AD from all other neurodegenerative etiologies with great sensitivity and specificity (Hampel, Buerger et al. 2004). $P\text{-}tau_{181}$ CSF measurement is able to differentiate AD patients from FTD patients with a sensitivity of 85% and a specificity more than 80% (Schoonenboom, Pijnenburg et al. 2004). These results are similar in differentiation of AD and all other neurodegenerative dementias (Hampel, Buerger et al. 2004). Furthermore this measurement improves distinction of AD and DLB patients (Parnetti, Lanari et al. 2001; Vanderstichele, De Vreese et al. 2006).

Other phosphorylated species of tau protein allow to improve accuracy of biological diagnosis. The $P\text{-}tau_{231}$ appears early during AD pathogenesis (Augustinack, Schneider et al. 2002). This form of tau in CSF differentiates patients with AD from control subject with high sensitivity and specificity (more than 90%) (Mitchell 2009), nevertheless its interest for differential diagnosis appears to be less important than $P\text{-}tau_{181}$ except for distinction of AD and FTD (Hampel, Buerger et al. 2004). Moreover $P\text{-}tau_{231}$ well reflects AD neuropathology because its CSF concentration is correlated with amounts of NFT found at the autopsy (Buerger, Ewers et al. 2006). Since this form of phosphorylated tau permits to identify AD within a population of patients presenting a mild cognitive impairement (MCI), it seems

particularly interesting for the early diagnosis (Brys, Pirraglia et al. 2009). The CSF P-tau$_{231}$ levels decrease with time during the AD process; this will enable to approach dementia severity (Hampel, Buerger et al. 2001). CSF P-tau$_{199}$ measurement and determination of its phosphorylation, that seems precociously involved during AD pathogenesis and NFT formation (Maurage, Sergeant et al. 2003), are able to discriminate AD patients from other degenerative dementias with a sensitivity and a specificity of 85% and 80% respectively (Itoh, Arai et al. 2001). Phosphorylation of tau protein on both amino acid 231 and 235 might be predictive of MCI conversion into AD (Arai, Ishiguro et al. 2000). In the same way, CSF level of P-tau$_{396/404}$ is elevated in AD compared to vascular dementia, and control subjects (Hu, He et al. 2002). Nevertheless these results have to be confirmed on patients with other degenerative disorders.

- Combination of etiological markers

The use of combinations of these CSF markers (Aβ_{42}, T-tau and P-tau$_{181}$) improves biological diagnosis performance for dementias in terms of differential or early diagnosis (Andreasen, Minthon et al. 2001; Riemenschneider, Wagenpfeil et al. 2002; Pijnenburg, Schoonenboom et al. 2004). These CSF biomarkers are also prognosis markers since a recent prospective study conduct on 150 AD patients for 5 years revealed that extreme CSF biomarkers levels (high T-tau and P-tau$_{181}$ and low Aβ_{42}) are associated with more aggressive diseases leading to an earlier death (Wallin, Blennow et al. 2010).

Recently, a clinical study of the Alzheimer Disease Neuroimaging Initiative Project has detected an Alzheimer disease signature in the CSF levels of, Aß$_{42}$ T-tau and P-tau181 in more than one third of cognitively normal subjects (De Meyer, Shapiro et al. 2010). This finding suggests that this CSF signature is present and detectable early during the neurodegenerative process. A recent study including 43 patients with a clinical dementia disorder and conducted in Sweden failed to show a concordance between T-tau and Aß CSF levels and pathological symptoms (Brunnstrom, Rawshani et al. 2010). Further, it has been suggested that low CSF Aß$_{42}$ may also be a marker of diffuse plaques in addition to fibrillar plaques. Indeed, Fagan and colleagues have studied the relationship between *in vivo* brain amyloid and CSF markers of proteins (T-tau, P-tau$_{181}$ and Aß$_{42}$) in cognitively normal individuals ranging in age from 43-89 years (Fagan, Mintun et al. 2009). In this study they have identified a new class of non demented individuals who present low CSF Aß$_{42}$ with cerebral amyloid deposition. The authors suggested that CSF Aß$_{42}$ drop prior to amyloid becomes detectable and may reflect the presence of diffuse plaques and/or oligomeric Aß species. These data demonstrate that CSF Aß$_{42}$ may be considered as a biomarker for plaque burden and prognosis providing the very earliest clue to identify preclinical AD.

Combination of CSF T-tau and Aβ_{42} allows to distinguish AD from FTD patients with both sensitivity and specificity of 85% (Riemenschneider, Wagenpfeil et al. 2002). Moreover the use of this combination permits to identify AD in MCI patients with sensitivity and specificity of 95% and 83% respectively (Hansson, Zetterberg et al. 2006). Interestingly, predictive value of P-tau$_{181}$ is better than T-tau for prediction of conversion of MCI in AD patients (Parnetti, Lanari et al. 2006). Association of 3 biomarkers is clearly useful to identify a degenerative process concordant with AD in atypical clinical presentation. A study with 9 patients presenting PCA revealed CSF levels of these 3 biomarkers similar to those observed in AD (Baumann, Duyar et al. 2010). In the same way, very recent prospective study conducted on 22 ACP patients demonstrated that a majority of patients with this syndrome (90%) have intrathecal biomarker levels compatible with AD (Seguin, Formaglio et al. in press).

Using of ratio T-tau/$A\beta_{42}$ increases the diagnosis specificity of AD compared with control subjects and other degenerative dementias (Gomez-Tortosa, Gonzalo et al. 2003; Kapaki, Paraskevas et al. 2003). Specificity of this ratio in differentiation of AD from vascular dementias is higher than 80% nevertheless this ratio is less efficient for differentiating AD and DLB (Gomez-Tortosa, Gonzalo et al. 2003). Finally, ratio T-tau/$A\beta_{42}$ could be early instructive since its increase permits to predict cognitive alterations in asymptomatic subjects within 8 years (Fagan, Roe et al. 2007). Similarly, the ratio P-tau_{181}/$A\beta_{42}$ appears as an important element for differential diagnosis. It allows to distinguish AD patients from those with FTD with a sensitivity and a specificity of more than 90% (de Souza, Lamari et al. 2010). Furthermore this ratio is able to discriminate patients with semantic dementia from AD patients with an excellent sensitivity of 98% and a specificity of 84%. Ratios with other forms of Aβ peptides such as $A\beta_{42}/A\beta_{38}$ and $A\beta_{42}/A\beta_{37}$ combined with T-tau CSF levels are able to differentiate AD from DLB with a sensitivity of 100% and a specificity of 92% (Bibl, Mollenhauer et al. 2006).

Since CSF T-tau levels in sporadic CJD are clearly elevated comparing to all other neurodegenerative disorders and P-tau_{181} is in normal concentrations, the use of P-tau_{181}/T-tau ratio seems interesting to identify CJD patients (Riemenschneider, Wagenpfeil et al. 2003). This ratio is inferior to 0.05 in CJD and superior to 1.25 in AD even in very early stages of the disease. Similar results were found with P-tau_{231}/T-tau (Buerger, Otto et al. 2006). It is important to note that CSF P-tau_{181} level in new variant CJD are higher than in sporadic CJD, and this inversely to T-tau CSF level (Goodall, Head et al. 2006). So, this ratio P-tau_{181}/T-tau is able to differentiate sporadic CJD from new variant with an elevated ratio. Nevertheless, biological levels overlap, particularly between new variant CJD and AD, thus does not permit to attain a differential diagnosis value.

Other combinations with different P-tau are useful for biological diagnosis. Combining CSF P-tau_{181} and P-tau_{231} enables to identify AD patients with a sensitivity of 94%, but a weak specificity of 66% confronting with all neurodegenerative disorders (Hampel, Buerger et al. 2004). More interestingly, ratio of P-$tau_{396/404}$ and T-tau is able to distinguish AD patients from others dementias with a sensitivity and a specificity of 96% and 86% respectively and have an excellent specificity (100%) in the distinction of AD and vascular dementias (Hu, He et al. 2002).

- Others potential biological markers

Numerous other molecules have been investigated as biological CSF markers and are liable to participate on etiological dementia diagnosis. We briefly deal with some of them which seem to supply advantage for differential and early diagnosis.

Implication of oxidative stress hypothesis and inflammation in AD or other neurodegenerative disorders opened some research ways. It is known that inflammation is associated with parenchymal Aß deposits in AD (Schmidt, Schmidt et al. 2002). Inflammation markers have been investigated in CSF to reveal potential inflammatory process linked to these pathological deposits. Inflammatory mediators such as chemokines might be increased into AD patients CSF (Galimberti, Schoonenboom et al. 2006; Galimberti, Schoonenboom et al. 2006). The concentration of classical complement cascade C1q subunit decreases in CSF of AD patients and is correlated with disease severity (Smyth, Cribbs et al. 1994). However, this intrathecal molecule only attests for microglial reactions met in AD and also during physiological aging (Lue, Walker et al. 2001; Schuitemaker, van der Doef et al. 2011). Similarly isoprostane CSF levels, an oxidative stress marker, clearly increase in biological fluid (CSF, blood and urine) proportionally to cognitive decline and might be an

early biomarker of AD in MCI patients (Pratico, Clark et al. 2000; Pratico, Clark et al. 2002). If these molecules seem interesting for understanding physiopathological mechanisms which occur in neurodegenerative disorders (oxidative stress and microglial activation particularly), these biomarkers cannot be a specific part of differential diagnosis since they are frequently involved during cerebral aging (Montine, Peskind et al. 2011).

Molecules with an implication in protein catabolism are present within CSF and their levels may reflect cerebral damages. An increase of the intrathecal neprisylin activity was observed and correlated to T-tau CSF level and cognitive decline in AD patients and patient with prodromal form of AD (Maruyama, Higuchi et al. 2005). Nevertheless this activity is modestly predictive for MCI conversion to dementia. Ubiquitine, another catabolic signal protein might be linked to aggregated tau proteins in the cerebral cortex and its CSF levels might increase in AD patients (Iqbal, Flory et al. 2005). Moreover ubiquitine CSF levels correlate with those linked with cerebral tau aggregates in AD (Kudo, Iqbal et al. 1994). Nevertheless, elevated ubiquitine CSF levels were observed in other non-AD dementias (Iqbal and Grundke-Iqbal 1997).

A protein largely present in CSF and blood, tranthyretin, has higher CSF levels in AD and MCI stage. CSF measurements can distinguish AD from control subjects with sensitivity of 100% and specificity of 93% (Lovell, Lynn et al. 2008). In the same way, CSF concentrations of the mitochondrial protein cytochrome c are increased in AD and permit to identify AD process in MCI patients with a sensitivity of 100% and specificity of 75% (Papaliagkas, Anogianakis et al. 2009). However, a specificity problem is present regarding other neurodegenerative dementia such as FTD (Ruetschi, Zetterberg et al. 2005).

Recently, Zhong and colleagues have quantified the levels and the activity in the CSF of ß-secretase (BACE 1), an enzyme involved in the cleavage of APP to Aß (Zhong, Ewers et al. 2007). An elevated BACE 1 activity was observed in CSF of MCI and AD subjects compared with normal controls. Moreover, this activity was correlated with CSF BACE 1 protein and Aß peptide levels. A more recent study showed that CSF BACE1 activity correlates with CSF $Aß_{40}$, total T-tau and P-tau_{181} levels but surprisingly not with intrathecal $Aß_{42}$ concentration (Mulder, van der Flier et al. 2010). These results are promising and suggest that BACE 1 could be a potential candidate as a biomarker of early-stage AD, but additional studies are necessary to confirm this hypothesis.

4. Peripheral biomarkers

Because the CSF collection by lumbar puncture is an invasive, expensive and time-consuming procedure, the detection of biomarker molecules in blood would be more widely applicable. However, in comparison with CSF biomarkers, efforts to discover reliable biomarkers for Alzheimer's disease in peripheral blood have not been successful and do not allow developing a solid diagnostic. The use of many different sensitive ELISA tests for the detection of $Aβ_{40}$ and $Aβ_{42}$ allowed the detection and quantification of Aß in human plasma (for review see Irizarry, 2004; Mehta, 2007). According to these studies, some controversy persists concerning the changes in blood Aß levels in relation with the severity of the disease suggesting that plasma Aß levels cannot allow differentiating sporadic AD from control cases. As the pool of circulating Aß includes multiple C-terminal truncated fragments, and possibly oligomeric species, it has been suggested that the use of of antibodies to various forms of Aß with different affinities may explain these differences. Nevertheless, the ELISA assay may not be an ideal method to measure Aβ levels in blood. Indeed, the two major

difficulties in the measurement of Aβ in plasma consist in the low concentration of the plasma peptide, needing a more sensitive quantification assay, and in the interaction of the beta peptide with different carrier proteins present in plasma, that can mask the epitopes of Aβ and interfere with the detection of the peptide (Kuo et al., 2000).

Nowadays, the development of more sensitive and more specific assays for the detection of plasma Aβ levels is needed for proposing plasma Aβ as a biomarker for diagnosis. To overcome the Aβ low levels different new promising strategies have been developed based on purification and concentration steps prior to the Aβ peptides analysis. Using a denaturing solid-phase extraction (SPE) combined with an ELISA assay, Lanz and co-workers (Lanz & Schachter, 2008) have compared the Aβ detection in plasma and CSF bio-fluids of normal individuals. But, while human CSF exhibit, in comparison with non-SPE samples, the most robust recovery (≥ 90%), the human plasma showed a lower recovery (between 40 and 60% in function of the extraction process used, guanidine or acid formic, respectively) suggesting that the use of a SPE step does not improve the detection of the protein comparatively to the non-denatured plasma. Slemmon and collaborators (Slemmon et al., 2007) have linked to the SPE a reverse-phase HPLC (SPE-HPLC) compatible with analysis by ELISA. In this study, the detection of Aβ peptides from the whole blood of six normal subjects was analyzed; by comparison with a native plasma, a significantly increase in the amount of total Aβ peptide was obtained after guanidine extraction and HPLC detection, with concentrations ranging from 100 to 165pg/ml. These results suggest that there is a pool of Aβ peptides in non-denatured plasma samples that is not accessible for the detection by ELISA assay probably due to their interactions with plasma proteins or to their aggregated state that could mask the epitopes.

Several studies on the detection of tau proteins in blood have been performed but failed to show a clear relationship to dementia diagnosis, thus, the correlation between increased tau brain levels and elevated CSF levels of tau in AD patients is not totally clear (Bitsch et al., 2002; Ingelson et al., 1999). Further research is needed to improve the specificity and the sensitivity of the assay in order to develop a robust method to measure tau protein levels in serum or plasma.

Recently, various plasma signalling proteins were proposed as potential candidates to develop a blood test for AD (Ray et al., 2007). O'Bryant and colleagues developed an algorithm in order to differentiate patients with Alzheimer disease from controls. In this study serum protein-based multiplex biomarker data from a large group of Alzheimer patients and controls were analyzed. Combined with age, sex, years of education and ApoE genotype, their logarithm model reached a diagnostic sensitivity and specificity of 94% and 84% respectively (O'Bryant et al., 2010).

In addition to CSF and blood, several other biological fluids are under investigation for the detection of AD biomarkers. Among these biological fluids, a special attention is accorded to saliva and urine. The study of Bermejo and colleagues (Bermejo-Pareja et al., 2010) found that saliva $A\beta_{42}$ is, in comparison with control subjects, significantly elevated in early stage AD patients while $A\beta_{40}$ levels remain unchanged within all the samples analyzed. Curiously the elevated saliva $A\beta_{42}$ levels were not observed in severe stages of the disease. This augmentation seems to be specific to AD and not to Parkinson disease. These results show that in combination with Aβ levels in brain, the saliva levels of $A\beta_{42}$ could be a potential biomarker for clinical AD.

In addition to the specific markers of AD disease (like Aβ peptides and tau proteins), there are non-specific markers like oxidative stress and neuronal inflammation molecules. An increased expression in AD patients of many inflammatory and pro-inflammatory cytokines has been widely documented. Inflammation in AD brains induces important microglia

activation and consequently production of free radicals responsible of an intense oxidative stress. So these inflammatory and oxidative mechanisms are involved in the aetiology of AD and can contribute to the neurodegeneration. However conflicting results exist about the pertinence of the use of these peripheral biomarkers. Among the inflammatory markers several cytokines have been measured in CSF or blood, such as interleukin 1-α and 1-β, interleukin-6, -10, -11 and -18, and tumor necrosis factor-alpha (for review see Casoli et al., 2010; Olson & Humpel, 2010). But the results are very divergent between the different groups. The same conclusions can be enunciated for chemokines. More studies are needed to clarify and determine the reproducibility of such inflammatory markers.
Oxidative stress parameters have also been investigated as AD biomarker. A biomarker of oxidative stress study (BOSS) coordinated by the NIH was performed in order to determine among 16 commonly studied biochemical products of oxidative stress which ones could been validated as *in vivo* biomarkers (kadiiska et al., 2005). This study was performed on plasma, blood and urine samples. Among these molecules, malondialdehyde (MDA), 8-hydroxy-2′-deoxyguanosine (8-OHdG) and F2-isopropanes were the most promising biomarkers of oxidative stress. F2-isopropanes, a product of lipid peroxidation, was the most extensively investigated in CSF of AD patients. These clinical studies showed comparatively with control subjects a significant increase of F2-isopropanes in damaged regions of AD brain and in CSF in patients in early stages of mild cognitive dementia (Montine et al., 1999, 2001; Pratico et al., 2000). In plasma and urine samples, the quantification of such marker yield to conflicting results.

5. Which techniques could be applied?

Hyperphosphorylated Tau proteins are currently used for AD diagnostics in CSF, as recently proposed by an international working group on Alzheimer Disease (NINCDS-ADRA working group) (Dubois et al., 2007). Moreover, there are no well-established and accepted biochemical tests available for therapeutic follow-up of these diseases. Their diagnosis requires thus high sensitivity and specificity. Sensitivity is needed for early diagnosis, when the biomarker is present at very low levels to ensure cost-effective therapeutic interventions before the disease has progressed to a stage where damages to the brain are irreversible. Specificity is needed to discriminate first, non-degenerative causes of dementia (e.g. vascular, alcoholic, psychiatric, metabolic, infective etc.) from degenerative forms and, second, to discriminate the different molecular aetiologies in order to propose an appropriate therapeutic treatment: for example, anticholinesterase drugs show certain efficacy in AD, but not in frontotemporal dementia (Musial et al., 2007).
Today, AD can be diagnosed with certainty only post-mortem with histopathological evaluation of amyloid plaques and NFTs. Ante-mortem, AD is diagnosed by clinical criteria. Clinical diagnosis included systematically before 2007 December the presence of dementia (McKhann et al., 1984). Before apparition of dementia symptoms, complaining patients suffer from memory trouble without social and professional effects; this clinical phase was usually called "amnestic mild cognitive impairment (MCI)" (Petersen et al., 1999). Many of these patients convert to AD whereas few of them convert to other neurodegenerative dementia or remain stable (Fischer et al., 2007). As recently proposed, using the combination of clinical, neuropsychological, imaging methods and CSF biomarkers, this clinical stage would be nowadays called preclinical AD and, more important, its detection would allow a better care for AD patients. Neuropsychological evaluation permits a more accurately defined diagnosis, but it is time consuming (half a day), is not available for all concerned

people, is still proposed at advanced stages of disease, and is not 100% accurate since there is a risk of overlaps among different aetiologies of dementia as frontotemporal degeneration (FTD), Lewy's body dementia (LBD), and potentially other dementias. Neuroimaging may permit exclusion of vascular pathology, detection of hippocampal atrophy and cerebral hypoperfusion (Tapiola et al., 2008; Xu et al., 2007). It must be noted that first AD lesions may coexist with vascular dementia in at least 50 % of cases. During the past years, great efforts have been made to identify reliable biomarkers for AD in body fluids of patients that are suitable for minimal invasive early diagnosis of AD, mainly cerebrospinal fluid (CSF) and blood. Recently, we've summarized all of the criteria needed to establish convenient technique with the following advantages (Dupiereux et al. (2009):

a. Low-cost
b. Rapid-fast
c. Good level of accuracy

6. Should Alzheimer's detection be associated to biomarkers of other diseases?

Other diseases could be associated with AD resulting in mixed dementias. Furthermore, unique pathologies could mimic others and it is difficult to make differences between given pathological events or to identify all neurodegenerative processes. Effectively, 15 to 50% of AD patients have Lewy bodies (neuropathological hallmark of DLB constituted by α-synuclein) associated with NFT and Aβ deposits after neuropathological examination (Hamilton 2000). Nevertheless, α-synuclein (α-Syn) is not always associated with this clinical phenotype (Parkkinen, Kauppinen et al. 2005). This pathological association seems to make part for cognitive alteration severity with a synergic effect (Clinton, Blurton-Jones et al. 2010). Mixed dementia may be more aggressive than pure AD (Kraybill, Larson et al. 2005). Since a low CSF α-Syn level was detected in CSF of patients with DLB and Parkinson disease comparing to other neurodegenerative diseases, an association of this measurement with typical CSF biomarkers of AD seems interesting (Tokuda, Salem et al. 2006; Kasuga, Tokutake et al. 2010). Moreover, oligomeric forms of α-Syn were detected in CSF and blood of patients with Parkinson disease (El-Agnaf, Salem et al. 2006). Nevertheless, several studies are conflicting since elevated or normal CSF levels were also observed in DLB patients (Mukaetova-Ladinska, Milne et al. 2008; Spies, Melis et al. 2009; Ballard, Jones et al. 2010). In the same way, we can postulate an association of typical CSF biomarkers of AD with prion CSF measurement since neuropathology of CJD is more intricate than we though it several years ago (Kovacs, Seguin et al. 2011).

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12

Phospo-PKCs in Abeta1-42-Specific Human T Cells from Alzheimer's Disease Patients

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1. Introduction

Alzheimer's Disease is the most common neurodegenerative dementia of older age. Accurate diagnosis of this condition has important prognostic and therapeutic implications. In the latter, Amyloid-Beta is thought to be produced in excess and subsequently deposited in the brain as plaques, forming the pathological hallmark of Alzheimer's Disease. Interestingly, B- and T-lymphocytes have been implicated in the disease processes being responsible of Amyloid-Bata1-42 peptide removal and activation of inflammation response. Amyloid-Baeta1-42-specific T-cells are present in Alzheimer's disease but not in other neurodegenerative conditions. By using multi-colour flow-cytometry it is possible to analyse cytokine production and Phosho-Protein-Kinase C expression of *in vitro* Amyloid-Beta 1-42 stimulated T-cells. It has been demonstrated that a subset of Amyloid-Beta1-42-specific T-cells, characterised by bright expression of Phosphorylated-Protein-Kinase C, distinguishes Alzheimer's Disease from other neurodegenerative conditions. Therefore, such a new marker might provide further prospective to the studies aimed at diagnosis of Alzheimer's disease and its discrimination from other forms of dementia.

2. T cell activation and lipidic-dependent signal transduction

T cells recognize antigen (Ag) as a peptide– major histocompatibility complex (MHC) on Ag-presenting cells (APC) such as dendritic cells (DC) through direct cell–cell interactions. The T cell antigen receptor (TCR) binds to the Ag peptide–MHC complex and triggers T cell activation by recruiting various signaling molecules. Early analysis of the signaling events related to T cell activation revealed that some intracellular proteins, such as phospholipase-C (PLC), are involved (Bunnell et al., 2002). PLC is a member of phosphoinositide family, including phosphoinositide lipids within cellular membranes and soluble inositol phosphates (Ips). In most stimulatory cells, the plasma membrane phosphoinositide, phosphatidylinositol(4,5)bisphosphate (PIP2) is a key precursor for both other phosphoinositides and soluble IPs. Many of these regulate distinct and overlapping

downstream effectors (Irvine & Schell, 2001; Alcazar-Roman & Wente, 2008; Resnick & Saiardi, 2008; Sauer et al., 2009; Shears, 2009; Sauer & Cooke, 2010). In particular, class I phosphoinositide 3-kinases (PI3K) phosphorylate PIP2 at the 3-position of its inositol-ring into phosphatidylinositol(3,4,5)trisphosphate (PIP3) after receptor stimulation (Vanhaesebroeck et al., 2005; Juntilla & Koretzky, 2008; Fruman & Bismuth, 2009). Receptor-induced PIP2-hydrolysis by phospholipases such as PLCg1/2 in lymphocytes generates the lipid diacylglycerol (DAG) and the soluble IP inositol(1,4,5)trisphosphate (IP3). PIP3, DAG, and IP3 have essential second messenger functions in many cells, including lymphocytes. It is now evident the importance of phosphoinositide signaling in T cells and highlight the importance of a recently identified, intriguing molecular interplay between second messenger lipids and their soluble IP counterparts. All phosphoinositides contain a hydrophobic membrane-embedded diacylglyceride and a hydrophilic solvent-exposed IP moiety. The inositol ring hydroxyl groups can be stereo-specifically phosphorylated by phosphoinositidekinases. Most phosphatidylinositol-bisphosphate in the plasma membrane of unstimulated cells is phosphorylated at the inositol 4- and 5-positions. PIP2 is an important second messenger, recruiting and regulating multiple signaling proteins (McLaughlin et al., 2002). Due to the constitutive PIP2 availability in resting cells, these PIP2-associated proteins likely maintain signaling pathways in a preactivation state (Han et al., 1998; Ang et al., 2007; Ceccarelli et al., 2007). Despite the importance of PIP2, much greater attention has been given to the products of PIP2 phosphorylation or PIP2 hydrolysis that are induced following receptor activation. PIP2 phosphorylation is mediated by PI3Ks. PI3Ks phosphorylate phosphatidylinositol (PI) into PIP and PIP2. PI3Ks are activated by most stimulatory receptors on lymphocytes including T- and B-cell antigen receptors (TCR, BCR), and co-stimulatory, Toll-like, and cytokine receptors (Vanhaesebroeck et al., 2005; Buitenhuis & Coffer 2009; Fruman & Bismuth 2009) and have important roles in T cell development and function (Sasaki et al., 2000; Okkenhaug et al., 2002; Okkenhaug et al., 2006; Patton et al., 2006; Swat et al., 2006; Alcazar et al., 2007; Matheu et al., 2007; Liu et al., 2009a; Liu & Uzonna, 2010; Soond et al., 2010). Taken together, immunoreceptor-induced PIP3 gneration is important for lymphocyte proliferation and differentiation (Juntilla & Koretzky, 2008; Buitenhuis & Coffer, 2009; Fruman & Bismuth, 2009). PIP3 mediates the cellular effects of PI3K activation by recruiting effector proteins binding to PIP3 (Haslam et al., 1993; Mayer et al., 1993), such as Akt (Protein kinase B) and Tec protein kinase families (August et al., 1997; Heyeck et al., 1997; Stokoe et al., 1997). In addition, Akt can also bind to PI(3,4)P2 (Cozier et al., 2004; James et al., 1996; Lemmon, 2008). Akt is particularly important during early T cell development (Juntilla et al., 2007; Juntilla & Koretzky, 2008). PIP2 is a substrate for another immunologically important enzyme, phosphatidylinositol-specific phospholipase-Cg (PLCg). PLCg hydrolyzes PIP2 into its hydrophobic and hydrophilic components, the membrane-lipid DAG and soluble IP3. Both are second messengers that regulate proteins through specific binding domains. The two mammalian PLCg isoforms, PLCg1 and 2, have partially overlapping expression patterns and functions (Wilde & Watson, 2001). T cells exclusively express PLCg1. T cell-specific PLCg1-deletion impaired thymocyte positive and negative selection, T regulatory cell development and function, TCR-induced peripheral T cell proliferation and cytokine production. Defective TCR activation of the MAP kinases Erk and Jnk, and of the transcription factors NFAT, AP-1, and NF-kB indicates the broad importance of PLCg1 in TCR signaling through several

pathways. Autoimmune disease symptoms show the physiological importance of PLCg1 in T cells (Fu et al., 2010). Severe blocks in T cell development and late onset autoimmunity in mice expressing a PLCg1-binding-deficient LAT allele indicate the importance of LAT interactions for PLCg1 function (Sommers et al., 2002; Sommers et al., 2005). Finally, severe defects in early hematopoiesis in chimeric mice generated with PLCg1-deficient embryonic stem cells suggest important PLCg1 functions in hematopoietic stem or progenitor cells (Shirane et al., 2001). In contrast, PLCg2-deficient mice are viable with specific defects in B cells, mast cells, dendritic cells, osteoclasts, and neutrophils (Wang et al., 2000; Graham et al., 2007; Cremasco et al., 2008; Epple et al., 2008; Cremasco et al., 2010). The membrane second messenger, DAG, propagates signals via membrane recruitment of cytosolic signaling proteins by binding to their C1 domains, cysteine-rich domains of approximately 50 amino acids. Several well-characterized DAG-effector families include Ras guanine-nucleotide-exchangefactors/releasing proteins (RasGRPs), protein kinase C-related kinases (PKCs, PKD), chimaerin Rho/Rac-GTPase-activating proteins (Yang and Kazanietz, 2007), Munc13 proteins (Betz et al., 1998), and diacylglycerol kinases (DGKs).

3. Protein Kinase C (PKC) and T cells

Noticeable, among different proteins participating to the lipidic-dependent signal transduction, is the role of PKCs involved in TCR activation. PKCs are a family of serine/threonine protein kinases involved in different lipidic-dependent signal transduction events. PKCs were identified, for the first time, by Nishizuka and colleagues in 1977. To date, different PKC isoenzymes have been described in several tissues and with differential cellular localizations (Saito and Shirai 2002). PKCs can be grouped into three categories according to the presence of motifs dictating cofactor requirements for their optimal catalytic activity. Whereas conventional [cPKCs: alpha, beta I-II (spliced variants) and gamma] and novel [nPKCs: delta, epsilon, ni and theta] PKCs bind DAG which stimulates kinase catalytic activity, atypical [aPKCs: zeta, iota/lambda] PKCs do not interact with DAG. cPKCs but not nPKCs also require for their activation Ca^{2+}.

Each PKC contains a highly homologous C-terminal catalytic domain and an N-terminal regulatory domain, which mediates cofactor binding and substrate accessibility. PKCs also are characterised by the presence of a DAG/phorbol-ester-binding C1 domain, defined by the presence of two repeated cysteine-rich zinc-finger motifs (C1A and C1B); it is functional in cPKCs and nPKCs, but not in aPKCs. The C2 domain mediates Ca^{2+} binding in cPKCs but differences in key residues abolish this function in nPKCs. aPKCs have single modified C1 domains. C3 and C4 domains are ATP- and substrate-binding lobes of the kinase core. The auto-inhibitory pseudosubstrate sequence (PS) present in the regulatory domain of all PKC isoenzymes interacts directly with the substrate-binding cavity in the catalytic domain, thereby sterically blocking access of substrates to the active site. The activation of signal transduction pathways, involving PKCs, leads to the hydrolysis of PIP2, and consequently to the formation of DAG and IP3. DAG binds to the PKC C1 domain; PKCs are activated by phosphorilation and can finally phosphorylate protein substrates. PKCs demonstrated broad substrate specificity *in vitro* related to several functions *in vivo*. Distinct roles of different PKC isoforms can be, at least in part, attributed to differences in their structures and to the different mechanisms modulating their activation (Figure 1). These different PKC roles are also evident in the context of intracellular immune cell signalling (Tan and Parker 2003).

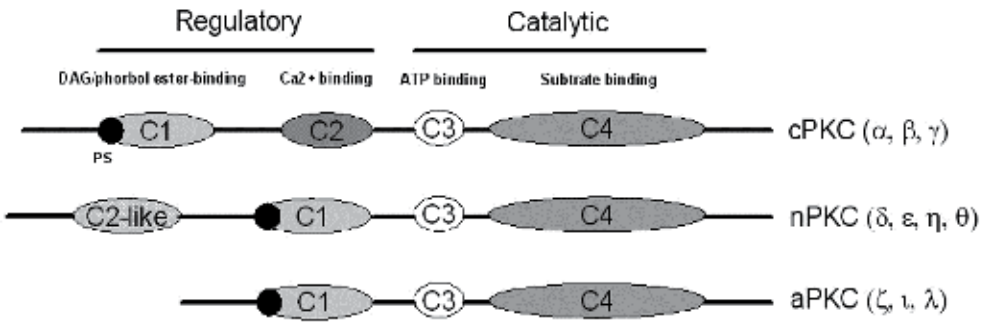


Fig. 1. PKC structure

The role of PKC in regulating T cell activation has been well characterised. The nPKC member PKC-theta which expression is largely restricted to T cells co-localised with the TCR was originally identified to play an important role in TCR-induced T cell activation (Baier 2003) (Isakov and Altman 2002). PKC-theta has also been involved in cell signalling events triggered by TCR engagement both *in vitro* in cell models and *in vivo* in knockout mice (Baier-Bitterlich, Uberall et al. 1996); (Lin, O'Mahony et al. 2000); (Bauer, Krumbock et al. 2000); (Sun, Arendt et al. 2000) (Pfeifhofer, Kofler et al. 2003). In particular PKC-theta mediates activation of the transcription factor activator protein-1 (AP-1) and of the nuclear factor κB (NF-κB) in response to TCR/CD28 co-stimulation in several T cell models (Baier-Bitterlich, Uberall et al. 1996); (Lin, O'Mahony et al. 2000); (Bauer, Krumbock et al. 2000). It has been demonstrated that PKC-theta activation could also be linked to nuclear factor of activated T cells (NFAT) signaling (Pfeifhofer, Kofler et al. 2003) (Figure 2). Beside the well-recognized role of PKC-theta for T cell receptor activation, other PKC isoforms seem to be involved in T cell signalling in an alternative or cooperative fashion.

Among others, PKC-alpha has been suggested to play a potential role in thymocyte developmen. PKC-delta has been reported to be possibly involved in T cell migration (Volkov, Long et al. 1998). Self-reactive B-cells normally undergo either clonal deletion or tolerance to self-antigens (B-cell anergy), which is essential for the prevention of autoimmune disease. The physiological role of PKC-delta, the closest related PKC member to PKC-theta, in the control of B-cell tolerance has recently been uncovered by characterization of PKC-delta-knockout mice generated independently by two laboratories (Miyamoto et al., 2002; Mecklenbrauker et al., 2002). Loss of PKC-delta in mice leads to significant splenomegaly and lymphadenopathy because of increased numbers of peripheral B-cells, although no noteworthy abnormalities are observed in T cells (Miyamoto et al., 2002). The mice die prematurely due to severe autoimmune disease, which is characterized by the detection of autoreactive antibodies, indicates that PKC-delta is essential for the prevention of autoimmune disease. Furthermore, PKC-delta deficiency prevents B-cell anergy, allowing maturation and differentiation of self-reactive B-cells, attributed to a defect in nuclear factor κB (NF-κB) activation, at least as judged by inefficient IκB degradation in the cytoplasm (Mecklenbrauker et al., 2002). Although the above studies suggest that PKC-delta is involved in negative regulation of proliferation, there is no consensus on the mechanism. Mecklenbrauker et al. reported that the B-cells from PKC-delta-deficient mice have normal responses to antigenic stimulation and thereby concluded that PKC-delta −/−

B-cells have a specific defect in the induction of anergy. In contrast, Miyamoto et al. showed that the proliferation of B-cells from PKC-delta −/− mice was increased in response to several mitogenic stimuli, suggesting a generalized enhancement of signalling events. Whereas NF-κB activation remained unaffected, increased production of the growth-promoting cytokine IL-6, as well as the DNA-binding activity of the nuclear factor IL-6 (NFIL-6) transcription factor, was detected in the PKC-delta −/− B-cells, suggesting PKC-delta might negatively regulate B-cell growth through transcriptional regulation of the IL-6 gene.

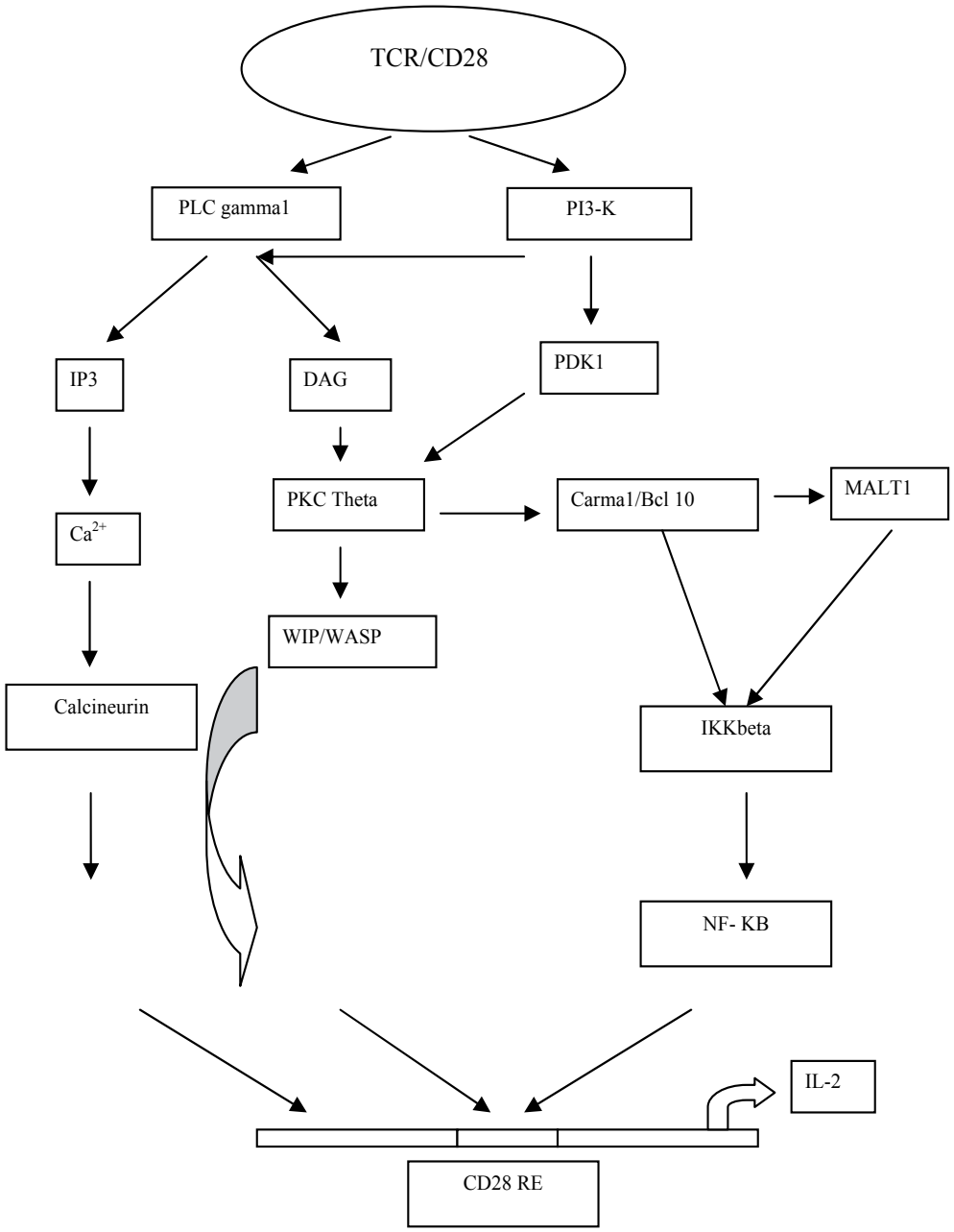
Intriguingly, PKC-zeta has also been implicated in the T cell-dependent immune response (Duran, Diaz-Meco et al. 2003); (Savkovic, Koutsouris et al. 2003). Targeted disruption of the PKC-zeta gene in mice indicates that the role of this aPKC within the immune system is also specific to B-cell function (Martin et al., 2000). B-cells from PKC-zeta-deficient mice showed increased spontaneous apoptosis, and impaired proliferation and survival in response to IgM cross-linking, whereas both peripheral T cells and thymocytes seemed to develop and proliferate normally. The defective survival of B-cells in these mice correlated with defects in the activation of extracellular-signalregulated kinase (ERK) (but not p38 MAPK or JNK) and the transcription of NF-κB-dependent genes, including Bcl-xL, IκB and IL-6. Furthermore, transcription of these NF-κB-dependent genes, but not NF-κB nuclear translocation, was inhibited in B-cells stimulated with IgM. PKC-zeta-null mice were unable to mount an optimal T cell-dependent immune response, in spite of the fact that, as adults, they exhibited no major defects in the subpopulations of B-cells, indicating that this is a post-B-cell maturation phenomenon. Although the possibility of a PKC cascade involving both PKC-beta and PKC-zeta has not been excluded, recent findings showed that PKC-zeta can regulate NF-κB via an IKK-independent pathway, by directly phosphorylating Ser^{311} of the pHealy et al., 1998 subunit (RelA) (Duran et al., 2003; Savkovic et al., 2003).

Therefore, modulation of the expression and phosphorylation of different PKC isoforms might play critical independent or complementary roles in the context of the better known PKC-theta-driven T cell signalling in different normal and pathological conditions. As far as AD is concerned, while a body of literature refers to a possible involvement of PKC signalling in brain and skin tissues of these patients (Alkon, Sun et al. 2007), scarce or no knowledge is available about the behaviour of the above reported PKCs in peripheral T cells from AD patients. Recently, it was reported that flow cytometric assessment of well defined bright P-PKC-delta and P-PKC-zeta T cell subpopulations (probably CD4+ T cells) after specific Abeta1–42 stimulation in the majority of AD patients, may refer to the development of T cell subpopulations reactive to Abeta1–42 and concomitantly expressing high levels of phosphorylated PKC-delta and PKC-zeta (Miscia, Ciccocioppo et al. 2009).

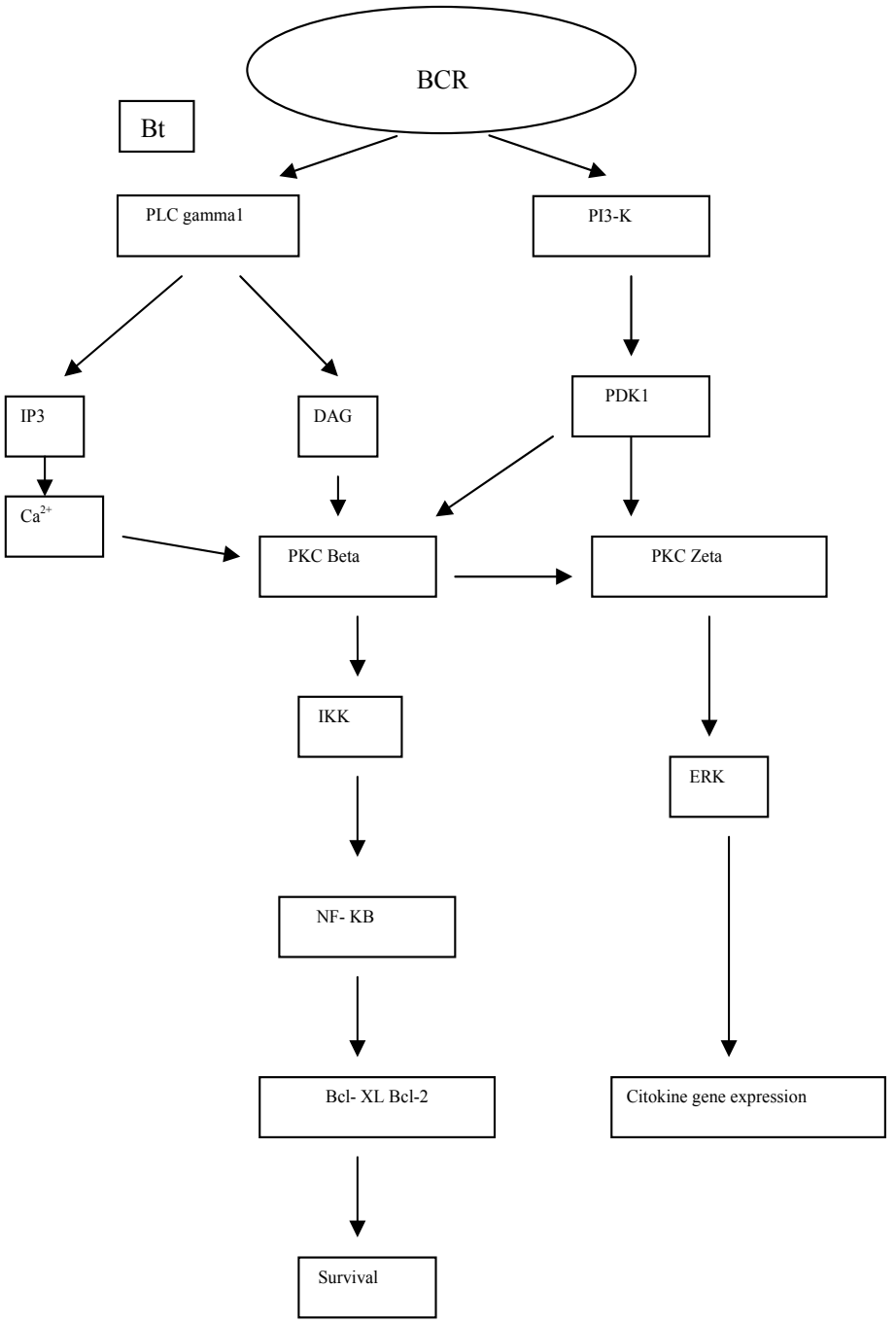
4. Inflammation and T cells in Alzheimer's disease

Normal aging in humans brings a progressive loss in memory and is often exacerbated by diseases such as Alzheimer's disease (AD). Although many underlying processes have been invoked, one common ground that links many factors associated with cognitive aging is neuroinflammation. Markers of inflammation are associated directly with deficits in cognitive function and with diseases that are risk factors for cognitive decline (Gemma C, 2010). Amelioration of brain inflammation with various treatments has beneficial actions on several indicators of impaired cognitive aging. Understanding how neuroinflammation

affects cognition may provide directions for useful interventions to prevent or treat an aberrant cognitive decline in older adults.



a)



b)

Fig. 2. Signal transduction pathways involving PKC in T (a) and B (b) cells

However, to better understand inflammation's role in disease, it is necessary to recognise that inflammation is a protective response of our body that occurs in response to an insult. In the case of infection, the immune system is activated to identify the foreign agent and neutralize it. This involves a series of events and requires the recruitment of a variety of immune cells. Throughout most of the body, cells known as macrophages, search for invaders, and then engulf and neutralizing them. The recognition of infectious non-self is mediated by a limited number of germline-encoded pattern-recognition receptors (PRRs), which trigger rapid responses. In the brain, supporting cells of the glial family comprise of astrocytes and microglia. Microglial cells, act as scavengers and are considered "the CNS professional macrophages". Microglia are myeloid lineage cells expressing a wide range of PRRs and for this reason they embody the innate immune response of the brain, as they provide the first line of defense whenever there is an injury. They engulf and eliminate dead neurons that have been damaged by injury or illness. However, they also secrete harmful neurotoxins and toxic oxygen free radicals in an attempt to neutralize foreign or undesirable substances. Unfortunately, sometimes the injurious event overwhelms the protective effect and inflammation may become self-perpetuating. This is the case of normal aging, but it is much more rampant in neurodegenerative diseases such as Alzheimer's, Parkinson's, which are characterized by exacerbated microglial activity. To date, the neurotoxic and neuroprotective roles of innate immune reactions in brain injury, ischemia, autoimmune and neurodegenerative disorders of the CNS, altogether solicits an intensively investigated and debated scientific research issue. However, despite considerable work in this area, much more points remain to be elucidated, notably cellular events regarding the early dysregulating events that activate brain inflammatory pathways. If it will be possible to target and harness these inflammatory processes toward therapeutic application, then cognition could be protected during aging and disease by early intervention against the negative consequences of inflammation.

It is now clear that inflammation plays an important pathogenethic role in Alzheimer's disease (AD). At onset of pathology, the inflammatory changes are probably linked to misfolding and the consequent accumulation of Amyloid beta (Abeta) peptide in the limbic and associative cortices of AD brains (Rogers, Webster et al. 1996); (McGeer and McGeer 1999; McGeer and McGeer 1999; McGeer and McGeer 2002). Many studies have demonstrated that such inflammation arises mainly from cellular (glial) sources within the central nervous system (CNS) rather than from external sources such as T cells. However, recent studies have suggested that systemic T cells, and in particular CD4+ T cells, can be recruited to the CNS to modify potential destructive local inflammation (Schwartz and Shechter 2010). As a consequence of increased damage to the blood–brain barrier (BBB) or in response to inflammatory signals T cells might more commonly cross the BBB and accumulate in AD brains (Rogers, Luber-Narod et al. 1988); (Togo, Akiyama et al. 2002). It remains to be determined whether brain penetration of T cells is involved in the etiopathogenesis of AD, or if it is simply an epiphenomenon.

5. Peripheral T cell responses in Alzheimer's Disease

In humans, naïve T cells typically express CD45RA on the surface. When naïve T cells encounter their antigen they become activated and a CD45 isoform switching from RA to RO occurs consequently (Dutton, Bradley et al. 1998). This isoform switching can thus be taken as a marker of human T cell "memory" (Figura 3).

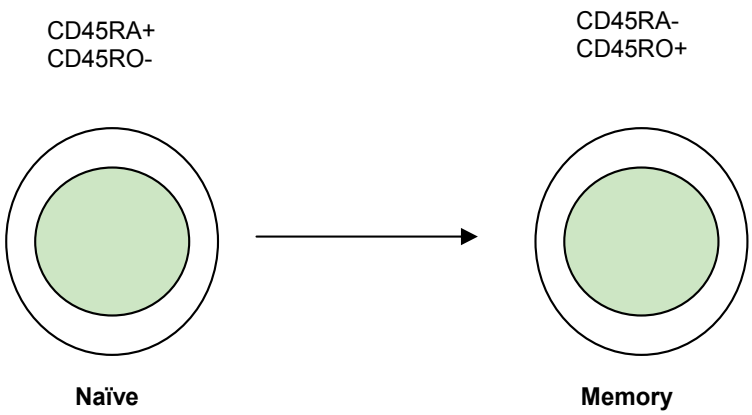


Fig. 3. Cell surface antigens on naïve and memory subsets

It has been demonstrated that CD45RO expression was increased in T cells from AD patients compared to controls, when isolated T cells were placed in culture (Lombardi, Garcia et al. 1999). Interestingly, Togo et al. reported the presence of CD45RO+ T cells in brains of AD patients (Togo, Akiyama et al. 2002). These findings demonstrated that T cells are indeed activated at some point during the clinical progression of AD. In an attempt to address this question, Lombardi et al. (Lombardi, Garcia et al. 1999) found an increase in CD4+ T cells and CD25+ T-regulatory cells in the AD group compared to healthy controls. Additional evidence of systemic T cell activation in AD comes from studies designed to measure Abeta auto-antibodies or Abeta-reactive T cells in AD patients and controls. Although antibody production is a B cell-dependent process, the response is supported by activated T cells. At least three studies to date found increased levels of circulating Abeta auto-antibodies in patients clinically diagnosed with dementia compared to non-demented controls (Nath, Hall et al. 2003) (Gruden, Davudova et al. 2004) (Mruthinti, Buccafusco et al. 2004). Using various peptides and specific assay systems to stimulate peripheral T cells from AD patients, it was also demonstrated that T cells specific for the fragment 1-42 of the Abeta peptide (Abeta1-42) can be detected in peripheral blood (Monsonego, Zota et al. 2003). More recently, by applying a slightly modified system than the one used by Monsonego et al (2003), an increased T cell reactivity to Abeta1–42 peptide in peripheral blood from AD patients respect to control subjects was seen (Miscia, Ciccocioppo et al. 2009).

6. T cell subsets in AD pathogenesis and diagnosis

In vitro studies have shown that IFN-gamma-treated microglia are efficient antigen-presenting cells (APCs) presenting Abeta and triggering Abeta-reactive T cell proliferation. Th1 Abeta-reactive cells become apoptotic after such stimulation, whereas Th2 cells stimulated by Abeta express the cardinal Th2 cytokines (Monsonego, Zota et al. 2003). It has been suggested that Th2-type Abeta-reactive T cells could be beneficial in AD by secreting cytokines which downregulate the proinflammatory environment. To date, the diagnosis of AD is based on neuropsychological examination using criteria such as insidious onset and progressive impairment of memory, as well as loss of other cognitive functions. The presence of plaques and tangles assessed after post mortem examination of brain tissue has been considered a strong marker for AD diagnosis (Hof 1997). Early inflammatory

processes, identified at AD onset, suggest that specific inflammatory biomarkers in the peripheral blood, such as increased levels of TNFalpha, CD40L and other pro-inflammatory cytokines might support the diagnosis (Akiyama, Barger et al. 2000; Speciale, Calabrese et al. 2007; Schwartz and Shechter 2010). A definitive role of biomarkers in clinical practice has not been unequivocally accepted. As a novel approach to identify specific AD biomarkers, it could be hypothesised that peripheral immune changes could be a suitable and specific AD biomarker. Several reports showed the existence of Abeta1-42-responding T cells in the peripheral blood of AD patients (Monsonego, Zota et al. 2003; Miscia, Ciccocioppo et al. 2009). The up-regulation of some PKC isoforms in T cells from AD patients has also been shown (Ciccocioppo, Lanuti et al. 2008; Miscia, Ciccocioppo et al. 2009). It could be observed that circulating T cells expressing high levels of P-PKC-delta and P-PKC-zeta after Abeta1-42-specific stimulation are present in AD patients. By applying a multicolour flow cytometry method, an association between some activation marker production, such as IL-2, INFgamma, TNFalpha and CD40L and up-regulation of P-PKC-delta and P-PKC-zeta levels following Abeta1-42 stimulation was found in peripheral blood from AD patients but not in healthy subjects. This suggests that T cells expressing bright levels of P-PKC-delta and P-PKC-zeta are Abeta1-42-specific, even if further characterisation of these T cells, in terms of phenotype and memory compartment, has to be addressed in future studies.

7. Discrimating AD from other forms of dementia: possible biomarkers?

After AD, Dementia with Lewy bodies (DLB) is the second most frequent cause of neurodegenerative dementia in the aged population. Neuropathologically, DLB is characterised by an accumulation of inclusion bodies (Lewy bodies) consisting of aggregated alpha-synuclein (Francis 2009). It is generally recognised that the clinical differentiation between AD and DLB is at best difficult (Geser, Wenning et al. 2005; McKeith, O'Brien et al. 2007). Thus, the identification of biomarkers would facilitate the differential diagnosis of AD and DLB. Even though Abeta deposition is identified in DLB brain (Town, Tan et al. 2005), there is no Abeta-triggered inflammatory activity in DLB. It could be postulated that Abeta1-42-specific T cells, expressing bright levels of P-PKC-delta and P-PKC-zeta are absent in DLB. In addition, preliminary experiments were carried out on some patients affected by two other different forms of amyloidopathies, used as controls: inclusion body myositis (IBM; n = 3) and cerebral Amyloid angiopathy (CAA; n = 5) patients. IBM is a rare, chronic and slowly progressing inflammatory myopathy, characterised by T cell invasion of muscle fibres (Askanas and Engel 2002). In IBM the peripheral accumulation of Abeta1-42 plays a critical role in skeletal muscle degeneration (Kitazawa, Green et al. 2006). CAA is instead characterised by predominant deposition of the Abeta1-40 fragment in cerebral blood vessels (Preston, Steart et al. 2003) and it is generally not associated with inflammation. P-PKC-delta and P-PKC-zeta bright T cell subsets were detectable in all IBM patients, while patients with CAA, mainly expressing the fragment Abeta1-40, do not show a similar T cell activation. These data indicate that Abeta1–42 does not induce P-PKC bright subpopulation in T cells from non-AD, neurodegenerative diseases.

8. Conclusion

All thoghether these hypotheses could demontrate that in AD patients, CD4+ Abeta1-42-specific T cells expressing high levels of P-PKC-delta and P-PKC-zeta could represent a

peripheral footprint of an Abeta1-42-mediated inflammation in the brain, related to protein deposition observed in AD. The presence of Abeta1-42-specific T cells, expressing bright levels of P-PKC-delta and P-PKC-zeta, might support clinical diagnosis of AD versus other forms of dementia.

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13

The Predictive Role of Hyposmia in Alzheimer's Disease

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1. Introduction

Loss of olfactory function starts at 60 years and become significantly worse after 70. In many cases olfactory disorders may be a consequence of a disease. Different types of olfactory deficit may be revealed by smell evaluation. *Anosmia* is defined as inability to perceive all odors (total) or some odors (partial). *Hyposmia* or *microsmia* is a decreased sensitivity to odors. *Dysosmia* is a distorted smell percpeption. *Olfactory agnosia* is defined as failure to identify odors in presence of normal detection and discrimination. Olfactory allucinations are named *phantosmias*.

Many common diseases may compromise the sense of smell, permanently or temporaneally. The range of diseases causing olfactory disorders varies from the common cold to neurodegenerative diseases. Most common causes of olfactory loss are local nasal diseases (allergic rhinitis, nasal polyposis, sinus disease), head trauma, viral and bacterial infections of upper airways. Some neurodegenerative diseases like Alzheimer's disease (AD) and various forms of Parkinson's disease (PD) are accompanied, even from their earliest stages, by olfactory disorders.

Dementia is defined by the American Academy of Neurology as a progressive and permanent decline in cognitive function and affects nearly 15% of people who live up to 65 years and 35% of those who reach the age of 85. The Alzheimer's Disease International (ADI) in Alzheimer World Report published in 2010 provides that an aging population with dementia - the most common form is AD which is currently estimated to affect 35,6 million of people - will nearly double in 20 years to reach 66 million in 2030 with a higher concentration in poor countries leading to enormous social costs.

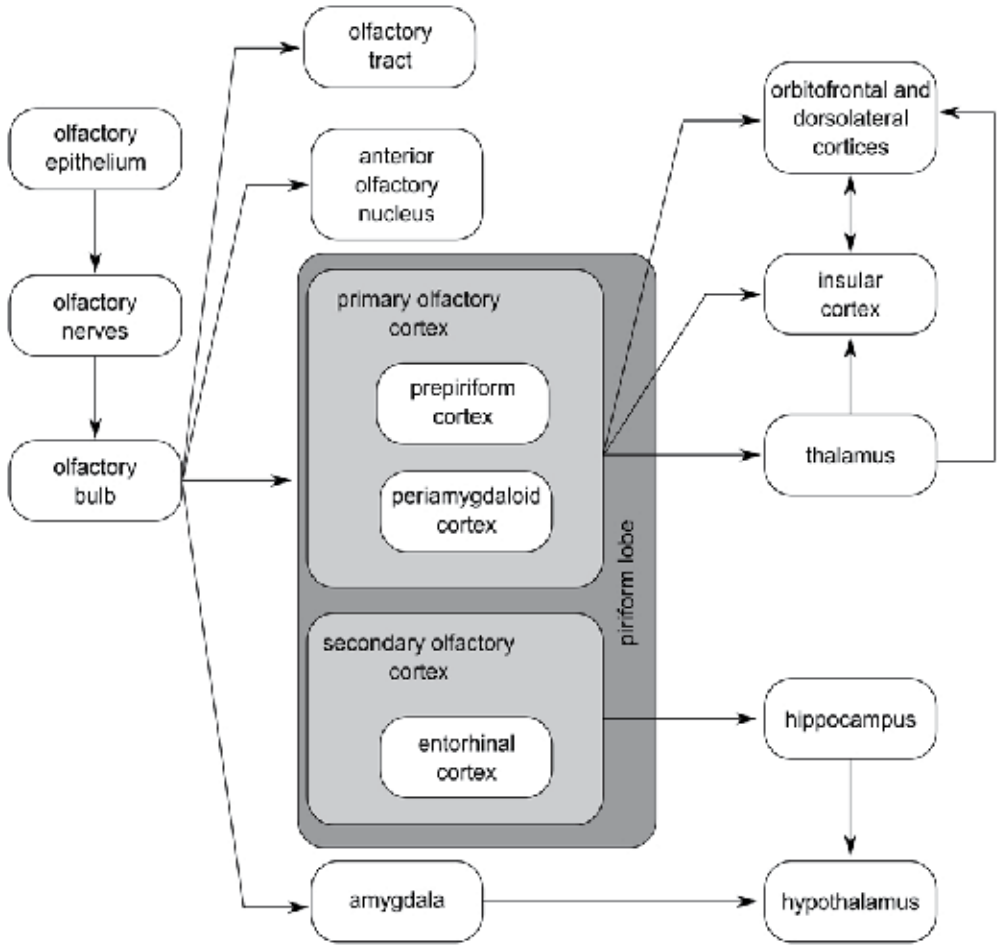
The research on AD is now oriented to an early diagnosis which is essential before the development of the irreversible and typical changes due to AD. In AD patients, a reduced capacity for olfactory detection, discrimination and identification is usually found and confirmed by several studies (Mesholam et al., 1998; Hawkes, 2003; Kovacs, 2004; Albers et al., 2006; Westervelt et al., 2007).

In this chapter we present a review on the predictive role of hyposmia in the early diagnosis of AD patients.

2. Central mechanisms of smell

Humans can detect more then 10.000 different odorants (Ressler KJ et al, 1994).

Odorants may be perceived through sniffing (ortonasal olfaction) and through the mouth (retronasal olfaction) (Heillmann S & Hummel T, 2004). The anatomical area covered by olfactory neuroepithelium is estimated between 100-400 mm^2 (Moran DT et al, 1982).
The human olfactory neuroepithelium is localized in the superior turbinate, in the dorsal areas of the nasal vault and in the superior part of the nasal septum. Olfactory mucosa is composed of olfactory sensory neurons (OSNs), supporting cells, basal cells and Bowman's glands.
The OSNs are bipolar cells with a dendrite that ends in a knob with 10-25 projecting cilia.
These cilia are covered by a layer of mucus which extends over the neuroepithelium and represent the site of sensory transduction. The cilia contain G-protein-linked receptors that bind to the odour molecules (Jones D & Reed RR, 1989): the expression of these receptors is still unclear.
These receptors are proteins encoded by a family of 1,000 genes in the mammals (Buck L & Axel R, 1991) while in the humans 60% of OR genes appear to be pseudogenes (Sosinsky A et al, 2000). The G-protein activates an adenylyl cyclase that converts ATP into the second messenger cyclic adenosine 3′ monophosphate (cAMP) which is the major messenger for olfactory signaling.
The cAMP opens a cyclic nucleotide-gated (CNG) channel. Cations (Na+ and Ca2+) entering through the CNG channels cause a membrane depolarization and generate an action potential which is propagated along the olfactory axon. The olfactory axon crosses the lamina propria and became an unmyelinated axon that penetrates the foramina of the cribriform plate and synapses in the glomerulus of the olfactory bulb where signals are integrated. In the glomeruli, the axons of the olfactory sensory neurons form synapses with the dendrites of the mitral and the tufted cells (second-order of neurons). The axons originated in the mitral and tufted cells leave the olfactory bulb and project to the olfactory tract, to the anterior olfactory nucleus, to the piriform lobe (prepiriform cortex, periamygdaloid cortex and entorhinal cortex) and to the limbic system (amygdala and hippocampus).
The prepiriform cortex and the periamygdaloid cortex represent the primary olfactory cortex while the entorhinal cortex, which represents the secondary olfactory cortex, receives olfactory fibres from the primary olfactory cortex (Graph 1).
When medication molecules come in contact with this specialized olfactory mucosa they are rapidly transported directly into the brain and achieved cerebrospinal fluid levels (faster than if the drug is given intravenously).
This concept of molecules transfer from the nose to the brain is referred to the so called nose-brain pathway and has implications when centrally acting medications such as sedatives, anti-seizure drugs and opiates are delivered nasally (Hussain AA, 1989; Dale O et al., 2002; Westin et al, 2006).
In this way the absorptive surface is not the intestinal mucosa so the drug is not subjected to hepatic metabolism and lead to early effects. Recently, Dale O (Dale O, 2010) indicated Intranasal fentanyl spray (INFS) as an effective treatment of breakthrough cancer pain; this formulation has been investigated as a new and convenient route of administration that may offer a rapid onset and short duration of analgesic effect.
Many studies based on the functional imaging in humans, point to the role of the piriform cortex in odor classification and differentiation (Li et al. 2006; Howard et al. 2009).



Graph 1. Central olfactory pathways of the olfactory system

The role of temporal lobe structures in olfactory memory was investigated (Dade LA et al., 2002) by the convergent approach of examination of odour learning and memory in patients who had undergone resection from a temporal lobe (including olfactory regions) for the treatment of intractable epilepsy, as well as studying these aspects in the healthy brain, through the use of the PET.

Some studies have also examined olfactory memory in patients with epilepsy before and after surgical intervention in different brain regions, and only subjects with resection within temporal lobe and orbitofrontal regions have shown impairments (West SE& Doty RL, 1995).

The piriform lobe may have an active role not only in odour perception but also in odour memory processing without hemispheric superiority among patients with resection from the left or right temporal lobe regions (Dade LA et al. 2002).

2.1 Diagnostic approaches for detecting olfactory disorders

An accurate evaluation starts with an history to establish the type of olfactory disorder (hyposmia, anosmia), the onset (rapid, slow) and progression, the presence of concomitant

nasal diseases, previous trauma and neurological symptoms, occupational exposure, medications, alcohol and tobacco consumption. A nasal examination is necessary to exclude signs of nasal diseases.

Sophisticated investigations like computed olfactometry and electrophysiological tests are available. Computed olfactometry provides the precision of the stimulus presentation and data collection but it is expensive and it requires a long time for administration. That is why it is restricted to specialized centers. The electrophysiological tests such as the odor event-related potentials evaluate the integrated electrical activity at the surface of the scalp but require a complex stimulus presentation and recording equipment.

For this reason in the last 20 years many practical and reliable psychophysical tests of olfactory function have been developed and largely diffused (Table 1).

Olfactory tests	Olfactory function tested
University of Pennsylvania Smell Identification Test (UPSIT)	Identification
Sniffin' Sticks (SS)	Identification, discrimination, threshold
Cross-Cultural Smell Identification Test (CC-SIT)	Identification
Quick Smell Identification Test (Q-SIT)	Identification
Odorant confusion matrix	Identification
Biolfa olfactory test	Identification
Brief Smell Identification Test (B-SIT)	Identification
Smell diskettes test	Identification
Smell Threshold Test (STT)	Threshold
T&T Olfactometer	Threshold
Olfactory Perception Threshold Test (OPTT)	Threshold
Sniff Magnitude test	Psycolfaction
12-item Odour Memory Test	Odorant Memory, discrimination

Table 1. Olfactory tests and respective olfactory function tested

Most of modern olfactory tests are brief and easy to use. Unilateral test with occlusion of the nostril controlateral to the tested side is preferred to evidence a monolateral anosmia which can be undervalued with a bilateral test.

In regard to cross-cultural differences in olfactory assessment, several olfactory tests have been proposed over the years to study smell function and its quantifiable parameters such as threshold, identification, discrimination and memory.

The *odor threshold* test measures the lowest concentration of a stimulus that can be discerned. Modern olfactory tests evaluate the detection threshold which is the lowest odorant concentration where such a presence is detected but not recognized (Stevens JC et al., 1988; Kobal G et al., 2000).

The *odor discrimination* test evaluates the ability to differentiate between odorants and requires the subject to decide whether two stimuli are similar or different (Kobal G et al., 2000)without requiring the identification.

The *odor identification* test evaluates the subject's ability to identify an odorant at the supra-threshold level. The multiple-choice identification test is the most sensitive and specific procedure to assess identification. In this type of test the subject identifies the stimulus from a list of odor names (Doty RL et al., 1984; Cain WS et al., 1988; Simmen G et al., 1999; Kobal G et al., 2000).
To evaluate *odor memory*, the subject is required to smell an inspection odorant and to select the same odorant from a set of alternatives choices after a delay period of 10, 30 or 60 seconds (Campbell IM et al., 1972).
Several threshold tests are available like the Smell Threshold Test (Doty RL, 2000), the odor threshold test of Sniffin' Sticks (Hummel T et al, 1997) and the T&T olfactometer (Toyota B et al. 1978).
In most cases a marked variability in threshold values depends on the techniques of stimulus presentation, interstimulus time, method of stimulus dilution, number of trials presented, confusion of the subject between detection and recognition of the stimulus.
Currently the most widely identification tests used are screening tests, as the Brief Smell Identification test (Doty et al., 1995) (B-SIT) and the Sniffin 'Sticks Screening Test (Kobal G et al.,1996;Hummel et al., 2001) (SSST) (Burghart GmbH, Wedel, Germany) and complete tests as the University of Pennsylvania Smell Identification Test (Doty et al., 1984) (UPSIT) (Sensonics, Inc., Haddon Heights, New Jersey, USA) and the Sniffin 'Sticks Extended Test (Hummel et al., 1997) (SSET). Therefore it is recommended a full assessment of respiratory function with naso-pharyngeal nasal endoscopy and rhinomanometry. After examination, further diagnostic investigations such as citology, CT or MRI of the nose and paranasal sinuses, may be prescribed to verify an insufficient ventilation in the presence of nasal or sinus diseases which make the olfactory test less reliable by substantially reducing its specificity and sensitivity.

2.1.1 Sniffin' Sticks

The SSST is a test of olfactory identification by the administration of 12 odors presented in felt-tip pens (sticks) (Hummel et al., 2001). Few hours prior to the test food intake is limited only to water. The subject is asked to identify among 4 written names of different odours the one smelled on a specific single odour stick. Based on the final score, adjusted per age and sex, subjects are classified in three categories: normosmic (>12) hyposmic (< 10) and anosmic (< 6). As opposed to neuropsychological tests the smell test is not influenced by the level of schooling. Our experience in administering the SSS test also showed that almost all of the test odors are familiar to Italian subjects except for cloves. In fact 23 of 102 normal subjects tested by us with the SSS test didn't know the cloves but they correctly identified it with the aid of the four possible answers.
The SSET provides for the assessment of olfactory identification, discrimination and threshold (Kobal et al., 2000).
The olfactory identification is evaluated with 16 odors which are presented to the patient using a 4-alternative forced-choice task with presentation of a list of 4 descriptors for each pen (normal value: ≥12 correct identifications).
The olfactory threshold is achieved by presenting the patient 16 triplets of sticks: only one of three sticks contains a smell, not the other two and the patient must recognize that stick smells unlike the other 2. The 16 odors presented to the patients for the threshold, are 16 dilutions of n-butanol. Odor threshold was represented by the mean of the last 4 out of 7 staircase reversals (normal values: >6 for men, >6.5 for women).

For the assessment of olfactory discrimination 16 triplets of odors are presented to the patient. In each triplet two sticks have the same smell and a stick has a different smell: the patient must recognize the stick with different smell (normal value: ≥11 correct discriminations). The execution time of SSET varies from 25 to 45 minutes.
The sum of the three scores in the evaluation of threshold, discrimination and identification gives us the total score (TDI score) and classifies the subject as normosmic (> 30.5), hyposmic (≤ 30.5) or anosmic (≤ 15.5)(Hummel et al., 2007).
The SS test is largely used to evaluate olfactory dysfunction in neurodegenerative disorders like Parkinson's disease (Daum RF 2000), Alzheimer and MCI (Peters JM et al., 2003).

2.1.2 UPSIT

The UPSIT is a scratch and sniff test used in North America since 1984 (Doty et al., 1984). The UPSIT is a multiple-forced-choice odor identification test available in 11 languages. For each odorant there are four alternative responses and the subject is required to choose one of these even if no smell is perceived. It requires 10 to 15 minutes to be administered. This test consists of 40 odorants at the supra-threshold level embedded in microencapsulated crystals in four booklets, each containing 10 odorants. Every odorant is located on brown strip that is "scratched" with a pencil. The UPSIT detects most olfactory disorders (anosmia, severe microsmia, moderate microsmia, mild microsmia) and also identifies malingerers on the basis of improbable responses. Malingerers avoid the correct response more often than expected on the basis of the chance (zero score detects a malingerer). The test-retest reliability is high (r=0.92) (Doty RL et al., 1989). Normative data for the UPSIT include a score on a scale of 0-40 to evaluate olfactory dysfunction and percentile ranks for men and women across the entire age span.
Some odorants of the UPSIT as root beer, skunk, fruit punch and pumpkin pie may be unfamiliar to patients outside of the USA. Although the UPSIT is a self-administered test, we underline the great importance of a ENT evaluation before each test administration because many pathologies involving nose and paranasal sinuses can interfere with the mechanical transport of the odorants to the olfactory areas with the consequent test failure.
The 40-item UPSIT has been used to test olfactory dysfunctions in many neurodegenerative disorders like AD (Doty RL et al., 1987; Hawkes C. 2003), MCI (Devanand DP et al., 2000, Wang QS et al., 2002), Parkinsonism (Hawkes C. 2003), multiple sclerosis (Doty RL et al., 1999).

2.2 General diseases causing olfactory disorders

Few studies have investigated the real prevalence of olfactory disorders in the population (Deems DA et al., 1991). According to a recent study the prevalence of measured olfactory impairment is 24.5% overall, but among elderly people it can be high as 70% (Murphy C et al., 2002). Disorders of the sense of smell are caused by conditions that interfere with the access of the odorant to the olfactory neuroephytelium (transport loss), injure the receptor region (sensory loss), or damage the central olfactory pathways (neural loss). Smell disorders may be typically intermittent or permanent. When the smell disorder is intermittent there are usually some conditions that interfere with the access of the odorant to the olfactory neuroephytelium and in these cases we suppose a transport loss. Most frequent causes of transport loss are nasal diseases and polyposis. The onset of hyposmia is more gradual and intermittent, the recovery is possible with an adequate medical or surgical treatment. Conversely, a permanent olfactory dysfunction may be secondary to an injure of

the receptor region when there is a sensory loss (like in prior upper respiratory infections and toxic exposure) or secondary to a damage of the central olfactory pathways in case of neural loss (like in head trauma). A temporary hyposmia often occurs with a prior upper respiratory infection but in a small percentage of cases olfaction never returns. Temporary or permanent hyposmia due to toxic exposure can occur through modification of neurotransmitter levels or anatomical damage to the olfactory receptor. Even a minor head trauma can produce a total anosmia and recovery occurs in fewer than 10 %, most occurring several months. Olfactory impairment is a common occurrence in aging and may be an early signal of neurodegenerative disease. Olfactory loss caused by aging and diseases effects both quality of life and personal safety. Aging as well as prior upper respiratory infections, head trauma (approximately 5-10% of adult patients with head trauma report olfactory loss to be in anosmic range) and nasal and/or sinus diseases lead to smell dysfunction but frequently the cause of the olfactory loss remains unknown (idiopathic smell dysfunction). Disorders of olfactory function have been also associated with the exposure to toxic chemicals, tobacco smoking, endocrine disorders (hypothyroidism, diabetes, Kallmann's syndrome, Cushing syndrome) and neurodegenerative diseases (Table 2). Among these, Alzheimer's disease (AD) is one of the earliest to be reported and studied in detail.

Nasal/sinus diseases
Head trauma
Prior upper respiratory infections (viral, bacterial)
Idiopathic
Toxic exposure (drugs, airbone compounds like metals, dusts, ecc.)
Neurodegenerative diseases
Congenital
Endocrine disorders (hypothyroidism, diabetes, Kallmann's syndrome, Cushing syndrome)
Tumors (olfactory groove meningioma, temporal lobe glioma, nasopharyngeal carcinoma)

Table 2. Main disorders associated with smell dysfunction

2.3 Clinical and pathological features of hyposmia in different CNS diseases

Hyposmia is one of the markers of a future cognitive decline but it is not specific for AD because it can also precede other neurological diseases, like PD and multiple sclerosis (Table 3).

Although genetic predisposition in undoubtedly relevant in AD (older people with Down syndrome inevitably developed AD) environmental agents cannot be ignored (Table 4).

Particular attention should be paid to recent theories suggesting the olfactory neuroepithelium as a major point of invasion by external pathogens such as viruses, ionized metals (cadmium, gold, manganese) and nanoparticles to the central nervous system (Charles et al., 1995; Itzhaki et al., 2004; Doty, 2008).

Alzheimer's disease	Mild Cognitive Impairment
Idiopathic Parkinson's disease	Guam Parkinson's disease-dementia complex
Dementia with Lewy bodies	Down syndrome
Amyotrophic lateral sclerosis	Huntington's disease
Multiple sclerosis	Motor neuron disease
Pallidopontonigral degeneration	Korsakoff's psychosis
Multiple system atrophy (type-P)	Friedreich's ataxia

Table 3. Neurodegenerative diseases associated with olfactory dysfunction

Viruses
Toxic metals (cadmium, iron, manganese)
Air pollutants
Herbicides (rotenone)
Defoliants

Table 4. Possible environmental agents implicated in the "olfactory vector hypothesis"

The "olfactory vector hypothesis" suggests that certain viruses could be transported directly from the olfactory epithelium to the olfactory bulb and the central regions of the limbic system, without intermediate synapses, not causing damage to the epithelium, but only using this route to reach the brain (Youngentoub et al., 2001).
The "olfactory vector hypothesis" may explain how some neurodegenerative diseases like AD and PD may be caused by external patogenes that damage the olfactory system and enter the brain through the nose possibly in accordance with genetically determined substrates (Youngentoub et al., 2001).
Different brain cells may be infected by viruses in the presence or absence of certain specific receptors to which the virus would bind. However the entry of a pathogen through the nose seems more feasible as a cause of PD than AD.

2.3.1 Alzheimer's disease

In the preclinical phase which can precede over decades the disease, the presence of typical lesions of AD as amyloid plaques and neurofibrillary tangles have been demonstrated, even at the level of brain areas involved in olfactory function. Until few years ago it was thought that the beta-amyloid, the first 42 aminoacids of the amyloid precursor protein (APP), was just an inactive storage without biological activity. Recent researches have shown that the Aβ-42 provides cellular cascades and it's an initiator of the degeneration of neurons. The hyperphosphorylation of tau protein from which neurofibrillary tangles originate would be determined by the action of Aβ42. Autoptic studies conducted in the olfactory neuroepithelium in the past proposed the hypotesis that the diagnosis of AD would be confirmed by the finding of typical lesions at this periferic level (Lovell et al., 1982; Talamo et al., 1989; Arnold et al., 2010). However, following studies showed that these changes were not specific for AD because they where similar to those found in other neurodegenerative

diseases and in control elderly subjects (Trojanowski et al., 1991; Kishikawa et al., 1994; Smutzer et al., 2003). The presence of amyloid plaques and neurofibrillary tangles has been widely described in all layers of the olfactory bulb and in the central olfactory pathway of patients with AD (Kovacs et al., 2001) with a significant association between the peripheral olfactory and cortical degenerative changes (Christen-Zaech S et al., 2003). However it is not yet determined whether the olfactory involvement first appears at peripheral levels, in the olfactory bulb or in the temporal cortex.

There is a strict relationship between the loss of cells in the anterior olfactory nucleus, the development of anosmia, the extent of neurofibrillary degeneration and the severity of AD. It has long been known that the typical lesions of AD are early and selectively localized in rhinencephalon. This is not surprising when one considers that the rhinencephalon plays a key role in all processes of memory and that the first symptom in AD, the most important and included in all diagnostic systems as deemed necessary, is the early deficit of memory.

Some authors point out in many patients with AD the presence of early anosmia with slowly progressive deterioration of cognitive disorder. According to some authors, the olfactory deficit in AD could be based on a genetic predisposition. The allele ε4 of apolipoprotein E gene is a known genetic marker of hereditary AD with a low prevalence and it is the subject of ongoing researches. Schiffman (Schiffman SS et al, 2002) found that at-risk relatives of AD patients had higher detection thresholds and decreased odor memory than control subjects with an ApoE-4 status not associated with at-risk status. Conversely Graves (Graves et al., 1999) showed an increased risk of cognitive decline in patients with olfactory deficit and allele ε4, greater in males but with a trend also evident in females.

The transentorhinal area is early impaired in AD and it would be the point of passage of the sensory cortical afferents to the hippocampus, followed by the involvement of the anterior parahippocampal cortex or the entorhinal area (Braak&Braak, 1998).

In general terms, therefore, the entorhinal cortex receives a constant flow of informations from cognitive and sensory associative areas, that move towards the hippocampus to recover in a consolidated form. These informations are transmitted to the associative areas where they are encoded in the form of memory traces.

In contrast with the hypothesis of early involvement of the transentorhinal area, Kovacs (Kovacs, 2001) argues that neurofibrillary tangles are present in the anterior olfactory nucleus prior to the first changes observed in the entorhinal cortex.

The primary olfactory cortex would also be less severely affected than medial orbitofrontal cortex (associative area) and there would be a correlation between the pathology of the olfactory bulb and some areas not involved in olfactory processes.

2.3.2 Parkinson's disease

PD is a progressive neurodegenerative disease characterized by a loss of dopaminergic neurons in the substantia nigra. In the majority of cases it's clinically diagnosed as idiopathic Parkinson's disease (IPD). In the IPD the impairment of smell has been well documented in the early stages with the use of psychophysical tests (Doty et al., 1992; Daum et al., 2000, Muller et al., 2002; Ponsen MM et al., 2004; Haehner et al., 2007) and olfactory evoked response (Barz et al., 1997).

Olfactory dysfunction is a non-motor symptom of PD which includes deficits in odor detection, discrimination and identification without any relationship with the duration or severity of parkinsonism and with the nigrostriatal dopamine depletion.

As confirmed by autoptical studies (Beach TG et al., 2009) the presence of α-synucleinopathy in the olfactory bulb predicts, with greater than 90% sensitivity and specificity, the existence of neuropathologically confirmed PD.
Recent findings support the association between anosmia and autonomic failure, like orthostatic hypotension, in sporadic PD so they might provide biomarkers of the pathogenetic process. (Kaufmann H et al., 2004; Goldstein D et al., 2010).
Once IPD has become clinically manifest, olfaction appears to be already severely compromised, which is in line with the absence of longitudinal changes of olfactory function during IPD progression (Herting et al. 2008).
In a recent study (Wattendorf E et al., 2009) gray matter atrophy was investigated using morphometric analysis of magnetic resonance images voxel-based morphometry (VBM) and it was related to psychophysically measured scores of olfactory function in early PD patients, moderately advanced PD patients and age-matched healthy controls. Cortical atrophy in olfactory-related brain regions (mainly in the right piriform cortex in early PD patients and in the right amygdala in moderately advanced PD patients) was shown in PD patients, but not in controls, and it was specifically correlated with the olfactory dysfunction.
A selective hyposmia in PD patients correlated with hippocampal dopamine innervation as shown by DAT (dopamin transporter) PET binding was also demonstrated (Bohnen NI et al., 2008). These findings support the hypothesis of selective deficits in odor identification correlating with dopaminergic activity in the hippocampus, an area related to cognitive and memory processing.
Dopamine replacement therapy, does not improve olfaction in PD, suggesting that hyposmia cannot be explained by dopamine deficiency alone (Doty RL et al.,1992).

2.3.3 Multiple sclerosis

Multiple sclerosis (MS) is a demyelinating disease of the central nervous system. In MS (Doty et al., 1999) the olfactory abilities decline progressively over the course of the disorder. The presence of olfactory dysfunction in MS is confirmed by studies with UPSIT and olfactory evoked potentials (Hawkes CH et al., 1997) and with Sniffin' Sticks (Fleiner F et al., 2010). An important role could be entrusted to the association of olfactometric and neuropsychological measurements with imaging.

2.3.4 Epilepsy

The presence of olfactory deficits is also well known in epilepsy. Chen (Chen C et al.2003) in a study on patients who underwent temporal lobectomy for medically intractable temporal lobe epilepsy (TLE) found olfactory auras in 12 (5.5%) patients. The conclusion of this study is that the mesial temporal sclerosis is the most common aetiology rather than tumors and that the mesial temporal structures, especially the amygdala, may play an important role in the genesis of olfactory auras.

2.4 The role of olfactory test in Alzheimer's disease

The olfactory identification tests are considered more sensitive and specific in distinguishing patients with AD from control subjects. Although it is not possible to determine how early the olfactory deficit appears in the course of AD, it is well established that its presence is an early and consistent element before the clinical diagnosis (Devanand et al., 2000; Royall et

al., 2002; Swan et al., 2002; Wilson et al., 2009). Some studies highlighted the increase in olfactory threshold in AD subjects rather than in the control group (Nordin et al., 1997) while other studies showed that the olfactory identification is compromised earlier than the perception.

Anosmia, alongside at least one allele of Apo-E 4 may be associated with a risk five times higher to develop a subsequent cognitive decline (Graves et al., 1999). Wilson et al. (Wilson et al., 2007a) in a recent study showed a deficit of olfactory identification inversely related to AD (in particular with NFT in the entorhinal cortex and hippocampus) in elderly patients. The autopsy outcomes suggested that in elderly the olfactory identification deficit is partly due to the accumulation of NFT in the primary olfactory cortex.

Hyposmia can predict the next start of mild cognitive impairment (MCI) in patients with a not yet measurable cognitive impairment (Wilson et al., 2007b) and an increased risk of AD was demonstrated in MCI patients with hyposmia associated with unawareness of the olfactory deficit (Devanand et al., 2000). Conversely Bahar-Fucks et al. suggest that unawareness of olfactory deficit does not improve the identification of patients with MCI progressing to AD (Bahar-Fuchs et al., 2011).

Hyposmia incurs with a latency of less than 10 years since the beginning of clinical manifestations of the disease in patients with AD and autosomal dominant mutation of the presenilin-1(Nee et al., 2001). Another explanation could be that the olfactory deficit is predictive only of sporadic forms of AD.

In a recent study (Luzzi et al., 2007) the smell was measured in patients with mild semantic dementia, with frontotemporal dementia, with corticobasal degeneration and with mild AD. As expected, patients with AD showed lower scores in the discrimination, denomination and visual recognition. Patients with semantic dementia showed a normal discrimination but a marked reduction in denomination and olfactory agnosia. In patients with frontotemporal dementia and corticobasal degeneration there was a slight deficit of naming and discrimination.

Compared to the olfactory tests the role of olfactory event-related potentials (OERPs) is considered useful in the diagnosis of AD (Morgan&Murphy, 2002).

The results of olfactory tests are supported by functional imaging techniques (CT, MRI, PET) which show a reduced activation of the central olfactory structures (Wang et al., 2010), mainly on the right side (Kareken et al., 2001) and an atrophy of hippocampus (Jack et al., 1992;Yousem et al., 2001) and olfactory bulb (Thomann et al., 2009).

Murphy et al. (Murphy et al., 2003) studied olfactory function (odor threshold and odor identification) and volumetric MRI measures of mesial temporal areas (hippocampus, the parahippocampal gyrus and the amygdala) in patients with probable AD. They found strong relationships between mesial temporal lobe volumes and olfactory functional measures, particularly between the left hippocampal volume and the performance on the odor identification task so they concluded a left-hemisphere prevalence for verbally mediated olfactory tasks.

2.5 The role of olfactory test in Mild Cognitive Impairment

In a recent study we highlighted the role of olfactory test in early diagnosis of dementia (Fusetti et al, 2010).

The early identification of those patients which can develop a dementia before its clinical appearance has become a priority since they could benefit from therapeutic and preventive

options. For this reason in our researches we selected patients with Mild Cognitive Impairment (MCI).

The term MCI defines a transient condition that occurs along the progression from normal aging to dementia and comprises a broad clinical spectrum of pre-dementia stages. Standard MCI diagnostic criteria are the following:

1. subjective symptoms of memory loss
2. pathologic performance in mnemonic testing in relation to age and level of schooling
3. normal activities of daily living
4. normal cognitive functions
5. absence of dementia
6. lack of other diseases which impair or may alter memory (Petersen et al., 2001).

The discriminant role of the olfactory test was determined by the outcomes of a study on a group of patients with amnesic Mild Cognitive Impairment (aMCI) which is the category with the higher risk of conversion in AD (Peters et al., 2003). aMCI progresses to AD with a prevalence of 15% annually and they are identified clinically with neuropsychologic testing to determine isolated memory loss.

In our study 29 patients diagnosed with aMCI were selected and reassessed at 18 months (T1) after the first visit (T0). Exclusion criteria were considered neuropsychiatric disorders different from aMCI (Parkinson's disease, schizophrenia, multiple sclerosis and depression), head trauma (with loss of consciousness greater than 15 minutes), maxillofacial surgery, rhinosinusitis, nasal polyposis, chronic obstructive pulmonary disease, asthma, active hepatitis, cirrhosis, chronic renal failure, vitamin B12 deficiency, alcohol and drug abuse, cerebral vascular accidents, insulin dependent diabetes mellitus, hypothyroidism and Cushing syndrome.

The patients underwent an assessment by the SSST and the SSET and a neuropsychological evaluation using the Mini Mental State Examination (MMSE) and the Mental Deterioration Battery (MDB).

The MMSE is a quick, simple and reliable screening test used to explore cognitive functions and to evaluate disease progression. However its applicability in differential diagnosis shows limitations as well, due to the low reliability in individualizing specific profiles of cognitive deficit. The MMSE requires 5-10 minutes and it provides a score between 0 and 30 points adjusted according to the age and years of education in the Italian population. The MMSE score >24 was used as cut-off to distinguish between MCI and early dementia.

The MDB discriminates with a high degree of accuracy AD patients from normal aging subjects and it provides qualitative informations on the cognitive deficit. The MDB is a battery including seven tests that measures memory function and other cognitive abilities. This test is easily reproducible and lasts between 45 and 75 minutes.

Further inclusion criteria were the diagnosis of "questionable demented" (score=0.5) according to the CDR. Depressive symptoms were rated using the GDS with a score > 6 as cut off to discriminate patients with depression. All patients underwent CT scan or MRI of the brain to assess the presence and degree of cerebral atrophy and causes of secondary dementia.

Patients with aMCI showed a lower score in the ability of olfactory identification and discrimination worse than 18 months from first visit. The percentages of normosmic, hyposmic, anosmic patients at T0 and T1 based on the mean TDI score are reported in Table 5. We demonstrated that all patients (100%) who developed AD were hyposmic at T0 while

of 20 patients who didn't develop AD 12 (60%) were hyposmic and 1 (5%) was anosmic based on TDI score.

aMCI patients	*T0*	*T1*
9 with AD, n (%) Score on TDI	9 hyposmic (100%)	9 hyposmic (100%)
20 without AD, n (%) Score on TDI	7 normosmic (35%) 12 hyposmic (60%) 1 anosmic (5%)	5 normosmic (25%) 14 hyposmic (70%) 1 anosmic (5%)

Table 5. Percentages of normosmic, hyposmic, anosmic patients at T0 and T1 based on the mean TDI score

The correlation between individual neuropsychological tests of the MDB and the 3 olfactory functions (threshold-discrimination-identification) evalueted with the SSET was examined with the Spearman correlation.

The most statistically significant correlations were found between olfactory discrimination and copying designs with elements of programming, between olfactory discrimination and verbal fluency, between olfactory discrimination and immediate recall of Rey's 15 words and between olfactory identification and delayed recall of Rey's 15 words.

The olfactory test has been shown to be sensitive and in some cases statistically more reliable of neuropsychological tests such as the MMSE. Another fact that emerges from the results of our study is that the olfactory test appears to allow a prediction of conversion to AD in aMCI who show a worsening of 'olfactory identification at follow-up. 9 of 29 patients with aMCI (31%) developed AD, and all had a worse olfactory deficit to 18 months from the first survey showing also unawareness of their olfactory deficit.

This finding confirms the results of Devanand stating that the olfactory deficit associated with unawareness increase the risk of progression to AD.

Our data show an early olfactory deficit in aMCI patients and suggest to introduce the study of the smell in early evaluation of aMCI patients. At follow-up we evidenced a more rapid cognitive than olfactory decline but they must be confirmed on the basis of further studies on a larger sample population. We also demonstrated a significant progression from hyposmia to anosmia in aMCI patients which have developed AD after 18 months. This suggested the validity of the olfactory test as a possible early diagnostic marker of AD. Moreover we found in the 20 aMCI patients that did not developed AD an increase of the mean MMSE score at the 18 months follow-up. These findings is in concordance with epidemiological studies which suggest that the progression of MCI is heterogeneous and may be reversible, stable or progress to AD (Ritchie K et al., 2001; De Carli C., 2003). Förster et al. have instead shown in patients with early AD studied with SSET and FDG-PET a correlation between the scores in the identification, discrimination and olfactory threshold and different areas of brain activation (Förster et al., 2010). In our studies we confirmed the accuracy of the olfactory test to find an impairment of the olfactory identification function in the early stages of AD.

3. Conclusion

The olfactory tests are a useful and reliable diagnostic aid for the identification of olfactory deficit. They are easy to use, with a low cost, they don't take too much time for the administration and they are not influenced by the level of education. The ideal test should overcome the problem of the different ethnic and currently the most widely used standardized tests in clinical practice and research are the UPSIT and the SS. For a first rapid evaluation the screening identification test can be used but in case of abnormal results or if cognitive disorders are suspected a complete test is strongly reccomended.

The only olfactory test is obviously not sufficient for the early diagnosis of AD but its high sensitivity is now accepted and should be included into a full battery of neuropsychological tests and other diagnostic aids (ApoE, PET or SPECT brain, functional MRI, etc..) commonly used to evaluate these patients.

The role of the ENT specialist in this area is very important as fundamental to ensuring the reliability of the olfactory test excluding with a careful selection of patients hyposmia secondary to malformations, acute and chronic inflammatory phenomena, allergy, exposure to irritants. We also recommend to avoid the self-administration expecially if the test is not for screening aims but is part of the clinical routine for diagnosis.

In conclusion, we can assert that the role of olfactory deficit in neurodegenerative diseases is still underestimated, mainly for the problem of an early and correct identification of a clinical disorder. Long-term studies can determine the real predictive value of hyposmia in AD and the efficacy of early treatment even on olfactory function.

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14

Retinal Nerve Fibre Layer Thinning in Alzheimer Disease

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1. Introduction

Alzheimer disease (AD) is a progressive neurodegenerative disorder characterised by impaired memory and cognitive function. A proportion of patients present with visual symptoms which could result from either anterior or posterior visual pathway dysfunction. Retinal and optic nerve abnormalities have in recent years been studied intensively in Alzheimer disease (AD) and are reviewed in this chapter.

2. Evidence of retinal nerve fibre layer thinning in AD

2.1 Histological evidence

Retinal ganglion cell (RGC) degeneration or optic neuropathy is one of the features of AD that has been identified in several histological, imaging, and electroretinogram (ERG) studies. Analyses of neuronal numbers in the RGC layer of severe AD patients and age-matched control subjects have revealed extensive neuronal loss throughout the entire retina in AD when compared with control eyes: the mean (RGC) number is 696,871 in AD which is significantly less than that of the controls (1,095,904) representing a loss of 36% ($p<0.004$) (Blanks et al., 1996b). The findings are in good agreement with other histological studies (Hinton et al., 1986; Sadun & Bassi, 1990; Trick et al., 1989) and compatible with diminished contrast sensitivity which may be secondary to afferent visual pathway dysfunction in AD patients (Crow et al., 2003). A post-mortem study revealed widespread optic nerve fibre degeneration with thinning of the retinal nerve fibre layer and reduced ganglion cell numbers in AD patients aged 76-89 years (Hinton et al., 1986), any potential correlation with the degree of cognitive impairment was not studied. In one study the axonal loss was prominent in the posterior part of the optic nerve suggesting that the process involved may be one of retrograde degeneration of the retinal ganglion cell axons (Sadun & Bassi, 1990). Neurons in the ganglion cell layer (GCL) in AD patients are reduced by 25-40% throughout the entire retina (Blanks et al., 1996b). The greatest density of RGCs is located in the macular region and the total numbers of ganglion cell fibres in the fovea/parafovea are reduced by 25% ($p < 0.001$) in AD as compared to normal eyes (Blanks et al., 1996a). The loss in the central retina is greatest in the temporal area, which is surprisingly different from that found in the periphery (Blanks et al., 1996a). The neuronal loss reaches its peak in the superior and inferior quadrants of the peripheral retina (Blanks et al., 1996b). Unlike what is found in normal ageing, the RGC loss in AD is not related to age (Blanks et al., 1996a, 1996b). It was also found that both small- and

large-diameter RGC fibres are affected equally throughout the retina in AD eyes in some studies (Blanks et al., 1996b; Curcio & Drucker, 1993) but only large M-cell degeneration has been identified by the others (Sadun & Bassi, 1990; Trick et al., 1989; Blanks et al., 1989, Miller, 1990). The total number of astrocytes in the GCL was found to be 16% greater in the AD patients than in controls but the increase did not reach statistical significance (Blanks et al., 1996b). The ratio of astrocytes to neurons in the GCL is significantly raised in the AD retinas, resulting from both an increase in astrocytes and the decrease in neurons (Blanks et al., 1996b). A study comparing patients with at least a 4 year history of severe AD aged 67-86 years and age-matched controls revealed no significant difference in the number of RGCs (Curcio & Drucker, 1993). This is not the only study to find no significant difference in RGC number between AD patients and controls (Davies et al., 1995). It is notable that there is no data whether AD patients in this review are familial or sporadic type.

2.2 Imaging evidence

By employing fundus photography, RNFL degeneration was observed in AD patients as compared to age-matched control subjects (Hedges et al., 1996; Tsai et al., 1991). A study utilizing scanning laser ophthalmoscopy (SLO) has demonstrated a reduction of optic nerve fibres in AD patients when compared to age-matched controls (Danesh-Meyer et al., 2006). However, another SLO study showed no difference (Kergoat et al., 2001). It was shown that there was no significant difference in mean overall RNFL thickness and RNFL thickness in each quadrant between the AD and age-matched controls. The patients selected for this study were classed as mild to moderate dementia with a range of mini mental-state examination of 11-29 (mean 21.57) and a mean duration of 3 years. Optical coherence tomography (OCT) has been recently developed and has found wide application in neurology and ophthalmology both in the clinic and in research (Jindahra et al., 2009). It has been employed to measure RNFL thickness in several AD studies. In AD eyes, the mean of overall peripapillary (Parisi et al., 2001, 2003; Iseri, et a., 2006; Paquet et al., 2007; Lu et al., 2010; Valenti, 2007) and macular RNFL thickness (Paquet et al., 2007) as well as the mean of total macular volume (Paquet et al.,2007) measured by OCT are lower when compared with age-matched control subjects. It has been proposed that the retinal involvement might have occurred early in the course of the disease as the peripapillary RNFL loss is identified in mild cognitive impairment (MCI) (Paquet et al., 2007). The reduction of RNFL thickness is statistically significant in MCI (Paquet et al., 2007), mild AD (Parisi, 2003; Iseri et al., 2006; Paquet et al., 2007), and moderate to severe AD (Parisi, 2003; Iseri et al., 2006; Paquet et al., 2007), compared to controls. No difference was found between the results observed in MCI and mild AD patients (Paquet et al., 2007) but the measurements of RNFL thickness seen in moderate to severe cases are significantly thinner than those in MCI cases (Paquet et al., 2007). The RNFL thickness was found to be thinner than the controls in all four quadrants in MCI (Paquet et al., 2007) and AD cases (Parisi et al, 2001, 2003; Paquet et al., 2007). However in one study the temporal quadrant was unaffected (Iseri et al., 2006) and significant thinning was found only in the superior quadrant in mild to moderate AD cases compared with controls in a study and a case report (Berisha et al., 2007; Valenti, 2007). Yet another study, the RNFL was found to be significantly thinner than in normal subjects in the superior and inferior quadrants in AD patients and hence the patients no longer showed the double peak RNFL pattern (Lu et al., 2010). The thinning in the nasal and temporal quadrants did not reach statistical significance (Lu et al., 2010). It seems that the retinal damage due to AD may be localized preferentially to the vertical quadrants (Lu et al., 2010)

and as such may be considered to mimic the pattern seen in glaucoma (Figure 1, Table 1 & 2). By using OCT it has been shown that the reduction in total macular volume is highly related to the severity of cognitive impairment (MMSE) (Iseri et al., 2006).

Study	n	Country	OCT	Age Mean (± SD)	MMSE mean (± SD)
AD(Berisha 07)	9 subjects	USA	0CT 3000	74.3(±3.3)	23.8(± 5.1)
AD(Parisi 01)	17 eyes	Italy	Stratus 0CT	70.37(±6.1)	16.4 (11.27-19.05)
AD (Iseri 06)	28 eyes	Turkey	0CT 3000	70.1(±9.7)	18.5(±6.3)
AD (Lu 10)	22 subjects	China	0CT 3000	73(±8)	N/A
Healthy (Savini 10)	8 eyes	Italy	Stratus OCT version 4.0	N/A	N/A
Healthy (Berisha 07)	8 subjects	USA	0CT 3000	74.3(±5.8)	29.5(±0.5)
Healthy (Parisi 01)	14 eyes	Italy	Stratus 0CT	N/A	N/A
Healthy ((Iseri 06)	30 eyes	Turkey	0CT 3000	65.1(±9.8)	29.4(±0.6)
Healthy (Lu 10)	22 subjects	China	0CT 3000	68 (±9)	N/A
Healthy (Bowd 00)	30 eyes/subjects	USA	0CT 2000	N/A	N/A
Healthy (Bock 10)	405 eyes/203 subjects	Germany	0CT 3000	N/A	N/A
Glaucoma (Bowd 00)	29 eyes/subjects	USA	0CT 2000	N/A	N/A
OHT (Bowd 00)	28 eyes/subjects	USA	0CT 2000	N/A	N/A
Glaucoma (Bock 10)	39 eyes/22 subjects	Germany	0CT 3000	N/A	N/A

Table 1. Demographic data of subjects in the studies shown in Figure 1 (N/A = not available).

Study	refractive error	best VA	IOP (mmHg)	perimetry	disc appearance
AD(Berisha 07)	(-6) to (+6)	>=20/60	14.4± SD 4.2	N/A	vertical CDR 0.5±0.2
AD(Parisi 01)	(-3) to (+3)	>=8/10	<18	N/A	N/A
AD (Iseri 06)	(-3) to (+3)	>= 5/10	<22	normal	normal
AD (Lu 10)	N/A	logMar 0.4±0.2 LE	15.4±1.2 LE	N/A	CDR 0.53±0.2 (LE)
		0.33±0.19 RE	15.5±1.3 RE		0.5±0.16 (RE)
Healthy (Savini 10)	(-3) to (+3)	>20/25	<21	N/A	normal
Healthy (Berisha 07)	(-6) to (+6)	>=20/60	13.3±SD 3.5	N/A	vertical CDR 0.49±0.13
Healthy (Parisi 01)	N/A	N/A	N/A	N/A	N/A
Healthy ((Iseri 06)	(-3) to (+3)	>= 5/10	<22	normal	normal
Healthy (Lu 10)	N/A	logMar 0.65±0.3LE	15.4±1.4 LE	N/A	CDR 0.38±0.1 (LE)
		0.66±0.22 RE	15.3±1.3 RE		0.35±0.1(RE)
Healthy (Bowd 00)	N/A	>=20/40	<=22	normal	normal
Healthy (Bock 10)	N/A	N/A	N/A	N/A	N/A
Glaucoma (Bowd 00)	N/A	>=20/40	>=24	abnormal	glaucomatous changes
OHT (Bowd 00)	N/A	>=20/40	>=24	normal	normal
Glaucoma (Bock 10)	(-10) to (+6)	N/A	<22	N/A	N/A

Table 2. Ophthalmic examination of subjects in the studies shown in Figure 1

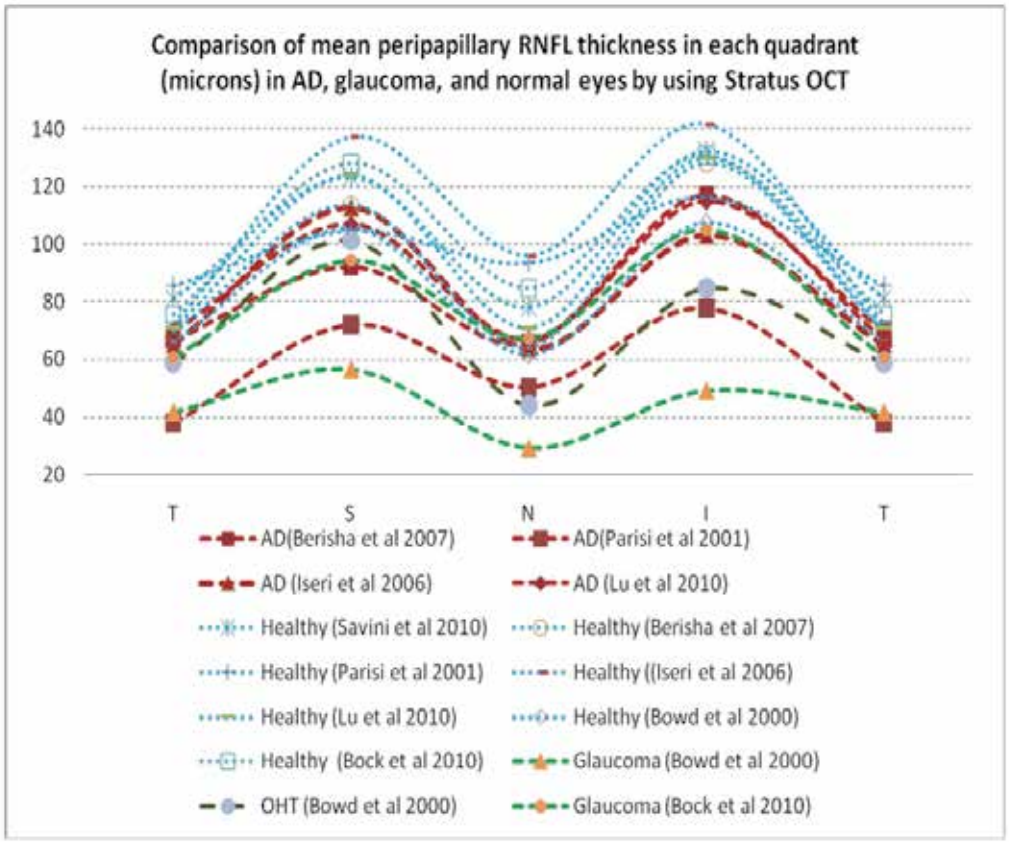

Fig. 1. A comparison of mean peripapillary RNFL thickness in each quadrant of AD, glaucoma, ocular hypertension (OHT) and normal eyes. See Tables 1 and 2 for details of the cohorts in each study.

2.3 Electrodiagnostic evidence

The results of electrodiagnostic testing are conflicting. ERG studies have failed to demonstrate changes in AD patients (Justino et al., 2001; Kergoat et al., 2001, 2002; Davies et al., 1995). Scotopic and photopic electroretinograms and oscillatory potentials in patients with mild Alzheimer disease were compared with normal individuals in one study (Justino et al., 2001). The amplitude and latency of a and b waves in mild AD patients were normal, reflecting intact function of the outer retina. The oscillatory potentials were also unremarkable in this study. Pattern electroretinogram (PERG) recordings have shown a significant delay in P50 and N95 implicit times and reduction in both P50 and N95 amplitudes in mild to severe AD when compared with the results obtained in control eyes (Parisi et al, 2001, 2003). This might indicate that the dysfunction lies in both ganglionic and preganglionic elements (Parisi et al, 2001, 2003). The delayed P50 and N95 implicit times and the reduced P50 and N95 amplitudes are significantly correlated with the reduced overall

mean of the RNFL thickness measured by OCT (Parisi et al., 2001, 2003). No significant difference was found in the latency of the pattern visually evoked potential (PVEP) P100 of AD patients and control subjects (Iseri et al., 2006). The normal PVEP responses revealed no evidence for any abnormality of primary visual cortex or of optic nerve function despite considerable RNFL loss (Iseri et al., 2006). However, some earlier studies did find abnormalities of the flash VEP (Wright et al., 1986; Norman et al., 1995) but probably not useful clinically (Coburn et al., 2003). However using PERG and PVEP, another study revealed a reduction in amplitude of N95 and increased latency of P100 wave in most AD eyes (Krasodomska et al., 2010).

2.4 Optic disc morphology in AD eyes

A large cup-to-disc ratio, thin rim area and volume are identified in AD eyes compared to age-matched normal eyes (Tsai et al., 1991; Danesh-Meyer et al., 2006). In one of these studies, the patients had a MMSE result of 21±4 taking an upper limit of the vertical cup-to-disc ratio as 0.42 gives a sensitivity of 0.45 and specificity of 0.84 (Danesh-Meyer et al., 2006). Pallor area to disc area ratio did not significantly differ between AD patients and normal subjects in one study (Tsai et al., 1991). However patients with a higher ratio had a higher Alzheimer disease assessment scale and longer duration of illness (Tsai et al., 1991). The changes are not in a uniform pattern for all AD patients (Berisha et al., 2007).

2.5 Information from Down syndrome

It has been shown that all adults with Down syndrome (DS) over 35-40 years old who had autopsies performed have AD pathology in their brains i.e., beta amyloid plaques and neurofibrillary tangles (Malamud, 1972). Amyloid precursor protein gene on the locus of the proximal part of the long arm of chromosome 21 is over-expressed in DS patients (Goldgaber et al., 1987), leading to AD development (Prasher et al., 1998). The DS brain pathology is comparable to AD brain and may be useful in further AD studies (Hof et al., 1995). Regarding visual functions, DS patients have impaired colour discrimination, stereoacuity, and contrast sensitivity, similarly to AD patients (Rocco et al., 1997). Moreover abnormal spatial vision in DS children has been detected without other ophthalmologic abnormalities (Suttle & Turner, 2004). A literature review of children with Down syndrome age 0-16 years revealed that refractive error, strabismus, poor acuity, nystagmus, and blepharitis were common ophthalmologic findings whereas cataract and glaucoma were less common (Creavin & Brown, 2009). A pattern reversal VEP study demonstrated significantly longer P100 latency and smaller amplitude in DS patients (16/36 cases) as compared to age-matched controls (Kakigi et al., 1993). By employing achromatic transient VEP, children with DS had small or undetectable N75 but normal latency as compared to normal developing children (Suttle & Turner, 2004). Patients with DS also responded abnormally to chromatic transient VEP (Suttle & Lloyd, 2005). As far as we are aware, there has been no current report about RNFL measurement in DS eyes.

3. Hypotheses to explain RNFL thinning in AD

Three hypotheses to explain retinal ganglion cell fibre damage in AD have been proposed.

3.1 Is the retina affected by AD pathology directly?

AD pathology might develop not only in the cortex but also in the retina, perhaps in the ganglion cell layer (Lu et al., 2010). Beta amyloid, amyloid associated proteins, tau and amyloid precursor protein are expressed in the human retina at the level of ganglion cells and fibres in older eyes and in the retinal pigment epithelium in retinitis pigmentosa and age-related macular degeneration (Löffler et al., 1995). However neurofibrillary tangles, senile plaques, and amyloid angiopathy have not been identified in the retina even in association with extensive neuronal loss (Leuba & Kraftsik, 1994; Blanks et al., 1989, 1996b). A further study identified neither neurofibrillary degeneration nor amyloid angiopathy in AD patients' retinas (Hinton et al., 1986). Glial fibrillary acidic protein (GFAP) localized to Muller cells and astrocytes in the GCL is increased in AD eyes; as is found in retinal injuries and in the AD brain (Blanks et al., 1996b). It indicates that the retinal degeneration is accompanied by a glial response as GFAP is a major cytoskeletal component of astrocytes (Blanks et al., 1996b). Amyloid beta or Abeta deposition was found mainly in the outer and inner plexiform layer in the retina of the APPswe/PS1DeltaE9 transgenic (tg) mouse model of Alzheimer disease (Perez et al., 2009). Likewise, Abeta plaques with increased retinal microvascular deposition of Abeta and neuroinflammation in Tg2576 mouse retinas were detected chiefly from the GCL to the inner plexiform layer and some plaques were also identified in the outer nuclear layer, the photoreceptor layer, and the optic nerve (Liu et al., 2009). Abeta deposits reduced with abeta vaccinations (Liu et al., 2009). Hyperphosphorylated tau was demonstrated in areas adjacent to the plaques (Liu et al., 2009). Furthermore, abeta deposition was observed in the cytosol of lens fibre cells along with equatorial supranuclear cataracts in AD patients as compared to age-matched controls (Goldstein et al., 2003). No supranuclear cataracts were identified in any normal individual in this study (Goldstein et al., 2003). Like AD, an evaluation of lens in patients with Down syndrome revealed supranuclear opacification with accelerated supranuclear abeta accumulation (Moncaster et al., 2010).

3.2 AD and glaucoma

Wostyn has proposed a link between glaucoma and AD suggesting that an abnormal high trans-lamina cribrosa pressure difference in AD eyes has led to glaucomatous optic neuropathy (Wostyn et al., 2009). Glaucoma is characterized by a progressive loss of RNFL and a resulting visual field defect. Elevated IOP is a strong risk factor but not all patients with glaucoma have high IOP (Johanson et al., 2008; Berdahl et al., 2008b). The subgroup is classified as normal tension glaucoma (NTG) (Johanson et al., 2008). The retinal ganglion cell fibres in NTG eyes might be vulnerable to normal IOP as it is relatively high in the NTG eyes. The cause of NTG is still unknown. Recent studies have revealed that AD patients may have a higher risk of developing glaucoma than normal subjects (Bayer et al., 2002a; Tamura, 2006) and that glaucoma in AD patients tends to be more progressive than glaucoma in non-AD cases (Bayer & Ferrari, 2002b). A case control study (Chandra et al., 1986), investigating all death certificates (1,930,627) for the United States in 1978, compared 7195 cases who had senile and presenile dementia as the cause of death with other patients who died from other conditions. Gluacoma was associated with these demented patients with odd ratio of 2.6. Early RNFL loss in glaucoma occurs in the temporal inferior and temporal superior regions (Hoyt et al., 1973; Pederson & Anderson, 1980; Tuulonen &

Airaksinen, 1991; Jonas et al., 1993) as found in some of the AD studies described above. Trans-lamina cribrosa pressure differences may have resulted in the glaucomatous like RNFL changes. A study (Jonas et al., 2003) revealed that lamina cribrosa forms a barrier between the intraocular space and retrobulbar space. The lamina cribrosa has been found to be thinner in glaucomatous eyes than in control eyes (Fig.2). The outer part of the cribrosa that is directly in contact with pia mater or indirectly with cerebrospinal fluid (CSF) was significantly thinner in the glaucomatous eyes as compared to the controls, and the shortest distance between the intraocular space and the CSF space was significantly less in the glaucoma patients. The optic disc is situated close to this area. Trans-lamina cribrosa pressure (the pressure gradient across the lamina cribrosa) is derived from the intraocular pressure minus the retrobulbar CSF pressure (Jonas et al., 2003). Normally the intraocular pressure is higher than that in the CSF. Abnormal pressure from either side of the lamina cribrosa may be involved in the pathogenesis of several ocular and neurological conditions. In vivo, high IOP glaucoma can damage the optic nerve head and very low IOP can cause swollen discs.

Reduced ICP in patients with normal tension glaucoma (NTG) could cause abnormal trans-lamina cribrosa pressure (Berdahl et al., 2008a). Trans-lamina cribrosa pressure was significantly greater in patients with primary open angle glaucoma (POAG) and NTG than in normal individuals (Ren et al., 2010). CSF pressure in severe AD patients tends to be disproportionately low (Silverberg et al., 2006) and therefore may create a situation where there is relatively high IOP in their eyes (still within a normal reference range). Glaucomatous-like RNFL changes may then be expected to occur. There is evidence of choroid plexus (CP) degeneration in AD brains and their CSF production is affected (Serot et al., 2003). The choroid plexus consists of villi covered by a single layer of ciliated cuboidal epithelium and extends through the lateral, 3rd, and 4th ventricles, acting as a blood-CSF barrier (Serot et al., 2003). It is contiguous with ependyma; produces CSF; synthesizes several molecules; and carries nutrients from blood to the CSF (Serot et al., 2003; Silverberg, et al., 2001). Two-thirds of the CSF secretion is derived from the CPs, the remainder coming from brain interstitial fluid drainage, which is produced by the capillary-astrocyte complex found in the blood brain barrier (Johanson et al., 2008). The production rate of the blood-CSF barrier is substantially greater than that of the blood brain barrier (Johanson et al., 2008). Another source of CSF production is likely to be ependyma lining the ventricles (Pollay & Curl, 1967). CSF reabsorption (Johanson et al., 2008) occurs along sleeves of subarachnoid spaces surrounding cranial nerves that enter the nose and eyes; through the cribriform plate, nasal mucosa, and cervical lymphatic system eventually. CSF is also drained along spinal nerve arachnoid pathways. Arachnoid villi in the dural sinuses absorb the CSF when ICP is elevated. CSF pressure (Johanson et al., 2008) is normally higher than venous pressure in the dural sinuses. It is steady when CSF formation and reabsorption are balanced. CSF pressure measured by lumbar puncture in a lateral recumbent position is directly proportional to CSF production rate and outflow resistance. CSF pressure is determined by hydrodynamic and haemodynamic parameters. Regarding hydrodynamic factors, reduced CSF production or increased outflow resistance will decrease CSF pressure. In ageing the CP epithelium becomes atrophic; its basement membrane thickens; and CSF secretion decreases by 50% (Serot et al., 2003). These changes in AD choroid plexuses appear more pronounced than in

Fig. 2. Top: histological section of the optic disc in a non-glaucomatous eye; bottom: histological section of the optic disc in a glaucomatous eye (periodic acid Schiff staining). Arrows: regions in the posterior lamina cribrosa in direct contact with pia mater and indirectly exposed to the CSF space. The lamina cribrosa is outlined in black lines and was thinner with greater posterior bowing in the glaucoma than the controls (Jonas, Berenshtein, & Holbach, Anatomic Relationship between Lamina Cribrosa, Intraocular Space, and Cerebrospinal Fluid Space, 2003). Permission to reproduce the figures has been granted by Investigative Ophthalmology and Visual Science.

normal ageing and additionally stroma fibrosis has also been demonstrated in AD (Serot et al., 2003). Abeta proteins have been detected in the choroid plexus in AD brain (Kalaria et al., 1996). Ig and C1q depositions are frequently found along the basement membrane of the plexus in AD brains, suggestive of immunological processes in this location (Serot et al., 2003). As a consequence the choroid plexus cannot function normally (Serot et al., 2003). In young adults, the CSF production rate is 0.4 ml/min or 500 – 600 ml per day, the CSF volume is 150 ml, and the CSF turnover rate is 4 volumes per day (Johanson et al., 2008). In AD patients, on the contrary, the CSF production rate is 0.2 ml/min, the CSF volume is 250 ml due to brain atrophy, and the CSF turnover rate is 1.2 volumes per day (Johanson et al., 2008). In addition to the affected CSF production, the resistance of CSF outflow in AD is becoming greater for there is evidence of abeta depositions in the meninges (Silverberg et al., 2003; Hamano et al., 1997; Kalaria et al., 1996). Further studies are needed to confirm low CSF pressure in AD patients; to establish a relationship between the severity of cognitive impairment, brain atrophy, ventricular volume, CP morphology with CSF pressure; and to establish whether the trans-laminar cribrosa pressure difference plays an important role in the pathogenesis of RNFL thinning in AD.

3.3 Retrograde trans-synaptic degeneration secondary to cortical pathology

Lastly, we hypothesize that the RGC loss in AD could be partly due to retrograde trans-synaptic degeneration. RGC loss following an occipital injury, which is a consequence of retrograde trans-synaptic degeneration of geniculo-cortical towards retino-geniculate pathways; and anterograde degeneration of cortico-geniculate pathway, has been identified in the visual pathway (Cowey, 1974; Mehta & Plant, 2005b; Jindahra et al., 2009; Bridge et al., 2011). Neuronal loss, neurofibrillary tangles (NFT) and senile plaques have been identified in several neocortex areas including primary visual cortex (Pearson et al., 1985; Leuba & Kraftsik, 1994). Senile plaques were also identified in the LGN (Leuba & Kraftsik, 1994; Leuba & Saini, 1995) along with NFTs and degenerating axons or threads in the white matter underlying area 17 (Leuba & Saini, 1993; Leuba & Saini, 1995), reflecting a spread of the degeneration along the cortico-geniculate axons (Leuba & Kraftsik, 1994; Leuba & Saini, 1995). In a study (Leuba & Saini, 1995), senile plaques were found more in the parvocellular layer of the dLGN than the magnocellular layer, the interlaminar zones, and the optic radiation. No neuritic degeneration (NFT, neuritic plague, and thread) was demonstrated in the LGN in this study, suggesting mainly amyloid deposition in this area. The finding was in good agreement with another that showed mild tau pathology in the LGN of AD patients (Dugger et al., 2011). In addition, senile plaques and NFTs were detected in the pyramidal and non-pyramidal cells in layer 5 and 6 of the primary visual cortex (Leuba & Saini, 1995). The degeneration in the visual cortex varied greatly among individuals possibly due to different AD subtypes as presented below. Neuronal loss, glial cell proliferation, NFT, and neuritic plaque (NP) deposition have been demonstrated in visual cortex, area 17 in particular, in AD patients with mean age of 76.1 +/- 8.1 years when compared with age-matched controls (Leuba & Kraftsik, 1994). The tangles in layer 5 are twice in number as in layer 3 in the occipital lobe except area 18 and the neuritic plaques are found in all layers in one study (Pearson et al., 1985). In a study (Hof et al., 1989), AD patients with Balint syndrome which is characterized by optic apraxia (impairment of target pointing under visual guidance), ocular apraxia (inability to shift gaze to a new visual target), and

simultagnosia (perception and recognition in a small part of visual field) had greater NFT density and NP area in all cortical layers of area 17 and 18 as compared to AD patients without Balint syndrome. The NFT density in the superior frontal cortex of AD with Balint syndrome appeared much less than that in AD without Balint syndrome. Patients with Balint syndrome from stroke did not show NFT or high NP numbers in the visual cortex. The mean number of Meynert cells in layer 5 and 6 of area 17 in AD with Balint syndrome was significantly lower than that in AD without Balint syndrome. This study suggested a disruption of occipito-parietal connections or dorsal stream in these AD with Balint syndrome cases. Another study used SMI32-immunoreactive staining technique which represented pyramidal neurons in a small subset of total neuronal population (Hof & Morrison, 1990). It had been shown that the Meynert cell (the largest SMI32-ir neurons) counts in area 17 and 18 were significantly lower in AD patients than in age-matched controls only in a small magnitude. The loss appeared more pronounced in temporal and prefrontal cortices. The neuronal loss was confined to area 4b in area 17 and layer 3, 6 in area 18. The findings might have indicated the degeneration of projections of Meynert cells in these regions to area V5 that is responsible for visuospatial skills. NFT in area 17 and 18 were less numerous than area 9 and 20 in AD patients (Hof & Morrison, 1990). NFT were dominant in layer 2-3 in the visual cortices whereas in layer 5 in area 9 and 20. NP were numerous in layer 2-4 with the greatest density in layer 4 of area 17 in layer 2-3 in area 18.

A study (Arnold et al., 1991) revealed the distribution of NFT and NP among 39 cortical regions in 11 AD patients, having mean age 80.2 years (range 63-88 years) and mean duration of disease 7.5 years (range 3-15 years). It had been shown that NFT in the limbic and temporal lobes were substantially higher than the frontal, parietal, and occipital lobes. NPs were evenly distributed throughout the cortex with the highest density in the temporal and occipital lobes. When comparing NFT among visual cortices namely area 17, 18, and 20 (inferior temporal gyrus) in 8 AD patients aged 48-82 years, the number of NFT was low in area 17 but progressively increased in area 18 and 20 respectively, which paralleled to the hierarchical visual organization (Lewis et al., 1987). NFTs were found predominantly in layer 3 and 5, which contained cortico-cortical and cortico-fugal projecting fibres (Lewis et al., 1987). A substantial number of NPs was identified equally in all three regions. They were present across all cortical layers (Lewis et al., 1987). A study (Kiyosawa et al., 1989) of AD patients with and without impaired visual functions i.e., figure copying, colour vision tested by isochromatic plates, and steropsis showed no change in their primary visual cortices in 18F-fluoro-2-deoxyglucose positron emission tomography (PET) as compared to the results in age-matched controls. Additionally AD cases with impaired visual functions showed significantly decreased glucose metabolism in visual association and inferior parietal areas compared with the controls. AD patients with good vision showed no significant change in these areas. No neuronal loss in area 17 of AD brains was demonstrated in another study (Mountjoy et al., 1983).

4. Conclusion

There is increasing evidence of RNFL thinning or RGC loss in patients with AD but the relationship between the degree of cognitive impairment and the degree of RNFL loss has not been established yet. There are a few possibilities that could explain the findings. These

include AD change in the retina, abnormal trans-lamina cribrosa pressure, and retrograde trans-synaptic degeneration. The degenerative changes in the brain and retina vary among AD patients because of different AD subtype, severity, and duration. It seems that the RNFL measurement has a good potential to be a monitoring tool in AD patients in the near future. Further investigations are required to understand more about AD pathology in these areas.

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Part 5

Potential Mechanisms of Neurodegeneration

15

Modulation of Signal Transduction Pathways in Senescence-Accelerated Mice P8 Strain: A Useful Tool for Alzheimer's Disease Research

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1. Introduction

Senescence-accelerated mouse (SAM) lines serve as models of aging and age-associated diseases. The SAMP8 strain has a shortened life span and early-onset manifestations of senescence with characteristic pathological features observed in elderly humans, including deficits in learning and memory. In brains of SAMP8 mice, the processing of amyloid precursor protein (APP) is altered, resulting in excess production and accumulation of amyloid-β peptide (Aβ), tau is hyper-phosphorylated, and oxidative stress is increased. These phenotypic abnormalities are quite reminiscent of the findings in human brains with Alzheimer's disease (AD). Mechanistically, metabolic pathways that are responsible for the generation of reactive oxygen species (ROS) are increased, while antioxidant systems are reduced in activity in the cerebral cortex of aged SAMP8 mice. Besides these structural and metabolic alterations, brains of aged SAMP8 mice exhibit neurochemical abnormalities such as altered signaling through G protein-coupled receptors for 5-hydroxytryptamine, acetylcholine, adenosine, dopamine, melatonin, glutamate and GABA, ion channel receptors, and nuclear hormone receptors (e.g. for all-trans-retinoic acid, cortisol or estradiol). Consequences include alterations in the levels of neurotransmitters, receptor numbers, receptor binding affinity, and second messengers. Of note is that in AD, G protein-coupled receptors and/or their corresponding signaling pathways are often impaired. Together, the observations in aged SAMP8 mouse brains provide convincing evidence that

this model serves as an excellent research tool for studying AD pathogenesis and strategies for treatment. Additionally, many of the pathological and neurochemical abnormalities in SAMP8 mice are linked to altered expression of genes that are integrally related to processes such as neuroprotection, signal transduction, protein folding/degradation, intracellular transport and immune response. Several studies have already utilized pharmacological or dietary measures to restore cognitive function and enhance neuroprotection in aged SAMP8 mice, suggesting that these approaches may have applications in the treatment of AD. This review compiles available data concerning the signaling pathways that are altered in SAMP8 mice, and compares the effects to known abnormalities in AD brains.

2. Senescence-Accelerated Mouse model hallmarks: the interest in SAMP8 strain

The senescence-accelerated mouse (SAM) model of accelerated senescence was originally established by Dr. Takeda at the University of Kyoto through phenotypic selection from a common genetic pool of the AKR/J strain. After continuous sister-brother mating to maintain the line under conventional conditions, some littermates were found to differ from the original offspring. This distinct subset of mice exhibited an unusual phenotype characterized by early-age onset senility and shortened lifespan. Five litters with this new phenotype were selected as progenitors of the senescence-accelerated-prone mice (SAMP), while 3 litters with normal ageing were selected as progenitors of the senescence-accelerated-resistant mice (SAMR) (Takeda et al., 1981). Fourteen SAMP and four SAMR litters were produced from the original breeding pairs (Takeda, 1999). In the SAMP mice, the first signs of premature aging were hair loss, reduced physical activity, ophthalmic disorders, and shortened lifespan (mean lifespan of 9.7 months versus 16.3 months for SAMR mice) (Takeda et al., 1994). These and other characteristic features of SAMP mice are summarized in Table 1.

The use of experimental models to study the beginning and the course of senescence and, in a pharmacological approach, to design drugs capable of delaying this process has been widely accepted. Some examples of these experimental approaches are the development of genetically modified organisms, which overexpress genes related to aging, chemical increase of free radicals, viral inoculation, pharmacological interference with some nerve and endocrine functions, and stress induction, among others. These are very useful approaches but present some disadvantages which are not within the scope of this chapter to describe. Moreover, in recent years there has been brought to light a need for improved animal models as a critical barrier in the study of several neurodegenerative disorders. In the particular case of AD study, a direct consequence of the knowledge of AD etiology is that scientists are not able to design an animal model that fully resembles AD pathology. Furthermore, time is not on our side; it has been estimated that nowadays AD affects at least 30 million people around the world, death occurs within 10 years of diagnosis (Lee & Chodosh, 2009), and predictions are not encouraging at all. In the year 2050 the number of ageing people will be on the order of two billion people (WHO data and statistics), most of them in developed countries, which would exponentially increase the number of Alzheimer's patients. Nowadays, the available animal models of AD are restricted to the overexpression of genes with specific mutations associated with early-onset familial AD, which only represents ≈5% of AD cases— considered by many researchers, although useful,

to be models with poor clinical relevance and limited predictive value. The use of SAM strains has allowed researchers to pursue a more biological approach to study senescence processes. As may be observed in the central column of Table 1, the main feature of all SAM strains is accelerated senescence, which is the reason why they are a widely used animal model to study geriatric disorders. It is necessary to establish the difference between *accelerated* senescence and *pathologic* senescence. The former is the biological phenomenon observed in living organisms as time goes by. In this case, the observer only reports a faster course of events when compared to physiologic senescence (which is also convenient from a technical point of view). The latter refers to a pathological phenomenon that alters aging process by interacting with biological processes, inducing *premature* senescence.

Strain	**Characteristics**	**Geriatric disorders**
SAMP1	Senile amyloidosis Impaired immune system Impaired auditory system Retinal atrophy Hypertensive vascular disease Contracted kidney Pulmonary hyperinflation	Presbycusis Atrophy of the retina Senile cataracts Senile lung
SAMP2	Senile amyloidosis Impaired immune system	Senile cataracts Senile lung
SAMP3	Degenerative arthrosis	Degenerative joint disease
SAMP6	Senile osteoporosis	Senile osteoporosis
SAMP7	Senile amyloidosis Thymoma	
SAMP8	Age-related learning and memory deficits Anxiety Impaired immune system Age-dependent deposition of amyloid β-peptide	Deficits in learning and memory Emotional disturbances Abnormal circadian rhythm Damage to the blood-brain barrier
SAMP9	Age-related cataracts	Senile amyloidosis
SAMP10	Brain atrophy Age-related learning and memory deficits Age-related depression	Deficits in learning and memory Forebrain atrophy Abnormal circadian rhythm
SAMP11	Age-relating thickening of tunic media of thoracic aorta Senile amyloidosis Contracted kidney	Diffuse medial thickening of the aorta

Table 1. Summary of the main features of different strains of SAMP model. From Butterfield & Poon, 2005 and Chiba et al., 2009.

During the past century, a considerable number of theories have been proposed to explain the nature of aging from the different points of view which are involved in it: molecular, cellular, tissue level, etc. This has made it a difficult task to achieve. Nowadays, the scientific

community is still debating which of these theories is the one to explain all the changes reported during aging, but although there is not a universally accepted theory of aging, the free radical theory (Harman, 1956) seems to be the one with the most adherents. This theory postulates that changes observed during aging are due to the direct action of free radicals, generated during cellular metabolism, over biomolecules. Hence, chemical oxidation of proteins, DNA, lipid membranes and other biomolecules induced by reactive oxygen species (ROS) leads to cellular dysfunction and aging in humans and animals. Furthermore, ROS damage has been described as the first pathologic event that occurs in the brain of AD patients (Nunomura et al., 2001) and in SAMP8 strain (Pallas et al., 2008). Following these facts, the SAMP8 strain seems to be a valid animal model for the study of AD as the molecular mechanism that generates age-related impairments (even if it is not the only one, Zhu et al., 2007) in both senescent mouse and AD patients appears to be the same.

SAMP8 strain has been extensively studied by comparing aged SAMP8 mice with aged SAMR1 mice, and also aged SAMP8 mice with young SAMP8 mice. In both cases, neuropathological, neurochemical, and, especially, behavioral/cognitive abnormalities found in aged SAMP8 mice are similar to the deficits observed in AD patients and in some genetically modified animals. Moreover, the chronology of AD symptoms and appearance of pathology are closer to abnormalities described in SAMP8 strain than to what is reported in other genetically modified animals (Pallas et al., 2008). Recently, SAMP8 strain has caught the attention of several investigators, owing to its unique characteristics, with special focus in their use as neurodegenerative models (Pallas et al., 2008; Takeda, 2009). This strain spontaneously develops a pathologic phenotype characterized by age-related disorders, such as learning and memory deficits, mood disorders, such as reduced anxiety-like behavior and depressive behavior, and abnormality of circadian rhythm. In addition, when APP cDNA from SAMP8 strain was sequenced, familial AD mutations were not found (Kumar et al., 2001), suggesting that the age-related disorder reported in this strain was probably not following the same pathways as observed in familial AD. Furthermore, Aβ levels found in SAMP8 mice seem to be closer to those observed in AD patients than what has been reported in genetically modified mice (Rosenberg, 2000). This is, precisely, SAMP8's main characteristic: the age-related phenotype is developed spontaneously and in almost the same order as reported in human beings. That is the reason why this animal model is a high value-added model; neurodegenerative observations reported in this model are not due to the introduction or the deletion of a gene(s) in an animal model but are directly induced by physiological processes.

Age-related deficits in SAMP8 strain have been known for a long time. Since their first description by Dr. Takeda, several laboratories have associated behavioral changes to age-related cognitive impairment. However, Dr. Takeda's laboratory was the first to globally describe age-related cognitive deficits in SAMP8 mice compared to SAMR1 age-matched controls (Miyamoto et al., 1986). They reported an age-associated increase in spontaneous motor activity, impairment in the acquisition of passive avoidance response at 2 months of age, and impairment in the acquisition of active avoidance response at 12 months of age. Alternative paradigms designed to test other behavioral tasks, such as spatial memory acquisition, are particularly useful to study age-related deficits, as memory and learning deficiencies are directly dependent on hippocampal function. These experiments, such as multiple T-maze and Morris water maze, showed an age-related impairment of spatial memory acquisition in 2-month-old SAMP8 mice. Nevertheless, these results turned controversial as other groups found different onset times for SAMP8 age-related

impairments in learning and memory depending on the test used (reviewed in Flood & Morley, 1998). Taken together, all these studies suggest that learning and memory tasks are impaired in SAMP8 mice at early age which will be detected at one or another age depending on the specific aspect of learning and memory measured by the experimental test used. These age-related cognitive deficits found at an early age in SAMP8 mice have been used by researchers to connect treatments focused on neuropathological abnormalities with improvements in physiologic functions such as learning and memory acquisition. The most relevant pathological defects found in SAMP8 mice will be discussed in this section.
In the section bellow, we will consider the main features that are known to occur early in the pathogenesis of AD and which the SAMP8 strain exhibits, such as increased oxidative stress, amyloid-β alterations and tau phosphorylation. Although not commented upon here, other features that have been described in AD pathogenesis have been also found in SAMP8 model as: gliosis (Lu et al., 2009), protein alterations in neurons and astrocytes (Diez-Vives et al., 2009), lower hippocampal PKC activity (Hung et al., 2001), and altered protein expression in the olfactory system (Poon et al., 2005c).

3. Pathological findings in SAMP8 mice

The unique characteristics of SAMP8 mice are not new, but recently this strain has drawn the attention of researchers [1] in the area of gerontological study of dementias as a result of its exclusive development of learning and memory deficits with age (Flood & Morley, 1998) and as a consequence of the clinical relevance obtained employing other commonly used AD models. In this section, we will summarize recent findings related to pathological data obtained from the SAMP8 strain that are also common in the pathophysiology of AD: damage induced by oxidative stress, Aβ deposition and hyperphosphorylation of tau protein.

3.1 Oxidative stress

Free radical-mediated damage to neuronal membrane components has been implicated in the etiology of Alzheimer's disease (AD) and aging; in fact, as has been previously noted, one of the earliest events to occur in AD is oxidative stress (Nunomura et al., 2001). Furthermore, loss of functional enzyme activity during aging had previously been associated with oxidative modification of enzymes (Oliver et al., 1987). Early studies focusing on the oxidative stress level of SAMP8 mice detected elevated levels of lipid peroxide and protein carbonyl in cerebral cortex of SAMP8 mice as young as 4 to 8 weeks old, compared to those of SAMR1 controls (Sato et al., 1996b). After this discovery, this group also confirmed that the oxidative events could be triggered by the loss of activity of anti-oxidative enzymes, such as catalase, and/or by the increase of activity of pro-oxidative enzymes, such as acyl-CoA oxidase (Sato et al., 1996a). These were the first reports that supported the fact that oxidative stress is one of the earliest events related to AD pathology that happen in the SAMP8 strain, and they lead to later papers that studied oxidative stress as a loss of functionality of anti-oxidative enzymes or as an increase in functionality of pro-oxidative enzymes.

[1] Searching for "senescence-accelerated mouse" in PubMed database reported 401 citations on March, 25 2011.

Other authors have complemented these studies using more powerful approaches, such as proteomics, to study the level of carboxylation of proteins in the SAMP8 strain. Therefore, higher protein oxidation and lipid peroxidation reported by Sato et al. were confirmed in 12-month-old SAMP8 mice (Poon et al., 2004a). Those oxidative stress markers were reduced not only when SAMP8 mice were treated with antioxidants, such as α-lipoic acid (Poon et al., 2005b), as would be expected, but also when antisense therapy directed to decrease APP expression was carried out (Poon et al., 2004b, 2005a). Both approaches are capable of reversing cognitive deficits in 12-month-old SAMP8 mice. Furthermore, a complete proteomics study including about 1700 proteins was carried out in SAMP8 and SAMR1 mice (Zhu et al., 2011). In this report there was found to be a group of proteins which were expressed in an age-dependent way and, interestingly, some of them had previously been found to be expressed in AD patients. One of these, Cu/Zn superoxide dismutase (De Leo et al., 1998), will be commented upon later in this chapter. Taken together, these reports related to oxidative stress have not only provided understanding of the mechanisms underlying memory and learning deficits but have also suggested potential therapies to counteract age-related dementia. Therefore, it has been demonstrated for the SAMP8 strain that age-related dementia observed in these animals is ROS-dependent, as the treatment with antioxidants improved learning and memory ability and reduced Aβ deposition (Shih et al., 2010).

Several studies have been carried out in the SAMP8 strain focused on physiologic enzymes with antioxidant properties that counteract ROS produced by oxidative metabolism, or on enzymes with pro-oxidant properties that support ROS production. The first of these was carried out a long time ago when SAM nomenclature had not yet been established. In this study, there was reported to be elevated activity of monoamine oxidase B (MAO-B) and decreased activity of superoxide dismutase (SOD) in SAM-P mice versus SAM-R mice (Nomura et al., 1989). After that, more detailed studies were performed specifically in the SAMP8 strain where there was reported to be a decrease in manganese superoxide dismutase (Mn-SOD) activity in 10-month-old SAMP8 mice (Kurokawa et al., 2001), and an age-related decrease in Cu/Zn superoxide dismutase (Cu/Zn-SOD) expression in 5 to 15-month-old SAMP8 mice (Zhu et al., 2011), as well as in activity of glutathione peroxidase in 12-month-old SAMP8 mice (Okatani et al., 2002). Furthermore, glutathione reductase, glutathione peroxidase and catalase activities were lower in 5-month-old SAMP8 mice than in aged-matched SAMR1 mice (Sureda et al., 2006).

Another family of enzymes involved in ROS damage is nitric oxide synthases (NOS) composed of three isozymes. Nitric oxide (NO) is a signaling molecule widely distributed in the nervous system whose increase has been related to a variety of neurodegenerative pathologies such as AD (Steinert et al., 2010). In SAMP8 mice an age-related increase of NOS activity has been reported which can be reduced by natural antioxidants (Inada et al., 1996). This age-related increase in NOS activity was not due to an increase in mRNA or protein levels (Ali et al., 2009). Although it has been previously reported that Aβ antisense treatment reduced oxidative stress markers (Poon et al., 2004b), recent studies suggest that regulation of the NOS family is complex, as Aβ antisense or antibody treatment further increased NOS activity, with an increase in inducible NOS (iNOS), and reduced neuronal NOS (nNOS). No changes were reported in endothelial NOS (eNOS) (Ali et al., 2009).

A summary of the reported variations in enzymatic activity of anti and pro-oxidant enzymes in the SAMP8 strain is presented in Table 2.

Anti-oxidative enzymes (decreased oxidative stress)	**Pro-oxidative enzymes (increased oxidative stress)**
Catalase ↓	Acyl-CoA oxidase ↑
Manganese superoxide dismutase ↓	Monoamine oxidase B ↑
Cu/Zn superoxide dismutase ↓	NOS ↑
Glutathione peroxidase ↓	
Glutathione reductase ↓	

Table 2. Summary of the variations reported in enzymes related to oxidative stress in the SAMP8 strain.

Some antioxidants, such as melatonin, have been used in SAMP8 mice to counteract oxidative damage. The effect of melatonin treatment on the activity of AD-related kinases has been described (Gutierrez-Cuesta et al., 2007). In addition, melatonin-treated SAMP8 mice reported elevated levels of activity of glutathione peroxidase, an anti-oxidant enzyme, improving indexes of lipid peroxidation and oxidative stress by a combination of melatonin scavenger activity and its ability to stimulate anti-oxidant enzymes (Okatani et al., 2002). Furthermore, as there is clear evidence of the relationship between oxidative stress and age-related dementias in SAMP8 strain, these mice have also been used to test the effects of some natural antioxidants in the diet. Natural antioxidants derived from plants, such as rose-flower extract, have been tested as antioxidant compounds in the SAMP8 strain. In this case, it was demonstrated that antioxidant treatment increased activity and expression of the anti-oxidant enzymes catalase and glutathione peroxidase, decreasing lipid peroxidation and therefore extending the lifespan of SAMP8 (Ng et al., 2005). The antioxidant effects of other plant extracts, such as Toona sinensis Roemor, have been also tested in the SAMP8 strain. The results obtained demonstrated that dietary supplemented animals presented decreased Aβ deposition and lower levels of antioxidant markers as well as higher levels of anti-oxidant enzymes, such as SOD, catalase and glutathione peroxidase (Liao et al., 2006). Recently, icariin, a flavonoid extracted from several plant species, was shown to improve age-related deficits in SAMP8 mice by decreasing NO levels and NOS activity, and by increasing glutathione peroxidase and SOD activities (He et al., 2010). Finally, the administration of lotus seedpod proanthocyanidins to SAMP8 mice decreased NO levels and lowered NOS activity while increasing glutathione peroxidase and SOD activity, which correlates whit an improvement in SAMP8 cognitive deficits (Gong et al., 2008)

Nevertheless, the literature related to oxidative stress in SAMP8 strain is not always conclusive. For example while Okatani et al. did not find age-related changes in SOD, either in SAMP8 or in SAMR1 strain (Okatani et al., 2002), Alvarez-Garcia et al. did not observe any age-related differences in activity of catalase and glutathione reductase, while they did in SOD (Alvarez-Garcia et al., 2006). Finally, Sureda et al. found decreased activity of glutathione reductase, glutathione peroxidase and catalase in SAMP8 mice (Sureda et al., 2006). In addition, other systems might also participate in brain oxidative stress processes. Recent studies have demonstrated that, as in AD brain, peroxide detoxification by astrocytes could play a role in age-related cognitive deficits. Astrocytes derived from SAMP8 mice were more susceptible to H_2O_2 induced damage than those derived from SAMR1 mice. Decreased peroxide detoxification was seen in SAMP8 mice (Lu et al., 2008). Taken together, these results remind us that there is still a long way to go before we completely understand

the role of oxidative stress in SAMP8 age-related cognitive deficits and, by extension, in AD pathology.

3.2 Amyloid-β deposition

Senile plaques are one of the hallmarks of AD and have become one of the main hypotheses for the development of this disease (Selkoe, 2000). This hypothesis is based on the fact that familial AD (≈5% of AD cases) may be caused by mutations in the amyloid-β precursor protein (APP) or by mutations in genes that participate in amyloid-β cleavage (presenilins-1/2). According to this hypothesis, the increase in Aβ peptide, which would result in an increase in the number of senile plaques, is the main factor that triggers the rest of the clinical and histopathological features reported in AD. Although this hypothesis has not been refuted, it has not been demonstrated, despite the vast knowledge acquired from models and clinical studies, and even most current reviews on this topic continue to include the words "hypothesis" or "speculation" (Marchesi, 2011).

Although the SAMP8 strain has been proposed as a model of AD, the chronology of appearance of Aβ deposits has been controversial for a long time. Furthermore, there was one main difference between these deposits and the ones found in humans: SAMP8 Aβ deposits were not stained using Congo red and Thioflavin S (Takemura et al., 1993), a classic method used to stain amyloid plaques (Kelenyi, 1967). This main difference has led some researchers to incorrectly consider the age-related dementia described in SAMP8 mice as independent of amyloid plaque deposition (Ashe & Zahs, 2010). In one of the first studies of Aβ deposition in SAMP8 strain, the authors Takemura et al. proposed that little differences in the primary structure of APP or in the processing enzymes could lead to different processed proteins that might be responsible for their different polymerization into the β-pleated sheet. Although possible, this explanation seems improbable since it has been demonstrated that SAMP8 APP protein shows high similarity to that of other species (99.2% homologous with that of mouse and rat (Kumar et al., 2001)). In addition, since its discovery in 1967, the staining method based on fluorophores, such as Congo red and Thioflavin S, has been modified in order to increase the resulting resolution (Sun et al., 2002).

Nevertheless, Aβ deposits in SAMP8 strain have been detected in AD-affected regions (including the hippocampus, medial septum, cerebral cortex and cerebellum) using a wide variety of antiserum, although until a few years ago the date of appearance of Aβ deposits was open to question. One of the first studies to demonstrate the appearance of amyloid plaques in young SAMP8 mice was published in 2006 (Liao et al., 2006), but it was not until 2010 that a complete study was carried out. In this research, Del Valle et al. performed a time course of Aβ deposition in SAMP8 strain, demonstrating that amyloid plaques could be observed in the hippocampus of 6-month-old SAMP8 mice and that they increased with age, while 15 months was needed to observe them in control SAMR1 mice (Del Valle et al., 2010).

As well as immunodetection, quantitative expression studies of AD-related genes were carried out. All this research points to an increase in Aβ levels in the SAMP8 strain. In this sense, an age-related increase of expression of APP gene in SAMP8 hippocampus has been detected which correlated with an age-related increase in APP protein levels (Morley et al., 2000; Poon et al., 2004a). In agreement with these findings, variations in the expression of other genes related to AD pathology have also been reported. In this sense, there have been studies of the expression of presenilin-1 (PS1) and presenilin-2 (PS2), two proteins that are included in the γ-secretase complex whose mutations give rise to an increase in

amyloidogenic cuts of APP observed in familial AD. In the SAMP8 strain an increase in the gene expression of both PS1 and PS2 has been reported (Wei et al., 1999; Kumar et al., 2009) which may be one of the causes of increased processing of APP that leads to Aβ accumulation. The expression of another familial AD marker, apolipoprotein E (ApoE), was also studied in the hippocampus of the SAMP8 strain (Wei et al., 1999). This biomarker was less expressed in SAMP8 mice compared to the SAMR1 strain. In contrast, ApoE allele ε4 expression is associated with increased susceptibility to AD. Although, the connection between AD and ApoE is not clear, some researchers postulate that ApoE could be the link between Aβ deposition and pathological alterations of the cerebral microvasculature in AD (Marchesi, 2011). Apolipoprotein A-II (ApoAII) is the precursor protein that induces mouse senile amyloidosis, a form of amyloidosis in which the severity of Aβ deposition increases with age. This has also been the focus of research in SAMP8 strain which expresses type A ApoAII, considered as moderately amyloidogenic (Higuchi et al., 1991). This finding made the SAMP8 strain an appropriate model system for the study of the mechanism(s) of age-related amyloid fibril formation. In conclusion, all the foregoing research suggests that every one of these findings could contribute to the age-related Aβ accumulation described in the SAMP8 strain.

In addition to all the data already considered, there are other factors that contribute to Aβ accumulation. Among these is the Aβ peptide rate of efflux from the brain to blood. There are some hypotheses concerning how this peptide could be transported across the blood brain barrier which will not be discussed here. What is clear, however, is that the higher the efflux rate of Aβ is, the less Aβ remains inside the central nervous system where it may accumulate. This phenomenon was studied by Banks et al., using radioactive labeled Aβ which was centrally administered to SAMP8 mice. This group demonstrated that Aβ efflux was impaired in SAMP8 mice, suggesting that Aβ-impaired efflux could be an early contributor to Aβ deposition in the SAMP8 strain (Banks et al., 2003).

As noted above, if the hypothesis that Aβ deposition is responsible for the cognitive/behavioral deficits observed in SAMP8 strain holds, then when Aβ accumulation is inhibited, independently of the technique employed, the reported cognitive and behavioral deficits should improve. This hypothesis is correct for APP inhibition in SAMP8 strain and has been validated by several reports. Some of these have been commented upon earlier in this chapter but the original work in which downregulation of APP was achieved using a specific oligonucleotide was performed by Kumar et al. (Kumar et al., 2000). After this report, other authors demonstrated that APP expression could be decreased using antisense APP oligonucleotides (Poon et al., 2004b), improving learning and reversing memory deficits. Recently, a new strategy, employing micro ribonucleic acids (miRNAs), which are post-transcriptional regulators of APP in SAMP8 mice and show different expression patterns in SAMP8 and SAMR1 mice, has been proposed (Liu et al., 2010b). These authors hold that as miRNAs (miR-16, miR-144, miR-195, and miR-383) negatively regulate APP expression in SAMP8 strain, the use of these miRNAs could help to improve cognitive deficits in the SAMP8 strain, although this hypothesis has not been tested yet. Furthermore, Aβ immunotherapy strategies were employed as well using Aβ-directed antibodies, injected intracerebroventricularly (Morley et al., 2002) or intraperitoneally (Zhang et al., 2011), with recovery in cognitive deficits in reported in the SAMP8 strain.

Although Aβ accumulation is associated with cognitive impairment, there are some animal models of AD whose age-related brain disorders are not related to amyloid plaques but rather to abnormal APP metabolism that could accelerate age-related brain disorders

independently of Aβ deposition (Ashe & Zahs, 2010). As can be deduced from all the evidence described here, this is not the case with the SAMP8 strain. In addition, there was a study recently carried out using a monoclonal antibody against Aβ oligomers on SAMP8 mice which makes it increasingly evident that the age-related deficits observed in SAMP8 strain are Aβ deposition-dependent. In this study, the administration of this specific antibody to SAMP8 mice improved learning and memory tasks and decreased Aβ oligomers and phospho-tau levels (Zhang et al., 2011).

3.3 Hyperphosphorylation of tau

The presence of neurofibrillary tangles, consisting of hyperphosphorylated tau protein, is another hallmark of AD, although formation of neurofibrillary tangles is considered a consequence of Aβ accumulation (Hardy & Selkoe, 2002). Tau is a highly soluble protein which is implicated in microtubule assembly and stabilization whose 6 known cerebral isoforms are translated from a single gene by alternative splicing. Although tau mutations have been involved in a variety of neurodegenerative disorders named familial tauopathies (Goedert et al., 1998), tau is not mutated in AD. Different tau functions are controlled by site-specific phosphorylation which affects microtubule binding. While kinase-mediated phosphorylation inhibits microtubule binding, phosphatase-mediated dephosphorylation restores microtubule binding. In AD pathology, aberrant tau hyperphosphorylation induces microtubule instability and promotes a toxic effect on neurofibrillary tangles surrounding neurons (Johnson & Stoothoff, 2004). Therefore, if the SAMP8 strain is a valid animal model for the study of AD, the pathology induced by tau phosphorylation should be similar in AD and in SAMP8 mice.

In contrast to Aβ accumulation, total tau quantity is not a critical factor in toxicity, as is kinase-mediated phosphorylation. Consequently, an increase in phosphorylated tau in SAMP8 (cortex, striatum and hippocampus, and to a lesser extent in cerebellum) has been detected compared with SAMR1, whilst levels of tau protein were maintained overall in the different areas (Canudas et al., 2005). Furthermore, elevated levels of phosphorylated tau protein were found in SAMP8 strain at an early age, in 5-month-old mice (Alvarez-Garcia et al., 2006), indicating that tau phosphorylation is an early event in the SAMP8 strain. Thus, the kinase(s) responsible(s) for the phosphorylation of tau protein in the SAMP8 strain needed to be studied in detail. Two of the serine/threonine kinases which phosphorylate human tau protein are cyclin-dependent kinase 5 (CDK5) and glycogen synthase kinase 3 (GSK3). When they were studied in detail, an elevated activity of CDK5 accompanied by an increase in expression was detected in cortex, striatum, hippocampus and, a lesser extent, in cerebellum (Canudas et al., 2005). However, GSK3β expression and activity were not altered in SAMP8 mice (Canudas et al., 2005). Yet chronic melatonin treatment in SAMP8 mice diminished tau hyperphosphorylation, reducing CDK5 and GSK3β activation and suggesting that these kinases and their downstream pathways participate in tau hyper-phosphorylation in SAMP8 mice (Gutierrez-Cuesta et al., 2007).

Besides the relationship between tau protein and AD-related kinases, a relationship that links oxidative stress and tau phosphorylation has also been established. We noted earlier the work of Gutierrez-Cuesta et al., which links chronic antioxidant treatment with reduced tau phosphorylation, probably due to a reduction in CDK5/GSK3β activation, in SAMP8 mice. This relationship is attested by the abundant bibliography that supports the antioxidant properties of lithium. Lithium treatment in SAMP8 mice decreased CDK5/GSK3β activation, reducing tau phosphorylation and providing neuroprotection to

SAMP8 mice (Tajes et al., 2008). However, to date GSK3 has not been seen to be involved in the antioxidant properties of lithium (Camins et al., 2009). All the data corroborates a chronology of appearance of AD-like pathology in the SAMP8 strain: as oxidative stress is considered an early event in AD, the use of antioxidants should improve subsequent events in this pathology.

Recently, it has been postulated that elevation of endogenous formaldehyde levels may be related to the pathogenic processes of neurodegenerative diseases. From this, it has been demonstrated that some aldehydes, directly related to lipid peroxidation, are not only able to enhance amyloid plaque formation (Chen et al., 2006), but also to induce misfolding of tau protein and formation of globular amyloid-like aggregates (Nie et al., 2007). Thus, in addition to all the available research pointing to SAMP8 as an appropriate model for the study of age-related disorders, it has been recently described that formaldehyde levels are significantly increased in the SAMP8 strain (Tong et al., 2011), which suggests that endogenous formaldehyde is related to aging and that SAMP8 strain could be a suitable system for the study of these neurotoxic aggregates that could play a role in the induction of tauopathies.

4. Neurochemical changes in SAMP8 mice: GPCR mediated pathways

G protein-coupled receptors (GPCRs) are a large superfamily of membrane-bound signaling proteins that are involved in the regulation of a wide range of physiological functions and which constitute the most common target for therapeutic intervention (Ma & Zemmel, 2002). Visual, olfactory and taste sensation, intermediary metabolism, cell growth, differentiation and many other phenomena are all influenced by GPCR signals (Luttrell et al., 2008). GPCR had been viewed as a simple on-off switch that used a single class of effector molecule: the heterotrimeric G proteins. However, converging lines of evidence demonstrate the existence of G protein-independent signal transduction and its unique biochemical and physiological effects (Rajagopal et al., 2010). This increasing complexity of molecular mechanisms responsible for GPCR signaling and its regulation, through interplay of positive and negative regulatory events that amplify the effect of a hormone binding the receptor or that dampen cellular responsiveness, should be tackled as a very promising field to develop new therapeutic approaches. Many G protein-coupled receptors and/or their corresponding signaling pathways, such as cholinergic, glutamatergic, serotonergic, adrenergic and peptidergic neurotransmitter systems, are deregulated in brain from AD cases. This deregulation could be responsible for an altered sequential cleavage of APP by the α-, β-and γ-secretases, which are regulated by GPCRs, and the determination of the extent of amyloid-β peptide generation. In turn, amyloid-β can directly or indirectly affect GPCR function (for a review see Thathiah & De Strooper, 2011). Different neurochemical changes have also been found in the SAMP8 brain, including modifications in signal transduction pathways mediated by G protein-coupled receptors, ion channel receptors and nuclear hormone receptors. These changes have been reported at different levels (e.g. neurotransmitter concentration, receptor number and/or affinity, second messenger generation). Several GPCRs have been analyzed in the SAM model. However, many available data deal with the effects on learning and memory deficits in SAMP8 mice due to the administration of different ligands with agonist activity on these receptors. In contrast, very few reports have tried to analyze the possible modulation of the receptor itself (i.e., quantity, affinity,

regulation, etc.). Available data on different GPCR pathways analyzed in AD and the SAMP8 model are summarized in Table 3.

4.1 Serotonin receptors

The 5-hydroxytryptamine (5-HT; serotonin) receptors belong to both the G protein-coupled and ligand-gated ion channel superfamilies. These have been divided into seven distinct families, or classes, according to structural diversity and the preferred effector mechanism. Some of these classes comprise multiple receptors, which share similar structural and effector properties while displaying very different operational profiles (Barnes & Sharp, 1999). The serotonergic system has been implicatred in learning and memory (Reis et al., 2009). A possible age-related change in serotonin (5-HT) receptor antagonist dose–response curves was analyzed in SAMP8 mice using a retention test on a T-maze footshock avoidance apparatus (Flood et al., 1996, 1998). 5-HT_1 and 5-HT_2 receptor antagonists, methiothepin and ketanserin, improved retention, but the dose needed to improve retention was at least 10 times greater in 12-month-old P8 mice than in 4-month-old mice, indicating that there was a reduction in serotonin receptor activity in the 12-month-old mice. Similar drug studies done in the hippocampus of 4- and 12-month-old mice found no age-related change in the dose–response curves of serotonin antagonists for improvement of retention (Flood et al., 1996). Therefore, these results suggest that with increasing age SAMP8 develops a dysfunction in septohippocampal functioning which may initially begin with a decrease in serotonergic activity that appears to be specific to the septum. In agreement with this, the serotonin uptake inhibition by indeloxazine hydrochloride [(±)-2[(inden-7-yloxy)methyl]morpholine hydrochloride], a cerebral metabolic enhancer with cerebral activating properties (Yamamoto, 1990), potentiated the serotonergic system in SAMP8 mice (Yamaguchi et al., 1998). In fact, 5-hydroxytryptamine (5-HT) levels are significantly lower in SAMP8 as compared to SAMR1 mice (Qiu et al., 2010). Similar serotonergic deficits have been reported in patients with AD, including loss of 5-HT_2 receptors in cerebral cortex (Blin et al., 1993), 5-HT_4 in hippocampus and frontal cortex (Reynolds et al., 1995) and 5-HT_6 in pyramidal cells (Lorke et al., 2006).

4.2 Acetylcholine receptors (muscarinic)

Muscarinic receptors mediate cellular response to the natural ligand acetylcholine (ACh). They enjoy widespread tissue distribution and are involved in the control of numerous central and peripheral physiological responses, as well as being a major drug target in human disease. This family of G protein-coupled receptors consists of five members designated M_1-M_5. Hence, M_2 and M_4-muscarinic receptors are able to couple to the pertusiss-toxin sensitive $G_{i/o}$ proteins, and M_1, M_3 and M_5-muscarinic receptors couple to $G_{q/11}$ proteins. However, the muscarinic receptor family can couple to a wide range of diverse signaling pathways, mediated or not by G proteins (Caulfield & Birsall, 1998; van Koppen & Kaiser, 2003). In the hippocampus of SAMP8 at 12 months, binding of [^{3}H]pirenzepine, a M_1 muscarinic receptor antagonist, was significantly lower than that in SAMR1. However, in the cerebral cortex, binding was higher in SAMP8 than in SAMR1 at 12 months (Nomura et al., 1997). In addition, SAMP8 mice show decreased release of acetylcholine (Qiu et al., 2010). Thus, there is a decreased cortical acetylcholine content in SAMP8 (Kitaoka et al., 2010). Moreover, there is a reported effect of dysfunctional teeth on age-related changes in the septohippocampal cholinergic system by assessing acetylcholine

(ACh) release and choline acetyltransferase (ChAT) activity in the hippocampus of young-adult and aged SAMP8 mice after removal of their upper molar teeth (molarless condition) (Onozuka et al., 2002). In this experimental model, significantly less KCl-evoked ACh release was seen in aged molarless SAMP8 mice compared with aged controls, whereas the molarless condition had no effect on young mice. In the control groups, the KCl-evoked ACh release decreased with aging, but the difference was not statistically significant. Moreover, in control mice, ChAT activity was higher in the young-adult group than in the aged mice, indicating an age-dependent decrease in hippocampal ChAT activity in SAMP8 mice. In addition, aged molarless mice showed a greater reduction in ChAT activity than age-matched control mice, whereas the molarless condition had little effect on young-adult mice (Onozuka et al., 2002). This decreased cholinergic activity results in a decreased ability of the SAMP8 mouse to learn and retain new information (Morley et al., 2002). In fact, M_1 mAChR is the most abundant subtype in the cortex and hippocampus, two major brain regions that develop amyloid plaques and neurofibrillary tangles (nFTs) in AD, and postsynaptic M_1 mAChRs play a major role in hippocampus-dependent short-term memory and memory consolidation (Anagnostaras et al., 2003), which is impaired in AD (Levey, 1996). Therefore, considerable efforts have been directed towards developing M_1 mAChR-selective agonists that are capable of restoring the cognitive deficits in patients with AD, in whom hippocampus M_1 level are reduced (Pakrasi et al., 2007) while they are increased in frontal cortex (Svensson et al., 1992).

4.3 Adenosine receptors

Adenosine is a nucleoside widely distributed in central and peripheral nervous system that exerts its actions through four types of receptors named A_1, A_{2A}, A_{2B} and A_3, all of them being GPCRs. A_1 and A_3 receptors are coupled, through $G_{i/o}$ proteins, to adenylyl cyclase activity inhibition, while A_{2A} and A_{2B} receptors are coupled to stimulation of the enzymatic activity, through G_s protein (Ralevic & Burnstock, 1998; Fredholm et al., 2001, 2005). Out of the four adenosine receptors, the A_1 subtype is the most abundant and widespread in the brain, where it plays a neuroprotective role because of its capacity to decrease the release of excitatory neurotransmitters, mainly glutamate (Dunwiddie & Masino, 2001). A_{2A} receptors are concentrated in the basal ganglia but they are also present throughout the brain, albeit in a considerably lower density. A_{2B} and A_3 receptors are the least abundant in the brain (Cunha, 2005). Adenosine receptor gene expression and the quantification of A_1 and A_{2A} proteins in plasma membranes were analyzed recently in SAMP8 and SAMR1 strains using 21- and 180-day-old animals (Castillo et al., 2009). Results show that mRNA coding adenosine A_1 and A_{2B} receptors is significantly increased in middle-aged versus young SAMR1 animals, suggesting an age-associated up-regulation in resistant control mice. However, these increases were not detected in SAMP8 animals. Concerning A_{2A} receptors, no significant differences were found between young and middle-aged animals, either in SAMP8 or in SAMR1. However, the level of A_{2A} mRNA expression was significantly lower in SAMP8 versus SAMR1. Finally, an increase in mRNA coding A_3 receptor was observed in both middle-aged SAMR1 and SAMP8 animals without significant differences between strains. Adenosine A_1 receptors were measured in plasma membranes from SAMR1 and SAMP8 mice by binding assay using [^{3}H]DPCPX, a selective A_1 receptor antagonist, as radioligand. Total adenosine A_1 receptor numbers in middle-aged SAMR1 decreased by 38%, suggesting a loss of receptors associated with aging. This decrease was not observed in

the SAMP8 strain. However, and of interest, total A_1 receptor level in SAMP8 was significantly lower than in SAMR1, with even fewer receptors detected than in middle-aged SAMR1 mice, suggesting that A_1 receptors are already altered at a very early age in SAMP8. No significant age-associated differences in Kd values were observed in any SAM strain. However, Kd values in SAMP8 strain were lower than in SAMR1, suggesting a higher receptor affinity in the former. This age-related loss of A_1 receptors in SAMR1 was then associated with an increase in the rate of synthesis of this receptor, probably as a compensatory mechanism to prevent the important loss of receptor detected at the membrane surface. In contrast, an age-related increase in adenosine A_{2A} receptors was observed in SAMR1 with no variation in SAMP8, by Western-blotting. The most important finding in the paper of Castillo et al. is that adenosine A_1 receptors, which have been described as being neuroprotective, are already severely decreased in very young SAMP8 mice (3 weeks old), suggesting a great alteration of these receptors in this aging model. Higher A_1 and A_{2A} receptor expression levels have been found in the frontal cortex of post-mortem brains of patients with AD (Albasanz et al., 2008). Interestingly, A_{2A}-deficient mice display improved spatial recognition memory (Wang et al., 2006), whereas in vivo A_{2A} overexpression leads to memory deficits (Gimenez-Lort et al., 2007). In agreement, pharmacological blockade of A_{2A} receptors by caffeine seems to reduce Aβ formation and the risk of developing dementia (Chen & Chern, 2011; Gelber et al., 2011).

4.4 Adrenergic receptors

Adrenoceptors are 7-transmembrane receptors which mediate the central and peripheral actions of the neurotransmitter noradrenaline (norepinephrine), and the hormone and neurotransmitter adrenaline (epinephrine). Adrenoceptors are found in nearly all peripheral tissues and on many neuronal populations within the central nervous system. Both noradrenaline and adrenaline play important roles in the control of blood pressure, myocardial contractile rate and force, airway reactivity, and a variety of metabolic and central nervous system functions. Based on both pharmacological and molecular evidence they are classified into three major types -α_1, α_2 and β- each of which is further divided into at least three subtypes (Bylund et al., 1994). Agonists for norepinephrine receptors required little or no change in the dose needed to improve retention in older SAMP8 (12- versus 4-month-old) mice on a T-maze footshock avoidance test (Flood et al., 1998), suggesting that this signaling pathway is not modified with aging in SAMP8 mice. In contrast, the level of total β-adrenergic receptors and, more interestingly, the relative ratio of β_1-/β_2-receptors, have been reported as altered in different brain regions from AD patients (Kalaria et al., 1989). Thus, there is an increase in β_2-receptors in prefrontal cortex and hippocampus which could be responsible for the amelioration in AD patients detected by blocking β-adrenergic receptors with selective antagonists (Yu et al., 2011). Thus, a similar modulation in SAMP8 mice must not be ruled out as no detailed studies have been performed to date.

4.5 Calcitonin receptors

The calcitonin peptide family comprises calcitonin, amylin, calcitonin gene-related peptide (CGRP), adrenomedullin (AM) and AM2, also known as intermedin. All of these peptides, ranging from 32 to 52 amino acids in length in humans, share structural similarities, and their truncation beyond the second cysteine residue generates antagonists. With the

exception of CGRP receptors, such modified forms of the native peptides are the only pharmacological tools currently available for characterizing these receptors. The receptor family for these peptides consists of two class B GPCRs, the calcitonin receptor (CR) and calcitonin receptor-like receptor (sometimes abbreviated as CRLR) for which pharmacological specificity is dictated by additional proteins, known as receptor activity-modifying proteins (RAMPs). These are integral parts of the receptor complex (Hoare et al., 2005). In male SAMP8 and SAMR1 mice, blood samples were collected monthly from 3 to 12 months of age. With advancing age, the plasma calcitonin (CT) levels decreased progressively in both SAMR1 and SAMP8. However, the curve of age-related changes in the plasma CT levels was lower in SAMP8 than in SAMR1 (Chen et al., 2004). Measurement of CT level in female SAMP8 mice at the age of 3, 6, 9, 12, and 15 months revealed that plasma CT level decreased with aging and/or ovariectomy. Plasma CT levels in the ovariectomized (Ovx) mice were significantly lower than those in the intact mice. In addition, the decrease of plasma CT level was moderated by long-term dietary antler supplementation in both Ovx and intact mice (Chen at al., 2007). Although no data were found concerning CT receptors in SAMP8 mice, a reduced level of the endogenous agonist (CT) could suggest reduced signaling through these receptors. In fact, a possible receptor upregulation could be expected in response to low CT levels as a compensatory mechanism. Interestingly, the amylin receptor is a putative target for the actions of Aβ in the brain. Thus, in primary cultures of human fetal neurons (HFNs), AC253, an amylin receptor antagonist, blocks the electrophysiological effects of Aβ. Moreover, in transgenic mice (TgCRND8) that overexpress amyloid precursor protein, amylin receptor is up-regulated in specific brain regions that also demonstrate an elevated amyloid burden (Jhamandas et al., 2011).

4.6 Chemokine receptors

Chemokines (chemotactic cytokines) comprise a family of related proteins according to structural criteria including overall amino acid sequence homology, length, conserved cysteine motifs and a common fold. They are involved in leukocyte trafficking, antimicrobial activity, HIV inhibitory activity, angiogenic or angiostatic activity, tumour-promoting or tumour-inhibiting activity, apoptosis or mitogenic activity, and the ability to modulate gene expression, T cell differentiation and phagocyte activation. Chemokines act by binding to 7-transmembrane domain, G protein-coupled receptors. There are 18 human chemokine receptors and over fifty distinct chemokines (Melik-Parsadaniantz & Rostene, 2008). An interesting region has been found on chromosome 4 of SAMP8 mice harboring multiple genes that were more highly expressed in SAMP8 than in SAMR1; some of these genes are chemokine (C-C motif) ligand 19 (Ccl19) and Ccl27. The RNA levels for these two genes in retina or hippocampus were higher in SAMP8 than SAMR1 (Carter et al., 2005). In addition, CCL2, a small cytokine belonging to the CC chemokine family that is also known as monocyte chemotactic protein-1 (MCP-1), has a significantly increased expression in old as compared with young SAMP8 mice, and this expression was significantly reduced after melatonin treatment. In SAMR1 mice no statistically significant differences between young and old animals were found. CCL2 expression was also elevated in old SAMP8 mice when compared with old SAMR1 mice (Cuesta et al., 2010). CCL2 is the main ligand for chemokine receptor CCR2, which is required for macrophage infiltration at sites of axonal injury in the hippocampus. CCL2 has been localized to mature amyloid plaques in the AD brain (Ishizuka et al., 1997).

4.7 Dopamine receptors

Dopamine receptor subtypes are named D_1-D_5 and belong to two subfamilies, D_1-like and D_2-like, based upon similarities in sequence, pharmacology and ability to stimulate or inhibit adenylyl cyclase activity mediated via coupling to $G_{\alpha s}$ or $G_{\alpha i/o}$ proteins (Beaulieu & Gainetdinov, 2011). Retention improvement by agonists for dopamine receptors was similar in older SAMP8 (12- versus 4-month-old) mice on a T-maze footshock avoidance test (Flood et al., 1998), suggesting that this signaling pathway is not modified with aging in SAMP8 mice. However, there is a decrease in the number of dopamine (DA) neurons and the associated ultrastructural changes in the neurons of the nigrostriatal system in SAMP8 as compared with SAMR1. This reduction is even greater in aged SAMP8 mice (8–10 months old) (Karasawa et al., 1997), suggesting a reduced DA level in SAMP8 worsened by age. The administration of 2,4-diamino-6-hydroxypyrimidine (DAHP), an inhibitor of GTP cyclohydrolase I, to inhibit DA and serotonin syntheses in young mice (2 months old) and aged mice (10 months old), revealed that DA turnover is lower in aged mice than in young mice (Karasawa et al., 1999). This DA level could be modulated by diet or MPTP administration. DA level was similarly and significantly increased in cerebellum at 8 and 12 months of age after diet restriction (Kim & Choi, 2000). In contrast, administration of MPTP can induce a marked decrease in striatal DA levels and a loss of dopaminergic neurons in the substantia nigra of SAMP8 mice (Liu et al., 2008). Moreover, old MPTP-SAMP8 mice show an earlier and more severe loss of dopaminergic neurons than young mice (Liu et al., 2010a). No data concerning modulation of dopamine receptors was found for SAMP8 mice. However, in frontal cortex from AD brain, expression of D_1, D_3 and D_4 receptors was severely reduced, D_2 was moderately reduced and D_5 was the only receptor subtype whose expression was increased in AD (Kumar & Patel, 2007). Also, in temporal lobe of AD brain these dopamine receptors have a reduced expression (Gahete et al., 2010). In addition, DA plasma level is significantly lower in AD patients (Umegaki et al., 2000).

4.8 $GABA_B$ receptors

The $GABA_B$ receptor is a G_i/G_o protein-coupled receptor heterodimer with two subunits, designated 1 and 2. Activation at this site produces neuronal hyperpolarization by increasing membrane K^+ conductance. The antispastic agent (-)-baclofen is a highly selective agonist for $GABA_B$ receptors (Martin et al., 2001). Saclofen, a $GABA_B$ receptor antagonist, had to be injected into the septum at a higher dose in 12- than in 4-month-old mice to improve retention in SAMP8 mice after training on footshock avoidance (Flood et al., 1998), suggesting a possible upregulation of $GABA_B$ receptor. However, a quantitative autoradiography study of $GABA_B$ and $GABA_A$ receptors in AD revealed fewer hippocampal $GABA_B$ receptors in stratum moleculare of the dentate gyrus, stratum lacunosum-moleculare and stratum pyramidale of CA1 (Chu et al., 1987a), and a significantly lower $GABA_B$ level in frontal cortex (Chu et al., 1987b), with no significant differences on affinity (Kd). Also, the GABA level in cerebrospinal fluid was significantly reduced in AD patients (Jimenez-Jimenez et al., 1998).

4.9 Melatonin receptors

There are three major plasma membrane receptors for melatonin within the brain. Two of these, MT_1 and MT_2, are GPCRs whose activation leads to decreased levels of cyclic AMP. The third, MT_3 or NQO2, is a quinone reductase with poorly understood in vivo function

(Reppert et al., 1996; Rios et al., 2010; Tan et al., 2007). However, other mechanisms of action could be possible as there are additional melatonin binding sites in the nucleus of many cell types (Filadelfi & Castrucci, 1996). Melatonin acts as a neurohormone affecting transcriptional events in the CNS (Kotler et al., 1998). Several studies have shown that melatonin levels are diminished in AD patients compared to age-matched control subjects. CSF melatonin levels decrease even in preclinical stages when the patients do not manifest any cognitive impairment (at Braak stages I-II), suggesting that the reduction in melatonin is an early marker for the first stages of AD (for a review see Cardinalli et al., 2010). MT_1 activation depresses CREB and stimulates ERK (Chan et al., 2002). MT_2 levels are depressed in AD (Savaskan et al., 2005). Changes in these signaling pathways may form the basis of the alteration in gene expression effected by melatonin. The linkage between reduced melatonin levels and accelerated aging has been reviewed elsewhere (Bondy & Sharman, 2007) and may be more than merely correlative. Levels of both MT_1 and MT_2 receptors are very high within the suprachiasmatic nucleus (SCN), the site of circadian rhythm regulation. However, although administration of melatonin in drinking water promotes the phase advance of light–dark cycle in senescence-accelerated SAMR1 but not SAMP8 mice, levels of expression of both MT_1 and MT_2 mRNAs in the SCN are identical in the two SAM strains (Asai et al., 2000).

4.10 Opioid receptors

The opioid peptide receptors are heterogeneous. Measures of antagonist affinities against various opioid agonists in different systems resulted in unambiguous evidence for heterogeneity of receptor types, and the eventual definition of the μ, δ and κ receptor types (Dawan et al., 1996). In vivo (+)-[^{3}H]SKF-10,047 binding to σ sites was examined in the hippocampal formation and cerebral cortex of SAMR1 and SAMP8, at 12 months of age. Binding levels expressed as fmol/mg of proteins indicated decreases in the levels of total (+)-[^{3}H]SKF-10,047 bound in the cortex of SAMP8 as compared with SAMR1, but not in the hippocampus, and in the levels of (+)-[^{3}H]SKF-10,047 non-specifically bound in both structures. However, bound-to-free ratios never significantly differed between the two substrains. The apparent reductions in the in vivo binding levels of SKF-10,047 tracer may thus not reflect decreased binding capacities at the σ sites. However, the learning impairment observed in 10- to 12-month-old SAMP8 could be significantly attenuated by two high affinity σ agonists, JO-1784 (igmesine) and PRE-084, administered subcutaneously in the mg/kg range, on several tests of mnemonic capacities (Maurice et al., 1996). δ-OR binding is decreased in the amygdala and ventral putamen, and μ-OR binding is decreased in the hippocampus and subiculum of post-mortem brain samples from patients with AD. In addition, there is an elevated hippocampal level of enkephalin, the ligand for these receptors, in the human AD brain (for a review see Thathiah & De Strooper, 2011).

4.11 Orexin receptors

The orexins (orexin-A and orexin-B, also known as hypocretin-1 and hypocretin-2) are neuropeptides derived from a single precursor expressed in a few thousand neurons restricted to the posterior lateral and medial hypothalamus. Orexin-A is the selective endogenous agonist for OX1 receptor, which is coupled to the activation of phospholipase C via a G_s/G_q protein (Zhu et al., 2003). Inactivation of the receptor has been shown to cause impaired spatial learning in anaesthetized rats (Akbari et al., 2006). The effect of post-

training intracerebroventricular administration of Orexin-A on retention in active and passive avoidance has been reported in young (4 months) and old (12 months) SAMP8 mice (Jaeger et al., 2002). Orexin-A improved retention in young and old SAMP8 mice. However, no data about orexin receptor levels have been reported to date. Of interest is the observation that Aβ produced by neurons and secreted into the brain interstitial fluid is modulated by orexin in transgenic (Tg2576) mice, which express a mutated form of human amyloid precursor protein (APP) (Kang et al., 2009). Hypocretin-1 levels were normal in AD, although fragmentation of daytime wake was elevated in those with low hypocretin-1 levels. Thus, lower hypocretin-1 levels may be permissive for, or a consequence of, increased wake fragmentation in AD (Friedman et al., 2007).

4.12 Parathyroid hormone receptors

Parathyroid hormone (PTH), parathyroid hormone-related protein (PTHrP), and tuberoinfundibular peptide of thirty-nine residues (TIP39) are endogenous ligands for the parathyroid hormone 1 and 2 receptors (PTH1, PTH2). PTH is a classic endocrine hormone essential for mineral homeostasis (Potts et al., 1995). With advancing age (3, 6, 9, 12, and 15 months), the plasma PTH level increased progressively in female SAMP8 (Chen et al., 2007). In male SAMP8 and SAMR1 mice blood samples were collected monthly from 3 to 12 months of age. With advancing age, the plasma PTH level increased progressively in both SAMR1 and SAMP8 (Chen at al., 2004). The increase in serum PTH level has also been reported in older people and has been associated with impaired cognitive function, although the predictive value of PTH for cognitive decline has not yet been fully investigated (Bjorkman et al., 2010).

4.13 Somatostatin receptors

The somatostatin receptor family consists of 5 subtypes (sst1-5) each differentially distributed throughout the CNS and periphery. These receptors bind with high affinity to the endogenous polypeptides somatostatin-14, somatostatin-28 and cortistatin, as well as many other synthetic ligands. Receptor activation stimulates multiple intracellular signaling mechanisms giving rise to many tissue functions such as inhibition of growth hormone (GH) release and modulation of neuronal activity (Moller et al., 2003). The protein expression profile changes in the frontal cortex brain of SAMP8 model by means of microarray and RT-PCR techniques has recently been analyzed (Chen at al., 2010). In this study, somatostatin gene expression was lower in 12-month-old as compared with 4-month-old SAMP8 mice. Interestingly, the administration of NNC 26-9100, an sst4 receptor agonist, to 12-month-old SAMP8 mice did not modulate the expression of the sst4 receptor and APP when compared to vehicle control mice after 28 days of chronic NNC treatment, but it did enhance learning and memory (Sandoval et al., 2011). In addition, the expression of somatostatin did not change during aging in the pancreas of male SAMP8 and SAMR1 mice as no significant differences were observed between old (10 month) and young (2 month) mice. However, the expression of somatostatin was higher in the pancreas of young SAMP8 mice as compared with young SAMR1 mice (Cuesta et al., 2011). The expression of sst2 and sst4 is reduced in the cortex of human patients with AD (Kumar, 2005). Moreover, somatostatin levels are also reduced in the CSF, cortex and hippocampus of patients with AD (Thathiah & De Strooper, 2011).

4.14 Thyrotropin-releasing hormone receptors

Thyrotropin-releasing hormone (TRH) is a tripeptide (pyroGlu-His-Proamide) that is synthesized in the hypothalamus and released into the hypothalamic-pituitary portal circulation to act on the pituitary. TRH is produced in many other tissues, especially within the nervous system, where it appears to act as a neurotransmitter/neuromodulator. TRH receptors (TRH_1, TRH_2) belong to the Class A GPCR family. TRH_2 receptor has not been found in humans (Straub et al., 1990). Subcutaneous injection of a sustained release formulation of thyrotropin-releasing hormone (TRH-SR) produced a sustained increase in immunoreactive plasma TRH levels up to about 4 weeks after dosing in 8-month-old SAMP8. Furthermore, TRH-SR significantly improved the impairment of water maze learning in SAMP8 mice (Miyamoto et al., 1994a). TRH-SR also ameliorates impairments of learning behavior and the emotional disorder in SAMP8. In addition, SAMP8 shows an age-dependent abnormality of circadian rhythms of spontaneous motor activity (SMA) and ingestive behavior compared with the SAMR1 control, with diurnal SMA and water intake in SAMP8 being higher than in SAMR1 (Miyamoto, 1994b). These results suggest a reduced TRH level in SAMP8 mice. TRH concentration was decreased in the AD hippocampus compared to normal elderly controls (Luo et al., 2002). However, no significant differences from non-neuropsychiatric controls were noted on TRH receptor levels within the hippocampus in AD; just a slight alteration was noted in the cortical amygdala in AD (Lexow et al., 1994).

4.15 PACAP receptors

Vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase-activating polypeptide (PACAP) are members of a superfamily of structurally related peptide hormones that includes glucagon, glucagon-like peptide, secretin and growth hormone-releasing hormone (GHRH). At least three receptors for PACAP exist in mammals, two of which are also high-affinity receptors for VIP (Harmar et al., 1998). In SAMP88 mice, PACAP is transported across the BBB in almost all regions of the brain, with the highest rates of transport found in the hypothalamus and hippocampus. This transport is lower in aged (12-month-old) than in young (2-month-old) mice. Of the differences between young and aged SAMP8 mice, the most interesting is the loss of the ability to transport PACAP into the olfactory bulb (Nonaka et al., 2002). Several studies have found abnormalities of the olfactory bulb of both SAMP8 mice (Ueno et al., 1998) and patients with Alzheimer's disease (Kovacs et al., 2001).

4.16 Metabotropic glutamate receptors

Glutamate is the main excitatory neurotransmitter in the central nervous system which has been implicated in several physiological and pathological processes (Conn & Pin, 1997). The different actions of glutamate are mediated through glutamate receptor binding, which has been classified into ionotropic and metabotropic. Metabotropic glutamate receptors (mGluRs) are coupled, through G proteins, to different effector systems, including phospholipase C (PLC) and adenylyl cyclase (AC). They have been classified into three groups on the basis of their pharmacological profile, molecular properties, and transduction mechanisms. Group I receptors ($mGlu_1$, $mGlu_5$) are coupled to PLC activation, through $G_{q/11}$ proteins, whereas groups II and III are coupled to AC inhibition, through $G_{i/o}$ proteins

(Conn & Pin, 1997). Preliminary results by our group suggest that mGluRs are impaired in SAMP8 mice (unpublished data). Interestingly, the mGluR/phospholipase C signaling pathway is impaired in the cerebral cortex in AD patients. Moreover, a decrease in mGluR specific binding correlates well with stage of AD-related changes (Albasanz et al., 2005). The efficacy of glutamatergic neurotransmission also relies on glutamate uptake and release from vesicles (Ishikawa et al., 2002; Wojcik et al., 2004). Vesicular glutamate transporters (VGLUTs), which include VGLUT1, VGLUT2 and VGLUT3, are responsible for the uploading of L-glutamate into synaptic vesicles and they are specifically required for exocytotic release (Reimer & Edwards, 2004). For glutamatergic synapses, a single functional vesicular glutamate transporter is both necessary and sufficient to fill a synaptic vesicle. However, elevated VGLUT expression increases the quantal size of a vesicle, and vesicles without VGLUT are empty (Daniels et al., 2006). Therefore, the expression level of VGLUTs determines the amount of glutamate that is loaded into vesicles and released, and thereby regulates the efficacy of neurotransmission (Ishikawa et al., 2002; Wojcik et al., 2004). Protein expression of VGLUT1, VGLUT2, VGLUT3 and synaptophysin (Syp), a marker of synapse (Kashani et al., 2008), tends to decrease in the hippocampus, and was significantly decreased in an age-dependent manner in the cerebral cortex of SAMP8 with age-related deterioration of learning and memory (Cheng et al., 2011), which could indicate that the glutamatergic synaptic transmission was weakened in the brain of aging SAMP8. Consistent with a dysfunction in the recycling of glutamate, there is a selective loss of vesicular glutamate transport in synaptic vesicles isolated from cerebral cortex synaptosomes from AD (Westphalen et al., 2003).

GPCR	AD	SAMP8
5-Hydroxytryptamine receptors	↓ 5-HT_{2A}, 5-HT_4, 5-HT_6	↓ serotonergic activity (5-HT_1, 5-HT_2)
	↓ 5-HT level	↓ 5-HT level
Acetylcholine receptors (muscarinic)	↓ hippocampal M_1	↓ hippocampal M_1
	↑ frontal cortical M_1	↑ cortical M_1
	↓ ACh release	↓ ACh release
Adenosine receptors	↑ frontal cortical A_1, A_{2A}	↓ A_1, ↑ A_{2A} (whole brain)
Adrenoceptors	↑ prefrontal cortical and hippocampal β_2AR	no apparent change
Calcitonin receptors	↑ amylin receptor (TgCRND8 mice)	↓ plasma calcitonin
Chemokine receptors	↑ CCL2	↑ hippocampal Ccl19, Ccl27 expression, ↑ CCL2
Dopamine receptors	↓ frontal cortical D_1, D_3, D_4, D_2 ↑ frontal cortical D_5 ↓ temporal lobe DR	n.d.

GPCR	AD	SAMP8
	↓ DA level	↓ DA level
$GABA_B$ receptors	↓ hippocampal and cortical $GABA_B$	n.d.
	↓ GABA level in CSF	n.d.
Melatonin receptors	↓ MT_2	= MT_1, MT_2 mRNA expression
	↓ melatonin level in CSF	n.d.
Opioid receptors	↓ δ-OR amygdala and putamen ↓ μ-OR hippocampus and subiculum	↓ cortical σ site = hippocampal σ site
	↑ hippocampal enkephalin	n.d.
Orexin receptors	= Hypocretin-1 level	n.d.
Parathyroid hormone receptors	= PTH level (but ↑ PTH with aging)	↑ PTH level with aging
Somatostatin receptors	↓ sst2, sst4	n.d.
	↓ somatostatin level	↓ somatostatin level
Thyrotropin-releasing hormone receptors	= hippocampal TRH receptors ↓ cortical amygdala TRH receptor	n.d.
	↓ TRH level	↓ TRH level
PACAP receptors	↓ PACAP expression	Altered transport through BBB
Metabotropic glutamate receptor	↓ mGluR in frontal cortex	not published
	↓ VGLUT	↓ VGLUT

Table 3. G protein-coupled receptors assessed in Alzheimer's disease (AD) and senescence-accelerated mouse P8 strain (SAMP8). n.d.: not determined. GPCRs that have been not investigated in SAMP8, or at least not found in literature, are the following: Anaphylatoxin, Angiotensin, Apelin, Bile acid, Bombesin, Bradykinin, Calcium-sensing, Cannabinoid, Cholecystokinin, Corticotropin-releasing factor, Endothelin, Estrogen (G protein-coupled) Formylpeptide, Free fatty acid, Frizzled, Galanin, Ghrelin, Glucagon receptor family, Glycoprotein hormone, Gonadotrophin-releasing hormone, Histamine, Hydroxycarboxylic acid, Kisspeptin, Leukotriene, Lysophospholipid, Melanin-concentrating hormone, Melanocortin, Motilin, Neuromedin U, Neuropeptide FF/neuropeptide AF, Neuropeptide S receptor, Neuropeptide W/neuropeptide B, Neuropeptide Y, Neurotensin, P2Y, Peptide P518, Platelet-activating factor, Prokineticin, Prolactin-releasing peptide, Prostanoid, Protease-activated, Relaxin family peptide, Tachykinin, Trace amine, Urotensin and Vasopressin and oxytocin receptors.

5. Conclusion

Senescence-accelerated mouse P8 (SAMP8) has many features that are known to occur early in the pathogenesis of AD such as increased oxidative stress, amyloid-β alterations, and tau phosphorylation. The neurochemical changes reported to date in SAMP8 mice may help in the study of AD pathogenesis as many of them are similar to what is found in AD (see Table 3). However, there is still a lot of work to be done in the analysis of the different receptor-mediated signaling pathways and their modulation in this animal model.

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Valosin-Containing Protein (VCP) Disease and Familial Alzheimer's Disease: Contrasts and Overlaps

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1. Introduction

Contrasts between two entities may be illuminating because of the emphasis on what each is not. Here we describe two proteinopathies producing brain neurodegeneration in mature adults, autosomal dominant valosin-containing protein (VCP) disease and Familial Alzheimer's disease (FAD) caused by presenillin-1 (PSEN1) mutations, illustrating both contrasting patterns of clinical presentation and known neuropathologic and imaging features, and points of congruence.

Mutations primarily in the ubiquitin binding domain of the VCP gene cause frontotemporal dementia as part of a rare but important disorder that also encompasses inclusion body myopathy, Paget disease of bone, and in some cases, motor neuron disease. The VCP dementia has onset in the 50s, characterized by abulia, expressive language loss, and executive dysfunction. The pattern of degeneration generally is anterior, in frontal and temporal lobes, involving neuronal nuclear inclusions of ubiquitin and TAR DNA binding protein 43 (TDP-43), but not amyloid or tau.

The most common mutations causing FAD occur in the PSEN1 gene. The associated dementia has onset in the late 40s, characterized by early memory loss and diffuse amyloid vasculopathy, and posteriorly distributed neuritic amyloid plaque and neurofibrillary tau pathology in medial temporal and parietal lobes, but not ubiquitin or TDP-43. Nonetheless, both VCP and PSEN1 pathologies have extensively documented abnormalities in similar protein processing pathways.

2. VCP Disease – IBMPFD

Hereditary inclusion body myopathy associated with Paget disease of bone and frontotemporal dementia (IBMPFD; OMIM 167320) is a unique and rare disorder associated with mutations primarily in the ubiquitin binding domain of the valosin-containing protein

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(VCP) gene (Watts et al. 2003; Watts et al. 2004). VCP, a member of the AAA-ATPase super-family, occupies the crossroads of many cellular functions including ubiquitin mediated protein degradation, cell cycle control, membrane fusion, and golgi reassembly (Kimonis and Watts 2005; Halawani and Latterich 2006). It is lethal as a homozygous deletion in mice (Muller et al. 2007), and an important regulator of neuronal and dendritic development (Rumpf et al. 2011).

Current theories concerning the pathogenesis of VCP disease include altered protein degradation via the ubiquitin-protosomal system (Kakizuka 2008; Dai and Li 2001; Wojcik, Yano, and DeMartino 2004), generalized endoplasmic reticulum (ER) dysfunction with altered protein trafficking (Weihl et al. 2006; Wojcik et al. 2006; Poksay et al. 2011), and combined activation and failure of inhibition of cell death pathways (Braun and Zischka 2008). Recently VCP has been implicated in the autophagy/lysosome process (Badadani et al. 2010; Ju et al. 2009; Ju et al. 2008; Ju and Weihl 2010a, 2010b; Tresse et al. 2010). These studies have suggested that VCP mutations cause failure of autophagosome fusion with lysosomes, resulting in accumulation of ubiquitin together with other autophagosome proteins LC3 and p62/sequestosome, in rimmed vacuoles, a hallmark of VCP muscle disease (Vesa et al. 2009, Ju et al. 2009; Tresse et al. 2010).

Certain mutations are also suspected to interrupt the integrity of the hexomeric ring structure of the active VCP complex (Halawani et al. 2009), and its interaction with its adaptors, e.g. p47, gp78 and Npl4-Ufd1 (Alzayady et al. 2005), although this finding has not been universally replicated (Weihl et al. 2007). Our group has confirmed that mutant VCP protein exhibit strongly altered co-factor interactions suggesting that imbalanced co-factor binding to p97 is a key pathological feature of IBMPFD and potentially of other proteinopathies involving VCP (Fernandez-Saiz and Buchberger 2010). Elevated ATPase activity associated with cellular protein mislocalization (Manno et al. 2010) is associated with VCP mutations. Recently studies revealed significant reduction in ATP level in hs.TER94A229E and hs.TER94R188Q drosophila models which may contribute to the neurodegeneration phenotype (Chang et al. 2011, Ritson et al. 2010).

The R155H VCP knock-in heterozygous mouse is a promising model demonstrating several typical clinical and molecular features of the disease including progressive weakness, vacuolization of myofibrils with centrally located nuclei, and cytoplasmic accumulation of TDP-43 and ubiquitin in brain as well as in myofibers (Badadani et al. 2010; Custer et al. 2010). It may prove to be very useful in translational research studies seeking therapies for VCP disease. Analysis of a Drosophila model has provided evidence that mutant VCP interacts abnormally with TDP-43 as a gain-of-function mechanism to cause redistribution of TDP-43 from its usual location in the nucleus to the cytoplasm (Ritson et al. 2010). These findings would be usefully replicated in the mouse model.

The clinical disorder typically presents in the early 40s with progressive proximal muscle weakness or with Paget disease of bone (PDB). Weakness is associated with rimmed vacuoles and inclusions on muscle biopsy in the majority of individuals; PDB is present in approximately half of affected individuals. Frontotemporal dementia (Table 1) becomes symptomatic later in a third of affected at a mean age of 55 years (Kimonis and Watts 2007; Kimonis, Fulchiero et al. 2008; Kimonis, Mehta et al. 2008; Kimonis and Watts 2005; Kovach et al. 2001). A small percentage of individuals have been identified with motor neuron disease (MND) phenotype (Johnson et al. 2010), Parkinson's disease (Johnson et al. 2010;

Rohrer et al. 2011), cardiomyopathy (Hubbers et al. 2007; Miller et al. 2009), liver disease (Guyant-Marechal et al. 2006), cataracts (Guyant-Marechal et al. 2006), hearing loss (Djamshidian et al. 2009), or corticospinal tract dysfunction (Kumar et al. 2010).

The VCP disease-associated dementia typically presents with frontotemporal phenotypes, e.g., altered social behavior, abulia, executive dysfunction, altered expressive language, and loss of semantic knowledge (Table 1). However, different families carrying the same VCP mutation may have a wide variation in clinical phenotype. For example, some families carrying the R159H VCP mutation may have an apparent high penetrance for the dementia phenotype (frequency 75-100%; van der Zee et al. 2009) but different average ages of onset (46 ±2 vs. 62 ±1 years). Other families with R159H may express high penetrance of PDB and IBM phenotype (100%) but demonstrate relatively low dementia frequency (20%; Haubenberger et al. 2005). The presenting dementia phenotype in R155C VCP may be behavioral variant FTD, an AD-like memory loss, or a non-specific cognitive dysfunction across several domains (Guyant-Marechal et al. 2006).

Some of this variability may have to do with the interest and specialty expertise of the clinics in which affected patients are seen, e.g., increasing the likely detection of FTD in a clinic dedicated to this sometimes difficult to diagnose disorder. The age at which the patient is seen and the length of follow-up will determine the presence and degree of cognitive and behavioral symptoms, and thus the likelihood of meeting criteria for a clinical diagnosis. Early memory symptoms may evolve into a more recognizable behavioral syndrome typical of FTD (Guyant-Marechal et al. 2006; Krause et al. 2007; van der Zee et al. 2009). Relative timing of the symptoms of FTD, PDB and IBM may also influence observed phenotypic frequencies – severe muscle disease with cardiomyopathy and respiratory failure might occur before dementia could be observed. Early dementia symptoms could be misinterpreted as a medical complication of severe respiratory or cardiac illness.

Nonetheless a substantial biologic variability across and within families with the same mutation, and across mutations, is well documented in VCP dementia. Potential explanations for variability are modifier genes, epigenetic mechanisms, and environmental exposures, the latter two possibilities as yet unexplored. A possible modifier gene is apolipoprotein-E. Possession of one or more APOE4 alleles was found to be associated with dementia in VCP disease, and increases risk for sporadic FTD in a dose-dependent manner (Bernardi et al. 2006; Mehta et al. 2007; Rosso et al. 2002). Tau haplotype was not associated with VCP dementia (Mehta et al. 2007), and VCP polymorphisms have not been found to be increased in the general population of patients with sporadic FTD (Schumacher et al. 2009).

Despite variability in clinical presentation, the qualitative pathologic changes are relatively uniform (Table 2). Post-mortem brains of individuals with VCP mutations reveal 75% have findings pathologically classified as frontotemporal lobar dementia ubiquitin type (FTLD-U), with abundant intranuclear ubiquitinated protein inclusions, dystrophic neuritis and rare cytoplasmic ubiquitin-positive inclusions (Forman et al. 2006; Kimonis, Fulchiero et al. 2008). Possible exception to this relative uniformity is the finding of vacuolar change in frontotemporal regions but not intranuclear ubiquitin pathology in three autopsies of R155C VCP mutation patients (Guyant-Marechal et al. 2006). This apparent anomaly may have a technical basis, since two of these subjects had increased frontal lobe ubiquitin immunoreactivity on Western blot.

Intranuclear inclusions of ubiquitin co-localized with TDP-43 are widespread and numerous

Author	Mut.	Dementia	Onset Age (n)	Clinical Dementia Onset	Muscle Disease	Paget Disease	Comments
Gidaro, 2008	R155C	2/3	52 (1)	bvFTD	3/3	1/3	Very mild PDB
Guyant-Marechal, 2006	R155C	7/10	57 (7)	bvFTD (4) AD-type (2) Possible FTD (1)	8/10	3/10	Later mutism in AD-type patients; myotonia 7/9; seizure/myoclonus 1/7; liver disease 7/10
Kim, 2011	R155C	3/3	56 (3)	Semantic dementia	2/3	2/3	-
Kalbe, 2011	R155C	-	45	Mild Frontal Impairment	1/1	0/1	Lowered frontal assessment scores
Schroder, 2005	R155C	1/1	47 (1)	Semantic dementia	1/1	0/1	Poor language comprehension and naming, abulia
Miller, 2009	R155H	8/18		bvFTD	18/18	0/18	Cardiomyopathy; sphincter dysfunction
Johnson, 2010	R155H	-	59	Mild Frontal Impairment	1/1	0/1	Lowered frontal assessment scores
Kumar, 2010	R155L	-	-	-	-	-	Corticospinal dysfunction in one
Djamshidian, 2009	G157R	2/4		Mild Frontal Impairment	2/4	4/4	Hearing loss
Bersano, 2009	R159C	1/1	68 (1)	bvFTD	1/1	0/1	No family history; suspected MND plus IBM
Haubenberger, 2005	R159H	0/4	-	No dementia	4/4	4/4	Mild muscle weakness; age >60 4/4
van der Zee, 2009	R159H	10/15	56 (8)	bvFTD (2), possible FTD (2)	5/15	7/15	Early memory, later behavioral in two; three families studied
Johnson, 2010	R191Q	-	50 (1)	Mild Frontal Impairment	4/4	0/4	4/4 ALS; Family history of FTD in two, PDB in one
Watts, 2007	L198W	1/4	50 (1)	Possible FTD	4/4	2/4	Early memory, later behavioral
Rohrer, 2011	I27V	1/2	55 (1)	bvFTD	0/2	2/2	Parkinsonism (1) progressive dysarthria (1)
Guyant-Marechal, 2006	R93C	6/6	58 (6)	bvFTD	3/6	3/6	-
Krause, 2007	R93C	1/1	60 (1)	Semantic dementia	1/1	1/1	Later behavioral syndrome
Guyant Marechault	R93C	6/6	58 (6)	bvFTD	3/6	3/6	-
Watts, 2007	N387H	2/3	46 (2)	bvFTD (1) memory (1)	2/3	0/3	-

Table 1. Dementia in VCP disease. Columns (left to right): 1. Reporting 1st author, 2. Mutation, 3. Number with dementia/ total reported, 4. Average dementia onset age (number reported), 5. Clinical Dementia type (number of each reported), 6. Affected with muscle disease/ total affected, 7. Affected with Paget disease of Bone/ total affected, 8. Additional comments

-			Region Pathology Intensity					TDP-43		VCP								
Author	n	Mut.	Front	Temp	Pariet	Occip	MT	Nu	Cyt	Nu	Cyt	Ubiq	Nfil	tau	aSN	Poly	bAm	Comments
Watts, 2007; Forman, 2006	1	L198W	+	++	-	+/-	++					+	-	-	-	-	-	AHC loss; NFT limbic
Schroder, 2005	1	R155C	++	++	+	+	+/-			+	+	+		-	-		-	Few AT8+tau
van der Zee, 2009	3	R159H	++	++				+				+						"cats-eye" inclusions;
Neumann, 2007; Forman, 2006	1	R155C	+	++	+	+		++		-	-	+		-	-		-	VCP Regional Intensity
Neumann, 2007; Forman, 2006	6	R155H	++	++	++	++	+/-	++	+/-	-	-	+		+/-	-		-	VCP Regional Intensity (4/6)
Guyant-Marechal, 2006	3	R155C	++	++	+					-	-	-		-	-		-	AHC loss, Ubiq. IR 2/3

Table 2. Neuropathology in VCP Disease. Columns (left to right): 1. Reporting 1st author, 2. Number reported, 3. Mutation, 4 - 8. Intensity of regional pathology (subjective, relative, not quantitative); MT – medial temporal), 9-10. TDP-43 Pathology (Nu – intranuclear, Cyt – cytoplasmic), 11-12. VCP Pathology, 13. Ubiquitin staining, 14. Neurofilament staining, 15. Tau Pathology, 16. Alpha-synuclein staining (aSN), 17. Polyglutamine Pathology, 18. Beta-amyloid staining, 19. Additional comments (AHC – anterior horn cell).

in cortical and basal ganglia, sometimes with a "cats-eye" curvilinear morphology (Neumann et al. 2007; Neumann, Tolnay, and Mackenzie 2009). Dystrophic neurites and cytoplasmic inclusions are relatively low in number in VCP disease brain and contain both proteins. TDP-43 appears to be depleted in normal neuronal nuclei (Neumann et al. 2007). The distribution of protein pathology and neuronal loss may be diffuse and include the occipital lobe, but when focal is predominant in the frontal and temporal regions, sometimes asymmetrically to right or left. The medial temporal lobe, particularly the dentate gyrus, is mostly spared. Occasional coexistent tau, alpha-synuclein, or amyloid pathology is detectible in some cases but this is not characteristic. Some authors have reported VCP within inclusions (Schroder et al. 2005), but others have found it only rarely in dystrophic neurites (Forman et al. 2006). Other pathologies, e.g., neurofiliment or polyglutamine, are absent.

TDP-43 has also been identified as the major disease protein in the ubiquitin-positive inclusions of sporadic and familial FTLD-U, including patients with the MND phenotype (Cairns, Neumann et al. 2007). These pathologic features overlap with those of amyotrophic lateral sclerosis. Anterior horn cell loss has been observed on spinal cord examination in some affected subjects with VCP mutations (Liscic et al. 2008), and the MND phenotype has been described as a dominant feature in a family carrying the R191Q VCP mutation (Johnson et al. 2010). In VCP disease, the pathologic classification best fits the description of FTLD-U, type 4 (Sampathu et al. 2006), distinguished by the intracellular localization of the inclusions, relative rarity of cytoplasmic inclusions and dystrophic neurites, and sparing of the medial temporal lobe, particularly the dentate gyrus. The question of whether the neuropathologic features in VCP disease with MND phenotype most resemble FTLD-U type 4 or FTLD-U types 2 and 3 associated with sporadic FTD with MND phenotype, characterized by abundant cytoplasmic inclusions, remains to be answered. Although rare, VCP disease may provide new insight into the molecular mechanism of TDP-43 proteinopathies caused by more common genetic alterations.

Imaging studies of the brain in VCP mutation carriers with cognitive alterations have also demonstrated variability (Table 3). However, few studies have been performed. The variability in part is due to use of differing imaging modalities: structural computed tomography and magnetic resonance imaging, and functional resting fluorodeoxyglucose positron emission tomography (regional glucose uptake; FDG-PET) and single photon emission tomography (regional perfusion; SPECT). These studies have been performed in different combinations and at different stages of cognitive impairment.

Imaging performed in the presence of subtle cognitive changes thought to presage dementia demonstrates no structural change (Kalbe et al 2011; Djamshidian et al 2009; Watts et al. 2007) and occasional subtle regions of glucose hypometabolism (Kalbe et al. 2011). In subjects with dementia, when present local cortical atrophies may be symmetric in the frontotemporal regions (Watts et al. 2007, Miller et al. 2009, Krause et l. 2007, Schroeder et al. 2005, Rohrer et al. 2011, van der Zee et l. 2009) or lateralized to the right or left with an anterior temporal emphasis (Kim et a. 2011). Other structural studies may show only generalized atrophy (Gidaro et al. 2008, Watts et al. 2007, van der Zee et al. 2009, Guyant-Marechal et al. 2006). Hypoperfusion (SPECT) and glucose hypometabolism (FDG-PET) generally correspond to the regions of greatest atrophy seen on structural imaging in the same patients.

Author	Mut	Imaging Modality	Focal Atrophy	Diffuse Atrophy	White Matter	Comments
Gidaro, 2008	R155C	CT	-	(1/1)	-	-
Watts, 2007	N387H	CT	(1/2)	(1/2)	-	"Pick's" diagnosed in one
Watts, 2007	L198W	MR	-	-	-	MR normal; SPECT mild frontal hypoperfusion
Miller, 2009	R155H	MR	(3/3)	-	(3/3)	Frontal atrophy; mild peripheral hyperintensities
Krause, 2007	R93C	MR	(1/1)	-	(1/1)	Severe frontal WM change; PET frontotemporal hypometab.
Schroder, 2005	R155C	MR	(1/1)	-	-	Frontotemporal atrophy
Rohrer, 2011	I27V	MR	(1/2)	-	-	Frontotemporal atrophy, SPECT parietal hypoperfusion
van der Zee, 2009	R159H	MR	(1/1)	-	(1/1)	Frontal and generalized atrophy; SPECT frontotemporal hypoperfusion
van der Zee, 2009	R159H	CT	-	(1/1)	(1/1)	SPECT bifrontal and diffuse hypoperfusion
van der Zee, 2009	R159H	FDG-PET	-	-	-	Frontotemporal hypometabolism
Kim, 2011	R155C	MR (3), FDG-PET (2)	(3/3)	-	(3/3)	Asymmetric frontotemporal atrophy L (2) R (1), corresponding PET hypometabolism in 2. WM change mild, focal.
Guyant-Marechal, 2006	R155C	MR/CT	-	(5/5)	(0/5)	SPECT frontal lobe hypoperfusion (?/5)
Johnson, 2010	R191Q	MR	-	-	-	Normal in (1/1) mild frontal impairment
Djamshidian, 2009	G157R	MR	-		-	Normal (1/1)
Kalbe, 2011	R155C	MR	-	-	-	Pre-dementia; Normal MR and FDG-PET
Kalbe, 2011	R155H	MR	-	-	-	Pre-dementia; Normal MR; FDG-PET hypometabolism L medial temporal (1/1)

Table 3. Imaging in VCP disease. Columns (left to right): 1. Reporting 1st author, 2. Mutation, 3. Modality used, 4. Presence of focal atrophy, (number/ total images reported), 5. Generalized/diffuse atrophy pattern (number/ total images reported), 6. Presence of white matter hyperintensities or other abnormalities (number/ total images reported), 8. Additional comments.

3. Familial Alzheimer's disease-PSEN1

Autosomal dominant familial Alzheimer's disease (FAD; OMIM 104300) is usually of early onset (EOAD; age < 65 years) and has been known for many years (Janssen et al. 2003). Alzheimer's original case description was reported because of the observed early onset of disease at age 51; before then "senile dementia" was thought only to occur in the elderly (Maurer, Volk, and Gerbaldo 1997). Most cases of FAD are attributable to mutation of the PSEN1 gene on chromosome 14 (OMIM 104311; Campion et al. 1999). The remaining cases are found in rare families harboring mutations in amyloid precursor protein (APP) on chromosome 21, in presenillin-2 (PSEN2) on chromosome 1, or with a currently unknown genetic substrate, including overlap with a small part of the Bell curve continuous with late onset AD (LOAD; Brickell et al. 2006).

Here the focus is on PSEN1-related FAD because it is by far the most frequent FAD type and hence more is known about these families. Presenilin-1 is an important component of the gamma-secretase that cleaves amyloid precursor protein (APP) and NOTCH. It is involved in adult neuronal stem cell differentiation (Gadadhar, Marr, and Lazarov 2011), early cortical development (De Gasperi et al. 2008; Wines-Samuelson and Shen 2005), endoplasmic reticulum calcium regulation (Coen and Annaert 2010), and autophagy (Lee et al. 2010). There are currently 194 known PSEN1 mutations (http://www.molgen.ua.ac.be /ADMutations). Nonetheless, wide phynotypic variability has been found across families with PSEN1 mutations, even those harboring an identical putative founder mutation (M146L; Bruni et al. 2010). Individuals with this mutation may demonstrate early memory loss or temporo-spatial disorientation typical of LOAD (58% of 50), but others present with apathy or executive dysfunction (42%). Regardless of clinical manifestations,

neuropathology consists of AD-typical neuritic plaques, neurofibrillary tangles, neuropil threads, and amyloid angiopathy, differing only in the regional distribution of this pathology, a distribution that determines phenotype, e.g., dysexecutive dysfunction is associated with dorsal frontal lobe pathology (Bruni et al. 2010). These observations suggest that a universal intrinsic pattern of molecular profile difference between, for example, frontal and parietal regions will not explain where or in what sequence AD pathology will manifest in persons with M146L PSEN1 mutations.

The spectrum of phenotypic and neuropathologic variation is even wider when different mutations are considered. For example a variant with dementia associated with spastic paraparesis is associated with several PSEN1 mutations: deletion in exon 9, insertion in exon 3, P436Q, R278K, G217R and L85P point mutations, and deletion of codons 83 and 84 in exon 4 (Verkkoniemi et al. 2000; Houlden et al. 2000; Moretti et al. 2004; Ataka et al. 2004; Assini et al. 2003; Smith et al. 2001; Norton et al. 2009). Neuropathology of these variants includes characteristic fluffy spheres of non-neuritic extraneuronal amyloid termed cotton-wool plaques (Houlden et al. 2000). In one patient with a small deletion in PSEN1 exon 12, parkinsonism, spasticity and dementia were the clinical features and neuropathologic examination showed cotton-wool plaques, cortical and subcortical Lewy bodies, and extensive amyloid angiopathy (Ishikawa et al. 2005). Prominent periventricular white matter hyperintensities associated with spastic paraparesis have been observed on MRI in two E280G and in four P284S PSEN1 mutation carriers (O'Riordan et al. 2002; Marrosu et al. 2006). Extensive amyloid angiopathy causing white matter ischemia could explain the paraparesis in these cases.

Clinical studies of PSEN1 mutation kindreds have reported widely variable age of onset, e.g., 28 years in a de novo M233L mutation carrier (Portet et al. 2003) and a range of onset within the same H163T mutation family of 44-65 years (Axelman, Basun, and Lannfelt 1998). Clinical findings can also include, prominent psychiatric symptoms (S170F mutation (Piccini et al. 2007); L392P (Tedde et al. 2000)), a behavioral variant frontotemporal dementia syndrome (bvFTD; L113P(Raux et al. 2000)), anomia (R278I(Godbolt et al. 2004)), seizures and myoclonus (S170F(Snider et al. 2005), cerebellar ataxia, intention tremor, and dysdiadochokinesia. Neuropathologic findings are generally robust depositions in the form of A-beta$_{1\text{-}42(3)}$ and A-beta$_{1\text{-}40}$ amyloid in vessels, sometimes extending into parenchyma and termed dyshoric vasculopathy, neuritic plaques, tau-laden neuropil threads, and hyperphosphorylated tau protein forming intraneuronal tangles within cortical neurons (Janssen et al. 2000; Janssen et al. 2001). Pathologic, brainstem and cortical Lewy bodies (Kaneko et al. 2007; Snider et al. 2005), and possibly Pick-type tauopathy has been found in carriers of the PSEN1 G183V and M146L mutations (Dermaut et al. 2004; Halliday et al. 2005). TDP-43 and ubiquitin are not seen.

A large kindred identified in Columbia, South America is the focus of an ongoing large scale study of AD in its earliest, pre-symptomatic stages, serving as a model for the much more frequent LOAD (>95% of all AD cases; Lopera et al. 1997; Acosta-Baena et al. 2011). The causative mutation is E280A. Onset age in the initial study was an average 47 years, but there was a wide range between 34 and 62 years. The average life span following diagnosis was 8 years (Lopera et al. 1997). Longitudinal follow-up has shown that the earliest detectible cognitive changes occur at average age 35 years, progressing through mild stages of impairment associated with memory complaints to dementia over approximately 15 years. Time from dementia to death is now estimated as 10 years, likely due to improved

methods of early detection and diagnosis as the study has developed (Acosta-Baena et al. 2011). Studies in this kindred using hexamethylpropyleneamine oxime SPECT has demonstrated decreased perfusion in hippocampus, posterior cingulate, and frontoparietal cortex in asymptomatic carriers (n=18) using t-scores based on a template derived from 200 normal subjects. Carriers with diagnosed AD dementia (n=16) had decreased frontal and parietal perfusion compared to normal non-carriers from the same kindred (n=23). The clear major advantages for the study of this kindred is its large size (449 identified mutation carriers), a cognitive phenotype that parallels LOAD, and the very high predictability of dementia in PSEN1 carriers. In contrast, LOAD has no genetic profile or multivariate model that can approach the predictive power of an autosomal dominant mutation.

4. Contrasts and overlaps

At the most general level cortical regions most affected by VCP-associated pathology are connected by the anterior 60% of the corpus callosum and the anterior commissure – the prefrontal, orbitofrontal, premotor and anterior temporal cortices. Anterior horn cells and muscle share the ubiquitin/TDP-43 pathology. Long tract findings are exceptional. The clinical syndromes associated with cortical dysfunction in these regions fall broadly into the class of frontotemporal dementias, and encompass behavioral, dysexecutive, expressive language, and semantic access symptom cores. In brain the characteristic ubiquitin/TDP-43 inclusions are neuronal intranuclear and rarely cytoplasmic or extracellular. The medial temporal lobe, particularly the dentate nucleus, is largely spared. Tau and amyloid pathology are not found. Imaging reveals commensurate frontotemporal atrophy, sometimes lateralized in correlation with the clinical syndrome, accompanied by hypometabolism and hypoperfusion in these anterior regions.

In contrast, cortical regions most affected by FAD PSEN1-associated pathology are connected across the posterior 40% of the corpus callosum and posterior hippocampal commissure – the parietal, superior and inferior temporal lobes and medial temporal lobes but generally sparing the primary occipital region. Neuropathology is described as quite dense and parallels that found in LOAD, e.g., include extracellular neuritic plaques, cytoplasmic fibrillary tangles, neuropil threads and amyloid angiopathy. The temporal lobe, particularly the medial portion is heavily affected. Ubiquitin and TDP-43 are absent. In many cases a classic AD clinical sequence of early memory loss followed by declines in other cognitive domains is described, particularly well documented in PSEN1 E280A families. Variants include EOAD with spastic paraparesis, characteristic "cotton wool" extracellular amyloid plaques and dense amyloid angiopathy. Involvement of the lower motor neuron has not been reported. Structural imaging reveals atrophic changes in temporal and parietal lobes, with hypometabolism, particularly in posterior cingulate and other parietal areas.

Both VCP disease and FAD PSEN1 are single-gene disorders producing dementia phenotypes similar to those seen much more frequently in sporadic disease. In both there is marked variation in phenotypic expression of the same mutation within and across families, and across mutations in the same gene, with overlapping presentations of the FTD or AD dementia phenotypes between genes in some cases. Both VCP and PSEN1 genes have dual roles in both CNS development and in maintenance of the mature nervous system, but produce neurologic dysfunction only in the adult associated with characteristic protein

accumulations. Finally both VCP and PSEN1 pathophysiologic alterations appear to overlap at several points within cellular protein processing and functional pathways, including protein trafficking in the trans-golgi apparatus, downstream in the ubiquitin-proteosome system, and autophagy (Table 4).

IBMPFD Disease: VCP Gene	Familial Alzheimer's Disease: PSEN1 Gene
• Autosomal dominant IBMPFD (OMIM 167320)	• Autosomal dominant FAD (OMIM 104300)
• Single-gene disorder produing dementia phenotype	• Single-gene disorder produing dementia phenotype
• Marked variation of phenotypic expression of the same mutation within and across families	• Marked variation of phenotypic expression of the same mutation within and across families
• Mutation in the valosin-containing protein (VCP) gene	• Mutation in the Presenillin1 (PSEN1) gene, an important component of gamma-secretase that cleaves amyloid precursor protein (APP) & NOTCH
• Currently over 20 known VCP mutations	• Currently over 194 known PSEN1 mutations
• Onset in the 50's	• Onset in the late 40's
• Characterized by abulia, expressive language loss, and executive dysfunction	• Characterized by early memory loss and diffuse amyloid vasculopathy
• Anterior, frontal and temporal lobes pattern of degeneration	• Amyloid plaque and neurofibrillary tau pathology in temporal and parietal lobes, but not ubiquitin or TDP-43
• Neuronal nuclear inclusions of ubiquitin and TDP-43, but not amyloid or tau	• PSEN1 has been implicated in adult neuronal stem cell differentiation, cortical development, ER calcium regulation, and autophagy
• Long tract findings are not described	• Cortical regions affected by FAD PSEN1 pathology are conncected across the posterior 40% of the corpus callosum and posterior hippocampal commissure
• VCP plays a role in ubiquitin-mediated protein degradation, cell cycle control, membrane fusion, and gogi reassembly	• Neuropathology includes extracellular neuritic plaques, cytoplasmic fibrillary tangles, neuropil threads and amyloid angiopathy
• VCP has been implicated in ubiquitin-proteasomal system, ER dysfunction, cell death and autophagy/lysosomal pathways	• Structural imaging reveals atrophic changes in temporal and parietal lobes
• Cortical regions affected by VCP pathology are connected by the anterior 60% of the corpus callosum and anterior commissure	
• Imaging reveals commensurate frontotemporal atrophy accompanied by hypometabolism and hypoperfusion in anterior regions	

Table 4. Neuropathologic Features and Points of Comparison: IBMPFD vs. FAD.

5. Conclusion

VCP disease and FAD PSEN1 appear to have commonalities at a fundamental level in that both involve altered polyfunctional proteins involved in specific overlapping functions, particularly autophagy, and have common downstream pathways, e.g., proteosomal. Yet the diseases are clearly distinct in most particulars, suggesting a principle of independent compartmentalization that may provide insights into both disorders.

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17

Neural Basis of Hyposmia in Alzheimer's Disease

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1. Introduction

At the beginning of twenty century Alois Alzheimer described the pathology that now bears his name (Alzheimer, 1907). Over a hundred years later, Alzheimer's disease (AD) is the most common cause of dementia in developed countries. Here eighteen million people are currently affected and the number of patients is expected to increase dramatically with the ongoing increase in the elderly population (Fotuhi et al., 2009, Mount & Downton, 2006). Because no suitable biomarkers are available, the diagnosis of AD remains inconclusive until postmortem pathological analysis, and physicians rely on behavioral manifestations to differentiate between AD and other conditions. For this reason firm diagnosis is generally only made at later stages of the disorder when treatment is purely palliative. These features make AD a social and economic challenge in developed countries (Wimo et al., 2010).

Clinically, AD is characterized by progressive loss of cognitive functions with specific deficits in episodic memory. Clinical diagnosis of is generally only made when cognitive deficits are sufficiently severe to cause dependent status of the patient (Nestor et al., 2004). Pathological analyses of AD brain have described two distinct types of proteinopathy in the frontal and temporal lobes involving the limbic system and the basal forebrain. The first type comprises aggregates of beta-amyloid peptide (Aβ) – a specific fragment of the amyloid precursor protein (APP), a plasma membrane protein. These aggregates accumulate in the extracellular space and give rise to senile plaques (SPs). SPs cause synaptotoxicity, neurotoxicity, oxidative stress and hypoxia (Peers et al., 2009, Selkoe, 2001, 2008). The second proteinopathy occurs in the cytosol. Hyperphosphorylation and abnormal aggregation of the microtubule-associated protein tau leads to the intracellular formation of neurofibrillary tangles (NFTs) which cause cytoskeleton destabilization and eventually cell death (Hernandez & Avila, 2008, Selkoe, 2001).

It has been widely reported that olfactory loss (anosmia and hyposmia) takes place in the early stages of AD, and before any detectable cognitive deficits are present. Interestingly, AD pathology extends throughout the limbic system and the basal forebrain, including the olfactory system (Braak & Braak, 1991). The human olfactory system includes peripheral sensory neurons in the olfactory epithelium; these send their axons across the cribriform plate of the etmoides bone to the olfactory bulbs. In the glomerular layer of the olfactory

bulbs their axons synapse with dendrites of the mitral and tufted cells which in turn project to the main olfactory cortex in the basal forebrain. The human olfactory system constitutes complex circuit connections including primary and secondary cortical areas that are connected, as represented schematically in Figure 1.

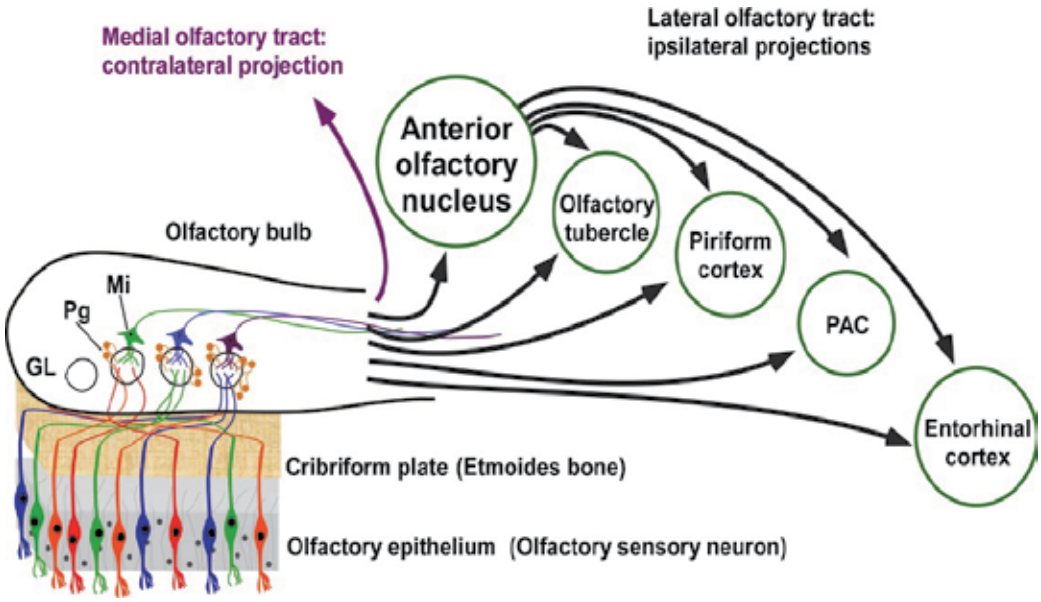

Fig. 1. Schematic diagram of the human olfactory system. GL, glomerular layer; Mi, mitral cell; PAC, periamygdaloid complex; Pg, periglomerular cell.

The progression of AD pathology has been divided into six stages according to the extent of NFT accumulation. Accumulation is first detected in the entorhinal cortex and hippocampus of the limbic system; this extends into the basal forebrain including the olfactory system (Braak & Braak, 1991, Price et al., 1991, Van Hoesen et al., 1991), and from the rostral entorhinal cortex, periamygdaloid cortex, and piriform cortex, to the olfactory tubercle, anterior olfactory nucleus and olfactory bulbs (Fig. 1). Tau pathology has also been described in the olfactory epithelium (Lee et al., 1993). Olfaction is affected in many psychiatric disorders in addition to AD, including Parkinson's disease, Huntington's disease, schizophrenia, senile dementia of Lewy body type, and depression (Atanasova et al., 2008,Kovacs, 2004). It has been widely reported over the past 25 years that olfaction is impaired in AD (Djordjevic et al., 2008, Doty et al., 1987, Mesholam et al., 1998, Murphy, 1999, Murphy et al., 1990, Serby et al., 1985, 1991), and olfaction has become a priority area in the search for biomarkers to establish an early diagnosis of AD and to facilitate early therapeutic intervention (Doty, 2003,Hampel et al., 2010,Hawkes, 2009,Wilson et al., 2009). It has been proposed that the early involvement of the entorhinal cortex and the hippocampus, regions that are tightly related to memory deficiencies (Nagy et al., 1996), could be also the cause of olfactory deficits (Wilson et al., 2007). However, other authors suggest that alternative olfactory areas, for example the posterior part of the piriform cortex, are the specific cause of olfactory deficiencies (Li et al., 2010). Nevertheless, the neural basis underlying hyposmia in the AD brain remain uncertain.

2. Materials and methods

We have studied the olfactory system in 19 AD cases and 7 age-matched controls from the Banc de Teixits Neurològics, Universitat de Barcelona-Hospital Clínic and the Banco de Tejidos/Fundación para Investigaciones Neurológicas, Universidad Complutense de Madrid. Mean ages (± standard derivation) in AD and controls were 77.68 ± 9.01 yr and 74.57 ± 4.47 yr, respectively. Tissue samples were fixed by immersion in paraformaldehyde 4% for one month at least. Then, samples were cryoprotected in 30% w/v sucrose and 50μm coronal sections were obtained using a sliding freezing microtome.

To study the early stages of disease development we employed a double transgenic mouse model of Alzheimer disease (App_{swe}/*Psen1*Δ9). Animals at 2, 4, 6 to 8 months of age (n = 4 homozygous and 4 control female mice per group; N = 32) were collected for analysis. Animals were anesthetized with a mixture of ketamine hydrochloride (Ketolar, Parke-Davis, Madrid, Spain, 1.5 ml/kg, 75 mg/kg) and xylazine (Xilagesic, Calier, Barcelona, Spain, 0.5 ml/kg, 10 mg/kg). Mice were transcardially perfused with saline solution followed by 4% w/v paraformaldehyde fixative (phosphate buffered; 0.1 M, ph 7.2). Brains were removed from skulls and cryoprotected in 30% w/v sucrose, and sectioned (50 μm) in the frontal plane (brains) or in the sagittal plane (olfactory bulbs) using a sliding freezing microtome.

In order to delimit areas of interest sections were stained by Nissl technique (Fig. 2A). Primary antibodies used for immunodetection were mouse anti-tau (tau 46, 1:800, Cell Signaling Technology, Beverly, MA, USA), rabbit anti-Aβ (1:250, Cell Signaling Technology), and goat anti-somatostatin D-20 (1:1000, Santa Cruz Biotechnology, Santa Cruz, CA, USA). Secondary antibodies were either biotinylated (anti-goat IgG, 1:2000, Vector Laboratories, Burlingame, CA, USA) or fluorescent-labeled (1:200, alexas 488 donkey anti-mouse, 568 donkey anti-rabbit, and 350 donkey anti-goat; Molecular Probes, Invitrogen, Carlsbad, CA, USA).

For quantification, somatostatin-positive cells were charted with an X-Y recording system (AccuStage, Minnesota Datametrics, MN, USA). Colocalization levels were measured by confocal microscopy using LSM 710 Zeiss confocal microscope (Carl Zeiss MicroImaging, Barcelona, Spain). Intensities of each fluorochrome were analyzed using the profile tool of the ZEN software (Zeiss).

One-way ANOVA followed by *post hoc* Bonferroni test ($p<0.05$) was used to estimate significant differences among markers and age groups.

3. Interneurons in the olfactory system

Interneurons constitute 20–30% of the neuronal population of the cerebral cortex and possess distinct morphological, electrophysiological and neurochemical characteristics (Ascoli et al., 2008,DeFelipe, 1997,Markram et al., 2004). Two primary features are common to all interneuron subpopulations. First, these cells are predominantly inhibitory interneurons which express γ-aminobutyric acid (GABA), an inhibitory neurotransmitter; and, second, their neuronal connectivity is predominantly restricted to the local region of the brain (DeFelipe & Farinas, 1992, Kawaguchi & Kondo, 2002).

Interneurons are tightly related to the pathoetiology of AD, and many reports have described the involvement of interneuron subpopulations in AD neuropathology (Attems et al., 2008, Brady & Mufson, 1997, Geula et al., 2003, Saiz-Sanchez et al., 2010, Solodkin et al.,

1996, Supnet & Bezprozvanny). It was recently reported that numbers of interneurons in the entorhinal cortex and hippocampus are significantly reduced in early AD stages (Koliatsos et al., 2006). Interneurons regulate synaptic signaling by pyramidal neurons and the loss of this regulation could produce deficits in learning and memory (Palop et al., 2003, Wallenstein & Hasselmo, 1997). Moreover, disregulation of olfactory information-processing due to loss of interneurons could underlie the hyposmia described in the early stages of the disease. This review focuses on four major types of interneurons based on their importance to AD etiology and brain calcium homeostasis: respectively cells expressing somatostatin, calbindin, calretinin or parvalbumin. We also discuss the distribution of different types of interneurons and their involvement with tau and β-amyloid pathology as revealed by confocal microscopy.

3.1 Somatostatin

The neuropeptide somatostatin is implicated in diverse functions in the central nervous system (Epelbaum, 1986, Viollet et al., 2008). Somatostatin is expressed in all olfactory areas. Recently, somatostatin has been linked with AD etiology because it is reported to act as a positive regulator of neprilysin, an enzyme which catalyzes the degradation of β-amyloid peptide (Saito et al., 2005). Somatostatin levels decline with aging (Lu et al., 2004) and are further reduced in AD (Davies et al., 1980). It has been proposed that the decline in somatostatin levels with age could explain the age-dependency of AD onset because reduced somatostatin would be expected to lead to downregulation of neprilysin activity, thereby predisposing to the accumulation of β-amyloid peptide (Hama & Saido, 2005).

Confocal microscopy of the olfactory system in AD brain has revealed that somatostatin is selectively reduced, by up to 50%, in olfactory areas such as anterior olfactory nucleus (AON). Moreover, the deficiency in somatostatin was predominantly associated with β-amyloid pathology rather than tau pathology (Figs 2, 3). These findings are in agreement with the theory of Hama & Saido that there is a tight relationship between somatostatin and β-amyloid. The AON is an important relay in olfactory information processing (Price, 1990). Two distinct portions of the AON can be distinguished in the basal forebrain – the medial AON and the lateral AON divided by the olfactory tract (Fig. 2A). The AON is an early site for the accumulation of tau protein (Fig. 2B) (Price et al., 1991) and is also targeted for β-amyloid deposition (Fig. 2C). Somatostatin-expressing cells in the AON possess typical bipolar interneuron morphology. Confocal analyses show that most somatostatin-cells expressing are not associated with tau pathology (Figs 2E, F and 3), and somatostatin-expressing cells are most commonly associated with β-amyloid or with β-amyloid plus tau (Figs 2F and 3).

In App_{swe}/*Psen1*Δ9 mice, somatostatin is expressed in all olfactory areas. Expression levels decline with age, and are most markedly reduced in the areas where AD initiates, for example the entorhinal cortex. As in the human AD brain, confocal analyses of the olfactory system of double transgenic mice revealed a correlation between somatostatin expression and β-amyloid pathology. Colocalization with β-amyloid peptide was very extensive and was evident in the youngest animals analyzed. Colocalization was seen in all olfactory areas with the exception of the olfactory bulb. Notably, colocalization of somatostatin-expressing interneurons with β-amyloid peptide was evident (Figs 4, 5), even in the absence of reduced levels of somatostatin expression.

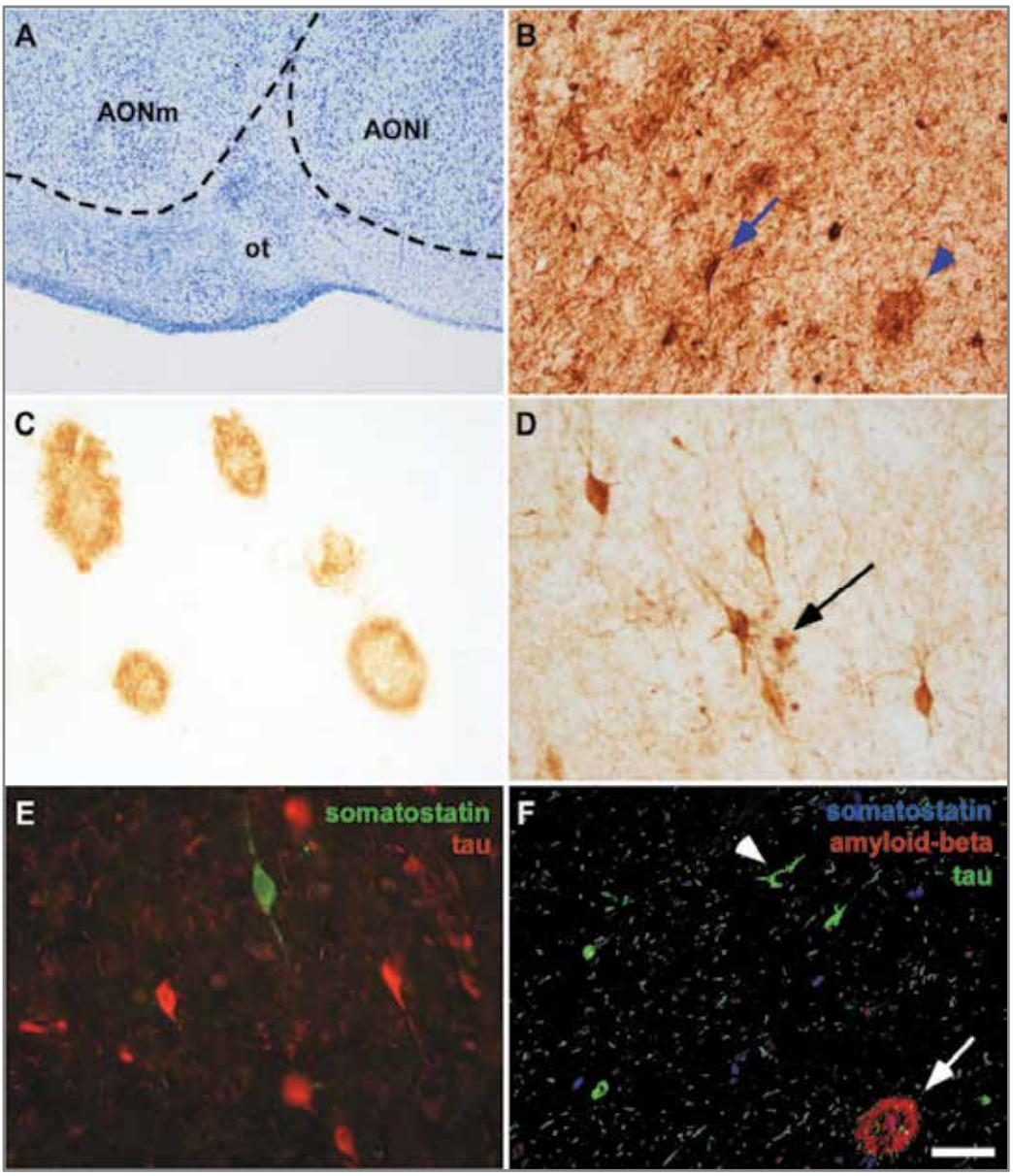

Fig. 2. Expression of somatostatin, tau and β-amyloid in the anterior olfactory nucleus (AON) in Alzheimer's disease. (A) Nissl staining of AON in the basal forebrain. (B) Immunohistochemistry of tau protein (blue arrow, dystrophic neuron; blue arrowhead, cellular debris). (C) β-Amyloid positive senile plaques in AON. (D) Somatostatin-expressing cell in the AON showing a dystrophic neuron (black arrow). (E) Double immunofluorescence for somatostatin (green) and tau protein (red). Confocal image of triple immunofluorescence for somatostatin (blue), β-amyloid (red) and tau (green). Note a neuron positive only for tau protein (white arrowhead) and a typical senile plaque (red). Scale bar: A = 400 μm, B & F = 80 μm, D, C & E = 40 μm.

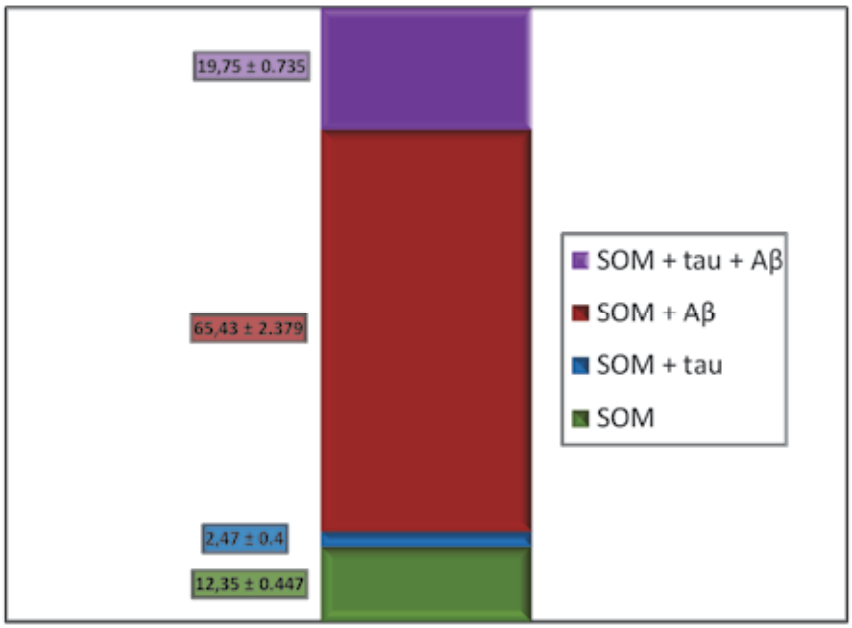

Fig. 3. Percentage of the three different types of colocalization of somatostatin-expressing cells (SOM) with tau protein (tau) and β-amyloid peptide (Aβ) in the human anterior olfactory nucleus.

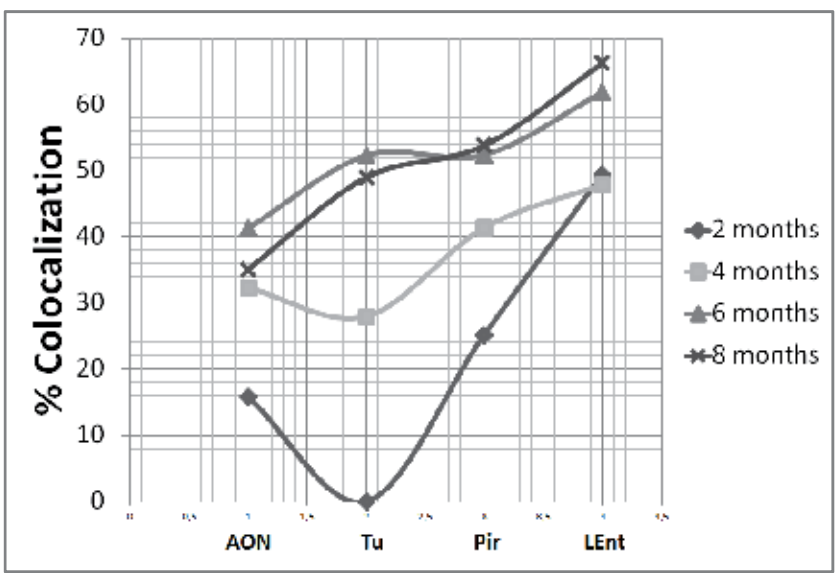

Fig. 4. Percentages of somatostatin and β-amyloid colocalization in the APPswe/PSEN1Δ9 mice olfactory system. Note that the external plexiform layer from the olfactory bulb is absent. AON, anterior olfactory nucleus; LEnt, lateral entorhinal cortex; Pir, piriform cortex; Tu, olfactory tubercle.

All olfactory areas showed a marked accumulation of β-amyloid deposits, but the extent of accumulation in olfactory tubercle (Tu) was less than in other areas (Fig. 5A–D). The greatest reduction in cells expressing somatostatin was seen in the external plexiform layer (EPL) (Fig. 5E,F) of the olfactory bulb, the piriform cortex (Pir) and the entorhinal cortex (Ent). In addition, the olfactory tubercle (Tu) and anterior olfactory nucleus (AON) both showed significant reductions in levels of somatostatin-positive cells. Different forms of colocalization were observed, including isolated cells (Fig. 5G), fibers and cell debris (Fig. 5H, I). Colocalization increased with age and was greater in caudal olfactory areas than in rostral areas. No colocalization was found in the external plexiform layer of the olfactory bulb where β-amyloid pathology was restricted to the granule cell layer (Fig. 5A,D) and was largely absent from the EPL.

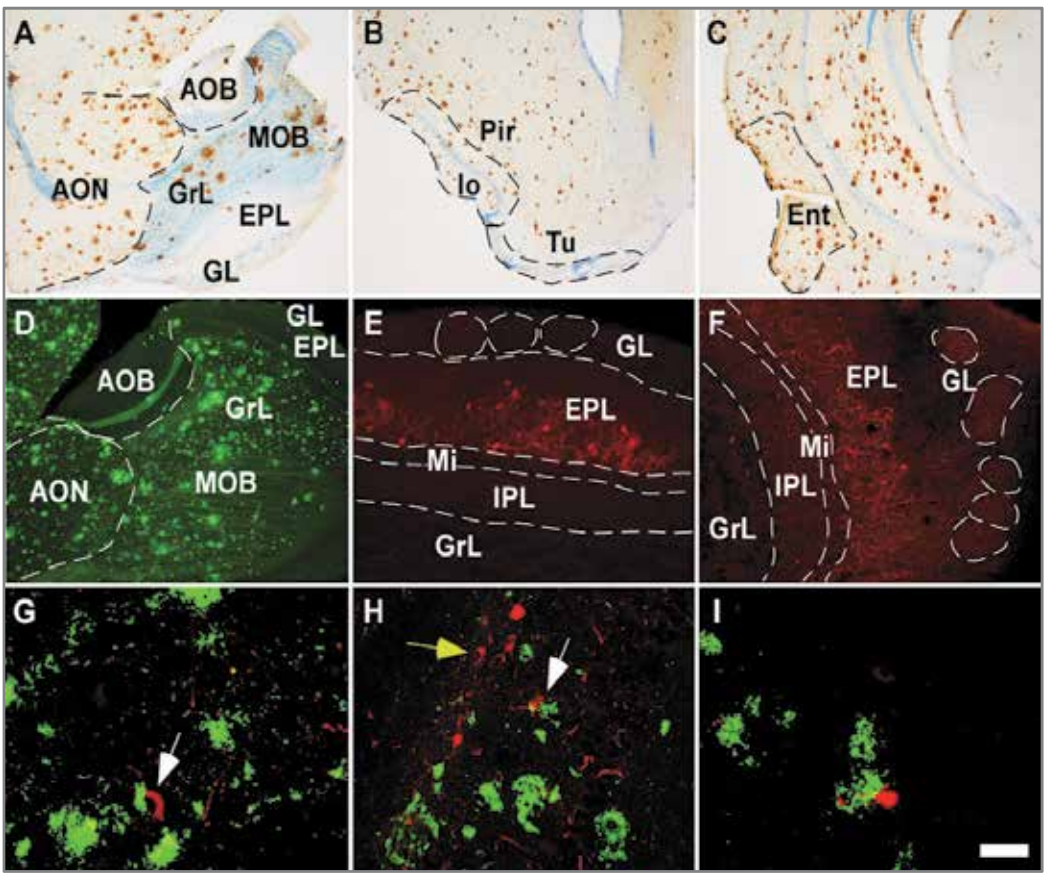

Fig. 5. Somatostatin and β-amyloid in the olfactory system of APPswe/PSEN1Δ9 mice. Green, β-amyloid; red, somatostatin. Immunohistochemistry for β-amyloid in the olfactory bulb (A), including anterior olfactory nucleus, piriform cortex and olfactory tubercle (B) and entorhinal cortex (C). Immunofluorescence in the olfactory bulb for β-amyloid (D) and somatostatin in a control mouse (E) and 6 months old transgenic mice (F). Confocal images demonstrating β-amyloid colocalization with somatostatin-expressing cells (G), fibers and cell debris (H, I). Scale bar: A, B, C & D = 400 μm, E & F = 80 μm, G & H = 40 μm, I = 25 μm

3.2 Calcium-binding proteins

Calcium is an intracellular second messenger that mediates physiological responses of neurons to chemical and electrical stimulation. In AD defective calcium homeostasis is thought to cause aberrant cellular metabolism and promote cell death (Heizmann & Braun, 1992, Mattson, 2007). Calcium has been related to changes in learning (Foster, 2007, Palop et al., 2003) and altered calcium regulation has been reported in AD brains before any cognitive deficits become apparent (Bezprozvanny & Mattson, 2008).

Our analysis focused on three interneuron subpopulations expressing three different calcium-binding proteins (CaBP): calbindin, calretinin and parvalbumin. All three proteins are expressed in the olfactory system, but with different distributions.

3.3 Calbindin

Most studies on calbindin D-28k have concluded that there is a general decline in levels in AD brain compared to controls (Ferrer et al., 1993, Iacopino & Christakos, 1990, Ichimiya et al., 1988).

In AD brain, calbindin D-28k is expressed widely throughout the olfactory system and is particularly abundant in key structures of olfactory processing such as the AON (Fig. 6A,B) and the piriform cortex (Fig. 6C). Especially evident is the pathological involvement of calbindin 28-Dk in the piriform cortex (Fig. 6C) where aberrant morphologies of calbindin-expressing dystrophic neuritis can be observed (Fig. 6D). Although calbindin-positive cells in the human olfactory system show some involvement with β-amyloid pathology, there was a stronger association with tau pathology (Fig. 6E, F).

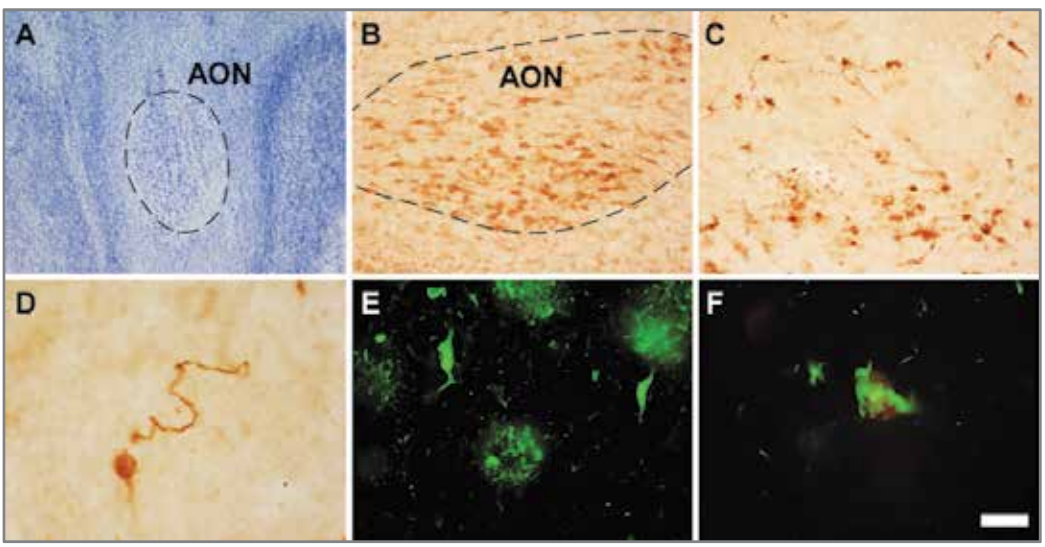

Fig. 6. Calbindin D-28k in the olfactory system of Alzheimer's disease brain. Green, tau protein and red, calbindin D-28k. (A) Nissl staining of the human anterior olfactory nucleus (AON). (B) Calbindin- expressing cell in the AON. (C) Calbindin- expressing cell in the piriform cortex (Pir). (D) Detail of a dystrophic neurite. (E) Tau pathology in Pir. (F) Calbindin-expressing cell with associated tau pathology. Scale bar: A = 160 μm, B & C = 80 μm, E = 40 μm, D & F = 25 μm.

3.4 Calretinin

Whereas calbindin D-28k is firmly associated with AD neuropathology, the involvement of calretinin in AD is more controversial (Brion & Resibois, 1994, Fonseca & Soriano, 1995, Hof et al., 1993, Sampson et al., 1997). Some authors propose that calretinin-positive cells are resistant to disease progression as a result of its capacity to buffer intracellular calcium levels. Furthermore, the potential role of calretinin in the neural basis of hyposmia remains unclear. We have studied the distribution of calretinin distribution in the human olfactory system and its involvement by tau and β-amyloid pathology.

Microscopy observations revealed that calretinin is present throughout the olfactory system. AD brain expression levels were found to be markedly reduced in olfactory areas such as AON (Fig. 7B) and Pir (Fig. 7C) relative to control brain (Fig. 7D). As with calbindin-expressing cells, calretinin-positive cells showed aberrant morphologies. In addition, these cells showed preferential involvement of tau pathology. In the olfactory bulb calretinin was found to be expressed in the periglomerular cells (Fig. 7A). It is interesting to note that sensory neurons from the olfactory epithelium send their axons to glomeruli in the olfactory bulb where they make synapses with dendrites of mitral cells. These synapses are regulated by periglomerular cells (Fig. 1). Involvement of periglomerular cells could therefore lead to disregulation of olfactory perception at the early stages of the disease.

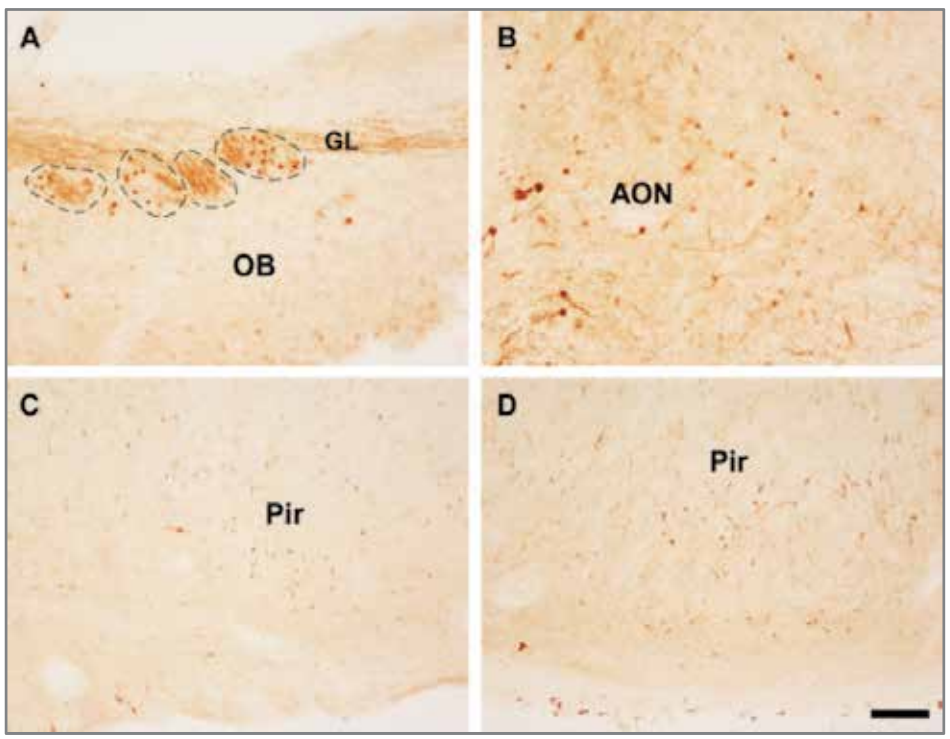

Fig. 7. Calretinin-expressing cells in the human olfactory system. (A) Periglomerular cells in the glomerular layer (GL) in the human olfactory bulb. (B) Calretinin-expressing cells in the anterior olfactory nucleus (AON). Expression of calretinin in (C) control and (D) Alzheimer's disease piriform cortex (Pir). Scale bar: A & B = 80 μm, C & D = 400 μm.

3.5 Parvalbumin

The third subclass of interneurons studied were those expressing parvalbumin. As with calretinin, the involvement of parvalbumin in AD is controversial. On the one hand it has been reported that there is an up to 60% decrease in the parvalbumin-positive cell population in AD hippocampus (Brady & Mufson, 1997) and entorhinal cortex (Mikkonen et al., 1999, Solodkin et al., 1996). On the other, no association was found between parvalbumin-positive cells and tau pathology in AD (Sampson et al., 1997).

In the human brain parvalbumin-expressing cells were present throughout the olfactory system and were particularly abundant in caudal olfactory areas such as piriform cortex (Fig. 8) and entorhinal cortex. In contrast to cells expressing calbindin- and calretinin, parvalbumin-positive cells showed physiological morphology, even at advanced stages of disease (Fig. 8A, B). Confocal images showed that parvalbumin-positive cells were predominantly associated with tau pathology (Fig. 8C); there was less evidence of involvement with β-amyloid pathology. As with calbindin- and calretinin-expressing cells, tau/NFT was the predominantly neuropathology associated with these calcium-binding proteins.

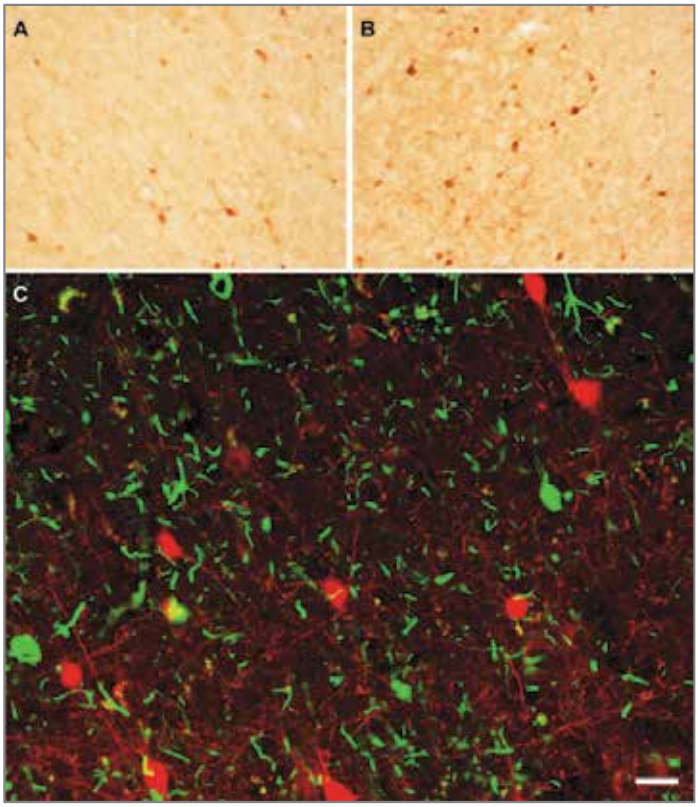

Fig. 8. Parvalbumin-expressing cells in the human olfactory system. Green, tau protein and red, parvalbumin. Immunohistochemistry of Alzheimer (A) and control (B) brain piriform cortex (Pir). (C) Confocal image of the Pir demonstrating the association of parvalbumin-expressing cells with tau pathology. Scale bar: A & B = 80 μm, C = 25 μm.

4. Discussion

In the present report we have studied the involvement of interneuron populations in the human olfactory system with AD neuropathology as revealed by two different disease markers: tau protein and β-amyloid peptide. Somatostatin-expressing interneurons in the olfactory system were preferentially associated with β-amyloid pathology in both AD brain and in *App*$_{swe}$/*Psen1*Δ9 mice. By contrast, interneurons expressing different calcium-binding proteins were predominantly associated with tau pathology. In transgenic mice, cells expressing Somatostatin-cell expressing was colocalized with β-amyloid pathology in the youngest animals examined, with the exception of the external plexiform layer, and the colocalization was evident even before disease-related reduction in the numbers of somatostatin cells.

The four subpopulations of interneurons were not randomly distributed within the olfactory system. Somatostatin-expressing cells were present in the olfactory bulb but were restricted to the external plexiform layer (EPL) (Fig. 5). Somatostatin was also present in other olfactory areas, particularly in the anterior olfactory nucleus (AON). Calbindin-expressing cells were present in the olfactory system, and were particularly abundant in the AON. Calretinin-positivity constitutes a specific marker for periglomerular cells which regulate the first relay of olfactory information from the olfactory epithelium to the olfactory bulbs (Fig. 1). Calretinin-expressing cells were also abundant in the piriform cortex (Pir) and the entorhinal cortex (Ent). Parvalbumin-positive cells were more abundant in the more caudal areas studied (Pir and Ent) and were only sparsely distributed in rostral olfactory areas.

The wide presence of these interneuron populations in olfactory structures and the severe and early involvement of these regions in AD neuropathology has focused attention on the role of these cells in the pathoetiology of AD. Generalized involvement of these cells and loss of interneuron populations and/or disregulation of their primary projection cells could underlie the olfactory deficits of AD patients. Loss of inhibitory regulation by γ-aminobutyric acid (GABA) could lead to altered firing patterns of projection neurons.

The olfactory system encompasses complex interconnections between several cortical areas (Fig. 1). Although it remains unknown whether dysfunction of specific interneuron populations in any given area could cause the olfaction deficits seen in AD, we report that there was selective association between different types of interneuron and AD neuropathology, as revealed by the two pathological markers employed in this study. However, it is not yet possible to relate these changes with specific olfaction deficits such as preferential or general anosmia, hyposmia or dysosmia. The specific contribution of each area in olfactory processing and how they are differentially affected during AD will need to be resolved before a specific olfactory test can be devised that could permit early diagnosis of AD.

5. Acknowledgements

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Edited by Suzanne De La Monte

The Clinical Spectrum of Alzheimer's Disease - The Charge Toward Comprehensive Diagnostic and Therapeutic Strategies is highly informative and current. Acknowledged experts in the field critically review both standard and under-appreciated clinical, behavioral, epidemiological, genetic, and neuroimaging attributes of Alzheimer's disease. The collection covers diverse topics of interest to clinicians and researchers alike. Experienced professionals and newcomers to the field will benefit from the read. The strengths and weaknesses of current clinical, non-invasive, neuro-imaging, and biomarker diagnostic approaches are explained. The perspectives give fresh insights into the process of neurodegeneration. Readers will be enlightened by the evidence that the neural circuits damaged by neurodegeneration are much broader than conventionally taught, suggesting that Alzheimer's could be detected at earlier stages of disease by utilizing multi-pronged diagnostic approaches. This book inspires renewed hope that more effective treatments could be developed based upon the expanding list of potential therapeutic targets.

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