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Alzheimer's Disease

The 21st Century Challenge

Edited by Jolanta Dorszewska and Wojciech Kozubski

ALZHEIMER'S DISEASE - THE 21ST CENTURY CHALLENGE

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and **Wojciech Kozubski**

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Notice

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Meet the editors

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Prof. Wojciech Kozubski, MD, PhD, is the head and chair at the Department of Neurology, University of Medical Sciences in Poznan, Poland. He graduated from the Medical School, Lodz, Poland, in 1980. From 1980 to 1983, he was a PhD student at the Department of Neurology, Medical School, Lodz. After completing his PhD degree, he became an assistant, an adjunct scientific worker, and an assistant professor at the same department. He attained his scholarship at the Academic Unit of Neuroscience of Charing Cross and Westminster Medical School, University of London (1987–1988), the Department of Neurology, Sackler School of Medicine, University of Tel-Aviv (1990), and the Department of Neurology, University of Trondheim, Norway (1990–1991). He has authored and coauthored over 290 papers (102 of them are original) concerning mainly the pathophysiology of migraine and related headaches, the pathophysiology of stroke and dementia, and the treatment of headaches and stroke. He is a coauthor and a coeditor of the *Handbook of Clinical Neurology for Neurologists* on brain tumors, affective diseases of the nervous system, and therapy in neurology; an editor of the *Handbook of Treatment in Neurology*; a coauthor and a coeditor of the *Handbook of Clinical Neurology for Medical Students*; and a coauthor of a monograph on stroke aphasia. He was the president of the Polish Neurological Society from 2011 to 2014 and the president of the Polish Headache Society from 1999 to 2001.

Contents

Preface

The progress of medicine and technology in the second half of the twentieth century has led to an increase in life expectancy. This phenomenon was accompanied by a higher incidence of diseases typical for old age, including Alzheimer's disease. It is known that the incidence of Alzheimer's disease is largely dependent on the patient's age. Alzheimer's disease is currently considered an incurable disease with an incomplete etiology and comprises a serious social and economic problem for many countries.

Nowadays, Alzheimer's disease is considered to be the most common dementia and leads to severe impairment of the patient, and even the final stage of the disease requires constant care of a mentally retarded older person. This book presents contemporary insights into the pathogenesis of Alzheimer's disease and currently used therapies that lead to slow the progression of the disease and delay its occurrence.

This publication sums up the knowledge of the genetic, biochemical, and immunological factors influenced in this dementive disease. It summarizes the pathophysiology observed both in Alzheimer's disease patients and in experimental studies. The book also contains the latest views on the molecular mechanism of dysfunction in this disease and life-long diagnosis.

We hope that this book may help in understanding the complex mechanisms of Alzheimer's disease pathogenesis and may be an inspiration to find factors to prevent this disease and to treat it effectively.

This book was created, thanks to the authors who wanted to share their scientific achievements in the field of basic and clinical research in dementive diseases.

The editors would like to acknowledge the authors from various parts of the world and all other people who helped with the production of this book for their participation in this publication.

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Chapter 1

Amyloid Beta Hypothesis: Attention to β- and γ-Secretase Modulators

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Additional information is available at the end of the chapter

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Abstract

The amyloid cascade hypothesis poses one possible explanation for the onset and progression of Alzheimer's disease (AD). With this respect, neurotoxic effect is attributed to soluble and diffusive amyloid-β (Aβ) oligomers. Aβ peptides are produced by proteolytic cleavage of the hydrophobic transmembrane portion of the amyloid precursor protein (APP) by successive action of β- and γ-secretases. Aβ peptides are generated in several isoforms, out of which the most pronounced are Aβ40 and Aβ42 being the major constituents of amyloid plaques found in AD patients' brains. Since the indisputable evidence pointed out to Aβ oligomers as toxic agents, several pathways to modulate or control the aggregation have been inspected. Given all these aspects, inhibitors of the β- and γ-secretases have gained the most attention. This chapter presents amyloid cascade hypothesis with current progress in the development of β- and γ-secretase modulators to counteract the Aβ burden.

Keywords: Alzheimer's disease, amyloid beta, neurodegeneration, amyloid precursor protein, β-secretase, γ-secretase, presenilin

1. Introduction

Despite the great progress in understanding pathogenetic and pathological processes associated with Alzheimer's disease (AD) in the last decade, the exact cause of AD still remains unrevealed. With the aim to clarify this cause, a number of hypotheses have been proposed, which involve, for example, the genetic hypothesis of AD based on malfunctioning variants of apolipoprotein E genes (*APOE*), the hyperphosphorylation of cytoskeletal proteins (especially of tau protein) or the theory of oxidative stress [1]. Importantly, AD is often explained by inflammatory processes in the brain, and metabolic processes leading to the formation and accumulation of the

beta-amyloid (Aβ) [2]. Among all these theories, the amyloid metabolic cascade or the amyloid hypothesis and posttranslational modification of tau protein are considered as the main pathophysiological theories elucidating the outbreak of AD, although none of them is able to sufficiently explain the diversity of the biochemical and pathological abnormalities associated with the developed AD [3].

According to the amyloid hypothesis, slow accumulation of extracellular senile plaques, composed of Aβ deposits, occurs in the beginning and further progresses into AD. On the other hand, a direct link between the toxic influence of Aβ, the impaired neuronal functions and the decline in memory functions still has not been fully clarified, but it is broadly accepted that Aβ undoubtedly plays a key role in the neuropathology of AD [4].

2. Amyloid precursor protein

Amyloid precursor protein (APP) is an integral membrane glycoprotein that is expressed in the brain and the central nervous system (CNS). APP can be cleaved by specific proteases in two different pathways: α-path and β-path [5]. In most cases, APP is cleaved in the α-path with the participation of enzymes α- and γ-secretases. The cleavage of APP by α-secretase proceeds in the way, which can be described as non-amyloidogenic one, while the cleavage in the β-way leads to formation of the toxic fragments of Aβ. In the case the non-amyloidogenic path, APP

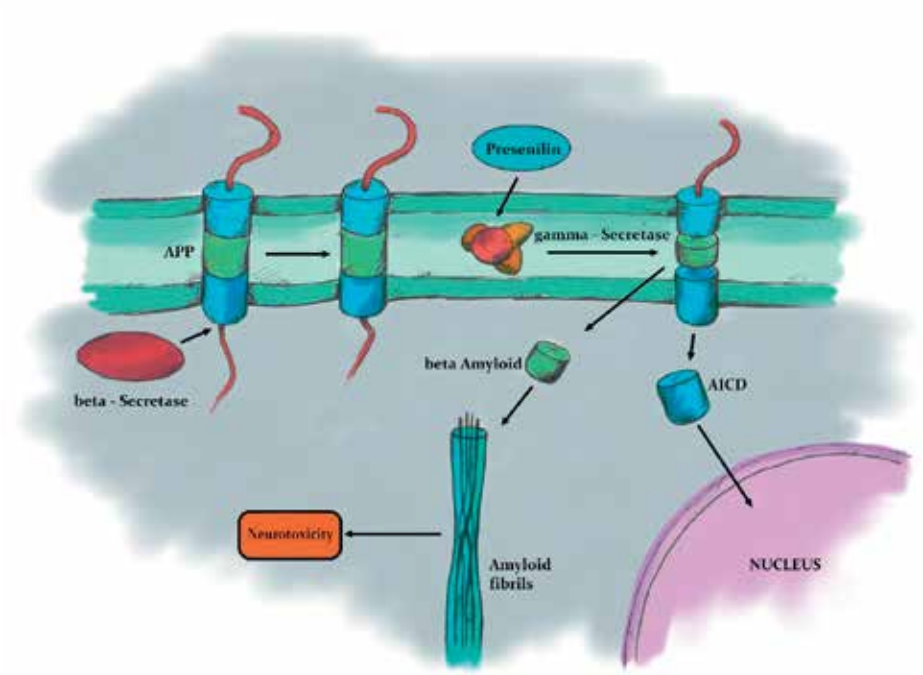

Figure 1. Scheme of the amyloidogenic processing of APP.

is cleaved by α-secretase to form a soluble extracellular fragment of sAPP-α and C83 fragment, which is split by γ-secretase, similar to the case of C99 [6]. However, the α-path does not release Aβ, but it leads to splitting out a short protein fragment p3. The exact physiological function of the fragment p3 has not been completely clarified yet [7]. In the course of the β-path, APP is first cleaved by the enzyme β-secretase (BACE-1) providing the *C*-terminal fragment of the length of 99 amino acids (C99) and a chain, which is transferred to the extracellular space. This remaining protein chain can be found in the literature under the acronym sAPP-β. Subsequently, C99 is cleaved by the activity of γ-secretase to short-length peptides consisting of 38–43 amino acids (referred to as Aβ) and the intracellular *C*-terminal domain (AICD). In most cases, formation of $A\beta_{1-40}$ mainly occurs, although a longer and more toxic form $A\beta_{1-42}$ sometimes can be also produced. However, recent findings also point to the fact that the production of Aβ can take place even within the proteolytic cleavage of APP along the α-path (**Figure 1**) [8].

3. Physiological function of amyloid precursor protein

Although APP is a part of the pathophysiological processes involved in AD, it is clear that the protein also carries out several natural physiological functions, particularly within the regulation of the synaptic transmission. It has been proved that transgenic mice with knock-out gene for APP exhibited an inability to transmit signals to the neuromuscular junction. Despite this fact, mice with upregulated expression of APP show better cognitive functions and spatial orientation. This is often rationalized by overproduction of AICD given by γ-secretase. The activity of APP is also put in a close connection with the metabolism of cholesterol. The neuroprotective character of APP was also demonstrated by suppression of the cyclin-dependent kinase 5 (CDK-5) activity in the process of tau hyperhosphorylation [9].

4. Pathological features of amyloid precursor protein

The pathological role of APP is generally associated with the amyloidogenic way of its splitting. In general, many mutations of APP cause the autosomal dominant form of AD with early onset. Interestingly, genetic mutations in the adjacent part of the β-site of the APP gene induce neuroprotective effects, because Aβ is then produced only in a small extent. On the other hand, an excessive expression of the mutated APP forms associated with FAD (a redox cofactor in a number of biochemical reactions) leads to a loss of sense of smell, without dissemination of amyloid plaques, though. This observation is in a line with the loss of sense of smell, which occurs in some patients in the early stages of AD [9].

5. β-secretase

β-secretase (BACE-1; also referred to as Asp2 or memapsin 2) is an enzyme that breaks down APP in the site called β into the *C*-terminal fragment, from which monomers of Aβ are

subsequently formed in the neurons. BACE-1 and the homologous BACE-2 are regulated differently and also control different processes. A disrupted intracellular calcium homeostasis may stimulate the genetic expression of BACE-1 *via* triggering the nuclear factor of activated T-cells of type 1 (NFAT1), which leads to over-production of Aβ. Expression of the BACE-1 can also be controlled by the level of $A\beta_{1-42}$ (but not by the $A\beta_{1-40}$) through some transcription factors. In addition, some plaques containing $A\beta_{1-42}$ even increase the levels of BACE-1 in the adjacent neurons just before their death [10]. The homologous enzyme BACE-2 shares 64% of the sequence identity with BACE-1. The action of BACE-2 is in many aspects similar to the activity of α-secretase. BACE-2 triggers a cascade of cleavage of APP by the non-amyloidogenic way. Its physiological function is associated with the organ pigmentation [11].

In order to clearly demonstrate the involvement of BACE-1 in the pathogenesis of AD, many prominent scientific groups worldwide dealt with developing a mouse model that had deactivated the gene for the production of BACE-1 (i.e., BACE-1 knockout (−/−) mice). At first, these strains of mice were viable, capable of reproduction, with the normal morphology of the body, without any obvious signs of damage of the tissues and normal blood picture [12]. This finding supported the idea that inhibition of BACE-1 can bring about the desired therapeutic effect without adverse effects. The results of this study also point to the fact that the related BACE-2 fails to offset the activity of BACE-1 in the formation of Aβ. It is interesting that hybridization of these BACE-1 knockout (−/−) mice with transgenic mice having the APP gene, which increasingly produce amyloid plaques, provided a generation, the newly born individuals of which did not exhibit the formation of Aβ, Aβ deposits or signs of memory impairment caused by production/accumulation of Aβ. As already mentioned, BACE-1 is located mostly in the presynaptic endings of neurons, where its physiological effects is assumed to occur. Over time, however, it was found that BACE-1 knockout (−/−) mice had impaired axonal conduction, experiencing hypomyelinization (i.e., disrupted formation of myelin, the substance that surrounds the axons and nerve fibers), memory disorders, disturbed neurochemical balance, pathological neurogenesis, astrogenesis, degeneration of neurons with increasing age, pathological changes in the retina and schizophrenic symptoms. All these discoveries observed in BACE-1 knockout (−/−) mice can serve as a model that reflects the potential adverse effects associated with the administration of BACE-1 inhibitors for normal animals or people [13].

The substrates subject to proteolysis by BACE-1 are in particular the membrane-bound proteins like APP. Many of these BACE-1 substrates undergo a process called ectodomain shedding (ectodomain is a part of a membrane protein which protrudes to the extracellular space), while at the same time, these substrates can be cleaved by proteases, called also disintegrins, and ADAM-related metaloproteases. The extent of cleavage of the substrate by ADAM related proteases or BACE-1 depends on the nature of the particular substrate. All the possible side effects caused by inhibition of BACE-1 thus may not be always exhibited, assuming that some substrates are hydrolyzed by another protease [14].

The homology between BACE-1 and BACE-2 gave rise to arguments that BACE-1 inhibitors may simultaneously inhibit non-selectively also BACE-2. For this reason, transgenic BACE-2 knockout (−/−) mice were developed to clarify the physiological role of BACE-2 and to explore the benefits offered by inhibition of this enzyme. Similar to the BACE-1 knockout (−/−) mice, the

BACE-2 knockout (−/−) mice showed the same phenotype. Double-knockout mice, that is, mice with deactivated genes for BACE-1 (−/−) as well as for BACE-2 (−/−), are not phenotypically very different from mice without the gene for the BACE-1, with the exception of an increased number of dying mice freshly after birth. The results of this study therefore assume that nonselective inhibitors of both subtypes of the enzyme BACE may be well tolerated at least from the perspective of the inhibition of BACE-2. The latest research has shown that BACE-2 is expressed in the pancreatic β cells and BACE-2 knockout mice exhibit an improved glycemic regulation due to the increased production of insulin. These findings imply the possible use of BACE-2 inhibitors for the treatment of diabetes mellitus of type 2 [15].

6. BACE-1 inhibitors in the treatment of Alzheimer's disease

Currently, BACE-1 inhibitors have an exclusive position regarding the therapeutic options for introduction into clinical practice to treat AD [16]. Their mechanism of action is based on reducing the levels of Aβ in the brain. Although several of these inhibitors had already reached clinical testing, there are still important questions to answer, for instance, about their safety, the optimum degree of inhibition of BACE-1 needed to achieve the desired therapeutic effect without the presence of side effects, and the stage of the disease when these compounds are to be indicated in order to achieve the greatest assets [17].

Aβ is produced by neurons in the brain, partly also by astrocytes and other glial cells, which are involved in the formation of this protein in particular during the stress conditions accompanying the AD development. For the production of Aβ, the activity of both enzymes, BACE-1 and γ-secretase, is necessary [10]. The biochemical processes involving the activity of these enzymes are often referred in the literature as the amyloid pathway. Importantly, modulation or inhibition of these enzymes can reduce the formation of Aβ in the brain of patients with AD. On the other hand, activation of the non-amyloidogenic pathway by supporting the α-secretase activity may also reduce the formation of Aβ and currently it is alternatively considered as a promising approach for therapy of AD. An important role for the accumulation of Aβ is also played by the genetic aspects of AD. Nowadays, more than 200 autosomal-dominant mutations in APP and presenilin (PS) have been identified which contribute to the occurrence of familial forms of AD [18]. Without any exception, all these mutations increase the production of all Aβ isoforms, in particular the toxic Aβ containing 42 amino acids ($A\beta_{1-42}$). An example might be seen in Swedish mutation of APP in the amino acids Lys670 and Met671, that is, the places where BACE-1 enzyme cleaves APP. This mutation results in higher proteolytic efficacy of BACE-1, which promotes an increased rate of the C99 fragment formation and thereby the total production of Aβ [19]. The *APOE-ε4* allele represents a major genetic risk factor for the development of AD with the late onset and it is also associated with an increased production and accumulation of Aβ. Similarly, mutation of ADAM10 metaloprotein, which is endowed with physiologically similar activity to that of α-secretase in neurons, causes the late onset of the AD by suppressing this enzyme activity, while the amyloidogenic cleavage of APP by BACE-1 prevails [20]. Recently, at least five different genes whose mutation contributes significantly to the increased formation of Aβ have been identified. Based on all

these mutations and their effects, we can conclude that Aβ is responsible for the pathogenesis leading to the breakout and development of AD. Accordingly, some mutations in APP can represent a protecting mean to suppress the progression of AD. For example, a mutation in the Ala673 region (so-called Ala673Thr mutation) causes a lower affinity of APP to BACE-1, bringing the production of Aβ reduced by up to 40% [21].

Extensive research is dedicated to the development of small molecule inhibitors of BACE-1, capable to act centrally. The first experimental inhibitors were derived from short fragments of APP, being therefore peptide derivatives. These differed from APP by modified amino acid sequence and increased metabolic resistance against cleaving by BACE-1. In *in vitro* conditions these bulky peptide derivatives showed high affinity for BACE-1, especially due to the fact that the active site of BACE-1 is so large that it is able to cleave very large substrates. The disadvantage of these derivatives is that they do not possess true drug-like properties, exerting low oral availability and short half-life in the plasma. Such drug candidates are quickly metabolized and have low permeability through the blood-brain barrier. For these reasons, the researchers have focused on the development of small BACE-1 inhibitors that have high affinity for this enzyme, but are small enough to penetrate through the blood-brain barrier and, at the same time, to exhibit suitable pharmacokinetic properties. In addition, these compounds must be lipophilic enough to permeate through the cytoplasmic and endosome membrane to block the active site of BACE-1 located inside of the lumen. A large number of these compounds, however, reached only a limited concentration in the brain because in most cases, they reached high efflux mediated by P-glycoprotein (P-glycoprotein is an ATP-dependent pump, which removes xenobiotics and protects the brain from the effects of these compounds) [22].

The latest generations of BACE-1 inhibitors are characterized by a good capacity to permeate through the blood-brain barrier, by a suitable pharmacokinetic profile, and the ability to induce reduction of the cerebral levels of Aβ. The result of the research is a panel of several inhibitors of BACE-1, which have entered various stages of clinical testing [23].

6.1. MK-8931

In 2012, the results of the first phase of clinical trials with inhibitor MK-8931 were presented (**Figure 2**), which had been performed in 88 healthy individuals aged between 18 and 45 years. The safety, tolerability, pharmacokinetic and pharmacodynamic parameters after single or repeated administration were experimentally determined. MK-8931 was generally well tolerated, and no severe side effects were observed. The main goal in this first phase was to determine whether MK-8931 was capable of penetration into the brain to inhibit the activity of BACE-1. Biomarkers monitoring the levels of $A\beta_{1-40}$, $A\beta_{1-42}$ and soluble fragment of APP (sAPP-β), which is formed by BACE-1, were intensively studied. MK-8931 significantly decreased the concentrations of cerebrospinal Aβ, depending on the dose administered, and even in repeated oral administration a reduction of Aβ in the CSF of up to about 90% has been observed. The plasma half-life of MK-8931 after a single administration was around 20 h, which assumes the dosing schedule within the range of a single daily dose. This was followed by a clinical study 1b, where the safety, tolerability, pharmacokinetics and pharmacodynamics in 32 patients with mild to moderate dementia of the AD type were determined.

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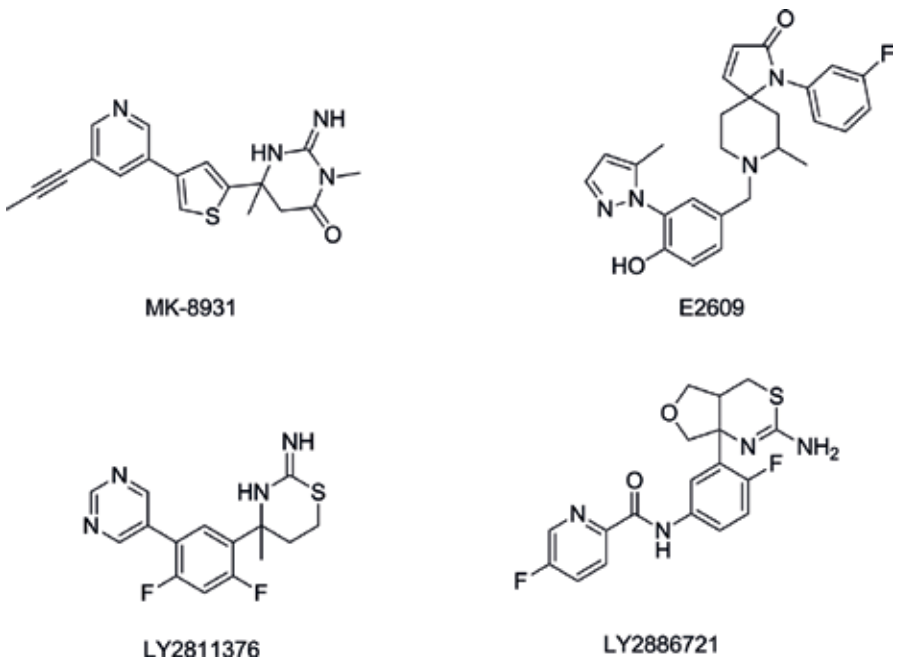

Figure 2. Chemical structures of BACE-1 inhibitors in clinical testing.

MK-8931 was applied in three different doses (12, 40 or 60 mg) and the effect was compared with the placebo over a period of 7 days. The markers of $A\beta_{1–40}$, $A\beta_{1–42}$ and sAPP-β were also monitored. As in the previous phase, decrease in the levels of Aβ, depending on the dose of the drug (for the $A\beta_{1–40}$ 57% (12 mg), 79% (40 mg), 84% (60 mg)) was observed and, in addition, without the presence of the more serious side effects. The results of this phase of clinical trials are especially important because the pharmacokinetic and pharmacodynamic properties of this BACE-1 inhibitor are not affected by the quantity of Aβ present in the brain of patients with AD. At the end of 2012, MK-8931 advanced to the clinical phase (II/III) with patients suffering from mild to moderate dementia of AD type. This substance was administered in dosages of 12, 40 and 60 mg and controlled with placebo in the total sample of 200 patients. According to the initial promising results, extension of the third phase of clinical trials by another 1960 patients with AD is expected. Further evaluation of MK-8931 is simultaneously monitored within the III phase of clinical testing on 1500 patients with AD. The results of both studies are to be expected in 2017–2018 [24].

6.2. LY2886721

A non-peptidic BACE-1 inhibitor LY2811376 (**Figure 2**), which was analyzed in a study with oral administration, demonstrated satisfactory pharmacokinetic and pharmacodynamic properties in animal models, which promoted the compound to the first phase of clinical trials. These clinical studies, however, were soon discontinued due to adverse reactions, in particular in the area of inflammation of the retina and the occurrence of stroke. Although all other studies

with the substance faded away, at present, LY2811376 has become a lead structure, which could be administered orally and reach its biological target behind the blood-brain barrier.

The molecule marked with LY2886721 (**Figure 2**) represents the next evolutionary generation of orally acting BACE-1 inhibitors, which has entered into the second phase of clinical trials. Compared to its predecessor LY2811376, the novel drug LY2886721 did not exhibit any side effects in the area of the retina and any stroke. During the first phase of clinical trials on 47 healthy volunteers, no adverse effects were observed in 14 days (different dosing schemes—repeated administration of 5, 15 and 35 or 70 mg single administration). The biological half-life fluctuated around 12 h, allowing the dosing once per day, when the drug holds the necessary biological effect even after substantial elimination from the body. Treatment with LY2886721 resulted in the reduction of the plasma and cerebrospinal levels of $A\beta_{1-40}$ by up to 74% (i.e., after the highest dose of 70 mg). Similar decreasing changes were detected in the cerebrospinal levels of $A\beta_{1-42}$ and sAPP-β, while the blood level of sAPP-α was increased, which is logically explainable by relative excess of α-secretase in comparison with BACE-1.

The second phase of clinical trials with LY2886721 was carried out in 130 patients with moderate to severe AD dementia type. This testing, however, was terminated because of liver abnormalities, but, presumably, this is not associated with inhibition of BACE-1 [25].

6.3. E2609

E2609 (**Figure 2**) is an orally available, nonpeptidic spirocyclic inhibitor of BACE-1, which induced a significant decline of brain levels of Aβ in preclinical studies. Based on this success, E2609 entered the first phase of clinical testing in which 73 volunteers, administered uniformly with increasing dose from 5 to 800 mg of the drug, and 50 volunteers, administered with different doses in the range of 25–400 mg, participated. The plasma half-life of E2609 is around 12–16 h, which again allows one-day dosing schedule. At the maximal single dose (400 mg), decrease of the cerebrospinal Aβ levels by up to 85% has been observed. The concentration of sAPP-β has been similarly reduced, while sAPP-α has been increased. Currently, the drug is in the third phase of clinical determinations [26].

7. γ-Secretase

γ-Secretase is a member of aspartic protease family that cleaves glycoproteins of type I including APP. Unlike β-secretase, γ-secretase has a regulated intramembrane proteolytic activity (RIP), thus, it breaks down domains inside of the cytoplasmic membrane. It is known that it breaks down multiple substrates, and to this day more than 50 such substrates, including APP, have been identified. Among these substrates are Notch, Jagged and Nectin-1α. The signal transmission by RIP is implemented so that the released intracellular domain is moved into the nucleus, as it is in the case of Notch, which regulates specific gene expression. Notch is therefore cleaved to Notch intracellular domain, NICD, which causes in the nucleus the mentioned regulation. In relation to AD, this signal pathway is interesting from the perspective of development and function of the nervous tissue.

Over the last few years, it has turned out that four main factors are responsible for the enzymatic activity of γ-secretase complex: presenilin, anterior pharynx-defective, presenilin enhancer 2 and nicastrin, which are described further in this chapter [27].

8. Inhibitors of γ-secretase in the clinical development

In recent years, a series of potential inhibitors of γ-secretase has been designed and synthesized. Unfortunately, most of them are not specific to cleaving APP with γ-secretase, and, like in the case of BACE-1, they prevent processing of other γ-secretase substrates that do not have any or at least no obvious role in the pathogenesis of AD. For these reasons, the inhibition of γ-secretase has been associated with serious side effects, which adumbrated the end for most drug candidates in clinical testing.

Historically, the first inhibitor of γ-secretase that underwent clinical studies was BMS-299897 (**Figure 3**) compound prepared by Bristol-Myers Squibb. In 2001, clinical trials of this molecule

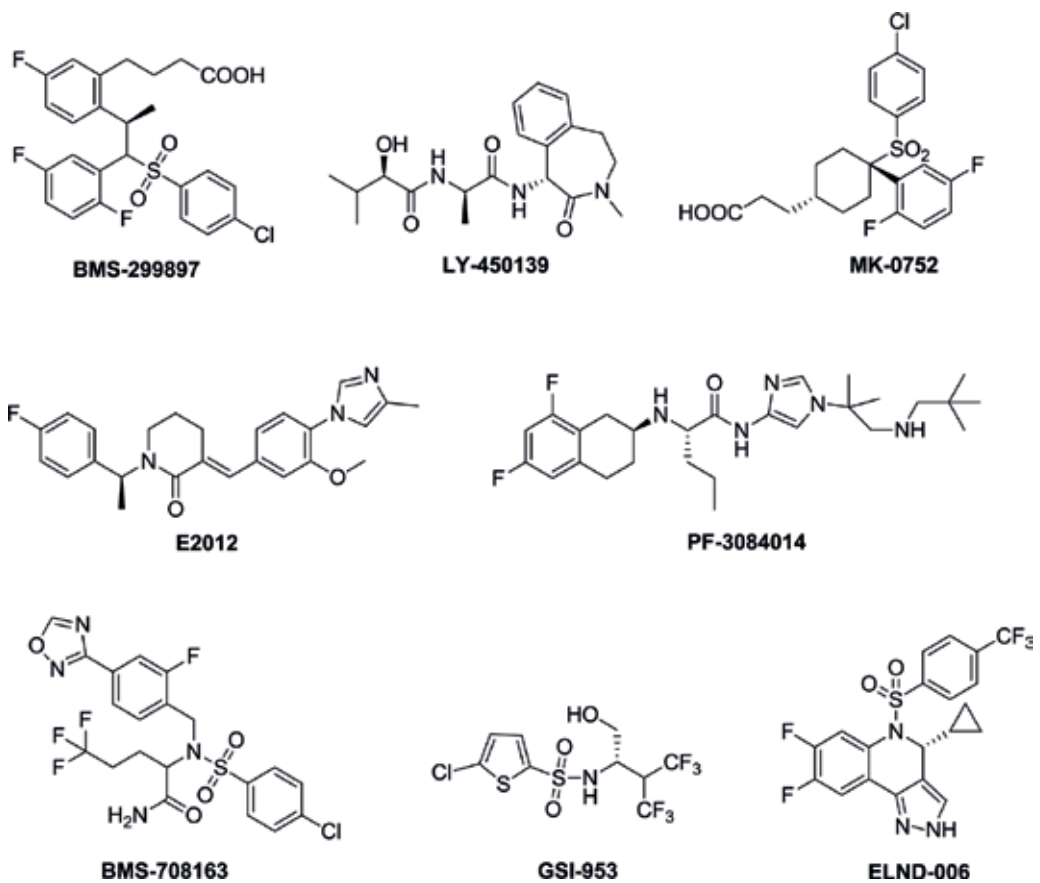

Figure 3. Inhibitors of γ-secretase in various stages of clinical testing.

began, but the results of this study have never been fully described. We only know that the next clinical trials have been terminated.

Six other inhibitors of γ-secretase are currently in various stages of clinical testing involving patients suffering from AD. As for the compounds LY-450139, MK-0752, BMS-708163, PF-3084014, GSI-953 and ELND-006, animal studies indicated that these substances reduced the brain levels of Aβ after oral or parenteral administration (**Figure 3**) [28].

8.1. LY-450139

LY-450139 (**Figure 3**), also known as semagacestat, is an inhibitor of γ-secretase developed by Eli-Lilly. It is a derivative of benzoazepinone with triple selectivity to inhibit the cleavage of APP with respect to the cleavage of Notch (APP: IC_{50} = 15 nM and Notch: EC_{50} = 49 nM). This derivative has undergone all three phases of clinical trials. When tested on experimental animals, it was found that LY-450139 has an effect on the level of Aβ in the brain, cerebrospinal fluid (CSF) and the plasma in mice, guinea pigs and dogs. A similar positive effect was achieved in the cerebrospinal fluid (CSF) of AD sufferers. However, due to the neurotoxicity detected in transgenic mice, gastrointestinal problems and an increased risk of developing skin cancer in humans, clinical testing was abandoned [29, 30].

8.2. MK-0752

This substance developed by Merck is a non-selective inhibitor of APP and Notch formation. In healthy volunteers, MK-0752 (**Figure 3**) administration led to reduction of $A\beta_{1-40}$ levels in the CSF. However, the drawback was the mentioned non-selectivity toward Notch cleavage and significant toxicity in humans. MK-0752 has reached only the first phase of clinical testing [31].

8.3. E2012

The drug E2012 (**Figure 3**) was developed by Eisai in cooperation with Torrey-Pines Therapeutics with the aim to reduce the levels of Aβ by modulating the γ-secretase without affecting the Notch. In mid-2006, the first phase of clinical testing has started, but in February 2007 it has been suspended due to the lenticular opacity observed in preclinical studies with rats. In the time of the study suspension, however, no health problems in humans were observed. In addition, the lenticular opacity has not appeared in later studies in monkeys. During a subsequent study, no eye toxicity was observed in rats, and, thus, the suspension of testing was repealed in April 2008. Currently, the drug is no longer the subject of research interests, anyway [32].

8.4. BMS-708163

The drug identified as BMS-708163 (**Figure 3**) is a benzene sulfonamide developed by Bristol-Myers Squibb. This molecule exhibits nearly 200× lower selectivity to Notch cleavage ($A\beta_{1-40}$: IC_{50} = 0.3 nM and Notch: EC_{50} = 58 nM). Animal studies, specifically in rats and dogs, have shown the ability of BMS-708163 to reduce the levels of Aβ in the brain and the CSF without the Notch-related gastrointestinal and lymphoid toxicity. Despite the fact that reduction of the Aβ level in the CSF has been observed in healthy volunteers, there is insufficient information on storing Aβ

plaques in the brain of transgenic mice, as well as on their behavioral changes. This inhibitor has passed the phase II of clinical development, but further testing is currently not being performed [33].

8.5. PF-3084014

PF-3084014 (**Figure 3**) is a new effective, aminotetraline based γ-secretase inhibitor developed by Pfizer, which does not affect Notch. In in vitro tests, the compound was evaluated as an uncompetitive and reversible inhibitor of human γ-secretase with IC_{50} = 6.2 nM. In studies on tissue cultures, it seems as a weak inhibitor of Notch with IC_{50} = 1915 nM. The ratio between the APP and Notch selectivity is roughly 1500. The merit of this compound is a sufficient penetration through the blood-brain barrier, long-term effect on reducing the Aβ levels and no rebound phenomenon for levels of Aβ in animal plasma. As in the case of BMS-708163, there is also a lack of data for this inhibitor about the storage of Aβ plaques in transgenic mice, as well as about their behavioral processes. PF-3084014 is currently introduced into the second phase of clinical testing [34].

8.6. GSI-953

This inhibitor, known also as begacestat, is a potent thiophene-related sulfonamide developed by Weyth. It is able to suppress the production of Aβ in low nanomolar concentrations in vitro (IC_{50} = 8 nM) and in vivo ($Aβ_{1–42}$: IC_{50} = 15 nM). Cellular studies on the Notch cleavage showed 15× higher selectivity of this molecule to inhibit preferably the cleavage of APP. It was found that GSI-953 (**Figure 3**) improves the memory functions in transgenic mice; however, it does not diminish the level of $Aβ_{1-40}$ in the CSF in people suffering from AD. This drug completed the first phase of the clinical trials, but the lack of its efficacy caused it to no longer be a subject of follow-up studies [35].

8.7. ELND-006

The inhibitor ELND-006 (**Figure 3**) developed by Elan Pharmaceuticals shows increased selectivity for inhibition of the APP cleavage (IC_{50} = 0.34 nM) with regard to Notch cleavage (IC_{50} = 5.3 nM). Therefore, it does not significantly affect Notch; it has a good penetration through the blood-brain barrier and can reduce the level of Aβ in the brain in transgenic mice. The disadvantage of this drug is the rebound phenomenon in the plasma of animals and lack of data on behavioral processes in animal models of AD. Clinical studies of the drug have been terminated because of severe hepatic adverse reactions, which presumably are not related to the mechanism of γ-secretase inhibition by the drug [36].

9. Presenilins 1 and 2

Presenilins (PSs) are membrane proteins encoded by two genes: PS1 and PS2. PS, nicastrin, anterior pharynx-defective (aph-1) and presenilin enhancer 2 (pen-2) form an active part of the γ-secretase complex, while PS form the catalytic core of the complex [18].

Presenilin-1 (PS1) and presenilin-2 (PS2) are considered as the key elements of the γ-secretase complex. The proteins are composed of 9 transmembrane domains containing 467 or 448 amino acids. These domains are autoproteolytically cleaved in the process endoproteolysis to form two ends, each of them having an active aspartate site, which create the catalytic γ-secretase complex site for Aβ. Anterior pharynx-defective (Aph-1) and presenilin enhancer 2 (Pen-2) act as cofactors in the active γ-secretase complex. Aph-1 is a transmembrane protein composed of seven subunits with *N*- and *C*-ends protruding into the lumen and the cytosol. It plays an important role in the initial formation of γ-secretase and carries out the enzymatic function in the final complex. Pen-2 is the smallest membrane protein with two transmembrane domains, in which both the *C*- and *N*-ends point to the lumen. Pen-2 holds an important role in stabilizing PS in the final step of γ-secretase building and also helps in endoproteolysis of presenilins [37].

Nicastrin has been described as the main protein that interacts with presenilins. This part of the γ-secretase complex contains 709 amino acids including glycoprotein with 1 large ektodomain and can serve as the substrate receptor of γ-secretase. Nicastrin is essential for the recognition and processing of the substrate, for the maturation of the γ-secretase complex and its transport to the cell surface [38].

In addition to the amyloidogenic fragment of APP (i.e., sAPPβ), γ-secretase breaks down also a variety of other transmembrane proteins (e.g. Notch). Mutation in PS1 often leads to an increase in the relative production of toxic $A\beta_{1-42}$ peptide, which is hydrophobic and is easily prone to aggregation. This process results in a cascade of pathological events, at the end of which a degenerative damage to neurons comes up. The hypothesis about the influence of PS1 mutations on the creation and subsequent aggregation of $A\beta_{1-42}$ was supported by the results of studies on transgenic mice with an increased production of APP, in which increased formation and accelerated storage of the Aβ deposits occurred. Moreover, the PS mutations always appear in different parts of the protein, so it can be hard to predict what toxic effect due to PS mutation will show up. In this context, however, it is possible that the loss of normal functions of the PS caused by one of the mutations closely correlates with the onset of pathological cascades leading to AD.

The most recent studies have pointed to the loss of function of PS, which is usually associated with the mechanism of AD development. In this respect, it was proved that mice with the knockout genes for both PS proteins exhibit degenerative disruption of the front part of the brain, without the formation and storage of Aβ, although cognitive dysfunctions arise as it is normally observed in AD with the appearance of Aβ in the brain. Similar symptoms can be found in frontotemporal dementia in humans, which is presumably caused by a mutation of the gene for PS1, when amyloidogenesis (i.e., formation of Aβ) does not occur. From the abovementioned information, it follows that neurodegeneration may proceed even without the formation of Aβ [39].

However, PS also plays an important role in many other physiological processes. These processes can be divided into those related with the activity of γ-secretase and those without a close connection with the activity of γ-secretase. It is interesting that some of the inhibitors of γ-secretase increase the production of $A\beta_{1-42}$ in low concentrations while reducing the formation of $A\beta_{1-40}$. A similar effect can be observed as a result of PS mutations [40].

10. Apolipoprotein E and other apolipoproteins

Apolipoprotein (APO) is a general term for denoting proteins which bind with lipids. They play an important role in the regulation of pathological manifestations caused by Aβ. APOE is the main representative of the APO present in the CNS, which is produced and secreted exclusively by astrocytes and microglia. APOE is involved in the transport of lipids between the cells in the CNS, where it physiologically induces the lipid homeostasis, repairs damaged neurons, supports synaptic transmission of excitation and separates specific toxins. The *APOE* gene is encoded by three alleles—*APO-ε2, APO-ε3* and *APOE-ε4*. These alleles differ in only two residues at positions 112 and 158. These small differences between the alleles, however, determine their different function. The isoform *APOE-ε2* carries out a neuroprotective function, while the isoform *APOE-ε4*, occurring in a population at about 14%, is associated with a number of diseases. Many studies point to the *APOE-ε4* allele as a risk factor associated with cognitive dysfunction and the onset of AD. The effect of *APOE-ε4* is regulated by cholesterol. The *APOE-ε4* variant has a function of chaperone in relation to the Aβ. The chaperone assists in structural formation of Aβ, but, in fact, it also increases the toxicity of Aβ. The consequences of the relation of *APOE-ε4* to Aβ were demonstrated on transgenic animals, when blocking the interaction of *APOE-ε4* with Aβ significantly reduced the accumulation of Aβ into amyloid deposits. The deposition degree of Aβ depends on the presence of the APOE alleles and descends in a series of *APOE-ε4* > *APOE-ε3* > *APOE-ε2*. Interestingly, the intake of sugary drinks leads to induction of the amyloidogenic process, to distortion of memory functions and increased levels of *APOE-ε4* [41].

11. Aβ and neurodegeneration

A number of studies show that Aβ plays a key role in the onset and progression of AD. But so far, it is still not clear whether the culprit of the onset of dementia is the soluble or insoluble form of Aβ and if the extent of the Aβ impact depends on the localization of this protein in extracellular or intracellular compartments. Current research has revealed a variety of processes in which Aβ plays an important role, for instance, mitochondrial dysfunction, oxidative stress, turmoil and disruption of the transfer function of the membrane. According to the amyloid hypothesis, deposition of Aβ in the brain is the primary cause and controlling force of the degeneration associated with AD, which involves formation of intracellular neurofibrillary tangles and induce the death of neurons [42].

12. Conclusion

AD is a complex neurodegenerative disease which is caused by a number of factors, both biological and environmental. Among these factors, one of the main elements is excessive production of Aβ via amyloidogenic processing of APP, and its subsequent storage in the brain. All of these processes lead to neuronal death, which initializes the outbreak

of dementia and AD with the early onset or sporadic forms with the late start. The genes encoding APP, BACE-1, PS1/2 and *APOE-ε4* thus play a crucial role in the pathogenesis of AD. Besides these genes, it is also worth noting the role of neprilysin and the insulin-degrading enzyme. Both neprilysin and the insulin-degrading enzyme are involved in the elimination of Aβ. The levels of both enzymes are decreased in the brains of patients with AD. Further biochemical, behavioral and clinical studies in this area are, however, necessary in order to develop an effective treatment, whether symptomatic or such that alters the course of the disease or hopefully even heal the disease. BACE-1 is an enzyme that initiates the proteolytic cleavage of APP into smaller fragments of Aβ. According to preclinical and clinical data, BACE-1 is a convenient therapeutic target for the treatment of AD. BACE-1 (−/−) knockout mice are viable but they exhibit a range of neurological symptoms which points to the fact that BACE-1 inhibitors may have serious side effects that are associated with the physiological function of this enzyme. In particular, development of new BACE-1 inhibitors represents a major challenge for the future since only a limited number of these drugs successfully entered clinical trials. From this perspective, the most promising compounds are MK-8931 or E2609 which have been promoted to the II/III phase, while the others are between I and II phases. All the drugs consistently induce a large decrease in spinal cord levels of Aβ, up to 90%. They are usually well tolerated; only testing of two inhibitors of BACE-1 was terminated because of serious side effects. The most discussed question remains to what extent it is beneficial to modulate the activity of BACE-1, and in what phase of the AD it is best to start the treatment [17]. Theoretical knowledge on the mutation of Ala673Thr further shows that 50% of BACE-1 inhibition results in a 20% reduction of the Aβ level. But it still remains unclear to what extent it is necessary to inhibit the activity of BACE-1 if the amyloid plaques are already formed. The amyloid plaques themselves can form many years before the clinical manifestation of the symptoms of dementia. However, in recent years, new theories have emerged posing Aβ on the crossroad [43]. Indeed, in some patients, the presence of Aβ in AD brain does not necessarily mean dementia will break out. Postmortem biopsy showed that older persons can have extensive amyloid burden without any signs of cognitive impairment. Note that it also remains unclear whether these individuals would have developed AD if they had lived longer. Be that as it may, it was proved that the presence of Aβ in cognitively normal persons was prone more rapidly to develop symptoms related to AD [44, 45]. Last but not least, it is not fully understood what the relationship between the quantity of the Aβ deposit and cognitive distortions really is. Nonetheless, everything should be more or less elucidated by the results of ongoing clinical trials, especially those on γ-secretase, which seems to be the most perspective biological target for therapy of AD.

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Chapter 2

Alzheimer's Disease: Beyond the Neuron

Aradhana Verma and Matthew Zabel

Additional information is available at the end of the chapter

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Abstract

This chapter describes the various systems beyond the central nervous system that are associated with Alzheimer's disease (AD). There is strong evidence to believe that while AD has symptoms of memory and cognitive impairment—undoubtedly domains of the central nervous system—the primary insult that causes this condition may arise systemically. We describe associations with the immune system, gut microbiome, and endocrine abnormalities that may be at play. Our goal is to incorporate a multi-system approach to understand the pathogenesis of AD. Our body does not function as soloed organ systems, and we hypothesize that the mechanisms described herein are similarly contributing to the progression of cognitive impairment in AD.

Keywords: microglia, inflammation, metabolism, diet, gut microbiota, amyloid

1. Introduction

No scientific problem has seen more heartbreak and frustration than the challenges of Alzheimer's disease (AD). This is not surprising—we are dealing with a disease that progressively degenerates a complex biological system. A century has passed since the symptoms were first recorded by Dr. Alois Alzheimer, yet we lack meaningful treatments. We propose that this is not a weakness of past research, but a misguided approach that focuses on specific aspects of disease pathogenesis centering within the brain and out of context from other systems involved. In other words, we suggest that the seemingly elusive nature of piecing together this tragic disease is due to viewing it through the lens of only one or two potential mechanisms at a time. Our goal is to synthesize several mechanisms into an explanation of disease pathogenesis that incorporates neurons, the immune system, and even the gastrointestinal tract and its microbial inhabitants. We will show that the pathology seen in AD is

a result of multiple hits contributed by systems within and outside the brain parenchyma and thus prompt the search for novel therapies that address the multi-organ etiology of AD pathology.

2. The amyloid cascade hypothesis

The most widely accepted theory of AD etiology is the amyloid cascade hypothesis [1], which maintains that overproduction and/or decreased clearance leads to extracellular aggregation of the presumably toxic amyloid-beta (Aβ) peptide. These extracellular Aβ aggregates act to increase neuronal kinase activity, resulting in phosphorylation of the microtubule-associated protein tau. Hyperphosphorylation of tau induces formation of intracellular aggregates known as neurofibrillary tangles and alters intracellular transport along microtubule tracks. This in turn abolishes neuronal communication, resulting in cell death in a spatially conserved pattern and producing deficits in networks that subserve memory and cognition. Aggregation of Aβ and tau is well-established pathological characteristics of AD brain tissue at autopsy. It is also known that in familial forms of AD, mutations in amyloid precursor protein (APP), Presenilin 1, or Presenilin 2 accelerate Aβ production and accumulation and lead to cognitive decline at a much earlier age. Presinilins function as part of the gamma secretase protein complex, one of three proteolytic enzymes responsible for cleaving APP into Aβ or nonaggregating amyloid peptides. Autopsy samples from brain parenchyma of patients with familial AD, which account for less than 1% of all AD cases, present with exorbitant Aβ and Tau accumulation similar to sporadic AD. Additionally, since the APP gene is located on chromosome 21, individuals with Down syndrome (trisomy 21) invariably develop AD-like dementia, also at a younger age than sporadic cases. This intuitively makes sense: an extra copy of APP on chromosome 21 will inevitably lead to the generation of more Aβ. However, it is highly uncertain to what degree familial AD and Down syndrome recapitulate the initial stages of sporadic AD, which accounts for the vast majority of AD cases. This is the core of the debate surrounding the amyloid cascade hypothesis: Is Aβ aggregation the start of AD or a downstream effect of an earlier insult? Additionally, and of considerable concern, to the day of writing this chapter, multiple immunotherapy clinical trials that target and clear Aβ as well as trials to block the activity of the secretases have failed to reverse cognitive loss and, in some cases, have accelerated it [2]. In this chapter, we will describe Aβ aggregation only as surrogate for the final common pathway of multiple disease mechanisms leading to the established end pathology of AD and not as a direct, initiating cause of clinical demise.

3. Microglia in brain homeostasis

3.1. Microglia and brain development

Microglia are the endogenous immune cells of the central nervous system. Over the past decade, the ontogeny of microglial cells has been controversial. Their developmental progression has

gone through several interesting iterations leading to our current understanding of how these peripherally derived cells come to reside in the central nervous system [3]. During development, myeloid precursors travel to the brain and then differentiate into microglia (CNS parenchymal macrophages). These tissue-specific macrophages make their way to the brain through the circulation from the embryonic yolk sac [4]. They grow concurrently with neurons, before the development of astrocytes and oligodendrocytes, participating in key neurodevelopmental events such as neurogenesis, synaptic pruning, and thus the development and remodeling of neuronal circuits. There is evidence that microglia need to adapt to their quickly changing environment and modify their functions as needed [5]. It seems logical, then, that aberrant or impaired microglial activation during development would be implicated in CNS disease later on in life.

Early brain development involves a vast amount of axon and synaptic growth—a process known as exuberant synaptogenesis. During early childhood and puberty, these synapses are slowly eliminated in a regulatory process called synaptic pruning. Interestingly, the mechanisms responsible for synaptic pruning are related to peripheral immune mediators such as major histocompatibility complex [6] and complement proteins [7, 8]. As described in a review by our group [9] and briefly summarized below, the reemergence of these molecules in the aging brain may lead to inappropriate synaptic pruning and uncontrolled neuroinflammation.

3.2. Microglia and AD

The role of microglia in the body is the story of Goldilocks. Much like the body's peripheral immune system, diseased or dystrophic microglia have diminished capacity to fight exogenous infections, clear endogenous cellular waste products, or promote homeostasis after an injurious insult. On the other hand, too much activation can severely harm the brain, much like how autoimmunity or graft rejection occurs in the periphery. In the brain, microglia contribute to Aβ clearance [10, 11]. However, the ability of microglial clearance appears to deteriorate and, in some cases, negatively change with age [12, 13]. At late stages of AD, microglia are thought to become overstimulated and paradoxically contribute to the disease by releasing proinflammatory cytokines in response to Aβ deposition [14, 15] or actively phagocytosing damaged, but live neurons [16]. Recent studies have consistently shown complement cascade proteins C1q and C3b—both normally associated with peripheral inflammation—upregulated on synapses induced by Aβ plaques in a mouse model of AD. Microglia then eliminated these C1q- or C3b-tagged synapses, leading to neurodegeneration and behavioral impairment [17, 18]. Immunohistochemistry studies reveal that Ig-positive neurons were C1q and C5b-9-positive and appeared degenerative [19]. These data suggest that neurons in AD brains are dying from an antibody-induced classical complement process. Additionally, newly discovered genetic risk factors are based on microglial phagocytosis, including CD33 [20], TREM2 [21, 22], and complement receptor 1 [23]. A full description of these mechanisms is out of the scope of this chapter, but the reader is encouraged to read more exhaustive reviews on this topic [24–26]. Nonetheless, it is a fascinating prospect that a peripherally derived cell plays such a large part in a central nervous system disease and that many of the processes used for brain development resurface to wreak havoc during degeneration. This shall segue into our next section discussing purely systemic mechanisms of AD pathogenesis.

4. Peripheral manifestations of a central nervous system disease

Over the past two to three decades, significant research effort has attempted to characterize the peripheral contributions to brain disease. This is a fascinating notion, considering the apparent impermeability of the central nervous system. However, even this impermeability depends on the environment to which the brain is subjected and may be under the influence of factors important during development. Unlike most other organs (with the exception of the retina and testes), the brain is highly susceptible to injury by chemical stressors normally present outside the confines of the blood-brain barrier (BBB). Neurons, despite their seemingly robust ability to work throughout the human lifespan, constant firing during that lifespan and frequent turnover of their signal transmitting elements (synapses), are a delicate class of cells. For this, neurons are accompanied by three other cell types termed glial cells, which are supportive in nature. These consist of the myelinating oligodendroglia, the jack of all trades astroglia and the aforementioned specialized immune cells of the CNS called microglia. All of these cells—count approximately 172 billion [27]—are separated from the nearly 500 miles of brain vasculature and capillary networks by the tight junction-lined and sealed BBB [28]. Most of the protection afforded to neurons is performed by the BBB, microglial cells, and astrocytes. Dysfunction of any of these components leads to some form of neuronal compromise. In this section, we will concentrate specifically on the BBB and microglia and how peripheral insults, including an unsuspecting role of the resident microflora, may influence their ability to protect neurons.

4.1. Systemic inflammation

Recent studies reveal that a cross-pollination between molecules thought to be exclusively involved with either the CNS or the immune system. Cytokines, complement proteins, and major histocompatibility complex (MHC) class 1 proteins have all been implicated in brain development [29–31] and neurological disease.

We know that bacterial, viral, fungal, and parasitic infections that target the CNS are associated with an increased risk of AD. These infections likely trigger a chronic, systemic inflammatory state in the CNS, leading to neurodegeneration. For example, it has been shown that a bacterial infection can induce amyloidosis and thus lead to the development of AD [32]. A recent study in mice showed that memory impairment after West Nile virus infection was dependent on microglia and complement-induced synaptic pruning within the CA3 region of the hippocampus [33]. However, the big question that many in the field of AD have asked is: What are the contributions of the immunological effectors that exist solely in the peripheral blood, and how do they wreak havoc within the tightly regulated brain parenchyma?

The start of this research began even before the discovery that established Aβ as the composition of the senile plaques that are the hallmark pathology seen in postmortem AD brains [34]. Eikelenboom and Stam found both immunoglobulins and complement proteins resided within senile plaques using basic immunoperoxidase techniques [35]. This study, along with continued confirmatory experiments led to the subsequent study of non-steroidal anti-inflammatory drugs

(NSAIDs) in randomized control trials [36–40]. Unfortunately, a relatively recent meta-analysis demonstrated no clinically significant slowing of AD progression when these data were aggregated [41]. However, many of the studies included in the meta-analysis were done well before the establishment of a thorough understanding of AD clinical progression [42, 43]. In other words, could it be that therapy needs to be initiated during prodromal clinical stages of the disease—a time when the pathology has not yet reached a saturation threshold and may be more effectively halted? A corollary to this is whether we should begin battling neurodegeneration even in the first years of life, as we will discuss below. These questions are being actively studied in current trials of both anti-amyloid and anti-inflammatory therapies.

Research still continues to produce good studies implicating a peripheral source of immunological and inflammatory mediators of disease. Of particular interest in this regard is a series of studies using a parabiotic model of AD pathogenesis. Villeda and colleagues demonstrated that connecting the circulatory systems of old and young mice could alter cognitive function in both groups, but in opposite directions [44]. For example, blood transferred from old to young mice reduced synaptic plasticity and neurogenesis and thus decreased spatial learning and memory and fear conditioning. In addition, the authors were able to isolate several chemokine differences between the two groups of mice (specifically CCL11) and when injected intraperitoneal or into the dentate gyrus of young mice, a similar decrease in cognitive function ensued. Conversely, and further proof of concept, the same group then exposed older mice to young blood and found a reversal of the effects seen in their previous study (i.e. increased dendritic spine density, stabilization of synaptic plasticity, and reversal of age-related cognitive dysfunction) [45]. This positive regulation also seems to be mediated by remodeling of the cerebrovasculature, which ultimately increases blood flow [46] and additionally lends credence to vasculopathic origins of neurodegenerative diseases.

Preclinical studies of this possible therapeutic modality in AD mouse models are ongoing and have so far shown some promise. For example, aged mice harboring an APP mutation that underwent heterochronic parabiosis to young wild-type mice or injection of young plasma showed a complete restoration of markers of synaptic function compared to old APP isochronic parabiotic mice [47]. Important to the overarching theme of this chapter is that these effects were independent of changes in amyloid between the groups, suggesting Aβ is not involved to the degree that the field often perpetuates. However, results of cognitive and behavioral testing were not as impressive suggesting more work will need to be done to determine the specific factors involved in the synaptic changes and thus the efficacy of this treatment option.

Another interesting set of data that supports a peripheral cause for AD comes out of the field of sepsis and critical care. Sepsis is an exacerbated and uncontrolled peripheral inflammatory response to an infectious agent via the release of proinflammatory cytokines such as IL-1 and TNF-alpha as well as complement proteins. Although sepsis is an acute event, it could be an enlightening lens through which to view the link between peripheral inflammation and cognitive dysfunction. For example, one study compared relatively young ICU patients (mean age 55) with and without sepsis and found that those who had survived sepsis 6 to 24 months prior demonstrated cognitive dysfunction equivalent to mild cognitive impairment on a battery of neuropsychological tests [48]. Additionally, volumetric magnetic

resonance imaging showed reduction in hippocampal volume in sepsis patients compared to nonsepsis patients, but no evidence of vasculopathy. Confounding factors such as depression, systemic infection that is not sepsis and quality of life were all controlled for. This was corroborated by a separate group that showed a decrease in whole brain volumes at least 3 months after sepsis, which was associated with long-term cognitive impairment at least 12 months post sepsis [49]. Another study in older individuals (mean age 77) demonstrated that patients with sepsis 3 years prior were three times more likely to become cognitively impaired compared to nonsepsis patients [50]. These data suggest that cognitive impairment persists several months to years after a peripheral blood insult, although it would be interesting to follow these patients even further, even to autopsy. Even studies looking at nonsepsis patients, systemic infections show that an increased infectious burden with common pathogens (including bacteria such as *Chlamydia pneumoniae* and *Helicobacter pylori* and viruses such as cytomegalovirus and herpes simplex viruses 1 and 2) conferred a higher risk of memory decline that is independent of vascular risk factors [51].

More recent studies have tried to delineate some of the molecular and cellular mechanisms of sepsis-induced cognitive decline, and many are unsurprisingly similar to those proposed for the etiology of AD. One very interesting study compared the neuropathology of a rat model of sepsis-associated encephalopathy to that of deceased patients with sepsis and found two patterns of brain damage: diffuse axonal injury and ischemic damage [52]. Pathologically, human sepsis specimens demonstrated Aβ-positive plaques and neurofibrillary tangles, which corresponded to increased levels of βAPP and altered axonal morphology in the rat model. Both pathological hallmarks were absent in control specimens of both humans and rats. Furthermore, MRI was able to demonstrate either diffuse axonal injury or ischemic brain injury in 9 of the 13 sepsis patients, although several of the patients were of advanced age making it difficult to determine if these lesions are truly a result of sepsis or a separate underlying pathology. However, this is a unique study, and larger numbers of patients with more quantitation would be of great value for future clinical management. This may be prudent sooner rather than later as a recent preclinical study has shown that statins may be beneficial in preventing this cognitive decline in mice with experimental sepsis-associated encephalopathy [53]. The authors showed that this cognitive protection (not necessarily prevention of death from sepsis) was due to reduction in peripheral and brain proinflammatory cytokines, oxidative stress, and even microglial activation, in addition to increased capillary density and subsequent increase in blood flow. These results coincide nicely with findings demonstrated in clinical studies, as discussed above.

4.2. Diabetes mellitus: the effect of peripheral blood glucose

To have a discussion linking peripheral inflammation and other peripheral stressors to brain disease, one must discuss the effect of diet and exercise on neuronal homeostasis. Just as AD has become an epidemic in the aging population, there is an increasing prevalence of obesity and type 2 diabetes mellitus (T2DM). T2DM is related to chronically elevated blood glucose. Both T2DM and metabolic syndrome are highly associated with aberrant insulin signaling. The association of AD with impaired insulin signaling suggests that a similar pathological pathway may be at play here.

Epidemiologic and basic science research has found a shared link between the pathophysiology of AD and T2DM. This is a difficult association to make since both conditions are common in aging. However, several key animal and human studies have shown that the connection may be deeper than just that of aging. Some have even suggested identifying Alzheimer's disease as type 3 diabetes mellitus.

Chronically elevated glucose levels are a known risk factor for dementia and Alzheimer's disease in individuals with and without a diagnosis of diabetes [54]. This literature highlights the various deleterious consequences of chronically elevated glucose on the aging brain. A 2015 study compared the brains of individuals with T2DM and those without T2DM to identify any possible effects on the brain. The brains of individuals with T2DM was associated with higher levels of total tau and phosphorylated tau in the CSF, suggesting an increased level of neuronal damage in the brain, although no significant association was made with regards to the brain Aβ load. The study concluded that T2DM may promote neurodegeneration by promoting tau hyperphosphorylation [55]. As with all studies between two separate conditions, we should be cautious if these types of studies demonstrate correlation or in fact a causation. More research is needed to support either conclusion.

On a mechanistic level, the insulin receptor and the insulin-like growth factor-1 (IGF-1) receptor have been found to be impaired in AD neurons, suggesting that CNS cells in persons with AD may be resistant to insulin signaling. One possible mechanism for the impaired signaling pathway is due to aberrant phosphorylation of Ser/Thr sites, IGF-1, and insulin receptor resistance. The increased levels of phosphorylation sites were found primarily in neurons with neurofibrillary tangles of AD brains [56]. A disruption of insulin signaling to the brain would have significant consequences to the brain as it could lead to a compromised source of energy. It would impair important neurotrophic and metabolic brain functions and contribute to AD pathology.

Switching gears from causes to treatment, recent studies have shown an interesting connection between therapeutic targets of T2DM and AD. Medications such as glucagon-like peptide-1 and glucose-dependent insulinotropic polypeptide that have shown to improve glucose control in patients with diabetes also show evidence of memory improvement in mice models of Alzheimer's disease. Amyloid plaque load, neuroinflammation, and oxidative stress have been shown to be reduced by these anti-diabetic drugs [57]. The results are still early, and it remains unclear if these treatments will demonstrate similar results in humans. Further clinical research and potential clinical trials will bring us one step closer to understanding the link between diabetes and Alzheimer's disease. Importantly, it may open doors for new, innovative approaches to treatment of AD and other forms of dementia.

The benefit of regular physical activity and exercise is clearly recognized in the neurological wellbeing of a population. Multiple cohort studies have found that high physical activity is associated with a reduced risk of AD and dementia [58–60]. The connection of T2DM and obesity with AD is a compelling reason to explore the effects of exercise since there is robust evidence that demonstrates the efficacy of exercise on reducing the progression of insulin resistance. Physical activity and exercise stimulates release of particular neurotransmitters and growth factors, specifically brain-derived neurotrophic factor (BDNF) and insulin-like

growth factor (IGF-1), and increases circulating testosterone levels. All of these effects have been shown to reduce the levels of Aβ in the brain, both by decreased production and increased clearance in the brain. The reduced Aβ was even found in individuals that carried the ApoE4 allele, which put them at greater risk for Alzheimer's disease [61]. These findings suggest the observation that Alzheimer's disease is linked to metabolism and the body's hormonal signaling system. The Aβ found in AD may be the result, but not the true culprit of the condition.

4.3. The microbiome

The human microbiome—the complement of microbial species (or microbial genes) and communities inhabiting the human organism—has been the subject of intense research interest in the context of brain development and dysfunction [62]. The influence of microflora on external and internal cues in brain development has been known for some time through population-based studies. As part of normal physiology, crosstalk between the gut and the brain plays a critical role in modulating brain homeostasis and behavior. Several neurological and psychiatric disorders (e.g. multiple sclerosis, Parkinson's disease, spinal cord injury, autism, and Alzheimer's disease) have been associated with dysbiosis or the disruption of normal gut flora. For example, children with late onset autism were found to have significantly more and different species of *Clostridium* in their fecal flora than control subjects without autism [63], and oral vancomycin improved several neurocognitive parameters when given to late-onset autistic children [64]. Experimental studies in rodents have shown that germ-free (GF) mice have increased serotonin, norepinephrine, and dopamine turnover and a decrease in their receptor levels, as well as reduced anxiety [65, 66]. Interestingly, changes to the microflora due to high-fat diet during pregnancy can have detrimental effects on the fetus when compared to normal chow diet [67]. Maternal obesity seems to also correlate with changes to the microflora (i.e. increase in *Bacteroides and Staphylococcus*) [68], which may predispose the mother to neurological disease and increase the risk of future neurodegeneration in her offspring. Additionally, gut bacterial infection early in life can alter memory formation in the young [69] and later in life, especially after a subsequent inflammatory insult [70].

At the cellular level of brain development, the resident microflora can alter the development and thus the permeability of the BBB. In GF mothers, BBB permeability was increased in the fetus [71]. Mice born to GF mothers demonstrated decreased BBB integrity beginning *in utero* with decreased levels of tight junction proteins in the hippocampus, frontal cortex, and striatum. Interestingly, pericyte coverage and vascular density were not altered in this model, but the authors did not investigate the role that GF status had on astrocyte physiology, which are an important cellular component of the BBB. The mechanism of decreased tight junction components was due to the lack of short-chain fatty acids (SCFAs) normally produced by commensal organisms. Considering the importance of the BBB in keeping neurotoxic molecules out of the brain parenchyma, this developmental flaw makes the brain vulnerable to a number of insults from the periphery increasing neuronal stress.

Of particular interest here is that SCFAs produced by bacteria in the gut also have the potential to inhibit Aβ aggregation in cell culture [72] and guide the proper development of microglial

cells, as discussed later. At the genetic level, Apolipoprotein E (ApoE)—one of the most important risk factors in AD—may play a role in selecting for a microflora more prone to generating SCFAs. For example, 5xFAD mice harboring the ApoE2 allele, which is considered protective, contained higher numbers of the *Ruminococcaceae* family of bacteria, which are known to produce high levels of SCFAs [73]. However, ApoE4 mice (the best characterized genetic risk factor for AD) contained higher levels of *Lactobacillaceae*, which are considered a pro-health microflora, making these results difficult to interpret, but may highlight the importance of SCFAs in CNS protection. As might be expected, the neutral ApoE3 mice contained a mixture of both families of bacteria. These results were independent of 5xFAD status.

An altered microbiome may be a source of proinflammatory molecules that are toxic to the brain. For example, in humans, it has recently been demonstrated that elderly patients with higher levels of Aβ based on ^{18}F-Florbetapir positron emission tomography (PET) contained higher levels of proinflammatory microbiota (e.g. *Escherichia* and *Shigella*), as well as proinflammatory cytokines, while also containing lower levels of anti-inflammatory microbiota (e.g. *Eubacterium rectale, Eubacterium hallii,* and *Bacteroides fragilis*) [74]. Interestingly, even cognitively impaired individuals without PET evidence of amyloidosis showed a similar increase in proinflammatory microbiota and peripheral cytokines and decreased anti-inflammatory microbiota, although the effect was smaller. This corroborated findings in the first PET study show that periodontal disease was associated with amyloidosis in AD-specific brain regions. However, the authors did not characterize the clinical characteristics of the study subjects, so it is difficult to know if these findings are relevant to cognitive decline. In addition, peripheral inflammation was implicated in the increased rate of cognitive decline in a cohort of mild to moderate AD patients who had periodontitis [75], which was not seen in patients without it, although the relative changes were not that robust. However, other studies have shown a positive relationship between the levels of TNF-α and immunoglobulins to periodontal bacteria in AD patients with periodontal disease that was absent in normal controls [76]. In fact, serum immunoglobulins to a wide variety of periodontal pathogens were present in patients before they converted to clinical AD [77], implying an increased risk of AD due to peripheral inflammation mediated by oral microflora.

Experimental and preclinical models of AD have also shown that changes to the microbiome have an effect on the progression of disease pathology. In the first study to show this, the authors used a well-characterized AD mouse model harboring the Swedish APP mutation and the PS1 tau mutation [78]. The experimental group (ABX) of these mice was given a cocktail of antibiotics after postnatal day 14 for the entirety of their lifespan. As might be expected, the ABX group had a distinctly different microbial profile than the control group, but also demonstrated a lower Aβ plaque burden and smaller plaque size. Additionally, insoluble levels of Aβ40 and Aβ42 were decreased, but soluble forms of these two peptides were actually increased, although it is not clear as to why this was. A subsequent study in the same model of AD, but with a different method of GF group generation, obtained similar findings of reduced Aβ plaque burden in GF-APP mice [79]. Interestingly, when the authors cross-colonized the GF-APP mice with the microbiota from the conventionally raised APP-PS1 group, the Aβ levels increased in the GF-APP group. Conversely, colonization of the GF-APP group with microbiota from wild-type mice (a separate group of mice conventionally raised

and without the APP-PS1 mutations) contained less Aβ pathology than conventionally raised APP-PS1 mice. This last set of data is congruent with human findings that the specific microbial populations involved in AD pathogenesis are more important than simply whether microorganisms are present or not. The authors attempted to demonstrate this idea by looking at the differences in microbial populations between conventionally raised APP-PS1 mice and GF-APP mice. However, because the variable being changed in this circumstance is the APP status, their results would suggest that APP mutation effects microbial diversity and not necessarily that microbial diversity effects Aβ generation. In other words, any mouse model starting with a mutation that increases Aβ levels in the first place has already conceded that an overproduction of Aβ is the cause of pathology in that model, which in humans has shown to be inaccurate for 95% of AD cases (i.e. the sporadic, non-Mendelian cases make up the vast majority of human cases). For now, thought, the data suggests that microbial products and the immune response to microbiota contribute to specific pathological outcomes implicated in AD—namely APP metabolism. Unfortunately, the experimental studies described in the previous paragraph lack a clinical surrogate. For example, the studies by Minter et al. and Harach et al. did not characterize neuronal degeneration or cognitive decline in their identical models, so we cannot know if there was any clinically relevant change to neuronal integrity. It is well known within the field of AD that neuronal degeneration is a better predictor of cognitive decline than is Aβ pathology.

One mechanism that may link the microflora with neurodegeneration involves the immune cells of the brain. As one might expect of a peripherally derived immune cell, a complex gut microbiota promotes microglial development, while the lack of rich microbiota leads to impaired microglial maturation, differentiation, and function. In the first of its kind, one study compared the immune responses and its association within the brain by studying GF mouse models [80]. Moreover, the same study found that the reintroduction of complex microbiota may largely, but not entirely, restore microglia. Interestingly, the authors of the study suggest that the wide complexity of the microbiota, not the bacterial load, is associated with restored microglial function.

This seems like a good time to revisit another interpretation of the amyloid cascade hypothesis put forth by Bishop and Robinson over two decades ago and, unfortunately, largely forgotten. They named it the bioflocculant hypothesis of AD [81]. It is an alternative way to look at the production of Aβ, not as much as the start of a pathological cascade, but as a way to halt the sequence of events beginning with a previous injury or stressor that leads to neurodegeneration. It views Aβ production as a response to exogenous insults since Aβ is produced after a variety of brain injuries [82–85]. They compare the production of Aβ, and subsequent aggregation into plaques, to a web constructed to trap any offending agents that may enter the brain in a pathological state. They convincingly describe a situation in which neurons may use the sticky properties of Aβ as a way to contain pathogens, toxic metals, or other products of blood in a trap much like a spider's web. It is then easy to imagine microglia as the spider in this scenario engulfing anything trapped within the web and disposing of it. We would add to this list of functions, a means to plug up holes in the microvasculature as might be seen

in the microbleeds of cerebral amyloid angiopathy. In support of their hypothesis, a recent paper demonstrated Aβ's role in trapping infiltrating bacteria (specifically *Salmonella typhi* and *Candida albicans*), which coaggregated in 5xFAD mice by binding to the bacterial cell wall via heparin-binding domains [86].

One could easily imagine such a scenario playing out in the etiology of AD starting even with risk factors present in the early years of life: (1) early embryonic changes to host microbiota may predispose a person to a leaky BBB and all of the consequences of that derangement later in life (**Figure 1a**). (2) BBB malfunction may either contribute to or coincide with the microbiota-dependent alterations to networks responsible for memory formation—AD is a disease of memory formation after all. (3) Although in its beginning phases of understanding, the SCFAs that are responsible for maintaining components of BBB tight junctions during development seem to also decrease the toxic effects of the Aβ peptide later in life. (4) The brain's immune cells, if not exposed to the appropriate milieu of microorganisms (and their metabolites such as SCFAs) during development, may be unable to protect the brain against invading pathogens in adulthood and/or contribute directly to inappropriate neuronal network remodeling in development and disease (**Figure 1a**, **b**). (5) Changes to the normal microflora during adulthood, either through systemic infection (e.g. sepsis, periodontal disease, or any other form of peripheral increase in the proinflammatory state) or antibiotic use, can increase the risk of conversion to AD, especially in the elderly (**Figure 1c**). (6) Lastly, all of these steps leading to neuronal demise are also dependent on the metabolic perturbations seen in disorders of glucose control and obesity (**Figure 1d**).

4.4. Role of probiotics and antibiotics

The gut microbiota-brain axis is still insufficiently understood. There is a need for more research to better identify the unique combination of microbiota that is implicated in the disease process. The logical next step would be the development of antibiotic or probiotic treatments with the goal of reducing the disease burden.

An important study to answer the question of the microflora's influence on AD pathology and cognitive function did so by feeding an AD mouse model a probiotic formulation rather than depleting them of bacteria [87]. The study authors found that cognitive dysfunction was ameliorated with the use of probiotics and this was dependent on reduction in peripheral proinflammatory cytokines, increased anti-inflammatory cytokines, and replenishment of autophagic and proteasomal function within neurons. These are two important ways for the body to regulate itself and remove old or damaged proteins. Aberrant proteasome function then leads to neurotoxicity and favors the development of misfolded proteins in the brain [88, 89]. In addition, several studies presented at this year's annual Neuroscience meeting using probiotics containing *Lactobacilli* and *Bifidobacteria* improved memory in several mouse models of AD [90]. Although it is early, these data lend credence to the importance of correcting the composition of the microflora after use of antibiotics and the possible importance of taking a probiotic to maintain both brain and overall health.

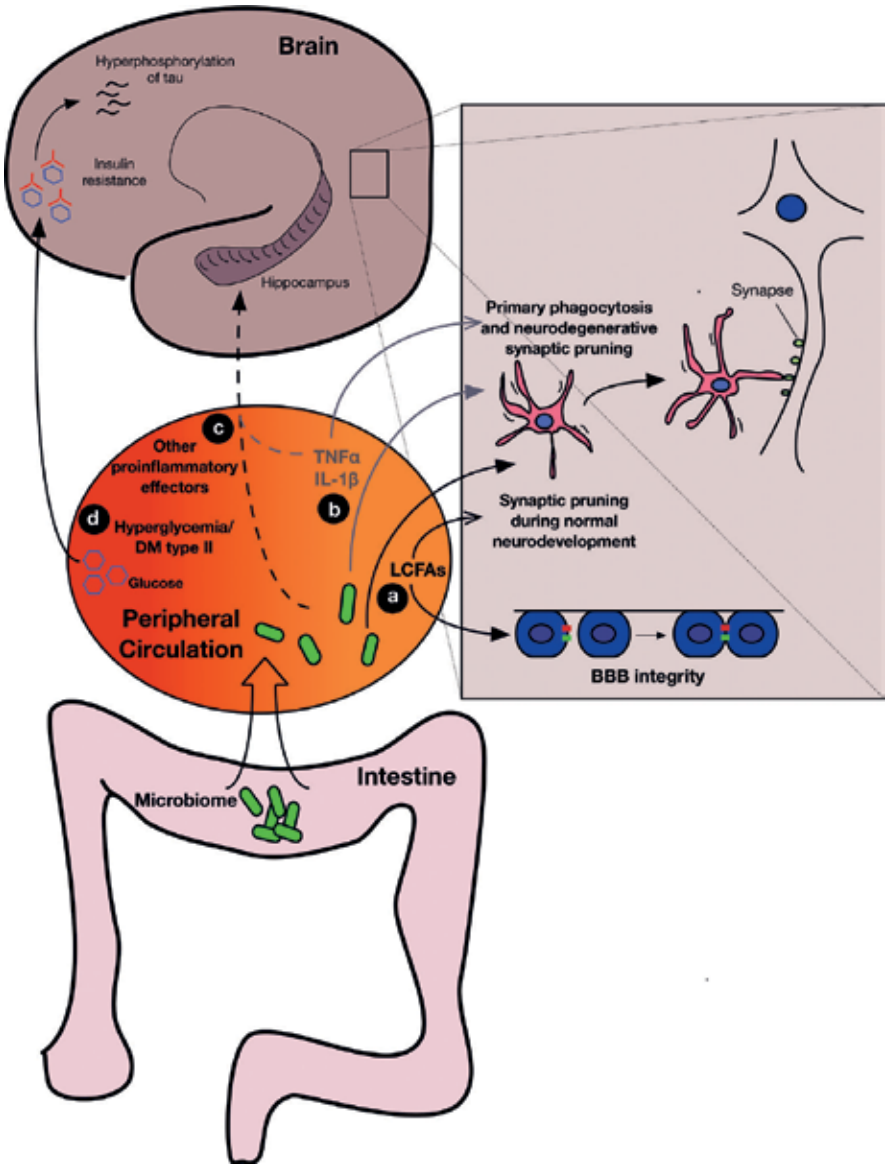

Figure 1. Summary figure of proposed involvement of the microbiome in brain development and dysfunction. **(a)** Short-chain fatty acids (SCFAs) produced as bacterial metabolites by anti-inflammatory bacteria exert their influence both on the development of the blood-brain barrier (BBB) and on the development of microglia. Dysfunction of either of these processes may lead to neurodevelopmental disorders early in life or neurodegenerative disorders in adults. On the other hand, proinflammatory bacteria are recognized by the immune system as such during a state of sepsis, which elicits the overproduction of proinflammatory cytokines. These effectors may **(b)** activate microglia in adults leading to aberrant synaptic pruning and primary phagocytosis of live neurons or **(c)** have a direct effect on memory forming networks during development as well as memory formation and/or retrieval in the adult. development as well as memory formation and/or retrieval in the adult. **(d)** Chronically elevated peripheral glucose levels may lead to insulin resistance and aberrant phosphorylation of the insulin receptor and concomitant hyperphosphorylation of the microtubule-binding protein tau, which is a hallmark pathology of the AD brain and correlates more specifically with the progression of neurodegeneration.

5. Conclusion

In this chapter, we described several concurrent mechanisms of AD pathogenesis, including the effects of systemic inflammation, metabolic dysfunction, and the gut microbiome. Since there seems to be no cure for AD and current established and experimental therapies are suboptimal at best, we suggest that more research should focus on minimizing peripheral inflammation and maintaining an anti-inflammatory complement of microbiota as early as possible. Targeting these two entities appears to positively affect the plethora of mechanisms implicated in AD (i.e. Aβ aggregation, tau hyperphosphorylation, microglial and complement activation, and BBB breakdown). There is reason to believe that AD arises from a manifestation of multiple hits within and outside of the central nervous system. A multi-system strategy will thus be most efficacious for prevention and treatment.

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Conflict of interest

The authors have no conflicts of interest to disclose.

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Chapter 3

Defining Microglial Phenotypes in Alzheimer's Disease

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Additional information is available at the end of the chapter

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Abstract

The concept of activated microglia being associated with neurodegenerative pathological structures in aging and Alzheimer's disease (AD) has been well established, but questions remain about how well are we defining "*what are microglia **actually** doing*" when we look at diseased or aged brains? Most studies of microglia in human AD brains have employed a limited set of antigenic markers, particularly the major histocompatibility complex protein HLA-DR and ionized calcium-binding adaptor molecule IBA-1, along with cellular morphological criteria, but in recent years, it has been appreciated that microglial responses are very heterogeneous depending on their surrounding environment—*every microglia might be different*. Initial observations on human brain microglia associated with plaques and tangles suggested that microglial inhibition with broad spectrum anti-inflammatory drugs should slow down AD pathology, but clinical trials did not show this approach to be effective. In this article, we will consider the needs, challenges and benefits for refining how microglia are defined as they associate with pathological proteins. This may aid in defining which ones are accelerating neurotoxicity and which ones are performing reparative/phagocytic functions. More complete definition of microglial phenotypes offers the potential of developing ***targeted*** anti-inflammatory approaches for this disease.

Keywords: neuroinflammation, pathology, immunohistochemistry, antibodies, activation, microglia

1. Introduction

Progress in developing new treatment approaches for Alzheimer's disease (AD) has been slow. The goals of identifying key pathological events early in the disease and preventing them from progressing further has been investigated for many approaches; including preventing amyloid

formation and/or aggregation [1, 2], preventing tau phosphorylation and aggregation [3], reducing mitochondrial dysfunction and production of reactive oxygen species with various anti-oxidants [4, 5], enhancing autophagy [6], reducing the consequences of abnormal lipid metabolism [7], and targeting inflammation and microglia [8] amongst others with limited effectiveness. Since the identification of increased inflammation in AD brains, first characterized more than 30 years by increased expression by microglia of HLA-DR [9], and subsequently for other macrophage proteins, including beta II integrins and immunoglobulin Fc receptors CD16, CD32 and CD64 [10, 11], ferritin [12, 13], CD68 [14, 15] amongst others, led to the development of the inflammation hypothesis for AD that suggested that inflammatory products were driving the loss of synapses and neurons in this disease [16]. If this were true, reducing inflammation should have shown great potential for treating AD, however; this approach has not been successful to date [17, 18].

The initial studies identified several features about the microglia associated with AD plaque and tangle pathology. There was increased expression of the MHC class II marker HLA-DR, and morphologically the microglia were different with thicker cell processes and enlarged cell bodies [19]. From these initial observations, it was concluded that the microglia were in a pro-inflammatory state and thus must be producing neurotoxic cytokines that would be accelerating the progression of disease. This hypothesis is illustrated in **Figure 1**, where in this illustration aggregated amyloid beta (Aβ) peptide, produced as an initial feature of AD, activates the microglia, which can produce cytokines such as tumor necrosis factor (TNF)-α or interleukin (IL)-1β that accelerate neuronal dysfunction. This scheme was supported by experimental studies showing that aggregated/fibrillar Aβ had strong proinflammatory-inducing properties on microglia, including activation of the NADPH oxidase complex of myelocytic

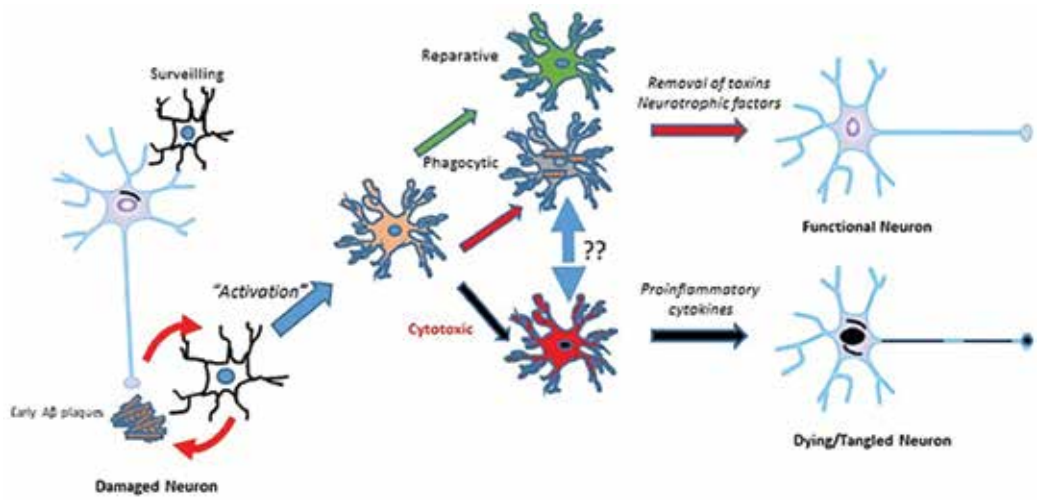

Figure 1. Microglia are performing multiple functions in Alzheimer's disease brains. How should microglia be classified in human brains. Scheme to illustrate the potential change in activation of microglia with development of disease. The morphologies of resting or surveilling microglia is established, but it is apparent that activated-cytotoxic-microglia in brain tissue may be indistinguishable from the activated-reparative or -phagocytic microglia. The observation of ameboid microglia in AD tissue without other pathology (infarcts/hemorrhage) involving lymphocyte infiltration is rare.

cells that leads to a burst of production of highly toxic reactive oxygen species [20, 21], as well as induction of many proinflammatory genes [22]. Another feature of this figure, which could be as important, is the other (non-proinflammatory) microglia that will be present in the AD pathological environment. There has been insufficient attention to describing these microglia, but they will play an essential role in phagocytosis of Aβ as well as performing other homeostatic functions. It has been hypothesized that one could distinguish between these microglia based mainly on cellular morphology; however there are limited data to support this. The presence of activated microglia in AD tissues provided the rationale for testing of "non-specific" non-steroidal anti-inflammatory drugs (NSAIDS) in AD patients. There had been supporting, though not unanimous, data that subjects with a history of long term anti-inflammatory drug usage also seemed to be protected from dementia [23–25]. Controlled drug trials of NSAIDs or related agents in AD patients have never shown significant effectiveness in slowing disease progression, and even these widely-used agents appear to have significant side effects in elderly subject. It has been argued that these trials failed because the disease was too far progressed to respond to therapy, but we will posit in this article that maybe our understanding of the unique features of inflammation in AD are still not complete enough for selecting appropriate drug treatments. This comes back to the central question of "*what are the activated microglia actually doing*" in the AD brain [17, 18]. Such studies need adequate numbers of quality tissue samples from control and AD cases. The presence of AD plaque and tangle pathology is also a common feature in aged brains without clinical dementia. These cases, called high pathology controls or high plaque non-demented, are particularly valuable for neuroinflammation studies as they provide material for studying what might be pre-AD stages of microglial activation. Being able to describe progression of inflammatory changes leading AD could be critical for identifying therapeutic targets.

2. Dichotomy of microglial function: do we know which microglia are producing damaging inflammation and which are performing phagocytosis of damaging abnormal proteins

Since the initial discoveries of activated microglia in AD and aging brains, the concepts and knowledge of what microglia are doing or could potentially be doing has progressed. The central role of microglia in brain, or macrophages in general, is to phagocytose and digest cellular waste products, which should include the extra-cellular Aβ that is deposited in AD and aging brains. An important question that is still unanswered today is "why are microglia not doing a better job of removing Aβ plaques?" Some concepts of microglial function in relation to AD came from transgenic mouse models using animals engineered to develop Aβ plaques in a manner similar to humans. These studies produced some conflicting results, but in general it was shown that microglia could be manipulated to achieve greater removal of plaque material, but in these mice, as in humans, microglial removal of plaque material is not efficient without some stimuli [26]. Again, we can ask does this apply to all microglia? Certain cytokine treatments affect microglia leading to reduced phagocytosis such that plaque material accumulates to a greater extent [27–29]. These studies illustrated how microglia can be

activated to be more or less efficient at Aβ removal [30, 31]. This was particularly shown in Aβ-peptide immunized mice, which had produced a specific antibody response to plaque material. The coating of plaques with anti-Aβ immunoglobulin appeared to promote phagocytosis through engagement of the microglial IgG Fc receptors. Overall, these studies showed that microglia of a particular phenotype have the potential to remove Aβ; similar observations have come from human pathological studies in certain subjects who had received the Aβ vaccine [14].

3. Schemes for defining microglial function: limitations of M1 and M2 activation state definitions

Phenotyping of macrophages and microglia progressed with the pioneering work of Gordon and colleagues who sought to develop schemes for classification of macrophages, first in mice and then in humans, by assigning activation states to the expression of different antigenic markers based on responses to defined activation stimuli [32, 33]. Much of this work employed gene expression profiling mRNA analysis since these techniques have fewer of the limitations associated with antibodies that will be discussed. These phenotyping schemes were also applied to microglia, both rodent and human. The scheme defined classical activation or M1 activation, as being the state of macrophages/microglia that have been stimulated with strong inflammatory agents such as lipopolysaccharide (LPS) and interferon gamma (IFN-γ). Such activated cells will be expressing increased levels of cytokines and enzymes such as TNF-α, IL-1β, IL6, matrix metalloproteinase (MMP)-3 and MMP-9. The corollary to this is alternative activation or M2, which defines the markers and products of cells responding to anti-inflammatory cytokines such as IL-4 or IL-13. These cells have a reparative/neurotrophic phenotype and can produce growth factors. Such reparative M2 microglia also show increased phagocytosis. The M2 scheme was further subdivided into M2a (responses to IL4 or IL13), M2b (responses to immune complexes in combination with IL-1β or LPS) and M2c (responses to anti-inflammatory IL-10, TGFβ or glucocorticoids). It was shown that increased expression of the scavenger receptor CD163, a marker for M2c was upregulated in microglia in AD and Parkinson disease dementia cases. This is the first study showing a type of alternative activation in AD tissues by immunohistochemistry [34].

Many studies have tried to apply these schemes to tissue microglia but their validity has been contested [35]. The schemes are dependent on using defined stimuli, while in the degenerating AD brain, there will be many different stimuli (Aβ and tau in different conformations, reactive oxygen, cytokines, bioactive lipids, ATP/ADP, DNA, etc.) that will account for the heterogeneity of microglia responses in tissue. In recent years, there has been criticism that the M1 and M2 scheme is not applicable for tissue microglia as such defined microglia do not seem to exist in brain [35]. This may be correct as the microenvironment around every plaque and every neuron will be different, but to attempt to profile microglia does require some form of scheme, even an imperfect one, to relate to function. It will also be proposed that the limitations of the M1 and M2 classification schemes could be due to technical reasons as much as biological reasons.

4. Practical issues involved in microglial phenotyping in human autopsy brains

Success in classifying microglia in postmortem human autopsy tissue sections is primarily dependent on the antibodies being used for this purpose, but also the manner in which the brain tissue being studied was preserved. Many published studies of microglial markers for immunohistochemistry have been restricted to antibodies that produce strong immunoreactivity on extensively fixed tissue sections. This is particularly true for HLA-DR, which is the most widely used for human microglial studies, as available antibodies can produce vivid results on a wide range of preserved brain tissue. The following references are the first for HLA-DR and the most recent, spanning 30 years of studies [36, 37]. The function of HLA-DR in AD microglia is still unclear. This protein functions to present processed antigens to T lymphocytes that are not present in the AD microenvironment. The signaling that leads to upregulation of HLA-DR in AD microglia has not been defined. In recent years, the marker IBA-1, which recognizes an actin-binding protein involved in cytoskeletal reorganization and cell motility, has also been extensively used to identify microglia because of the availability of robust staining antibodies [38]. IBA-1 antibodies seem to recognize all microglia with limited upregulation in activated microglia, though this interpretation is also dependent on observations related to microglial morphology. The use of antibodies that produce strong results in tissue sections may have biased our understanding of microglial function in disease as many other antigenic markers are present, but suitable antibodies to reveal them in tissue are not available. Most useful markers of function are cell-surface glycosylated proteins whose antigenicity become significantly affected by fixation conditions and also by the degree of glycosylation. The most widely available tissues for many researchers are tissue blocks that have been formalin-fixed for extended periods and then embedded in paraffin (FFPE); this process includes treatments with xylene. These preservation methods strongly affect the ability of antibodies to recognize many antigens, but in particular cell-surface glycoproteins. The numbers of antibodies that are effective at antigen recognition on FFPE tissue are a small percentage of available antibodies. In addition, the use of FFPE tissue usually requires the application of antigen retrieval techniques for most antibodies to work; there are a range of these methods but their successful application is dependent on operator skill and can lack reproducibility. As mentioned, the applicability of M1 and M2-like schemes to classify microglia in human brain samples has been criticized as many of the classification antigens have not been proven in tissue microglia [35], however such schemes may have been prematurely discarded due to the lack of panels of antibodies functional on available brain tissue samples.

4.1. Previous studies of microglial functional proteins in AD

Since the initial studies of increased HLA-DR expression by microglia in AD brains, in areas associated with pathology [9, 19, 36, 39–42], expression of a range of macrophage markers have been applied to AD brain tissues. These include beta II integrins (CD11a, b, and c and CD18—complement and phagocytic receptors), immunoglobulin Fc receptors (CD16, CD32, CD64) [11], lipopolysaccharide receptor CD14 [43], macrophage colony stimulating factor receptor-1 (CSF-1R; CD115) [44], type B scavenger receptor CD36 [45, 46], ferritin [47], signal regulatory

protein beta-1 (SIRPβ-1) [48] and progranulin [49]. The markers CD43 and TMEM106B were shown to be downregulated in AD microglia compared to controls [50, 51]. This represents an incomplete list due to space limitations but many of these markers are related to phagocytic function rather than cytotoxicity. Ferritin has unique properties in relation to microglial activation as it is a ubiquitous iron transport protein but in tissue seems to selectively identify a population of activated microglia [12, 13]. To directly demonstrate potential cytotoxicity, the demonstration of increased levels of cytokines in microglia is needed. Over the last 30 years, there have been few studies using immunohistochemistry to profile cytokines in tissue sections. A series of studies by Griffin and colleagues showed IL-1α-expressing microglia were associated with different types of plaques and tangles. Diffuse neuritic and non-neuritic plaques had the most IL-1α positive microglia, while dense core neuritic and non-neuritic plaques had significantly few IL-1α positive microglia. These results suggest that this population of microglia were involved at early stages of plaque formation [52]. Use of this marker demonstrated that IL-1α positive microglia were involved in the generation of neurofibrillary tangles in the parahippocampal gyrus [53]. In another study, it was shown that IL-1β and TNF-α could be localized to microglia in human AD tissue [42]. The limited numbers of studies do highlight the technical difficulties of detecting secreted proteins such as cytokines. Griffin and colleagues employed FFPE tissue for immunohistochemistry. We have attempted a number of times using our short-fixed microtome cut sections to localize cytokines to tissue and have never been successful. As these molecules are secreted rather than membrane localized, it is possible the hard fixation involved in FFPE is needed to anchor them, and then antigen retrieval to allow antibody access. With short fixed brain tissues materials, these soluble proteins might not be adequately fixed *in situ* for localization.

4.2. Selecting antibodies for microglial phenotyping

The whole field of human brain immunohistochemistry has several limitations when it comes to selection of suitable antibodies needed to reveal location of proteins of interest. Firstly, the antibody, usually a monoclonal antibody of mouse or rabbit origin, thus specific to an epitope representing a small portion of the target protein, has to be able to show specificity—namely it is actually recognizing the target protein *in situ* and not cross reacting with other proteins. Secondly, the antibody, if it can be validated to recognize the target protein in tissue, its specificity and sensitivity can be affected by the fixation conditions. In our experience, the study of microglial antigens with a wider range of antibodies has been less problematic using brain tissue fixed for a short period (48 h) in paraformaldehyde (not formalin) and then cryoprotected and sectioned using a freezing microtome. This process avoids the harsh treatments involved in paraffin embedding of tissue. Over the years, we have successfully identified microglial proteins CD87 [54], RAGE, CD33 [55], TREM-2 [56], TLR-2, -3, -4 along with HLA-DR, IBA-1, CD68 in AD tissues.

Our experience with antibodies when using these tissues identified some features that help increase the chances of successful immunolocalization. One company—R & D Systems—Biotechne, Minneapolis, MN—have produced many of their antibodies using a relatively unique strategy for the industry. Many of their antibodies were prepared from proteins of interest expressed in eukaryotic cells. These proteins will be glycosylated in a similar manner

to what might be expected in human tissues. In addition, the immunizing proteins usually cover the majority of the native protein, and thus preserve some of the secondary protein structure that affects antigenicity, along with containing multiple antigenic epitopes. These proteins produce antibodies with immunogenicity superior to the strategy of many companies that use short synthetic peptide sequences of 10–20 amino acids as immunogens, and then conjugated to a carrier prior to animal immunization. Our experience with R & D Systems affinity purified polyclonal antibodies has generally been favorable for use on lightly-fixed tissues. These antibodies will contain a range of epitopes that can increase the likelihood of identifying epitopes on proteins not severely affected by fixation. The use of large protein antigens to prepare polyclonal does have some drawbacks as there is the potential for cross reactivity with other related proteins. Quality control is dependent on being able to carry out protein absorption of antibody to show removal of tissue reactivity, along with western blot detection of specific protein bands.

Figure 2 (panels A and B) illustrates our experience using an R & D Systems antibody to Toll-like receptor (TLR)-3 (AF1487) to identify microglia in AD brains, and an R & D Systems polyclonal antibody to CD206 (AF2535), which failed to identify microglia (panels D and E). The TLR-3 polyclonal antibody could identify structures in human brain microglia (**Figure 2**, panels A and B). One comment is that if western blots are carried out using complex material such as brain material, the presence of other protein bands, besides the full length protein should be anticipated (**Figure 2**, panel C). Most functional proteins are either cleaved during their normal function, for example loss of leader sequences, cleaved to produce secreted forms, or broken down as part of cellular metabolism. Interestingly, a monoclonal antibody to TLR-3 produced with the same immunizing protein could not stain microglia in tissue, but this antibody will be specific for only a single epitope present in the immunizing protein. We have had similar experience with an R &D systems antibody to CD206 (**Figure 2**, panels D and E), also known as macrophage mannose receptor, produced against a eukaryotic cell expressed protein. This protein has been defined as a prototypical marker for M2a alternative activation as its expression is induced in the presence of IL-4. We used this antibody to determine if there was evidence for alternative activation microglia in human brains. Using this antibody, which on western blots could detect specific bands on brain samples, did not identify microglia in any of the control or AD tissue sections we stained. Noticeable however was the strong CD206 staining of round cells (perivascular macrophages) located within or around the vessels present in the brain sections. This is similar to a published finding [34]. This seems to indicate alternative activated macrophages are common in vessels, while alternative activated microglia are not present in neuropil. In human brains, identifying expression of inflammatory associated molecules at the RNA or protein level using brain homogenates need to be interpreted with caution as significant numbers of blood cells can be trapped within the tissue [57]. Confirmation of findings by immunohistochemistry is needed when making observations relevant to microglia. The absence of alternative activated markers in AD brain samples was confirmed for the CD200 receptor (CD200R). This is a myeloid specific receptor that is activated by the ligand CD200 to induce anti-inflammatory signaling. We showed that it was induced by IL-4 and IL-13 and fit the classical definition of an M2a marker, similar to CD206. Using several antibodies, including R&D Systems polyclonal antibody (AF3414) and a custom peptide antibody, we could not localize CD200R

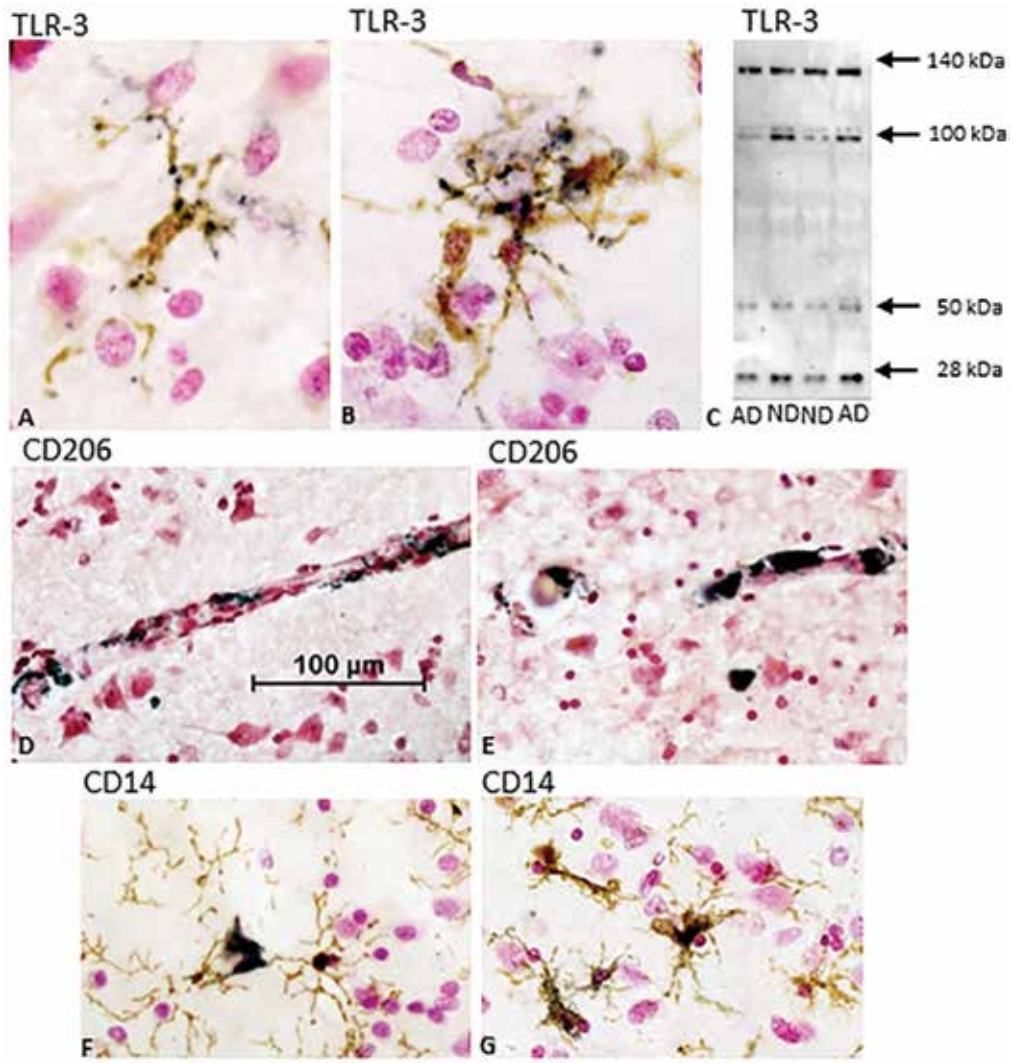

Figure 2. Approaches to microglial phenotyping in Alzheimer's disease brains. (A) and (B) Immunohistochemistry for a new marker for microglia (toll-like receptor-3: TLR-3 in human brains. Double immunostaining for TLR-3 (purple) colocalizing with IBA-1 microglia in (A) non-demented control middle temporal gyrus and (B) Alzheimer's disease case. See text for further explanation. These findings were obtained using R&D Systems antibody (AF1868). (C) Western blot of human brain samples for TLR-3. This panel illustrates that protein bands other than full length peptides can be present in biological samples. (D) and (E) Absence of alternatively activated microglia expressing CD206 in ND (D) or AD (E) temporal cortex brain sections but positive expression in perivascular/vascular macrophages. (F) and (G) The proinflammatory marker CD14 does show increased expression by microglia in AD cases (purple) colocalizing with IBA-1 immunoreactivity brain Strong positive staining is present in perivascular/vascular macrophages (purple) is also a feature.

immunoreactivity to brain microglia even though protein and mRNA expression of CD200R are detectable in human brains [58].

One marker that seems to have been overlooked in microglial profiling in tissue is CD14, the LPS co- receptor. This receptor is a classical M1-like activation marker with upregulation

associated with proinflammatory activation. There has only been a single study describing microglial immunohistochemistry in human AD brains for CD14 [43]. Using short fixation tissue, we reexamined expression of this marker (**Figure 2**, panels F and G). It is strongly expressed by most vascular macrophages of all cases (**Figure 2**, panel F—ND case), but increased expression was readily detectable in subsets of IBA-1 microglia in AD cases (**Figure 2**, panel G—purple). As CD14 can bind Aβ with proinflammatory activation through interaction with TLR-2 or TLR-4, increased CD14 expression could be a more used marker for defining cytotoxic microglia.

4.3. Profiling TREM-2 microglia in human brains

This discussion is of particular relevance for considering microglial phenotyping of TREM-2 positive microglia. Considerable interest in the role of TREM-2 in AD has spurred new interest in neuroinflammation and AD. A single nucleotide polymorphism (SNP) in the TREM-2 gene (rs75932628) that results in a mutation in the TREM-2 protein (R47H) can increase the risk of developing AD by 2- to 11-fold depending on the population studied [59, 60]. Mutations in TREM-2 or its adaptor protein DAP12 were first identified in Nasu-Hakola disease, which leads to early onset dementia amongst other symptoms [61]. The mutation appears to lead to loss of function of the TREM-2 protein, whose normal function is to promote phagocytosis of apoptotic neurons through binding to heat shock protein 70 (hsp70) or different conformations of lipids. There have been few studies of immunohistochemistry of TREM-2 in human AD brains, which appears mainly due to lack of robust antibodies for pathological work. We published one of the first studies that showed plaque- and tangle-associated microglia were positive for TREM-2 [56]. In this study, we had to screen a number of antibodies for specificity and sensitivity in human brain tissue. The best results were obtained with an R&D Systems polyclonal antibody to TREM-2 (AF1828) prepared using a eukaryotic cell expressed protein corresponding to 75% of the protein and to the complete extracellular domain. A recent study of TREM-2 expression in AD frontal cortex using an antibody prepared using a peptide corresponding to N-terminal amino acids 29–59 of human TREM2 (ab175262, Abcam, Cambridge, MA) showed specificity by western blots, but these authors presented no data on TREM2 immunohistochemistry [62]. TREM-2 expression is restricted to dendritic/myeloid cells and is high in brain microglia. Specificity of commercial antibodies has been an issue, but also the sensitivity of detection. Two studies have concluded that TREM-2 was not expressed by microglia in brain, but both studies employed FFPE tissue samples with antigen retrieval [63, 64]. One study showed that the R&D antibody was specific for TREM-2, similar to our published work, but they could not demonstrate microglial TREM-2 immunoreactivity [63]. Similar to our previous studies, we employed lightly fixed brain tissues that were not paraffin-embedded [56]. With these sections, we could demonstrate specific TREM2 localization to microglia [56]. Our finding is reasonable as TREM-2 has been localized to plaque-associated microglia in AD model transgenic mice [65].

5. Does expression of antigen correlate with identifiable function

How does antigen expression relate to demonstrated microglial function? With the exception of HLA-DR and IBA-1, most studies of microglia in human brains have not been adequately

replicated. Immunohistochemistry is not a quantitative technique due to the amplification technologies used along with the non-availability of standards for comparison, but the studies by Boche and colleagues using semi-quantitative measures deserve mention [10, 14, 66, 67]. Using a large series of brain samples and the expression of different markers, including CD64, MSR-A, CD68, HLA-DR and IBA-1, it was shown that microglia could be subtyped depending on their profile. These studies set the standards for microglial profiling in human brains. These studies employed two measures for analysis; the area of immunoreactivity and the numbers of positive cells. These studies attempted to assign phagocytic function or activation function to the microglia in relation to the type of pathology. One interesting observation was the lack of significant correlation between expression levels of these different antigens by microglia. These markers are related to different functions, with CD64, MSR-1, and C68 being related to phagocytosis, HLA-DR with antigen presentation and IBA-1 with microglial motility. Studies of TREM-2 and CD33 in AD brains demonstrated upregulated expression in AD brains, but both receptors induce inhibitory signaling when activated [55, 56]. There is evidence that upregulation of such activated microglial proteins encountered in disease tissue could be to have an inhibitory effect on inflammation, not amplifying inflammatory responses.

5.1. Where are the non-activated microglia in AD or aging brains?

In recent years, gene discovery methodology (RNA sequencing, microarrays, single cell sorting) has been applied to studies of microglia. One particularly interesting marker identified is the purinergic ADP/ATP receptor P2RY12. This was shown to be highly expressed by microglia (human and rodent) compared to macrophages. In addition, it appears to be a marker of non-activated microglia as expression becomes rapidly downregulated upon inflammatory activation with LPS [68]. P2RY12 has been defined as a M2 marker as it is unregulated in vitro in human microglia by treatment with IL-4 [69]. A common concept over the years about neuroinflammation and AD is that widespread proinflammatory activation is a significant and extensive feature. The expression that the brain is on fire has been used more than once in review articles of AD inflammation. If inflammation is widespread in pathological affected tissues, one would expect that P2RY12 expression would be very low or absent in AD brains. Our preliminary findings show that this is not the situation; there were many P2RY12 microglia in regions with severe AD pathology. Although western blot and mRNA studies showed no difference in expression of this gene between AD and control samples, however by immunohistochemistry, there was a very specific distribution of P2RY12 positive and negative microglia in brains in relation to pathological structures.

6. Future directions

The potential benefits for complete definition of microglial phenotypes by immunohistochemistry in human brains could be significant. The development of effective inflammation-focused therapies for AD requires the identification of therapeutic targets that are relevant

to the human disease, not to models of disease in a transgenic animal or cell culture. AD is a uniquely human disease of the elderly, with pathology having developed for years before dementia becomes observable. In transgenic models, disease pathology can develop over weeks. There have been many agents that can reverse plaque development and inflammation in AD mice models that have failed to be effective in humans. There are many challenges involved in human focused studies, but the benefits of having human disease targets validated in human tissue could involve significant saving in time and resources from pursuing the wrong approaches. Immunohistochemistry is not considered a state of art technique in the twenty-first century as the technologies have not much changed in 30 years, but ultimately it is required to show that gene discovery findings are valid. The need for large numbers of high quality human tissue samples has been one limitation, but this can be addressed by collaborative studies. Brain tissue that has been consistently prepared with appropriate clinical and pathological records allow studies involving progressive changes in pathology from negligible to severe rather than the less useful classification of control or AD. There is also a need for improved expectations on the performance of antibodies. The performance and reproducibility of antibodies in all biological experiments has been a growing concern [70], but ultimately it is the responsibility of the experimenter/pathologist to determine the suitability of antibodies used to make a unique observation. The field of neuroinflammation in AD has been reinvigorated with discoveries about TREM-2 having a direct link to AD risk. To be able to reliably identify TREM-2 positive microglia in brain is needed to fully understand its role in sporadic AD, and validate the large numbers of model studies that have proposed therapeutic strategies for AD focused on TREM-2.

7. Conclusions

Microglia represent approximately 10% of the total cell population in human brain, but it is now appreciated how complex their responses are to pathological stimuli and for maintaining healthy neurons. Treating pathological inflammation in AD with broad spectrum agents (e.g., cyclooxygenase inhibitors) may do more harm than good. If the microglial responses to pathology are highly dependent on the microenvironment; for example one microglia may be producing excess TNF-α while an adjacent one is attempting to remove the pathological stimuli, then treatments need to be targeted appropriately. This will only be possible if the microglia actively involved in AD can be adequately profiled.

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Chapter 4

Mitochondrial Link Between Metabolic Syndrome and Pre-Alzheimer's Disease

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Additional information is available at the end of the chapter

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Abstract

There is much evidence to demonstrate that the presence of the metabolic syndrome (MetS) is associated with an increase in the incidence of pre-Alzheimer's disease. The possible underlying mechanisms linking pre-Alzheimer's disease and MetS are still unclear. This study summarizes and discusses the potential mechanisms involved in pre-Alzheimer's disease under MetS conditions, including an increased brain oxidative stress, brain inflammation, brain mitochondrial dysfunction, hyper-phosphorylated tau protein, and amyloid beta production. This report focuses on brain mitochondrial alterations in cases of pre-Alzheimer's disease where MetS is also extant. The data from *in vitro*, *in vivo,* and clinical studies are included. In addition, potential interventions against pre-Alzheimer's disease in conjunction with MetS are summarized and discussed.

Keywords: mitochondria, brain, cognitive impairment, obesity, oxidative stress, inflammation

1. Introduction

1.1. Metabolic syndrome, pre-Alzheimer's disease, and brain mitochondria

According to the consensus statement of the International Diabetes Federation, metabolic syndrome (MetS) is defined as abdominal obesity plus any two of four factors including raised triglycerides, reduced high-density lipoprotein (HDL) cholesterol, raised blood pressure, and elevated fasting plasma glucose [1]. The risk factors of MetS include genetic factors, physical inactivity, and too high a calorie intake or poor diet [2, 3]. It has been postulated that insulin

resistance is the main contributor toward MetS. Insulin resistance is a pathological condition, in which target tissues cannot take up glucose into the cells at the physiological insulin level. It is characterized by hyperinsulinemia with euglycemia. MetS is often represented by an obese-insulin-resistant condition. It can lead to the development of not only cardiovascular diseases but also stroke [4] and neurodegeneration [5]. In addition, data from clinical trials have indicated that hyperinsulinemia during insulin resistance is related to cognitive decline in elderly adults [6, 7]. MetS has been induced in several animal models to enable the investigation of the mechanisms responsible for the adverse effects of the MetS condition on cognitive impairment. MetS has been induced in animal models by using high-fat/high-calorie diet consumption. Interestingly, previous studies have investigated the effects of long-term high-fat diet (HFD) consumption on metabolic and brain dysfunction [8, 9]. Those data demonstrated that the consumption of a HFD for 8 weeks caused obese-insulin resistance or MetS, as indicated by central obesity, hyperinsulinemia, dyslipidemia, and raised blood pressure [8, 9]; however, cognitive impairment and brain insulin resistance were observed later at the end of 12 weeks of HFD consumption [8, 10]. Those findings suggest that the metabolic disturbance preceded cognitive dysfunction in induced MetS

Pre-Alzheimer's disease or mild cognitive impairment (MCI) is a condition of memory decline but does not significantly affect the normal function of a person's life [11]; however, Alzheimer's disease (AD) is an irreversible chronic neurodegenerative disease and it is the most common type of dementia [12]. The presence of neurofibrillary tangles and amyloid beta deposition in the brain is hallmarks of AD [12]. Recent studies have shown that the incidence of AD has increased in MetS subjects [13–15]. Those findings suggest that there is a possible connection in the pathogenesis between MetS and AD. Data from a clinical study suggest that oxidative stress is a key component that regulates the development of AD in MetS subjects [15]

Mitochondria are known as the major source of oxidative stress [16]. Brain mitochondrial dysfunction was observed in several pathological conditions, including MetS and AD [17–22]. That dysfunction causes increased oxidative stress [10] and leads to the activation of several stress kinases [19]. Subsequently, a raised oxidative stress impaired brain insulin receptor function [23], inhibited insulin-degrading enzymes and increased beta-secretase activity [23, 24], resulting in increased hyperphosphorylated tau and amyloid beta deposition in the brain [19]. Therefore, brain mitochondrial dysfunction could be an important feature in AD pathogenesis in the MetS condition. Furthermore, the elevation of oxidative stress caused the imbalance of brain mitochondrial dynamics [25]. Mitochondrial dynamics are a key process for the maintenance of cell life and death through the balancing of mitochondrial fission and fusion [26]. In the physiological status of the brain, mitochondrial dynamics enables mitochondria to recruit subcellular components, exchange substrates between mitochondria, and control mitochondrial shape [26]. Recently, it has been proposed that brain mitochondrial dynamic imbalance is another mechanism that is involved in the brain pathogenesis of MetS and AD [27, 28]. Examples from the recent research are as follows: (1) several studies have reported that levels of Dynamin-related protein 1 (Drp1) and mitochondrial fission 1 (Fis1), markers of mitochondrial fission, were increased in the brains of MetS and AD animals [29, 30], leading to neuronal apoptosis [29]; (2) mitochondrial fusion protein levels were decreased in the brains of both MetS and AD animals [29, 30]. Therefore, a mitochondrial dynamic imbalance may play an important role in cognitive dysfunction in MetS and AD [26, 30]

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2. The implications of metabolic syndrome on brain mitochondria and its association with the development of AD: *in vivo* studies and clinical studies

2.1. MetS condition from a high-fat diet-induced obese-insulin-resistant model

Obese-insulin resistance is characterized by body weight gain and peripheral insulin insensitivity [20–22, 31–34]. These characteristics are similar to those seen in the MetS condition in humans. In addition to peripheral insulin resistance, brain insulin resistance has also been reported in the obese condition in rats [20–22, 31, 34]. A diet containing 60% E from fat is considered to be a high-fat diet (HFD), and it has been widely used to induce obese-insulin resistance in rodents [20–22, 31–33]. In some studies, it has been found that HFD consumption increased plasma cholesterol and free fatty acid levels [20–22, 31, 32, 34]. However, the plasma glucose level was not increased, but hyperinsulinemia was observed following HFD consumption even after long-term consumption of a HFD (12 months), indicating a pre-diabetic state [20, 31–34].

HFD consumption between 16 weeks and 12 months caused brain mitochondrial damage, including an increased mitochondrial ROS production [20–22, 31, 34], a reduced mitochondrial membrane potential [19, 31, 34–36], and an impaired mitochondrial morphology as indicated by an increased mitochondrial swelling [20–22, 31, 33]. Furthermore, HFD reduced adenosine triphosphate (ATP) production [34]. Although several studies suggested that HFD caused brain mitochondrial dysfunction, Jorgensen et al. reported that HFD did not impair brain mitochondrial function even when the rats were given a HFD for 12 months. Therefore, the effects of a HFD on brain mitochondrial function still need to be elucidated.

There are several studies which have shown that brain mitochondrial damage could impair cognitive function and synaptic plasticity [20–22, 31, 33, 34]. Various cognitive tests have been used such as the Morris water maze (MWM), novel object recognition (NOR), novel object smelling (NOS), and Y-maze test. The MWM and Y-maze are tests for hippocampal-dependent learning process, including the acquisition of spatial memory and long-term spatial memory [36]. NOR and NOS are used to assess non-force driving and spontaneous memory [35, 37].

Rats and mice fed on a HFD for 16–20 weeks had an increased time to reach the platform and a decreased time in the target quadrant and crossing target number, compared with normal diet (ND)-fed animals, when cognitive function was assessed using the MWM [20–22, 31, 34]. Furthermore, recognition index was decreased in HFD-fed mice, compared to ND-fed mice [34]. Mice fed on a HFD for 12 months did not indicate an impaired discrimination index following the NOS test, but there were decreased percentage correction alterations in the Y-maze test [33]. These accumulative data suggested that the consumption of a HFD caused obese-insulin resistance, brain mitochondrial dysfunction, and synaptic dysplasticity, possibly leading to cognitive dysfunction. However, no study has demonstrated brain mitochondrial dysfunction with elevated AD markers such as Aβ levels and hyperphosphorylated tau in HFD-fed animals. This suggests that obese-insulin resistance can lead to the development of brain mitochondrial dysfunction and cognitive impairment or MCI or pre-AD without AD symptoms. Data regarding the effects of HFD-induced obese-insulin resistance on brain mitochondria and its association with the development of AD are shown in **Table 1** and are summarized in **Figure 1**.

Study model	Major findings					Refs
Animal/diet/duration	**Metabolic parameters**	**Mitochondrial parameters**	**Cognitive function**	**AD marker**	**Interpretation**	
Wistar rats/HFD (60% E fat) or ND (20% E fat)/16 weeks	• ↑ BW, insulin, HOMA, cholesterol • ↔Glucose • ↓Peripheral insulin sensitivity • ↓Brain insulin signaling	• ↑ ROS • ↓ MMP ○ ↑Swelling	*MWM* • ↑Time to reach platform • ↓Time in target quadrant *Synaptic plasticity* • ↓LTP	N/A	HFD-induced obese-insulin resistance leads to synaptic dysplasticity and brain mitochondrial dysfunction and finally results in cognitive decline.	[20–22, 31]
C57BL/6 mice/HFD or ND/20 weeks	• ↑BW, insulin, HOMA, FA, cholesterol • ↓Peripheral insulin sensitivity • ↓Brain insulin signaling	• ↑ROS • ↓MMP • ↓ATP	*NOR* • ↓Recognition index *MWM* • ↓Time in target quadrant • ↓Crossing target number	N/A	HFD-induced obese-insulin resistance leads to brain mitochondrial dysfunction and cognitive dysfunction.	[34]
C57BL/6 mice/HFD (60% E fat) or ND (12% E fat)/12 months	• ↑ BW, insulin • ↔Glucose	• ↑Swelling (elongated mitochondria)	*NOS* • ↔Discrimination index *Y-maze test* • ↓% Correct alterations *Synaptic plasticity* • ↓Synaptic density	N/A	HFD-induced obese-insulin resistance leads to synaptic dysplasticity, brain mitochondrial dysfunction and cognitive dysfunction.	[47]
Wistar rats/HFD (60% E fat) or ND (13% E fat)/12 months	• ↑BW, insulin, HOMA, FA • ↔Glucose	• ↔ State 3, 4 respiration • ↔ RCR	N/A	N/A	HFD-induced obese-insulin resistance does not impair brain mitochondrial function.	[32]

Abbreviations: BW, body weight; HOMA, homeostasis model assessment; HFD, high-fat diet; ND, normal diet; ROS, reactive oxygen species; MMP, mitochondrial membrane potential; RCR, respiratory control ratio; MWM, Morris water maze; LTP, long-term potentiation; NOR, novel object recognition; NOS, novel smell recognition; N/A, not assessed.

Table 1. Implications of obese-insulin resistance on brain mitochondria and its association with the development of Alzheimer's disease.

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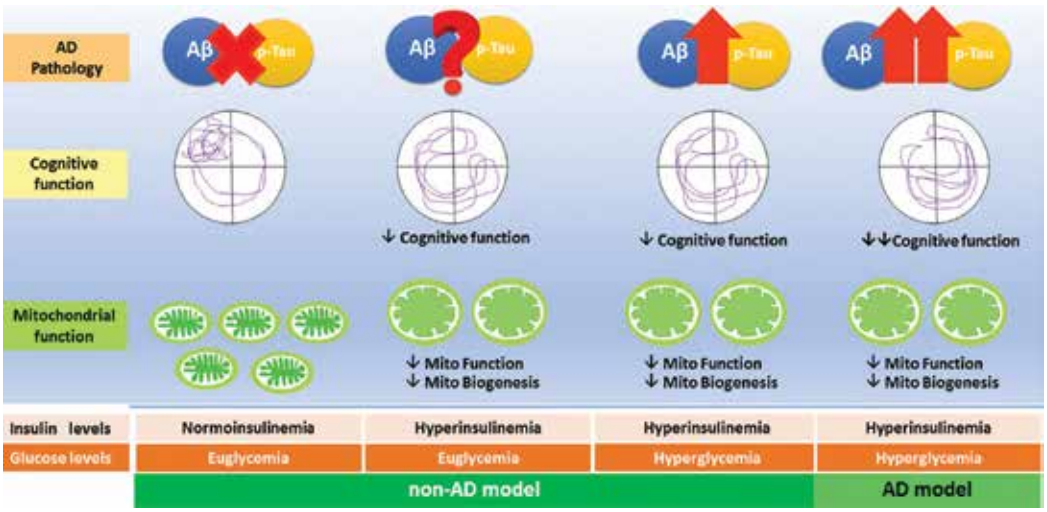

Figure 1. The effects of insulin resistance and T2DM on brain mitochondria and their association with the development of Alzheimer's disease in non-AD and AD models.

2.2. Type 2 diabetes mellitus model

Type 2 diabetes mellitus (T2DM) is diagnosed when hyperglycemia is observed along with insulin resistance [38–41]. In order to create a T2DM animal model, a combination of a HFD with low-dose streptozotocin, and a high-calorie diet were used [38–41]. Both regimens caused hyperglycemia in rodents [38–41]. Similar to obese-insulin-resistant models, T2DM animals are also found to develop brain mitochondrial damage [38–41].

Beside the effects of T2DM on oxidative stress and mitochondrial membrane depolarization, nuclear respiratory factor 2 (NRF2) levels were reduced in the brains of T2DM mice [38]. NRF2 acts as an antioxidant and detoxifying enzyme and helps to reduce oxidative stress in mitochondria [42]. Therefore, a decreased level of NRF2 directly impairs the brain mitochondrial redox system, which leads to the reduction of brain mitochondrial antioxidant capacity. In addition, a previous study showed a decrease in brain mitochondrial numbers in T2DM mice [41]. The possible explanation may be due to a decrease in NRF2 in the brain of T2DM mice, in which NRF2 regulates brain mitochondrial biogenesis [43]. These data indicate that T2DM caused brain mitochondrial damage and brain mitochondrial dysfunction, resulting in an increased brain oxidative stress.

Consistent with the findings from obese-insulin-resistant animals, T2DM animals also developed brain mitochondrial dysfunction with cognitive impairment, quantified using the MWM test, as indicated by an increased escape latency and time in target quadrant and a decreased crossing target number [39, 41]. Also, T2DM rats had decreased percentage correction alterations and total distance, when the abilities of these animals were investigated using the Y-maze test [39]. T2DM also affected brain synaptic plasticity proteins, as indicated by reducing postsynaptic density protein 95 (PSD95) and synaptosomal-associated protein 25 (SNAP25) levels [38]. However, T2DM did not affect synaptophysin protein levels [38].

In the T2DM model, brain mitochondrial markers were evaluated along with the changes in AD markers. It is interesting that T2DM rats developed AD signs, specifically that the levels of AD markers, including Aβ42 and hyperphosphorylated tau, were significantly increased in T2DM rats, when compared with non-T2DM rats [39, 41]. In addition, acetylcholine esterase enzyme activity was increased, and ACh levels were decreased in the brains of T2DM mice [39]. These data suggested that T2DM rats had impaired brain mitochondrial dysfunction and synaptic plasticity, leading to cognitive dysfunction and showed increased AD markers. Interestingly, those findings indicated that AD was developing in the T2DM condition. Contrary to the findings from animal studies, when Loo et al. investigated the effect of T2DM on mitochondrial function in human mononuclear cells, their data showed that T2DM did

Study model	**Major findings**					**Refs**
Animal/diet/ duration	**Metabolic parameters**	**Mitochondrial parameters**	**Cognitive function**	**AD marker**	**Interpretation**	
SD rats/HFD (60% E fat) + STZ (30 mg/kg, i.p.) or ND + citrate buffer/11 weeks	• ↑BW, insulin, glucose • ↓Peripheral insulin sensitivity • ↓Brain insulin signaling	• ↓Mito number • ↑ROS • ↓MMP	*MWM* • ↑Escape latency • ↓Crossing target number	• ↑Aβ42	T2DM causes brain mitochondrial dysfunction, increases levels of AD markers, and cognitive dysfunction.	[41]
C57BL/6 mice/ HFD (60% E fat) or ND (10% E fat)/10 weeks	• ↑Glucose	• ↑ROS • ↓MMP • ↓ATP	*MWM* • ↑Escape latency • ↓Time in target quadrant *Y-maze test* • ↓% Correct alterations • ↓Total distance	• ↑pTau/ Tau • ↑AChE activity • ↓ACh	T2DM causes brain mitochondrial dysfunction, increases levels of AD marker, and cognitive dysfunction.	[39]
Wild-type mice/ sucrose(20%) solution or control (water)/7 months	• ↑BW, insulin, HbA1c	• ↓RCR • ↓MMP • ↓ATP • ↓NRF2	*Synaptic plasticity* • ↓PSD95 *SNAP25* • ↔Synaptophysin	N/A	T2DM causes mitochondrial dysfunction and impairs synaptic plasticity.	[38]
T2DM patients compared to healthy controls	N/A	• ↔ROS • ↔MMP • ↔ATP	N/A	N/A	T2DM is not associated with mitochondrial dysfunction.	[40]

Abbreviations: BW, body weight; STZ, streptozotocin; T2DM, type 2 diabetes mellitus; HFD, high-fat diet; ND, normal diet; ROS, reactive oxygen species; MMP, mitochondrial membrane potential; Ach, acetylcholine; AChE, acetylcholine esterase; ATP, adenosine triphosphate; RCR, respiratory control ratio; NRF, nuclear respiratory factor; MWM, Morris water maze; N/A, not assessed.

Table 2. Implications of type 2 diabetes mellitus (T2DM) on brain mitochondria and its association with the development of Alzheimer's disease.

not affect mitochondrial function [40]. Their findings showed that T2DM affected regional mitochondria, but not systemic mitochondria. Data regarding the effects of T2DM on brain mitochondria and its association with the development of AD are shown in **Table 2** and are summarized in **Figure 1**.

3. The implications of high-calorie diet consumption on brain mitochondrial function and brain function in an AD model: *in vivo* studies

Two AD animal models, including 3xTg AD mice and APPswe/PS1dE9 mice, have been used to investigate the implications of high-calorie diet consumption on brain mitochondrial function

Study model	**Major findings**					**Refs**
Animal/diet/ duration	**Metabolic parameters**	**Mitochondrial parameters**	**Cognitive function**	**AD marker**	**Interpretation**	
3xTgAD mice/HFD (60% E fat) or ND (12% E fat)/12 months	- ↑ BW - ↔ Glucose, insulin	• ↔Mito number • ↔Mito morphology	*NOR* • ↔Discrimination index *Y-maze test* • ↔ % Correct alterations *Synaptic plasticity* • ↔ Synaptic number	• ↔Aβ42	Obesity did not alter brain mitochondria and AD markers AD model.	[33]
APPswe/PS1 dE9 mice/HFD (45% E fat) or ND/12 weeks	• ↑BW • ↔Brain insulin signaling	• ↓PGC1α • ↔NRF1,2 • TFAM	N/A	• ↑APP • ↓ADAM10 • ↓IDE • ↔BACE1 • ↑Cortical soluble Aβ40, Aβ42 insoluble Aβ42	Obesity increased AD markers, but did not alter brain mitochondrial biogenesis in AD model.	[17]
APPswe/PS1 dE9 mice/HFD (45% E fat) or ND/24 weeks	• ↑BW, insulin, glucose • ↓Peripheral insulin sensitivity • ↓Brain insulin signaling	• ↓PGC-1α • ↓NRF1,2	*NOR* • ↓Discrimination index	• ↔APP p-Tau/Tau • ↓IDE • ↑Cortical insoluble Aβ42	Obesity increased AD markers, impaired brain mitochondria biogenesis, and cognitive function in AD model.	[18]

Study model	Major findings					Refs
Animal/diet/ duration	**Metabolic parameters**	**Mitochondrial parameters**	**Cognitive function**	**AD marker**	**Interpretation**	
Sucrose (20% sucrose) fed mice or 3xTgAD mice fed with ND	N/A	• ↔RCR • ↔MMP • ↔ATP • ↔NRF2	*Synaptic plasticity* • ↔PSD95, synaptophysin, SNAP25	N/A	T2DM and AD mice exhibited similar phenotypes as regards brain synaptic plasticity and brain mitochondrial function.	[38]

Abbreviations: AD, Alzheimer's disease; HFD, high-fat diet; ND, normal diet; BW, body weight; PGC, peroxisome proliferator-activated receptor gamma; NRF, nuclear respiratory factor; TFAM, mitochondrial transcription factor A; RCR, respiratory control ratio; MMP, mitochondrial membrane potential; ATP, adenosine triphosphate; PSD, postsynaptic density protein; SNAP, synaptosomal-associated protein; NOR, novel object recognition; N/A, not assessed.

Table 3. Implications of high-calorie diet consumption on brain mitochondria and brain function in an Alzheimer's disease model.

and brain function. 3xTg AD mice cells with the mutations Thy-1.2-driven APP-Swedish and tau P301L were co-injected into a homozygous PS1M146V knock-in background. This type of AD mice had parenchymal plaque by 6 months of age combined with tau pathology by 12 months of age [44]. In APPswe/PS1dE9 mice, APP/PS1 animals co-express a Swedish (K594 M/N595 L) mutation of a chimeric mouse/human APP (Mo/HuAPP695swe), together with the human exon-9-deleted variant of PS1 (PS1-dE9), which leads to an increase in human Aβ peptide secretion in the brain of APPswe/PS1dE9 mice [17, 18].

There is only one study that has compared the brain mitochondrial function between T2DM and AD animal models. The investigators reported that both T2DM and AD mice had similar degrees of brain mitochondrial dysfunction, decreased synaptic plasticity proteins levels, and raised AD markers [38]. Those findings indicated that AD pathology was developed in T2DM animals, with an involvement of brain mitochondrial dysfunction.

The provision of a HFD to AD mice resulted in a different outcome depending on a genetic background of the AD mice. In 3xTg AD mice, the provision of a HFD led to increased body weight, but did not alter plasma glucose and insulin levels, compared to 3xTg AD mice given an ND [33]. In addition, the brain mitochondrial number and brain mitochondrial morphology, as well as cognitive function and AD markers were not affected by the HFD [33]. The data from this study suggested that T2DM did not alter brain mitochondria, cognitive function, or AD markers in 3xTgAD mice. By contrast, the consumption of a HFD led to a markedly decreased brain mitochondrial biogenesis and aggravated cognitive impairment in APPswe/PS1dE9 mice [17, 18]. Furthermore, a HFD aggravated AD pathogenesis in APPswe/PS1dE9 mice, as indicated by increased cortical soluble and insoluble Aβ, and decreased insulin-degrading enzymes [17, 18]. Data regarding the effects of consumption of a high-calorie diet on brain mitochondrial function and brain function in the AD model are shown in **Table 3** and are summarized in **Figure 1**.

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4. Therapeutic approaches on rats with the MetS condition specific to brain mitochondrial dysfunction and its association with the development of AD

Several studies have used various interventions on brain mitochondria and described their associations with the development of pre-AD. In this report, we have separated these interventions into three categories: (1) antidiabetic drugs, (2) traditional medicine, and (3) other drugs.

4.1. Antidiabetic drugs

Several studies have demonstrated the beneficial effects of antidiabetic drugs on insulin sensitivity and brain mitochondrial function [21, 31, 45]. Our previous study found that the sodium glucose cotransporter 2 (SGLT2) inhibitor, which is a new antidiabetic drug, could decrease metabolic disturbance, brain mitochondrial ROS production, brain mitochondrial membrane potential change, brain mitochondrial swelling, synaptic dysplasticity and cognitive decline in HFD-fed rats [21]. In addition, the incretin-based drugs such as sitagliptin and vildagliptin, dipeptidyl peptidase-4 (DPP-4) inhibitors, also had beneficial effects on the improvement of insulin sensitivity, brain mitochondrial function and cognitive function in HFD-fed rats [21, 31, 45]. Another incretin-based drug, liraglutide, a glucagon-like peptide-1 (GLP-1) agonist, also improved insulin sensitivity and decreased brain mitochondrial swelling [45]. All of these findings indicated that the antidiabetic drugs could reduce peripheral and brain insulin resistance, leading to improvement in cognitive function and synaptic plasticity and were associated with improved brain mitochondrial function. However, there is still lack of evidence showing the effects of antidiabetic drugs on AD markers. Data pertinent to the effect of antidiabetic drugs on brain mitochondrial dysfunction and their association with the development of pre-AD in the MetS condition are shown in **Table 4**.

4.2. Traditional medicine

Several studies have shown the beneficial effects of traditional medicine on brain mitochondrial function in HFD-fed, T2DM and AD rat models [20, 34, 39, 41]. Naringin, a citrus flavonoid, can improve insulin sensitivity and decrease brain mitochondrial ROS production, brain mitochondrial membrane potential change, brain mitochondrial ATP production, and cognitive decline in HFD-fed mice [34]. Furthermore, our previous studies found that garlic extract reduced peripheral and brain insulin resistance, brain mitochondrial ROS production, brain mitochondrial membrane potential change, and brain mitochondrial swelling, leading to improved cognitive function in HFD-fed rats [20]. The ZiBuPiYin recipe (ZBPYR), a traditional Chinese medicine, reduced brain mitochondrial ROS production, increased brain mitochondrial membrane potential change, increased brain mitochondrial number, and decreased cortical insoluble Aβ42, leading to improved cognitive function in T2DM mice [41]. *Dendropanax morbifera* (Araliaceae), a herbal medicine in Asia, improved peripheral and brain insulin sensitivity, decreased brain mitochondrial ROS

Study model	Major findings					Refs
Animal/ interventions/ duration	**Metabolic parameters**	**Mitochondrial parameters**	**Cognitive function**	**AD marker**	**Interpretation**	
HFD-fed rats/ SGLT2 inhibitor (1 mg/kg) or vehicle/4 weeks	• ↓BW • ↑Peripheral insulin sensitivity • ↑Brain insulin signaling	• ↓ROS • ↑MMP • ↓Swelling	*MWM* • ↓Time to reach platform • ↑Time in target quadrant *Synaptic plasticity* • ↑LTP	N/A	SGLT2 inhibitor reduced peripheral and brain insulin resistance, improved brain mitochondrial function, and improved cognitive function and synaptic plasticity in obese-insulin-resistant rats.	[21]
HFD-fed rats/ vildagliptin (3 mg/kg) or vehicle/3 weeks	• ↔ BW • ↓Insulin HOMA	• ↓ROS • ↑MMP • ↓Swelling	*MWM* • ↓Time to reach platform • ↑Time in target quadrant	N/A	DPP-4 inhibitor reduced peripheral and brain insulin resistance, improved brain mitochondrial function, and improved cognitive function in obese-insulin-resistant rats.	[31]
HFD-fed rats/ sitagliptin (30 mg/kg) or vehicle/3–4 weeks	• ↔ BW • ↓Insulin HOMA	• ↓ROS • ↑MMP • ↓Swelling	*MWM* - ↓ Time to reach platform - ↑ Time in target quadrant	N/A	DPP-4 inhibitor reduced peripheral and brain insulin resistance, improved brain mitochondrial function, and improved cognitive function in obese-insulin-resistant rats.	[31, 45]
HFD-fed rats/ liraglutide (0.6 mg/kg) or vehicle/3 weeks	• ↔ BW • ↓Peripheral insulin sensitivity	• ↓Swelling	N/A	N/A	Liraglutide reduced peripheral resistance and improved brain mitochondrial function in obese-insulin-resistant rats.	[45]

Abbreviations: HFD, high-fat diet; ND, normal diet; DPP-4, dipeptidyl peptidase 4; SGLT2, sodium glucose transporter 2; BW, body weight; ROS, reactive oxygen species; MMP, mitochondrial membrane potential; MWM, Morris water maze; NA, not assessed.

Table 4. Potential effects of antidiabetic drugs on brain mitochondria and their association with the development of Alzheimer's disease.

production, increased brain mitochondrial membrane potential change, and increased brain mitochondrial ATP production, leading to a decrease in cognitive decline in HFD-fed mice [39]. All of these traditional medicines contain flavonoid and phenolic compounds which have antioxidant properties, and it is thought that these properties may play an important role in the improvement of insulin sensitivity and brain mitochondrial function, leading to improved cognitive function. Data regarding the effect of traditional medicine on brain mitochondrial dysfunction and its association with a delay in the development of pre-AD in association with MetS are shown in **Table 5**.

Study model	Major findings					Refs
Animal/ interventions/ duration	**Metabolic parameters**	**Mitochondrial parameters**	**Cognitive function**	**AD marker**	**Interpretation**	
HFD-fed rats/ naringin (100 mg/kg) or vehicle/20 weeks	• ↓BW • ↑Peripheral insulin sensitivity • ↑ Brain insulin signaling	• ↓ROS • ↑MMP • ↑ATP	*NOR* • ↑Recognition index *MWM* • ↑Time in target quadrant • ↑Crossing target number	N/A	Naringin reduced peripheral and brain insulin resistance, improved brain mitochondrial function, and improved cognitive function in obese-insulin-resistant rats.	[34]
HFD-fed rats/garlic extract (200, 500 mg/kg) or vehicle/3 weeks	- ↔ BW • ↑Peripheral insulin sensitivity • ↑ Brain insulin signaling	• ↓ROS • ↑MMP • ↓Swelling	*MWM* • ↓Time to reach platform • ↑Time in target quadrant	N/A	Garlic extract reduced peripheral and brain insulin resistance, improved brain mitochondrial function, and improved cognitive function in obese-insulin-resistant rats.	[20]
HFD-fed rats/*Dendropanax morbifera* (20 and 50 mg/kg) or vehicle/10 weeks	• ↑Peripheral insulin sensitivity	• ↓ROS • ↑MMP • ↑ATP	*MWM* • ↑Time in target quadrant *Y-maze* • ↑Alternation behavior • ↑Total distance	N/A	*Dendropanax morbifera* reduced peripheral and brain insulin resistance, improved brain mitochondrial function, and improved cognitive function in obese-insulin-resistant rats.	[41]
HFD-fed mice + low-dose STZ/Chinese medicine ZiBu PiYin recipe or vehicle	• ↔ BW • Peripheral insulin sensitivity	• ↓ROS • ↑MMP • ↑Mito number	*MWM* • ↑Time in target quadrant • ↑Crossing target number	• ↓Cortical insoluble Aβ42	Although Chinese medicine did not improve peripheral insulin resistance, it improved brain mitochondrial function, improved cognitive function, and reduced AD marker in T2DM rats.	[39]

Abbreviations: HFD, high-fat diet; ND, normal diet; BW, body weight; ROS, reactive oxygen species; MMP, mitochondrial membrane potential; NOR, novel object recognition; MWM, Morris water maze; NA, not assessed.

Table 5. Potential effects of traditional medicine on brain mitochondria and its association with the development of Alzheimer's disease.

4.3. Other drugs

The other therapies such as fibroblast growth factor 21 (FGF21), hydroxytyrosol 2-(3,4-dihydroxyphenyl)-ethanol, and mitochondrial fission inhibitors also had beneficial effects on brain mitochondrial function in HFD-fed, T2DM and AD models. Our previous study found that an endocrine hormone, FGF21, decreased metabolic disturbance, brain mitochondrial ROS production, brain mitochondrial membrane potential change, brain mitochondrial swelling, synaptic dysplasticity, and cognitive decline in rats with MetS induced by the consumption of a HFD [22]. Hydroxytyrosol 2-(3,4-di-hydroxyphenyl)-ethanol, a major antioxidant phenol in olive oil, ameliorated mitochondrial dysfunction, reduced mitochondrial carbonyl protein, and enhanced superoxide dismutase 2 expression in AD mice. However, this drug did not affect Aβ accumulation in these AD mice [46]. The mitochondrial fission inhibitor, mdivi-1, improved synaptic plasticity and was associated with improving brain

Study model	**Major findings**					**Refs**
Animal/interventions/ duration	**Metabolic parameters**	**Mitochondrial parameters**	**Cognitive function**	**AD marker**	**Interpretation**	
HFD-fed rats/FGF21 (0.1 mg/kg) or vehicle/20 weeks	• ↓ BW • ↑Peripheral insulin sensitivity • ↑ Brain insulin signaling	• ↓ ROS • ↑ MMP • ↓ Swelling	*MWM* • ↓ Time to reach platform • ↑ Time in target quadrant *Synaptic plasticity* • ↑ LTP	N/A	FGF21 reduced peripheral and brain insulin resistance, improved brain mitochondrial function and cognitive function in obese-insulin-resistant rats.	[22]
APP/PS1 mice/ hydroxytyrosol (50 mg/kg) or vehicle/8 weeks	N/A	• ↑OXPHOS I, IV • ↑ PGC1-α	N/A	↔ Aβ levels	Hydroxytyrosol improved mitochondrial function and increased mitochondrial biogenesis in T2DM mice.	[46]
db/db mice/mdivi-1 (50 mg/kg) or vehicle/2 weeks	N/A	• ↑Mito density • ↑OXPHOSI • ↑ ATP	*Synaptic plasticity* • ↑ LTP	N/A	Mitochondrial fission inhibitor improved brain mitochondrial function and brain synaptic plasticity in T2DM mice.	[30]

Abbreviations: HFD, high-fat diet; ND, normal diet; BW, body weight; ROS, reactive oxygen species; MMP, mitochondrial membrane potential; OXPHOS, oxidative phosphorylation; PGC, peroxisome proliferator activated receptor gamma; ATP, adenosine triphosphate; LTP, long-term potentiation; N/A, not assessed.

Table 6. Potential interventions on brain mitochondria and their association with the development of Alzheimer's disease.

mitochondrial function and biogenesis via increasing mitochondrial density, OXPHOS I, and ATP production in T2DM mice [30]. All of these findings indicated that the SGLT2 inhibitor, vildagliptin, liraglutide, FGF21, naringin, garlic extract, and *D. morbifera* improved not only peripheral insulin sensitivity but also brain insulin sensitivity, brain mitochondrial function, and cognitive function. Hydroxytyrosol, Mdivi-1, and ZiBuPiYin improved brain mitochondrial function and cognitive function. However, only ZiBuPiYin reduced levels of AD markers, possibly resulting in improved cognitive function.

Data regarding the effect of other drugs on brain mitochondrial dysfunction and the association of this dysfunction with the development of pre-AD in the MetS condition are shown in **Table 6**. The summarized therapeutic approaches on brain mitochondria and their association with the development of AD are shown in **Figure 2**.

In addition, previous studies showed that acetylcholine (Ach) levels of AD brain were lower than that of healthy brain [6, 12]. Therefore, acetylcholinesterase inhibitors (AChEs) are commonly used for the symptomatic treatment of AD patients. Previous *in vivo* study and *clinical study* demonstrated that AChEIs have an effect on mitochondrial function (REFs). For example, (1) Donepezil (AChEI) attenuated brain mitochondrial dysfunction by reducing calcium-induced brain mitochondrial swelling and reduced mitochondrial Aβ40 and Aβ42 accumulation in AD mice, leading to improved cognitive function in AD mice [47]. (2) A clinical study by Casademont et al. showed that rivastigmin, AChEI, enhanced mitochondrial electron transport chain function as indicated by increased complex I and complex III of mitochondrial oxidation and increased enzymatic activities of complexes II, III, and IV

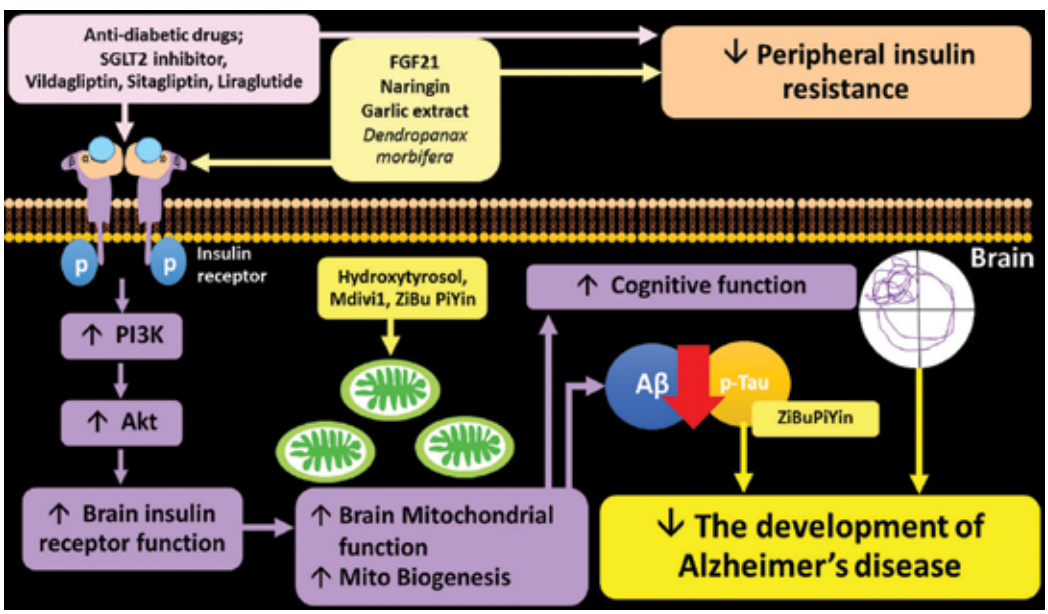

Figure 2. Summarized therapeutic approaches on brain mitochondria and their association with the development of Alzheimer's disease. Abbreviations: SGLT2, sodium-glucose transporter 2; PI3K, phosphoinositide 3 kinase; Akt, protein kinase B; FGF 21, fibroblast growth factor 21.

Study model	Major findings					Refs
Models/ interventions/ duration	**Metabolic parameters**	**Mitochondrial parameters**	**Cognitive function**	**AD marker**	**Interpretation**	
APP/PS1 (APPswe/ PS1dE9)/Donepezil (1 mg/kg) or vehicle/2 months	N/A	• ↓Ca2+ induced mitochondrial swelling	*T maze* • ↑%Accuracy *Elevated plus maze* • ↑Entry number into open arm • ↑Time in open arm	- ↓ Mito Aβ 40,42	Donepezil attenuated brain mitochondrial swelling, reduced brain Aβ accumulation, and improved cognitive function in AD mice.	[47]
AD patients/ rivastigmin (6–12 mg/ day)/6 months	N/A	• ↑Oxidation of complex I and III • ↑Enzymatic activities of complex II, III and IV • ↔Mitochondrial content	N/A	N/A	Rivastigmin enhanced mitochondrial electron transport chain function in AD patients.	[48]
Wistar rats/tacrine (15 mg/kg)/8 h	N/A	• ↓ Complex I, IV, V activity	N/A	• ↓AChE	Tacrine impaired brain mitochondrial function in rats.	[49]

Abbreviations: AChE, acetylcholinesterase; APP, amyloid beta precursor; AD, Alzheimer's disease; Ca^{2+}, calcium; Aβ, amyloid beta; N/A, not assessed.

Table 7. The effects of acetylcholine esterase inhibitors (AChEIs), the standard drugs for AD treatment, on brain mitochondrial function and their association with the development of Alzheimer's disease.

in the lymphocytes from AD patients [48]. (3) By contrast, tacrine, AChEI, impaired brain mitochondrial respiratory complex I, IV, and V activities in XX model [49]. Therefore, further studies are required to provide more evidence to support the effects of AChE inhibitor on brain mitochondrial function in AD patients as well as in the metabolic syndrome subjects. Those findings of AChEIs on brain mitochondrial function and their association with the development of Alzheimer's disease are summarized in **Table 7**.

5. Clinical implications

The presence of MetS is associated with an increase in the incidence of pre-AD. The possible underlying mechanisms involved in the association of pre-AD in MetS are still unclear. From this study, we concluded that mitochondrial dysfunction could be an important feature of AD pathogenesis in MetS. In addition, the findings indicate that the intervention which improved brain mitochondrial function led to improved cognitive function. These findings provide

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information regarding the role of mitochondria in the underlying mechanisms of pre-AD in MetS and offer important insights for future research on interventions that aim to improve the quality of life in MetS patients with AD.

6. Conclusion

In this study, the accumulated data led to the conclusion that although cognitive decline and brain mitochondrial dysfunction were observed in obese-insulin-resistant rats, AD was not developed during the pre-diabetic state. In addition, markers indicating the presence of AD were observed in T2DM subjects. Treatment with antidiabetic drugs, traditional medicine, FGF21, and mitochondrial fission inhibitors effectively improved brain mitochondrial function and cognitive function in rats with induced MetS.

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Declaration of interest

The authors declare that there are no conflicts of interest.

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Chapter 5

Lithium and Alzheimer's Disease: Experimental, Epidemiological, and Clinical Findings

Janusz K. Rybakowski

Additional information is available at the end of the chapter

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Abstract

Alzheimer's disease (AD) represents one of the greatest health-care challenges of the twenty-first century. Besides known pathologies such as intracellular accumulation of neurofibrillary tangles and extracellular deposition of amyloid-beta plaques, other factors, such as dysregulated GSK-3 activity, mitochondrial dysfunction, inflammation, and oxidative stress, have been shown to play a role in the pathogenesis of AD. Over the last two decades, the evidence accumulated for a neuroprotective effect of lithium, as an important mechanism of this ion in mood disorders, reflected by an increase in cerebral gray matter volume in lithium-treated subjects. Neurobiological mechanisms of lithium neuroprotective actions may also be relevant to the pathogenesis and treatment of AD, and they will be delineated. In most epidemiological studies, a negative association between lithium use and dementia has been shown, including two most recent papers regarding a concentration of lithium in drinking water. In this article, the results of initial studies using lithium in the treatment of dementia and showing some promise will also be presented. Therefore, considering the current paucity of treatments for the AD, further testing of lithium as a disease-modifying treatment in this illness may be warranted.

Keywords: Alzheimer's disease, dementia, lithium, neuroprotection, glycogen synthase kinase-3

1. Introduction

Alzheimer's disease (AD) represents one of the greatest health-care challenges of the twenty-first century. Nearly 50 million people over the age of 60 years are presently diagnosed with AD worldwide, and the projected figure is estimated to be 130 million in 2050 [1].

The main pathology of the AD includes the intracellular accumulation of neurofibrillary tangles, connected with abnormal tau protein phosphorylation, and extracellular deposition of amyloid-beta (Aβ) plaques. Such changes may be present even several years before symptom manifestation. However, in recent years, the evidence has been accumulated that some other factors may be both pathogenic and playing a role in the progress of the illness. They include, among others, dysregulated glycogen synthase kinase-3 (GSK-3) activity, mitochondrial dysfunction, inflammation, and oxidative stress [2].

Over the last two decades, the evidence accumulated for neuroprotective effects of lithium as important mechanisms of this ion in mood disorders. These effects were reflected by an increase in cerebral gray matter volume in lithium-treated subjects and also related to a possible influence of lithium on some pathogenic mechanisms operating in the AD. Such neurobiological mechanisms of lithium which may be relevant to AD pathogenesis, and treatment will be characterized in the first part of this article. They make lithium a candidate for use as a therapeutic drug in this illness [3]. In the recent decade, a negative association between lithium use and dementia has been shown in most epidemiological studies, including two most recent papers regarding a concentration of lithium in drinking water. In this article, preliminary studies of using lithium in the treatment of mild cognitive impairment (MCI) and AD that show some promise will also be presented.

2. Neuroprotective effects of lithium: relevance to pathology of Alzheimer's disease

The neuroprotective effect of lithium in bipolar patients has been reflected in neuroimaging studies, starting in 2000, when Moore et al. [4] in a research letter to the Lancet suggested a lithium-induced increase in human brain gray matter. Since then, several researches on this topic have been published. The prefrontal cortex, anterior cingulate, and hippocampus made the brain structures most frequently shown to be influenced by either short- or long-term lithium treatment. The results of cross-sectional and prospective studies on this issue were recently reviewed by Hajek and Weiner [5].

Among cross-sectional studies, the most frequently reported pattern was larger gray matter volumes in patients currently treated with lithium compared to those currently not on lithium. The association between lithium treatment and higher gray matter volume was reported regardless of mood state and diagnostic subtype [5]. Our research showed that bipolar patients receiving lithium had larger hippocampal volumes than non-lithium patients and comparable to healthy controls [6].

In a prospective study, Monkul et al. [7] performed a voxel-based morphometry analysis in healthy persons receiving lithium in therapeutic doses for 4 weeks. They found a significant increase in gray matter in the left and right dorsolateral prefrontal cortices and the left anterior cingulate region. Yucel et al. [8] made neuroimaging study in BD patients receiving lithium up to 2 months and for 2–4 years showing a bilateral increase in hippocampal volume in both groups. Moore et al. [9] extended their results published 9 years earlier as they found that an increase in total gray matter volume in the prefrontal cortex of depressed bipolar subjects after 4 weeks of lithium administration was significant only in lithium responders.

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The neuroimaging studies were also performed to compare lithium with valproate, carbamazepine, and antipsychotics, given to BD patients. In a paper of Lyoo et al. [10] including 22 BD patients treated with either lithium or valproate, the gray matter increased in lithium group, with maximum effect at weeks 10–12 which was still evident at 16 weeks of treatment. Such an increase was also associated with positive clinical response. On the other hand, patients receiving valproate did not show any significant changes in gray matter volume. Germana et al. [11] in their study of 74 remitted bipolar patients receiving long-term prophylactic treatment with lithium, valproate, carbamazepine, or antipsychotics observed that the volume of gray matter in some brain structures (the right subgenual anterior cingulate gyrus, the left postcentral gyrus, the hippocampus/amygdala complex, and the insula) was greater in patients receiving lithium than all other pharmacological treatments.

Thus, there is reasonable evidence showing that lithium administration can result in an increase of brain gray matter volume both in healthy subjects and in patients with BD which may be associated with its neuroprotective effect at a clinical level. The replicated substantiation for this has not been demonstrated for any other mood-stabilizing drug. The mechanism of the increase is not clear. The MRI changes are probably not related to the effect of lithium on tissue water or magnetic properties. Since the studies of magnetic resonance spectroscopy showed the association between lithium treatment and increased N-acetyl aspartate, localized in neurons, this may suggest an effect of lithium on neurons, which may involve an increase in the number of neurons, dendritic arborization, or neutrophil [5].

Several biochemical targets have been involved in the neurotrophic and neuroprotective effect of lithium which may be relevant to its possible disease-modifying treatment in the AD. The most important include the increased expression of neurotrophins [mainly brain-derived neurotrophic factor (BDNF)], the inhibition of glycogen synthase kinase-3 (GSK-3), modulation of the phosphatidylinositide (PI) cascade, inhibition of the protein kinase C (PKC), and increased expression of the B-cell lymphoma 2 (Bcl-2). As a result of such actions, lithium increases cell survival by promoting neurogenesis in the adult brain and by inhibiting cell death (apoptosis) cascades [12].

BDNF belongs to the neurotrophin family, along with nerve growth factor (NGF) and neurotrophin-3 (NT-3), NT-4, NT-5, and NT-6. These neurotrophins play an important role for the survival and function of neurons. BDNF regulates the activity of various neurotransmitters, e.g., glutamate, gamma-aminobutyric acid, dopamine, and serotonin. Experimental studies showed that lithium enhances the BDNF system. In clinical studies, lithium treatment increases the blood level of BDNF [13].

GSK-3 modulates a number of cellular processes, among others, cell apoptosis, and the inhibition of GSK-3 results in an antiapoptotic effect. GSK-3 is also a key enzyme in the metabolism of amyloid precursor protein and in the phosphorylation of the tau protein, the main pathological processes in AD. Lithium inhibits GSK-3 activity, and the evidence for this has accumulated in recent two decades, using various experimental models. Therefore, the GSK-3 can be considered as one of the most important therapeutic targets of lithium, and the GSK-3 inhibition by this ion can make an essential mechanism of its therapeutic action in mood disorders [14]. In experimental studies, using the cultures of rat neurons, it was shown that lithium reduces GSK-3 mRNA [15]. In mutant tau transgenic mice with neurofibrillary pathology, lithium delays the progress of neurofibrillary tangles, and in the *Drosophila* fly, adult-onset model of the AD, lithium alleviates

amyloid-beta pathology. Both these effects are thought to be obtained by lithium inhibition of the GSK-3 [16, 17]. About the GSK-3 inhibition by lithium, the effect of this ion on autophagy regulation should also be indicated, the signaling pathway of which is associated with the mammalian target of rapamycin (mTOR) [18].

The PI pathway plays a role in signal transduction mechanisms connected with the action of multiple neurotransmitters. Lithium significantly influences this pathway which resulted in the inositol-depletion hypothesis of lithium action, as an essential therapeutic mechanism in mood disorders. Lithium inhibits the inositol monophosphatase (IMPase) and many other phases of the PI pathway [12]. The effect of lithium on the PI pathway is also connected with enhancing autophagy by the mTOR-independent pathway [18].

Protein kinase C (PKC) is an enzyme associated with the PI pathway and plays a role in the action of many neurotransmitters and other cellular mechanisms. It has been found that lithium inhibits the activity of PKC that may contribute to its regulation of intracellular signaling and increasing neuroplasticity [12].

Bcl-2 is a protein playing a significant role in cellular resilience and plasticity, among others, by inhibiting apoptosis. Experimental studies demonstrated an increase of Bcl-2 in the brain by lithium treatment. Enhancing by lithium the expression of Bcl-2-associated athanogene (bag-1) augments the antiapoptotic effect, by mitigating glucocorticoid receptor nuclear translocation [12].

Morris and Berk [19] suggested some additional mechanisms of lithium action which may be important in the treatment of AD, such as the effects on mitochondrial function, calcium homeostasis, inflammation, microglial activation, glutamate excitotoxicity, and oxidative stress. Most of these processes are connected with the mechanisms described above.

Lithium produces a significant increase in mitochondrial performance in human brain tissue, the main factors of such effect being the inhibition of GSK-3 and activation of mTOR [20]. This cation also desensitizes brain mitochondria to the damaging effects of calcium influx [21] and increases mitochondrial levels of Bcl-2 [22]. As peripheral and neuro-inflammation, together with the chronic activation of microglia, constitutes an important element in the development of the AD, there is evidence that lithium can ameliorate various aspects involved in the pro-inflammatory response. These include the generation of tumor necrosis factor-alpha and interleukin-1 beta by microglia, and this effect is obtained via the inhibition of GSK-3 [23]. Lithium also exhibits a protective effect against the development of glutamate neurotoxicity, which is a consequence of chronic microglial activation, and this effect is due to the upregulation of BDNF [24]. In clinical conditions, lithium administration causes a decrease in markers of oxidative stress such as catalase and superoxide dismutase [25].

3. Epidemiological studies of lithium in Alzheimer's disease

The results of population studies of an association between lithium and dementia were reviewed by Donix and Bauer [26]. Data from large cohort and most case–control studies suggest an association between lithium treatment and dementia risk reduction or reduced dementia severity.

In their publication in 2005, Dunn et al. [27] reported that among 19,328 participants selected from a General Practice Research Database, more subjects with dementia were treated with Li compared to control subjects without dementia. However, mood disorders are the most frequent indication for Li treatment and also belong to the strongest risk factors for dementia, and as this study did not control for compliance/optimal treatment, it may have simply detected the increased risk of dementia in mood disorders. Terao et al. [28] investigated clinical records of 1423 outpatients at a university psychiatric department and compared patient treated with lithium to age- and the gender-matched control group who had never been prescribed with lithium. Patients who had previously received lithium and/or were currently on lithium had significantly better Mini Mental State Examination (MMSE) scores than the control patients. Nunes et al. [29] studied the occurrence of AD in 66 elderly BD patients assessed during euthymia, receiving long-term lithium therapy, and in 48 age-matched patients who were not recently taking lithium. The percentage of patients with dementia was 19% in the first group and 7% in the second group. The diagnosis of the AD was made in three patients (5%) receiving lithium and in 16 patients (33%) who were not taking lithium, which suggests that lithium treatment may reduce the prevalence of AD in patients with bipolar disorder. Angst et al. [30] studied subjects with bipolar disorder (N = 220) and major depressive disorder (N = 186) followed from 1965 to 1985, receiving long-term treatment with lithium, clozapine, or antidepressants. In the whole group, the prevalence of dementia showed a significant association with age. However, when an analysis of the 88 patients with dementia was performed, the association with age was lost, and there was a trend to an inverse correlation between lithium administration and the severity of dementia.

Two papers coming from the University of Copenhagen employed the Danish nationwide register of lithium prescriptions. In the first one, a comparison was made for the diagnosis of dementia or AD between 16,238 persons who had purchased lithium at least once during inpatient or outpatient treatment and 1,487,177 persons from the general population who had never bought lithium. Those who had bought lithium at least once had the 1.5-fold higher rate of dementia than the persons not taking lithium. However, those who continued treatment with lithium had the rate of dementia decreased to the same level as that for the general population. Such a decrease was exclusive to lithium because persons receiving anticonvulsant drugs had the risk of dementia increased with the duration of treatment [31]. The second study followed up 4856 patients which received a diagnosis of a manic or mixed episode or bipolar disorder at their first psychiatric contact for the period of 1995–2005 (103.6/10000 person-years). The percentages of patients receiving given drug were as follows: lithium 50.4%, anticonvulsants 36.7%, antidepressants 88.1%, and antipsychotics 80.3%. During the follow-up period, 216 patients were diagnosed with dementia. It was found that a reduced rate of dementia in BD patients was connected with long-term treatment with lithium. On the other hand, such a phenomenon was not observed with continued treatment with anticonvulsants, antidepressants, or antipsychotics [32].

In 2015, Gerhard et al. [33] examined the association of lithium and dementia risk in a large claim-based US cohort of publicly insured older adults with bipolar disorder (n = 41,931), including individuals ≥50 years who did not receive dementia-related services during the prior year. Each follow-up day was classified by past-year cumulative duration of lithium use.

Authors, year, ref. no.	Design of study	Results
Dunn et al.[27]	9954 lithium-treated patients and 9374 not on lithium, selected from a General Practice Database	Lithium treatment associated with increased dementia risk
Terao et al. [28]	1423 psychiatric outpatients from a university clinic	Current or previous lithium treatment associated with better performance on MMSE
Nunes et al. [29]	66 elderly patients with bipolar disorder receiving lithium and 48 matched patients without lithium	Reduced AD prevalence associated with lithium treatment
Angst et al. [30]	220 subjects with bipolar disorder and 186 subjects with unipolar depression followed for 20 years	Reduced dementia severity among patients receiving lithium
Kessing et al. [31]	16,238 persons who had purchased lithium in the past and 1,487,177 not using lithium	Reduced risk for dementia associated with the continued use of lithium
Kessing et al. [32]	4856 patients with bipolar disorder, followed for 10 years	Reduced risk for dementia associated with the continued use of lithium
Gerhard et al. [33]	41,931 patients >50 years with bipolar disorder, followed for 3 years	Reduced risk of dementia with the long-term (10–12 months) use of lithium
Kessing et al. [34]	73,731 patients with dementia, 733,653 control subjects	Decreased incidence of dementia in subjects exposed to >10 μg/L of lithium in drinking water
Fajardo et al. [35]	Changes in AD mortality between 2000 and 2006, and 2009 and 2015 and lithium in drinking water	Increase in AD mortality negatively associated with lithium concentration in drinking water

Table 1. Epidemiological studies of lithium and dementia.

Compared with nonuse, 301–365 days of lithium exposure was associated with significantly reduced dementia risk No corresponding association was observed for shorter lithium exposures or any exposure to anticonvulsants that may suggest that long-term lithium treatment may reduce dementia risk in older adults with bipolar disorder.

Recently, two papers appeared studying a relationship between lithium in drinking water and dementia. Kessing et al. [34] performed a Danish nationwide, case–control research, studying an association between the municipality of residence and measurements of lithium in drinking water. The data were obtained from all patients between 50 and 90 years of age who had a diagnosis of dementia during hospitalization, from 1970 to 2013. A total of 73, 731 patients with dementia and 733, 653 controls were included in the study. Lithium exposure was statistically significantly different between patients with a diagnosis of dementia and controls, and a nonlinear association was observed. Compared with individuals exposed to 2.0–5.0 μg/L, the incidence rate ratio of dementia was decreased in those exposed to more than 15.0 μg/L and 10.1–15.0 μg/L and increased with 5.1–10.0 μg/L. Similar patterns were found for Alzheimer's disease and vascular dementia as outcomes. In the second study, Fajardo et al. [35] examined the relationship between trace levels of lithium in drinking water and changes in AD mortality

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across several Texas counties. 6180 water samples from public wells since 2007 were obtained, and changes in AD mortality rates were calculated by subtracting aggregated age-adjusted mortality rates between 2000 and 2006 from those between 2009 and 2015. The authors found that the age-adjusted AD mortality rate was significantly (+27%) increased over time. Changes in AD mortality were negatively correlated with trace lithium levels, and statistical significance was maintained after controlling for most risk factors except for physical inactivity, obesity, and type 2 diabetes. Furthermore, the prevalence of obesity and type 2 diabetes positively correlated with changes in AD mortality but also negatively correlated with trace lithium in drinking water. The results suggest that trace lithium in water may be negatively linked with changes in AD mortality, as well as obesity and type 2 diabetes, which are important risk factors for AD.

The chronological arrangement of epidemiological studies on lithium and dementia is presented in **Table 1**.

4. Clinical studies of lithium in MCI and AD

In 2008, Macdonald et al. [36] first attempted to assess the safety and feasibility of prescribing long-term lithium (up to 1 year) to 22 elderly people with mild to moderate Alzheimer's disease (AD) in an open-label study. A comparison group not receiving lithium therapy was matched for cognition and age. The mean duration of treatment for 14 patients who discontinued prematurely was 16 weeks and for those continuing treatment at the end of the study was 39 weeks. The reason for discontinuation in three patients was possible side effects which disappeared on stopping therapy. The intensity of side effects did not differ between patients discontinuing therapy and the subjects remaining in the study. Two patients receiving lithium died; however, in neither case the treatment with lithium was related to the cause of death. The lithium and non-lithium groups were not different as to deaths, drop outs, or change in MMSE.

In 2009, the first randomized lithium trial in patients with mild AD appeared [37]. Seventy-one patients were randomized to receive either lithium (0.5–0.8 mmol/l) (n = 33) or a placebo for (n = 38) 10 weeks. The results obtained showed that there were no differences as to global cognitive performance, as measured by the ADAS-Cog subscale, depressive symptoms, as well as plasma activity of GSK-3 and disease biomarker concentrations in the cerebrospinal fluid (CSF), between lithium and placebo groups [42]. However, interesting results were obtained by an analysis of a single site subsample (Tübingen) containing 27 patients, 13 of which were randomized to lithium and 14 to placebo. In AD patients treated with lithium, in comparison to placebo-treated patients, a significant increase of BDNF serum levels and a significant decrease of cognitive impairment measured by the ADAS-Cog sum scores, inversely correlated with lithium serum concentration, were found [38].

Two Brazilian studies performed in 2011 and 2013 brought about some promising results. Forlenza et al. [39] employed lithium in placebo-controlled trial of 45 patients with amnestic mild cognitive impairment (MCI), randomized to lithium (n = 24) or placebo (n = 21) for 12 months. They found that lithium treatment (0.25–0.5 mmol/l) was associated with significantly better performance on the cognitive subscale of the Alzheimer's Disease Assessment

Scale and with a significant decrease of P-tau protein in cerebrospinal fluid (CSF). In the second study, Nunes et al. [40] assessed the effect of a microdose of 300 μg lithium, given to AD patients in one daily dose, for the period of 15 months. During this time, the group receiving lithium microdose showed no decrease in performance in the MMSE test. On the other hand, such a decrease was observed in the control group.

In a meta-analysis performed by Matsunaga et al. [41], three clinical trials including 232 participants that met the study's inclusion criteria were identified. The results obtained suggested that lithium significantly decreased cognitive decline (standardized mean difference = −0.41) as compared to placebo. There were no significant differences in the rate of attrition, discontinuation due to all causes or adverse events, or CSF biomarkers between treatment groups.

5. Conclusions

There is robust and highly replicated evidence for positive association between Li treatment and gray matter volumes. There has also been a strong experimental background for biochemical underpinnings of lithium's neuroprotective effect that may have possible relevance for therapeutic action of this ion in the AD. A negative association between lithium use and dementia confirmed in most epidemiological studies, including the recent ones on lithium in drinking water, has also been quite substantial. All the same, the results of using lithium in the treatment of AD involve some methodological and clinical issues, which complicate the interpretations. One must acknowledge the heterogeneity of studies regarding of methodology, duration of intervention, dose regimen, and also outcome variables. Nonetheless, three of the four available studies meta-analyzed by Matsunaga et al. [41] suggested some benefits from lithium treatment on amnestic mild cognitive impairment or early stages of the AD, including effects on illness biomarkers.

Despite the wide range of supporting evidence, the neuroprotective effects of lithium are mostly neglected and little known outside of the mood disorders field. However, considering the current paucity of treatments for neurodegenerative disorders, we cannot afford to let the research into neuroprotective effects of lithium come to a halt. The evidence presented in this chapter would warrant further testing of lithium as a disease-modifying treatment for the AD.

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Chapter 6

Mild Cognitive Impairment

Marina Janelidze and Nazibrola Botchorishvili

Additional information is available at the end of the chapter

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Abstract

Mild cognitive impairment (MCI) refers to cognitive decline from a previous level of functioning, both subjectively and by objective evidence. MCI is an intermediate stage of cognitive impairment between the normal cognitive aging and dementia. The concept of mild cognitive impairment originally evolved with an intention to characterize the pre-dementia phase of cognitive impairment. MCI is a known risk factor for dementia. Patients with MCI may represent an optimal target population for pharmacological and non-pharmacological interventions. The following chapter provides an overview of the concept of mild cognitive impairment, epidemiological data, current diagnostic criteria, clinical approach and management of MCI.

Keywords: mild cognitive impairment, prodromal dementia, mild neurocognitive disorder, cognitive decline, Alzheimer's disease

1. Introduction

Mild cognitive impairment (MCI) represents an intermediate stage of cognitive impairment between the normal cognitive aging and dementia. The development of the concept of MCI was stimulated by the clinical awareness of the existence of an intermediate level of cognitive impairment that was not captured by any clinical definition on the one hand and by the rising awareness of dementia as an important area of public health on the other [1]. The concept of MCI permits timely identification of patients at high risk of developing dementia, thus opening a potential therapeutic window and increasing the significance of controlling modifiable risk factors.

2. History of the concept of mild cognitive impairment

During the last few decades various terms and definitions have been proposed to determine intermediate stage between normal aging and dementia. In 1962, V.A. Kral first described two types of age related cognitive changes in his works. One of them is "benign senescent forgetfulness" (BSF), which is characterized by mild and non-progressive memory decline and presumably implies non-specific histopathological changes in the brain. The second form, "malignant senescent forgetfulness" (MSF) includes progressive cognitive and behavioral changes which involves specific brain histopathology [2]. Introduction of the term "benign senescent forgetfulness" was the first attempt to conceptualize MCI [3].

In 1986, the working group at National Institute of Mental Health proposed the diagnosis of age-associated memory impairment (AAMI) to identify age-related memory changes [4]. The concept was based on the comparison of older persons to young adult norms on a variety of memory tests. The idea was criticized by the WHO and International Psychogeriatric Association, because it included only memory assessment and did not imply diversity of age-sensitivity of memory tests [5]. The concept of AAMI did not develop further. Alternatively, in DSM-IV it evolved in the term aging-associated cognitive decline (AACD) [5, 6]. The AACD diagnosis is similar to age-associated memory impairment. However, the AAMI diagnosis is based on a less comprehensive evaluation which takes into account memory function only [7]. Subjects are classified as having AAMI if they score 1 SD below the mean of younger adults (not people of their own age) in a standardized memory test [4].

In 1989, Blackford and La Rue proposed modified version of age associated memory impairment, "late life forgetfulness" (LLF) [8]. LLF was defined as slight deterioration of memory compared with aged-match persons, but the absence of Dementia [5].

Before the introduction of MCI concept elderly persons with cognitive complains who were not demented were categorized as having questionable dementia [5]. In the 1980s, global clinical staging scales have been developed to classify the wide spectrum of cognitive dysfunction in geriatric population. Among them Global Deterioration Scale (GDS) and Clinical Dementia Rating (CDR) are the most frequently used [9]. These scales differentiate a type of cognitive impairment which is intermediate between dementia and normal cognition function. Subjects with GDS 3 or CDR stage 0.5 are classified as having "questionable," "borderline" or "preclinical" AD. Other terms, such as "minimal dementia," "limited cognitive disturbance," "isolated memory loss," "mild cognitive disorder," "mild neurocognitive disorder" and "cognitive impairment-no dementia" (CIND) have been used to reflect the similar intermediate level of cognitive performance [9].

The term "cognitive impairment no dementia" was introduced in the Canadian Study of Health and Aging [10]. It was a multicenter study evaluating epidemiological aspects of cognitive impairment among Canadians aged 65 and older. In this study individuals with some degree of cognitive decline, who did not meet criteria for dementia, were classified as having "cognitive impairment no dementia". Cognitive impairment no dementia is a broad concept and involves cognitive decline of any etiology, including delirium, alcoholism, drug addiction, depression, psychiatric disorders [11]. The prevalence of CIND among the Canadian elderly was reported to be twice that of all dementias combined [5, 10].

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At the same time, in the medical literature of dementia and aging, the term "mild cognitive impairment" has emerged. In 1988 Reisberg and colleagues used this term to characterize subjects with the Global Deterioration Scale Score 3 [12]. The GDS is a seven-point rating instrument for the staging of the magnitude of cognitive and functional capacity from normal aging to severe dementia [13]. Points in GDS range from 1 to 7. A score 3 indicates mild but obvious cognitive decline leading to difficulties in handling complex situations and tasks - e.g. lack of orientation while traveling to unfamiliar places, failure to recall names of new acquaintances, concentration deficit, troubles with retaining large amount of information, word and name finding deficit.

In 1995, in an observational study of aging at Mayo clinic, R.C. Petersen and colleagues adopted mild cognitive impairment as an independent diagnostic entity to categorize persons with memory complaints, who were not demented, retained global cognitive function and daily living skills, but scored below the age-adjusted norms on memory tests (Although, it was in 1991, when the term MCI first appeared in the headline of the article by Flicker et al., "Mild Cognitive Impairment in the elderly: predictors of dementia") [14, 15].

Petersen R.C. and colleagues provided Mayo clinic criteria for mild cognitive impairments: 1. Subjective complaint on memory disturbance (preferably supported by the informant); 2. Objective evidence of memory deficit: 3. Generally preserved cognitive functions; 4. Intact activities of daily living; 5. Absence of dementia [16].

In 2001, the American Academy of Neurology (AAN) incorporated new diagnostic criteria in a guideline on mild cognitive impairment. The AAN criteria for MCI were as follows: 1. An individual's report of his or her own memory problems, preferably confirmed by another person; 2. Measurable, greater-than-normal memory impairment detected with standard memory assessment tests; 3. Normal general thinking and reasoning skills; 4. Ability to perform normal daily activities [17]. Early detection and monitoring of persons with mild cognitive impairment was recommended, due to the high risk of progression to dementia.

Based on clinical observations, it became clear that mild cognitive impairment is not limited to memory loss. In 2003, the first key symposium was held in Stockholm, with the aim to integrate clinical and epidemiological perspectives on the topic of mild cognitive impairment [18]. The proposed MCI criteria were no more focused on memory impairment alone and included the following features: 1. The person is neither normal nor demented;

2. There is evidence of cognitive deterioration shown by either objectively measured decline over time and/or subjective report of decline by self and/or informant in conjunction with objective cognitive deficits; 3. Activities of daily living are preserved and complex instrumental functions are either intact or minimally impaired.

At the same time a comprehensive classification of MCI was proposed that categorizes individuals by the type or domain of cognitive deficit (memory vs. non-memory such as language, visuospatial, speed of processing or executive function) and the extent of the deficits (single domain vs. multiple domains). Based on these criteria, four MCI subtypes have been proposed: Amnestic MCI-Single Domain (a-MCI-sd), Amnestic MCI-Multiple Domain (a-MCI-md), Non-Amnestic MCI Single Domain (na-MCI-sd) and Non-Amnestic MCI-Multiple Domain (na-MCI-md) [19].

Presumably, the cognitive phenotype of MCI (a-MCI vs. na-MCI) and the number of cognitive domains affected (single vs. multiple) determine the future outcome of mild cognitive impairment. Amnestic single or multiple domain MCI is supposed to be precursor of Alzheimer disease, whereas persons with na-MCI likely progress to a non-AD dementia, such as dementia with Lewy bodies, fronto-temporal dementia, Huntington's disease or Parkinson-Dementia [20] (**Table 1**).

In the fifth edition of Diagnostic and Statistical Manual of Mental Disorders (DSM-V) the term dementia is replaced by the term neurocognitive disorder. DSM-V recognizes the pre-dementia stage of cognitive impairment and defines it as mild neurocognitive disorder (NCD) [21, 22]. Diagnostic criteria for mild NCD are almost identical to MCI criteria and include the following: 1. Clinical concern raised by the patient or an informant, or observations made by the clinician; 2. Cognitive impairment in one or more cognitive domains preferably relative to appropriate normative data for that individual; 3. Preservation of functional independence and 4. No dementia

In 2011, National Institute of Aging (NIA) and Alzheimer's Association (AA) developed diagnostic criteria for symptomatic pre-dementia phase of Alzheimer's disease [23]. The working group proposed two sets of criteria: core clinical criteria and clinical research criteria incorporating biomarkers. The NIA-AA criteria for MCI due to AD are as follows:

1. **Concern regarding a change in cognition.** Concern about a cognitive decline compared to the previous level should be obtained from the patient, form an informant, or from a clinician observing the patient.
2. **Impairment in one or more cognitive domains.** Evidence of dysfunction in one or more cognitive domains (memory, executive function, attention, language, and visuospatial skills) should be objectively demonstrated.

MCI subtypes and etiology			
Amnestic single domain	**Amnestic multiple domain**	**Non-amnestic single domain**	**Non-amnestic multiple domain**
Memory only	Memory plus ≥ 1 of the following: *Language* *Attention* *Executive function* *Visuospatial function* *Processing speed*	One of the following: *Language* *Attention* *Executive function* *Visuospatial function* *Processing speed*	>1 of the following *Language* *Attention* *Executive function* *Visuospatial function* *Processing speed*
Alzheimer's disease	**Alzheimer's disease** **Vascular dementia**	**Frontotemporal dementia**	**Lewy body dementia** **Vascular dementia**

Table 1. MCI classification.

3. **Preservation of independence in functional abilities.** Individuals with MCI usually experience some difficulties with handling complex situations, such as performance of financial operations, cooking, shopping. They may need more time, be less efficient, or make more errors during such activities. Nevertheless, they preserve functional independence.

4. **Not demented.** Observed cognitive dysfunction is usually mild and doesn't affect social or occupational activities. Objective demonstration of intra-individual change of cognitive function via the history or clinical assessment is required for the diagnosis of MCI.

The *Clinical Research Criteria* which incorporate the use of biomarkers, are intended to be used only in research settings to assess the underlying etiology of MCI and the likelihood of progression to dementia. According to the NIA-AA recommendations, concomitant application of clinical and research criteria can increase the diagnostic certainty. For this purposes 2 main types of biomarkers are investigated: Biomarkers of beta-amyloid deposition and biomarkers of neuronal injury/neurodegeneration. Biomarkers of beta-amyloid accumulation are: low CSF concentration of amyloid β42 and PET (positron-emission tomography) evidence of amyloid deposition. Biomarkers of neuronal injury are: High concentration of Tau/ Phosphorylated protein in CSF; Hippocampal, or medial temporal lobe atrophy on MRI, and temporoparietal/ precuneus hypometabolism or hypoperfusion on PET or SPECT [23, 24].

Based on biomarkers we can assess the risk of development of Alzheimer's disease. Currently, CSF $A\beta_{42}$ and tau measures, the ratio of CSF tau/$A\beta_{42}$, PET amyloid measures, and other biomarkers of neuronal injury such as hippocampal atrophy and temporoparietal hypometabolism have all been shown to predict progression of MCI to dementia [5, 24].

- The evidence of positive Aβ biomarker and a positive biomarker of neuronal injury indicate a high likelihood that the MCI syndrome is due to AD. In addition, individuals with this biomarker profile are more likely to decline or progress to dementia due to AD in relatively short periods.
- The probability that MCI is due to AD is moderate in cases in which one of the biomarkers is positive and the others have not been or cannot be tested.
- In a situation, where the biomarkers are negative, the likelihood of development of AD is low.

3. Epidemiology of mild cognitive impairment

Since MCI imposes a health burden of its own and increases the risk of dementia, it is important to reliably estimate the prevalence of MCI around the globe [25]. However, reported prevalence of MCI significantly differs across studies and ranges between 3 and 54% [5]. It is thought that this difference can be explained by the difference in research methodology, such as employed diagnostic criteria for MCI, variability of used neuropsychological tests, selected cut-off scores (≥1 SD or ≥1.5 SD), subjects of trials - population based or clinic based. Some of the variation may be associated with regional and/or ethnic differences. For example, MCI prevalence in India is 5 times higher than in China, despite standardization for age, sex

Study	Country	Years	N	Age	Prevalence
Purser et al.	US	1981–1991	3673	74	24.7
Lopez et al.	US	1991–1999	3608	≥65	18.8
Solfrizzi et al.	Italy	1992–1995	4521	73.4	3.2
Ravaglia et al.	Italy	1994–1996	34	≥ 65	7.7
Pioggiosi et al.	Italy	1999–2004		≥ 90	32.4
Huang et al.	China	2005	920	≥ 55	3.0
Choi et al.	Korea	2005–2006	1215	≥ 65	32.9
Choi et al.	Korea	2005–2006	1215	≥ 65	32.9
Artero et al.	France	2008	6892	≥ 65	42.0
Manly et al.	US	2008	1313	≥ 65	28.3

Table 2. Selected epidemiological studies in MCI.

Study	Country	Years	N	Age	Incidence
Larrieu et al.	France	2002	1265	≥ 65	9.9/1000
Busse et al.	Germany	2003	684	≥ 75	8.5/1000
Trevo et al.	Finland	2004	550	60–76	25.9/1000
Trevo et al.	Finland	2004	550	60–76	25.9/1000
Solfrizzi et al.	Italy	2004	2963	≥75	56.5/1000
Palmer et al.	Sweden	2004	379	≥75	34–168/1000
Carraciolo et al.	Sweden	2008	1070	≥75	13.7/1000 a MCI
Manly et al.	US	2008	1800	≥65	2.3–5.1%
Luck et al.	Germany	2010	2331	≥65	18.8

Table 3. Selected epidemiological studies in MCI.

and education [25, 26]. According to Einstein aging study, prevalence of MCI in the same geographical zone is higher in Negroid population compared with Caucasians. According to Mayo clinic study of aging, MCI prevalence was 16%, among them 11.1% was amnestic MCI and 4.9% non-amnestic MCI [5]. Single domain amnestic MCI was the most frequent type, based on Mayo clinic study of aging. MCI prevalence is increasing with age, is more frequent in males and *APOE e3e4* or *e4e4* allele carriers. The estimated prevalence of mild cognitive impairment in non-demented cohort of 65 years old or older in the Cardiovascular Health Study was 19% and it increased with age [27].

Recently an international consortium—Cohort Studies of Memory in an International Consortium (COSMIC) harmonized data from 11 studies from USA, Europe, Asia and Australia and applied

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uniform diagnostic criteria to more reliably estimate MCI prevalence across different geographical and ethno-cultural regions. They have applied three different diagnostic criteria, such as performance in the bottom 6.681%, Clinical Dementia Rating of 0.5 and Mini-Mental State Examination (MMSE) score of 24–27. Prevalence rates before standardization varied between 5.0 and 36.7%. These estimates were reduced with all definitions ranging between 1.8 and 20.7%. The lowest crude prevalence (5.9%) was obtained with the first definition and highest (12%) with MMSE score of 24–27 [25] (**Table 2**).

The overall incidence of MCI based on various trials is in range of 21.5 to 71.3 per 1000 person/year and significantly depends on age. In addition, cardiovascular disease, stroke, Diabetes type 2, Negroid and Hispanic ethnicity are associated with high frequency of MCI. The incidence of aMCI is lower in most of the studies and ranges from 8.5 to 25.9 per 1000 person-years [5, 25, 28, 29] (**Table 3**).

4. Clinical diagnosis of mild cognitive impairment

All patients with suspected MCI should undergo detailed physical, neurological, cognitive, psychological and functional status evaluation. It is important to identify potentially reversible causes of MCI, such as depression, thyroid diseases, vitamin B12 and foliate deficiency. Special attention should be given to the prescription history. Some medications, including sedatives, narcotic pain medications, anticonvulsants or anticholinergics have potential to affect cognitive function. An accurate *neurological* assessment is essential to determine potential etiology of cognitive impairment [13, 30].

For the accurate diagnosis it is highly important to interview patient's family member or close acquaintance, which is familiar with their functioning in daily activities, requiring planning, organization and communication skills. Ideally, an informant should know the patient for years to adequately recognize deterioration from a baseline of functioning. Information received from different sources should be integrated properly [13].

Clinician should be aware, that cognitive impairment is often accompanied by anxiety, which interferes with cognitive performance; therefore, interview should be held in relaxed and conversational manner.

Examiner should inquire about patient's ability to handle technical devices. For example, patients with MCI can drive cars normally, but they might experience episodes of disorientation when they are driving in an unknown environment, or have a tendency to make wrong turns. Patients with MCI can have particular difficulties while planning a trip or social activities and they might need more time to perform complex activities that require planning and organization [13].

Information should be collected about patient's ability to manage financial operations. Individuals with MCI may require more time to perform monetary transaction, or periodically make careless mistakes.

Cognitive assessment should be performed at the end of the interview, preferably without an accompanying person. Objective demonstration of cognitive dysfunction is obligatory to

diagnose MCI. Therefore, examiner should conduct one or more cognitive batteries. Cognitive assessment should incorporate memory, attention, executive function, language and visuospatial function evaluation in order to precisely differentiate MCI subtypes. There is no consensus on the type and number of neuropsychological tests that should be used to assess individuals with MCI. Various cut-off points are used to define abnormal cognitive performance (1.0, 1.5 and 2.0 SD). Commonly a deterioration cut-off point of 1.5 SD is adopted. There is no single recommended "gold standard" battery, but rather a set of valid cognitive tests [31]. Commonly used tests are represented in the **Table 4**.

Cognitive screening tests are helpful in clinical practice as a first step to evaluate patients with MCI, followed by formal neuropsychological assessment in selected cases. Andrew J Larner has reviewed data from several diagnostic test accuracy studies [32]. Summarized data on diagnostic validity are shown in the **Table 5**.

In 2016, a workgroup meeting was held at the Institute of Memory Impairments and Neurological Disorders of the University of California, Irvine, USA with the aim to provide recommendations for the diagnosis of mild cognitive impairments. According to the recommendations, workup with standard laboratory tests, neuropsychological assessment, and structural brain imaging is required to diagnose MCI. Assessment of cognitive performance with specific cognitive tests should be considered by the clinicians when delivering the MCI diagnosis. patients should be provided with a written summary of the diagnosis and treatment recommendations that include referral to appropriate supportive services and other local resources; Amyloid imaging may allow a physician to give the patient additional information about potential causes of MCI, improve prognostic information, and reduce the ambiguity and uncertainty associated with the diagnosis. Communication of negative scan results should include that patients with MCI who have a negative scan results remain at risk for dementia and that negative scans, while informative, do not indicate a specific diagnosis or unambiguously signify the absence of disease. Negative amyloid imaging result reduces the possibility that MCI is due to Alzheimer's disease. It also reduces the risk of MCI progression to dementia. Although the likelihood of underlying Alzheimer's disease or any

Cognitive domains	Tests
Memory and learning	Rey Auditory Verbal Learning Test
	Logical Memory Subset of WSM-R
	The New York University Paragraph Recall Test
	Buschke Cued Recall Selective Reminding Test
Language	Semantic and phonemic fluency
Executive function	Trial-Making test
Praxis	The Rey-Osterreith complex figure
	Mimicking the use of objects and Symbol gestures of communication (e.g. inserting a sheet of paper into an envelope; the correct one hand movements designed to wave "goodbye"; cutting a sheet of paper with a pair of scissors; and brushing teeth)

Table 4. Selected cognitive instruments.

Cognitive screening tests	Cut-off	Sensitivity	Specificity
Mini Mental State Examination (MMSE)	≤22/30	1.00	0.28
Mini Mental Parkinson (MMP)	≤20/32	0.92	0.61
Montreal Cognitive Assessment (MoCA)	≥26/30	0.93	0.60
Test your memory (TYM)	≤42/50	0.79	0.54
Mini-Addenbrooke's cognitive examination (M-ACE)	≤25/30	1.00	0.43
Mini-Addenbrooke's cognitive examination (M-ACE)	≤21/30	0.77	0.82
Six item cognitive impairment test (6CIT)	≤9/28	0.66	0.70

Table 5. Selected screening tools in MCI [32].

other neurodegenerative disease can't be fully excluded. Positive amyloid PET scan results in patients with MCI are associated with an increased risk for developing AD dementia. It is important to discuss the risk for cognitive and functional decline and the need for additional monitoring and planning in these patients. Volumetric brain imaging and detailed neuropsychological examination in combination with PET scan results help clinician to determine MCI prognosis and outcome [33].

5. Neuroimaging of mild cognitive impairment

Early radiological studies in MCI were focused on the assessment of the entorhinal cortex (ERC) and hippocampus. Volume of the ERC and the hippocampus in MCI patients tends to be smaller and is either intermediate between normal controls and patients with AD, or similar to AD. Some studies demonstrated higher sensitivity of the entorhinal cortex compared with hippocampal volume. The annualized rate of the hippocampal and entorhinal cortex atrophy has been shown to be more prominent in the MCI patients relative to normal controls [5, 34].

Apart from medial temporal lobe atrophy, decrease in gray matter volume was reported in the lateral temporal, parietal, and frontal lobes, amygdala, fusiform gyrus, cingulate, parietal and occipital lobes and insula. Several studies have documented that the whole brain volume loss rate is associated with objective cognitive decline over time.

Apostolova et al. followed a cohort of MCI subjects clinically and neuropsychologically for 3 years. They found that smaller hippocampal volumes predict conversion of MCI to AD and patients with MCI who convert to AD have greater atrophy in the CA1 and subiculum regions of the hippocampus [35].

Several studies reported significant alterations on diffusion weighted MR imaging (DWI) measures in the hippocampus, thalamus, posterior cingulum (PC) and several regions in posterior white matter in MCI patients. Kantarci et al. found that on the follow up, elevated hippocampal diffusivity predicts MCI progression to AD better than hippocampal volumetry [5, 36].

Study by Delano-Wood et al. showed that diminished white matter integrity of PC was strongly predictive of MCI status. Additionally, patients with amnestic MCI demonstrated lower PC white matter integrity relative to those with non-amnestic MCI [5, 34].

FDG-PET ([18F]-2-fluoro-2-deoxy-D-glucose-positron emission tomography) studies have found substantial reduction in brain activity in some cortical regions (HC limbic system, medial thalamus, and posterior cingulate). These findings are consistent with structural MRI findings [5, 37].

SPECT studies have reported reduced cerebral blood flow (CBF) in the parietal cortex, posterior cingulated cortex and precuneus in persons with MCI. Longitudinal SPECT studies showed that the presence of AD-like hypoperfusion in the posterior posterior cingulate cortex of patients in MCI was predictive of conversion to AD [37].

Accumulation of amyloid-β (Aβ) fibrils in the form of amyloid plaques is a neuropathological hallmark of dementia caused by AD. Amyloid deposition appears an early event in AD, possibly occurring up to 20 years before clinical symptoms. Amyloid imaging has become one of the central biomarkers of AD and predictor of cognitive decline. There is evidence that a positive amyloid PET scan result in patients with MCI will help in predicting conversion to AD. Amyloid-PET may help to differentiate between different etiologies of cognitive dysfunction and in the future it may help to appropriately select patients for anti-amyloid therapy [5].

6. Treatment of mild cognitive impairment

The aim of MCI treatment is to reduce existing clinical symptoms or to delay progression of cognitive dysfunction and prevent dementia. Unfortunately, at present there is no effective pharmacological therapy of mild cognitive impairment. Clinical trials on the effectiveness of Cholinesterase inhibitors didn't prove that they can delay the onset of Alzheimer's disease (AD) or dementia in individuals with MCI. Cooper et al. performed systemic review of studies on MCI treatment. They summarized results of 9 clinical trials on Cholinesterase inhibitors. Reduction in incidence of Alzheimer's disease has not been proven with 4 high quality trials (two evaluated galantamine, one donepezil and one rivastigmine). In one of the trials donepezil and galantamine showed improvement in global cognitive functioning. However, global cognition did not improve in other five large trials of Cholinesterase inhibitors. Donepezil improved immediate memory and delayed progression to AD in MCI patients with depression without affecting their symptoms of depression [38].

In a 2-year, double-blinded, placebo-controlled study, 232 MCI patients were administered 16 mg. galantamine combined with 20 mg. memantine, galantamine only, or a placebo. The amnestic MCI subgroup in the treatment arm combining galantamine and memantine demonstrated a significant positive effect on cognition. Discontinuation of galantamine, but not memantine led to a decline in cognitive functioning [38].

Ginkgo biloba is a natural medicine widely used to enhance memory. Yang et al. conducted meta-analysis of randomized clinical trials on Ginkgo biloba in treating mild cognitive impairment or Alzheimer's disease. Data from 21 trials with 2608 patients have been analyzed [39].

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Compared with conventional medicine alone, Ginkgo biloba in combination with conventional medicine was superior in improving Mini-Mental State Examination (MMSE) scores for patients with Alzheimer's disease and mild cognitive impairment. When compared with placebo or conventional medicine in individual trials, Ginkgo biloba demonstrated similar but inconsistent findings. Adverse events were mild.

The Ginkgo Evaluation of Memory (GEM Study) study was a randomized, double-blind placebo-controlled multicenter trial, which was held in 2000–2008 years in the United States. Out of 3069 participants of the clinical trial, most of them (n=2587) didn't have cognitive dysfunction, and 15.7% (n=482) were diagnosed with mild cognitive impairment on the basis of Peterson's criteria. After completion of the 6 -year observation period no significant effect of Ginkgo biloba on the incidence of dementia could be demonstrated [40].

There is an evidence, that inflammation plays an important role in the pathophysiology of Alzheimer's disease. Several epidemiological studies showed negative association between usage of anti-inflammatory nonsteroidal medications and development of Alzheimer's disease. For example, the Canadian Study of Health and Aging involving 5276 cognitively normal subjects demonstrated that there is an association between NSAID use and a lower incidence of AD and cognitive impairment no dementia (CIND) [38].

One large multicenter study on the efficacy of the COX-II inhibitor in preventing dementia has been conducted. In the trial participated 1457 subjects with mild cognitive impairment, half of them were taking Rofecoxib, approximately for 4 years. Trial revealed significantly high frequency of Alzheimer's disease in the group that used Rofecoxib [38].

A randomized, double-blind, placebo-controlled trial of Triflusal in patients with amnestic mild cognitive impairment reported no significant effect of Triflusal treatment on cognition, although it was associated with a reduced risk of conversion to AD [38].

Centrally acting angiotensin-converting enzyme inhibitors (CACE-Is) have demonstrated positive effect on cognitive function in a study including 361 patients with AD, vascular dementia, or mixed dementias, regardless of blood pressure levels at the time of their hypertension diagnosis.

Piridebil is an antagonist of dopamine receptors. Based on experimental trials it increases acetylcholine release in hippocampus and frontal cortex. Piribedil improved cognition over 3 months in individuals with MMSE of 21–25, in one small placebo controlled study.

The role of B vitamins was studied in few clinical trials. However, the data does not yet provide adequate evidence of an effect of vitamins B on general cognitive function, executive function and attention in people with MCI. Similarly, B vitamins are unable to stabilize or slow decline in cognition, function, behavior, and global change of AD patients.

Twelve-week treatment with dietary supplementation containing an oily emulsion of docosahexaenoic acid (DHA)-phospholipids demonstrated considerable improvement in cognitive function in 25 elderly patients with MCI in a randomized controlled study. Studies support the effectiveness of omega 3 fatty acids such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) on cognitive function, depressive symptoms and general functioning in persons with MCI [41].

Cochrane review on the use of vitamin E in the treatment of mild cognitive impairment and AD did not identify evidences that alpha-tocopherol prevents MCI progression or that it improves cognitive function in people with MCI due to AD. However, there is moderate quality evidence from a single study that it may slow functional decline in AD [42].

Meta-analysis of prospective trials revealed, that Mediterranean diet reduces risks of development of Alzheimer's disease and also progression of mild cognitive impairment into dementia. Mediterranean diet could potentially exert neuroprotective effects via different mechanisms, such as reduction of inflammation and oxidative stress.

Non pharmacological treatment of MCI involves management of modifying risk factors, social and cognitive rehabilitation and physical activity.

There is growing evidence that cognitive interventions may be associated with small cognitive benefits for patients with MCI and dementia. Based on recent trials, computer training program has particular positive effect on cognition and mood. Cooper at al. reviewed two long term group psychological intervention studies. They found that 20 sessions of memory training, reminiscence, cognitive stimulation, psychomotor recreation and social interaction improved global cognition on a primary outcome in a single, very small, 6-month placebo-controlled trial. However, another trial including ten sessions of memory training, psycho education and relaxation did not improve recall on secondary outcomes in one small 6-month trial [38].

Mayo clinic professionals created a MCI intervention program called Healthy Action to Benefit Independence and Thinking (HABIT). HABIT is a 10-day (50 hours) multi-component program offered to individuals with mild cognitive impairment. The program builds on existing strengths and recognizes that procedural memory can be utilized to promote the highest level of function and independence. The program includes five essential components: Individual memory compensation training; Group supportive therapy; Yoga; Brain fitness; Wellness education. Preliminary program evaluation data suggests positive impact on self-efficacy outcomes for patients and caregivers, as well as positive impact on patient functional outcomes [43].

Exercise has been associated with positive effects on neuronal survivability and function, neuroinflammation, vascularization, neuroendocrine response to stress, and brain amyloid burden. It also helps to improve cardiovascular risk factors. Ohman et al performed systematic review of selected 22 trials examining the effect of physical exercise on cognitive performance. According to the review of studies on older subjects with MCI reported some positive effect of physical exercise on cognition, mainly on global cognition, executive function, attention and delayed recall. However, most studies performed in older subjects with dementia showed no effect of exercise on cognition [39].

7. Prognosis of mild cognitive impairment

Mitchell and Shiri-Feshki analyzed 41 high-quality cohort studies. They have found that the annual conversion rate (ACR) from MCI to dementia is approximately 5–10% and most people with MCI will not progress to dementia even after 10 years of follow-up [39]. The cumulative risk over 10 years ranged between 30 and 50%, depending on whether the studies that were analyzed used a definition of MCI that included subjective memory complaints.

Other meta-analyses of long-term (5–10 years) studies reported lower annual conversion rates of 3.3–4.2% and cumulative conversion rate ~31% over 10 years. In fact, a substantial percentage of individuals with MCI actually revert to normal. Sujuan et al. found that the annual reversion rate from MCI to normal cognition was substantially higher (18.6%) than the annual progression rate from MCI to dementia (5.6%) in a study spanning between 1992 and 2009 [44].

Studies suggest that common factors related to MCI reversion include genetics (i.e., fewer APOE ε4 alleles), preserved global functioning, subtype of MCI (i.e., non-amnestic single domain), cognitive functioning (i.e., higher standard scores on cognitive assessments), and neuroimaging (i.e., larger hippocampal volumes) [45]. Huey et al. found that single-domain executive MCI has a better outcome than amnestic MCI and that executive dysfunction in multiple-domain MCI does not independently increase the risk of progression to dementia [46].

It has been shown that as many as 30% of people with MCI have potentially treatable causes of cognitive decline. The most common of these include hypothyroidism, vitamin B12 deficiency, vascular disease, normal pressure hydrocephalus, and subdural hematoma. Another study concluded that changing the risk factors for stroke and treating depression may have contributed MCI reversion to normal [47].

Nevertheless, the proportion of patients with MCI who convert to dementia still remains significant and it is important to identify factors that facilitate progression for adequate prevention and application of both pharmacological and non-pharmacological therapies. Adequate and on timely identification of MCI in definite cases can help to plan effective strategies for prevention of progressive cognitive decline.

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Conflict of interest

We have no conflict of interest to declare.

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Chapter 7

Challenges in Dementia Studies

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Additional information is available at the end of the chapter

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Abstract

Alzheimer's and other neurodegenerative diseases are generally incurable and often difficult to diagnose accurately. Yet early and accurate diagnosis of a neurodegenerative disease can potentially contribute to more effective treatment. Hence research efforts are moving towards early identification of high risk subjects and prevention of disease progression with biomarkers. Unfortunately dementia and biomarker studies are hampered by variables such as drop outs, challenges in comparing data sets, discordant biomarker sets, availability of histopathological confirmation at death, validity of cognitive testing, and nonlinear fluctuations in cognitive domains as disease progresses in vivo in subjects. This chapter is an assessment of the challenges in the early diagnosis of dementia, as well as a presentation of the issues faced in conducting dementia and biomarker studies.

Keywords: Alzheimer's disease, dementia, mild cognitive impairment, ageing, early diagnosis, biomarkers, research

1. Introduction

Although dementia is a priority for research globally, dementia studies are very complicated to design [1, 2]. Patents have a time limit which might expire prior to completing a trial, thus complicating contracts with a pharmaceutical company to use their drugs. Drug studies may involve issues related to the use of biomarkers which have not been validated for such use, like disclosure of biomarker results to participants. The treatment target for best outcome is still unestablished, and there are no guarantees that any treatment will work. In addition the odds of success are poor based on a string of crushing defeats so far [3, 4]. Pharmaceuticals pull out of trials because of the price and risk of not succeeding. Due to the slowly progressive nature of dementia, there is a huge time-lag between the commencement of trials and obtaining results. Dementia covers a multitude of specialities, including neurologists, geriatricians, nuclear medicine physicians, radiologists, psychogeriatricians, pathologists, and psychologists. Collaboration with colleagues from different sub-specialities and with regulatory agencies is needed to successfully conduct studies.

In any diagnostic entity, there is increased heterogeneity the earlier it is addressed, and so mild cognitive impairment (MCI) is a challenging population to study due to the heterogenous phenotypes, etiologies and prognosis, both cross-sectionally and longitudinally. Furthermore, similar symptoms can often be attributed to multiple different causes, each to varying degrees. Although there is a good amount of consistency between MCI studies themselves, increased heterogeneity in the actual early disease states does result in differences in outcome between MCI studies. The new research criteria for MCI due to Alzheimer's disease (AD) is an attempt to eventually move beyond highlighting MCI as a major risk factor for AD to operationalizing the prognostication of cognitive impairment in clinical settings.

This chapter considers the methodological issues, challenges and assumptions that need to be taken into consideration when evaluating dementia and biomarker studies.

2. Challenges in data acquisition and analysis

2.1. Challenges in recruiting participants for dementia studies

Longitudinal studies are better at establishing causal directions than are cross-sectional studies. However it is not easy to recruit MCI participants, especially for a longitudinal dementia study [5]. Factors affecting eligibility for enrolment include lack of awareness of the trial, lack of benefits to the participant, stringent enrolment criteria which may exclude many people, older age of study volunteers, co-morbidity factors, disability, lack of mobility, requiring the cooperation of a partner or carer, transportation, administration of medication, too many tests, and intensive monitoring of the individual's condition and progress. In general, dementia trials usually take at least 5–6 years to discover whether a drug works or not, due to slow enrolment [6, 7]. Ramifications of this include slow development of potential new treatment, increased costs associated with clinical trials, and impact on the reliability of trial results due to changes which include scanners, investigators, personnel, and economic cycles.

In order to improve internal validity, studies may seek to make recruitment criteria more stringent so as to reduce the heterogeneity typically seen in a memory clinic. Yet in order for studies to be more relevant to clinicians, they also need to be anchored clinically, which means recruitment criteria cannot be too tough for participants to be enrolled. One way to increase the number of volunteers is to simplify recruitment enrolment criteria and screening processes. By being less stringent on suitable subjects for recruitment, more can be eligible for enrolment which helps to encourage referrals from clinicians.

2.2. Leveraging data sets

The support for small studies with less statistical and mathematical rigour to detect or demonstrate a response may be just as important as large randomised controlled trials to validate a response. Justifying resources to be spent on designing and running a study first requires more than just a good idea, but also supporting data from smaller studies, as well as available timeframe and interest. While big studies are often desirable for improving validity, relatively smaller longitudinal studies may be no less significant in exposing a scientific law, if data was

collected and analysed the right way. We should remember that the modern science of genetics was founded on cross breeding yellow and green peas and their offsprings, at a time when many competing theories were making headway.

Research efforts are moving towards early identification of high risk subjects and prevention of progression. In the preclinical space, there is not yet a lot of longitudinal biomarker data. Longitudinal data provides important knowledge of biomarkers in predicting and monitoring cognitive and functional decline. To make the most of the limited data, use of both familiar as well as more sophisticated statistical techniques is required. There is a need for equations and formulas that can embrace heterogeneity without being too complex.

The Cox regression survival analysis is one statistical approach that can distill the heterogeneity of MCI aetiologies to determine independent risk factors for MCI conversion to AD. Cox regression is a survival analysis statistical technique that enables the simultaneous comparison and adjustment of the effects of several risk factors (i.e. the predictor variables or covariates) of an unwanted event occurring. It can also accommodate covariates that are dichotomous, continuous, and even if they might change in value. The required inputs are: time to an unwanted event of interest, the unwanted event of interest, and the predictor variables. The result is expressed as hazard ratios, which is the proportion of an unwanted event of interest between groups at an instantaneous moment in time. According to the Cox regression model, the hazard for an individual is a fixed hazard for any other individual. By inputting all known variables (risk factors) in a study cohort into the Cox model, we can adjust for all of them simultaneously.

2.3. Source of subjects, where and when the study was conducted

The source of subjects is a significant point that affects rates of conversion to AD [8]. People seeking specialist care for memory loss are more selected compared with people in the community who happen to have some memory problems [9]. Different studies have different aims and designs, and different methods to operationalize criteria [7]. Cognitive complains can be spontaneous, yet not routinely elicited in some cases; and clinical assessments can be standardised in some cases but based on more subjective clinical judgement in others.

Recruitment sites are an important consideration in designing studies. Cohorts at different sites are demographically different in some ways, so academic sites perform differently from commercial sites. Some cohorts like the Australian Imaging Biomarkers and Lifestyle healthy control cohort are Apolipoprotein E ε4 (E4) enriched [10]. The Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort consists of 398 MCI subjects, who were mostly white and highly educated, had intermediate cognitive measures and cerebral spinal fluid (CSF) biomarker levels between the ADNI controls and AD groups [11], and there was also a high proportion of E4 carriers.

MCI cohorts recruited today may not be entirely relevant to tomorrow's world. Secular changes influence the predictive value of cognitive performance in dementia. For example, in the Flynn Effect [12], massive gains in IQ of Americans were observed between 1932 and 1978. Humanity seems to gain skills that make IQ tests outdated. Lifestyle technology development like software apps may further leverage our function and so delay residential care.

2.4. Challenges in comparing data sets

Retrofitting criteria and statistical models developed from experience with one cohort to another that has different demographic characteristics will end up with varying outcomes, not to mention the different combinations of measurements, cut-offs, number of subjects, and length of follow-up between samples that will further compound the variability of results [13–17].

Validity is gained when results are repeatable. Power is gained when shared data is combined. Sometimes data sets are easily comparable. For example, the ability of 3.0-Tesla (T) and 1.5-T scanners to track longitudinal atrophy in AD and MCI patients using tensor based morphometry are both similar and powerful enough to detect atrophy longitudinally [18], so it may not matter much that one cohort had their magnetic resonance imaging (MRI) on a 1.5-T scanner and another cohort had their MRI on a 3.0-T scanner. However in dementia studies, combining data sets is not a trivial issue. Comparing results from different studies that have used different methodologies is rather difficult. Combining data from different scanners introduces noise. Different positron emission tomography (PET) or MRI scanners have different scanner and software combinations. Inter scanner variability is excluded if all cross-sectional and longitudinal scans are performed on the same scanner—but this is not practical.

Lack of standardisation threatens to hamper the comparison and replication of results, increase analytical variability, and complicate the evaluation of methods [7]. Different methods of biomarker analyses give varying degrees of precision [19]. Drop outs or missing data are dealt with differently. Time lag between receiving a clinical diagnosis of subjective cognitive impairment (SCI) or MCI and enrolment differs between studies. If the time lag between diagnosis and recruitment is long, this might make one SCI or MCI cohort have more stable subjects, and so less likely to progress to a dementia subtype. Different population norms are used for neuropsychological tests, and different batteries of neuropsychological tests are used.

Given that the stability of cognition can be affected by many factors in the short term, it is important to consider what variables are corrected for when we read published studies. As mentioned above, a down side to robustly designed studies which are generally informative as they control for many factors, is that they may not simulate routine clinical practice well.

2.5. Drop outs and their risk factors

Drop outs in research studies due to relocation and loss of interest should be classified as random dropouts. However drop outs from MCI studies are not entirely random [20]. Traditional survival analysis assumes censored observations are non-informative and ignorable [21]. Yet death alters the probability of observing dementia.

Risk factors for cognitive and functional impairments in MCI can also be risk factors for dropping out early from MCI studies causing potential bias in the sample. For example, E4 is a risk factor for progression from a clinical dementia rating (CDR) of 0.5 to a CDR of 1 and above and a risk factor for cardiovascular mortality [22]. Heart failure is a risk factor for progression from mild cognitive to severe cognitive impairment, and for functional decline [23]. Stroke is a risk factor for non-amnestic cognitive and functional decline [24].

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A joint modelling approach can potentially reduce the bias which attenuates the effect of neuropathology on cognitive decline. This bias occurs if non-random drop outs are excluded from analyses, or if the last observation carried forward method is used.

3. Diagnostic challenges

3.1. Accuracy of diagnosis

The dementia field is filled with many contradictory ideas and controversies. Accuracy of dementia diagnoses has been an unresolved challenge. For example, in the religious orders study involving over 1000 nuns, the majority of cases particularly in those over 85 have AD pathology as well as several other pathologies [25]. Of the phenotypes that look like clinically probable AD, some had Lewy bodies or other predominant neurodegenerative disorders at autopsy.

3.2. Volatility of clinical outcomes

Diagnosing during the pre-dementia stages is challenged by fluctuations in cognitive ability over long periods of time [26]. In short term MCI studies, outcomes are rather volatile, such that one can revert to normal, remain MCI with improvement or deterioration in cognitive abilities, convert to dementia, improve after deteriorating further, or deteriorate again after improving. For example, in the Rochester Minnesota longitudinal study, as high as 35% of MCI reverted to normal when followed long enough [27]. However two-thirds of these ultimately progressed again to MCI or dementia. In the Pittsburgh longitudinal health study after over a decade of follow-up, a small percent return to normal after being diagnosed with MCI [28].

One way to account for the observed volatility is the rigid way disease and states are categorised. By taking a disease continuum and subjecting it to arbitrary boundaries, patients are likely to bounce in and out of them. Another cause of volatility is the random fluctuation of cognitive test scores up to half a standard deviation. Someone vulnerable near the cut-off could be having a good day and so their scores may be considered to be within the normal range, or having a bad day and so their scores may be considered to be within the MCI range. This variability of performance from day to day is not a trivial matter because it predicts future decline over and beyond cognitive performance [29]. Consecutive clinical information should be taken more seriously as it may discount initial diagnoses.

The entire trajectory of cognitive decline in one at risk of AD is not necessarily due solely to AD. To date only up to half of cognitive decline can be accounted for by neuropathology seen on autopsies of brains, e.g. AD, micro and macro infarcts, Lewy bodies, TDP-43, pre-synaptic proteins, and neuronal density and locus [30]. Pathology may trigger events or formation of other pathologies, thus causing people's brains to differ in how they respond to the predominant neurodegenerative pathology. For example, mixed AD with Lewy Bodies will have more variability in their cognition due to attention impairment [31].

3.3. The paradox of Alzheimer's disease biomarker validation studies

High quality studies validating the diagnostic utility of biomarkers involve blinding of clinicians to the biomarker results when making a clinical diagnosis, and blinding assessors of the biomarkers to the clinical diagnoses. However the diagnosis of clinically probable AD using standard criteria has an error rate of at least 20%, and definite diagnosis requires confirmatory pathology [32]. Hence no biomarker study can outweigh the quality of the clinical diagnosis even if double blinding is the gold standard. Unblinding a clinician to an amyloid PET scan result introduces circularity in the validation of the amyloid PET scan. However doing so has value as it may actually improve the certainty of an AD diagnosis or correct a wrong diagnosis of AD.

3.4. Qualitative versus quantitative approach to diagnosis

The ability to accurately diagnose the clinical group to which a subject belongs is a crucial first step for appropriate management, and for clinical trial design. Categorising participants into MCI subtypes is heavily reliant on cross-sectional performance on neuropsychological tests as compared with a matched normal cohort. However, clinical assessment rather than quantitative variables takes precedence in assigning individuals into a dementia subtype. The problem with basing the MCI criteria on objective scores is that objective scores which are arbitrarily defined are required to support the subjective complains of symptoms which fluctuate. This system of categorising MCI helps to define MCI subgroups to facilitate research studies, but adds confusion when applied to assessing individuals. It has been observed in the ADNI cohorts that study variables have significant overlap between clinical groups, and that groups differ more qualitatively than quantitatively [33].

3.5. Conundrums in dementia studies

Even with histopathological confirmation of a definite AD diagnosis at death, it can be argued that there is always a degree of circularity in testing the predictive utility of any individual biomarker or clinical marker in high risk subjects for conversion to AD, unless each factor is not associated with each other. For example, if subjects are recruited from different sites, then regrouped by biomarker profile, those recruited from tertiary memory clinics are likely to both progress to AD faster and have positive biomarker or clinical marker profiles, whatever biomarker or clinical marker is used. Therefore in testing predictive utility for conversion to AD, comparing between at least two or more biomarkers or clinical markers, may enhance study quality.

All dementia neuropathological studies are designed based on neuropathologies we currently know how to identify. Neuropathologies that we do not know how to identify due to limitations in current histopathological staining techniques are pathologies that are not studied. Should they in fact be clinically relevant, we are unable to know this.

In order to test the concept that early intervention before disruption of neuronal integrity is key in successful therapy, subjects will have to be recruited at a stage where there is minimal disruption of neuronal integrity. However, if these subjects are recruited at too early stages of disease, they may not decline for the same reason that they are recruited, so results may be negative and they are not considered to have a disease but a syndrome. Having to recruit subjects with a syndrome but not a disease classification makes it harder to apply for research

funding. If subjects are recruited after downstream processes have began, even though there is minimal disruption of neuronal integrity at enrolment, the treatment may not work. Yet it is easier to raise money when subjects are considered to have a disease.

3.6. Discordant biomarker results

Phenotypes can range between being atypical to being unambiguous. Clinical labels lose credibility when challenged by biomarker evidence which are themselves not perfect. It is possible for an amyloid PET scan to be positive and the CSF Aβ level to be high, and vice versa. It is possible for tracer uptake to be concentrated only on one brain region unilaterally. It is possible for tracer uptake to increase rapidly between serial scans within a relatively short space of time. It is possible for tracer uptake to decrease between serial scans. False negatives, albeit rare, have been reported with Pittsburgh compound B (PiB) scans [34]. Even pathological confirmation, which is the gold standard, is not an exact science. Conflicting biomarkers add complexity to diagnosis and prognostication. It is important to apply Bayesian logic (i.e. post-test probability is affected by pre-test probability and the robustness of the test) when considering differentials.

3.7. Clinical diagnosis versus clinical deterioration

Clinical diagnosis does not necessarily predict deterioration over time. It is appropriate to conclude that having a positive amyloid scan will result in AD patterns of deficits developing, but this does not exclude significant co-morbid conditions from becoming the predominant contributing factor in cognitive or functional decline. Older persons may be living long enough to accumulate another threat to the body. Thus neurodegenerative pathologies may be more relevant in pre-terminal decline than terminal decline. Death is a competing risk for seeing the clinical syndrome develop, even though the pathology is there.

4. Principles and challenges in cognitive testing

4.1. Introduction

Cognitive tests demonstrate cognitive performance. They should be considered an adjunct tool in the assessment and management of an underlying neurodegenerative condition. All tests are based on paradigms on how we learn information. In order to detect deficits, tests are designed to push people until they make errors. A low score does not diagnose dementia. A high score does not exclude dementia. A single score cannot be considered in isolation.

Confidence that cognitive tests accurately reflect subject cognition is important. Tests require a wide response distribution and evenness of scale to enable sensitive detection of clinical changes and assessment of the degree of deficits. Sensitivity to cognitive disease and change over time, enables tracking of disease progression, evaluation of treatment effectiveness, and maintains focus on the symptoms and disease of interest. Measures should be able to capture deficits, have low noise, and relate to biological markers. Characterising early presenters based on neuropsychological test performance should be detailed enough to make sense, but not overly precise—otherwise it can paradoxically complicate assessment and follow-up.

Data is currently lacking in how well tests track with amyloid. Longitudinal examination of different trajectories of cognitive decline over time can validate specific biomarker profiles, help to elucidate underlying mechanisms of disease, and predict clinical outcome. The challenge in observational studies is to be selective yet inclusive of tests that can be operationalised in all participants, and sensitive enough to track changes [7]. Regulatory agencies require that measures are well experienced and understood [35]. Application of technology can enable easier tailoring of cognitive and functioning assessment protocols to meet the needs of unique populations or settings, and extend the possibility of administering assessments and delivering interventions remotely [36].

Cognitive tests cannot extract specific unimodal factors alone. They all extract broad based processes. No neuropsychological test is orthogonal because testing is affected by many processes, like allocation of attention resources, language and executive function. All tests should be empirically derived from actual patients, then refined to improve sensitivity, reduce variability, and simplify use. When developing a test, having some overlap between measures to ensure concurrent validity is worthwhile, but there should not be too much correlation either. Some tests are more highly predictive than others. For example the semantic interference test was highly predictive of decline from MCI to dementia over an average 30 month period compared with standard memory tests such as memory for passage and visual reproduction [37].

4.2. The importance of pattern recognition

Cognitive testing is not specific for a neuropathology. External manifestations of results are due to a combination of neuropathology and cognitive reserve. Patterns of deficits on different sub-scores are important for the assessment of underlying pathology, so better testing approaches should distinguish between memory and non-memory cognitive domains. The possibility of a neurodegenerative disease is raised when there is a typical cerebral pattern of spread [38–41]. This possibility is reduced when there is no overlap between deficit patterns on sub-scores and neurodegenerative subtypes. For example, since living items is the most impaired semantic category in AD, relatively poorer scores in this category compared with others raises the odds of AD. The pattern of scores should be interpreted in context to the patient's situation, e.g. poor education, culturally and linguistically diverse background, comorbidities, conditions of the testing environment, hearing aids, glasses, tester, etc.

4.3. Difficulties with cognitive testing

Cognitive measures may not be able to detect subtle changes or effects of underlying neuropathology due to cognitive reserve, ceiling effect, or floor effect. Cognitive measures should be sufficiently sensitive and specific to detect the effects being tested for, while being clinically meaningful at the same time. Delayed logical memory or face-name tests are examples of tests that can well detect amyloid deposition in the brain [42, 43].

Cognition is a heterogeneous construct, so while more sensitive and precise measures may emerge, there will be limits to applying them across different cohorts. Reference norms differ for different patient groups. For example, IQ-adjusted norms are used to predict progressive cognitive decline in highly intelligent older individuals [44]. People who have individualised strategies for learning (that is, those with high cognitive reserve) will do much better in

general, so neuropsychological testing can be quite noisy. Non-memory tests are generally less predictive of dementia in those with more education. Neuropsychological screening tools like the mini-mental state examination are cultural and language biased even with the use of an interpreter [45]. Efficacy can be limited by ceiling effects and variability in subject performance over time. Cognitive testing may be more subjective than biomarker measurements as results can be influenced by the behaviour of persons conducting or taking the test, fatigue of the patient, and time of the day. Cognitive testing is susceptible to attention deficits, so delirium, depression, and distress can result in scores in the dementia range.

4.4. Non-linear decline trajectory

Cognitive decline in ageing and dementia follow a non-linear trajectory [46]. However, during short time intervals of only 2–3 years, changes may appear to be linear. Acceleration over time (i.e. the non-linearity) is usually clearly seen with data points 7 years and beyond. Cognitive scales may be sensitive to early changes but do not work well later, or sensitive to changes in the later stage and do not work well earlier. While considerable work needs to be conducted to establish which tasks are sensitive at particular stages of the preclinical period, the rule of thumb is that the earlier the test, is the less precise it is. Still there is an increasing interest in developing tools to detect the earliest manifestations of cognitive decline in order to prescribe remediation strategies or measure effectiveness of treatment approaches. The more sensitive the measure, the less numbers are needed in a trial.

4.5. Composite scoring

Composite testing smooths individual scores to better average the overall score. A simple approach by deriving composite scores from combining different tests can enable more equality of different tests, reduce noise and facilitate a statistically more simple analysis of relationships between cognitive domains like memory and imaging data. This would simplify studies that make comparison between groups.

The best neuropsychological test batteries are not necessarily the longest or the most comprehensive. A certain degree of precision is required, but there may be no need to be overly precise. People do dread having their neuropsychological deficits pointed out, and it can be emotionally difficult for them to sit through a battery of tests. The size of a battery matters not as much as the quality of the precision of the battery in detecting degrees of cognitive deficits.

One way to validate such neuropsychiatric composite scores is to see if similarity of results can be obtained from different cohorts. Memory composite scores like the ADNI-Mem have been found to be comparable with other memory measures in the prediction of cognitive change over time, and could also differentiate changes over time. Such composite scores were associated with neuroimaging parameters [47].

4.6. Serial scoring and practice effects

Serial assessments enable better cognitive evaluation than cross-sectional assessment. For example, the trajectory pattern of serial scores helps to differentiate between dementia and delirium. While serial assessments are better than cross-sectional assessments, they become subjected to

practice effects. Practice or re-test effects occur in non-demented adults [48]. They involve episodic memory in learning test content, procedural non-declarative learning for familiarisation with task procedures, and anxiety reduction by desensitisation. Practice effects are not necessarily a nuisance as they themselves comprise a test. For example, one study showed that the loss of short-term practice effects portends a worse prognosis after 1 year in patients with MCI [49]. When the Cogstate was repeated four times a day, having attenuated practice effect in non-demented participants detects MCI [50, 51].

5. Principles and challenges in biomarker use

5.1. Introduction

A biomarker is any identifiable biological measurement that can be objectively measured; that accurately represents underlying pathology associated with disease, like blood, CSF, or imaging; and that changes with risk or expression of disease. Biomarkers in dementia measure directly, the neuropathology that is primarily responsible, like the amount of β-amyloid (Aβ) plaques in the Alzheimer's disease brain (e.g. CSF $A\beta_{42}$ and Aβ amyloid PET), and indirectly, their downstream effects, like the amount of neuronal damage (e.g. CSF tau and volumetric MRI) or synaptic dysfunction (e.g. FDG PET). Biomarkers should not be confused with genetic risk factors, e.g. Apolipoprotein E ε4 polymorphism.

The diagnostic goals of biomarkers in dementia are to ensure significant neuropathology is present or not present in people at risk of developing dementia, so as to increase confidence in making a dementia subtype diagnosis like AD or non-AD in atypical cases, to reduce subject numbers in clinical studies, and to reduce heterogeneity in a study cohort. The prognostic goals of biomarkers are to assess risk and proximity of future decline by serving as surrogate outcome measures to demonstrate effects on downstream targets of neurodysfunction and neurodegeneration, to help define the disease stage, and reduce trial duration. The theragnostic goals of biomarkers are to serve as end point measures to prove engagement of disease modifying treatment with Aβ plaques, and to select drug of choice.

Due to the added value that biomarkers bring, they enable us to hypothesise in a much more rigorous way how we conduct dementia studies. For example, the development of disease-modifying anti-amyloid therapies is now assisted by in vivo cerebral Aβ imaging to reduce the sample size by better selection of eligible volunteers for trials and to evaluate the efficacy of treatment. Biomarkers can help in planning which drugs are safe for AD drug trials by seeing if there had been some unexpected outcome in the brain. This would potentially improve safety, minimise cost which will in turn enable more drugs to be trialled while avoiding unsafe ones. Nonetheless, at this point in time, biomarkers are not used routinely in most clinical settings in dementia management. On top of limited access or support from current clinical guidelines, no neurodegenerative disease modifying drugs are currently licenced for routine use. However, should disease-modifying therapy become available, the issue of expanding infrastructure to meet the demands for biomarkers will be a subject of further debate. The potential for the usefulness of biomarkers is fully dependant on whether or not a cure for AD or non-AD dementias can be found.

The fundamental consideration with any assessment approach in dementia, whether with clinical bedside tests or with biomarkers is how precise a measure is in determining what it is meant to be detecting. To be used as surrogates for clinical measures, biomarkers need to be validated as reflecting clinical and/or pathological disease processes, taking into account the phase of disease where they have a high degree of specificity and sensitivity [52, 53]. Standardising procedures will reduce measurement errors in clinical trials. They should apply similarly to everyone no matter what race, language or culture they come from. Ideally, the biomarkers and clinical markers must be strongly associated, yet independent of each other, in order to be used as recruitment criteria and as outcome measures, yet avoiding circularity. However validating the relationship between biomarker change and cognitive outcome is an imperfect science. Considerable challenges remain in establishing the relationship between biological and cognitive measures throughout the chronology of the preclinical phase of AD.

A measurable biomarker needs to be operable clinically, have significant clinical implications if results are positive, and have clinical utility in terms of improving confidence in diagnosing, prognosticating or guiding treatment options. Unlike cognitive assessments, biomarkers offer more objective results and are considered complimentary to memory testing. They are highly valued for their ability to detect underlying structures or neuropathology in vivo. However the evaluation of biomarkers is an expensive endeavour, and cannot be carried out without collaboration between pharmaceuticals and public institutions.

The reproducibility of biomarker results can be affected by many factors. For example, discrepancy of biomarkers and cognitive tests can happen because of a plateau of biomarkers prior to cognitive change. Individual biomarkers of amyloid PET, MRI, FDG PET, and CSF in the ADNI cohort vary in their rate of change during disease progression, such that they fit better in sigmoidal models than linear models [54]. An ideal biomarker should have a sensitivity, specificity, as well as positive and negative predictive values above 80% for whatever is it supposed to be testing for [55, 56]. Biomarkers are expensive. Risks, benefits and costs have to be discussed with the patient.

5.2. Operationalisation challenges

The challenges in operationalising biomarkers for clinical practice are: standardization of techniques; harmonising practices between settings; and developing infrastructure for community access to access them. In applying biomarkers in the clinical setting, we need to consider the noise and variability factors, whether these are going to present a critical issue when it comes to trying to apply this in cross-sectional or longitudinal evaluation. Different biomarkers provide different levels of certainty, are sensitive and specific at different disease stages and in different disease subtypes. Cross-sectional data of single time-point measures have less predictability than multiple measurements for seeing progression and outcomes in longitudinal data, which then in turn limits on-going participation. For most biomarkers, biomarker progressions are more associated with cognitive decline than baseline values [57]. This suggests that clinical trials which require recruiting at-risk subjects could be improved by using progression rather than baseline values in biomarkers to enrich the study subjects. Further studies are warranted to estimate the incremental effectiveness of improving clinical trial statistical power by using biomarker progression criteria.

Biomarkers should only offer additional information which we are unable to obtain during routine history-taking, physical examination, and investigations. Their use is more appropriate when there is some uncertainty in the clinical picture. All test results must be carefully interpreted in the context of a patient's clinical presentation. All tests have inherent limitations, so over-reliance on any test without first considering relevant clinical information is likely to lead to either over- or under-diagnosis, with potentially negative consequences. Hence we need to exercise our clinical judgement to consider how additional information helps in improving the probability of a dementia subtype diagnosis or in guiding treatment. Over-emphasising biomarkers at the expense of appreciating the context of an individual case may end up inappropriately prioritising less important aspects of a case.

Until an effect on a particular biomarker is reasonably likely to predict clinical benefit by widespread evidence based agreement, it should not be used routinely as a surrogate outcome measure in AD. The specific potential benefits of biomarkers as individuals transit from normal to SCI, SCI to MCI, or MCI to dementia states need to be identified and measured. Although further validation for currently available biomarkers is still required, advancement in the biomarker field is currently approaching a plateau, as there is still no biomarker break-through that can capture processes upstream to Aβ accumulation.

Finally, it is wrongly assumed that biomarkers are just as sensitive and specific for detecting neuropathology across the age range and across the disease stage. For example, since the standardised uptake value ratio (SUVR) is calculated using cerebellar grey matter as the reference region, in late to advanced stages there will be amyloid build-up causing reduction of SUVR. This has implications for longitudinal studies. The general reduction in amyloid load after the plateau with ageing may falsely suggest that treatments are working.

5.3. Cerebral spinal fluid biomarkers

CSF tau levels increase because of tau leaking from neuronal injury, and CSF Aβ levels decrease possibly because Aβ is crystallising in the cortices. The potential benefits of using cerebral spinal fluid biomarkers in AD research studies and prevention trials are the ability to: identify the presence of AD pathologies in the absence of cognitive symptoms; evaluate therapeutic target engagement; stage disease pathology; track progression of disease pathology; evaluate potential therapy-related disease modification; cost effectively assess multiple analytes in a single sample; and allow for better trial design with fewer subjects, shorter duration, and assessment of effects on the underlying disease pathologies.

CSF biomarkers are currently not routinely recommended for individual use in clinical practice. The disadvantage of CSF is that it requires a lumbar puncture. Not everyone is willing to have one, and also there is increased use of anticoagulation treatment in the elderly. Hence is it not suitable for population studies. Other challenges in the use of CSF include the lack of protocol and assay standardisation, sub-optimal assay reproducibility, difficulties in defining normal vs. abnormal cut-off values, misperception regarding safety, tolerability and utility of CSF collection and analysis, and the need for assay development and validity in the presence of a therapeutic agent, especially with antibody-based therapies. Agreement between CSF Aβ and florbetapir in ADNI subjects is reasonable but not great ($\kappa = 0.72$) cross-sectionally and longitudinally [58].

An analysis of within-site and inter-site assay reliability across seven centres using aliquots of CSF from normal control subjects and AD patients showed the coefficient of variation was 5.3% for Aβ, 6.7% for t-tau, and 10.8% for p-tau within centre, and it was 17.9, 13.1 and 14.6% for Aβ, t-tau, and p-tau respectively between centres [59]. The reason for the inter-laboratory precision is not well understood.

5.4. Cut-offs

Determining the threshold of a positive or negative biomarker result is arbitrary to some extent, and can be problematic. Yet it may significantly influence categories and outcomes. The essential difference between MCI and those considered to have normal cognition is evidence of objective impairment on cognitive test scores, even though cut-off scores are arbitrarily defined.

Different approaches to determining cut-offs yield different degrees of positives, and form a band of intermediates close to where the cut-offs are. A case can be made for cut-offs to be modified by age rather than by merely depending on a simple number, but this will increase complexity in the analyses. Examples of cut-off approaches include clustering analysis, 95th percentile, iterative outlier approach, absolute cut-off (e.g. SUVR over 1.50 for PiB scans), and greater than control mean plus two standard deviations.

CSF may be abnormal before PET and the discordance of low CSF $A\beta_{42}$ levels with PiB depends on the cut-offs for both [60]. Cases with discordance of both biomarkers are usually cases where one or both biomarker results are around the cut-off.

Cut-offs can have implications in the design of AD trials. Lower cut-offs for amyloid positivity ensure the sample subjects are more likely to have AD, and high cut-offs might avoid exposing individuals to the risks of treatment with little chance of benefit.

6. Ethical challenges in the disclosure of biomarker results

6.1. Introduction

By and large, the medical community tends to blur the distinction between that which is kept strictly for research, and that applied in routine clinical practice. At present, the boundaries between current research guidelines in dementia research and clinical practice are not distinct. Research criteria have a strong potential to impact clinical practice, such that terminologies used in research settings easily become adopted into routine clinical practice.

Biomarkers in dementia give risk information only, and results can be inconclusive. Until a cure is developed, the distance between advancements in diagnosis and treatment continues to grow. A positive result is not a diagnosis. Not all with positive biomarker results will develop AD. Potential harms with study participation include confusion over inconclusive results, being given wrong diagnoses, stigmatisation, exploitation, discrimination, negative affective reactions [61], escalation of insurance premiums [62], loss of the right to drive, additional work conditions, and over-protection by law which can disadvantage employers.

6.2. Disclosure of biomarker results

Disclosure of AD biomarker results is an important consideration in dementia trials. Study designs that reveal increased risk may facilitate willingness to participate [63]. People participate in studies because by knowing, they may potentially lower their risk, so they may give their time and effort [64]. Similarly investigators are more in favour of disclosing scan results to MCI than to healthy controls [65]. Communicating AD risk information has wide-ranging ethical, psychological, behavioural, and social implications. People have different views about whether or not they actually want to learn the results. Periodic assessments of mood and well-being, providing access to appropriate care if there are problems, and presence of a designate partner for support are important considerations for participation in studies.

The practice in ADNI has been not to disclose biomarker results to participants. Yet being in the Anti-Amyloid Treatment in Asymptomatic Alzheimer's (A4) study means that a participant is declaring that he has a positive amyloid PET scan. No disclosure would be needed in the A4 study if it was designed as a three-arm randomised control trial with normal controls. However this would require greater sample sizes escalating costs and complicating the informed consent process.

Although biomarker use had been limited to research, clinicians in tertiary care are often involved in biomarker research, and have an interest in the biomarker result to guide management of their patients. Before biomarkers were officially approved for routine clinical use, specialist clinicians were already applying biomarkers results informally in clinical practice with the informed consent of their patients [65]. It was openness for accumulating such experiences that drove thinking and enabled planning in biomarker validation studies. Clinicians are motivated to refer their patients for biomarker research studies, and patients are motivated to participate, when they can benefit from obtaining a copy of the results even if the biomarkers are not validated.

The more opportunities there are to use biomarkers in the clinical setting, the more we are going to find cases of amyloid PET scans showing intermediate levels of amyloid in the brain, particularly as cases requiring biomarkers to improve the diagnostic work-up tend to present with some degree of diagnostic dilemma. While these cases are the hardest to diagnose, they are also potential opportunities to further our understanding.

Both positive and negative biomarker results can benefit patients and families. A negative result brings relief, and unnecessary further clinical testing is avoided. A positive result when handled well enables early decision making when participants still have capacity, efficient channelling of resources, and it also encourages healthy lifestyle change.

6.3. Evidence-based disclosure practice

The problem with AD is not merely whether one has plaques in the brain or not, or whether people will want to know if they have the disease, but also how long do they have before they have to move into residential care, and if they do have the disease whether they can be eligible for costly drug treatment. One other consideration is what people will do once they get that

information. While disease modifying treatment is currently only available by participating in drug trials and may offer a glimmer of hope, it does have side effects and is not guaranteed to work. Clinicians need to be sensitive to the negative impact breaking bad news can have on patients, and be ready to provide support, like disease counselling. Regardless of whether patients want to know, the disease will progress, and confidently diagnosing AD will help them and their relatives make firm plans.

The need to mitigate the potential harm must be balanced by the patient's right to know their result. Cognitive biases in affective forecasting may over- or under-estimate reactions to negative events. Empirically validated methods of disclosing risk information can inform practice and policy, and avoid speculation of how long and how intensely negative reactions will last following disclosure. The full long term downstream effects of finding out and of how individuals and families interpret and handle the information is not known, so these people should be followed to observe the effects of disclosure.

One study that followed 148 cognitively normal people participating in a randomised clinical trial of genetic testing for Alzheimer's disease for 1 year after risk assessment and E4 disclosure showed that those tested as positive were 5.76 times more likely to have altered their long-term care insurance than those who did not receive E4 genotype disclosure [62]. Nonetheless the broader literature suggests that receiving a diagnosis of MCI or AD did not increase depression or anxiety in patients nor their carers in the short term, and anxiety often decreased [66]. One study that assessed the impact of genetic risk assessment on adult children of people with AD showed a slight increase in the impact of event between E4 carriers and non-carriers at 6 weeks, but the effect washed out at 6 months [67]. Hence E4 status can be revealed safely to patients without risk of long-term depression or anxiety.

7. Final word

Other than finding a cure, promoting healthy brain ageing is also important. This can be done by determining and promoting those factors that promote longevity and healthy brain ageing. Promotion involves staying mentally and physically active, staying socially engaged, and controlling cardiovascular risk factors like weight, blood pressure, cholesterol, and blood sugar, quitting smoking and having a balanced diet.

The need to be persistent, to innovate and to move forward is urgent despite numerous challenges. Whether we choose to address the conundrums or ignore them because of technical difficulties, the tsunami of the dementia epidemic will hit us in a few short years. Fortunately the dementia field has been very motivated. In spite of the numerous challenges in developing new models of understanding, diagnostic criteria, clinical markers, biomarkers, treatment, and improving diagnostic accuracy, the field is marching towards addressing, and intervening in, AD in its early stages.

Finally, attention to the nuances and caveats, and applying little tweaks in study designs can improve efficiency and study quality, reduce risk, and shed new insights.

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Conflict of interest

No conflict of interest to declare.

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Chapter 8

Passive Immunotherapy in Alzheimer's Disease

Philip J. Dolan and Wagner Zago

Additional information is available at the end of the chapter

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Abstract

The development of therapeutics for the treatment of Alzheimer's disease (AD) has been challenged with a myriad of obstacles: an evolving and incomplete understanding of disease etiology and progression, challenges with early diagnosis, multifactorial genetic and environmental factors that contribute to patient variability, and the cost of conducting lengthy clinical trials. One approach that has garnered a significant amount of attention and resources for its potential as a disease modifying approach is passive immunotherapy directed at clearing amyloid-β (Aβ) species, a pathological hallmark of Alzheimer's disease. While passive immunotherapeutic trials directed at Aβ have not yet demonstrated clinical benefit, they have prompted important advances in the application and understanding of biomarkers, patient selection, novel functional readouts, and safety monitoring. Application of these lessons has enabled more recent clinical trials to incorporate better trial designs and refine inclusion criteria to optimize patient population enrollment. In addition, new passive immunotherapy targets emerging in the clinic have emerged, as well as novel technologies to enhance future antibody therapeutics. Taken together, the advances in research and clinical science have prepared the passive immunotherapy field to advance emerging promising disease modifying treatments in AD.

Keywords: amyloid-β, tau, passive, immunotherapy, Alzheimer's disease

1. Introduction

Alzheimer's disease is a progressive neurodegenerative disease that clinically presents as a gradual onset of dementia, beginning with mild cognitive and functional deficits, leading eventually to an inability to carry out everyday tasks. Alzheimer's disease and other dementias have a reported worldwide prevalence of approximately 42 million people, with an age-standardized rate of 761 per 100,000 [1]. Current therapeutics are limited to symptomatic

approaches, such as acetylcholinesterase inhibitors and NMDA receptor (NMDAR) antagonists, which aim to enhance the function of unaffected neurocircuitry but do not target the underlying cause of the disease, thus there is a desperate need for approved disease modifying therapies.

Alzheimer's disease is characterized by the dual pathological hallmarks of extracellular senile plaques and neurofibrillary tangles, composed of the amyloid-β (Aβ) peptide and tau protein, respectively. In addition, the primary familial forms of the disease are caused by mutations that directly affect Aβ homeostasis [2]. Due to both the pathological and genetic link to disease initiation, Aβ has been a prominent target for the development of disease-modifying therapeutics.

One such therapeutic approach is anti-Aβ immunotherapy. Active immunotherapy approaches utilize either the ability of the immune system to raise polyclonal antibodies against a therapeutic composed of an Aβ sequence-derived antigen and adjuvant, while passive immunotherapy approaches treat a patient with monoclonal antibodies with known antigen binding capabilities. While a large amount of research and development has been carried out regarding active immunotherapy towards AD targets [3], this chapter will focus on passive immunotherapy in AD, with the goal of describing what has been learned from past clinical studies, and what lessons may be applied to future efforts.

2. Aβ

2.1. Mechanisms of Aβ pathophysiology

The primary component of senile plaques is Aβ, a small peptide derived from the amyloid precursor protein (APP). In AD, Aβ is formed via sequential cleavage of APP by β-secretase [4] and the presenilin-1 (PS1) subunit of γ-secretase [5], respectively. This results in peptides of varying length, ranging from 38 to 43 amino acids [6], of which $A\beta_{1-42}$ is the most amyloidogenic [7]. A central tenet in the understanding of causative factors of AD is the amyloid cascade hypothesis [8], which holds that the pathological increase of amyloidogenic Aβ in AD is a central initiating event in disease, that precedes and initiates a cascade of events that lead to other pathologies such as the formation of neurofibrillary tangles, inflammation, oxidative stress, neuronal dysfunction, and cell death [9]. While the amyloid cascade hypothesis has been challenged since first proposed [10, 11], there is abundant evidence from *in vitro* and *in vivo* studies confirming the significant role Aβ plays in inducing neurotoxicity, synaptic dysregulation, and pathology.

Degeneration of cultured neurons by treatment with aggregated forms of Aβ has been observed in multiple laboratories, and appears to correlate with extent of aggregation [7, 12]. Strong evidence indicates that soluble aggregated forms of Aβ might exert direct toxicity to neurons [13–15] through a variety of mechanisms, including (but not limited to) disruption of plasma membranes [16], dysregulation of mitochondrial function and dynamics via direct interaction [17], and excitotoxicity [18]. Confirming the centrality of Aβ's role in neurotoxicity,

myriad transgenic mouse models expressing mutant APP or APP/PS1 recapitulate many AD phenotypes, including plaque pathology, synaptic dysfunction, decreased cognition, neuroinflammation, and neuronal loss (reviewed in [19]).

One of the earliest mouse models of Aβ plaque deposition was the PDAPP mouse (Line109). These transgenic mice exhibit high human APP expression (>10-fold higher than endogenous levels), which is accompanied by extracellular Aβ plaque deposition, development of neuritic dystrophy, gliosis, and loss of synaptic and dendritic structures in the hippocampus [20]. The PDAPP mouse model was instrumental to demonstrate that therapies developed to clear Aβ deposits could potentially ameliorate functional deficits. Schenk and colleagues were the first to develop an active immunization approach using aggregated $A\beta_{1-42}$ [21], which resulted in prevention of plaque formation in mice immunized before the development of pathology, and more importantly demonstrated that the induced polyclonal response can promote plaque clearance in aged PDAPP mice via phagocytosis by resident microglia. This breakthrough was later extended by administering the anti-N-terminal Aβ monoclonal antibody (mAb) 3D6 directly to PDAPP mice (passive immunotherapy); antibodies crossed the blood-brain barrier (BBB), localized to pathological features, and induced the opsonization and clearance of senile plaques in a microglia-dependent manner [22]. These preclinical findings validated Aβ-directed passive immunotherapy as a potential therapeutic strategy for AD.

2.2. Aβ passive immunotherapy in the clinic

The first Aβ immunotherapy clinical trial utilized active vaccination with $A\beta_{1-42}$ (AN1792) and was halted during Phase IIa due to the appearance of meningoencephalitis, likely due to the infiltration of T-cells in the brain as a result of the presence of T-cell epitope(s) in the antigen, which contained the full-length $A\beta_{1-42}$ peptide [23]. However, long-term follow-up indicated that patients that developed an immune response displayed modest but significant sparing of function, as assessed by the Disability Assessment for Dementia (DAD) and the Dependence scale [24]; in addition, autopsy of a patient immunized with AN1792 without meningoencephalitis displayed an absence of plaque pathology at autopsy and the presence of Aβ-reactive microglia, indicating that AN1792 was successful at engaging phagocytes to remove plaques [25].

Concerns for safety in active Aβ vaccination trials shifted most development efforts to passive immunotherapy, which carries less risk of an inflammatory response to drug. An overview of clinical Aβ antibody efforts described in the following text is listed in **Table 1**.

2.2.1. First-generation Aβ passive immunotherapies

Bapineuzumab, directed at the N-terminus of Aβ, was the first monoclonal antibody therapy developed to target Aβ in AD. It was first tested in a phase I study in AD patients with single ascending doses ranging from 0.5 to 5 mg/kg administered every 13 weeks to evaluate safety, tolerability, and pharmacokinetics (PK) [26]. A significant safety finding of this study was the presence of vasogenic edema (VE) in the highest-dose cohort: 3/10 patients

Name	Epitope	Most recent clinical phase	References
First-generation Aβ passive immunotherapeutics			
Bapineuzumab	1–6	PhIII (terminated)	[22]
Solanezumab	16–26	PhIII	[33, 41]
Ponezumab	35–40	PhIIa (terminated)	[32, 42]
Second-generation Aβ passive immunotherapeutics			
Crenezumab	16–26 (aggregate-selective)	PhIII	[41]
Gantenerumab	3–11, 18–27	PhIII	[34]
BAN-2401	Protofibrils	PhII	[36]
Aducanumab	N-terminus	PhIII	[37, 38]

Table 1. Past and current Aβ antibody therapeutics.

displayed these abnormalities, two of whom were asymptomatic. Due to the observation of VE at 5 mg/kg a dose regimen ranging from 0.15 to 2 mg/kg, administered every 13 weeks for 18 months was selected for the multiple ascending dose phase II trial [27]. In the phase II trial, study completers that received all 6 planned infusions displayed significant improvements in DAD score and the Alzheimer's Disease Assessment Scale-Cognitive (ADAS-cog), though this effect was not observed in the intent-to-treat population. VE was observed in ~10% of bapineuzumab treated patients (half of whom were asymptomatic), in comparison to 0% of the placebo group; the appearance of VE was dose-dependent and appeared early during the course of treatment. Interestingly, the majority (10/12) of VE cases occurred in carriers of the *APOε4* allele, a risk factor for aggressive AD [28].

Two phase III trials for bapineuzumab were completed to evaluate efficacy in patients with mild to moderate AD who were either *APOε4* carriers or non-carriers in separate trials, with a lower dose regimen in the carrier trial [29]. These trials did not meet the co-primary cognitive and functional endpoints, though CSF phospho-tau, a proposed biomarker of neurodegeneration in AD, did decrease in both studies and positron emission tomography-Pittsburgh B (PET-PIB) imaging revealed less amyloid pathology in the *APOε4* carrier group treated with bapineuzumab compared to placebo. One important finding is that of the subgroup that underwent PET-PIB imaging, 6.5% of *APOε4* carriers and 36.1% of non-carriers did not have detectable PET-PIB signal at trial entry, raising concerns about misdiagnosis and improper subject selection in the trials. While these studies did not succeed in meeting primary endpoints, they did provide information to guide future trials, particularly in understanding MRI abnormalities, such as VE and microhemorrhages.

During the course of the phase III trials, the observation that VE and microhemorrhages correlated with anti-amyloid dose levels was more pronounced in *APOε4* carriers, and were normally transient and asymptomatic [30] led to the formation of an Alzheimer's

Association-led workgroup composed of industry and academic experts to advise the FDA on potential routes to monitor VE and microhemorrhages. The term amyloid-related imaging abnormalities (ARIA) was adopted to address the spectrum of MR imaging abnormalities observed with anti-amyloid therapies, spanning from sulcal effusion and vasogenic edema seen on FLAIR MRIs to hypointensities (hemosiderin deposits) on T2* MRI. The ARIA terminology was further subdivided to ARIA-E (sulcal effusion and edema) and ARIA-H (hemosiderin deposits) [31]. Recommendations from the workgroup included (a) standardization of technical and monitoring practices for MRI, (b) exclusion from trials of patients with preexisting ARIA-H, and (c) monitoring of symptoms potentially associated with ARIA. The adoption of these standards, and the understanding that ARIA is largely a short-lived treatment related effect inherent to many anti-amyloid therapies, opened the possibility of testing higher and more frequent drug administration regimens with appropriate patient safety monitoring.

In parallel with bapineuzumab, two additional anti-Aβ passive immunotherapies underwent contemporaneous clinical trials: Ponezumab, directed at the C-terminus of Aβ, underwent Phase I and IIa trials, but was discontinued after Phase IIa [32]. Solanezumab, directed at an internal epitope of Aβ and hypothesized to function by binding soluble species in the CNS and periphery, failed a phase III trial in mild AD patients [33], and a trial conducted in prodromal patients was discontinued. However, it is currently being tested in genetically-defined Alzheimer's disease populations, with results expected in 2021 (clinicaltrials.gov; Identifier: NCT02008357).

2.2.2. Second-generation Aβ passive immunotherapies

Whereas the first generation of Aβ therapeutic mAbs differed in binding to distinct antibody domains (N-, mid-, and C-terminus), the second generation are intended to primarily bind specific conformations and aggregation states. Gantenerumab, currently in two phase III trials for mild and prodromal AD, binds a discontinuous epitope consisting of the N-terminus and an internal epitope, implying a unique conformational binding specificity (clinicaltrials.gov; Identifiers: NCT01224106, NCT02051608) [34]. Crenezumab, currently in phase II and phase III trials for autosomal dominant AD and prodromal-to-mild AD, respectively, is reported to selectively bind soluble and insoluble aggregates, but not monomers (clinicaltrials.gov; Identifiers: NCT01998841, NCT03114657) [35]. In contrast to other therapeutic mAbs, crenezumab is engineered on an IgG4 backbone to reduce effector function, and microglial-mediated phagocytosis of Aβ deposits is not anticipated. BAN-2401, is in clinical development in a large phase II study in early AD patients; is proposed to selectively bind Aβ protofibrils (clinicaltrials.gov; Identifier: NCT01767311) [36].

A promising antibody candidate from this group that is currently in the clinic is aducanumab. Aducanumab is a human mAb that selectively targets soluble aggregates and fibrils, and binds the N-terminus of Aβ. Preclinical studies demonstrate that the chimeric form of aducanumab peripherally administered to an APP transgenic mouse (a) crosses the BBB and binds to plaques (b) reduces calcium overload in neurons [37], and (c) reduces plaque burden in a dose-dependent manner [38]. An interim report from a double-blind, placebo controlled

phase Ib study revealed a dose-dependent decrease of amyloid PET signal that corresponded with significant slowing of cognitive decline at 52 weeks at the highest dose level, 10 mg/kg [38]. While ARIA was reported at a similar frequency compared with previous trials, adherence to guidelines formalized by the Alzheimer's Association ARIA working group [31] allowed for higher and more frequent dosing, potentially contributing to the positive results seen in these early studies. Aducanumab is currently in phase III trials in prodromal early AD patients, with endpoints and patient populations informed by the successful phase Ib study [39]. Interestingly, enrollment for these phase III clinical trials was recently increased by approximately 15% due to patient variability in the primary functional endpoint [40].

3. Tau

While most passive immunotherapy clinical trials in AD have been directed at Aβ, key discoveries regarding tau function and contribution to disease mechanisms have prompted significant efforts directed towards tau. Hyperphosphorylated and aggregated tau protein are the main component of neurofibrillary tangles (NFTs), which, together with Abeta plaques, are considered a primary hallmark in Alzheimer's disease. Because of its intracellular localization, tau deposits have historically been thought to be unavailable to immunotherapeutic treatments. However, results outlined in this section indicate the potential for targeting tau through a passive immunotherapeutic approach.

3.1. Tau biology and pathophysiology

Since the discovery that NFTs are composed of the microtubule-associated protein tau [43–45], many efforts have been devoted to elucidating molecular mechanisms of tau pathophysiology. Tau is an intracellular microtubule binding protein, which is involved in the regulation of microtubule stability and dynamics. In the brain, tau exists principally as six different isoforms, which vary in the absence or presence of N-terminal acidic repeats and a microtubule repeat; these differences are due to the splicing in or out of exons 2, 3, and 10 [46]. In normal physiological situations, the specific ratio of tau isoforms is developmentally regulated, likely due to the changing needs of microtubule fluidity versus stability throughout development and maturity [47].

Tau is an intrinsically disordered, natively-unfolded protein [48] whose physiological function is tightly regulated by post-translational modifications—principally via phosphorylation, which regulates microtubule binding affinity [49, 50]. In the AD brain, tau aggregates to form hyperphosphorylated NFTs and inclusions, composed of paired-helical and straight filaments [51]. In contrast to the intrinsically disordered nature of monomeric tau in solution, these structures adopt an ordered structure composed of a β-sheet core comprised of central residues, surrounded by a disordered coat comprised of the C- and N-termini of the molecule [52]. In AD, the appearance of tau pathological features positively correlates with dementia and disease progression [53, 54], leading to the hypothesis that the formation of tau pathology is a primary causative agent in the development of AD.

While the stereotypic appearance and progression of tau pathology down the perforant pathway—the neurocircuit from the entorhinal cortex to the hippocampus—has been described [55, 56], the molecular mechanisms underpinning this observation had remained elusive. Neurons in the performant path have long been known to be selectively vulnerable to insult such as hyperactivity [57] and expression of AD-related presenilin mutations [58], but the discovery that, when injected into the brain parenchyma, tau from a mutant mouse could simulate the formation of tau aggregates in a previously healthy animal [59] allowed the possibility that this progression may be mediated by aggregated and misfolded forms of the protein. This was strikingly confirmed in mice with tau expression restricted to the entorhinal cortex: in these mice, tau pathology propagated from the region of expression to distant efferent neurons [60, 61], demonstrating that direct cell-cell contact was not required for propagation, and that the pathological signal could be spread trans-synaptically. The demonstration that tau itself was present in interstitial fluid [62], could be secreted from neurons [63], and passed between cells [64] and neurons [65] provided evidence that tau species themselves could be directly transmitted between neurons *in vivo*, providing a potential mechanistic basis for the propagation of tau pathology. Although tau and Aβ are likely associated with different pathophysiological processes in Alzheimer's disease, the presence of pathogenic extracellular tau species could theoretically also be targeted by immunotherapeutic approaches, in this case by a different mechanism of action: interception/sequestration and prevention of cell-to-cell transmission.

3.2. Tau passive immunotherapy

An overview of preclinical and clinical tau antibody efforts described in the following text is listed in **Table 2**.

Pioneering tau immunotherapy studies demonstrated that immunization with phospho-tau peptides (phosphorylated at Ser396/404) in two different tau transgenic lines raised anti-tau antibodies, which immunohistochemically stained the brains of P301L-tau transgenic mice. In addition, active immunization resulted in reductions in tau pathology. The mice also displayed improved performance in motor tasks [66, 67]. Purified anti-tau antibodies from

Name	Epitope	Most recent development phase	References
MC1	7–9, 313–322	Preclinical	[69, 70]
BIIB092/BMS986168	17–28	PhII (recruiting)	[85, 88]
ABBV-8E12	25–30	PhI open label extension	[81, 82]
Cis mAb	*Cis*-pT231	Preclinical	[74]
RO7105705	pSer409	PhII (recruiting)	[71]
PHF1	pSer396/404	Preclinical	[67, 69, 70]
TOMA	Tau oligomer	Preclinical	[76]

Table 2. Tau clinical and preclinical antibodies discussed in this chapter.

immunized mice were peripherally injected into naïve transgenic mice and localized to neurons in the brain displaying tau pathology, demonstrating their ability to cross the blood-brain barrier (BBB) and localize to their target. In a separate study performed by the same lab, passive administration of the mAb PHF1, directed at the Ser396/404 phosphoepitope, also resulted in reductions in tau pathology in mice compared to isotype control [68]. The findings from this series of studies were proposed to be due to two potential mechanisms: (a) antibody-mediated clearance of extracellular tau deposits and (b) intracellular uptake of tau antibodies. The efficacy of passive immunotherapy using PHF1, as well as the conformational antibody MC1, were also confirmed in independent labs [69, 70], bolstering early evidence of this novel promising therapeutic avenue.

An antibody targeting a different phosphoepitope, pSer409, also shows promise in preclinical models; however, conclusions regarding the mechanism of antibody function were considerably different than those proposed in the initial active and passive studies described in the prior paragraph. In this study, a highly selective mAb was able to bind tau phosphorylated at Ser409 and specifically bind AD brain tissue. The mAb was shown to neutralize oligomer-induced neurotoxicity; however, the neutralization activity of the antibody was reduced in mixed neuron-microglial cultures. Antibody engineered with reduced effector function (REF) maintained neutralization activity in mixed neuron-microglial cultures, while the wild-type anti-pSer409 antibody did not prevent neurotoxicity and in fact promoted the release of pro-inflammatory cytokines from microglia [71]. Both wild-type and REF variants of the antibody prevented the progression of tau pathology in the tau P301L mouse, leading the authors to conclude that phagocytic clearance of tau structures was not a contributing mechanism of action to efficacy in the transgenic mouse model. In addition, the lack of FcR message found in isolated neurons prompted the conclusion that receptor-mediated uptake did not occur. The antibody examined in this report has been developed into a therapeutic candidate, which is currently in clinical development (clinicaltrials.gov; Identifier: NCT03289143).

Additional studies have been conducted to identify and target post-translationally modified forms of tau to explore effects of antibody treatment. One compelling approach targets a unique structural isoform of tau induced by phosphorylation of tau at T231. Phosphorylation of tau at T231 occurs during disease progression; the prolyl isomerase Pin1 normally binds and converts the pT231/Proline motif from a toxic *cis* form to a soluble nontoxic *trans* form [72]. A mAb targeting *cis* but not *trans* pT231-tau detects pathology during mild cognitive impairment (MCI) [73]. In addition to AD, this post-translational signature (as well as others) appears in the brains of traumatic brain injury (TBI) patients. When administered peripherally in a murine TBI model carried out in tau transgenic mice, the *cis*-pT231 tau antibody prevented the spread of tauopathy and cortical LTP deficits, and improved performance in the elevated plus maze, which was correlated to TBI-induced disinhibition behavior in patients [74]. Another effort targeting disease-specific forms of tau is centered around developing antibodies that bind soluble oligomeric tau—hypothesized to be the most toxic form of the molecule [75]—and have minimal binding to monomeric or mature NFTs [76]. Tau oligomer-specific monoclonal antibodies (TOMAs) were dosed via intracerebroventricular (i.c.v.) infusion to tau P301L mice. Strikingly, a single i.c.v. injection reduced

tau oligomers and histopathology, and rescued deficits in rotarod and spontaneous alternation tests. Examination of serum revealed oligomeric tau and antibody/antigen complexes, suggesting peripheral clearance as a mechanism of action [77].

Informed by studies indicating the potential for the propagation of tau pathology across cell membranes [64], as well as the demonstration of trans-synaptic transmission *in vivo* [60, 78], an independent effort to discover tau antibodies that interrupted cell-to-cell transmission yielded phosphorylation-independent antibodies that blocked uptake of tau aggregates to cultured cells [79, 80]. When administered to tau transgenic mice centrally via an Alzet minipump, these antibodies slowed the advance of tau pathology, as measured by immunohistochemical and biochemical means [79]. One of the efficacious antibodies used in this report, HJ8.5, was used in a peripheral administration model to further explore its potential as a therapeutic agent [81]. HJ8.5 is a high affinity anti-N terminal mAb that recognizes residues 25–30, which are present on all splice isoforms of tau. In this study, P301S tau transgenic mice were dosed intraperitoneally over a 3-month period with 10 or 50 mg/kg of HJ8.5. The high dose cohort displayed decreases of insoluble tau, AT8 staining, and thioflavin S staining. In addition, this cohort exhibited improvements in sensorimotor function compared to isotype control and low-dose cohorts. The preclinical efficacy profile, as well as the concordance of *in vivo* data with mechanistic *in vitro* studies, propelled the humanized analogue of this antibody into the clinic (clinicaltrials.gov; Identifier: NCT03391765) [82]. Interestingly, a separate effort focused on discovering antibodies and epitopes important for uptake and transmission determined that while N-terminal antibodies could indeed block uptake of recombinant and AD patient-derived tau, there were other epitopes with potentially more potent function, notably antibodies binding C-terminal to the acidic inserts [83].

A key component of the amyloid cascade hypothesis is that Aβ aggregation induces, either indirectly or directly, fibrillization of tau as well as other disease processes (reviewed in [84]). The finding that extracellular secreted and truncated forms of tau (termed eTau) could regulate Aβ levels demonstrated a potential upstream role of tau in relation to Aβ, complementary to the amyloid cascade hypothesis. In this study, secreted eTau was isolated from iPSC neurons derived from patients with AD; treatment of neurons with eTau displayed increases in secreted Aβ, and these increases could be prevented via application of eTau-binding antibodies such as MC1 and IPN002, which recognizes residues 17–28. Aβ levels were not affected by PHF1 antibody, as the PHF1 epitope is not present in eTau. This finding was recapitulated in transgenic P301L-tau mice; peripheral treatment with IPN002 resulted in reductions in Aβ in the interstitial fluid and cortical tissue [85]. These findings were recently confirmed by a different group using mAbs that target very similar N-terminal tau epitopes; in these studies, behavioral improvements as well as decreases in Aβ were noted in mice transgenic for mutant forms of presenilin, APP, and tau [86, 87]. IPN002 has been developed into a clinical therapeutic and is undergoing clinical trials as BIIB-092/BMS986168 (clinicaltrials.gov; Identifier: NCT03068468) [88].

Though the success of preclinical studies with tau antibodies has provided sufficient rationale to begin exploration in the clinic, a greater understanding of the full range of factors involved in tau toxicity and the mechanisms of action of tau passive immunotherapy are needed. These

mechanisms may be different than those proposed for Aβ immunotherapy. There remain conflicting details from the studies presented here, such as the relative contribution of microglial-mediated phagocytosis, the relative importance of eTau-mediated Aβ production, the extent of trans-synaptic transmission in transgenic mice with widespread expression in the brain, and the optimal epitope to target. Gaining a clearer understanding of these factors continues are a current research focus.

Clinical trials with Aβ immunotherapies have demonstrated the importance of proper clinical diagnosis, patient selection, sensitive cognition tests, and effective biomarkers to monitor efficacy and disease progression. Though some general commonalities may exist in the clinical design of Aβ and tau passive immunotherapy trials, there are substantial differences in the targets and any potential clinical development approaches. In contrast to Aβ, there are a number of non-AD tauopathies such as progressive supranuclear palsy (PSP) [89] and frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17) [90] that may provide alternative clinical development pathways to test novel tau-directed therapeutic approaches. In contrast to AD, these diseases present pathological signatures composed almost uniformly of tau and neurofibrillary tangles; in addition, FTDP-17 is an autosomal dominant disorder, genetically validating the causative role of tau. Diagnosis of these and other tauopathies have historically been made solely based on clinicopathology; due to the difficulty of diagnosis from to the overlap of symptomologies with other neurodegenerative disorders, as well as the lack of clear biomarkers, diagnosis is only confirmed at autopsy [91]. Modern tau PET imaging agents are currently under clinical investigation [92]; while early generations of tau PET tracers displayed nonspecificity and suboptimal binding and PK characteristics, the newest class of tracers display improved specificity, PK properties, and may allow for improved diagnosis in tauopathies as well as an ability to monitor tau pathology in AD clinical trials [93].

4. New targets and technologies

4.1. Targeting the immune system in AD

The vast majority of passive immunotherapeutic approaches in AD have targeted Aβ and tau; this is a natural outcome of the primacy of these proteins as the principal pathological hallmarks of the disease. The association of mutations of APP (and proteins that modulate its generation, such as presenilin-1) to familial AD, and the high degree of correlation between tau pathological development and cognition, strengthen the validity of these two proteins as important causative disease agents. However, new approaches, primarily targeting immunomodulatory proteins, are also currently under development.

The presence of neuroinflammatory processes and signatures in AD has been well established, but the exact role they play in disease etiology, or whether neuroinflammation has a primarily protective or harmful role, has not been clear (reviewed in [94]). Studies examining the complement cascade have helped to understand this duality. The synaptic pruning activity carried out by microglia is regulated by complement [95]. The initiating protein of the

classical complement cascade, C1q, is enriched in the developing mouse CNS and localizes to synapses; genetic ablation of this protein results in misregulated innervation due to increased presence of synapses [96]. While C1q is normally downregulated after development, it is elevated in normal aging [97] and disease, including AD [98]. In a transgenic APP mouse, C1q localizes to synapses, and is required for pathological synapse loss. Treatment of C1q knockout mice with oligomeric Aβ displayed no synaptic loss, indicating that C1q is a required mediator of Aβ-induced toxicity. Interestingly, an anti-C1q antibody rescued Aβ-induced synaptotoxicity *in vivo*, and LTP impairment *in situ*, when compared to isotype control [99]. These data hinted at the promise of C1q immunotherapy to provide protective benefits by neutralizing a key mediator of Aβ-induced microglial overactivation, which results in synaptic loss. The anti-C1q antibody used in this study has been developed into a human therapeutic, and is beginning clinical trials (clinicaltrials.gov; Identifier: NCT03010046) [100].

The mounting evidence of involvement of the adaptive immune system in restraining the advance of AD pathology has opened the possibility of directing passive immunotherapies to the periphery, which considerably eases the challenge of achieving sufficient drug exposure in the CNS to affect pathology. Microglia resident in the brain are known to be recruited to sites of injury such as senile plaques, but the finding that peripherally-derived bone marrow stem cells are able to enter the CNS, and differentiate into microglia [101, 102], was the first direct evidence that repopulation and recruitment of microglia from the periphery was an active process. This finding was extended to AD mouse models with the finding that peripherally-recruited microglia are mobilized by Aβ, recruited to the site of senile plaques, and are able to clear plaques via phagocytosis [103]. The protective role of these immune cells in the presence of AD-like pathology was confirmed with the observation that (a) knocking out the chemokine receptor CCR2 in an APP-transgenic mouse resulted in decreased recruitment of monocytes to Aβ plaques [104], and (b) the specific ablation of bone-marrow derived cells via diphtheria-toxin receptor expression resulted in increased Aβ plaques [105]. Furthermore, increasing trafficking of macrophages by inhibiting the normally immunosuppressive regulatory T-cells through pharmacologic or genetic methods results in reduced Aβ pathology [106].

Elucidation of the biology of inhibitory signaling pathways and proteins such as Programmed cell death protein 1 (PD-1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), known as immune checkpoints, led to the development of antibody therapeutics for use in cancer (reviewed in [107]). These therapies function by neutralizing immune checkpoints and activating T-cells, which prompts antitumor activity. The characterization of checkpoint signaling pathways, along with the findings that peripheral immune cells modulate AD-like pathology in a regulatory T-cell (T_{reg})-dependent manner, has prompted examination of the PD1/PD-L1 axis in AD. In a recent study, AD transgenic mice were treated with an anti-PD1 antibody to blockade the PD1/PD-L1 axis. Remarkably, checkpoint blockade in this model resulted in substantial rescue of performance in a behavioral assay of memory and cognition after a single dose, and mice exhibited decreases in Aβ pathology with only two dose administrations [108]. The effect on pathology was observed even in mice with profound amyloid burden. While the findings of a profound effect on functional measures after such a short dose regimen are very exciting, they should be taken with a note of caution. A follow-up study, carried out by three pharmaceutical companies using three transgenic models and numerous

PD-L1 antibodies was attempted to recapitulate these results. Despite peripheral immune activation, in all instances neither reductions in Aβ pathology nor infiltration of peripheral monocytes were detected [109]. Further studies are needed to elucidate the potential of checkpoint modulation.

4.2. Increasing blood-brain barrier (BBB) penetrance for passive immunotherapeutics

A significant barrier in the development of passive immunotherapeutics for AD is the low percentage of circulating antibody that crosses the BBB. Animal studies have indicated that ~0.1–0.5% of IgG enters the CSF from the periphery [110, 111], which is borne out by preclinical [112] and clinical [113, 114] data obtained with antibodies tested for use in AD. This has led to trials with increasing amounts of antibody administered to patients ([82]; clinicaltrials.gov, Identifier NCT03318523) with the hope of delivering sufficient amounts of antibody to the CNS to achieve a clinical effect. There are, however, indications that concentrations of antibodies are higher in brain parenchyma than what is present in CSF. The chimeric form of aducanumab reported brain:plasma AUC ratios when tested in a transgenic APP model of 1.3% [38]. This is in agreement with the finding that the concentration of protein analyte present in the interstitial fluid is approximately 10-fold higher than in the ISF [62, 115]. This could be due to the rapid turnover of CSF volume [116] compared to ISF, longer elimination times of antibodies in brain parenchyma compared with CSF, or increased residence time due to target-mediated binding. Nevertheless, methods and technologies to increase BBB penetrance of biomolecules urgently need to be applied to antibodies and other proteins.

One of the more promising approaches to increase penetrance of protein therapeutics into the brain utilize endogenous receptors that transcytose between the brain and periphery, such as transferrin receptor (TfR) [117], insulin receptor [118], and LDL receptor-related protein 1 (LRP1) [119]. Protein engineering approaches feature fusion of the therapeutic molecule to proteins, ligands, or peptides that bind these receptors and facilitate transcytosis across the BBB (reviewed in [120]). One of the best understood receptor-mediated delivery systems is the use of TfR, though a similar path has been taken in the development of technologies that utilize insulin receptor. Increased brain uptake of transferrin/antibody fusion proteins were detected in rats [121], though the relatively large size (~80 kDa) of full-length transferrin make this impractical for biotherapeutic use. The detection of increased transcytosis of anti-TfR antibodies and antibody fragments [122, 123], and later advances in antibody generation technologies, enabled bispecific antibodies that bind TfR as well as target [124]. As understanding of the transcytotic properties of TfR binding moieties have increased, so has the understanding of how best to incorporate properties to ensure delivery to the brain. For example, reducing TfR affinity improves delivery, as a low affinity anti-TfR moiety will release from the receptor faster than a high affinity moiety [124]. As receptor-binding fusions enter the clinic, further questions regarding safety and distribution changes brought about by higher CNS concentrations will need to be continually addressed [125, 126]. Work continues to identify receptors that may be useful for increasing BBB concentrations of antibodies to allow engagement with wider range of drug targets [127, 128].

5. Conclusions and future perspectives

AD provides a monumentally challenging drug development landscape. The uncertainty about disease etiology, variability in patient genetics and disease progression, and difficulties in early diagnosis are all but a noncomprehensive list of hurdles to developing effective drugs. Though development of therapeutics to slow or halt AD disease progression, including passive immunotherapeutics, have not yet yielded clinical benefit, the prospect of applying lessons learned in the clinic towards validated targets such as Aβ and tau provides optimism for future success. In addition, our understanding of the mechanisms of other principal contributing factors to disease progression will provide a variety of new targets to explore. Combined with advances in drug technology to increase the availability of biomolecules in the CNS, these clinical and biological advances offer great promise around future success in treating AD.

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Conflict of interest

PJD and WZ are employees of Prothena Biosciences.

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Alzheimer's disease was discovered over 100 years ago and still belongs to incurable neurological diseases; its pharmacotherapy is considered to be ineffective. This book presents contemporary views on the genetic, biochemical, and immunological determinants of this disease.

This book also concerns the issue of Alzheimer's disease prevention through lifestyle and physical activity. Moreover, it describes the therapies used in Alzheimer's disease to slow the progression of the disease and delay its onset. Subsequently, the authors discuss experimental and clinical trials used now and in the near future.

We hope that this book will help the readers to understand the complex mechanism leading to the development of Alzheimer's disease and indicate effective ways to prevent this disorder.

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