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Spinocerebellar Ataxia

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Contributors

Thorsten Schmidt, Jeannette Hübener, Jana Schmidt, Luis Velázquez-Pérez, Roberto Rodríguez Labrada, Hans-Joachim Freund, Georg Auburger, Cornelius Boerkoel, Hok Khim Fam, Miraj Chowdhury, Luis Velazquez-Pérez, Luis Pereira De Almeida, Yoshihisa Takiyama, Haruo Shimazaki, Stefan Strack, Ronald Merrill, Andrew Slupe, Conceição Bettencourt, Manuela Lima, Jácome Bruges-Armas, José Gazulla, María Tintoré, Cristina Andrea Hermoso-Contreras

Notice

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Meet the editor

Born in 1957, Dr. José Gazulla performed his medical studies at the University of Navarra, and received training in Neurology at the Hospital Universitario Marqués de Valdecilla, in Santander (Spain). He worked at the Hospital General de Teruel and Hospital San Jorge, in Huesca, before joining the Service of Neurology of Hospital Universitario Miguel Servet, in Zaragoza (Spain). His deployment as a general neurologist has not impaired his interest in the degenerative diseases of the nervous system, especially of the cerebellum, spinal cord and peripheral nerves. Deeply interested in neurological semiology and pathophysiology, as well as in genetics, imaging and molecular biology of central and peripheral nervous system disorders, his interest in the field of degenerative ataxias became evident from early in his professional career. As a result of this, a number of reports concerning the clinical manifestations, imaging, neurochemistry and pharmacological treatment of the degenerative ataxias have been published, in collaboration with a small number of close collaborators.

Contents

Preface

The purpose of this book has been to depict as many biochemical, genetic and molecular advances as possible, in the vast field of the spinocerebellar ataxias. In addition, potential lines of pharmacological treatment in spinocerebellar ataxia type 3, enumerated by Professor Luis Pereira, are complemented by a chapter in which the pharmacological trials of the cerebellar ataxias have been reviewed in depth. Clinical manifestations of the spinocerebellar ataxias are also included in the text, like the description by Dr. Luis Velázquez-Pérez of those in spinocerebellar ataxia type 2, and the exhaustive review about eye movement abnormalities in cerebellar disease, written by Dr. Rodríguez-Labrada.

Dr. Jose Gazulla
Service of Neurology,
Hospital Universitario Miguel Servet,
Zaragoza,
Spain

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Model Systems for Spinocerebellar Ataxias: Lessons Learned About the Pathogenesis

Thorsten Schmidt*#, Jana Schmidt* and Jeannette Hübener*
Eberhard-Karls-University Tuebingen, Medical Genetics
Germany

1. Introduction

Model systems are important tools for the investigation of pathogenic processes. Especially for diseases with a late onset of symptoms and slow progression, like most spinocerebellar ataxias (SCA), it is time-consuming or even impossible to analyze all aspects of the pathogenesis in humans. Due to the reduced lifespan of model organisms, it is possible to study disease progression in full within a reasonable timeframe and due to the shorter generation time of most model organisms more individuals can be generated and analyzed, thereby strengthening the reliability of data via an increased number of replicates. Detailed studies of the histopathology can only be performed as endpoint analyses in humans, but with the help of an animal model, multiple time points can be analyzed throughout the course of the disease. In addition, model systems allow not only for the reduction of time from idea to results but also reduce the complexity due to their smaller genome sizes, less genes, nonredundant pathways, and a simpler nervous system.

Before using a specific species to model a disease it is of interest to check whether the proteins affected in humans are conserved within the respective model organism in order to increase the probability that binding partners and other keyplayers, involved in the pathogenesis of this disease, are likewise conserved. For those SCA which are caused by polyglutamine (polyQ) expansions, the respective affected genes are conserved in most organisms used as models (Table 1). Especially the proteins affected in SCA2, SCA6 and SCA17 are conserved with high similarity down to even yeast. This is not surprising as the TATA-binding protein (affected in SCA17) or a subunit of a voltage-dependent calcium channel (affected in SCA6) are important proteins for cellular maintenance. Although polyQ repeats are comparatively frequent in drosophila (Alba et al., 2007), only the repeat region of the TATA-binding protein is conserved. For most other non-mammalian model organisms, the respective orthologues are smaller and the polyQ repeats itself or even including the whole surrounding domains are not conserved. For analyses of SCA, various model systems have been employed. From the worm (*Caenorhabditis elegans*) and the fly (*Drosophila melanogaster*) all the way to mammals, i.e. the mouse (*Mus musculus*), model systems have

* All three authors contributed equally to this work
# Corresponding author: Thorsten Schmidt, Ph.D.; University of Tuebingen; Medical Genetics; Tuebingen; Germany; Email: Thorsten.Schmidt@med.uni-tuebingen.de

made important contributions to the understanding of disease progression and will be important tools for the first line tests of potential treatment strategies.

This review aims to sum up the model systems used for the investigation of SCA and especially focuses on the lessons learned from these models about the pathogenesis of SCA. We also compare commons and differences in the results obtained using these animal models and highlight the species-specific advantages and possible problems associated with the use of this species as a model organism.

2. Lessons learned from non-mammalian models of SCA

2.1 Lessons learned from worm models

The nematode *Caenorhabditis elegans* is frequently used as a model organism, primarily because of its anatomic and biochemical simplicity as well as its genetic tractability. The worm genome encodes orthologues for about 65% of all known human disease genes. Moreover, it allows for easy and rapid establishment of transgenic lines, thus facilitating screening and characterization of human disease-causing mutations *in vivo*. Overall it is an often used model organism to analyze pathological features of neurodegenerative diseases (Huntington's disease, Parkinson's disease or Alzheimer's disease) (reviewed in Driscoll and Gerstbrein, 2003 and Brignull et al., 2006b). Except for ataxin-7, the worm contains orthologues for all SCA caused by polyQ expansion. Interestingly, for SCA *C. elegans* strains have only been generated and characterized for SCA2 and SCA3 (Ciosk et al., 2004; Khan et al., 2006; Kiehl et al., 2000; Rodrigues et al., 2007; Teixeira-Castro et al., 2011).

In the field of polyQ diseases (e.g. HD or SCA) the formation of aggregates, and therefore, the transition of polyQ proteins to their toxic forms is not well understood. Due to its transparency, *C. elegans* is especially suitable to address this question. PolyQ proteins can be attached to a fluorescent protein (e.g. GFP, YFP, CFP) and the dynamics of aggregate formation both within individual cells and over time can be examined throughout the worm lifespan. Transgenic lines can be rapidly generated by feeding *C. elegans* wildtype strains with genetically transformed bacteria or by microinjection of manipulated DNA into the germline. The worm's life-cycle of about 2 to 3 weeks under suitable living conditions is short. This allows studying the aggregate formation of many different constructs with various polyQ lengths, with or without flanking sequences of the endogenous protein and under control of a wide range of different promoters. When expressed in the body wall muscle of *C. elegans*, even short polyQ stretches (with less than 40 Qs) without any flanking sequences from endogenous proteins tend to aggregate in old worms indicating a balance of different factors including repeat length and changes in the cellular protein-folding environment over time (Morley et al., 2002). In neurons, however, the pathogenic threshold turned out to be about 35-40 repeats, which correlates well with the human disease. This means that in comparison with muscle cells, neuronal cells have a higher aggregation threshold (Brignull et al., 2006a). By way of contrast, the analysis of aggregation in the protein context of (full-length) ataxin-3 revealed that only a highly expanded polyQ stretch (Q130) was able to induce the formation of aggregates in the cytoplasm and nucleus of neuronal cells in transgenic *C. elegans* lines. Non-expanded (Q14, Q17) and even pathological expanded polyQ stretches (Q75, Q91) were diffusely distributed within neurons

	SCA1	SCA2	SCA3/MJD	SCA6	SCA7	SCA17
Human (*Homo sapiens*)	**ATXN1 815 aa (6-39 Q)**	**ATXN2 1313 aa (14-32 Q)**	**ATXN3 361 aa (12-40 Q)**	**CACNA1A 2512 aa (4-18 Q)**	**ATXN7 945 aa (7-18 Q)**	**TBP 339 aa (25-43 Q)**
Mouse (*Mus musculus*)	Atxn1 791 aa (2 Q) 89 %	Atxn2 1286 aa (1 Q) 91 %	Atxn3 355 aa (6 Q) 87 %	Cacna1a 2368 aa (no polyQ) 93 %	Atxn7 867 aa (5 Q) 87 %	Tbp 316 aa (13 Q) 95 %
Zebrafish (*Danio Rerio*)	atxn1a 781 aa (no polyQ) 52 %	si:dkey-165i4.1 1112 aa (no polyQ) 66 %	atxn3 266 aa (no polyQ) 71 %	cacna1ab 2338 aa (no polyQ) 79 %	LOC100001490 918 aa (1 Q) 55 %	tbp 302 aa (6 Q) 91 %
Fly (*Drosphila melanogaster*)	Atx-1 230 aa (no polyQ)	Atx2 1084 aa (no polyQ) 30 %	n. o.	cac 1850 aa (no polyQ) 63 %	n. o.	Tbp 353 aa (8 Q) 68 %
Worm (*Caenorhabditis elegans*)	K04F10.1 299 aa (no polyQ)	ATX2 959 aa (no polyQ)	atx-3 317 aa (no polyQ) 38 %	unc-2 2087 aa (no polyQ) 55 %	n. o.	tbp-1 340 aa (no polyQ) 59 %
Yeast (*Saccharomyces cerevisiae*)	n. o.	PBP1 722 aa (no polyQ)	n. o.	CCH1 2039 aa (no polyQ)	SGF73 657 aa (no polyQ)	SPT15 240 aa (no polyQ) 79 %

Table 1. Orthologues of the affected proteins in spinocerebellar ataxias caused by polyglutamine expansions. For each protein, its name, size in aa, number of polyglutamine repeats and % of sequence identity (if specified in HomoloGene) is listed. Sizes of human proteins depend on polyglutamine repeat number. Orthologues were selected according to Rubin et al. (2000), Ciosk et al. (2004), Khurana and Lindquist (2010) and Tsuda et al. (2005) as well as using HomoloGene (Sayers et al., 2011); Repeat numbers according to Schöls et al. (2004). n. o., no orthologue; no polyQ, the polyglutamine repeat expanded in humans is not conserved in the respective orthologue

without aggregation (Khan et al., 2006; Teixeira-Castro et al., 2011). In a truncated protein of ataxin-3, however, just 63Q are sufficient for aggregation mainly in the perinuclear region but rarely in the nucleus (Khan et al., 2006). These results are in line with observations made in mouse models, where a truncated form of the polyQ expanded protein induced more aggregates and a more progressive neurological phenotype than the full-length protein (Ikeda et al., 1996).

C. elegans is also a useful organism for studying the normal distribution and function of polyQ proteins both during development and throughout the full lifespan. For example, a SCA2 transgenic model, which expressed the *C. elegans* orthologue of the human ataxin-2 gene under the control of the endogenous promoter, revealed a strong expression of ataxin-2 in the central nervous system of adult worm, but also allowed the detection of ataxin-2 even in the early embryo, beginning around the 4-cell stage (Kiehl et al., 2000). Likewise, the expression of the worm orthologue of the human ataxin-3 was strongly detected during the late embryogenesis and during all stages of postnatal development. Interestingly, ataxin-3 was not only detected in the central nervous system (in the neuronal dorsal and ventral cord as well as in neurons of the head and tail) but was also observed in the spermatheca, vulval muscle, hypoderm, coelomocytes and body muscles (Rodrigues et al., 2007).

Using knock-out strains or knocking down expression of polyQ proteins with a siRNA loaded diet has provided another method for the study of polyQ distribution and function. The knockdown of ataxin-2 by siRNA results in reduced numbers of eggs and developmental arrest whereas the knock-out of this gene was embryonically lethal (Kiehl et al., 2000). In comparison, the knock-out of ataxin-3 results in viable animals, which show no obvious morphological abnormalities as well as normal lifespan and behaviour (Rodrigues et al., 2007) but a significantly increased resistance to stress (Rodrigues et al., 2011).

Aside from protein distribution *C. elegans* has been used to study synaptic function (Khan et al., 2006) and to perform genome-wide RNAi-based genetic screens to identify modifiers (Poole et al., 2011). Such a RNAi screen identified that the aggregation of pure polyQ repeats was enhanced by factors involved in RNA metabolism and protein synthesis (leading to an increased production of misfolded proteins) as well as factors involved in protein folding, transport and degradation (leading to decreased protein clearance) (Nollen et al., 2004).

Invertebrate models, like *C. elegans*, are also particularly useful models for first-line screenings of possible therapeutic compounds, especially in late-onset neurodegenerative diseases such as SCA. The useful nature of *C. elegans* in such screenings was demonstrated in 2007 when a first drug screening for Huntington's disease was published. Voisine et al. developed a so called food clearance assay by exploiting that *C. elegans* can easily be cultured in solution. For this assay, wildtype *C. elegans* were incubated in *E. coli* liquid culture to determine the optimal drug concentration. The optical density was used to measure the consumption of *E. coli* (food source) to indicate the growth or survival of *C. elegans*. Drugs in the established concentrations were then used to treat worms with a polyQ expanded huntingtin (Htn-Q150) and analyzed using a starvation assay (by measuring the presence or absence of GFP expression in neurons). In this assay, a HDAC inhibitor (Trichostatin, TSA) was able to suppress neurodegeneration and LiCl decreased polyQ-induced neurodegeneration, while NaCl had no effect (Voisine et al., 2007).

Although no single model organism is able to recapitulate all features of a human disease, *C. elegans* models have proven to be a very good starting point. Worm models allow answering research relevant questions *in vivo* in an easy to handle and "low-cost" organism, before generating a more complex and expensive, but also more comparable model to human diseases, like mouse models.

2.2 Lessons learned from fly models

A big advantage of disease models involving *Drosophila melanogaster* is the so called GAL4-UAS system (Brand and Perrimon, 1993; Fischer et al., 1988). A specific promoter controls the expression of the transcription factor GAL4 which binds the UAS (upstream activating sequence) in the responder construct containing the gene of interest. The use of different promoter GAL4-lines, thereby, allows controlling the expression strength and/or directing the expression of the disease–causing gene to different organs or cell types. A frequently chosen promoter is the mainly eye specific gmr-GAL4 driver (Freeman, 1996) directing the transgene to the flies eyes. *Drosophila* eyes are highly organized structures thereby allowing a macroscopic observation of the degeneration of (visual) neurons without the need of even preparing and staining brain sections. The high reproducibility, the simple breeding and the ease of analyzing neurodegeneration macroscopically make *Drosophila* models the ideal tool for the screening for and analysis of factors influencing neurodegenerative events in SCA. However, not all genes causing SCA are conserved in flies, e.g. there are no natural orthologues for ataxin-3 and ataxin-7 in *Drosophila melanogaster*. However, the *CACNA1A*, the affected gene in SCA6, as well as *ataxin-1* (Tsuda et al., 2005) and *ataxin-2* seem to be conserved albeit with only reduced homology (Rubin et al., 2000) as the CAG repeat is missing in these genes. This lack of endogenous genes excludes any knock-in or knock-out approaches and at first sight questions the chance of successful generation of transgenic models for these diseases as relevant binding partners for the affected proteins may also not be conserved. Interestingly, the sole overexpression of the *Drosophila* orthologue of ataxin-1 (*dAtx-1*) induced a similar phenotype than the overexpression of human ataxin-1 (*hATXN1*) although *dAtx-1* misses more than 60 % of *hATXN1* amino acids including the polyQ repeat (Tsuda et al., 2005). Not even a polyQ expansion is required as a high level of *hATXN1* with normal repeat length (30Q) caused neuronal degeneration (Fernandez-Funez et al., 2000). This data indicates that both *Drosophila* and human ataxin-1 are "intrinsically toxic at high levels" (Lu and Vogel, 2009). Likewise, the overexpression of *dAtx2*, the *Drosophila* orthologue of human ataxin-2, caused developmental defects and degeneration of tissues (Satterfield et al., 2002). As well the loss of dAtx2 had comparable effects, stressing the importance of maintaining normal ataxin-2 activity (Satterfield et al., 2002).

Analyses using *Drosophila* connected pathogenic mechanisms in SCA1, SCA2, and SCA3 and identified ataxin-2 as a potential key player both in SCA1 and SCA3 (Al-Ramahi et al., 2007; Lessing and Bonini, 2008): In both cases, the overexpression of *dAtx2* enhanced the neurodegeneration caused by ataxin-1 and ataxin-3, respectively, and downregulation of *dAtx2* had the opposite effect. Comparable observations were made even for a non-polyQ disease, amyotrophic lateral sclerosis (ALS) (Bonini and Gitler, 2011). This influence of *dAtx2* seems to be linked to the conserved PAM2 motif (PABP-interacting motif 2) within ataxin-2 which mediates the interaction of ataxin-2 with the Poly(A)-binding protein (PABP) (Lessing and Bonini, 2008) implicating ataxin-2 in the regulation of translation of specific mRNAs (Satterfield and Pallanck, 2006). The general importance of protein domains apart from the

polyQ repeat were first addressed using pure polyQ repeats which proved to be toxic in *Drosophila* in expanded, but not in normal lengths (Marsh et al., 2000). However, adding as few as 26 additional amino acids (such as addition of a myc and a FLAG tag) and even more, adding the surrounding amino acids of a full protein is able to even neutralize the toxic effect of expanded polyQ repeats (Marsh et al., 2000).

Drosophila models were also used to assess the relevance of the intracellular localization of the affected protein: Ataxin-2 is normally a cytoplasmic protein and the occurrence of intranuclear aggregates in SCA2 patients is still controversial as both the presence and absence of nuclear aggregates have been described (Huynh et al., 2000; Koyano et al., 2000). However, the intracellular localization of dAtx2 strongly influences the phenotype in flies. While nuclear dAtx2 induces strong neurodegeneration, the phenotype of flies with cytoplasmic dAtx2 is much milder (Al-Ramahi et al., 2007).

As SCA are neurodegenerative disorders, with ubiquitous expression of the disease causing gene in humans, glial cells are usually not the main focus of interest. However the choice of different driver lines allows for the analysis of glial vs. neuronal expression of the disease-causing genes in *Drosophila*. Data suggest that the effect of glial expression of the transgene is more pronounced than of neuronal expression (Kretzschmar et al., 2005).

Another strong advantage of *Drosophila* as a model organism is the suitability for large-scale screens for modifying factors. Such screens for ataxin-1, ataxin-3 or even pure polyQ repeats identified somehow expected proteins involved in protein folding (like chaperones) and protein degradation (components of the ubiquitin-proteasome system and autophagy) (Bilen and Bonini, 2007; Fernandez-Funez et al., 2000; Kazemi-Esfarjani and Benzer, 2000; Latouche et al., 2007). In addition, these screens gave insight into further mechanisms relevant for polyQ disease pathogenesis like cellular detoxification, protein transport, transcriptional regulation and RNA and miRNA processing (Bilen and Bonini, 2007; Bilen et al., 2006; Fernandez-Funez et al., 2000; Latouche et al., 2007). The identification of *muscleblind* (*mbl*) as a modifier of an SCA3 fly model drew attention to the role of CAG repeat RNA in the pathogenesis of SCA3 (Li et al., 2008) and led to the conclusion that not only the expanded polyQ repeat but also the RNA coding for it has an effect on the pathogenesis of polyQ diseases at least in *Drosophila*. *Muscleblind* is known to be involved in Myotonic dystrophy caused by aberrant RNA containing massive CUG expansions (Jiang et al., 2004). The expression of an untranslated CAG repeat caused neurodegeneration in *Drosophila*. This toxicity was mitigated just by the interruption of the pure CAG repeat by replacing it with a CAACAG repeat (Li et al., 2008). These results were in line with previous data for a non-polyQ SCA, SCA8, also caused by non-coding RNA. Both a normal and an expanded CAG repeat led to neurodegeneration in a fly model of SCA8 (Mutsuddi et al., 2004). Interestingly, a screen for modifiers of this phenotype caused by non-coding RNA (containing expanded CAG repeats) pointed to several pathways which were also identified as modifiers of a phenotype caused by (translated RNA coding for) expanded polyQ repeats (Mutsuddi et al., 2004). Taken together, disease models in *Drosophila* facilitated both the identification and further analysis of multiple factors and mechanisms involved in the pathogenesis of SCA.

3. Lessons learned from mammalian models of SCA

In contrast to disease models in the worm or the fly, mouse models resemble pathogenic processes in humans much closer than their non-mammalian counterparts. For example the

brain structure of mice is much closer to that of humans than those of flies or worms and mechanisms of special importance for late-onset diseases like SCA, e.g. gene expression changes during aging (Bishop et al., 2010), are better conserved. In particular, mouse models allow analyzing aspects of the disease which cannot be analyzed in simpler organisms. Although behavioural analyses are possible in *C. elegans* and *Drosophila* models, they are rather basic compared to more sophisticated behavioural tests possible with mouse models which even allow for e.g. fear and spatial learning analyses (Huynh et al., 2009).

3.1 Lessons learned from knock-out mouse models

In mouse models, it is possible to selectively inactivate a specific gene-of-interest via gene targeting. There is a large amount of insight to be gained from generating such knock-out models and a lot of information has been uncovered about the functional roles of specific genes in mammalian biology (Capecchi, 2005). To learn about the native function of genes affected in SCA knock-out mice were generated for SCA1, 2 and 3. All mice were viable, fertile and had a normal lifespan with no severe ataxic phenotype or neurodegeneration (SCA1: Matilla et al., 1998; SCA2: Kiehl et al., 2006; Lastres-Becker et al., 2008; SCA3: Schmitt et al., 2007; Switonski et al., 2011), providing evidence that loss-of-function is not the primary cause for ataxic symptoms in these disorders. However, these mice served to give indications for normal functions of the respective knock-out genes. For *ataxin-1*, the gene affected in SCA1, a role in learning and memory was identified (Matilla et al., 1998) and its function as a transcriptional co-regulator was elucidated (Goold et al., 2007). Knocking out the *ataxin-2* gene led to adult-onset obesity and reduced fertility (Kiehl et al., 2006; Lastres-Becker et al., 2008a) as well as hyperactivity and abnormal fear-related behaviour (Huynh et al., 2009). In *ataxin-3* knock-out mice increased levels of ubiquitinated proteins were detected reflecting its function as a deubiquitinating enzyme (Schmitt et al., 2007). However, in a second SCA3 knock-out model changes in the ubiquitination level were not observed. The authors suggested compensational effects as the cause for this opposing result (Switonski et al., 2011). Other analyses on SCA3 knock-out mice were able to show a protective function of ataxin-3 in the heat shock response pathway (Reina et al., 2010).

In contrast to only mild effects observed with the deletion of genes responsible for polyQ products, the knock-out of genes affected in non-polyQ SCA resulted in severe ataxic phenotypes. The deletion of the *Klhl1* gene which is mutated in SCA8 led to the loss of motor coordination due to degeneration of Purkinje cell function (He et al., 2006). The analysis of mice showing signs of a severe autosomal recessive movement disorder revealed a deletion in the inositol 1,4,5-triphosphate receptor (ITPR1 gene) as the cause of the observed symptoms. Knowing that the gene correlated to SCA15 in humans maps to the *ITPR1* genomical region, it was possible to identify a deletion in this gene as the cause of this autosomal dominant disorder (van de Leemput et al., 2007).

Taken together, the analyses of SCA knock-out mice demonstrated a toxic gain-of-function as the cause for SCA due to polyQ expansions, whereas for non-polyQ SCA loss-of-function seems to be the primary mechanism of pathogenesis.

3.2 Lessons learned from classical transgenic mouse models for SCA

Transgenic mouse models gave insight into various pathogenic mechanisms in SCA. Here, we review three examples: Lessons learned about the cell-type specificity of neuro-

degeneration, the aggregation and localization of the affected protein as well as transcriptional dysregulation caused by expanded polyQ proteins.

3.2.1 Lessons learned about the cell-type specificity of neurodegeneration

A classical transgenic mouse model is generated by using a specific promoter typically controlling the expression of a cDNA construct of the respective gene-of-interest. The effect of expressing different transgenes in a specific subgroup of neurons can be nicely compared among several proteins affected in SCA as the Purkinje-cell-specific promoter (Pcp2/L7 promoter) (Vandaele et al., 1991) was used for the generation of transgenic mice for SCA1 (Burright et al., 1995), SCA2 (Huynh et al., 2000), SCA3 (Ikeda et al., 1996), SCA7 (Yvert et al., 2000) and SCA17 (Chang et al., 2011), respectively. In the SCA1, SCA2 and SCA17 mouse models the expanded full-length transgene causes a strong degeneration of Purkinje cells (Burright et al., 1995; Chang et al., 2011; Huynh et al., 2000). By contrast, in the SCA7 mouse model, the sole expression of full-length *ataxin-7* with 90 Q induced a behavioural phenotype, but only mild degeneration of Purkinje cells in quite old mice (Yvert et al., 2000). Ironically, the expression of full-length *ataxin-7* (92 Q) in most neurons except for Purkinje cells (Garden et al., 2002; La Spada et al., 2001) or even just in Bergmann glia cells (Custer et al., 2006), led to a strong degeneration of Purkinje cells (Custer et al., 2006). Likewise, when a full-length ataxin-3 protein with 79 Q was expressed using the same promoter, no phenotype was induced. Only a fragment containing not more than a few amino acids surrounding the expanded polyQ repeat was able to induce a phenotype (Ikeda et al., 1996). These data demonstrate that Purkinje cells in transgenic mice seem to be more vulnerable by a repeat expansion within *ataxin-1*, *ataxin-2* and *ataxin-17*, than by an expansion within *ataxin-3* and *ataxin-7*, thereby –at first sight- nicely replicating the situation in humans where Purkinje cells are strongly affected in SCA1 (Cummings et al., 1999a), SCA2 (Lastres-Becker et al., 2008b) and SCA17 (Rolfs et al., 2003), but the loss of Purkinje cells can be observed but is not so prominent in SCA3 patients (Rüb et al., 2002a; Rüb et al., 2002b). In SCA7, however, Purkinje cells are typically affected (Holmberg et al., 1998), thereby possibly indicating that the pathogenic processes leading to Purkinje cell death in SCA7 differ from those in SCA1, SCA2 and SCA17.

3.2.2 Lessons learned about the aggregation of polyQ proteins and their localization

A common feature of polyQ as well as other neurodegenerative diseases is the accumulation of insoluble proteins in neurons, a feature recapitulated by most model systems of these disorders. Despite this fact the role of these so called neuronal nuclear inclusions (NIIs) in the pathological processes of polyQ diseases is still controversially discussed but it is known that these structures are associated with pathogenesis. Analysis of a *C. elegans* model of SCA3 directly linked the formation of aggregates to neuronal dysfunction (Teixeira-Castro et al., 2011), whereas several opposing results in mouse models exist. Observations in transgenic mouse models for SCA1, SCA2, SCA3 and SCA6 (Boy et al., 2010; Cummings et al., 1999b; Huynh et al., 2000; Klement et al., 1998; Silva-Fernandes et al., 2010; Watase et al., 2008) reveal that the development of a pathological phenotype is independent of the formation of inclusions excluding large aggregates as a primary cause for neuronal dysfunction. Even more, evidence exists for a protective role of inclusion bodies (Bowman et al., 2005). Inclusions in human SCA patients and respective mouse models stain positive for

ubiquitin and other components of the ubiquitin-proteasome-system (UPS) (Bichelmeier et al., 2007; Cummings et al., 1998; Holmberg et al., 1998; Klement et al., 1998; Koyano et al., 1999; Paulson et al., 1997; Schmidt et al., 2002; Watase et al., 2002; Yvert et al., 2000) pointing to an involvement of this protein degradation system in the clearance of proteins with expanded CAG repeats. In *C. elegans* it was observed that expanded polyQ tracts impair the functions of UPS (Khan et al., 2006). In brains of SCA3 patients a marked misdistribution of proteasomal subunits was detected leaving only a subpopulation of neurons with the possibility to form functional proteasome complexes (Schmidt et al., 2002). Comparable results were obtained for SCA1 patients and transgenic mice (Cummings et al., 1998) and further studies revealed that an impairment or altered function of the ubiquitin and the proteasomal degradation system could contribute to the SCA1 pathogenesis (Cummings et al., 1999b; Hong et al., 2002). Data gained using a knock-in model, though, excluded an impairment of the ubiquitin-proteasome-system as a major neuropathological cause of SCA7 (Bowman et al., 2005).

The mechanism which leads to the formation of aggregates is not well understood. It has been proposed that proteolytic cleavage of polyQ-containing proteins is required for aggregate formation, because polyQ-containing fragments are predominantly found in NIIs. Another indication for the cleavage hypotheses is the detection of protein fragments in brains of mouse models for SCA3 (Goti et al., 2004), SCA7 (Garden et al., 2002) and SCA17 (Friedman et al., 2008) as well as human SCA patients (Garden et al., 2002; Goti et al., 2004). As possible protein cleavage enzymes, caspases or calpains are under controversial discussion. For ataxin-3, calpain (Haacke et al., 2007; Koch et al., 2011) and caspase cleavage was analyzed *in vitro* (Berke et al., 2004; Pozzi et al., 2008). It was shown that a C-terminal fragment of ataxin-3 containing the polyQ stretch leads to a more progressive phenotype (Ikeda et al., 1996), but also an N-terminal fragment without the CAG repeats can cause SCA3 symptoms (Hübener et al., 2011). In addition, mice expressing a fragment of the TATA-binding protein (affected in SCA17) exhibit a more severe phenotype (Friedman et al., 2008) than those expressing a full-length protein (Friedman et al., 2007). These studies suggest that cleavage of the affected protein is important for the pathogeneses of polyQ SCA. Although neuronal nuclear inclusions (NIIs) are a common feature of polyQ diseases, in some SCA the affected protein is normally localized in the cytoplasm. For this reason, the question arose whether the intracellular localization of the affected protein is of relevance for the pathogenesis of SCA. For an polyQ expansion within an ectopic protein context (Jackson et al., 2003), for ataxin-1 (Klement et al., 1998) and for ataxin-3 (Bichelmeier et al., 2007) it was demonstrated that the nuclear localization of the affected protein is a requirement for the manifestation of symptoms. Mice in which the respective protein was kept in the cytoplasm typically had less and smaller aggregates and milder or even almost no behavioural phenotype. For SCA1, Emamian et al. (2003) even went one step further demonstrating that although the nuclear localization of ataxin-1 is required, it is not sufficient to induce a phenotype. A serine residue close to the endogenous NLS within ataxin-1 (S776) was required additionally for the induction of a phenotype (Emamian et al., 2003).

3.2.3 Lessons learned about transcription dysregulation

Transcriptional dysregulation is a common feature of most polyQ diseases, but the underlying mechanisms which cause the differential regulation remain unknown. Many

proteins affected in polyQ diseases are functioning as transcription factors/cofactors or at least interact with transcription factors: TBP (SCA17) is a general transcription factor, ataxin-7 is a part of a transcriptional co-activator complex and both ataxin-1 and ataxin-3 interact with various transcription factors (Helmlinger et al., 2006).

Especially for SCA1, the molecular basis of transcriptional dysregulation and therefore its influence on the pathogenesis is thoroughly studied. Transcriptional dysregulation mediated by ataxin-1 has been attributed to the interaction with the polyQ binding protein 1 (PQBP1). This interaction interferes with the cellular RNA polymerase-dependent transcription (Okazawa et al., 2002). Microarray analyses of SCA1 knock-in and knock-out mice revealed differential expression of proteins involved in calcium signaling (Crespo-Barreto et al., 2010). In SCA3 and SCA7, components of the NIIs are transcriptionally dysregulated, including subunits of the proteasome and heat shock proteins (Chou et al., 2010; Chou et al., 2008). Several other transcription factors such as CREB (cAMP response element binding protein) and HDAC proteins and therefore histone deacetylation is often differential regulated in polyQ diseases (McCampbell et al., 2000; McCullough and Grant, 2010). For this reason, treatment studies using HDAC inhibitors such as sodium butyrate were performed (Chou et al., 2011; McCampbell et al., 2001). In several studies, transcriptional dysregulation is associated with the degeneration of specific neurons: for SCA17, a downregulation of TrkA (nerve growth factor receptor) is linked to Purkinje cell degeneration (Shah et al., 2009), or for SCA1 an interaction of ataxin-1 and PQBP1 and therefore transcriptional dysregulation leads to selective neuronal loss in the cerebellum (Okazawa et al., 2002).

3.3 Lessons learned from YAC, BAC and knock-In mouse models

In the process of generating classical transgenic mice it is only possible to insert cDNA randomly into the animal genome, not allowing for controlling the expression of the pathogenic gene in the native genetic environment at endogenous levels or excluding alternative splicing events. Therefore, different techniques have been developed to overcome these limitations and to generate models which more closely resemble human disease conditions. One strategy was the use of a yeast artificial chromosome (YAC) containing a large fragment of the human *MJD1* locus for the generation of a model for SCA3 thus enabling the expression of a full-length *ataxin-3* gene with the endogenous regulatory elements needed for cell specificity and endogenous levels of expression (Cemal et al., 2002). Mice with expanded CAG tracts showed mild and slowly progressing cerebellar symptoms with nuclear inclusions and cell loss in specific brain regions closely resembling main features of the SCA3 disease in humans (Cemal et al., 2002). A likewise approach was used to generate a model for SCA8. Moseley *et al.* (2006) used a bacterial artificial chromosome (BAC) to control the expression of the SCA8 locus encoding a non-expressed transgene. If they would have used just a classical transgenic construct without 116 kb of flanking sequences they may not have observed that the construct is indeed expressed in both directions encoding both a non-translated RNA containing a CTG expansion as well as a polyQ containing protein expressed from the opposite strand (Moseley et al., 2006).

A different more widely used strategy in the generation of SCA mouse models is to take advantage of homologous recombination techniques leading to knock-in models. This allows for endogenous levels of expression in proper spatio-temporal patterns (Yoo et al.,

2003). The first knock-in model generated for SCA1 targeted an expanded CAG tract of 78 repeats to the endogenous *ataxin-1* mouse locus. These mice reflected genetic repeat instability observed in human SCA1 patients, but showed only mild behavioural changes in late life with no clear neuropathological changes (Lorenzetti et al., 2000). From this first attempt the conclusion was drawn that the short lifespan of mice seems to be a limiting factor and that the longer exposure of the mutant protein in humans might be necessary for the development of neuronal dysfunctions. This drawback can be overcome by either overexpression of mutant proteins or by the use of extremely long CAG tracts to produce neurodegeneration (Yoo et al., 2003; Zoghbi and Botas, 2002). Therefore, in the next knock-in model for SCA1, more CAG repeats (154 repeats) were used and this model then indeed resembled main features of the human SCA1 disease (Watase et al., 2002). Analyzing these mice it was also shown that there is no direct relationship between the degree of somatic instability and the selective neuronal toxicity (Watase et al., 2003), but that the selective neuropathology rather arises from alterations in the function of the ataxin-1 protein (Bowman et al., 2007). Furthermore, these mice served to demonstrate that a partial loss-of-function contributes to the SCA1 pathogenesis (Bowman et al., 2007; Crespo-Barreto et al., 2010; Lim et al., 2008). SCA6 knock-in mice with up to 84 (hyperexpanded) CAG repeats in the *CACNA1A* gene (encoding for a calcium channel subunit) gave evidence against the assumption that the SCA6 pathogenesis is caused by alterations of channel properties and rather indicated that it is due to the accumulation of mutant calcium channels (Saegusa et al., 2007; Watase et al., 2008). In infantile cases of SCA7 expansions of 200-460 CAG repeats were documented (Benton et al., 1998; van de Warrenburg et al., 2001) and knock-in mice with 266 CAG repeats indeed reproduced hallmark features of the infantile disease (Yoo et al., 2003). Using this knock-in model it was shown that polyQ nuclear inclusions seem to have a protective role against neuronal dysfunction, that an impairment of the ubiquitin-proteasome-system can be excluded as a major neuropathological cause (Bowman et al., 2005) and that SUMOylation influences the aggregation process of ataxin-7 (Janer et al., 2010). A most recent publication reported on the attempt to generate the first knock-in mouse model of SCA3, but due to unexpected splicing events ended up creating another SCA3 knock-out model (Switonski et al., 2011) showing some of the difficulties which may occur generating animal models.

3.4 Lessons learned from an alternative strategy to generate mouse models

An alternative approach for the generation of animal models is the use of viral injections. By using lentiviral vectors it was possible to overexpress wildtype or polyQ expanded ataxin-3 in brain regions of adult wildtype rats. An expression of polyQ-expanded ataxin-3 in the substantia nigra, an area affected in SCA3, led to the formation of ubiquitinated ataxin-3 positive aggregates, loss of dopaminergic markers and an apomorphine-induced turning behaviour. If polyQ expanded ataxin-3 is overexpressed in the striatum or cortex, regions previously not linked to SCA3 pathogenesis, by the lentiviral-based system it results in accumulation of misfolded ataxin-3 and loss of neuronal markers especially in the striatum (Alves et al., 2008b). Using the lentiviral vector system it is also possible to co-express ataxin-3 with knock-down vectors or other proteins and to analyze direct effects in specific brain regions. For example a co-expression of expanded ataxin-3 with beclin, an autophagic protein, led to stimulation of autophagic flux, clearance of mutant ataxin-3 and neuroprotective effects (Nascimento-Ferreira et al., 2011).

3.5 Treatment approaches using mouse models

At the moment, curative treatment for SCA is not possible. Only treatments directed towards alleviating symptoms are available (Duenas et al., 2006). Therefore, one or the most important goal in the research of SCA is the development of a cure.

The basic question of whether any treatment -if available- would be able to even reverse symptoms already manifested was addressed using conditional mouse models. With these models which allow to turn off the pathogenic trangene expression using the Tet-off system it was possible to demonstrate that already developed symptoms of SCA1 and SCA3 indeed can be reversed (Boy et al., 2009; Zu et al., 2004). Inhibiting or reducing the production of pathogenic proteins could therefore be a powerful tool in the therapy of dominant neurodegenerative diseases. Using the RNA interference (RNAi) technology (Mello and Conte, 2004) to inhibit the expression of mutant ataxin-1 in a mouse model of SCA1 led to improved motor coordination, restored cerebellar morphology as well as resolved ataxin-1 inclusions demonstrating the *in vivo* potential of this strategy (Xia et al., 2004). RNAi knockdown was also successfully used for a selective allele-specific silencing of mutant ataxin-3 showing to mitigate neuropathological abnormalities in a lentiviral-mediated model of SCA3 (Alves et al., 2008a; Alves et al., 2010) and may be a possible treatment approach. As protein misfolding and impaired protein degradation is implicated in the pathogenesis of polyQ SCA and other related diseases that present with intracellular inclusion bodies, supporting the correction of these alterations might be a therapeutic strategy. In this manner it was possible to show that crossbreeding of SCA1 transgenic mice with mice overexpressing a molecular chaperone leads to the mitigation of the SCA1 phenotype (Cummings et al., 2001).

In addition to genetic approaches, some of the published mouse models have already been used to test the effect of different compounds on the movement phenotype, neuronal loss and aggregate formation: Lithium carbonate enhanced the motor performances and improved spatial learning, but had neither an effect on the distribution and formation of aggregates nor did it improve the lifespan of the SCA1 knock-in mice (Watase et al., 2007). A treatment approach using lithium chloride in a *C. elegans* model for Huntington's disease, however, was beneficial (Voisine et al., 2007). A dietary supplementation with creatine improved survival and motor performance and delays neuronal atrophy in the R6/2 transgenic mouse model of Huntington's disease. In a SCA2 transgenic mouse model, however, creatine extended the Purkinje cell survival, but was not able to improve or delay ataxic symptoms (Kaemmerer et al., 2001). Two promising studies were performed using transgenic models for SCA3: The HDAC inhibitor sodium butyrate (SB) delayed the onset of ataxic symptoms and improved the survival rate by reversing polyQ induced histone hypoacetylation and transcriptional repression (Chou et al., 2011). In addition, a rapamycin ester (also called temsirolimus or CCI-779) which inhibits the mammalian target of rapamycin and upregulates the protein degradation by autophagy, reduced the number of aggregates and improved the motor performance (Menzies et al., 2010). In a study using a SCA2 mouse model, the Ca^{2+} stabilizer dantrolene was able both to alleviate motor symptoms and to reduce the loss of Purkinje cells in this model (Liu et al., 2009). Another group used a specific mouse model, the so called rolling mouse Nagoya, which has been suggested as an animal model for some human neurological diseases such as autosomal dominant cerebellar ataxia (SCA6). This model was treated with talrelin, a synthetic

analogue of the thyrotropin-releasing hormone (TRH) which alters the metabolism of acetylcholine and dopamine and therefore activates the dopaminergic system. Talrelin significantly elevated the cerebellar dopamine and serotonin levels of mice and improved the locomotion phenotype (Nakamura et al., 2005).

Other therapeutical attempts are based on the functional restoration of affected cell populations. Expanded ataxin-1 causes the degeneration of Purkinje cells thereby also negatively effects the synthesis of the insulin-like growth factor–I (IGF-I) a factor promoting Purkinje cell development (Fukudome et al., 2003). Administering this factor to SCA1 transgenic mice (SCA1[82Q]) intranasally led to significant improvement of motor coordinative abilities as well as to partial restoration of Purkinje cell survival (Vig et al., 2006). Using the same SCA1 transgenic model, improved motor skills and a higher Purkinje cell survival rate was reached after grafting neural precursor cells into the cerebellar white matter (Chintawar et al., 2009). Although there is a long way from successful treatment approaches in animal models to clinical application, the recent results give hope that treatment of SCA will be possible in the future.

4. Commons and differences between SCA models in worms, flies and mice

It is self-evident that the data acquired in different model organisms especially those obtained in non-vertebrate compared with those from vertebrate models cannot be identical. However, if results obtained in a specific model are to be translated to the situation in humans one would expect that basic mechanisms in the pathogenesis of SCA are conserved among species. Previous studies revealed that many pathogenic mechanisms are indeed comparable among species, however, also indicated that there are some differences between model organisms (Table 2). Orthologues of ataxin-2 can be found all the way down to simple organisms and even in yeast (Table 1). However, the knock-out of ataxin-2 gave rise to contradictory results among model organisms: The knock-out of the endogenous SCA2 gene in the worm and the fly is embryonic lethal. In contrast to that, SCA2 knock-out mice are viable and showed no developmental defects. Further analyses of SCA2 knock-out worms demonstrated that ataxin-2 is functioning during development, since the knockdown by RNAi results in developmental arrest.

These results indicate that the function of homologous proteins as well as the interaction of different proteins in special pathways is not conserved in the species analyzed (Kiehl et al., 2006; Kiehl et al., 2000; Lastres-Becker et al., 2008a; Satterfield et al., 2002). Since in *C. elegans* the polyQ repeats in all orthologous genes are not conserved, one could assume that much shorter repeat expansions than e.g. in the mouse may already give rise to a phenotype. However, the exact opposite seems to be true: Within the full-length context of a protein, much higher polyQ repeat numbers are required to be toxic (Khan et al., 2006; Teixeira-Castro et al., 2011). Proteins with polyQ repeats are frequent in *Drosophila* (Alba et al., 2007), but these repeats are generally encoded by interrupted rather than pure CAG repeats and, therefore, more resistant to expansion (Alba et al., 2001). This could lead to the assumption that pure CAG repeats may behave unstable in *Drosophila* as observed in human SCA patients and mouse models (Boy et al., 2010; Kaytor et al., 1997; Lorenzetti et al., 2000). However, CAG repeats seem to be perfectly stable in *Drosophila* even within a challenging genomic context (Jackson et al., 2005) pointing to a specific protection mechanism against repeat expansion in *Drosophila*.

	Caenorhabditis elegans	*Drosophila melanogaster*	*Mus musculus*
knock-out	SCA2: lethal (1) SCA3: viable (2)	SCA2: lethal (3)	SCA1/2/3/8: viable (4-9)
overexpression of pure polyQ causes phenotype	yes (10; 11)	yes (12)	yes (13; 14)
truncated protein requires less repeats to induce phenotype	yes (15)	yes (16)	yes (13)
full-length protein causes phenotype	yes (≥ 130 Q) (15; 17)	wt: no or mild exp: strong (3; 18-22)	wt: no exp: mild to strong (23-26)
instability of repeats		no (27)	SCA1/3: yes (28-30) SCA2: no (24)
repeat numbers causing phenotype	≥ 130 Q (15; 17)	≥30 Q (18)	≥ 30 Q (18; 24; 26)
increasing repeat length intensifies phenotype	yes (15; 17)	yes (3; 18-22)	yes (23-26)
formation of aggregates	yes (15; 17)	yes (31)	yes (33; 25) no (24; 32) late (30; 34)
neurodegeneration/ neuronal loss		yes (18; 19; 35)	wt: no exp: mild to strong (13; 23; 24; 36)
switching-off led to reversal of symptom		yes (31)	yes (30; 37)
transgene leads to reduced lifespan	yes (17)	yes (31)	yes (25)

References: (1) Kiehl et al., 2000; (2) Rodrigues et al., 2007; (3) Satterfield et al., 2002; (4) Matilla et al., 1998; (5) Kiehl et al., 2006; (6) Lastres-Becker et al., 2008a; (7) Schmitt et al., 2007 ; (8) Switonski et al., 2011; (9) He et al., 2006; (10) Brignull et al., 2006a ; (11) Morley et al., 2002; (12) Marsh et al., 2000; (13) Ikeda et al., 1996; (14) Ordway et al., 1997; (15) Khan et al., 2006; (16) Lu and Vogel, 2009; (17) Teixeira-Castro et al., 2011); (18) Fernandez-Funez et al., 2000; (19) Al-Ramahi et al., 2007; (20) Warrick et al., 1998; (21) Warrick et al., 2005; (22) Moseley et al., 2006; (23) Burright et al., 1995; (24) Huynh et al., 2000; (25) Bichelmeier et al., 2007; (26) Friedman et al., 2007; (27) Jackson et al., 2005; (28) Kaytor et al., 1997; (29) Lorenzetti et al., 2000; (30) Boy et al., 2009; (31) Latouche et al., 2007; (32) Silva-Fernandes et al., 2010; (33) Cummings et al., 1999b ; (34) Watase et al., 2008; (35) Lessing and Bonini, 2008; (36) Aguiar et al., 2006; (37) Zu et al., 2004

Table 2. Exemplary phenotypical features of human SCA patients compared among model organisms. For clearness, only examples for the respective phenotypic features are listed. The table is not intended to be exhaustive. (wt, normal repeat; exp, expanded repeat).

5. Conclusion

Multiple successful attempts generating transgenic animal models for SCA were performed in different species. While each model organism has its own advantages and disadvantages, all animal models contributed to the knowledge about the pathogenesis of SCA. The transparency of *C.elegans* together with the simplicity to generate transgenic models as well as the option to study neurodegeneration even macroscopically by targeting the gene of interest to the *Drosophila* eye make smaller organisms like the worm or the fly especially suitable for the screening of compounds or genetic modifiers. Since many pathologic mechanisms in SCA are conserved in these models, there is a high probability that results obtained in worms and flies can be translated to mammals. Although unsuitable for large-

scale (genetic and compound) screening approaches, mouse models are the ideal tools for verification of screening results in mammals. Viral injections now even allow a comparatively rapid analysis without the need of breeding or even generating transgenic mice. Especially to answer questions which require brain structures closer to humans or for analyses of ataxic movement or even emotional phenotypes, mammalian models are required. Taken together, model organisms are indispensable tools for the analysis of pathogenic mechanisms important for SCA *in vivo*.

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7. References

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2

Non-Mendelian Genetic Aspects in Spinocerebellar Ataxias (SCAS): The Case of Machado-Joseph Disease (MJD)

Manuela Lima[1,2], Jácome Bruges-Armas[1,3] and Conceição Bettencourt[1,2,4]
[1]*Genetic and Arthritis Research Group, Institute for Molecular and Cell Biology (IBMC), University of Porto, Porto*
[2]*Center of Research in Natural Resources (CIRN) and Department of Biology, University of the Azores, Ponta Delgada*
[3]*Serviço Especializado de Epidemiologia e Biologia Molecular, Hospital de Santo Espírito de Angra do Heroísmo*
[4]*Laboratorio de Biología Molecular, Instituto de Enfermedades Neurológicas, Fundación Socio-Sanitaria de Castilla-La Mancha, Guadalajara*
[1,2,3]*Portugal*
[4]*Spain*

1. Introduction

Monogenic disorders of Mendelian nature, defined as those resulting of mutation at a single locus, and in which the observed alteration is both necessary and sufficient for phenotypic manifestation (Gropman & Adams, 2007), constituted, until recently, the main target of gene-finding studies. Mendelian or otherwise "simple" phenotypes are frequently referred in the scientific literature in opposition to the "complex" ones; the designation of "Mendelian", therefore, should reflect the occurrence of such diseases in accordance with simple, predictable family patterns, with a single locus determining its manifestation. It has, however, become very evident that even in the case of individual causative genes, the associated phenotypes can display attributes which result in non-Mendelian patterns of the trait or disease whose expression is being considered (Gropman & Adams, 2007; Sherman, 1997). In practical terms, this implies that the number of diseases for which the respective phenotypes can be explained by the effect of mutations at a single locus is dramatically diminishing (Gropman & Adams, 2007). Several diseases, initially characterized as monogenic, are now known to be modulated by a yet undetermined number of loci. Incomplete genotype-phenotype correlations observed in a large number of diseases have, therefore, forced the widening of the monogenic model, to allow the accommodation of the remaining factors, which can potentially explain the spectrum of the phenotypic variability (Badano & Katsanis, 2002). The incompleteness of the genotype-phenotype correlations seen in such situations confirms that the product of the primary mutation is imbedded in a highly complex system, in which polymorphic variation, mutations at other loci as well as environmental variables modulate the differences observed amongst individuals (Van Heyningen & Yeyati, 2004).

Factors that produce atypical or irregular patterns of inheritance are frequently referred as "complicating factors". The understanding of the mechanisms on the basis of such patterns is pertinent not only at a theoretical level, but also due to implications in terms of diagnosis and genetic risk assessment, conditioning, furthermore, the ability to predict the initiation and course of disease (Haines & Pericak-Vance, 2006; Van Heyningen & Yeyati, 2004). On the other hand, in the context of research related with the identification of deleterious genes, such irregularities can imply, amongst other aspects, severe obstacles in the interpretation of pedigrees, as well as difficulties in the selection of families (*e.g.*, in the context of linkage studies). Several of such complicating factors are frequently cited, namely clinical variability, expressivity, pleiotropism, anticipation, incomplete penetrance, age-dependent penetrance and meiotic drive. Underlying these observations are mechanisms such as allelic and locus heterogeneity, presence of modifier genes, intergenerational instability, somatic instability, genomic imprinting and *de novo* mutations (Gilchrist et al., 2000).

Amongst the several diseases with a known causative mutation but which, nevertheless, display complicating features are those associated with triplet repeat expansions. Four classes of triplet repeat disorders are usually considered, based on the location of the repeat motif in 5´or 3´untranslated regions, in introns or in coding regions (revised in Bettencourt et al., 2007). Polyglutamine (Poly-Q) disorders are part of this "expansion disorders" group, being caused by a CAG repeat expansion in the coding region of the respective causative genes; enclosed within this group are several spinocerebellar ataxias (SCAs), namely SCA1, SCA2, SCA3, SCA6, SCA7, SCA17 as well as dentato-rubro-pallido-luysian atrophy (DRPLA). Poly-Q diseases exhibit atypical features, difficult to integrate in the classic mendelian expectations (Tsuji, 1997). Machado-Joseph disease (MJD/SCA3) is considered the most frequent SCA worldwide (Cagnoli et al., 2006; Paulson, 2007; Schöls et al., 2004); using MJD as a paradigm, this review aims to explore complicating, non-Mendelian aspects of Poly-Q SCAs, from a perspective that synthesizes the current knowledge concerning such complicating factors as well as their implications at several levels, namely at the level of genetic counseling (GC).

2. Machado-Joseph disease: general perspective

Machado-Joseph disease, also known as spinocerebellar ataxia type 3 (MJD/SCA3 - MIM 109150) is an autosomal dominant neurodegenerative disorder. Described as a disorder of adulthood, with an average age at onset rounding 40 years (Coutinho, 1992), this disease has, nevertheless, reported onset extremes of 4 (Carvalho et al., 2008) and 70 years (Coutinho, 1992). Average survival time is of 21 years (Coutinho, 1992; Kieling et al., 2007). MJD is characterized by a complex and pleiotropic phenotype, involving the cerebellar, oculomotor, pyramidal, extra-pyramidal and peripheral systems. The high clinical variability observed in this disorder has led to its systematization into three clinical types, which can occasionally be present in a single family (Coutinho & Andrade, 1978).

MJD was originally described in North American patients of Azorean ancestry, residing in the United States (Nakano et al., 1972; Rosenberg et al., 1976; Woods & Schaumburg, 1972). The history of its initial description, as three distinct clinical entities, clearly reflects the high phenotypic variability that characterizes this disorder, whose unification was dependent of the identification, in a single family, of the different clinical forms that were described in the original reports (Coutinho & Andrade, 1978). The common ancestry of the three families

that were described between 1972 and 1976 (known as Machado, Thomas and Joseph), has largely conditioned the interpretations about the origin of the disease, initially considered as Azorean, and designated as "Azorean disease of the nervous system" (Romanul et al., 1977). The molecular screening of MJD's causative mutation allowed, afterwards, the differential diagnosis, which has led to an epidemiological profile clearly distinct from the one obtained on the basis of clinical criteria alone (Lopes-Cendes et al., 1996). In Portugal, MJD represents about 58% of the families with dominant ataxias (Vale et al., 2009). In the Azores, more precisely in the small island of Flores, the disease achieves the highest values of prevalence reported worldwide (Bettencourt et al., 2008a; Lima et al., 1997).

Two main studies have addressed the issue of the worldwide origin of the MJD mutation. Gaspar et al. (2001), using flanking and intragenic markers, have identified two main haplotypes in 94% of the families studied. In the Azores, these two haplotypes were present, and were related with the islands of higher prevalence (Flores and São Miguel), indicating that two mutational events were responsible for the presence of MJD in the families of Azorean origin, a result previously disclosed by genealogical analysis (Lima et al., 1998). Aiming to determine the origin, age and dispersion of these two main mutational events, Martins et al. (2007) have conducted a more extensive haplotype analysis, which revealed that the TTACAC lineage, the most frequently found in the expanded alleles of patients from all over the world, achieved its highest variability in Asia (specifically in the Japanese population). A "Short Tandem Repeat" (STR) based haplotype was identified in this population and an approximate age of 5774±1116 years was suggested.

The MJD locus was mapped to 14q24.3-q32 in 1993 (Takiyama et al., 1993). In the following year, Kawaguchi and colleagues (Kawaguchi et al., 1994) isolated and characterized a cDNA clone, designated as MJD1a, identifying MJD's causative mutation as an expansion of a CAG motif in the coding region of the *ATXN3* gene. Initially described as containing 11 exons, *ATXN3* spans a genomic region of around 48 kb, with the CAG tract located in exon 10, at 5' (Ichikawa et al., 2001). Two novel exons were identified recently, in a study that used information from cDNA clones of Azorean MJD patients and controls (Bettencourt et al., 2010a). In the MJD locus, normal chromosomes have from 12 to 44 CAG repeats, whereas in patients, usually heterozygous, the number of repeats in the mutated allele consensually ranges between 61 and 87 (Maciel et al., 2001). Intermediate alleles are rare and, as a result, their behavior is poorly understood. For example, in a family described by Maciel and colleagues (Maciel et al., 2001), an allele with 51 repeats apparently was not associated with the disease. On the other hand, alleles of intermediate size have been associated with the MJD phenotype (*e.g.*, Padiath et al., 2005; Van Alfen et al., 2001); in the study of Van Alfen et al. (2001), four symptomatic family members presented CAG tract sizes between 53 and 54 repeats. Although rare, the cases of intermediate alleles imply that the distinction, initially very clear, between normal and mutated alleles has become much more difficult to establish. Alleles with around 50 repeats seem, in certain cases, to act as fully penetrant, a scenario that remains, nevertheless, rare (Paulson, 2007). In Portugal, and despite the high prevalence of the disease, a study of nearly 2000 chromosomes from the general population, representing all Portuguese districts, failed to detect the presence of intermediate alleles (Lima et al., 2005). Normal and pathogenic repeat size ranges are not definitive, and gathering of up-dated information should be a concern of laboratories offering molecular testing.

The *ATXN3* gene is ubiquously expressed in neuronal and non-neuronal tissues; it encodes for ataxin-3, a protein with an approximate molecular weight of 42kD, in its native form. In the neurons, ataxin-3 is found essentially in the cytoplasm (Paulson et al., 1997). Five products of the *ATXN3* gene, referring to transcripts of different sizes, were described by Ichikawa and colleagues (Ichikawa et al., 2001). More recently, the sequence of 56 distinct transcripts, generated by alternative splicing, was described, and the high transcriptional variability of MJD's causative gene has been demonstrated (Bettencourt et al., 2010a). Ataxin-3 belongs to the family of cysteine-proteases; structurally it is composed by 339 aa, to which a variable number of glutamines is added (Poly-Q tract) (Kawaguchi et al., 1994). This protein is composed by a Josephine domain (JD), located in its N-terminal portion, containing, at its C-terminal, two or three ubiquitin-interacting motifs (UIMs) and the Poly-Q tract. It has been proposed that the native form of the protein acts as a deubiquitinating enzyme in the ubiquitin-proteossome pathway (revised in Bettencourt & Lima, 2011). Therefore, evidence concerning the proprieties of ataxin-3 suggests that this protein participates in cellular pathways related to quality control of proteins (see, amongst others, Schmitt et al., 2007). The involvement of the normal form of the protein in the regulation of transcription has also been suggested (Chou et al., 2008).

In the MJD locus, the presence of an expanded allele leads to a protein enriched in glutamines. The Poly-Q tract expansion should lead to a gain of neurotoxic function of the corresponding protein and, as a consequence, to neuronal death, by a process which remains, nevertheless, incompletely understood. Models of pathogenesis include the formation of toxic oligomers of ataxin-3, as well as aberrant protein-protein interactions, which disrupt normal cellular functions; revisions on the aspects of MJD's pathogenesis can be found, amongst others, in Paulson (2007) and Katsuno et al. (2008).

Notwithstanding the fact that MJD constitutes a relatively well defined clinical entity, its clinical diagnosis can, in many situations, be difficult to establish. Such is the case, when the disease is at its initial stage and minor, but more specific signs are absent. Moreover, in apparently isolated cases, or in cases that appear associated to a less common geographic distribution, a clinical diagnosis can also be hard to establish with certainty (Lopes-Cendes et al., 1996). Therefore, in the differential diagnosis of MJD, molecular testing, available after the identification of the causative mutation, has become of major importance (Maciel et al., 2001). Furthermore, predictive testing (PT), as well as prenatal diagnosis (PND) became available (Sequeiros et al., 1998). More recently, pre-implantation genetic diagnosis (PGD) is also feasible (Drüsedau et al., 2004). The program of PT and GC, available for MJD in Portugal since the end of 1995, was based on the previous experience with Huntington's disease (HD) (Sequeiros, 1996). This program is ongoing in the Azores since 1996, and its impact in patients and families is periodically revised (Gonzalez et al., 2004; Lima et al., 2001).

Presently there is no effective pharmacological approach for SCAs. Specific drugs have been prescribed to minimize some of the symptoms, such as ataxia or dystonia (reviewed in Bettencourt & Lima, 2011). Cell and animal models have also been fundamental in the understanding of the pathogenesis and gene therapy search *(e.g.,* Gould, 2005); the use of interference RNA and the administration of antisense oligonucleotides showed promising results according to Alves et al. (2008; 2010) and Hu et al. (2009), respectively.

3. Complicating factors in MJD

Several aspects of non-compliance with the Mendelian expectations can be readily recognized for MJD. Variation in the age at onset, variability in clinical presentation, presence of anticipation as well as repeat instability (somatic and germ line), have been described as the main complicating factors in MJD (Tsuji, 1997). Other factors, which will be referred, are also noteworthy.

3.1 Clinical variability

As previously referred, MJD is characterized by a complex phenotype, which is highly variable amongst patients. The recognition of its high degree of clinical variability has led to the proposal of Coutinho & Andrade (1978), in which patients could be classified into three clinical types. According to Coutinho (1992), type 1 has an early onset (average of 24 years) and a more rapid progression of symptoms, being characterized by pyramidal signs and dystonia. Type 2 is the most frequent, and occurs around 40 years of age, being dominated by ataxia and ophtalmoplegia, with or without pyramidal signs. Type 3 has a latter onset (average of 47 years) and progresses slowly, with amyothrophies. The three clinical types can, occasionally, be present in the same family. A fourth type, a rare presentation that associates parkinsonism to cerebellar signs (Suite et al., 1986), and a fifth type, associated with spastic paraplegia (Sakai & Kawakami, 1996), have also been proposed. Notwithstanding the fact that some clinical features, if present, can help in the differential diagnosis of MJD (*e.g.*, ophthalmoplegia, bulging eyes or face and tongue fasciculations), phenotypic overlapping with other SCAs has implications for GC and PT. Therefore, at-risk individuals entering the PT program must have an affected close relative with a definitive molecular diagnosis - "mutation-positive" familial control (Sequeiros et al., 2010).

Variation in age at onset, evidenced by its reported extremes (4 and 70 years) described by Carvalho et al. (2008) and Coutinho (1992), constitutes a particular aspect of the clinical variability of this disorder. A significant, but partial, negative correlation between the size of the expanded allele (and thus, the extension of the Poly-Q tract) and the age of appearance of symptoms explains between nearly 50 to 75% of the variation in the age at onset, depending on the analyzed series of patients (*e.g.*, Maciel et al., 1995; Maruyama et al., 1995). The size of the mutated allele also correlates with the frequency of particular clinical signs, such as pyramidal signs, which are more frequent in patients with larger repeats (Takiyama et al., 1995). In the Azorean series of patients, for example, the number of CAG repeats, determined in genomic DNA and in mRNA, explains 68% and 67% of variation in the age at onset, respectively (Bettencourt et al., 2010b). The incompleteness of the correlation observed between the size of the CAG tract and the age at onset implies that such information cannot be used for counseling purposes; whether allele sizes should be disclosed in the molecular test report is still being debated (Sequeiros et al., 2010).

The reported incompleteness of the genotype-phenotype correlation, observed in MJD as well as in other SCAs, confirms the involvement of non-CAG factors in the explanation of the phenotype, that can either be genetic or environmental (van de Warrenburg et al., 2005). For MJD, the hypothesis that an important fraction of the residual of the disease onset (after accounting for the CAG repeat size) is of genetic nature has been reinforced (DeStefano et al., 1996; van de Warrenburg et al., 2005). The few studies that attempted to

identify MJD modifiers have used candidate gene approaches; Jardim et al. (2003), in a study that considers the effect of the CAG tract at several expansion loci (namely SCA2, SCA6 and DRPLA), only found a correlation between the severity of fasciculations and the size of the CAG tract at the SCA2 locus. Recently, Bettencourt and colleagues (2011) found a significant association between the presence of the *APOE* ε2 allele and an earlier onset in MJD.

3.2 Gene dosage effect

The reduced number of homozygous patients described for MJD makes any generalization, concerning the impact of gene dosage on clinical presentation, hard to perform. The few cases described in the literature, nevertheless, reinforce the fact that the phenotype is more severe and the onset is earlier in homozygous carriers of the mutated allele (*e.g.*, Carvalho et al., 2008; Lerer et al., 1996). This indicates that gene dosage is an important determinant of the onset. The increased severity observed in homozygous is common in dominant diseases, which do not follow the Mendelian expectation of phenotypic overlapping between homo and heterozygous. In specific populations, known to have a particularly high prevalence of MJD, such as the Azorean island of Flores, the possibility of mating between carriers of the MJD mutation must be taken into consideration when planning GC sessions.

3.3 Incomplete and age-dependent pattern of penetrance

In MJD pedigrees, skipped generations are rarely observed. Coutinho (1992) refers that the majority of such cases can be explained by a premature death of the obligate carrier, in relation to the average onset of the disease. Other factors, such as migration, can further prevent the confirmation of the disease status in the obligate carrier. With an estimated value of 98%, the MJD gene penetrance presents an age-dependent pattern, which implies that the *a posteriori*, or residual risk, differs considerably depending on the age of the individual. Residual risk tables constitute, in this context, important tools for GC (Bettencourt et al., 2008a), since they allow the geneticist to estimate the probability that an asymptomatic at-risk individual has to develop the disease at a certain age.

3.4 Intergenerational instability of the CAG tract

Within the Poly-Q disorders, anticipation is more marked for DRPLA, SCA2, SCA7 and HD (Paulson, 2007; Takiyama et al., 1999). In MJD, however, the aggravation of symptoms, and the decrease in the age at onset in successive generations, is also observed. On the basis of anticipation in MJD is the instability of the *ATXN3* gene region containing the expanded CAG tract, which, during cellular division, can lead to alterations in the size of the repeat tract, resulting in expansions or, more rarely, in contractions. Although the decrease in the age at onset is highly related with the increase in the size of the CAG tract, families with a higher degree of anticipation than it would be expected for each repeat unit increase were identified (Takiyama et al., 1998).

Germline mosaicism has been consistently described for MJD (*e.g.*, Cancel et al., 1995). The tendency for the increase of the repeat size is more marked in male than in female meiosis; processes specific to sperm or oocyte development should be involved in such differences.

Maciel et al. (1995) reported that the size of the repeat tract varies in 55% of transmissions; from these, 78% correspond to expansions and 22% to contractions.

Several factors have been implicated as modulating the intergenerational instability in MJD, such as the sex of the transmitting parent as well as the intragenic environment (see, amongst others, Igarashi et al., 1996; Maciel et al., 1999). The results obtained by Takiyama et al. (1997) suggests the presence of an inter-allelic association involved in the instability of the CAG tract, trough yet poorly known mechanisms. Other inter-allelic and *cis* factors have also been studied by Martins et al. (2008); these authors have concluded that distinct origins of the mutation (established on the basis of haplotypes constructed using intragenic SNPs) present different behaviors on what concerns repeat instability. Evidences gathered so far support the presence of a mechanism associated to DNA repair, rather than associated with replication, on the basis of meiotic instability observed in this locus (Martins et al., 2008).

Little is known about the mutational process that leads to the emergence of repeats within the pathological size. It has been postulated that a mutational bias, in favor of expansions, exists in trinucleotide repeat loci, suggesting that the upper end of the normal allele distribution would provide a "reservoir" from which expanded alleles would be generated (Rubinsztein et al., 1994). The hypothesis that normal alleles of larger size could constitute such a reservoir was not supported by a study of nearly 2000 chromosomes of a representative sample of the general Portuguese population (Lima et al., 2005). On the contrary, the report from Lima and colleagues (2005), shows that the allelic distribution was skewed towards smaller size alleles. In a subsequent study, Martins et al. (2006) have integrated not only the analysis of the CAG repeat, but also information on haplotypes built using intragenic and flanking markers; the conclusions indicate that a multistep mechanism is on the basis of the evolution of the CAG repeats in MJD, originated either by gene conversion or DNA slippage.

3.5 Somatic mosaicism

The instability of the expanded polyglutamine-coding (CAG)n tracts during mitotic cell division can lead to changes in repeat length, either contractions or more frequently expansions, resulting in certain populations of cells carrying different repeat sizes. For MJD, the somatic mosaicism has been described by several authors (see, amongst others, Cancel et al., 1995; Lopes-Cendes et al., 1996; Maciel et al., 1997).

In the central nervous system (CNS), the pattern of mosaicism for mutated alleles is similar in the several structures, with the exception of the cerebellar cortex, which presents slightly reduced tracts (Cancel et al., 1998; Hashida et al., 1997). In non-neuronal tissues, the instability is lower in muscle (Tanaka et al., 1999). The studies conducted have consistently failed to demonstrate a correlation between the degree of mosaicism and the selective neuronal vulnerability (Cancel et al., 1998; Ito et al., 1998). The pattern of mosaicism in genomic DNA is maintained in mRNA, and the variation in the size of the CAG repeat in mRNA is also not relatable with the severity of the pathological involvement of the several tissues (Ito et al., 1998).

Somatic mosaicism contributes to the limitations in the precision of sizing the MJD repeat motif, since it originates differences in (CAG)n length among subpopulations of lymphocytes as well as between lymphocytes (where length is usually measured) and CNS

cells (revised in Lima et al., 2006). Thus an error of ±1 CAG repeat is considered as acceptable (Maciel et al., 2001; Sequeiros et al., 2010).

3.6 Segregation distortion

Alterations to the Mendelian proportions in the segregation of the *ATXN3* alleles were firstly highlighted by Ikeuchi and colleagues, in 1996. These authors suggested the occurrence of "meiotic drive" to justify the observation of an excess of affected descendents of MJD patients, a fact hardly explainable by the Mendelian principle of random segregation of alleles (Ikeuchi et al., 1996). Their results pointed to the existence of segregation distortion, in the male meiosis. This issue, however, is far from consensual. A single-sperm typing performed in Japanese patients, by Takiyama et al. (1997) indicated a preferential transmission of mutated alleles. On the other hand, a similar study by Grewal et al. (1999), which used sperm samples from patients of French origin, failed to report the presence of segregation distortion. Using a methodology based on the analysis of pedigrees of patients belonging to Azorean MJD families, Bettencourt et al. (2008a) also investigated the presence of segregation distortion in the transmission of mutated *ATXN3* alleles. According to that study, segregation is done in agreement with the expected Mendelian proportions.

The behavior postulated for the wild-type and the mutated *ATXN3* alleles is not necessarily comparable. Nevertheless, studies with normal individuals have also been conducted, aiming to contribute to the understanding of this issue (Bettencourt et al., 2008b; MacMillan et al., 1999; Rubinsztein & Leggo, 1997). Rubinsztein and Leggo (1997), in a segregation study of MJD alleles in normal heterozygous individuals, reported the presence of segregation distortion only when the transmitting parent was a female, with the smaller allele being preferably transmitted. Results from another study, by Bettencourt et al. (2008b), followed the same trend, with a preference for the transmission of the smaller allele. These last authors also reinforced the importance of the genotypic constitution of the sample being analyzed, which may act as a confounding factor in the detection of segregation distortion.

4. Conclusion

Poly-Q diseases occur as a result of a mutation at the respective causative genes, representing, from that perspective, simple, monogenic diseases. Several aspects of complexity are, nevertheless, present in this group of disorders. Many of the complicating factors present are displayed by MJD and were approached here; the majority of them have implications for patients management and, therefore, its understanding is of major importance.

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3

Spinocerebellar Ataxia with Axonal Neuropathy (SCAN1): A Disorder of Nuclear and Mitochondrial DNA Repair

Hok Khim Fam, Miraj K. Chowdhury and Cornelius F. Boerkoel
University of British Columbia
Canada

1. Introduction

Spinocerebellar ataxias (SCAs) are a group of progressive and irreversible neurological diseases affecting gait and movement coordination. Many result from cerebellar degeneration or the impairment of a portion of the neuroaxis that contributes to cerebellar inflow or outflow (Embirucu et al., 2009). In the cerebellum, the dysfunction and death of Purkinje cells, granule cells or interneurons can cause SCA. Molecular mechanisms for this pathology include polyglutamine tract expansion (SCA1, SCA2, SCA3), flawed basal transcription (SCA17) and defective DNA repair (ataxia telangiectasia, spinocerebellar ataxia with axonal neuropathy (SCAN1) and ataxia with oculomotor apraxia type 1) (Hire et al., 2010).

The mechanism by which defective DNA repair causes neuronal dysfunction and death is not yet fully understood, but damage to the nuclear and mitochondrial genomes underlie each potential explanation. Dysfunction of nuclear DNA repair enzymes results in nuclear DNA damage that impedes transcription and also induces programmed neuronal death (Fishel et al., 2007). Dysfunction of mitochondrial DNA repair enzymes leads to mitochondrial DNA damage that impairs mitochondrial gene expression causing mitochondrial dysfunction, oxidative stress and subsequently programmed neuronal death (Bender et al., 2006). Accumulation of DNA breaks within the neuronal nuclear genome has also been proposed to initiate expression of cell-cycle activators as a cellular response to repair genomic damage through replication-dependent mechanisms; however, these neurons are frequently unable to establish a new G0 quiescent state and this in turn activates neuronal death mechanisms (Kruman et al., 2004). Lastly, besides direct affects on the neurons, defective DNA repair also indirectly induces neuronal death by causing dysfunction of glia, which have trophic interactions with neurons and modulate neurotransmitter levels at synapses (Barzilai, 2011; Lobsiger and Cleveland, 2007).

For the purposes of this review, we focus on SCAN1, an autosomal recessive DNA repair disorder caused by the p.His493Arg active site mutation in tyrosyl-DNA phosphodiesterase 1 (Tdp1), an enzyme that enables DNA repair by processing blocked 3′ DNA termini. This mutation impairs this activity and also predisposes to the formation of Tdp1-DNA adducts.

Since the loss of Tdp1 activity predisposes to both nuclear and mitochondrial DNA damage, this review focuses on understanding SCAN1 etiology from the perspectives of DNA repair and mitochondrial dynamics.

2. Spinocerebellar ataxia with axonal neuropathy (SCAN1)

The only reported SCAN1 patients are from an extended Saudi Arabian family having nine affected individuals (Takashima et al., 2002). SCAN1 is characterized by normal intelligence and a late-childhood onset progressive cerebellar ataxia and peripheral neuropathy. Initial features include ataxic gait, gaze nystagmus and cerebellar dysarthria. As the disease advances, the affected individuals develop impaired pain, vibration and touch sensation in the hands and legs and eventually a steppage gait and pes cavus. With further progression of their cerebellar, motor and sensory symptoms, affected individuals become wheelchair-dependent in early adulthood (Hirano et al., 1993). Magnetic resonance imaging studies show cerebellar atrophy, especially of the vermis (Takashima et al., 2002). Nerve conduction studies show decreased amplitudes characteristic of axonal neuropathy. These clinical findings suggest a disease of large, terminally differentiated, post-mitotic neurons, especially those of the cerebellum, dentate nuclei, anterior spinal horn and dorsal root ganglia.

Currently, there are only symptomatic treatments for SCAN1. Physical therapy is recommended for maintaining activity. Prostheses, walking aids and wheelchairs are recommended for improving mobility. In addition, based on studies of cells from SCAN1 patients and animal models, SCAN1 patients should avoid exposure to genotoxic agents such as camptothecin, irinotecan, topotecan, bleomycin and radiation (Hirano et al., 2007).

Clinically, SCAN1 can be considered in the differential diagnosis for individuals who have 1) a slowly progressive cerebellar ataxia with onset in late-childhood or adolescence, 2) peripheral axonal neuropathy, 3) no signs of oculomotor apraxia and 4) no evidence of extraneurologic features such as telangiectasias, cancers or immunodeficiency. Supportive findings include increased serum cholesterol and decreased serum albumin (Takashima et al., 2002). The only known genetic defect causing SCAN1 is the c.1478A>G mutation in *TDP1*. This missense mutation, which encodes the p.His493Arg amino acid alteration, can be detected by DNA sequencing or by digestion of the PCR product with *Bsa*AI (Hirano et al., 2007; Takashima et al., 2002).

3. DNA repair mechanisms and progressive neurodegeneration

As exemplified by SCAN1, many DNA repair defects cause progressive neurodegenerative disease (Table 1) (Barekati et al., 2010; Sahin and Depinho, 2010). Neurons are particularly vulnerable to the accumulation of unrepaired DNA lesions because they are long-lived, post-mitotic and not readily replaced.

DNA lesions arise as a consequence of endogenous or exogenous genotoxic insults. However, the seclusion of central neurons, which are frequently more severely affected than peripheral neurons, by the blood-brain barrier suggests that the DNA lesions arise predominantly from endogenous genotoxic insults, particularly the oxidative damage arising from mitochondrial dysfunction (Harman, 1972, 1981)

Repair of DNA lesions is mediated by four major DNA repair pathways: double-strand break repair (DSBR), mismatch repair (MMR), nucleotide excision repair (NER), and base excision repair (BER). DSBR corrects double-strand breaks (DSB) in the DNA backbone; MMR corrects mismatches of normal bases; NER repairs bulky helix distorting DNA lesions, and BER repairs damage to a single nucleotide and handles single-strand DNA breaks (SSB). Dysfunction of each of these DNA repair processes causes or has been associated with progressive neurodegenerative disease (Table 1) (Jeppesen et al., 2011).

Gene(s)	DNA Repair Defect	Clinical Syndrome	Main Symptoms
SETX	Defective DSBR	Ataxia with oculomotor apraxia type 2	Cerebellar atrophy Axonal sensorimotor neuropathy Oculomotor apraxia Elevated serum concentration of alpha-fetoprotein
ATM	Defective DSBR	Ataxia telangiectasia	Progressive ataxia Defective muscle coordination Dilation of blood vessels in skin and eyes Immune deficiency Predisposition to cancer
MRE11	Defective DSBR	Ataxia telangiectasia-like	Slowly progressive cerebellar ataxia Ionizing radiation hypersensitivity
XPA, XPF, XPG, POLH, ERCC1-4, DDB2	Defective NER	Xeroderma pigmentosum	Sensitivity to sunlight Slow neurodegeneration Skin cancer
ERCC6, ERCC8	Defective NER and TC-NER	Cockayne's Syndrome	Sensitivity to sunlight Growth retardation Neurological impairment Progeria
ERCC2, ERCC3, GTF2H5	Defective NER	Trichothiodystrophy	Sensitivity to sunlight Dystrophy Short brittle hair with low sulfur content, Neurological and psychomotoric defects
TDP1	Defective BER	Spinocerebellar ataxia with axonal neuropathy 1	Progressive degeneration of post-mitotic neurons
APTX	Defective BER	Ataxia with oculomotor apraxia type 1	Slowly progressive cerebellar ataxia, followed by oculomotor apraxia Severe primary motor peripheral axonal motor neuropathy
ALS2, SETX, SOD1, VAPB	Defective BER	Amyotrophic lateral sclerosis	Progressive degeneration of motor neurons Muscle weakness and atrophy
C10orf2	Defective mitochondrial DNA repair	Infantile-onset spinocerebellar ataxia	Muscle hypotonia Loss of deep-tendon reflexes Athetosis

Table 1. DNA repair enzymes with mutations causing neurodegenerative disease. NER: Nucleotide excision repair, TC-NER: Transcription-coupled nucleotide excision repair, MMR: Mismatch repair, BER: Base excision repair, DSBR: Double-strand break repair, (Embirucu et al., 2009; Katyal and McKinnon, 2007; Subba Rao, 2007)

3.1 Double-strand break repair

DSBR corrects DNA double-strand breaks (DSBs) induced by exogenous sources such as ionizing radiation and genotoxic compounds or by endogenous sources such as reactive oxygen species, replication fork collapse, and errors of meiotic recombination (Ciccia and Elledge, 2010). The two major DSBR pathways in mammalian cells are homologous recombination (HR) and non-homologous end-joining (NHEJ). HR allows high fidelity repair of DSBs during DNA replication by using the intact sister chromatid as a template, whereas NHEJ allows for the error-prone repair of DSBs by modifying and ligating the two DNA termini of a DSB without using an undamaged template. HR is restricted to the late S to G2/M phase of the cell cycle when a sister chromatid is available in proliferating cells, whereas NHEJ operates throughout the cell cycle and can repair DSBs in differentiated cells. Therefore, since the mature nervous system is predominately post-mitotic cells, NHEJ is the major DSBR pathway in the postnatal brain.

Two NHEJ disorders with progressive neurodegeneration of the postnatal brain are ataxia telangiectasia and ataxia telangiectasia-like disorder. The neurological symptoms of ataxia telangiectasia are an early childhood onset of ataxia that generally leads to wheel chair dependence before adolescence. The neurological symptoms of ataxia telangiectasia-like disorder are similar to those of ataxia telangiectasia but of later onset and slower progression. For both disorders, the neurodegeneration is characterized by the loss of cerebellar granule and Purkinje cells.

3.2 Mismatch repair

MMR removes base–base mismatches and insertion-deletion loops that arise during DNA replication and recombination. Base–base mismatches are created when errors escape DNA polymerase proofreading, and insertion-deletion loops arise when the primer and template strand in a microsatellite or repetitive sequence dissociate and re-anneal incorrectly causing the number of microsatellite-repeat units in the template and in the newly synthesized strand to differ. Interestingly, expression of MMR components is not limited to replicating cells but is also observed in non-replicating postnatal neurons suggesting that this pathway plays a role in maintaining the genomic integrity of differentiated cells too (Ciccia and Elledge, 2010).

Consistent with a function in differentiated cells, studies of the Huntington trinucleotide repeat (CAG) in mice have shown somatic age-dependent repeat expansion that is suppressed by deficiency of some MMR components and is triggered by DNA glycosylases of the BER pathway (Kovtun et al., 2007; Owen et al., 2005). The relevance of the MMR pathway to trinucleotide repeat expansions of the human neurological disorders remains undefined however.

3.3 Nucleotide excision repair

In human cells, recognition of bulky helix distorting DNA lesions leads to the removal of a short single-stranded DNA segment surrounding and including the lesion. This creates a single-strand gap in the DNA that is subsequently filled during repair synthesis by a DNA

polymerase using the undamaged strand as a template. NER can be divided into two sub-pathways, global genomic NER (GG-NER) and transcription-coupled NER (TC-NER). GG-NER and TC-NER differ in the recognition of the DNA lesion but subsequently use the same excision mechanism. GG-NER recognizes and repairs DNA lesions anywhere in the genome, whereas TC-NER only resolves lesions in the actively transcribed DNA strand (de Laat et al., 1999).

Two NER-associated disorders, xeroderma pigmentosum and Cockayne syndrome, feature progressive neurodegeneration (Kraemer et al., 1987). About 30% of xeroderma pigmentosum patients have neurological symptoms that include abnormal motor control, ataxia, peripheral neuropathy, dementia, brain and spinal cord atrophy, microcephaly and sensorineural deafness. In contrast, nearly all Cockayne syndrome patients have progressive neurological disease characterized by demyelination in the cerebral and cerebellar cortex, calcification in the basal ganglia and cerebral cortex, neuronal loss, sensorineural hearing loss and decreased nerve conduction (Nance and Berry, 1992). The progressive neurodegeneration in both xeroderma pigmentosum and Cockayne syndrome are attributable to apoptotic cell death (Lehmann, 2003).

3.4 Base excision repair

BER corrects the most common forms of DNA damage by recognizing, excising and replacing a broad spectrum of specific forms of DNA modifications including those arising from deamination, oxidation and alkylation. It is initiated by a distinct lesion-specific mono- or bi-functional DNA glycosylase and completed by either of two sub-pathways: short-patch BER (SP-BER) that replaces one nucleotide or long-patch BER (LP-BER) that replaces 2–13 nucleotides (Frosina et al., 1996).

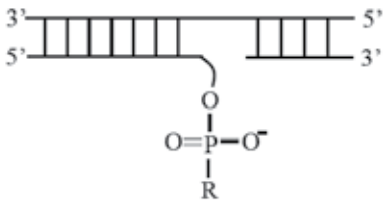
The BER proteins are also responsible for repairing DNA SSBs. SSBs are some of the most common lesions found in chromosomal DNA and arise via enzymatic cleavage of the phosphodiester backbone or from oxidative damage or ionizing radiation. Examples of enzymatic cleavage causing SSBs include those arising during BER (Connelly and Leach, 2004) and during DNA topoisomerase I (Topo I) activity (Pommier et al., 2003).

Ataxia with oculomotor apraxia type 1 and SCAN1 are both associated with defects in the repair of SSBs, specifically the processing of obstructive termini (Table 1). The neurodegenerative features of ataxia with oculomotor apraxia type 1 include progressive cerebellar atrophy, late axonal peripheral motor neuropathy, ataxia and oculomotor apraxia. The features of SCAN1, which is caused by a mutation of *TDP1*, have been described above.

4. Tdp1 function

TDP1 encodes tyrosyl-DNA phosphodiesterase 1 (Tdp1), a 608 amino acid enzyme that contains a bipartite nuclear localization sequence and two conserved HxKx4Dx6G (G/S) HKD (histidine-lysine-arginine) signature motifs. The two HKD motifs form a single symmetrical active site characteristic of the phospholipase D superfamily and catalyze a phosphoryl transfer that is common to enzymes in this superfamily (Interthal et al., 2001). The HKD motifs are very important for the catalytic function of Tdp1. Tdp1 enables SSBR

and DSBR by removing obstructing compounds linked by a phosphodiester bond to DNA 3′ termini and complements the 5′-phosphodiesterase function of TTRAP (Tdp2) (Cortes Ledesma et al., 2009; el-Khamisy and Caldecott, 2007; Zhou et al., 2009). Tdp1 endogenous substrates include 3′ tyrosine-DNA phosphodiester moieties, phosphoglycolates, mononulceosides and tetrahydrofurans, and exogenous substrates include 4-methylphenol, 4-nitrophenol and 4-methylumbelliferone (Figure 1) (Dexheimer et al., 2008; Interthal et al., 2005a). Tdp1 has the highest affinity for the 3′ tyrosine-DNA phosphodiester moieties, which are characteristic of Topo I-DNA intermediates (Dexheimer et al., 2010).



Physiologic R
Proteolysed Topo I, Tyrosine, Glycolate, Tdp1, Tetrahydrofuran and Mononucleoside

Non-physiologic R
4-methylphenol, 4-nitrophenol and 4-methylumbelliferone

Fig. 1. Substrates of Tdp1. Tdp1 can remove both physiologic substrates and non-physiologic substrates. R = Substrates.

During repair, replication, transcription, recombination and chromatin remodeling, Topo I relaxes superhelical tension by nicking DNA to allow controlled rotation of the broken DNA strand around the intact strand. After DNA relaxation has occurred, a nucleophilic attack by the DNA 5′ hydroxyl group on the phosphotyrosyl linkage between Topo I and the 3′ end of the DNA at the nick usually religates the DNA, and the Topo I dissociates. However, DNA damage such as abasic sites, nicks, and mismatched base pairs frequently impede removal of Topo I from the DNA by causing a misalignment of the 5′ hydroxyl end of the DNA that prevents it from acting as a nucleophile. (Pommier et al., 1998; Pommier et al., 2003; Pourquier and Pommier, 2001) Additionally, the 3′-Topo I-DNA intermediate can become unduly long-lived if Topo I binds oxidative base lesions (Interthal et al., 2005b). These trapped or long-lived Topo I-DNA covalent intermediates can then be converted to irreversible DNA breaks when the DNA replication machinery or RNA polymerase collides with the Topo I-DNA complex (Hsiang et al., 1989; Tsao et al., 1993; Wu and Liu, 1997)

Clearance of the trapped or stalled 3′-Topo I-DNA intermediates occurs via SSBR or DSBR if the SSB is converted to a DSB by collision of the DNA replication machinery with the trapped or stalled 3′-Topo I-DNA intermediate. Following recognition of the break, the trapped or stalled Topo I is proteolytically cleaved leaving a peptide bound to the 3′ end of the DNA by the phosphodiester bond formed between the DNA and the Topo I active site

tyrosine (Tyr723). Tdp1 then acts on the phosphodiester bond and removes the obstructing Topo I peptide from the 3′ terminus (Debethune et al., 2002; Liu et al., 2002). The Tdp1 reaction removes the peptide from the DNA by an S_N2 nucleophilic attack of His263, which resides in the first HKD motif, on the phosphodiester bond; Tdp1 is then released from the DNA by the catalytic activity of His493, which resides in the second HKD motif (Figure 2).

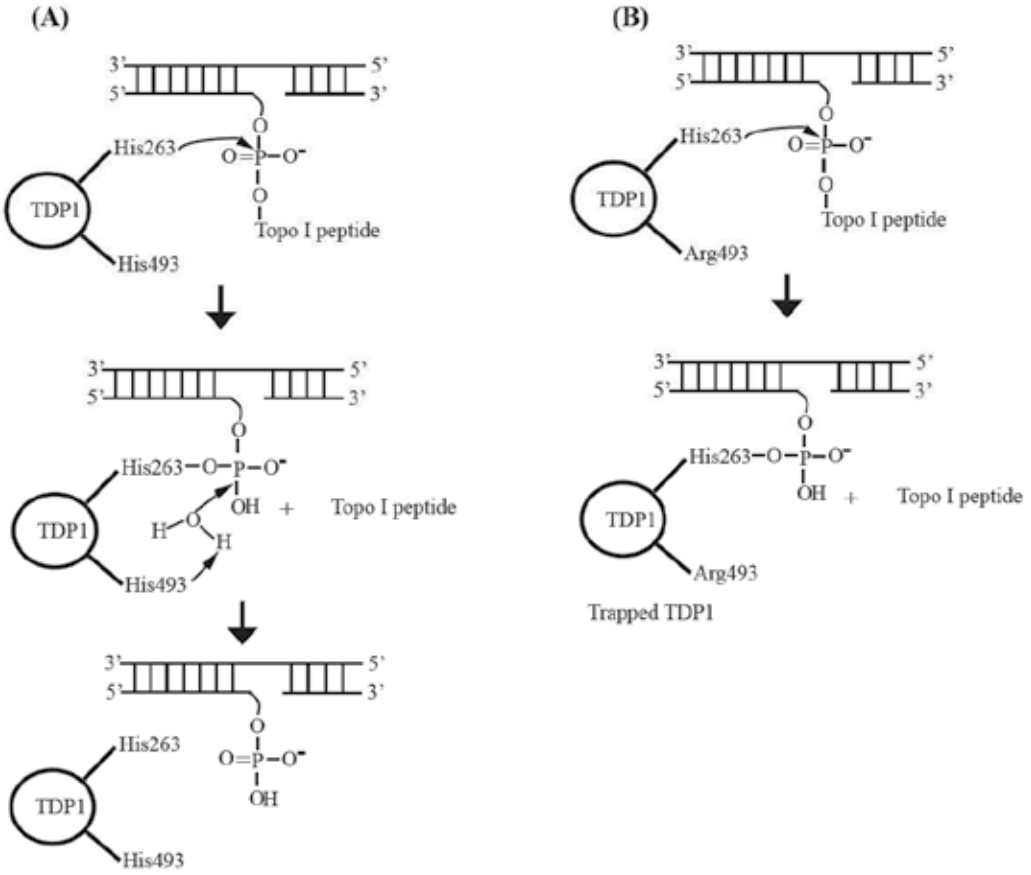


Fig. 2. Mechanism of Tdp1 catalytic activity. (A) Wild type Tdp1 removes proteolysed Topo I and forms a covalent intermediate with DNA before His493 of the second HKD motif excises Tdp1 from DNA through a nucleophilic substitution. (B) In SCAN1, the mutated Tdp1 (p.His493Arg) removes proteolysed Topo I but remains trapped on DNA and leads to accumulation of Tdp1-DNA adducts.

4.1 Tdp1 and nuclear DNA repair

Within the nucleus, Tdp1 is a component of the SSB multi-protein repair complex containing PARP1, LIG3α, XRCC1 and PNKP (Das et al., 2009). This repair complex is activated after proteasomal degradation of stalled Topo I (Zhang et al., 2004). PARP1 is an important regulator of the SSBR/BER pathway as it enhances the recruitment of DNA repair proteins. PARP1 hydrolyzes NAD^+ to catalyze the synthesis of ADP-ribose units onto glutamate

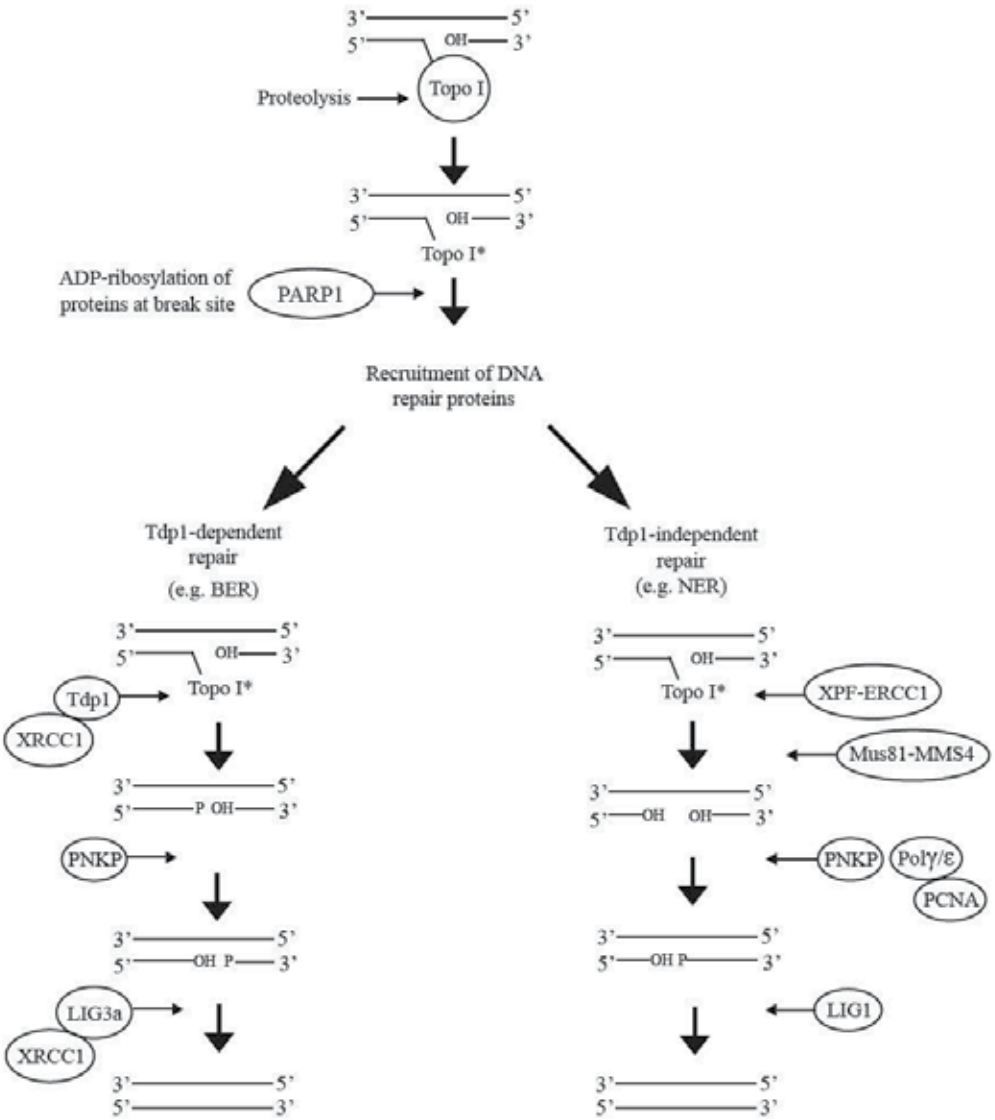


Fig. 3. Tdp1-dependent and Tdp1-independent pathways for the removal of Topo I-DNA covalent complexes. After Topo I is trapped on the DNA, proteolysis of Topo I occurs. The remaining Topo I peptide can be removed by either Tdp1-dependent pathway or Tdp1-independent pathways. Topo I* = Topo I peptide.

molecules of acceptor proteins. The addition of poly-ADP ribosyl (PAR) polymers onto histones promotes the relaxation of chromatin, while SSBR proteins such as XRCC1 and Lig3a are electrostatically attracted to PAR and are thus recruited to the site of DNA damage (El-Khamisy et al., 2003; Krishnakumar and Kraus, 2010). It is thought that XRCC1 acts as a molecular scaffold for the binding of Tdp1 and Lig3a and stabilizes the enzyme complex in the processing of Topo I derived SSBs. Processing of the 3′ tyrosine-DNA phosphodiester moieties by Tdp1 leaves a 3′-P terminus that is converted to 3′-OH by the phosphatase action of PNKP. The kinase activity of PNKP phosphorylates the 5′-OH terminus, allowing gap filling by DNA polymerase β (Polß), and finally the DNA nick is sealed by lig3a with the aid of the XRCC1 scaffold (Figure 3).

How Tdp1 processes obstructing 3′ overhangs on DNA DSBs has not been fully defined. The dependence of Tdp1 processing of DSB termini on the autophosphorylation activity of the NHEJ component DNA-PK suggests that Tdp1 contributes within the NHEJ pathway and that DNA-PK modulates the accessibility of DNA ends enabling Tdp1 to accomplish the processing necessary for eventual end-joining (Zhou et al., 2009).

One redundant DNA repair activity for Tdp1 is the nucleolytic removal of several DNA bases beginning upstream of the stalled Topo I. This is mediated by 3'-flap endonuclease complexes in the nucleus such as Mus81-MMS4 and XPF-ERCC1 that cleave at the 3'-flap created by stalled Topo I to enable short-patch gap filling. In comparison to Tdp1 processing, however, this mechanism is error-prone and less efficient.

4.2 Tdp1 and mitochondrial DNA repair

Besides its role in the repair of nuclear DNA, Tdp1 also plays a role in mitochondrial DNA (mtDNA) repair (Das et al., 2010). Mitochondria are membrane-enclosed organelles that generate most of the ATP via the electron transport chain at the inner mitochondrial membrane. This process leads to the generation of reactive oxygen species, and while mitochondria have various antioxidant enzymes to deactivate these highly reactive molecules, they do not constitute a perfect defense. This inevitably exposes the mtDNA to high levels of oxidative stress, particularly since the mtDNA is located in close proximity to the inner mitochondrial membrane and lacks protective histones (Ames et al., 1993). Consequently, the levels of oxidative base damage in mitochondrial DNA are 2–3 fold higher compared to nuclear DNA (Hudson et al., 1998), and the damage is more extensive and persistent than in nuclear DNA (Yakes and Van Houten, 1997).

Several DNA repair activities and pathways that function in the nucleus have also been identified and characterized in mammalian mitochondria. These include BER, MMR, and some components of DSBR (Larsen et al., 2005). Given the prevalence of small lesions generated by oxidative stress in the mitochondria, BER is the predominant mtDNA repair pathway. Mitochondrial BER proteins are not encoded by the mitochondrial genome; rather they are mitochondrial versions of nuclear-encoded proteins (Larsen et al., 2005). Among these is Tdp1, which could participate in the repair of oxidative mitochondrial DNA damage via resolution of 3′-phosphoglycolate obstructive termini and processing the apurinic/apyrimidinic (AP) sites arising from the DNA glycosylase removal of DNA lesions such as 7,8-dihydro-8-oxoguanine (8-oxoG). These two abilities of Tdp1 are also shared by APE1, although the relative contribution of each protein to either process is unclear.

Additionally, as there is a mitochondrial Topo I (mtTopo I) that has 71% identity and 87% similarity with the nuclear Topo I (Zhang et al., 2001), we hypothesize that the high level of mtDNA lesions predisposes to generation of long lived or trapped mtTopo I-DNA complexes similar to those in the nucleus and that the removal of mtTopo I peptides from mtDNA is also a function of mitochondrial Tdp1.

5. The molecular basis of SCAN1

In SCAN1, the p.His493Arg mutation in the second HKD motif of Tdp1 affects the active site of the protein and reduces its catalytic activity by 25-fold (Interthal et al., 2005b; Takashima et al., 2002). This alteration both decreases the processing of Topo I-DNA adducts and impairs the intermolecular reaction that would ordinarily release Tdp1. These Tdp1-DNA adducts can only be removed by wild-type Tdp1 (Figure 2) (Interthal et al., 2005b). This finding suggests that SCAN1 might arise, at least in part, from accumulation of the Tdp1-DNA adducts and the inability of the cell to remove $Tdp1^{H493R}$ in a timely manner (Dexheimer et al., 2008; Hirano et al., 2007).

Currently, the molecular basis of SCAN1 and the reason that mice deficient for Tdp1 do not develop ataxia are incompletely understood. Although there are no prominent tissue differences in gene expression nor evidence of positive selection of the Tdp1 protein (as reported in the Selectome database) between human and mouse (Proux et al., 2009), two observations suggest possible explanations for SCAN1 pathogenesis and the lack of ataxia in Tdp1-deficient mice. First, human Tdp1 is predominantly expressed in the cytoplasm of the neurons predicted to be affected in SCAN1, whereas mouse Tdp1 is predominantly expressed in the nucleus of the analogous neurons. Second, *in vitro* and cell culture experiments show that the p.His493Arg Tdp1 forms long lived Tdp1-DNA adducts (Hirano et al., 2007; Interthal et al., 2005b); therefore, development of SCAN1 may be dependent on this "mutagenic" property of p.His493Arg Tdp1.

5.1 Mitochondrial dysfunction model

The prominent cytoplasmic expression of Tdp1 in human Purkinje, dentate nucleus, anterior horn, and dorsal ganglion neurons suggests a cytoplasmic function for Tdp1. Given the mitochondrial localization of cytoplasmic Tdp1, this suggests 1) that the majority of Tdp1 in these neurons functions in the mitochondria and 2) that SCAN1 may be arising from mitochondrial dysfunction secondary to loss of mtDNA integrity. In contrast, the low expression of Tdp1 in the cytoplasm of these neurons in mice would suggest that Tdp1 plays a minor role in maintenance of the mouse mitochondrial genome or that the analogous neurons have less mtDNA damage in mice than in humans.

The human cerebellum contains post-mitotic neurons with a large mitochondrial population. Despite the non-proliferative nature of cerebellar neurons, the biogenesis of mitochondria and the maintenance of mitochondrial integrity are of central importance for survival of these neurons (Chen and Chan, 2009). The closed circular mitochondrial genome predisposes it to helical tension during mitochondrial replication, which is resolved by mtTopo I. In the nucleus, binding of Topo I to 8-oxoG rearranges the active site of Topo I and stabilizes it in an inactive conformation (Lesher et al., 2002). If the same occurs in mitochondria with mtTopo I, which encounters a much higher level of 8-oxoG, then Tdp1

will be critical for resolution of these lesions in mtDNA. Hypothetically, a repair process analogous to that in the nucleus would resolve these long-lived complexes: 1) protease cleavage of mtTopo I, 2) Tdp1 mediated cleavage and release of the mtTopo I peptide to leave an 8-oxoG 5′overhang and a 3′-phosphate, and 3) SP-BER. SP-BER would proceed by PNKP removal of the 3′-phosphate and OGG1 removal of the 5′ 8-oxoG lesion, followed by mitochondrial polymerase-ϒ filling in the missing nucleotides and Lig3a ligating the DNA strand (Figure 4a).

Based on these observations, we hypothesize that the processing of trapped or long-lived mtTopo I-DNA intermediates is hindered in cells from SCAN1 patients by the reduced catalytic activity of p.His493Arg Tdp1. Additionally, we hypothesize that mitochondria lack DNA repair pathways redundant for the activity of Tdp1 since 3′end processing flap endonucleases that could resolve mtTopo I-DNA adducts have not been detected in mitochondria (Liu et al., 2002). In this model, trapped or long-lived mtTopo I-DNA intermediates interfere with mitochondrial transcription and contribute to mtDNA damage. In turn, both transcriptional dysfunction and genomic instability cause mitochondrial dysfunction and thereby poor cellular health. Relative to other brain neurons, cerebellar neurons may be highly sensitive to this mitochondrial damage since they have a lower tolerance for mitochondrial dysfunction (Chen et al., 2007; Hakonen et al., 2008) (Figure 4b).

In summary, therefore, the dysfunction of mitochondrial Tdp1 may contribute to the pathogenesis of SCAN1. Also, the absence of ataxia in Tdp1 deficient mice may arise because mouse cerebellar and spinal cord neurons have a lower requirement for Tdp1 processing of damaged mtDNA (Hirano et al., 2007).

5.2 Tdp1 neomorph model

The formation and accumulation of Tdp1-DNA adducts by the mutant p.His493Arg Tdp1 causes increased DNA breaks in cells expressing this mutant Tdp1 (Hirano et al., 2007) and thus suggests that p.His493Arg Tdp1 acts as a mutagen. *In vitro*, wild type Tdp1 is the only identified enzyme that can remove the mutant Tdp1 from the DNA (Interthal et al., 2005a). However, *in vivo* it is possible that nuclear Tdp1-DNA adducts are processed by a DNA repair mechanism such as HR that is present in proliferating unaffected cells but not in affected quiescent neurons. Alternatively, there may not be an alternative repair pathway but simply replacement of cells that die from accumulated Tdp1-DNA adducts in proliferating tissues and a failure of replacement for non-proliferating neurons.

As an extension of this hypothesis, one might consider that both mitochondrial dysfunction and the neomorphic properties of p.His493Arg Tdp1 contribute to the pathogenesis of SCAN1. The repair of damaged DNA is costly, and if the costs exceed cellular energy resources (ATP/NADH), then cell death results (Zong and Thompson, 2006). In this context, a mechanism that could lead to cell death is the depletion of NAD^+ and ATP reserves by the over-activation of PARP1 due to accumulating SSBs created by $Tdp1^{His493Arg}$-DNA adducts in the nucleus and mitochondria. In the context of compromised mitochondria, such depletion of cellular energy reserves, which triggers permeabilization of the outer mitochondrial membrane and the release of cytochrome c and apoptosis-inducing factor

(AIF) (Wang et al., 2009), would occur at a lower threshold in affected versus unaffected cells of SCAN1 patients (Wang et al., 2011) (Chen and Chan, 2009). In this model, the neurons with the least energy reserves would be most sensitive and, unlike proliferating cells, difficult to replace (Figure 4c).

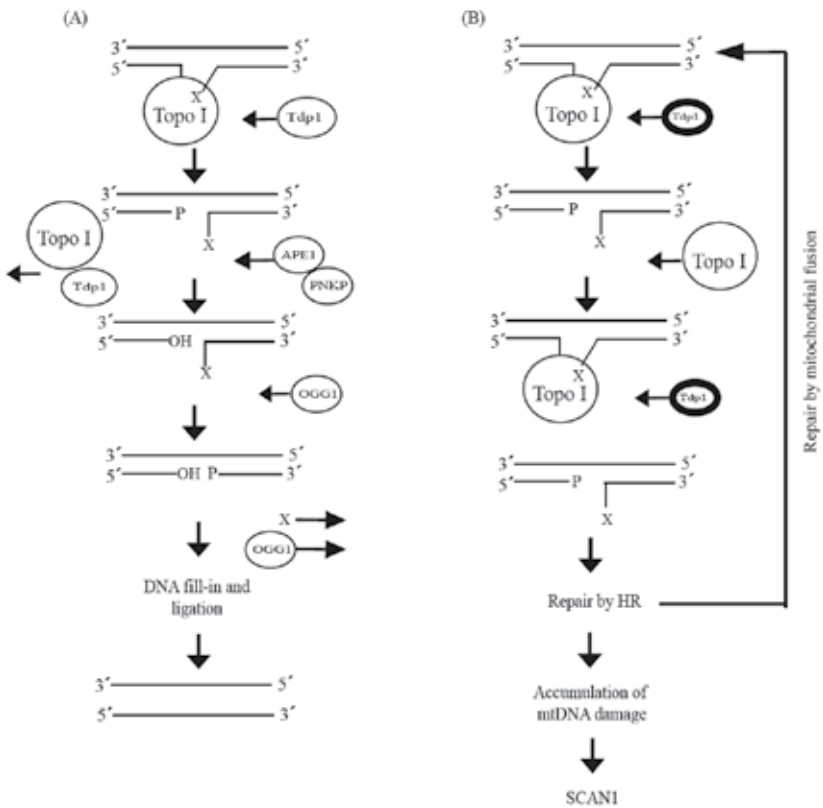


Fig. 4. A and B.

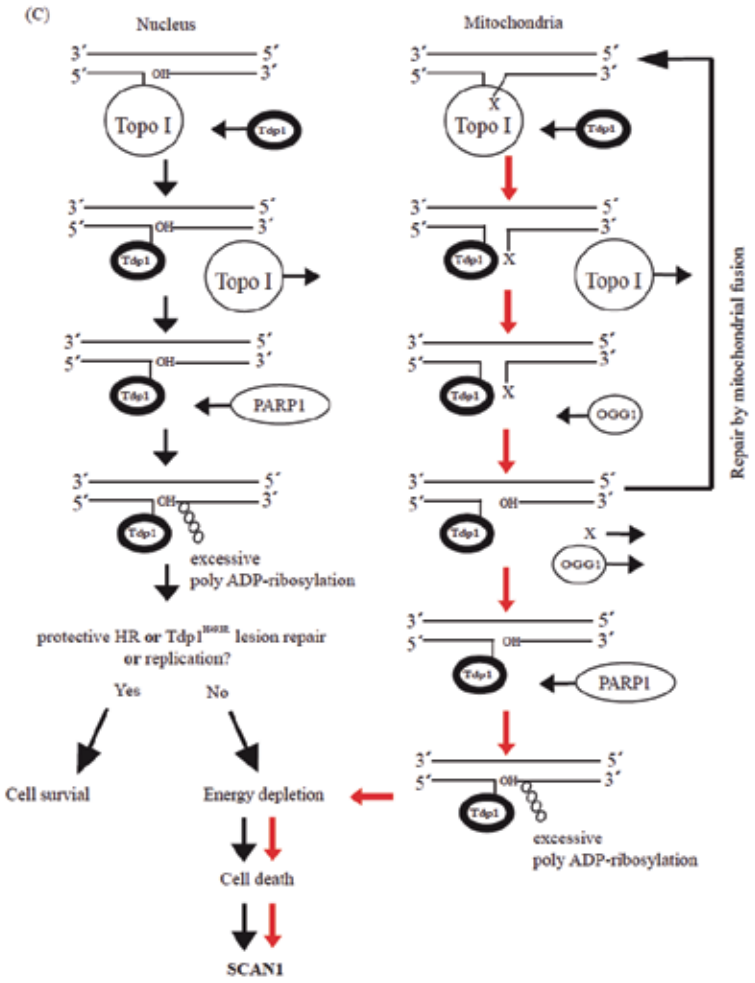


Fig. 4. Models for the pathobiology of SCAN1. (A) Putative Tdp1 function in the mitochondria. Wild type Tdp1 removes the residual peptide from stalled mtTopo I complexes. Interfering DNA lesions are processed by OGG1 and by PNKP or APE1. Religation synthesis would then proceed by mitochondrial short patch-BER. (B) The mitochondrial dysfunction model. In SCAN1, p.His493Arg Tdp1 (in bold) removes peptides derived from Topo I-DNA complexes at a severely compromised rate. This sluggish repair leads to a higher steady-state level of mtDNA SSBs and DSBs. To bypass the lack of Tdp1, error-prone repair may generate mtDNA deletions which impair mitochondrial function leading to cell death and SCAN1. (C) The Tdp1 neomorph model. Mutagenic p.His493Arg Tdp1 is trapped on DNA, and since wild type Tdp1 most efficiently repairs p.His493Arg Tdp1-DNA adducts, the unresolved p.His493Arg Tdp1-DNA adducts in cells from SCAN1 patients will lead to much higher steady state levels of nuclear and mitochondrial DNA SSBs. This could cause neuron death both by SSB-induced programmed cell death and by cellular energy depletion secondary to mitochondrial dysfunction. The energy depletion would be accentuated by the increased energy requirement for DNA repair as exemplified by PARP1 mediated poly ADP-ribosylation. X = interfering lesion.

6. Future directions

The discovery of Tdp1 in mitochondria places the pathobiology of SCAN1 in a new light although whether specific mitochondrial pathology is relevant to the pathogenesis of SCAN1 remains to be elucidated. To that end, the generation of Tdp1^{His493Arg} mice will enable a thorough investigation of the physical and molecular characteristics of SCAN1.

Equally important is the precise elucidation of Tdp1 function in DNA processing. Research in mice and yeast has deciphered much about Tdp1 function but much remains to be discovered. Tdp1 orthologues have been described in 29 organisms, most recently in the plants *Arabidopsis sp.* and *Medicago sp.*, where Tdp1 repair of Topo I-induced damage is consistent with its role in mammalian cells (Lee et al., 2010; Macovei et al., 2010). As the time and cost of DNA sequencing continues to decline and techniques for probing the genome become more accessible to biologists, the study of emerging model organisms will provide valuable insights into the evolutionary conservation of Tdp1. This approach would allow more detailed evaluation of Tdp1 from an evolutionary perspective and enhance our mechanistic understanding. For example, this might enlighten us as to why the *Drosophila melanogaster* homologue *glaikit* appears to have a function distinct from that of the mammalian and plant Tdp1 homologues (Dunlop et al., 2000; Dunlop et al., 2004)

The study of SCAN1 has also defined Tdp1 as a reasonable drug target for other diseases. The absence of neurological disease in Tdp1 deficient mice and the adolescent onset of SCAN1 in humans suggest that Tdp1 could be inhibited briefly without severe adverse consequences. Since Tdp1 increases resistance to the Topo I poisons used as anticancer agents, these findings suggest that a combination therapy of Topo I poisons with Tdp1 inhibitors might enhance the efficacy of the Topo I poisons as anticancer drugs (Marchand et al., 2009).

7. Conclusion

Mitochondrial dysfunction is not yet the *sine qua non* of SCAs, but it is increasingly reported in neurodegenerative diseases. Besides the SCAs, mitochondrial dysfunction has been reported as contributing to the pathobiology of aging, Alzheimer disease, Parkinson disease, Huntington disease and amyotrophic lateral sclerosis. Much of this work has focused on mitochondrial-derived reactive oxygen species; however, the contribution of mitochondrial fusion and fission to neuronal health and disease as well as other mitochondrial processes remain to be explored (Lin and Beal, 2006; Westermann, 2010). SCAN1 is emblematic of the interplay between the nuclear and mitochondrial genomes and how dysfunction in both organelles can jointly contribute to disease. This dual nuclear and mitochondrial pathobiology will need to be taken into consideration in experimental design as well as in the classification and clinical management of neurologic disorders.

8. Acknowledgements

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4

Eye Movement Abnormalities in Spinocerebellar Ataxias

Roberto Rodríguez-Labrada and Luis Velázquez-Pérez
Centre for the Research and Rehabilitation of Hereditary Ataxias, Holguin Cuba

1. Introduction

Spinocerebellar ataxias (SCAs) are a heterogeneous group of autosomal dominant neurodegenerative disorders characterized by a progressive cerebellar syndrome, variably associated to signs of brainstem involvement, pyramidal or extrapyramidal manifestations and cognitive dysfunctions, among other features that confer a remarkable wide range in phenotypes (Harding, 1983; Durr, 2010).

SCAs are associated with at least 31 different genetic loci, but the responsible gene is known in only 19 of them. Causative mutations include coding CAG expansions leading to a long polyglutamine (polyQ) tract in the respective proteins (SCA1, 2, 3, 6, 7 and 17), non-coding trinucleotide or pentanucleotide expansions (SCA8, 10, 12 and 31), as well as conventional mutations (SCA5, 11, 13, 14, 15/16, 20, 27 and 28) (Durr, 2010). The worldwide prevalence of SCAs is estimated near to 5-7 cases per 100 000 inhabitants but it can be higher in some regions due to foundational effects such as SCA2 in Holguín, Cuba (Velazquez-Pérez et al., 2009a) and SCA3 in Azores islands, Portugal (Vale et al., 2010).

Oculomotor disturbances are prominent features of SCA patients as result of cerebellar and brainstem neurodegeneration (Zee et al., 1976; Pula et al., 2010). The study of eye movement abnormalities give us valuable tools to search disease biomarkers because they can be easily accessible to clinical and/or electrophysiological evaluations and their dynamic properties and neurobiological basis are well known (Leigh & Kennard, 2004; Leigh & Zee, 2006; Reilly et al., 2008). The focus of this chapter is to review the state of the art of the eye movement deficits in SCAs, emphasizing in the usefulness of these features as disease biomarkers.

2. Brief overview of eye movements

Eye movements contribute to the clear vision stabilizing images on the retina, especially against movements of the head and body, capturing and keeping particular stimuli on the fovea and aligning the retinal images in the two eyes to guarantee the single vision and stereopsis. These functions can be achieved by 5 basic types of eye movements. For example, the image stabilization on the retina is promoted by the vestibulocular and optokinetic reflexes; the foveation occurs thorough the saccadic and smooth pursuit movements, whereas the binocular alignment is guaranteed by the vergence eye movements (Bruce & Friedman, 2002).

Eye movements differ in many aspects, such as their velocity, reaction time, reflexivity/volitional degree and their neurobiological substrate (Sparks, 2002). Nevertheless all have generic kinematic properties and share a common final path represented by three cranial nerve nuclei and the three pairs of eye muscles that they control (Bruce & Friedman, 2002; Leigh & Zee, 2006). Cranial nerve III (oculomotor) innervates superior, inferior and medial rectus muscles as well as the inferior oblique muscle, whereas troclear (IV) and abducens (VI) nerves innervate the superior oblique and lateral rectus respectively (Leigh & Zee, 2006).

Main features and neurophysiological bases of the 5 basic types of eye movements will be briefly addressed as follow.

2.1 Vestibulocular reflex (VOR)

The vestibulocular reflex (VOR) is elicited by the vestibular system in response to body/head rotations and consists in compensatory eye movements in opposite direction to body/head movement to guarantee the image stabilization on the retina (Aw et al., 1996). VOR depends of two neural circuits: *a)* Basic three neurons circuit and, *b)* Neural integrator circuit.

In the basic three neurons circuit, the head/body rotations are detected and transduced by vestibular ganglion neurons in the semicircular canal. Then, the transduced information is projected to neurons of the vestibular nuclei, located in the pons, and from there to oculomotor neurons (OMN) in one of the three oculomotor nuclei. Nevertheless, the three neurons circuit by itself is unable to adequately stabilize the image on the retina because it only generates phasic innervations of the oculomotor muscles, causing the return of the eye back to the central position due to the pulling of elastic forces. The neural integrator serves to exactly overcome this elastic force producing tonic innervations of oculomotor muscles. It is located in the *prepositus hypoglossi* and medial vestibular nuclei, which receive projections from the vestibular nuclei and have recurrent connections onto themselves. Some vestibular afferents go directly to the floculus/parafloculus cerebellar lobe, which is involved in VOR adaptation (Bruce & Friedman, 2002).

2.2 Optokinetic reflex (OKR)

When head/body rotations are very large and continued the VOR is depressed and thus it is complemented by the optokinetic reflex (OKR), in which the speed and direction of a full-field image motion is computed to develop eye movements with two phases, an slow phase that alternates with resetting quick phase (Tusa & Zee, 1989). Pathway underlying OKR includes the nucleus of the optic tract, which receives visual motion signals from the contralateral eye and striate/extrastriate cortical areas. This information is send to the vestibular nuclei and to the inferior olivary nucleus, and then to the flocular/paraflocular Purkinje cells via their climbing fibers (Bruce & Friedman, 2002).

2.3 Saccadic eye movements

Saccades are ballistic, conjugate eye movements that redirect fovea from one object of interest to another, allowing to explore accurately the visual scenes. For that, the saccadic system processes information about the distance and direction of a target image from the

current position of gaze. Saccades are the fastest eye movements, reaching up to 600^0/s. There are close relationships between saccadic peak velocities, durations and amplitudes, which represent the saccadic main sequence (Bahill et al., 1975, Ramat et al., 2007).

Behaviourally, the saccades may be classified as reflexive guided saccades and intentional or volitional saccades. The first ones are evoked by the suddenly appearing targets, whereas the second ones, called also higher-order saccades, are made purposely, involve high cognitive processing and include voluntary, memory guided and delayed saccades as well as antisaccades (Müri & Nyffeler, 2008; Leigh & Kennard, 2004).

The neural basis of saccadic eye movements system comprises some cortico-cortical and cortico-subcortical networks (Müri & Nyffeler, 2008). Visual information processed in the primary visual cortex is send to higher cortical areas, such as parietal eye field (PEF) and frontal eye field (FEF), which are involved in the preparation and triggering of reflexive and intentional saccades respectively (Pierrot-Deseilligny, et al., 2004). These cortical areas project their output directly or through the basal ganglia, to superior colliculus, a midbrain structure that identifies the target in retinotopic coordinates, generates trigger signal to the brainstem premotor oculomotor circuitry and encodes the magnitude and direction of the desired eye movement. This information is projected to the cerebellum, via a pontine pre-cerebellar nucleus, which guarantees the saccadic accuracy. Premotor burst neurons (PBN) for horizontal saccades lie within the paramedian pontine reticular formation (PPRF) while burst neurons for vertical and torsional saccades lie within the rostral interstitial nucleus of the medial longitudinal fasciculus. Saccade-related cerebellar areas include the oculomotor vermis (lobules VI and VII) and the caudal region of the fastigial nucleus which send saccade commands to the contralateral PBNs leading the activation of motorneurons and oculomotor muscles related with the desired saccadic movement (Leigh & Zee, 2006; Robinson & Fuchs, 2001; Prsa & Their, 2011; Voogd et al., 2011).

2.4 Smooth pursuit movements

Smooth-pursuit eye movements enable us to maintain the image of a moving object relatively stable on or near the fovea by matching eye velocity to target velocity (Leigh & Zee, 2006). Smooth pursuit performance is optimal for target speeds ranging between 15^0/s and 30^0/s but pursuit velocity can reach up to 100^0/s (Lencer & Trillenberg 2008; Bruce & Friedman, 2002). Smooth pursuit system is closely related to other oculomotor systems such as OKR and saccadic system. In fact, the small position errors raised when the tracking velocity is not properly matched to the target are corrected by saccadic movements named "catch up" saccades (Lencer & Trillenberg, 2007).

Neuronal pathways for smooth pursuit movements involve a complex network of cortical and subcortical structures. Extrastriate visual area V5 (divided into middle temporal visual area (MT) and the medial superior temporal visual area (MST)) play a crucial role for motion perception and smooth pursuit control. This area receives visual motion information from the primary visual cortex in a retinotopic and ipsilaterally organized fashion. The MT area encodes image motion in a retinal coordinate system whereas MST area converts the signals into a spatial coordinate system. The signals generated in the V5 area are projected to other cortical areas in the parietal and frontal lobes. Among them, the frontal eye field (FEF) is involved in the generation of oculomotor command for smooth pursuit. Both visual motion

signals and oculomotor commands are relayed to oculomotor parts of the cerebellum, through the dorsolateral and medial pontine nuclei. Smooth pursuit-related areas of the cerebellum comprise the paraflocculus, the flocculus, the oculomotor vermis and the uvula, which control the initiation and maintenance of smooth pursuit. Finally, the cerebellar output is projected, via the vestibular nuclei, to the oculomotor nuclei (Lencer & Trillenberg, 2007; Mustari et al., 2009).

2.5 Vergence eye movements

Vergence eye movements are disjunctive movements that provide the binocular alignment in response to changing fixation target distances, requiring that both eyes point in contrary directions (Zee & Levi, 1989). Vergence movements are elicited by retinal disparity (when a fixation target is not on both foveae) and retinal blur (when a target is not in focus) and are closely related to the lens accommodation and pupillary reflexes. Although the neural basis of vergence eye movements are not well understood, it is known that both the retinal disparity and the retinal blur signals are processed by cortical visual areas such as primary visual cortex (V1) and an anterior region of the FEF. Additionally, it is presumed an important role of the oculomotor nucleus (III) for vergence movements, due to its known relation to lens accommodation and pupillary reflexes (Vilis, 1997; Bruce & Friedman, 2002). The cerebellum is involved in the processing of dynamic vergence eye movements (Sander et al., 2009). Cerebellar regions related with these disconjugate eye movements lie on the dorsal paraflocculus, and the floccular lobe, which project to the lateral portion of the posterior interposed nucleus (Voogd et al., 2011).

2.6 Oculomotor disturbances

Oculomotor disturbances can be topographically classified as peripheral or central disturbances. Peripheral abnormalities result from lesions in the oculomotor muscles or nerves, whereas the central disturbances are caused by lesions in the brainstem, cerebellum or other higher-level centers (Karatas, 2009). Oculomotor signs of cerebellar impairment include pathological nystagmus such as downbeat, rebound and periodic alternating nystagmus, as well as abnormal pursuit, VOR/OKR abnormalities and saccadic dysmetria (Robinson & Fuchs, 2001; Strupp et al., 2011). Whereas, brainstem involvement produces slowed vertical, torsional or horizontal saccades, ophthalmoplegia, VOR/OKR impairments and gaze-evoked nystagmus (Rüb et al., 2008, Strupp et al., 2011). Affectations in the basal ganglia can lead to reduced ability to initiate voluntary eye movements and to suppress unwanted saccades, in addition to deficits in memory-guided saccades, eye-head coordination and eye-hand coordination (Hikosaka et al., 2000; Shires et al., 2010). Frontal cortex lesions produce prolongation of saccadic latency, impaired ability to make saccades to remembered target locations and errors on the antisaccade task, as well as delayed initiation of smooth pursuit and increase of catch up saccades (Pierrot-Deseilligny et al., 2004; Thurtell et al., 2007; Karatas, 2009).

3. Oculomotor findings of spinocerebellar ataxias

3.1 Spinocerebellar ataxia type 1 (SCA1)

The main eye movement abnormalities of SCA1 patients include saccadic dysmetria, gaze evoked nystagmus and depressed smooth pursuit (Matilla-Dueñas et al., 2008). Saccadic

hypermetria is observed in majority of the cases, appears at an early stage of the disease and progresses quickly (Klostermann et al., 1997; Rivaud-Pechoux et al., 1998; Buttner et al., 1998). The overshoot of saccades may reach values greater than 30% in comparison with normal subjects (Buttner et al., 1998).

Brainstem oculomotor signs such as saccadic slowing or ophthalmoparesis are observed in 74% (Schmitz-Hübsch, et al., 2008). Reduction of saccade velocity can be detected in mildly affected patients and it is accentuated with the disease duration. Advanced patients may show ophthalmoparesis or severe saccadic slowing, so that saccadic hypermetria is less noticeable in comparison to early stages (Klostermann et al., 1997). Abnormal prolongation of saccadic latency occurs in 67% of cases (Buttner et al., 1998), whereas the execution of the antisaccadic task shows increased error rates, suggesting the presence of neurodegenerative changes in the frontal cortex (Rivaud-Pechoux et al., 1998).

Reduced gain of smooth pursuit and OKN is noticed in 92% of SCA1 cases with the lowest smooth pursuit gains in comparison to SCA2 and SCA3 patients and comparable values of OKN gains to SCA2 (Burk et al., 1998). The progressive saccadic slowing causes the diminution of catch up saccades during visual tracking, leading to decrease of the smooth pursuit amplitudes on advanced disease (Buttner et al., 1998, Klostermann et al., 1997). Regarding vestibular functions, SCA1 patients are usually characterized by reduced VOR gains, which distinguish this SCA subtype from SCA2 but neither from SCA3 nor SCA6 (Burk et al., 1998; Buttner et al., 1998).

No oculomotor abnormalities of SCA1 patients correlate with the number of CAG repeats (Burk et al., 1999; Rivaud-Pechoux et al.,1998), suggesting that they are not under significant genetic control but are more dependent on disease duration.

3.2 Spinocerebellar ataxia type 2 (SCA2)

The most common oculomotor sign in patients with SCA2 is a significant reduction in horizontal saccadic eye velocity owing to brainstem involvement. This feature called attention to Wadia and Swami when made the first report of SCA2 in 1971, so that they described the disease as "*a new form of heredofamilial spinocerebellar degeneration with slow eye movements*" (Wadia & Swami, 1971). Several clinical and epidemiological studies have confirmed the high frequency of this saccadic alteration in more than 80% of cases (Velazquez-Pérez et al., 2009a; Orozco et al., 1989; Cancel et al., 1997, Wadia et al., 1998; Schmitz-Hübsch, et al., 2008).

The first electronystagmographical evaluation of SCA2 patients was conducted by Kulkarni & Wadia in 1975 who found a relative decrease of saccadic velocity up to 25% in comparison with controls (Kulkarni & Wadia, 1975). Furthermore, comparative studies of oculomotor phenotypes among patients with cerebellar ataxias demonstrated that saccadic slowing is more prominent in SCA2 patients in comparison with SCA1, SCA3, SCA6 (Burk, et al; 1999; Buttner et al., 1998; Rivaud-Pechoux et al., 1998) and late onset cerebellar ataxia (Rufa & Federighi, 2011) giving an important diagnostic value to this oculomotor feature for SCA2.

A comprehensive electronystagmographical study developed in 82 SCA2 Cuban patients showed little overlap between maximal saccadic velocity (MSV) values of SCA2 patients and controls. This study demonstrated a high sensitivity for SCA2 diagnosis assessed by a

receiver operating characteristic (ROC) yielding an area under the curve of 0.99. The most important finding of this work was the significant influence of the number of CAG repeats, but not of disease duration, on saccadic velocity (Figure 1). According to this relationship, patients with larger expansions showed more saccadic slowing, identifying the saccadic velocity as the main variable endophenotype of the SCA2, which is under strong genetic control and therefore it may be considered as a sensitive biomarker for the study of polyglutamine toxicity. Also, MSV was negatively correlated with the total score of a cerebellar ataxia scale suggesting its association with the severity of the cerebellar syndrome (Velázquez et al., 2004). Other study performed in Cuban SCA2 patients revealed a closer relationship between the saccadic velocity and the visuomotor learning capabilities assessed by a prism adaptation task (Fernandez-Ruiz, et al., 2007).

A preliminary follow-up evaluation of saccadic slowing after one year in 30 SCA2 patients revealed no significant changes of MSV (Seifried et al., 2004). Nevertheless, other follow-up study during a larger period time it is being conducted in a large Cuban SCA2 cohort.

The saccadic slowing appears during the presymptomatic stage of the disease only for 60° target amplitude, but asymptomatic subjects carrying full-penetrant CAG expansions (≥36) show reduced MSV values even for 30°. In fact, the MSV reduction is stronger in carriers of large expansions. This preclinical feature progresses insidiously and it correlates with predicted time to clinical manifestation, which classifies this variable as a preclinical biomarker of high values for diagnosis and prognosis of the disease (Velázquez-Pérez et al., 2009b).

The neuroanatomical basis of this disorder has been elucidated by post-mortem studies that demonstrated the marked loss of excitatory PBN in the PPRF (Buttner-Ennever, et al., 1985; Geiner et al., 2008), the structure that coordinates the horizontal saccades (Leigh & Zee, 2006). Early, Gierga et al, 2005 had reported a significant neuronal death in the abducens (cranial nerve VI) and oculomotor nucleus (cranial nerve III), which innervate the oculomotor muscles responsible for eye movements in the horizontal plane (Leigh & Zee, 2006).

Hypometric saccades to extreme gaze positions are usual in SCA2 patients (Velázquez, 2008), nevertheless for short target amplitudes the saccade accuracy is maintained, although some patients can make hypermetric saccades. It has been suggested that as SCA2 patients having slow saccades that are no longer ballistic, visual feedback might be continuously available during the movement execution to guide the eye to its target rendering accurate short saccades (Federighi et al., 2011).

A recent electronystagmographical study in 110 SCA2 patients demonstrated the significant prolongation of saccadic latency in 46% of SCA2 patients. This variable was neither influenced by the CAG repeats, disease duration nor ataxia score, but it was close related with the neuropsychological performance of frontal-executive tasks, which highlights the saccadic latency as sensitive biomarker of cognitive disorders in SCA2 (Rodríguez-Labrada, et al., 2011a). Additionally, SCA2 patients show increased antisaccadic error rate (Rivaud-Pechoux et al., 1998). The delayed saccade onset and antisaccadic deficits could be explained by the severe gyral atrophy and neuronal loss in the frontal lobes and neurodegenerative changes in caudate nucleus and substantia nigra (Orozco et al., 1989; Durr et al., 1995; Estrada et al., 1999; Gierga et al., 2005), as well as deficits in the processing

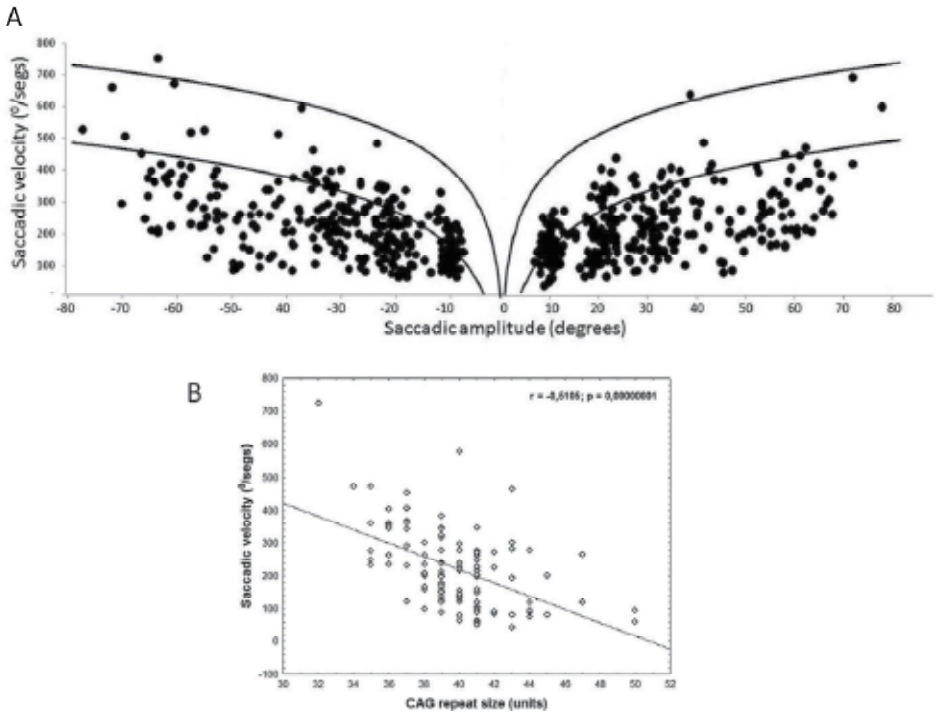
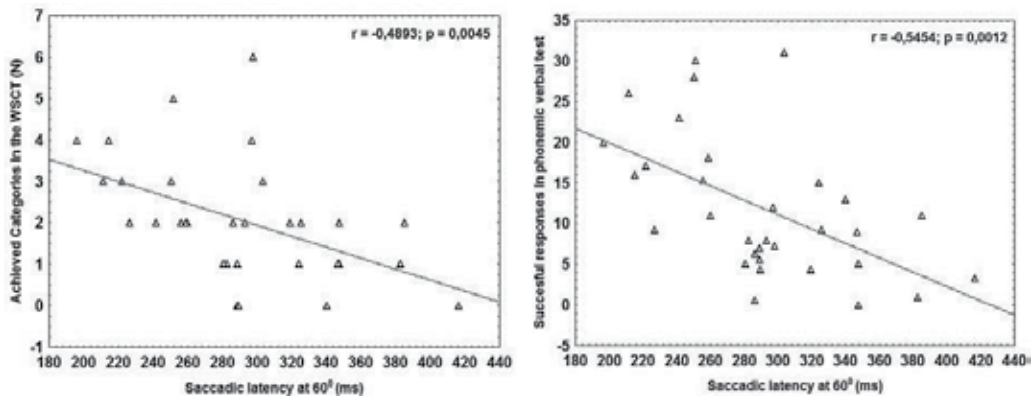


Fig. 1. Saccadic slowing in SCA2. A) Relationship of saccadic velocity and amplitudes in SCA2 patients. Show the significant reduction of saccadic velocity in almost all subjects. Dark lines represent the saccadic velocity ± 2 SD of controls. B) Influence of CAG repeat size on the saccadic velocity.

of visual information (Kremlacek et al., 2011) or in the visual-spatial attention (Le Pira et al., 2002).



(A) (B)

Fig. 2. Saccadic latency correlates with frontal-executive dysfunctions in SCA2 patients. Correlation analyses of saccadic latency with achieved categories in the Wisconsin sort card test (WSCT) and the number of correct responses in the phonemic verbal test.

Other oculomotor alterations include ofthalmoplegia, which usually appears at advanced disease in the 45% of the cases, although the severe saccadic slowing might overlook the frequency of ofthalmoplegia in SCA2. These patients have mild reduction of smooth pursuit gain in correspondence with the atrophy of cerebellar floculus (Ying et al., 2006) and the decrease of catch up saccades. The physiological and pathological nystagmus are very rare in SCA2 due to impaired ability to produce saccadic corrective phases. Some SCA2 patients have VOR responses with reduced gain (Burk et al., 1999; Rivaud-Pechoux et al., 1998; Buttner et al., 1998).

Saccadic eye movements have also been used to evaluate the efficacy of therapeutical alternatives in Cuban SCA2 patients, such as neurorehabilitation (Rodríguez et al., 2008) and oral supplementation with zinc-sulphate (Velázquez-Pérez et al, 2011a). In both cases the saccadic latency decreased significantly after the therapies, but saccadic velocity and dysmetria were unchanged.

For SCA2, the oculomotor function has not only evaluated in wake state, since the density of rapid eye movements (saccadic) during REM sleep was recently assessed. Both symptomatic and presymptomatic subjects show a marked decrease in this parameter, which is negatively correlated with the ataxia score in the patients (Velázquez-Pérez, et al., 2011b; Rodríguez-Labrada et al., 2011b). These findings suggest the usefulness of saccadic density during REM sleep as progression marker of the disease and reflect the extension of the oculomotor brainstem involvement to the sleep.

3.3 Spinocerebellar ataxia type 3 (SCA3)

Pathological nystagmus are prominent oculomotor signs of SCA3 patients. The frequency of gaze evoked and rebound nystagmus is approximately 90% (Jardim et al., 2001) being higher than those in SCA1, SCA2 and SCA6. Square wave jerks are usually reported in SCA3 subjects, unlike SCA1 and SCA2 individuals (Buttner et al, 1998; Burk et al., 1998). This oculomotor sign results from cerebellar disease and consists in small, horizontal, saccade-like movements that lead the eye away from the target trajectory and, after a delay, bring it back onto the target (Leigh & Zee, 2006).

Decreased VOR gain can be detected in majority of SCA3 patients and correlates with the CAG repeats, suggesting the pathologic involvement of the vestibular nuclei in the lateral brainstem. Furthermore, these patients show reduction of smooth pursuit and OKR gains with a presentation frequency above 70% in both cases (Buttner et al, 1998; Burk et al., 1998).

Upon saccades, the main abnormality is saccadic dysmetria. Nevertheless, there are apparently conflicting data regarding the predominant type of dysmetria. Buttner et al., 1998 reported hypermetric saccades in 86% of the cases, while Rivauld-Pechoux et al., 1998, observed a predominance of hypometric (56%) over hypermetric saccades (18%). The disagreement can be explained by differences in the clinical stage of studied patients. In fact, the 81% of the patients recruited by Rivauld-Pechoux and colleagues had a moderate to severe motor disability, which could explain the higher prevalence of saccadic hypometria.

Different to SCA2 and SCA1, decreased saccadic velocity is not a common feature of SCA3 patients (Burk et al., 1999; Rivaud-Pechoux et al., 1998; Buttner et al., 1998). This oculomotor feature appears in advanced disease, perhaps in correspondence with the degenerative

changes seen in the raphe interpositus nucleus (Rub et al., 2003), a key structure of the brainstem premotor network that contains the omnipausas neurons, a group of cells that play an important role in determining the size of the velocity command for saccades, beside their well-known role as gating saccades (Miura & Optican, 2006). Also, internuclear and nuclear ophthalmoplegia is observed in 53% and 10% of the cases respectively. The latter is associated with a more severe disease course (Jardim et al., 2001).

Finally, the prolongation of saccadic latency occurs late in few cases (14%) (Buttner et al., 1998) and the performance in the antisaccadic paradigm shows an increase in the number of errors (Rivaud-Pechoux et al., 1998).

3.4 Spinocerebellar ataxia type 6 (SCA6)

Oculomotor function of SCA6 patients is characterized by signs of cerebellar and vestibular impairments such as horizontal and vertical nystagmus, abnormal smooth pursuit, saccadic dysmetria and abnormal VOR (Buttner et al., 1998; Christova et al., 2008; Bour et al., 2008). In comparison with other SCAs, the spontaneous downbeat nystagmus and square-wave jerks have the higher incidence in SCA6 subjects, whereas gaze-evoked nystagmus, rebound nystagmus and periodic alternating nystagmus are common features too (Buttner et al., 1998; Colen et al., 2008; Kim et al., 2010).

Patients with SCA6 have the more severe pursuit, OKN and VOR-fixed deficits among other polyglutamine SCAs but these oculomotor signs are not directly associated to CAG repeats or disease duration (Buttner et al., 1998). Vertical pursuit is impaired more than horizontal whereas downward pursuit more than upward (Bour et al., 2008).

The pattern of saccadic dysmetria in SCA6 is variable since these patients can show both hypometric and hypermetric saccades (Buttner et al., 1998; Bour et al., 2008). Although the decrease of saccadic velocity is not a prominent sign in these patients, it has been reported a mild saccadic slowing in some subjects both for the horizontal and vertical planes (Bour et al., 2008). These findings suggest functional extracerebellar impairment in the saccadic system and therefore are opposed to the paradigm of SCA6 as a "pure cerebellar syndrome." In fact, the screening of non-ataxia signs reveals a 25% of brainstem oculomotor signs (Schmitz-Hübsch, et al., 2008). In these patients the saccadic latency is normal (Buttner et al., 1998).

In 2009, Christova and co-workers studied the eye movement's abnormalities in both symptomatic and asymptomatic SCA6 cohorts and noticed that square-wave jerks, saccadic abnormalities and depressed smooth pursuit can be detected even before the disease onset. Among them, the square-wave jerks were the most prominent with an apparition frequency of 80% (Christova et al., 2008).

3.5 Spinocerebellar ataxia type 7 (SCA7)

The major saccadic alteration in this SCA is the slowing of saccades, together with saccadic dysmetria (Miller et al., 2009; Manrique et al., 2009). The decrease in saccadic velocity in SCA7 is associated with marked pontine atrophy that characterizes these patients from early stages of the disease and progresses to produce significant external ophthalmoplegia in patients with longer disease history (Bang et al., 2004; Martin et al., 1999). These alterations

may precede cerebellar and retinal manifestations and are among the earliest signs of the disease (Oh et al., 2001). In addition, some cases have difficulties to initiate the saccadic eye movements and may develop gaze evoked nystagmus (Miller et al., 2009; Manrique et al., 2009).

3.6 Spinocerebellar ataxia type 17 (SCA17)

The patients with SCA17 show hypometric saccades in correspondence with the marked reduction of Purkinje cells in the cerebellum (Hubner et al., 2007). The saccadic hypometria is increased with disease duration but neither with ataxia score nor the number of CAG repeats. In 26% of cases, there are transient saccadic decelerations and accelerations causing hypometric saccades with multiple steps. Clinical assessments have reported normal (Nakamura, 2001) or slowed saccades (Rolfs et al., 2003), although the hypometria or prematurely terminated saccades may conduce to the erroneous classification of slowed saccades. In these patients, the saccadic latency is normal, while antisaccades have a significant increase in the error rate (Hubner et al., 2007).

Smooth pursuit abnormalities in SCA17 patients include decrease of initial eye acceleration, which appears even in the asymptomatic and mildly affected SCA17 mutation carriers, reduced steady state velocity and prolongation of smooth pursuit latency. Smooth pursuit gain decreases with the disease duration and ataxia score, whereas the latency prolongation correlates positively with the ataxia score. Gaze-evoked nystagmus is not a prominent feature in SCA17 patients (Hubner et al., 2007).

3.7 Other spinocerebellar ataxias

With the exception of polyglutamine expansions SCAs, the oculomotor function of remaining SCAs has not been systematically studied while most of data result for clinical assessment. SCA5 is characterized by eye abnormalities owing to cerebellar impairments such as downbeat nystagmus and impaired smooth pursuit movements (Ranum et al., 1994; Ikeda et al., 2002). Similar features occur in SCA8, in addition to saccadic dysmetria (Day et al., 2000; Koob et al., 1999), and SCA10 (Zu et al., 2000; Grewal et al., 2002; Lin & Ashizawa, 2005). SCA11 is associated with horizontal and vertical nystagmus as well as jerky pursuit (Worth et al., 1999), while approximately one third of SCA12 patients can develop saccadic slowing, abnormal smooth pursuits or pathological nystagmus (Worth et al., 1999, Fujigasaki et al., 2001). Besides, in subjects affected with SCA13 is usual to observe horizontal nystagmus (Stevanin et al., 2005; Waters & Pulst, 2008).

Regarding SCA14, the main oculomotor disturbance is the hypermetria of downgaze and horizontal saccades, even from the early stages of the disease. Additionally, upwards gaze evoked nystagmus are common in patients with longer disease duration. Smooth pursuit movements and VOR are also impaired (Yamashita et al, 2000; Brkanac et al, 2002a; Fahey et al., 2005). Eye movement abnormalities of SCA15/16 and SCA18 include nystagmus for all these SCA subtypes associated to saccadic dysmetria in the first one (Miyoshi et al., 2001; Brkanac et al, 2002b; Gardner et al., 2005). In addition, hypermetric saccades into downgaze and lateral gaze are detected in some patients with SCA20 (Knight et al., 2004).

SCA22 patients show nystagmus and impaired smooth pursuit with intermittent corrective saccadic (Chung et al., 2003), while in SCA23 the ocular dysmetria and slowed saccades can

be noted (Verbeek et al., 2004; Verbeek, 2009). SCA25, SCA26 and SCA27 are characterized by pathological nystagmus in some patients, associated with slow eye movements in SCA25, abnormal pursuit in SCA26 and saccadic dysmetria in SCA27 (van Swieten, et al., 2003; Stevanin et al. 2004; Yu et al., 2005). SCA28 patients develop gaze-evoked nystagmus at early disease, while subjects with advanced disease have slowed saccades and ophthalmoparesis with frequency estimates of 60% and 80% respectively (Cagnoli et al., 2006). SCA29, which overlap with SCA15, is characterized by bilateral horizontal nystagmus (Dudding et al., 2004). In the case of SCA30, hypermetric saccades and gaze evoked nystagmus can be detected (Storey et al., 2009), as well as abnormal pursuit in SCA31 (Ishikawa et al., 2004). Finally, in a new SCA subtype recently identified by Wang et al., 2010 in two Chinese families, it was observed ocular dysmetria as main oculomotor sign.

4. Conclusions

Eye movement abnormalities are among the most common phenotypic manifestations of patients with SCAs. The most prominent oculomotor feature is the presence of pathological nystagmus in almost all subtypes, which is generally associated to abnormal smooth pursuit, saccadic dysmetria, impaired VOR/OKR, saccadic slowing and ophthalmoplegia. These oculomotor phenotypes are useful, but not determinant, for the differential diagnosis of SCAs. For example, the early and severe saccadic slowing with rare pathological nystagmus distinguishes SCA2 from SCA1, SCA3, SCA6, SCA17 and other SCA subtypes, whereas the marked abnormalities of smooth pursuit, VOR and OKR; in association with pathological nystagmus and rare saccadic slowing may help to define a SCA6 phenotype. Nevertheless, the notable overlapping of oculomotor features between SCA subtypes implies the requirement of other clinical criteria or the genetic testing for sensitively discriminating among these diseases.

The study of eye movement abnormalities allows the identification of several biomarkers useful in the clinical and research practice of SCAs. Some of the oculomotor disturbances precede the ataxia onset, being important preclinical markers to detect the early stages of the neurodegenerative process, to evaluate the genetic susceptibility of the asymptomatic relatives and to identify individuals close to ataxia onset for enrollment in preventive clinical trials and as potential outcome variables in these same trials. As most of the oculomotor abnormalities of SCAs are significantly accentuated with the advance of the disease, these can be used in monitoring clinical progression and therefore to assess the response to symptomatic treatments at short, medium or long term. The number of CAG repeats influences significantly on the saccadic slowing in SCA2 and the reduced VOR gain in SCA3 classifying these oculomotor features as sensitive biomarker of genetic damage, useful to evaluate the effect of modifying factors and therapeutic alternatives on the polyglutamine toxicity.

Despite the above, still is necessary to deep more into the study of oculomotor function in SCAs. For example, vergence movements have not been studied, in spite of the known role of the cerebellum in these eye movements (Robinson & Fuchs, 2001) and the correspondent vergence deficits in patients with circumscribed cerebellar lesions (Sender et al., 2009). Moreover, further neuropathological, imaging and transcranial magnetic stimulation studies are required to focus the oculomotor system in order to provide more

insight on eye movement abnormalities and its potential role as therapeutic biomarkers in SCAs.

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5

Spinocerebellar Ataxia Type 2

Luis Velázquez-Pérez[1], Roberto Rodríguez-Labrada[1],
Hans-Joachim Freund[2] and Georg Auburger[3]
[1]*Centre for the Research and Rehabilitation of Hereditary Ataxias, Holguín,*
[2]*International Neuroscience Institute, Hannover,*
[3]*Section Experimental Neurology, Dept. Neurology,*
Goethe University Medical School, Frankfurt am Main,
[1]*Cuba*
[2,3]*Germany*

1. Introduction

The autosomal dominant cerebellar ataxias (ADCA) are a clinically, pathologically and genetically heterogeneous group of neurodegenerative disorders caused by degeneration of cerebellum and its afferent and efferent connections. The degenerative process may additionally involves the ponto- medullar systems, pyramidal tracts, basal ganglia, cerebral cortex, peripheral nerves (ADCA I) and the retina (ADCA II), or can be limited to the cerebellum (ADCA III) (Harding et al., 1993).

The most common of these dominantly inherited autosomal ataxias, ADCA I, includes many Spinocerebellar Ataxias (SCA) subtypes, some of which are caused by pathological CAG trinucleotide repeat expansion in the coding region on the mutated gene. Such is the case for SCA1, SCA2, SCA3/MJD, SCA6, SCA7, SCA17 and Dentatorubral-pallidoluysian atrophy (DRPLA) (Matilla et al., 2006).

Among the almost 30 SCAs, the variant SCA2 is the second most prevalent subtype worldwide, only surpassed by SCA3 (Schöls et al., 2004; Matilla et al., 2006; Auburger, 2011). The disorder was first recognized in India in 1971 by Wadia and Swami, who was intrigued by the early and marked slowing of saccade movements, associated to the cerebellar syndrome (Wadia & Swami, 1971). Contemporarily, in Cuba some neurologists were describing many families coming from the north-east region of the country with the same distinct clinical picture (Vallés et al., 1978). Subsequent epidemiological surveys in this Cuban region, Holguín province, focusing on the causes of the highest SCA2 prevalence rate worldwide found evidence for a founder effect (Orozco et al., 1989; Auburger et al., 1990; Velázquez-Pérez et al., 2001, 2009a).

2. Epidemiology

The collective worldwide prevalence of SCAs is estimated at about 6 cases per 100,000 people, although much higher figures have been reported in particular populations (Schöls et al., 2004). In the case of SCA2, the global prevalence is unknown because the most of the

few existing epidemiological studies have been performed in isolated geographical regions with families not large enough for linkage analysis. Nevertheless, large SCA2 families have been found in India, Martinique, Australia, Tunisia, Germany, Italy, Mexico, Poland and especially in Cuba (Klockgether, 2007; Sulek-Pitkowska, et al., 2010, Velázquez-Pérez et al., 2009a).

SCA2 represents 87% of ADCAs in Cuba, with a national prevalence rate of 6.57 cases per 10^5 inhabitants. If asymptomatic at-risk individuals are included in the prevalence analysis, the prevalence rate increases up to 28.51 cases per 10^5 inhabitants, with remarkable values in various eastern provinces, especially in Holguin (Figure 1A). In fact, there are regions in this province where the prevalence reaches higher values such as Baguanos municipality (141.7 per 10^5 inhabitants) (Figure 1B) (Velázquez-Pérez et al., 2009a).

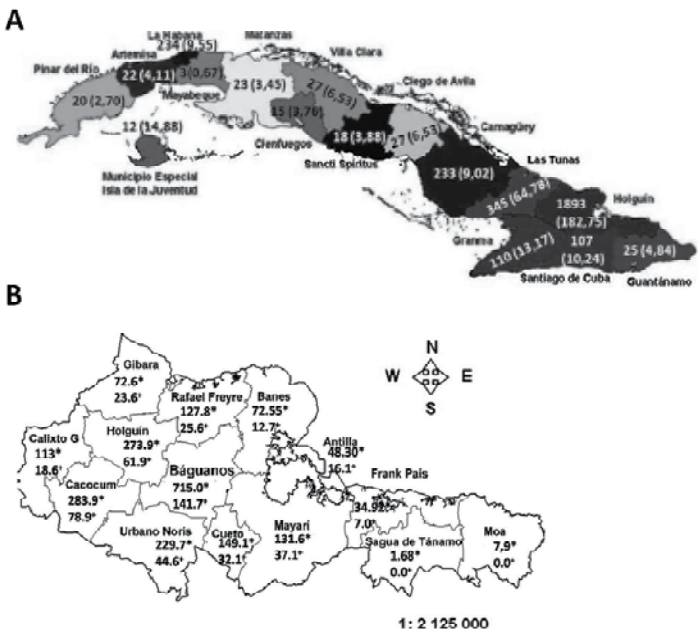


Fig. 1. Epidemiological picture of SCA2 in Cuba. A) Number and prevalence (in parenthesis) of SCA2 mutation carriers in all Cuban provinces. B) Prevalence rates of SCA2 patients (+) and SCA2 mutation (*) in Holguin province, Cuba (2006–2007).

3. Clinical features

The clinical picture of SCA2 includes a cerebellar syndrome in all patients, characterized by ataxic gait, cerebellar dysarthria, dysmetria and dysdiadochokinesia (Orozco et al., 1989; Orozco-Diaz et al., 1990). Patients also exhibit abnormal tandem stance (95%), slow saccadic eye movements (91%), limited voluntary ocular movements (88%), loss of vibration sense (73%), areflexia or hyporeflexia (77%) after initial hiperreflexia and abnormal swallowing (76%) (Velazquez-Pérez et al., 2009a).

Autonomic abnormalities (urinary dysfunction, hypohidrosis, constipation, and sexual dysfunction) are present in 78% of the cases and are accentuated in late stages of the disease

(Sánchez-Cruz, et al., 2001; Velázquez-Pérez et al., 2009a, Montes et al., 2010), together with dysphagia, ophthalmoplegia and distal amyotrophy. Sleep disturbances are frequent complaints of SCA2 patients and their relatives. The most prominent sleep disorders are restless legs syndrome (Trojano et al., 1998; Schöls et al., 1998; Abele et al., 2001; Irazno et al., 2007), muscle cramps, insomnia and reduced dream recalls (Velázquez-Pérez et al., 2011a).

Other clinical manifestations of SCA2 are the cognitive dysfunctions, which include frontal-executive impairment, verbal short-term memory deficits as well as reduction of attention and concentration (Storey et al., 1999; Reynaldo-Arminan et al., 2002; Bürk et al., 1999a; 2003). Although neuropsychological pattern of cognitive disturbances of SCA2 patients not necessarily resembling dementia, some studies have reported high frequency of demented patients (Durr et al., 1995, Burk et al., 1999a), but in the SCA2 Cuban population this neuropsychological state is rare (Reynaldo-Arminan et al., 2002; Orozco et al., 1989; 1990). Depression/anxiety/suicide attempts are found in a third of cases (Reynaldo-Arminan et al., 2002). In comparison to other SCAs, the frequency of slowed ocular movements, postural and action tremor and hyporeflexia are distinctive features of SCA2 (Schöls et al., 1997).

Extrapyramidal manifestations are common in SCA2 patients. Myoclonuses are reported in 13.7% whereas dystonia is present in 14.2%. Chorea may appear in approximately 7%. These symptoms are accentuated in patients with larger CAG repeats. Parkinsonian signs appear in some patients with low-range expansions containing CAA interruptions (Gwinn-Hardy et al., 2000; Payami et al., 2003; Lu et al., 2004; Charles et el, 2007). Among these manifestations, resting tremor (14,9%) and rigidity (7,9%) are the most common (Schmitz-Hubsch, et al., 2008). Recently it was reported an unusual case of SCA2 presenting as an ataxia-parkinsonism-motor neuron disease syndrome in a 46-year-old Brazilian man with 40 CAG repeats in the SCA2 gene (Braga-Neto et al., 2011).

The age at onset varies from 3 to 79 years (mean 33). Usually, the first symptom of the disease is the gait ataxia (97%), followed by the cerebellar dysarthria (3%). However some extracerebellar manifestations may occur a decade or more before the onset of gait instability or dysarthria, such as painful muscle cramps in the calf, sleep disturbances, problems with hand writing (Globas et al, 2008), as well as autonomic alterations, consisting in constipation (19.4%) and pollakiuria (17.7%) (Montes-Brown et al, 2011). In the Cuban SCA2 population the anticipation of clinical manifestation age in successive generations is observed in 80% of transmissions, usually upon transmission from an affected father (Velázquez-Pérez et al., 2009a).

Clinical features develop progressively with an increase in cerebellar syndrome, saccade slowing, and other features which confine the patients first to a wheelchair and following to a bed, where they die approximately 15–20 years after the initial symptoms. Nevertheless patients with larger CAG repeats have earlier age at onset, more saccadic slowing, axial tremor, pyramidal-dystonic-choreic signs, mental deficit and in general a faster progression to death (Filla et al 1999, Cancel et al 1997; Schöls et al., 1997; Sasaki et al., 1998; Filla et al., 1999; Velázquez-Pérez et al., 2009a) and the total disease duration from onset to death may vary between 6 and 50 years (Klockgether et al., 1998; Maschke et al., 2005). Also, the female gender is associated with shortened survival (Klockgether et al., 1998). The main cause of death is bronchopneumonia (63%), followed by bronchial aspiration and cardiovascular incidents, among others (Velázquez-Pérez et al, 2011b).

Pediatric-onset SCA2 is associated with large CAG expansions. Infantile phenotype includes rare symptoms such as retinitis pigmentosa, myoclonus-epilepsy, tetraparesis, developmental delay and facial dysmorphism (Babovic-Vuksanovic et al 1998; Rufa et al., 2002; Tan et al., 2004; Di Fabio et al., 2011). Ramocki and coworkers describe a female child who met all developmental milestones until age 3 years, deterioration of expressive language, comprehension, memory, graphomotor skills, and dysarthria. Cranial nerve examination showed bilaterally restricted lateral gaze with oculomotor apraxia (Ramocki, et al., 2008). Abdel-Aleem and Zakiwith reported a male child with progressive extrapyramidal manifestations, developmental delay, slow eye movements and cognitive impairment, trophic changes, vasomotor instability and dysphagia (Aleem and Zakiwith, 2008)

4. Molecular genetics

The underlying mutation of SCA2 consists in the unstable expansion of the trinucleotide repeat $(CAG)_8CAA(CAG)_4CAA(CAG)_8$ within the ATXN2 gene exon 1 located on chromosome 12q24.1. This repeat encodes a polyglutamine (polyQ) tract in the protein ataxin-2 (Gispert et al., 1993; Pulst et al., 1996; Imbert et al., 1996; Sanpei et al., 1996). In normal individuals, the trinucleotide repeat length varies and contains between 13 and 27 units. Intermediate expansions between 28 and 33 units may predispose the individual to an elevated risk for the motor neuron disease ALS or the Parkinson plus syndrome PSP (Elden et al., 2010; Ross et al., 2011). The prevalence of large normal alleles potentially acting as unstable premutation is particularly high in the Cuban province Holguín (Velázquez-Pérez et al., 2009a). Family planning can be aided by presymptomatic molecular genetic diagnostics, but care has to taken to offer psychological treatment together with the genetic counseling.

Pathological alleles in SCA2 have more than 32 CAG repeats, although the repeats range between 32 and 36 units has incomplete penetrance (Pulst et al., 1996; Cancel et al., 1997; Geschwind et al., 1997). The most frequent expanded allele is 37 (72%). The expanded alleles have lost interrupting CAA-triplets, a factor thought to promote the length instability. Expansions occur in 89% and contractions in 11% of the offspring of affected patients. Paternal transmissions show higher variability in repeat lengths compared with the maternal transmissions. (Velázquez-Pérez et al., 2009a). The presence of CAA interruptions in expanded alleles appears to predispose to a phenotype with Parkinson or with motor neuron disease (Charles et al., 2007; Kim et al., 2007; Modoni et al., 2007; Corrado et al., 2011, Yu et al., 2011), although both CAG and CAA code for glutamine, indicating that the neuronal population affected by the pathogenesis is determined by RNA toxicity rather than protein toxicity.

As in other polyQ diseases, in SCA2 the age at onset and symptom severity correlate inversely with the length of the trinucleotide repeat, which accounts for ~80% of variance, whereas the remaining variability suggests the existence of modifier genes, genetic polymorphisms, epigenetic factors and unknown environmental determinants modulating age of onset (Velázquez-Pérez et al., 2009a). Supporting the above mentioned, long normal CAG repeats in the CACNA1A (Pulst et al., 2005) and RAI1 genes (Hayes et al., 2000) as well as the 10398G polymorphism in the mitochondrial complex I gene (Simon et al., 2007) are associated with earlier manifestation age, also in the Cuban SCA2 population.

4.1 The physiological role of ataxin-2 in cell biology

The ataxin-2 protein (ATXN2) is a polypeptide containing 1312 amino acids encoded by 25 exons of the SCA2/ATXN2 gene encompassed within 130 kiloBases of genomic DNA (Sahba et al., 1998), with at least five human isoforms produced by allelic splicing (Nechiporuk et al 1998; Affaitati et al., 2001; Lastres-Becker et al., 2008a) and an expression in many organs, but only selected neurons of the brain (Huynh et al., 1999). It is phosphorylated, but not glycosylated (Turnbull et al., 2004). Currently, the function of ATXN2 is not clear, but several lines of evidence evoke its involvement in RNA metabolism. For example, the protein have sequence motifs related to mRNA processing, most of ATXN2 is associated to polyribosomes, at the rough endoplasmic reticulum (Satterfield and Pallanck, 2006; van de Loo et al., 2009), and this polypeptide interacts with RNA binding proteins such as A2BP1 and PABPC1 (Shibata et al., 2000; Ralser et al., 2005a; Satterfield and Pallanck, 2006).

Interestingly, ATXN2 and its orthologues in other organisms relocalize during periods of cellular stress to mRNP granules where mRNA is stored during translation repression, promote the formation of these stress granules and inhibit cell growth (Swisher and Parker, 2010; Nonhoff et al., 2007). Furthermore, the expression of ATXN2 is induced by specific stressors (Klinkenberg et al., submitted) and ATXN2 levels increase with old age (Huynh et al., 1999). The indirect effects of ATXN2 on RNAs appear to be mediated partially by its interactor DDX6, a RNA helicase (Nonhoff et al., 2007). Also the formation of P-bodies, mRNP granules implicated in RNA degradation, appears to depend on ATXN2, which may localize to these structures and influence the microRNA-mediated deadenylation of silenced RNAs (Nonhoff et al., 2007; Kozlov et al., 2010). There is preliminary evidence that ATXN2 co-sediments and co-localizes with neuronal mRNPs which are responsible for the transport of mRNAs to synaptic sites of local protein synthesis, and indeed ATXN2 is thought to modulate mRNA translation similar to its yeast orthologue Pbp1 (Siddiqui et al., 2007). Thus, ATXN2 might be important for stimulus-dependent local mRNA translation and influence in this way both synaptic strength and long-term potentiation, an electrophysiological finding which was indeed detected in ATXN2-knock-out mice in the amygdala, but not in the hippocampus (Huynh et al., 2009).

Some ATXN2 is also demonstrable at the plasma membrane, and within its protein sequence several proline-rich domains are able to interact with SH3-motif containing proteins. Such an interaction was demonstrated for endophilin A, CIN85 and Src, three components of the endocytosis complex that modulates trophic factor signaling through receptor tyrosine kinases (Ralser et al., 2005b; Nonis et al 2008). In these reports, ATXN2 was found to antagonize the internalization of the receptor for Epidermal Growth Factor. Interestingly, two other neurodegenerative disease proteins are also interactors of this complex, namely Huntingtin and Parkin, which was shown to ubiquitinate ATXN2 directly and to rescue ATXN2-toxicity (Ralser et al., 2005b; Huynh et al., 2007). Furthermore, the deficiency of ATXN2 in knock-out mice was observed to modulate the levels of insulin receptor, resulting in insulin resistance, altered fat metabolism and obesity (Kiehl et al., 2006; Lastres Becker et al., 2008b). Interestingly, the protein family A2D which shares sequence homology with ATXN2 also shows interaction with the cytoplasmic domain of the thrombopoietin and the erythropoietin membrane receptors which lack intrinsic tyrosine kinase activity, but is also internalized to modulate downstream events of cytokine signaling (Meunier et al., 2002). Of

course, this physiological influence of ATXN2 on trophic signaling may be important for neural atrophy in SCA2. Finally, recent evidence suggests a localization and role of ATXN2 in the nucleus, acting as interactor of the transcriptional regulator ZBRK1 (Hallen et al., 2011).

4.2 ATXN2 role for different diseases

SCA2 is thought to be caused by a toxic gain-of-function of the ATXN2 protein, but it is not clear to which degree the physiological function of ATXN2 is enhanced and to which degree unspecific toxic effects such as the aggregation of polyQ domain proteins dominate in the pathogenesis. Since polyQ expansions in different disease proteins affect different neuronal populations, and since the overexpression of wild-type ATXN2 and its orthologues in lower species, which lack the polyQ domain completely, is neurotoxic, the specific properties of ATXN2 regarding expression, subcellular localization and interactors seem to be relevant in disease. Intermediate-length expansions of the ATXN2 trinucleotide repeat below the threshold of SCA2 manifestation were shown to have a pathogenic role, increasing the individual risk to manifest the motor neuron degeneration disease ALS (Amyotrophic Lateral Sclerosis) and the basal ganglia degeneration disease within the Parkinson-plus group of disorders PSP (Progressive Supranuclear Palsy) (Elden et al., 2010; Daoud et al., 2011; Ross et al., 2011; Sorarù et al., 2011; Lee et al., 2011; van Damme et al., 2011). The RNA metabolism function of ATXN2 may explain this phenomenon, since ALS pathogenesis appears to be mediated mainly by altered mRNA processing (Lagier-Tourenne et al., 2010). ATXN2 gain-of-function also potentiates toxicity of ATXN1 and ATXN3 (the SCA1 and SCA3 disease proteins, respectively) and even toxicity of Tau (the frontotemporal lobar degeneration disease protein) in the fly model (Shulman and Feany, 2003; Al-Ramahi et al., 2007; Lessing and Bonini, 2008; Elden et al., 2010). Conversely, reducing ATXN2 levels is sufficient to mitigate the neurotoxicity triggered by TDP-43, ATXN1 and ATXN3 (Al-Ramahi et al., 2007; Lessing et al., 2008; Elden et al., 2010) in yeast and flies, indicating that these effects are mediated by the physiological function of ATXN2, but not by the polyQ domain which characterizes human ATXN2 and is not conserved until mouse.

Large expansions of ATXN2 were reported to exert a profound effect on intracellular calcium levels through specific binding to the carboxy-terminal region of the type 1 inositol 1,4,5-trisphosphate receptor (IP(3)R1), an intracellular Ca(2+) release channel (Liu et al., 2009), an effect mediated by ATXN2 at its major localization in the cytoplasm.

Several lines of evidence suggest that other alterations of the physiological ATXN2 function influence additional neuron populations and diseases. In neuroblastoma tumors, an upregulation of ATXN2 was found to be a decisive factor to induce apoptosis of the aberrant cells and spontaneous tumor remission (Wiedemeyer et al., 2003). In individuals who reached an age over 100 years, a single nucleotide polymorphism within ATXN2 intron 1 contributes to the genetic signature of exceptional longevity. Moreover, in the general human population the same ATXN2 intron 1 polymorphism determines high blood pressure levels (Levy et al., 2009; Newton-Cheh et al., 2009; Sebastiani et al., 2010).

4.3 Animal models

Animal models have been useful tools to study the polyQ expansion diseases, in particular the brain tissue of early stage pathology. Specifically ATXN2 orthologues are highly

conserved until Saccharomyces cerevisiae, permitting high-throughput genetic screens into the function of ATXN2 and revealing the role of ATXN2 as a risk factor for TDP-43 toxicity and motor neuron degeneration (Elden et al., 2010). Again, Drosophila melanogaster studies demonstrated the association of dATX2 with PABP and with polysomes (Satterfield and Pallanck, 2006). The use of RNA interference in Caenorhabditis elegans demonstrated an essential role of the atx-2 gene for early embryonic development (Kiehl et al., 2000).

Taking advantage of the mouse as an organism with genetic versatility and with similarity to man in brain structure, two transgenic models of SCA2 have been generated to date. The first one was produced by Huynh et al., 2000, who reported the use of the murine PcP2 (L7) promoter to direct a strong overexpression of the human ATXN2 gene with an expanded allele of 58 CAG repeats specifically to the cerebellar Purkinje neurons. Using the rotarod test, they found that the animals became ataxic at 26 and 16 weeks for the heterozygous and homozygous transgenic mice, respectively. Also, they described progressive incoordination and morphological alterations of Purkinje cells in this animal model. In 2005, Aguiar and coworkers (Aguiar et al., 2006) generated transgenic mouse lines overexpressing the full-length human ATXN2 gene with 75 CAG units under the control of the human self promoter. A neurological phenotype was reported after 12 weeks for heterozygous and 6 weeks for homozygous mice.

5. Imaging

Magnetic resonance imaging shows early cerebellar and brainstem atrophy (Figure 2) with marked involvement of the cerebellar cortex and the pons/inferior olive region in SCA2, in excellent agreement with the traditional neuropathological nomenclature of olivopontocerebellar atrophy (OPCA). Also, frontotemporal atrophy is observed in advanced disease (Bürk et al., 1996; Giuffrida et al., 1999). Voxel-based morphometry studies have revealed the atrophy of the cerebellar and brainstem white matter as well as the symmetric loss of gray matter in the cerebellar vermis (Brenneis et al., 2003; Brenneis et al., 2005; Della Nave et al., 2008a, b, Goel et al., 2011). Positron emission tomography (PET) studies showed a reduced regional glucose metabolism in the cerebellum, brainstem and parietal cortex, which may occur years before the clinical onset of SCA2 (Inagaki et al., 2005). PET analyses also revealed the loss of striatal dopamine transporter function with nigrostriatal atrophy, similar to the pattern observed in idiopathic Parkinson's disease (Boesch et al., 2004; Wüllner et al 2005; Inagaki et al., 2005). Imaging by proton magnetic resonance spectroscopy demonstrated the loss of choline-containing compounds in SCA2 cerebella, suggesting the decreased production and/or the loss of cell membranes as well as the reduced synthesis of precursors of acetylcholine. The same study demonstrated the increase of lactate levels in the cerebellum suggesting an impairment of glycolysis and mitochondrial function (Boesch et al., 2001).

6. Neuropathology

The macroscopic examination of nervous structures in *post-mortem* samples of SCA2 patients shows a significant atrophy of the cerebellum, brainstem, frontal lobe, as well as pallor of the midbrain *substantia nigra* and a reduction of the cerebral and cerebellar white matter. Microscopically, the cerebellum is characterized by an early and marked neuronal loss in Purkinje cell layer with reduction in the number of dendritic arborizations and torpedo-like deformations of their axons. The number of granular neurons is diminished, usually toward

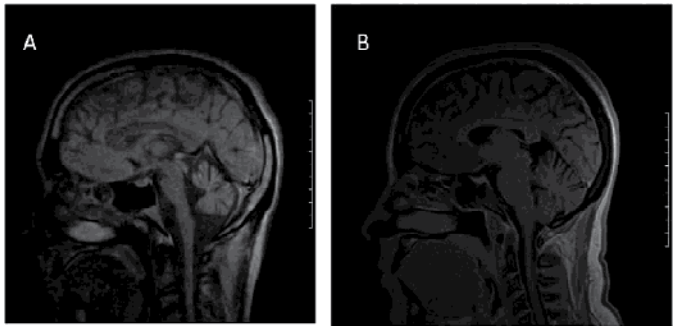


Fig. 2. MRI scans of a SCA2 patient (age 40 years; disease duration 8 years, CAG repeat size 39 units, SARA score 19) (A) and a healthy control (age 40 years, SARA score 0) (B). Note the severe atrophy of cerebellum and brainstem in the SCA2 subject.

late stages of the disease whereas the dentate nucleus is relatively spared. Parallel fibers are sparse and no climbing fibers are observed in the Purkinje cell dendritic trees (Orozco et al., 1989; Ihara et al., 1994; Estrada et al., 1999; Ying et al., 2005). In the brainstem, the most noteworthy microscopic findings are the marked loss of inferior olivary neurons in addition to the degeneration of pontine and other precerebellar brainstem nuclei (Rüb et al., 2005a; Lastres-Becker et al 2008a). The neuropathological evaluation of brainstem and cranial nerves shows that oculomotor, somatomotor, somatosensory, auditory, vestibular and autonomic nuclei are notably affected by neuronal loss and their associated fibers are atrophied and undergo demyelinization (Rüb et al., 2004a, 2004b; Rüb et al., 2006; Gierga et al., 2005; Hoche et al., 2008). Another important neuropathological marker of SCA2 is the notable reduction of neurons of the substantia nigra in the mesencephalon and the extensive degeneration of several thalamic nuclei, such as the reticular, fasciculosus, ventral anterior and posterior, lateral geniculate body and the anterior nuclei (Rüb et al., 2003a, 2003b, 2005b). In the spinal cord, an early and progressive demyelination of the posterior and spinocerebellar columns together with neuronal loss in cuneate and gracile nuclei, dorsal roots and ganglia as well as a reduction of motor neurons, usually in the cervical level and the Clarke's column, are observed (Rüb et al., 2007). Demyelination is severe (Armstrong et al., 2005). The selective neurodegeneration of large neurons affecting multiple regions of the brain with some glial inclusions is quite similar to the pattern of multiple-system atrophy (MSA) (Yagishita and Inoue 1997; Berciano and Ferrer 2005). Polyglutamine inclusion bodies appear to be much less prominent than in Huntington's disease or in SCA3 (Huynh et al., 2000; Uchihara et al., 2001; Koyano et al., 2002; Pang et al., 2002). Also, it is observed a significant loss of giant Betz pyramidal cells in the primary motor cortex. (Hoche et al., 2010). A recent study suggested that either the age at onset or the CAG repeat expansions influence on the distribution pattern of SCA2 neurodegeneration (Ishida et al., 2011).

7. Neurochemistry

The neurochemical findings in SCA2 patients were first recognized by Orozco and co-workers in 1989, (Orozco et al., 1989) who called attention to the significantly decrease of dopamine metabolites such as 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in cerebrospinal fluid (CSF), likely as result of neuronal depletion in the substancia nigra of autopsied patients. However, the mean concentration of Gamma-amino-butyric acid (GABA), as well as metabolites of noradrenalin and serotonin were similar to

normal subjects. Additionally, N-acetyl-aspartate and glutamate are markedly reduced in these patients (Oz et al., 2010).

A pathologically relevant biochemical finding is the significant reduction of zinc, iron and copper levels in the CSF and serum of Cuban SCA2 patients. The reduction of zinc levels could be associated with phenotypic features such as nerve conduction slowing, cognitive dysfunction, and immune-depression at final stages of the disease and could accentuate the dysfunction of cerebellar circuits, based on the important role of this element in the control of synapses in the cerebellum (González et al., 2006). Furthermore, most biomarkers of the antioxidant-prooxidant balance are significantly modified in Cuban SCA2 patients with an increase in malondialdehyde (MDA) as evidence of lipid peroxidation, as well as signs of oxidative damage to protein and DNA and significant reduction of the reduced glutathione (GSH). Also, the activity of glutathione S-transferase (GST), superoxide dismutase (SOD) and catalase (CAT) are depressed in these patients with a disruption of the balance CAT/SOD (Velázquez-Pérez et al, 2003; Almaguer, et al., 2005). A third interesting finding is the decrease of erythropoietin levels in the CSF with a compensatory increase of this molecule in the serum of Cuban SCA2 patients, suggesting the existence of reduced capabilities of neuroprotection in the nervous system (Velazquez-Pérez et al., 2011b). We believe that these biochemical features may contribute to the high phenotypic variability of SCA2 and that they could constitute potential therapeutical targets to design future clinical trials.

8. Neurophysiology

8.1 Nerve conduction and electromyography studies

The most common electrophysiological finding in SCA2 patients is a predominantly sensory axonal neuropathy, expressed by the early and progressive reduction of sensory amplitudes, suggestive of dorsal root ganglionopathy. These alterations are associated with slowing of nerve conduction as sign of demyelination. The progression rate of sensory axonal neuropathy is notably accentuated in patients with large CAG expansion sizes. Motor nerve conduction parameters are usually normal, but in patients with 10-15 years of disease duration it is possible to observe a reduction of motor amplitudes (Kubis et al., 1999; van de Warrenburg et al., 2004; Velázquez-Pérez et al., 2007, 2010). Electromyographical findings reveal motor unit potentials (MUP) with light polyphasic alterations, increased amplitudes and isolated contraction pattern in the first stage of the evolution. In advanced stages of the disease signs of denervation can appear (fibrillations and fasciculations) and the contraction pattern becomes simple oscillations, indicating the loss of motor neurons in the anterior horn of the spinal cord (Velázquez-Pérez et al, 2009b).

8.2 Somatosensory evoked potentials (SSEP)

Tibial nerve SSEPs are characterized by a marked prolongation of the P40 component and central conduction time latencies. In the median nerve SSEP there is a latency prolongation of N20 and N13 components in addition to a reduction of amplitude of Erb potentials. In almost all cases, the SSEPs show abnormal morphology and reduced reproducibility. These alterations get worse quickly in patients with larger CAG repeat number and may be detected even in presymptomatic subjects (Velázquez-Pérez et al., 2007, 2008).

8.3 Brain Stem Auditory Evoked Potentials (BSAEP)

BSAEPs have poor reproducibility and unstable morphology in 95% of the patients, in addition to the increase of latency of the waves III and V and the prolongation of the I–III interpeak interval. These abnormalities are common in patients with disease duration above 10 years but the abnormal reproducibility and morphology can be detected since preclinical stage (Velázquez-Pérez et al., 2007, 2008).

8.4 Visual Evoked Potentials (VEP)

VEP are frequently normal in SCA2 patients, but some patients in advances stages of the disease have prolonged P100 latencies with normal amplitudes. These findings reflect the integrity of the visual pathway in Cuban SCA2 patients, allowing us to distinguish SCA2 from other spinocerebellar ataxias such as SCA1, SCA3 and in particular SCA7 (Velázquez-Pérez et al., 2007, 2008).

8.5 Event-related evoked potentials (ERPs)

ERPs revealed prolongation of visual P300 latencies in 40% of cases with a significant correlation of this variable with the disease duration and clinical affectation (Kremlacek et al., 2011).

8.6 Motor evoked potentials

The study of the corticospinal tract by transcranial magnetic stimulation in SCA2 patients reveals an increase of central motor conduction time and motor threshold. Also, intracortical facilitation may be reduced and the induced cortical silent period prolonged. The progression of these abnormalities is dependent on the disease duration and ataxia severity. They probably reflect the reduced excitability of the motor cortex, disturbed conduction along the pyramidal tract and the loss of facilitatory influences of the cerebellum on the primary motor cortex (Yokota et al., 1998; Restivo et al., 2000, 2004; Schwenkreis et al., 2002)

8.7 Electrooculography

The main oculomotor abnormality in SCA2 is the slowing of horizontal saccadic movements, which is probably the result of early pontine brainstem degeneration. This feature is electrooculographically detectable in 99% of the patients and in several presymptomatic subjects. The maximal saccade velocity is negatively correlated with the polyQ expansion and the ataxia score, but is not significantly influenced by the disease duration. (Rivaud-Pechoux et al., 1998; Bürk et al., 1999b; Velázquez-Pérez et al., 2004, 2008, 2009c). The prolongation of saccadic latency is observed in 46% of the cases, reflecting the cortical/subcortical involvement in SCA2. Although this saccadic feature is not directly influenced by the CAG repeats or the disease duration it is close related with the frontal-executive dysfunctions, identifying it as a promising cognitive biomarker (Rodríguez-Labrada et al., 2011a). Additionally, SCA2 patients showed saccadic dysmetria reflecting the cerebellar involvement (Velázquez-Pérez et al., 2008) although saccades made for short target amplitudes are usually accurate due to the visual feedback might be continuously available during the slow movements (Federighi et al., 2011). Furthermore, gain measurements in smooth pursuit movements and horizontal optokinetic nystagmus are

slightly reduced in SCA2 patients, whereas the vestibulo-ocular reflex is normal (Buttner et al., 1998).

8.8 Videopolysomnography and electroencephalography

Sleep disorders are common complaints of SCA2 patients, fundamentally towards the final stages of the disease. Clinically, the most prominent findings are a restless legs syndrome and muscle cramps, which appear in 45 % of the cases. Patients with REM (rapid eye movements) sleep behavior disorder; bruxism and excessive daytime sleepiness are scarse. The polysomnographical evaluation reveals a reduction of REM sleep with decreased REM density in 70% of patients. These REM sleep abnormalities appear before the disease onset and their progression rates depend on ataxia severity and disease duration. (Velazquez-Pérez et al., 2011a; Rodríguez-Labrada et al., 2011b). REM sleep without atonia appears in 31% of SCA2 patients and showed a significant correlation with the ataxia score and CAG expansions (Velazquez-Pérez et al., 2011b). Periodic legs movements (PLMs) are also observed, in the 38% of SCA2 patients (Figure 3). They are directly associated with the clinical severity of the disease and their progression rate is notable (Velazquez-Pérez et al., 2011a). Other less prominent sleep abnormalities are the decrease of sleep efficiency, increase of arousal index and central apnea index. (Boesch et al., 2006; Tuin et al., 2006).

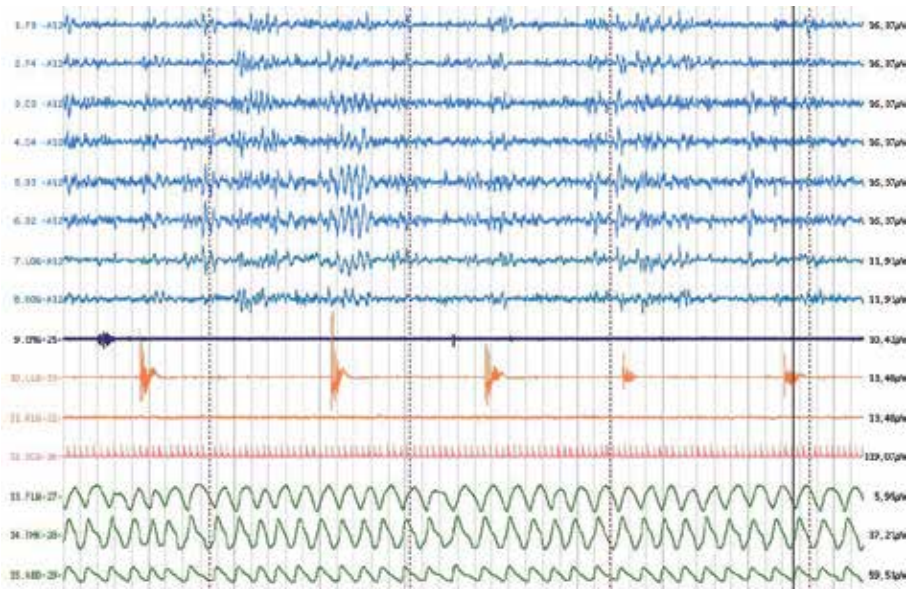
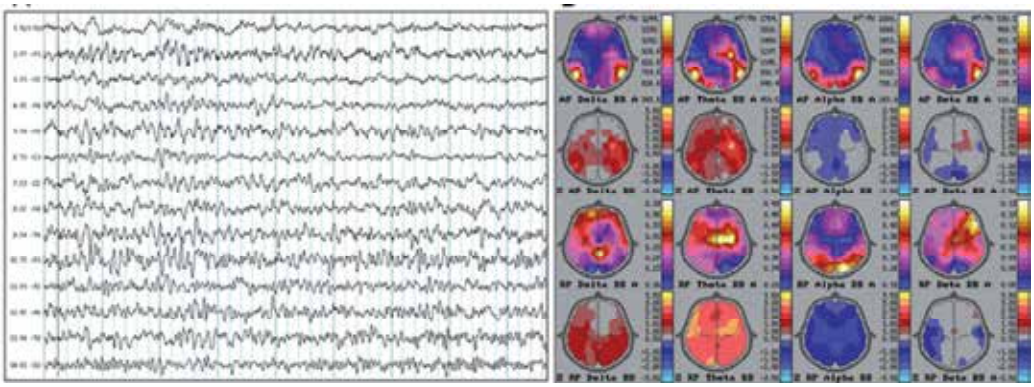


Fig. 3. Two-minute epoch of stage 2 sleep showing periodic leg movements in a SCA2 patient with 44 years old, 12 years of disease duration, 39 CAG repeats in the SCA2 gene and ataxia score in 15 units.

The conventional EEG in SCA2 patients shows a predominantly diffuse theta activity with reduced reactivity to eye opening in 72 % of the cases. In the brain electrical activity mapping a significant increase of absolute power for the theta band with reduction of absolute power for the alpha band is observed (Figure 4).



(A) (B)

Fig. 4. Conventional (A) and quantitative (B) EEG from a SCA2 patient with age 40 years, 10 years of disease duration, a repeat expansion to 40 CAG in the SCA2 gene and an ataxia SARA score of 17.

8.9 Other neurophysiological alterations

The study of autonomic control of cardiovascular function by heart rate variability (HRV) in a large group of SCA2 patients reveals the presence of cardiovascular autonomic dysfunction associated to SCA2 (Pradhan et al, 2008; Montes-Brown et al., 2010). Additionally, SCA2 patients show a significant impairment of olfactory threshold, identification and discrimination capabilities. The score of the *University of Pennsylvania smell identification test* (UPSIT) is significantly reduced and it correlates positively with ataxia score but it is not influenced by the age, age at onset, disease duration and CAG repeats (Velázquez et al, 2006).

The prism adaptation task let us identify the impaired adaptation decrement. This alteration is accentuated in patients with larger expansions. Also, the deterioration in the adaptation correlates with the motor performance and saccade velocity, suggesting that structures that degenerate in this disease may contribute to both adaptation and motor performance (Fernandez-Ruiz et al, 2007; Velázquez-Pérez et al, 2009d).

9. Early preclinical signs

The earliest subclinical sign appears even 15 years before the onset of ataxia by the slowing of horizontal saccades at 60^0 of target displacement, with amplitudes and latencies normal. This electrophysiological abnormality is accentuated in subjects with larger CAG repeats and reflects probably the early dysfunction or degeneration of paramedian pontine reticular formation (Velázquez-Pérez et al., 2009c). This alteration is followed by the reduction of REM sleep percentage with decreased rapid eyes movements' density, which may precede the ataxia onset by 10 years although its progression during this stage is insidious (Rodríguez-Labrada et al., 2011b). Other preclinical alterations include decrease of sensory amplitudes (Velázquez-Pérez et al., 2010), increased P40 latency (Velázquez-Pérez et al., 2007), motor performance deficits, shown by the prism adaptation task (Velázquez-Pérez et al., 2009d) and reduced capabilities to identify specific odors in a sensible smell

identification test (UPSIT). The comprehensive analysis of these early signs in SCA2 suggests the necessity for revisit the current criteria to define the disease onset delineating the boundaries between presymptomatics and symptomatic states.

10. Therapeutical options

Till now, there is no specific treatment for SCA2. Physiotherapy and neuropsychological rehabilitation have palliative effects on motor and cognitive symptoms. Therefore, Cuban SCA2 patients receive a specialized neurorehabilitation program (Pérez-Avila et al., 2004) since 1998, which has been applied to more than 400 patients and has allowed some recovery of motor, cognitive and antioxidant functions in about 75% of the treated patients (Rodríguez et al., 2008).

Regarding clinical trials, few studies have been conducted. For example, muscle cramps are successfully treated with magnesium and levodopa treatment alleviates the parkinsonian signs in SCA2 patients (Lastres-becker, 2008a), whereas severe myoclonus at advanced stage could be dramatically improved by piracetam (De Rosa et al., 2006). Recently, a randomized, double-blind, placebo-controlled pilot trial using riluzole resulted effective to SCA2 and other subjects with cerebellar ataxias (Ristori et al., 2010). Additionally, a double-blinded and placebo-controlled clinical trial with 50 mg zinc sulphate in 36 Cuban SCA2 patients was effective in increasing the zinc levels in serum and CSF of treated subjects and some benefit of this treatment for the cerebellar syndrome, the peripheral neuropathy and the restoration of antioxidant functions was apparent (Velázquez-Pérez et al., 2011c).

Deep brain stimulation with novel patterned low-frequency stimulation (PLFS) was effective in localizing the tremor generator at a subthalamic-thalamic electrode position, suppressing a coarse postural tremor for several postoperative years in one case (Freund et al., 2007; Barnikol et al., 2008).

11. Conclusions

In conclusion, although we have learnt much since SCA2 was described as a distinct clinical entity (Wadia and Swami, 1971) and since its cause was identified and genetic counseling became available (Imbert et al 1996; Pulst et al., 1996; Sanpei et al 1996), until today we have only taken the first steps towards understanding the pathogenic mechanisms and validating neuroprotective therapies.

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6

Machado-Joseph Disease / Spinocerebellar Ataxia Type 3

Clévio Nóbrega[1] and Luís Pereira de Almeida[1,2]
[1]*CNC - Center for Neurosciences & Cell Biology, University of Coimbra;*
[2]*Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal*

1. Introduction

Spinocerebellar Ataxia type 3 (SCA3) or Machado-Joseph disease (MJD) is one of the most common polyglutamine (polyQ) diseases, which comprise a group of inherited neurodegenerative conditions characterized by the pathological expansion of CAG trinucleotide repeats in the translated regions of unrelated genes. The expansion of a (CAG) tract in the coding region of the causative gene *MJD1*, translates into an expanded polyglutamine tract that confers a toxic gain of function to the ataxin-3 protein. The mutant protein form has 55-84 consecutive glutamines, in contrast to the normal ataxin-3, which carries 10-51 glutamines.

MJD is a fatal disease of the central nervous system (CNS) and a dominant neurodegenerative disorder of adult onset, characterized by a wide range of clinical symptoms, including gait and limb ataxia, peripheral neuropathy, bulging eyes, ophthalmoplegia, postural instability, dystonia, amyotrophy, dysarthria, nystagmus, lingual fasciculation's, facial myokymia and, in some cases, parkinsonism. The expression of mutant ataxin-3 is widespread, although neurodegeneration in MJD has been described in particular brain regions such as the cerebellum, brainstem, substantia nigra, pontine nuclei and striatum. A hallmark of the disease is the presence of neuronal intranuclear inclusions of mutant ataxin-3. The genetic basis of MJD is well described, however, the molecular basis is still poorly understood and controversial. Several pathogenesis mechanisms have been proposed for MJD (as well for other polyQ diseases), which could be explored as potential therapeutic approaches to MJD. Decreasing the expression of mutant ataxin-3 through gene silencing has been shown to be one of the most promising therapeutic approaches to MJD. However, several others are presently under investigation, such as the inhibition of protein cleavage, and the induction of autophagy, as well as strategies based on neuroprotection or regulation of transcriptional dysfunction. The main aim of this chapter is to review the current knowledge about MJD/SCA3, including a short review of clinical and neuropathological aspects of MJD and a particular focus on the pathogenesis and potential therapeutic strategies for the disease.

2. Machado-Joseph disease

Machado-Joseph disease (MJD) or spinocerebellar ataxia type 3 (SCA3) is the most common autosomal subtype of ataxia worldwide (Coutinho and Andrade, 1978; Rosenberg, 1992; Ranum et al., 1995; Schols et al., 2004). It is caused by the unstable expansion of a CAG repeat in the *MJD1* gene, which translates into a polyglutamine tract within the ataxin-3 protein (Takiyama et al., 1993; Kawaguchi et al., 1994). This neurodegenerative disorder of adult onset was named after Antone Joseph and William Machado, of Portuguese Azorean origin, who migrated to USA. MJD was subsequently identified in Brazil, Japan, China, Australia and many other countries. In the islands of the Azores, namely São Miguel and Flores, MJD reaches the highest prevalence (1:140 in the small island of Flores) reported worldwide (Sudarsky and Coutinho, 1995).

3. Clinical and physiological features

MJD is characterized primarily by cerebellar ataxia and pyramidal signs variably associated with a dystonic-rigid extrapyramidal syndrome or peripheral amyotrophy (Lima and Coutinho, 1980; D'Abreu et al., 2010). The clinical hallmark of MJD is progressive ataxia, a dysfunction of motor coordination that can affect gaze, speech, gait, and balance (Taroni and DiDonato, 2004). Other clinical manifestations include external progressive ophthalmoplegia, dystonia, intention fasciculation-like movements of facial and lingual muscles, as well as bulging eyes. Progressive ataxia, hyperreflexia, nystagmus, and dysarthria may occur early in the disease (Lima and Coutinho, 1980; Sudarsky and Coutinho, 1995).

MJD type	Age of onset	Prevalence	Symptoms
I	5-30 years		Limb and gait ataxia, severe dystonia, pyramidal signs, progressive external ophthalmoplegia. Fast progression of symptoms
II	≈ 36 years	The most common	Ataxia, pyramidal deficits and progressive external ophthalmoplegia
III	≈ 50 years	The second most common	Limb and gait ataxia, with marked pyramidal signs. The progressive external ophthalmoplegia can or not manifest. This type has a moderate progression and can evolve to one of the other types
IV	38-47 years	In patients with the fewest CAG-repeats expansion	Slow progressive parkinsonism, responsive to the L-DOPA treatment, fasciculations and peripheral neuropathy
V			Marked spastic paraplegia with or without cerebellar ataxia. This type is usually mis-diagnosed as hereditary spastic paraplegia (HSP)

Table 1. Classification of MJD according to symptoms, prevalence and age of onset.

Recent clinical data has demonstrated increased incidence of non-motor symptoms, which include cognitive and psychiatric disturbances, olfactory dysfunction, and sleep disorders (Rub et al., 2008). Levodopa-responsive parkinsonism symptoms resembling Parkinson's disease were also reported (Gwinn-Hardy et al., 2001). MJD patients present attention and

executive dysfunctions, and mildly depressed mood (Klinke et al., 2010). Based on clinical manifestations, MJD was divided into four sub phenotypes (Riess et al., 2008), which in some cases during the progression of the disease can evolve from one type to the other (Fowler, 1984). Recently, an additional MJD type (V) has been proposed based in a homozygous 33-years old patient of Portuguese/Brazilian descent (Lysenko et al., 2010) (Table 1).

4. Neuropathological features

The neuropathological alterations of MJD in the brain consist of widespread neuronal degeneration affecting multiple neuronal systems and not confined to the cerebellum, brain stem, and basal ganglia (Rub et al., 2008). The neuropathology involves cerebellar systems (particularly dentate nucleus and pontine neurons), substantia nigra, and cranial nerve motor nuclei, with relative preservation of cerebellar cortex, particularly Purkinje cells and inferior olive (Sudarsky and Coutinho, 1995; Durr et al., 1996; Yamada et al., 2008). However in some cases, loss of granule and Purkinje cells was found in the cerebellum, mainly in the vermis (Munoz et al., 2002). A marked degeneration of Clarke's column nuclei and vestibular and pontine nuclei is observed (Durr et al., 1996). Marked neuronal loss is also observed in the anterior horn of the spinal cord, and motor nuclei of the brainstem (Rub et al., 2008). Involvement of cerebellar cortex, autonomic ganglia and striatum were also confirmed in MJD (Yamada et al., 2001; Paulson et al., 1997b; Alves et al., 2008b). Recent data based on neuroimaging techniques (magnetic resonance imaging – MRI, and quantitative 3-D volumetry) confirmed a severe atrophy in MJD patients in the whole brainstem (midbrain, pons, and medulla), whole cerebellum, cerebellar hemispheres and cerebellar vermis, putamen and caudate nuclei (Schulz et al., 2010). Significant correlation of both brainstem and cerebellar atrophy with CAG repeat length, age, disease duration and degree of disability has also been recently reported (Camargos et al., 2011). Furthermore, an inverse relationship has been found in MJD patients between posture, gait and limb kinetic subscore (assessed by the Scale for Assessment and Rating Ataxia) and the brainstem and cerebellar hemispheric volumes (Jacobi et al., 2011).

5. The *MJD1* gene

MJD is associated with an unstable expansion of a CAG tract in the coding region of the *MJD1* gene localized on chromosome 14q32.1 (Takiyama et al., 1993; Kawaguchi et al., 1994). *MJD1* encodes ataxin-3, a polyubiquitin-binding protein whose physiological function has been linked to ubiquitin-mediated proteolysis (Burnett et al., 2003; Donaldson et al., 2003; Doss-Pepe et al., 2003; Scheel et al., 2003; Chai et al., 2004; Durcan et al., 2011). The mutation results in an expanded polyglutamine tract at the C-terminus of ataxin-3 (Kawaguchi et al., 1994; Durr et al., 1996). The CAG repeats in the *MJD1* gene range from 10 to 51 in the normal population and from 55 to 87 in MJD patients (Cummings and Zoghbi, 2000; Maciel et al., 2001; Gu et al., 2004; Padiath et al., 2005). This high threshold of pathogenicity is a special characteristic of this disorder, since in most other polyglutamine disorders trinucleotide repeats over 36 to 40 become pathogenic. There is an inverse correlation between the age of onset and the number of CAG repeats, as is the case for other polyglutamine disorders (Maciel et al., 1995; Maruyama et al., 1995; Globas et al., 2008).

6. The ataxin-3 protein

Ataxin-3 is a modular protein with an overall molecular weight of 42 kDa, containing a conserved N-terminal Josephin domain (Masino et al., 2003; Scheel et al., 2003; Albrecht et al., 2004), followed by two ubiquitin-interaction motif (UIM) domains and the polyglutamine repeat region (Figure 1). Alternative splicing of the *MJD1* gene has been shown to result in the production of different isoforms of ataxin-3 varying at the C-terminal portion of the protein (Goto et al., 1997), one of these containing a third UIM domain after the polyglutamine region (Ichikawa et al., 2001). Fifty-six alternative splicing variants of the ataxin-3 mRNA were recently identified, from which 50 had not been previously described, and 26 were only found in MJD patients (Bettencourt et al., 2010). Alternative splicing of ataxin-3 sequences distinct from the trinucleotide repeat may alter the properties of the encoded polyglutamine disease protein and thereby perhaps contribute to selective neurotoxicity (Harris et al., 2010). The protein is expressed in various tissues, suggesting that it plays an important role in eukaryotic cells (see Matos et al., 2011 for an extensive revision of putative ataxin-3 functions).

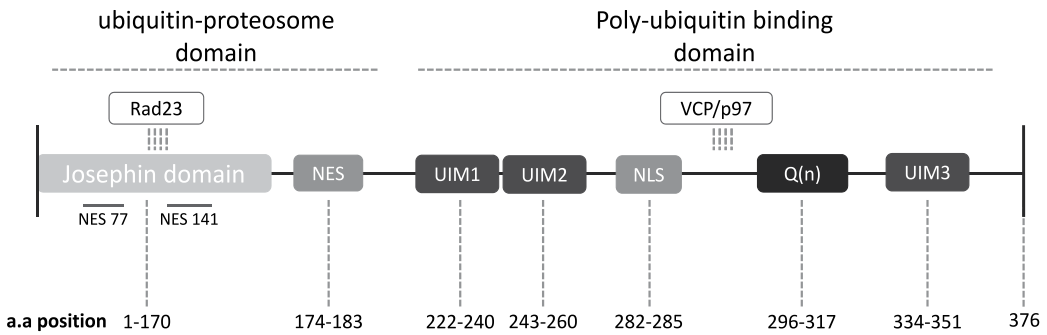

Fig. 1. **Structure of the ataxin-3 protein.** Ataxin-3 is mainly composed of a highly conserved N-terminal domain (Josephin), encoding a predicted ubiquitin-specific protease with the catalytic triad of amino acids (Cys14, His119, and Asn136), a nuclear export signal (NES), followed by a flexible C-terminal tail with 2 or 3 ubiquitin-interacting motifs (UIM), a nuclear localization signal (NLS) and the polyglutamine stretch ($Q_{(n)}$). Rad23 and VCP/p97, the two most frequently described interacting partners of ataxin-3, bind to the Josephin domain and the C-terminal region of the protein, respectively.

Regarding subcellular localization, ataxin-3 has been detected both in the nucleus and in the cytoplasm (Paulson et al., 1997a; Trottier et al., 1998; Ichikawa et al., 2001). A putative nuclear localization signal (NLS) has been identified upstream the polyglutamine repeat region at position 282 (Tait et al., 1998; Albrecht et al., 2004), and shown to have a weak nuclear import activity (Antony et al., 2009). Furthermore, two nuclear export signals (NES) with significant activity were identified in ataxin-3: NES 77 (177-Y99) and NES 141 (E141-E258) (Antony et al., 2009). Ataxin-3 its actively imported to and exported from the cell nucleus, and this nuclear export activity could also be dependent on a motif localized at is N-terminal region (Rodrigues et al., 2007; Macedo-Ribeiro et al., 2009), which is coherent with the hypothesis of the presence of a nuclear export signal (NES 174) following the Josephin domain (Albrecht et al., 2004).

Although the precise cellular role of ataxin-3 and how it is altered upon polyglutamine expansion is presently unknown, ataxin-3 was shown to be a polyubiquitin-binding protein (Donaldson et al., 2003; Doss-Pepe et al., 2003), interacting via the first two UIM domains with K48-linked tetraubiquitin chains (Burnett et al., 2003; Chai et al., 2004). Several lines of evidence suggest that ataxin-3 plays a major role in the ubiquitin proteasomal system, by interacting with ubiquitin and an ubiquitin-like protein called NEDD8 (Ferro et al., 2007). Ataxin-3 was reported to bind and hydrolyze polyubiquitin chains in vitro (Burnett et al., 2003). Recently, it was shown that ataxin-3 deubiquitinates parkin directly (Durcan et al., 2011). The same study argued that compared with wild-type ataxin-3, MJD-linked polyQ-expanded mutant ataxin-3 is more active, possibly owing to its greater efficiency at DUB K27- and K29-linked Ub conjugates on parkin. Ataxin-3 has been also shown to be involved in the regulation of the proteasome by interacting with various substrates (Wang et al., 2006, 2007; Rodrigues et al., 2009). Ataxin-3 deubiquitinating activity is thought to contribute to proteasomal degradation of ubiquitinated proteins by removing the poly-ubiquitin chains from substrates prior to digestion (Boeddrich et al., 2006; Winborn et al., 2008; Todi et al., 2009; Scaglione et al., 2011). Ubiquitination and deubiquitination enzymes help to control neuronal fate determination, axonal path finding and synaptic communication and plasticity (see Todi and Paulson, 2011 for a review). Altogether, these data imply that ataxin-3 modulates ubiquitin-dependent mechanisms, having an active role in the ubiquitin-proteasome pathway.

7. Nuclear inclusions

In MJD, mutant ataxin-3 aggregates into intranuclear inclusions (NIIs) with many affected neurons exhibiting more than one inclusion body, both in and outside areas affected by neurodegeneration (Paulson et al., 1997b; Schimdt et al., 1998; Rub et al., 2006a, b). Aggregates are also found in the cytoplasm of neurons in several affected areas (Hayashi et al., 2003), and in axons within fiber tracts (corpus callosum, the nigrostriatal tract, the olivocerebellar fiber, and others) known to undergo neurodegeneration in MJD (Seidel et al., 2010). The presence of these NIIs is a hallmark of neurodegeneration in the brains of MJD patients (Figure 2A), and to all the CAG repeat diseases except for the spinocerebellar ataxia type 6 (SCA6) (Paulson, 1999; Schols et al., 2004; Soong and Paulson, 2007). NIIs are eosinophilic round structures and vary in size from 0.7 to 3.7 μm. Ultra structurally, NIIs are non-membrane bound, heterogeneous in composition, and contain a mix of granular and filamentous structures. Both normal and expanded ataxin-3, and ubiquitin are components of NIIs of affected neurons in MJD patients (Paulson et al., 1997a), as well as other proteins, including heat shock proteins (HSPs) and transcription factors (Hayashi et al., 2003; Perez et al., 1998; Yamada et al., 2001). Ataxin-2, the protein that upon polyglutamine expansion causes spinocerebellar ataxia type 2 – SCA2, and the TATA box binding protein (TBP) were also found in NIIs of the pontine neurons of MJD patients (Uchihara et al., 2001).

The NIIs in MJD are distributed in many neurons covering a wide range of central and peripheral nervous system regions, including the cerebral cortex (Figure 2B), thalamus and autonomic ganglia (Schilling et al., 1999). The exact role of NIIs in neuronal cell death of MJD patients remains unclear and controversial (Bates, 2003; Michalik and Broeckhoven, 2003; Yamada et al., 2008). However, as NIIs are present in degenerated as well as spared brain regions in advanced MJD patients, NIIs are not thought to be directly pathogenic in

affected nerve cells (Rub et al., 2006b). In the other polyglutamine disorders the cytotoxicity of NIIs is also controversial. Several studies raised the possibility that NII formation may be a cellular reaction to reduce the toxic effect of mutant proteins (Klement et al., 1998; Saudou et al., 1998; Cummings et al., 1999). On the other hand, other studies revealed that the presence of transcription factors in NIIs (Yamada et al., 2001; Shimohata et al., 2000a,b), may induce secondarily transcriptional abnormalities in cell nuclei, resulting in slowly progressive neuronal degeneration.

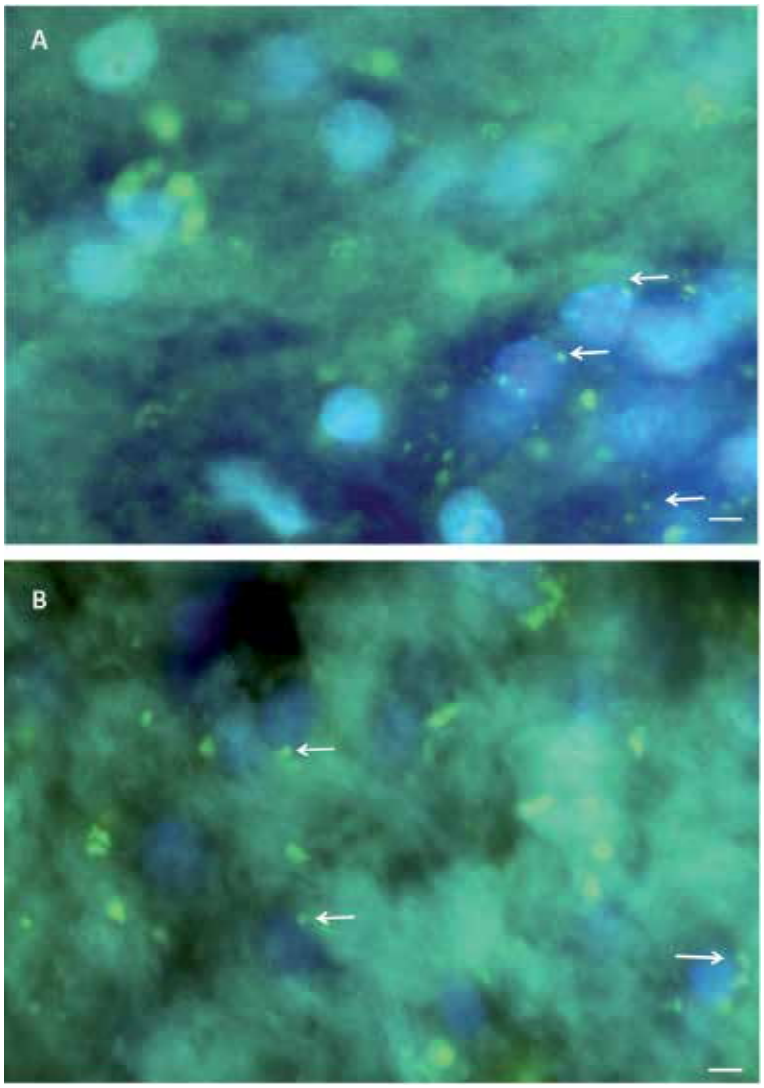

Fig. 2. **Intranuclear inclusions in the striatum of Machado Joseph disease patients.** (A) Fluorescence analysis shows ataxin-3 reaction intranuclear inclusions (green) in the neurons of the striatum of postmortem brain samples of MJD patients (white arrows). (B) Fluorescence microscopy analysis shows ataxin-3 intranuclear inclusions (green) in neurons of the cortex of postmortem brain samples of MJD patients (white arrows). Scale bar: 40μm.

8. Pathogenesis

The genetic basis of MJD is well described, however, the molecular basis is still poorly understood and controversial. It is widely accepted that polyglutamine diseases may share pathogenic mechanisms. In this section several pathogenic mechanisms that could be implicated in MJD are reviewed (Figure 3).

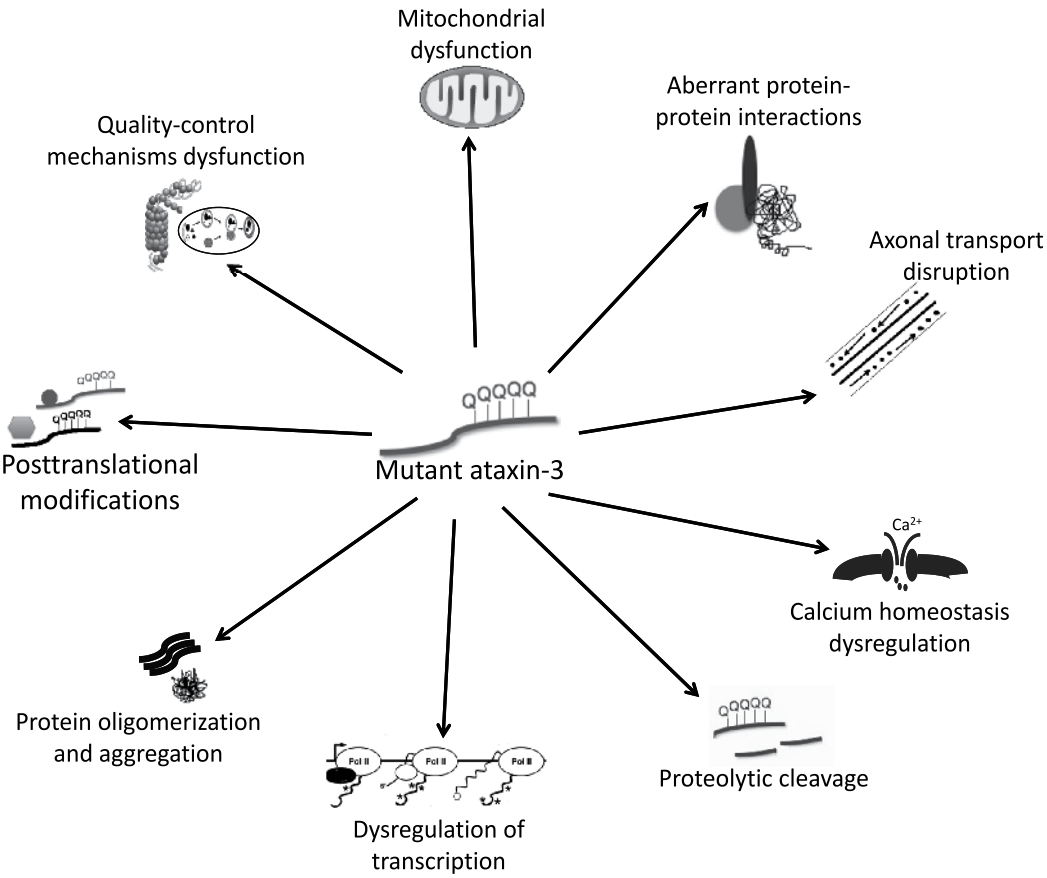

Fig. 3. **Mechanisms of pathogenesis in Machado-Joseph disease**. Several events and mechanisms could contribute to pathogenesis in MJD and other polyglutamine diseases. The presence of mutant ataxin-3 with an expanded tract in the cellular environment, triggers several events that lead to neurodegeneration in selective areas of the brain. For the neuronal cytoxicity and dysfunction several mechanisms related to the toxicity of the expanded polyglutamine stretch are important such as the oligomerization and aggregation, the formation of toxic fragments or posttranslational modifications. Furthermore, the normal function of ataxin-3 in the cell could contribute to the impairment of UPS in MJD, and thus contribute to a dysfunction in cellular quality-control mechanisms. Other mechanisms could also be important to MJD pathogenesis, such as dysregulation of transcription, mitochondrial dysfunction, aberrant protein-protein interactions, calcium homeostasis dysregulation and axonal transport disruption.

8.1 Toxicity of the polyglutamine stretch

A common feature of polyglutamine diseases is the deposition of insoluble intracellular ubiquitinated inclusions containing the misfolded disease protein (Paulson, 1999). These inclusions have long been suspected to be pathologic structures in polyglutamine diseases (Ross, 1997; Martindale et al., 1998; Yamada et al., 2000). Although this correlation is controversial and unclear (Bates, 2003; Michalik and Broeckhoven, 2003; Yamada et al., 2008), the NIIs could physically impair axonal transport or nuclear function (Morfini et al., 2005). Furthermore, the NIIs recruit other proteins, transcription factors and proteasome subunits (Chai et al., 1999a,b), underlying misfolding events that may be critical to pathogenesis (Paulson, 1999; Goti et al., 2004; Jana and Nukina, 2004; Taylor et al., 2002).

Polyglutamine monomers of ataxin-3 acquire β-strand conformations that have been shown to be cytotoxic in cultured cells (Nagai et al., 2007), assembling into oligomers (Bevivino and Loll, 2001; Takahashi et al., 2008), both of ataxin-3 as well as other polyglutamine monomers (Stott et al., 1995; Lathrop et al., 1998; Tanaka et al., 2001; Thakur and Wetzel, 2002), and can also simultaneously dissociate into monomers (Schaffar et al., 2004). Thus, it seems that β-stranded polyglutamine monomers are important for pathogenesis in MJD and other polyglutamine diseases, however its contribution to neurotoxicity is still controversial.

In several neurodegenerative disorders, including Alzheimer's disease, Parkinson's disease, prion diseases, and polyglutamine diseases, including MJD, oligomers of causative proteins have been proposed to be the most toxic structures (Walsh et al., 2002; Kayed et al., 2004) and candidates for a pathogenic intermolecular structure. Polyglutamine oligomers, in particular, have been shown to induce greater toxicity than polyglutamine monomers or inclusion bodies in differentiated neurons (Takahashi et al., 2008). This and other findings support the hypothesis that polyglutamine oligomers may have a crucial role in cytotoxicity (Poirier et al., 2002; Sanchez et al., 2003; Kayed et al., 2003; Ross and Poirier, 2005; Behrends et al., 2006).

The proteolytic cleavage of mutant protein may produce smaller toxic fragments containing an expanded polyglutamine tract, in this way facilitating the entry of cytoplasmic polyglutamine proteins into the nucleus. These toxic cleavage fragments upon release undergo the conformational change required for aggregation formation (Wanker, 2000; Ross et al., 2003). The misfolded expanded fragments may interact with full-length ataxin-3, possibly inducing a misfolding event in the polyQ tract of ataxin-3, which facilitates its stable incorporation into the fibrillar aggregates (Ikeda et al., 1996; Haacke et al., 2006). The proteolytic fragment has been proposed to be a product of caspase enzymes (Wellington et al., 1998; Berke et al., 2004), of autolytic cleavage (Mauri et al., 2006) or of calpains (Haacke et al., 2007). This toxic fragments hypothesis was also proposed for other polyglutamines diseases (Walsh et al., 2005), namely Huntington disease (Goldberg et al., 1996; Schilling et al., 2006) and spinocerebellar ataxia type 7 (SCA7) (Young et al., 2007; Takahashi-Fujigasaki et al., 2011). The mutant ataxin-3 mjd1a putative–cleavage fragment was identified in permanent clones of a transfected cell line (Yamamoto et al., 2001), transgenic mice and MJD patient's brains (Goti et al., 2004). Nevertheless, some controversy remains as other studies failed to identify the proteolytic fragments of ataxin-3 (Cemal et al., 2002; Berke et al., 2004; Chou et al., 2006). Recently, it was reported that the presence of a 259 N-terminal ataxin-3 fragment (without the polyglutamine stretch) was sufficient to induce MJD neurological phenotype in mice (Hubener et al., 2011).

The toxicity of causative gene products in MJD and other polyglutamine diseases has been proposed to be influenced not only by the polyglutamine stretch but also by the post-translational modification of amino acid residues outside the polyglutamine stretch, including phosphorylation (Fei et al., 2007; Tao et al., 2008; Mueller et al., 2009), acetylation (Li et al., 2002; Evert et al., 2006; Chou et al., 2011), ubiquitination (Matsumoto et al., 2004; Jana et al., 2005; de Pril et al., 2007), and sumoylation (Ueda et al., 2002; Shen et al., 2005). These modifications might result in aberrant interactions with other proteins or modification of the properties of causative proteins, including the stability or tendency to form toxic structures.

8.2 Protein interactions

The importance of expanded polyglutamine protein in disease progression is important, however, the toxicity of expanded polyglutamine protein does not fully explain the selective neuronal degeneration in MJD and in other polyglutamine diseases. Mutant ataxin-3 is widely expressed in the brain (Paulson et al., 1997a), even in areas with no significant neuronal degeneration. Thus, the normal function of ataxin-3 or interactions with other proteins in each neuronal subpopulation might explain its selective toxicity (Takahashi et al., 2010). Normal ataxin-3 is found in nuclear inclusions of different polyglutamine diseases, particularly in spinocerebellar ataxia type 1 – SCA1, SCA2, Dentatorubral-pallidoluysian atrophy, (Uchihara et al., 2001) and in neuronal intranuclear hyaline inclusion disease (Takahashi et al., 2001). It is also found in Marinesco bodies under stressful conditions and aging in human and non-human primates brains (Fujigasaki et al., 2000; Fujigasaki et al., 2001; Kettner et al., 2002).

Ataxin-3 recruitment to inclusions raises the possibility that normal ataxin-3 and ubiquitin-mediated pathways may be involved in cellular reactions against stress and misfolded proteins (Fujigasaki et al., 2001). In a *Drosophila* model normal ataxin-3 suppressed the neurotoxicity of mutant ataxin-3 by an ubiquitin-mediated mechanism in association with the proteasome (Warrick et al., 2005). However in a MJD lentiviral rat model the overexpression of normal ataxin-3 did not mitigate the mutant ataxin-3 induced neurodegeneration and even aggravated inclusion generation (Alves et al., 2010).

Several studies have revealed the importance of protein-protein interactions in understanding the normal function of the disease-causing protein (Steffan et al., 2001; Yoshida et al., 2002; Chen et al., 2004; Goehler et al., 2004; Ravikumar et al., 2004; Kaytor et al., 2005; Tsuda et al., 2005). Recently, the normal activity of ataxin-2 was shown to be important to MJD neurodegeneration, suggesting that toxicity of one polyglutamine disease protein could be modulated by the normal activity of another (Lessing and Bonini, 2008). The protein-protein interaction and alteration of the activity of causative proteins was also reported for other neurodegenerative disorders and is therefore an important subject of research (Lim et al., 2006; Zoghbi and Orr, 2009; Elden et al., 2010).

8.3 Dysregulation of transcription

Expanded polyglutamine proteins tend to accumulate in the nucleus, where the high concentration of solutes creates favorable conditions for interaction with transcriptional factors or cofactors (Yamada et al., 2000; Lim et al., 2008). Furthermore, many of the proteins

affected by polyglutamine expansion, such as ataxin-1 or ataxin-2 either interact or function as transcription factors (Fernandez-Funez et al., 2000; Lim et al., 2006; Lastres-Becker et al., 2008) suggesting that transcriptional dysregulation may be a central feature of the neurodegenerative mechanism in the polyglutamine disorders (Steffan et al., 2001; Nucifora et al., 2001; Minamiyama et al., 2004; La Spada et al., 2001; Hughes et al., 2001; Yamada et al., 2000; Lim et al., 2008; Godavarthi et al., 2009; Yamanaka et al., 2008, Riley and Orr, 2006). Accordingly, the transcription factor TBP and transcription co-factor CBP were shown to be incorporated into nuclear inclusions of polyglutamine-expanded ataxin-3 (McCampbell et al., 2000). Thus, it is possible that mutant polyglutamine ataxin-3 causes transcriptional dysregulation and resulting neurotoxicity. Downregulation of mRNA levels of genes involved in glutamatergic signaling and signal transduction, but no neurological phenotype, were reported in a MJD transgenic mouse expressing ataxin-3 with 79 CAG repeats in brain regions affected in the disease. This suggests the involvement of transcriptional abnormality in initiating the pathological process of MJD, with expanded ataxin-3 disrupting the normal pattern of gene transcription and contributing to cerebellar dysfunction and ataxia (Chou et al., 2008).

8.4 Ubiquitin-proteasome system dysfunctions

Cells produce a large amount of misfolded proteins, thus protein degradation systems like the UPS or autophagy are crucial to maintain cellular function and viability. A dysfunction in the UPS leads to the accumulation of misfolded proteins, resulting in dysfunction and cell death in neurons. The normal function of ataxin-3 has been linked to protein surveillance pathways (Chai et al., 2004). Ataxin-3 acts as polyubiquitin-binding protein, recruiting poly-ubiquitinated substrates through a carboxy-terminal cluster of ubiquitin interaction motifs (Burnett et al., 2003; Raoul et al., 2005). A loss of mutant ataxin-3 function could affect the UPS and in that way enhance neuronal degeneration and death. Moreover, mutant ataxin-3 nuclear inclusions are ubiquitinated and contain proteasome components, suggesting that the UPS may be disrupted by expanded protein (Paulson et al., 1997b; Chai et al., 1999b).

8.5 Autophagy impairment

There are strong evidences that proteins with a mutant polyglutamine tract are inefficiently degraded by the UPS but could be degraded by macroautophagy, a mechanism with a crucial role in degradation of insoluble aggregate-prone proteins and essential for neuronal survival (Cuervo, 2004a, b; Williams et al., 2006). Recently, our group has shown that important autophagy proteins are sequestered by mutant ataxin-3 inclusions in an MJD lentiviral model and abnormally accumulate in MJD patient's brain (Nascimento-Ferreira et al., 2011). As it happens with the UPS system a disruption in the autophagy system could enhance neurodegeneration and cell death induced by mutant ataxin-3. Accordingly, impairments in the autophagy pathway have been reported in other neurodegenerative diseases (Shibata et al., 2006, Pickford et al., 2008; Crews et al., 2010), as well as a decrease of activity with ageing (Cuervo, 2004b; Vellai, 2009).

8.6 Mitochondrial dysfunction

There is growing evidence that mitochondrial dysfunction may play important roles in neurodegeneration (Knott et al., 2008), and could be implicated in the pathogenesis of MJD

(Yu et al., 2009) and other polyglutamine diseases (Browne et al., 1997; Panov et al., 2002; Cui et al., 2006). In addition, mitochondrial dysfunction has been implicated in ageing, which is a major risk factor of progressive neurodegenerative diseases. Oxidative stress is induced by reactive oxygen species (ROS) or free radicals, and increasing with age, and possibly diminished capacity to deal with oxidative stress may cause modification of cellular macromolecules and lead to cell damage.

8.7 Impairment of axonal transport

The function and survival of neurons demands continuous axonal transport of mRNA and proteins. Several studies suggest that axonal transport disturbance is an attractive hypothesis that could explain the vulnerability of neurons (Gunawardena et al., 2003; Szenbenyi et al., 2003; Caviston et al., 2007). However, currently there is no sufficient evidence to confirm this hypothesis in polyglutamine diseases. Recently, the presence of inclusions in axons was identified in several brain regions of MJD patients affected by neurodegeneration (Seidel et al., 2010). It was hypothesized that the presence of axonal inclusions could be detrimental to axonal transport mechanisms and thereby contribute to degeneration of nerve cells in MJD.

8.8 Dysregulation of intracellular Ca^{2+} homeostasis

Intracellular Ca^{2+} homeostasis is important for the function and survival of neurons, and it has become clear that cellular Ca^{2+} overload, or perturbation of intracellular Ca^{2+} compartmentalization, can cause cytotoxicity and trigger either apoptotic or necrotic cell death (Orrenius et al., 2003). Several studies proposed that deranged Ca^{2+} signaling might play an important role in Huntington's disease (Tang et al., 2003; 2005; Bezprozvanny and Hayden, 2004; Wu et al., 2006). Abnormal Ca^{2+} homeostasis has been reported in mitochondria isolated from lymphoblast's from patients and from brains of the YAC72 HD mouse model (Hodgson et al., 1999; Panov et al., 2002). This Ca^{2+} role could also be important in other polyglutamine diseases, as it is generally assumed that many of these diseases share a common pathogenic mechanism (Cummings and Zoghbi, 2000; Gusella and MacDonald, 2000; Zoghbi and Orr, 2000; Gatchel and Zoghbi, 2005). Accordingly, recent evidence suggests that abnormal neuronal Ca^{2+} signaling might also contribute to pathogenesis in SCAs (Bezprozvanny, 2009; Kasumu and Bezprozvanny, 2010). In MJD, data also suggest that deranged neuronal Ca2+ signaling plays a significant role in pathology onset and progression (Chen et al., 2008). Mutant ataxin-3 has been shown to specifically bind to and activate an intracellular calcium channel, similar to huntingtin. Moreover, long-term feeding of MJD-transgenic mice with a Ca^{2+} stabilizer (dantrolene) alleviated age-dependent motor coordination deficits and prevented neuronal loss in pontine nuclei and substantia nigra regions (Chen et al., 2008).

9. Therapeutic strategies in MJD

Expansion of the polyglutamine tract of ataxin-3 initiates a cascade of events that include the accumulation of insoluble inclusions and culminates in degeneration of specific neurons. The strategies that can be used to treat MJD or other polyglutamine diseases can be grouped into five main approaches: i) reducing the levels of expanded proteins, ii) preventing mutant ataxin-3 cleavage, oligomerization and aggregation, iii) activating the

clearance mechanisms, iv) targeting a specific cellular mechanism and v) promoting neuroprotection (Figure 4).

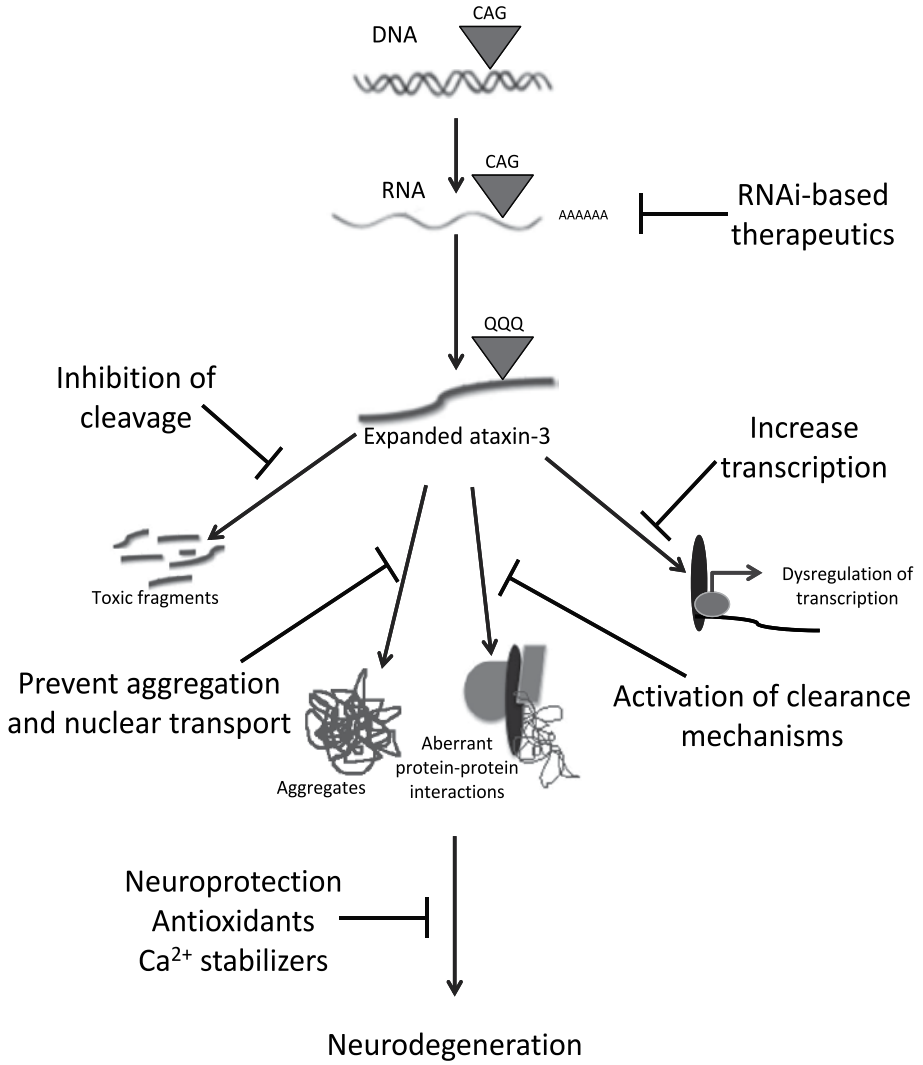

Fig. 4. **Potential therapeutic strategies to Machado-Joseph disease.** Expansion of the polyglutamine tract of ataxin-3 initiates a cascade of events that culminates with the accumulation of insoluble inclusions and degenerations in selected neurons. The strategies that can be used to treat MJD or other polyglutamine diseases can be grouped into five approaches: i) reducing the levels of expanded proteins (using gene silencing by RNAi-based strategies), ii) preventing mutant ataxin-3 cleavage, oligomerization and aggregation (inhibiting proteolysis, using aggregation inhibitors or preventing the nuclear transport), iii) activation of the clearance mechanisms (upregulation of UPS and autophagy), iv) targeting a specific cellular mechanism (increase transcription, stabilize Ca^{2+} homeostasis or inhibit oxidative stress) and v) neuroprotection strategies (using drugs, proteins or factors to protect neurons).

9.1 RNA interference-based therapeutics

Although several approaches could be envisioned to treat MJD and other polyglutamine diseases, the most direct solution to counter these diseases pathogenesis is to reduce the expression of the mutant allele (Kim and Rossi, 2007). RNA interference (RNAi) is a powerful tool for selective knockdown of gene expression. Gene silencing by RNAi has been successfully used to downregulate the expression of mutant genes and rescue phenotype in various neurodegenerative diseases, including Huntington's disease (Harper et al., 2005; Rodriguez-Lebron et al., 2005; DiFiglia et al., 2007, van Bilsen et al., 2008; Lombardi et al., 2009; Pfister et al., 2009), familial forms of amyotrophic lateral sclerosis (ALS) (Raoul et al., 2005; Ralph et al., 2005; Azzouz, 2006), SCA1 (Xia et al., 2004), and MJD (Miller et al., 2003; Alves et al., 2008a, 2010; Hu et al., 2009).

However, a major problem of gene silencing may be the lack of discrimination between normal and mutant forms of the causative protein. In some diseases partial silencing of normal protein could be tolerated; for example in HD transgenic animal models silencing of mutant huntingtin and 75% of endogenous protein led to behavioral enhancement (Boudreau et al., 2009). However, it has been reported that in cellular MJD models absence of wild-type ataxin-3 leads to cytoskeletal disorganization and increased cell death (Rodrigues et al., 2010). This would suggest that for some polyglutamine disorders it might be prudent to preserve the wild-type protein, as prolonged full knockdown of normal protein function could be harmful. This would demand specific targeting of the mutant allele for RNAi.

It was first demonstrated in cell models that RNAi species could be engineered to specifically silence the causative genes while preserving the wild-type, which differed in a single nucleotide (Miller et al., 2003). More recently, our group showed both in vitro and in a rat model of MJD that lentiviral-mediated silencing of the mutant human ataxin-3 was efficient and selective, allowing preservation of wild-type ataxin-3 (Alves et al., 2008a). Specific silencing has also been later reported to SNPs targeting ataxin-7 in SCA7 (Scholefield et al., 2009) and huntingtin in Huntington's disease (Zhang et al., 2009; Hu et al., 2009). This allele-specific silencing of ataxin-3 significantly decreased the severity of the neuropathological abnormalities associated with the disease by targeting a single nucleotide polymorphism (SNPs) that is present in more than 70% of the patients with MJD (Stevanin et al., 1995; Gaspar et al., 1996). These data support the therapeutic potential of RNAi for MJD. However, this therapy would benefit ~70% of MJD patients at best. Whether silencing not discriminating between wild type and mutant alleles would be safe and effective was recently investigated, by either overexpressing or silencing wild-type ataxin-3 in a rat model of MJD. It was shown that (i) overexpression of wild-type ataxin-3 did not protect against MJD pathology, (ii) knockdown of wild-type ataxin-3 did not aggravate MJD pathology and that (iii) non-allele-specific silencing of ataxin-3 strongly reduced neuropathology in a rat model of MJD. These findings indicate that therapeutic strategies involving non-allele-specific silencing to treat MJD patients may also be safe and effective (Alves et al., 2010).

9.2 Preventing the cleavage of ataxin-3

In MJD, it was proposed that production of a cleavage fragment of mutant ataxin-3 contributes to neurotoxicity (Ikeda et al., 1996; Goti et al., 2004; Colomer-Gould, 2005;

Haacke et al., 2006). Thus, blocking the proteases involved in ataxin-3 cleavage and decreasing the concentration of the cleavage fragment bellow a critical level in the brain could be an effective strategy for MJD treatment. This approach has been used for other neurodegenerative diseases, including Alzheimer (Citron, 2004) and Huntington's diseases (Ona et al., 1999; Gafni et al., 2004) and therefore could also be a therapeutic strategy for MJD (Tarlac and Storey, 2003). Nevertheless, the natures of the protease and of the cleavage fragment still need investigation.

9.3 Acceleration of the degradation of misfolded proteins

The acceleration of the proteolysis mechanisms (UPS and autophagy machinery) could promote mutant ataxin-3 degradation and probably prevent or delay the MJD progression. Overexpression of chaperones has been shown to aid in the handling of misfolded or aggregated polyglutamine-expanded ataxin-3 and suppress polyglutamine aggregation with a parallel decrease in toxicity (Chai et al., 1999b). Thus the induction of such molecular chaperones can be envisaged as a strategy for therapy of polyglutamine diseases (Nagai et al., 2010; Robertson et al., 2010). Accordingly, the use of chemical chaperones such as the organic solvent dimethyl sulfoxide – DMSO, cellular osmolytes glycerol, trimethylamine N-oxide – TMAO, and ectoine reduce aggregate formation and cytotoxicity induced by truncated expanded ataxin-3 (Yoshida et al., 2002), alters subcellular localization of inclusions and reduces apoptotic cell death induced by mutant ataxin-3 (Furusho et al., 2005).

It was also shown that overexpression of UPS-related factors or proteins (e.g. E64 or CHIP) increase ubiquitination and degradation rate and decrease aggregation and cell death (Matsumoto et al., 2004; Jana et al., 2005; Miller et al., 2005). Therefore, overexpression of these proteins could be a molecular approach for therapy of MJD. It was shown that CRAG (guanosine triphosphatase) acts as an activator of promylocytic leukaemia protein-associated ubiquitin ligase and leads to the degradation of polyQ through the ubiquitin-proteasome pathway (Qin et al., 2006). Because the expression levels of CRAG decrease in the adult brain (Qin et al., 2006), it was suggested that a reduced level of CRAG could underlie the onset of polyglutamine diseases. In fact, lentiviral-mediated overexpression of CRAG in Purkinje cells of a transgenic mice model extensively cleared polyQ aggregates and re-activated dendritic differentiation, resulting in a striking rescue from ataxia (Torashima et al., 2008). It was also suggested that the activity of normal ataxin-3 could provide a therapeutic approach to MJD, enhancing the cellular pathways in which it participates (Warrick et al., 2005). However, in a lentiviral-based rat model for MJD as well as in double-transgenic mice, the overexpression of normal ataxin-3 did not decrease the pathological abnormalities induced by mutant ataxin-3 (Alves et al., 2010; Hübener et al., 2010).

Another possible therapeutic approach to MJD and to other polyglutamine diseases could be the up-regulation of autophagy, leading to a selective clearance of the mutant protein. Rapamycin, an activator of the autophagy pathway alleviated neurodegeneration in *Drosophila* and in a transgenic mouse model of HD. However, this drug failed to prolong life span in a mouse model (Ravikumar et al., 2004). In MJD, it was recently shown that the administration of a rapamycin esther improves motor coordination in a transgenic model of MJD (Menzies et al., 2010). The rapamycin esther reduced the number of aggregates in the

brains of transgenic mice and decreased the levels of cytosolic soluble mutant ataxin-3, while endogenous wild-type protein levels remained unaffected.

Recently, our group showed that lentiviral-mediated overexpression of beclin-1, a crucial protein in early and late steps of autophagy, led to a stimulation of autophagic flux, mutant ataxin-3 clearance and overall neuroprotective effects in neuronal cultures and in a lentiviral-based rat model of MJD (Nascimento-Ferreira et al., 2011). The same study found an abnormal expression of endogenous autophagy markers, accumulation of autophagosomes and decreased levels of beclin in the brain of MJD patients. Overall, these data suggest that up-regulation of UPS or autophagy can be a therapeutic option for MJD and for other polyglutamine diseases.

9.4 Inhibition of nuclear transport

It has been shown that ataxin-3 translocates to the nucleus, and that the polyglutamine expansion is not essential for this transport (Tait et al., 1998). The resulting presence of ataxin-3 in the nucleus has been shown to drastically aggravate the pathology in Machado-Joseph disease (Bichelmeier et al., 2007). Therefore, inhibition of nuclear transport may slow the disease progression, and might be sufficient to ameliorate the disease symptoms, and thus could be explored as therapeutic approach for MJD (Breuer et al., 2010).

9.5 Prevention of protein misfolding, oligomerization and aggregation

Protein misfolding, oligomerization, and formation of insoluble inclusions represent a common physiological response to pathogenic proteins. Thus, different research groups have developed high-throughput screening assays aiming at the discovery of molecules with selective binding affinities for polyglutamine expanded proteins, with the ability to modulate their pathogenic properties and potential therapeutic applications (Desai et al., 2006; Lansbury and Lashuel, 2006). Several compounds have been identified as potential inhibitors of polyglutamine aggregation (Heiser et al., 2000, 2002; Apostol et al., 2003; Sanchéz et al., 2003; Tanaka et al., 2005; Wolfgang et al., 2005; Herbst and Wancker, 2006). The prevention of aggregation and oligomerization by polyglutamine disease can also be promoted by modulation of molecular chaperones (Nagai et al., 2010; Roberston et al., 2010). The Hsp90 inhibitor geldanamycin suppresses aggregation of polyQ-expanded mutant huntingtin through induction of endogenous molecular chaperones (Sittler et al., 2001). In MJD Drosophila models, it was shown that the administration of a less toxic derivative of geldanamycin suppresses polyQ-induced neurodegeneration through the induction of multiple endogenous molecular chaperones (Fujikake et al., 2008).

Another therapeutic approach involves the use of small peptides or molecules with the ability to modulate protein folding, stabilize proteins in their native conformation, and prevent or inhibit aggregation (Tanaka et al., 2005). Several compounds proved to be suitable in preventing polyglutamine proteins aggregation, mainly for Huntington Disease (Table 2). In a screening of 16,000 compounds a small molecule (IC_{50}) that inhibits polyglutamine aggregation in HD neurons and suppresses neurodegeneration *in vivo* was found (Zhang et al., 2005). In a MJD *Drosophila* model a tandem repeat of the polyglutamine binding peptide QBP1, which preferentially binds to polyglutamine stretches, has been shown to decrease aggregate formation and rescue survival (Nagai et al., 2003). More

recently a high-content chemical and RNAi screening in a Drosophila primary neuronal culture of HD model identified several compounds that suppress mutant huntingtin aggregate formation (Schulte et al., 2011).

Compound	Disease tested	Study
Geldanamycin	Huntington Disease	Sittler et al., 2001
17-(allylamino)-17-demethoxygeldanamycin (17AAG)	Machado-Joseph Disease	Fujikake et al., 2008
Congo red	Huntington Disease	Frid et al., 2007
C2-8	Huntington Disease	Chopra et al., 2007
Trehalose	Huntington Disease	Tanaka et al., 2005
GW5074	Huntington Disease	Schulte et al., 2011
Juglone	Huntington Disease	Schulte et al., 2011
Radicicol	Huntington Disease	Schulte et al., 2011
Rapamycin	Huntington Disease	Schulte et al., 2011
Rapamycin esther	Machado Joseph disease	Menzies et al., 2010
Camptothecin	Huntington Disease	Schulte et al., 2011
Etoposide	Huntington Disease	Schulte et al., 2011
Ouabain	Huntington Disease	Schulte et al., 2011
Proscillaridin A	Huntington Disease	Schulte et al., 2011
Ethacrynic acid	Huntington Disease	Schulte et al., 2011
IC_{50}	Huntington Disease	Zhang et al., 2005

Table 2. Compounds that have shown to prevent or inhibit polyglutamine proteins aggregation.

9.6 Targeting transcriptional dysfunction

Polyglutamine-expanded ataxin-3 (as other polyglutamine expanded proteins) has been shown to repress transcription. Ataxin-3 acts through distinct mechanisms involving both the polyglutamine-containing C-terminus and the N-terminus of ataxin-3 (Li et al., 2002). Transcriptional dysregulation has been suggested to play a central role in neurodegenerative mechanisms of the polyglutamine disorders (Chou et al., 2008). The overexpression of transcription factors that interact with polyglutamine diseases reduces the cytotoxicity of mutant proteins (Dunah et al., 2002; Taylor et al., 2003). Moreover, it was shown that the use of several reagents that increase transcription reduce the toxicity of expanded polyglutamine (Steffan et al., 2001; Ferrante et al., 2003, 2004; Hockly et al., 2003; Gardian et al., 2005; Shimohata et al., 2005). Recently, it was shown that regulation of transcriptional activity through an inhibition of histone hypoacetylation (Chou et al., 2011) might be a promising therapeutic intervention for MJD. Histone acetylation, which is controlled by histone acetyltransferase and histone deacetylase (HDAC), plays an important role in regulating transcriptional activity (Kurdistani et al., 2004). The H3 and H4 histones were hypoacetylated in the cerebellum of MJD transgenic mice, which displayed transcription downregulation and ataxic symptoms. Daily administration of a HDAC inhibitor (sodium butyrate) reversed histone hypoacetylation and transcriptional downregulation in the cerebellum of the MJD transgenic mice, delaying the onset of ataxic symptoms, ameliorated the neurological phenotype and improved the survival rate of the mice (Chou et al., 2011).

9.7 Targeting the calcium homeostasis

It has been shown that deranged calcium signaling might play an important role in MJD pathology (Chen et al., 2008). The same study found that feeding a MJD transgenic mice with dantrolene, a clinically relevant stabilizer of intracellular Ca^{2+} signaling, improved motor performance and prevented neuronal cell loss in pontine nuclei and *substantia nigra* regions. Therefore, calcium-signaling stabilizers such as dantrolene may be considered as potential therapeutic drugs for the treatment of MJD patients.

9.8 Targeting mitochondrial dysfunctions

Several studies have shown that administration of antioxidants ameliorates motor deficits and prolongs survival in transgenic mouse model of HD (Ferrante et al., 2002). Moreover, drugs that improve transcriptional regulation of genes necessary for energy metabolism also improve HD motor phenotype (Hathorn et al., 2011). In MJD, evidences point to a role of mitochondrial dysfunction in MJD pathogenesis (Yu et al., 2009). Decreased mitochondrial DNA copy numbers were found in mutant cells stably transfected with ataxin-3 with 78 CAG repeats and in MJD patients, compared to normal controls. Furthermore, mitochondrial DNA depletion was higher in MJD patients compared with that in normal individuals. Overall, mutant ataxin-3 may influence the activity of enzymatic components to remove O_{2^*} and H_2O_2 efficiently and promote mitochondrial DNA damage or depletion, which leads to dysfunction of mitochondria (Yu et al., 2009). Therefore, therapies targeting mitochondrial dysfunction in MJD should be further investigated.

9.9 Neuroprotection

The possibility of administration of drugs or molecules with neuroprotective properties in neurodegenerative diseases has also been explored. Many research groups have investigated the use of neurotrophic factors for therapy of polyglutamine disorders over the last decade (Bensadoun et al., 2000; de Almeida et al., 2001; Zala et al., 2004; Xie et al., 2010). In HD the BDNF supply to striatal neurons is compromised. Therefore delivery of this factor has been investigated as a replacement therapy for the missing factor (Zuccato et al., 2001). BDNF replacement was later shown to enhance the motor phenotype (Canals et al., 2004), and BDNF overexpression prevented loss and atrophy of striatal neurons and motor dysfunction (Xie et al., 2010), both in in HD transgenic mice.

Studies in mouse models of Alzheimer's and Parkinson's diseases found that caffeine could alleviate pathological signs and behavior deficits in these neurodegenerative disease paradigms, by antagonizing A2A adenosine receptors (Arendash and Cao, 2010; Prediger, 2010; reviewed in Cunha and Agostinho, 2010). Moreover, administration of caffeine and other stimulants in orexin/ataxin-3 transgenic narcoleptic mice induced an increase in motor activity but the effects on neuropathology remain to be investigated (Okuro et al., 2010) and should be further investigated in MJD models.

Several evidences suggest that neuroprotective compounds could be also explored as a therapeutic strategy in MJD and the drug ability of some of these compounds may contribute to earlier access of patients to much needed disease-modifying therapies.

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7

Spinocerebellar Ataxia Type 12 (SCA 12): Clinical Features and Pathogenetic Mechanisms

Ronald A. Merrill, Andrew M. Slupe and Stefan Strack
University of Iowa Carver College of Medicine
USA

1. Introduction

Spinocerebellar Ataxia 12 (SCA12) is a rare disease that was first identified in a family in the United States. Patients suffered from classical spinocerebellar ataxia symptoms with an age of disease onset ranging from 8-55 years. A trinucleotide (CAG) repeat expansion was confirmed in all the affected individuals. The CAG expansion mapped to the 5′ untranslated region (UTR) of the PPP2R2B gene. This gene encodes a regulatory subunit, Bβ, of the heterotrimeric protein phosphatase 2A (PP2A). The function of this particular PP2A complex is not well understood, and the underlying molecular mechanism of SCA12 remains unclear. Additional pedigrees have been identified throughout the world but SCA12 remains a rare disease. In this chapter we will discuss the clinical manifestation of the disease and the known functions of the PP2A regulator Bβ.

2. Molecular genetics and Incidence

SCA12 is defined as an autosomal dominant cerebellar ataxia (ADCA) of otherwise unknown cause concurrent with a CAG repeat expansion within chromosome 5q31-33 upstream of the PPP2R2B gene (Holmes et. al., 1999). The PPP2R2B gene product, termed Bβ, is a neuron specific regulatory subunit of the heterotrimeric PP2A (Strack et. al., 1998). PP2A has been shown to play an essential role in many cellular functions (Janssens & Goris, 2001). The CAG repeat expansion associated with SCA12 was first identified through an unbiased repeat expansion detection study and found to occur within the noncoding region of the PPP2R2B gene (Holmes et. al., 1999). The nonpathalogical range of allele expansion is quite large (7-45 repeats) and is highly dependent on ethnic background (Fujigasaki et. al., 2001; Holmes et. al., 1999). The lower extreme of the range of pathological allele expansion has been established as 51 repeats. As is common to all ADCA disorders, inheritance of SCA12 follows an autosomal dominant pattern wherein a CAG repeat expansion of pathological length in just one allele is sufficient to induce the SCA12 disease state. Unlike other neurodegenerative diseases associated with a CAG repeat expansion, such as Huntingon disease, the number of CAG repeats associated with SCA12 does not correlate with the age of disease onset (Srivastava et. al., 2001). In addition, nondirectional vertical instability in the length of the expanded allele has been observed, however its clinical significance is unknown (Srivastava et. al., 2001). One individual has been identified with

pathological repeat expansions in both alleles; however, due to the young age of this patient, it is unclear what effect homozygosity will have on the disease phenotype (Bahl et. al., 2005).

The world-wide incidence of SCA12 is quite low. Nonetheless, SCA12 has been identified across the globe in independent populations. The results of ADCA population screens that have examined the CAG repeat of the PPP2R2B gene are summarized below (Table 1), regardless of whether a SCA12 pathological CAG repeat expansion was identified. The well characterized SCA12 patient populations will hereafter be referred to as the American, Indian, Italian and Chinese cohorts when referencing the work by Holmes, et. al. (1999) and O'Hearn, et. al. (2001); Fujigaski, et. al. (2001), Srivastava, et. al. (2001) and Bahl, et. al. (2005); Brusco et al. (2002) and Brussino, et. al. (2010); and Jiang, et. al. (2005-1), Jiang, et. al. (2005-2) and Wang, J., et. al. (2011).

Study	County	Affected families (individuals)	Pathalogical $(CAG)_n$ repeat expansion (range)	Healthy Population $(CAG)_n$ repeat expansion (range)	Age range in years of disease onset (mean)
(Holmes, 1999) & (O'Hearn, 2001)	United States	1 (10)	66 - 78	7 - 28	8-55 (34)
(Fujigasaki, 2001)	Indian	1 (9)	55 - 61	9 - 45	39-41 (40)
(Srivastava, 2001)	Indian	5 (6)	55 - 69	7 - 31	26 - 50 (37.2)
(Bahl, 2005)	Indian	20 (81)	51 - 69	8 - 23	26 - 56 (40.2)
(Brussino, 2010)	Italian	2 (3)	57 - 58	NA	45-60 (52)
(Jiang, 2005 - 1)	Chinese	1 (NA)	NA	NA	NA
(Wang, J., 2011)	Chinese	1 (9)	51-52	NA	34
(Brusco, 2002)	Italian	0	NA	8 - 21	NA
(Jiang, 2005 - 2)	Chinese	0	NA	NA	NA
(Silveira, 2002)	Portugal and Brazil	0	NA	8 - 28	NA
(Tsai, 2004)	Taiwan	0	NA	7 - 25	NA
(van de Warrenburg, 2002)	Netherlands	0	NA	NA	NA
(Worth, 2001)	United Kingdom	0	NA	7 - 30	NA
(Cholfin, 2001)	United States	0	NA	9 - 22	NA

Table 1. Summary of SCA12 descriptions available in the primary literature.

3. Clinical features

At present, SCA12 confirmed by genetic testing remains a very rare illness. However, as genetic testing, including whole genome sequencing, becomes common practice, the true incidence of SCA12 may prove to be much higher among previously categorized ADCA patients of unknown cause. Indeed, among a cohort of ADCA patients in India the incidence of SCA12 has proven to be much higher than in other geographical locales (Bahl et. al., 2005; Srivastava et. al., 2001). Given this observation, those who encounter ADCA patients should be aware of SCA12 and develop an index of suspicion informed by careful history taking, detailed neurological examination and deliberate laboratory testing.

As SCA12 has only been recognized as a distinct pathology for the last decade and, at present, only a very few patients have been described in the primary literature, an

appreciation for the natural history of the disease is still evolving. By careful consideration of those cases that have been well characterized in the American, Indian, Italian and Chinese cohorts, a clinical picture of the SCA12 patient will be developed here. The descriptions provided here are intended to inform the clinician who encounters ADCA patients of unknown cause and to guide clinical decision-making.

3.1 Patient reported history of illness

Early in the course of the disease the prototypical SCA12 patient will present with postural and action tremor of the upper limbs. Age of onset of this tremor is highly variable with a range between 8 and 55 years, but seems to cluster primarily between the third and fifth decade of life (Brussino et. al., 2010; Fujigasaki et. al., 2001; Holmes et. al., 1999; O'Hearn et. al., 2001; Srivastava et. al., 2001). The first manifestations of the action tremor of the upper limbs have been described by patients as difficulty with activities requiring fine motor coordination, such as writing, as well as difficulties with activities requiring gross motor coordination such as attempting to hold and purposefully manipulate objects like a cup (Fujigasaki et. al., 2001; O'Hearn et. al., 2001). Observers describe the tremor as slowly progressive in nature with an increase in amplitude and involvement of the head and neck have been observed over the course of a decade (O'Hearn et. al., 2001). The action tremor of the upper limbs as the harbinger of the disease is unique to SCA12 and differentiates SCA12 from other ADCA disorders (Schols et. al., 2004; Teive, 2009). This tremor is not, however, universal among SCA12 patients, and its absence does not rule out SCA12 (Srivastava et. al., 2001; Wang, J et. al., 2011). Presentation of the upper limb action tremor is very similar to that of essential tremor and has previously been misdiagnosed as such early in the SCA12 course (O'Hearn et. al., 2001). Differentiating the SCA12 associated upper limb action tremor from isolated essential tremor requires an appreciation of the complete constellation of SCA12 associated symptoms as well as a family history consistent with ADCA.

3.2 Neurological examination

The time elapsed since disease onset has been reported to directly correlate with the number of neurological abnormalities (O'Hearn et. al., 2001). The examination of an SCA12 patient should therefore be informed by the patient reported history. To fully characterize the constellation of symptoms associated with SAC12 early in the course of the disease, care should be taken to elicit mild neurological abnormalities that may otherwise be subclinical in nature. Characterizing the gross neurological deficits present late in the course of the disease can serve to chart disease progression.

3.2.1 Motor skills deficits

As indicated above, the action tremor associated with SCA12 is one of the earliest hallmarks of the disease. Action tremor features include postural and kinetic properties, as well as a low frequency (3 Hz)(O'Hearn et. al., 2001), and are similar to a tremor subset associated with cerebellar lesion termed "cerebellar postural tremor"(Hallett, 1991). As such, the postural features of the tremor can be elicited in the clinical setting by asking the patient to maintain their arms in an outstretched position and observing for limb tremor. The kinetic features of the tremor can be assessed by having the patient engage in a goal-directed movement of the upper limbs, such as finger-to-nose testing. Tremor should disappear

completely while the upper limbs are at rest and not maintaining position against the force of gravity.

Loss of motor coordination due to cerebellar dysfunction associated with SCA12 manifests when the patient engages in a number of activities. During finger-to-nose testing, rather than smooth, rapid, accurate movements, the SCA12 patient will display slow, hesitant, inaccurate movements consistent with upper limb dysmetria. Further, the SCA12 patient has been reported to be unable to engage in rapid alternating movements (dysdiadochokinesia) such as alternating between turning the palms or the back of the hand face up (O'Hearn et. al., 2001). Motor deficits also disrupt speech and can result in dysarthria (O'Hearn et. al., 2001; Srivastava et. al., 2001).

Parkinsonain features have also been described in SCA12 patients from the American Cohort. These manifest as paucity of spontaneous movements, mild bradykinesia, upper limb rigidity and postural anteroflexion (O'Hearn et. al., 2001).

A great deal of heterogeneity has been observed in the symptoms of SCA12 patients from different ethnic backgrounds. Unique to the Indian cohort, facial myokymia has also been described in a small number of SCA12 patients (Srivastava et. al., 2001). Although the proband of the Chinese cohort developed generalized ataxia during the third decade of life, action tremor has not been observed (Wang, J et. al., 2011).

3.2.2 Gait abnormalities

The ataxic gait of the SCA12 patient has been described as being very similar to that observed in other diseases with cerebellar dysfunction. The SCA12 patient maintains stability by adopting a broad based stance. Parkinsonian features have also manifest in the gait among individuals of the American Cohort (O'Hearn et. al., 2001). Initiation of movement is delayed. Steps have been described as hesitant, small and slow. When turning, the SCA12 patient has been described as engaging in an "en bloc" approach. A mild ataxic phenotype can be exaggerated by having the patient maintain a tandem gait, wherein the patient walks in a straight line with the heel of the front foot touching the toes of the back foot at each step.

3.2.3 Cranial nerve assessment

With the exception of oculomotor nerve (CNIII) abnormalities, the cranial nerves are largely intact and function without deficit in the SCA12 patient. Horizontal nystagmus has been described and may represent an early manifestation of the disease (Fujigasaki et. al., 2001; Holmes et. al., 2003; O'Hearn et. al., 2001; Srivastava et. al., 2001). In addition slow saccades and broken pursuit have been described in SCA12 patients from the Indian cohort (Fujigasaki et. al., 2001; Srivastava et. al., 2001).

3.2.4 Assessment of reflexes

Diffuse hyperreflexia has been described for SCA12 patients from the American, Indian and Italian cohorts (Brussino et. al., 2010; Fujigasaki et. al., 2001; O'Hearn et. al., 2001; Srivastava et. al., 2001). A return of primitive reflexes in the otherwise mature SCA12 patient has also been described. These reflexes include an extensor plantar response (positive Babinski sign), grasp reflex, rooting reflex and glabellar blink reflex (Myerson sign).

3.2.5 Mental Status

Psychiatric disorders have been reported to occur concurrently with SCA12. Anxiety and depression have been reported in members of the American cohort, but not the Indian or Italian cohorts (Brussino et. al., 2010; O'Hearn et. al., 2001; Srivastava et. al., 2001). Whether these disorders result as a direct consequence of the SCA12 disease process or represent an individual response to the presence of the disease is unclear. Paranoid delusions have also been reported in one SCA12 patient (O'Hearn et. al., 2001). A decline in cognition has been described in SCA12 patients two to three decades after initial onset of the disease (Fujigasaki et. al., 2001; O'Hearn et. al., 2001).

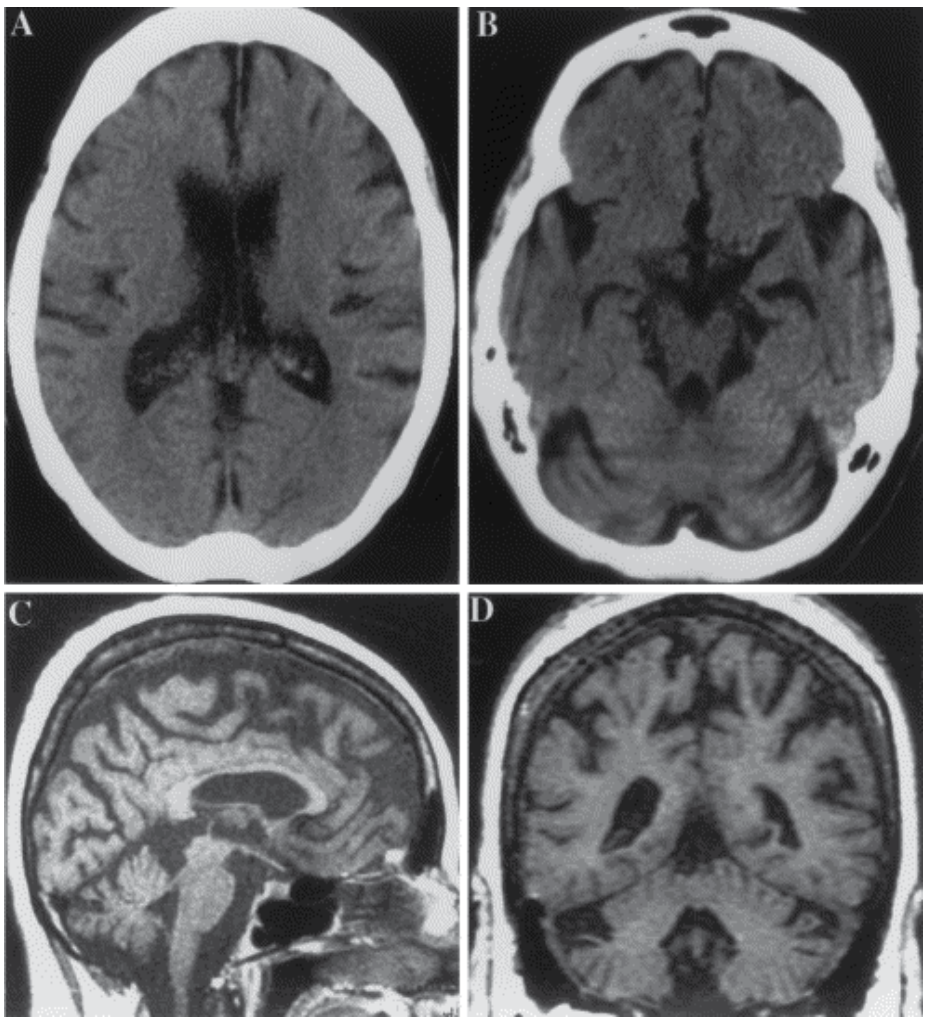

Fig. 1. Neuroradiologic images from two patients with spinocerebellar ataxia type 12. (A,B) Coronal computed tomography of the proband at age 62 years reveals cerebellar and diffuse cerebral cortical atrophy. (C) (sagittal), (D) (coronal): T-1 weighted magnetic resonance images of a 59-year-old affected woman also shows cerebellar and cortical atrophy. Reproduced from Holmes et. al. (2001), with permission from Elsevier Science.

3.3 Neuroimaging studies

Computerized tomography (CT) and magnetic resonance imaging (MRI) studies of symptomatic SCA12 patients reveal that mild to moderate cerebellar and cortical atrophy is a near universal finding of the disease (Brussino et. al., 2010; Fujigasaki et. al., 2001; O'Hearn et. al., 2001; Srivastava et. al., 2001; Wang, J et. al., 2011). An example of these findings from imaging studies performed on members of the American cohort of SCA12 patients is shown (Figure 1). The cerebellar vermis appears to be more vulnerable to atropy than the cerebellar hemispheres (O'Hearn et. al., 2001). Atrophy of subcortical structures has not been described. Additional characterization by single-proton emission computed tomography (SPECT) revealed metabolic deficiencies in atrophic cortical areas; however, the value of this test is uncertain in the symptomatic patient (Fujigasaki et. al., 2001). Proton magnetic resonance spectroscopy has been used to demonstrate neurometabolic and microstructural changes in the SCA12 patient (Brussino et. al., 2010), and this technique represents a noninvasive method that may longitudinally describe the asymptomatic SCA12 patient.

3.4 Genetic testing

Genetic testing for the presence of CAG repeat expansion is available. The reader is directed to the GeneTests Laboratory Directory available online (http://www.ncbi.nlm.nih.gov/sites/GeneTests/lab) for a list of available testing centers. The small sample size of affected individuals currently identified has left the question of penetrance of the disease open. Therefore, a great deal of care should be exercised when interpreting the results of a genetic test from an asymptomatic patient.

3.5 Medical management

Currently, management of SCA12 is limited to providing symptomatic relief for the action tremor. Treatment of the SCA12 action tremor is very similar to that provided for essential tremor. A reduction in tremor amplitude has been achieved with beta-blockers and barbiturates (O'Hearn et. al., 2001). When appropriate, pharmacological relief for symptoms associated with the disease such as depression and anxiety should be offered to the SCA12 patient.

4. PPP2R2B gene regulation and protein function

4.1 PP2A and B regulatory subunit

Protein phosphorylation is the most common posttranslational modification of proteins, and it plays a role in nearly every cellular function. The addition of phosphate is mediated through a large group (>500) of enzymes called kinases and requires ATP as a substrate. The reverse reaction is mediated by a smaller number of protein phosphatases in which, in most cases, specificity is provided through the formation of multimeric protein complexes. One of the most abundant protein phosphatase is PP2A, which is an essential, ubiquitously expressed phosphatase that targets phospho-serine and phospho-threonine. PP2A exists as a heterotrimer composed of one member of four diverse families of regulatory subunits (B), a scaffolding subunit (A) and a catalytic subunit (C) (Figure 2). Humans express 4 families of

regulatory subunits termed B, B′, B′′, and B′′′, which determine both cellular localization and substrate specificity (Slupe et. al., 2011). The B family, also known B55, consists of 4 distinct genes (α, β, γ, δ) that encode proteins containing a highly conserved core WD40 domain, which has propeller like structure, with over 90% amino acid identity among the family members (Figure 2B). The Bβ regulatory subunit is encoded by the PPP2R2B gene, which has several splice-variants that are expressed exclusively in neuronal tissue.

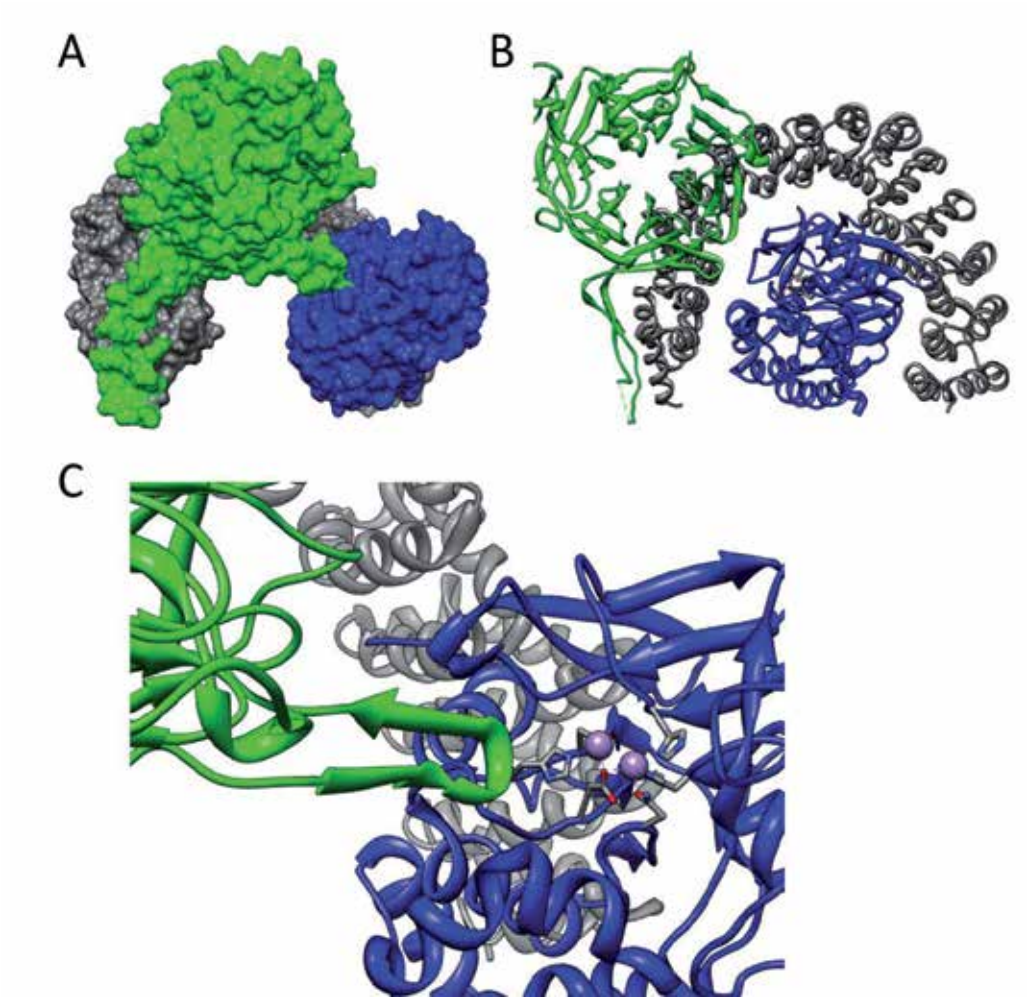

Fig. 2. Models of PP2A/Bα prepared from PDB 3DW8. The subunits of the heterotrimeric complex are color coded with the catalytic subunit (C) in blue, the scaffold subunit (A) in gray, and the regulatory subunit (B) in green. A, "top-down" view of the heterotrimer suface. B, "end-on" view of the heterotrimer ribbon diagram. C, Close of view of the PP2A active site highlighting infiltration of a regulatory subunit loop into the catalytic cleft.

4.2 Gene structure and expression

The exon arrangement of the PPP2R2B gene is highly conserved among mammals and spread over more than 500,000 base pairs (Dagda et. al., 2003; Schmidt et. al., 2002). Exon 1.1 and 1.2 are alternatively expressed first exons containing the ATG start site for the splice variants Bβ1 and Bβ2, respectively. These first exons, which contain the unique amino-termini, are spliced to common exons 2-9 that encode the WD40 domain found in all the B family of regulatory subunits (Figure 3) (Dagda et. al., 2003). At the mRNA level, Bβ1 and Bβ2 are expressed prominently in brain tissue, and Bβ1 can also be found in the testis (Dagda et. al., 2003). At the protein level, western blot analysis indicates that the Bβ1 is exclusively expressed in brain tissue and not in the testis, despite the high mRNA expression in that tissue. Closer analysis of specific brain regions has shown high levels of the Bβ1 protein throughout the brain (Strack et. al., 1998).

4.2.1 Transcriptional regulation

The CAG trinucleotide repeat expansion associated with the SCA12 disease is situated just upstream of the transcriptional start site of the Bβ1 specific exon 1.1. A recent study identified the apparent transcriptional regulators for basal expression of the Bβ1 promoter and the effect of the CAG repeat on basal expression (Lin et. al., 2010). Luciferase assays using deletions of the Bβ1 promoter and chromatin immunoprecipitation assays reveal that

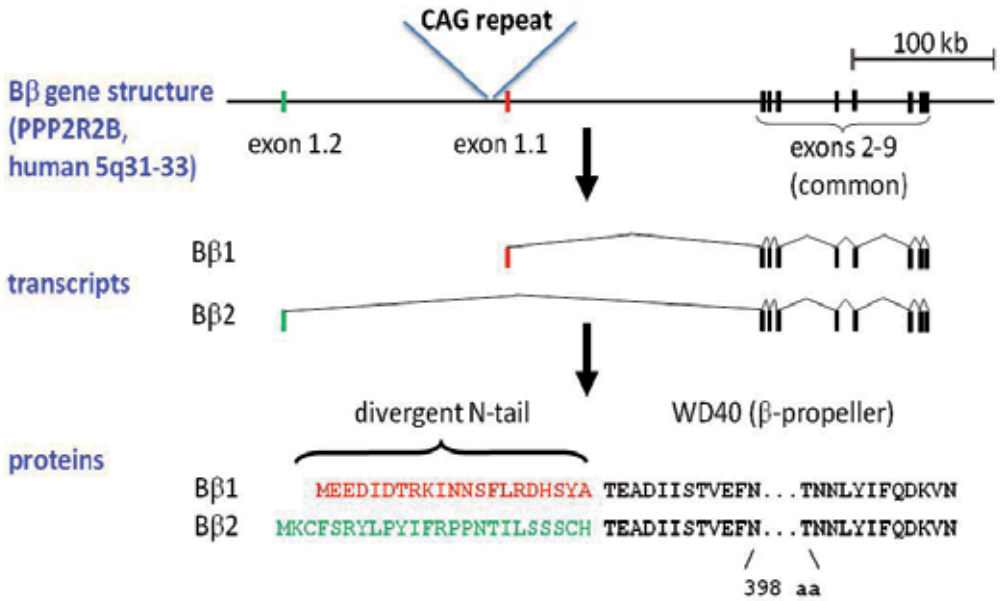

Fig. 3. Schematic representation of PPP2R2B gene structure, splice variant transcripts and proteins. The gene structure shows the CAG repeat expansion location, the Bβ1 (exon 1.1; red) and Bβ2 N-terminal coding sequences (exon 1.2; green). Transcripts and proteins indicate the Bβ1 (red) and Bβ2 (green) splice variant specific transcripts and encoded proteins. Modified from Dagda et. al. (2003).

CREB1, SP1 and TRAP4 bind to and regulate the Bβ1 promoter. Higher luciferase activity is seen in neuronal cell lines and correlates well with the known Bβ1 neuronal expression. Interestingly, increasing the size of the CAG repeat in the Bβ1 promoter increased the promoter activity two-fold. The increased activity is specific to the expansion of the CAG and not a result of changing the spacing of promoter since no change is seen in an AT expansion control (Lin et. al., 2010). A normal length CAG repeat does appear to be important for basal promoter activity since decreasing the number of CAG repeats reduced the promoter activity (Chen et. al., 2009). Independent studies conducted in Japan and Taiwan found that patients suffering from Alzheimer's disease had an increased likelihood of having a reduced number of CAG trinucleotide repeats compared to healthy control subjects (Chen et. al., 2009; Kimura et. al., 2011). Overall, these studies have identified important aspects of the PPP2R2B transcriptional regulation and help to discriminate between the role of the CAG repeat in providing basal transcriptional activation and the pathological effects of increasing or decreasing the trinucleotide repeat number.

A recently identified Japanese autosomal dominant cerebellar ataxia raises more uncertainty about the role of PPP2R2B gene in SCA12. The disease locus for this new ataxia included the PPP2R2B gene but contained no CAG expansion (Sato et. al., 2010). Additionally, all exons and intron/exon borders were sequenced for the entire PPP2R2B gene, including the both first exons (1.1 and 1.2), and no mutations were discovered. Several neuronally expressed genes are within the identified locus and may contain the genetic insult resulting in the ataxia (Sato et al 2010). This does raise the possibility that some of the effects of the CAG expansion in the PPP2R2B gene may be mediated through dysregulation of other nearby genes and not just changes in Bβ gene expression.

4.2.2 PPP2R2B regulation and cancer

Another important form of regulation of Bβ1 occurs in colorectal cancer (CRC) wherein developed cell lines show a decrease or complete absence of Bβ1 expression (Tan et. al., 2010). Furthermore, gene array comparisons of matched patient-derived mucosa controls and CRC tumors indicate a significant decrease in Bβ1 expression in 90% of the tumors. The loss of Bβ1 expression is mediated through hypermethylation of a CpG island that occurs in the Bβ1 promoter. Aberrant methylation of the PPP2R2B gene also appears to be important in breast cancer, as seen in recent reports (Dejeux et. al., 2010; Muggerud et. al., 2010). Finally, an intronic SNP of the PPP2R2B gene, with unknown functional consequence, is correlated with improved prognosis in a breast cancer cohort (Vazquez et. al., 2011). These studies clearly indicate that regulation of the PPP2R2B gene is important in multiple cancers and may provide additional insight into the function of the PPP2R2B gene.

4.3 Protein function

The Bβ1 and Bβ2 splice variants encode proteins that share a common WD40 repeat domain that mediates the recruitment of the A and C subunits of PP2A to make a functional trimeric protein phosphatase. The Bβ1 and Bβ2 proteins differ only in the first 21 and 24 amino acids, respectively, but this leads to a dramatic difference in the protein distribution within the cell.

4.3.1 Bβ1 protein function

Bβ1 has a cytoplasmic distribution and overexpression in cultured primary neurons does not change the morphology, survival or sensitivity to toxic treatments (Figure 4) (Dagda et. al., 2008). Overexpression of Bβ1 in a neuroblastoma cell line does result in increased autophagy (Cheng et. al., 2009). In CRC the loss of Bβ1 following methylation of the CpG island leads to aberrant phosphorylation of several proteins, including the oncogene c-myc. Reexpression of Bβ1 in a colorectal cell line decreases xenograft growth (Tan et. al., 2010). This represents the first described pathway regulated specifically by a Bβ1 containing PP2A trimer. Since some of the proteins regulated by Bβ1 in CRC are also expressed in neuronal tissues, it may be of interest to examine whether the Bβ1-mediated changes in phosphorylation also play a role in SCA12.

4.3.2 Bβ2 protein function

The Bβ2 N-terminus encodes a mitochondrial targeting sequence that results in recruitment of the trimeric PP2A enzyme to the outer mitochondrial membrane (OMM) (Dagda et. al., 2003). In primary hippocampal neurons, PP2A-mediated phosphatase activity at the OMM, through recruitment by Bβ2, results in mitochondrial fragmentation and increased basal death and sensitivity to neurotoxic insults (Figure 4) (Dagda et. al., 2005; Dagda et. al., 2008). Expression of Bβ2 mutants, that either do not target to the OMM or cannot recruit the A and C subunits, prevents the mitochondrial fragmentation and increased neuronal death (Figure 4) (Dagda et. al., 2008). Epitasis experiments indicate that the PP2A/Bβ2-mediated mitochondrial fragmentation precedes and is obligatory to the increased neuronal cell death (Dagda et. al., 2008). An additional study, utilizing neuroblastoma cells, confirmed the increased sensitivity of cells expressing Bβ2 but implicated an increase in autophagy as the culprit in the increased cell death (Cheng et. al., 2009).

Mitochondrial dysfunction is a hallmark of several neurodegenerative diseases, including Alzheimer disease. It can therefore be postulated that the CAG trinucleotide repeat expansion, which is known to increase Bβ1 promoter activity, amplifies both Bβ1 and Bβ2 expression. The Bβ2 upregulation may lead to increased mitochondrial fragmentation and increasing mitochondrial dysfunction in SCA12. Indeed, several other ataxias involve mitochondrial dysfunction. In patients suffering from SCA7, both liver and skeletal muscle biopsies show abnormal mitochondria (Han et. al., 2010). Heterozygous knockout mice for AFG3L2, a mitochondrial-targeted AAA-protease, develop abnormal mitochondria with decreased function and are a model of SCA28 (Maltecca et. al., 2009). Finally, in clinical trials pharmacological treatments with idebenone, an antioxidant thought to counteract mitochondrial dysfunction, have shown some promise in treatment of the genetic neurological disorder Friedreich ataxia (Marmolino, 2011). These examples highlight some of the ataxias associated with mitochondrial dysfunction and exemplify why mitochondrial dysfunction could be an important aspect of SCA12.

4.4 Animal models of SCA12

While characterization of the PPP2R2B gene products has suggested possible pathogenic mechanisms, animal models of SCA12 are urgently needed to test the predictions of the in

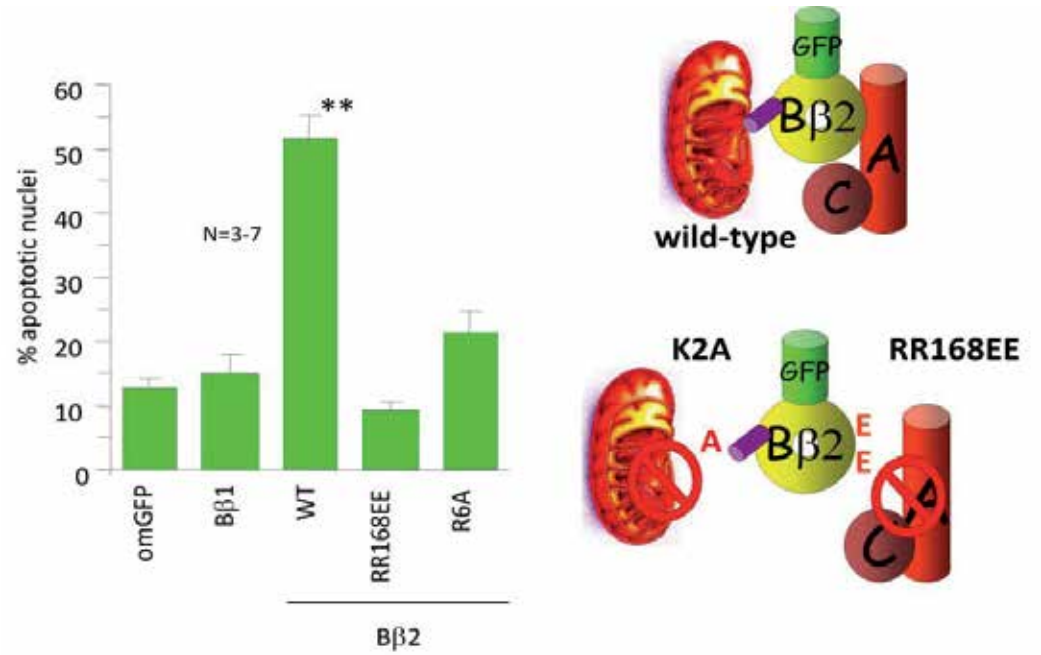

Fig. 4. Mitochondrial targeting of PP2A/Bβ2 is neurotoxic. Hippocampal neurons were transfected with the indicated GFP fusion proteins (om, outer mitochondrial; WT, wild-type) and scored for apoptotic nuclei. Bβ2 mutants that block mitochondrial localization (R6A) or AC dimer recruitment (RR168EE) also block apoptosis induction. Modified from Dagda et. al. (2008).

vitro studies discussed above. A recently developed fly model of SCA12 does display some neuropathies that may be homologous to the human disease (Wang, YC et. al., 2011). In this model, Drosophila overexpresses the human Bβ2 or tws, the fly homolog of Bβ, which results in a dramatic increase in neuronal apoptosis and, for the highest level of tws, a decrease in fly life span. Overexpression of tws results in mitochondrial fragmentation and dysfunction, observed as an increase in reactive oxygen species (ROS) production. Expression of superoxide dismutase 2 or antioxidants treatments reduces ROS production and attenuates the effects of tws overexpression. How the neuropathies and their reversal by pharmacological treatments seen in the fly SCA12 model relate to the human disease remains to be seen.

5. Conclusion

The CAG trinucleotide repeat expansion that occurs in the PPP2R2B gene is now well established as the cause of the autosomal dominant SCA12. This is a rare disease that shows a classical ataxia phenotype. The CAG repeat occurs in the promoter of a neuronally expressed protein, Bβ1, and expansion of the CAG results in increased Bβ1 promoter activity. Aberrant expression of Bβ1 also correlates with several cancers. Expression of another neuronal splice variant of PPP2R2B, Bβ2, increases neuronal death, but its role in SCA12 remains unknown. Despite the identified PPP2R2B gene functions, the underlying molecular basis of the SCA12 disease is not known. Animal models are needed to address the complexity of SCA12 and develop potential therapeutic treatments. The fly model of SCA12 does show mitochondrial dysfunction and recapitulates some neuron specific cell death (Wang, YC et. al., 2011); however, the development of a mammalian model system will likely be required to understand the molecular basis of SCA12 pathogenesis.

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8

Autosomal Recessive Spastic Ataxia of Charlevoix-Saguenay (ARSACS): Clinical, Radiological and Epidemiological Aspects

Haruo Shimazaki[1] and Yoshihisa Takiyama[2]
[1]*Division of Neurology, Department of Internal Medicine, Jichi Medical University, Tochigi*
[2]*Department of Neurology, Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi, Chuo-City, Yamanashi, Japan*

1. Introduction

Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) (OMIM #270550) was originally found among inhabitants of the Charlevoix-Saguenay region of Quebec (Bouchard et al., 1978). ARSACS patients in Quebec show uniform phenotypes characterized by early-onset spastic ataxia, peripheral neuropathy, retinal hypermyelination, hand or foot deformities, and normal mentality. In 2000, the *SACS* gene, which is responsible for ARSACS, was identified in Quebec patients (Engert et al., 2000). Since then, ARSACS has been reported worldwide, especially in the Mediterranean area (El Euch-Fayache et al., 2003; Criscuolo et al., 2004; Grieco et al., 2004; Richter et al., 2004) and Japan (Ogawa et al., 2004; Takiyama, 2006). More *SACS* gene mutations were also identified in other areas (Takiyama, 2007; Ouyang et al., 2008; Vermeer et al., 2008; Gerwig et al., 2010). Meanwhile, ARSACS in non-Quebec patients, especially in Japanese ones, showed marked clinical heterogeneity, i.e., there were patients without spasticity (Shimazaki et al., 2005; Hara et al., 2007; Shimazaki et al., 2007), without retinal hypermyelination (Hara et al., 2007), and with decreased mentality (Shimazaki et al., 2005; Yamamoto et al., 2005; Shimazaki et al., 2007; Hara et al., 2005). The clinical spectrum of the sacsinpathies will expand with the identification of more SACS gene mutations (Gomez, 2004).

We herein review the epidemiology, genetics, clinical phenotypes, radiological and pathological findings in ARSACS cases carrying mutations of the *SACS* gene.

2. Epidemiology

2.1 Quebec

ARSACS is the most common of all inherited spastic ataxias, 320 affected patients having been identified in Quebec (Bouchard et al., 1998). In Quebec, most of the patients' families originate from the Charlevoix and Saguenay-Lac-St. Jean (SLSJ) regions. These regions have a population of about 300,000 inhabitants today that share a limited number of French

ancestors who settled first in the Charlevoix region back in the seventeenth and early eighteenth centuries. ARSACS affects 1/1519 individuals in Charlevoix and 1/1952 in the Saguenay-Lac-St. Jean region, where the carrier frequency was estimated to be 1/22 for the 1941-1985 period (De Braekeleer et al., 1993).

2.2 Non-Quebec

SACS gene identification has enabled us to find ARSACS patients worldwide outside Quebec: Tunisia (El Euch-Fayache et al., 2003) in 2003, Italy (Criscuolo et al., 2004; Grieco et al., 2004)in 2004, Japan (Ogawa et al., 2004) in 2004, and Turkey (Richter et al., 2004) in 2004. More cases were then reported in Spain (Criscuolo et al., 2005), France (Anheim et al., 2008), Belgium (Ouyang et al., 2008), the Netherlands (Vermeer et al., 2008), Germany (Gerwig et al., 2010), Maritime Canada (Guernsey et al., 2010), and Morocco and eastern Europe (Baets et al., 2010). In eastern France, ARSACS was identified in two index patients among 102 autosomal recessive cerebellar ataxia (ARCA) ones (Anheim et al., 2008), meanwhile among 43 Dutch ARCA patients, 16 with mutations in the *SACS* gene were identified (Vermeer et al., 2008). In Japan, 17 Japanese ARSACS families have been discovered on SACS gene analysis so far (Ogawa et al., 2004; Hara et al., 2005; Shimazaki et al., 2005; Yamamoto et al., 2005; Ouyang et al., 2006; Yamamoto et al., 2006; Okawa et al., 2006; Hara et al., 2007; Takado et al., 2007; Shimazaki et al., 2007; Kamada et al., 2008; Tsugawa et al., 2009; Haga et al., 2011; Miyatake et al., 2011; Komure et al., 2006). ARSACS might be the second most frequent ARCA next to ataxia with oculomotor apraxia 1 (AOA1) in Japan. Figure 1 shows the geographical distribution and numbers of ARSACS families with *SACS* gene mutations in Japan. We could not find apparent regional accumulation of ARSACS families in Japan. Table 1 lists the previously identified SACS gene mutations that we could confirm in the 17 Japanese ARSACS families. The mutations were unique ones for each family except for one missense mutation (W3248R) found in two unrelated families.

Fig. 1. Regional distribution of ARSACS families in Japan.

ARSACS families show a nationwide distribution in Japan. The numbers in circles are the numbers of families identified in the regions.

Amino acid substitutions	Exon	family	references
W3248R	10	2	Ogawa, 2004, Takiyama, 2006
K2931fsX2952	10	1	Hara, 2005
F1054S	10	1	Shimazaki, 2005
G1734fsX1736	10	1	Yamamoto, 2005
S2058fsX2076	10		
V1231del	10	1	Komure, 2006
P3559L	10		
Q1345X	10	1	Okawa, 2006
R4325X	10	1	Yamamoto, 2006
C395fsX407	8	1	Ouyang, 2006
D687fsX713	8		
R2119X	10	1	Hara, 2007
L308F	8	1	Takado, 2007
D1996fsX1999	10	1	Shimazaki, 2007
N161fsX175	7	1	Kamada, 2008
L802P	10		
G1257X	10	1	Tsugawa, 2009
R3788fsX3820	10		
Y138X	6	1	Haga, 2011
K1755fsX1775	10		
R3636X	10	1	(unpublished)
S4007F	10	1	Miyatake, 2011

Table 1. Previously identified *SACS* gene mutations in Japanese patients.

3. Genetics

3.1 Gene structure and pathological mutations

The *SACS* gene was originally reported to consist of a single gigantic exon spanning 12,794bp encoding an 11,487bp open reading frame (ORF), which represents the largest exon and the largest ORF within an exon found in any vertebrate (Engert et al., 2000). Recently, eight new exons located upstream of the gigantic one were found (Ouyang et al., 2006). More recently, one more upstream non-coding exon was found (Genbank NG_012342, 13-

MAR-2011). The *SACS* gene comprises ten exons with a 13,737 bp ORF encoding 4579 amino acids (Figure 2A).

The *SACS* gene is predicted to encode a 520-kDa multidomain protein, sacsin. The region near the C-terminus exhibits sequence similarity to the J-domain (DnaJ motif) of heat shock protein (HSP) 40 proteins (Parfitt et al., 2009), and the higher eukaryote and prokaryote nucleotide-binding (HEPN) domain (Grynberg, Erlandsen, and Godzik, 2003) (Figure 2B). A ubiquitin-like domain was identified at the N-terminus of sacsin (Parfitt et al., 2009)(Figure 2B). A sacsin repeating region (SRR) is present in triplicate at the N-terminus of sacsin (Anderson, Siller, and Barral, 2010). A xeroderma pigmentosum complementation group C-binding (XPCB) domain (Kamionka and Feigon, 2004) upstream of the DnaJ domain is also predicted.

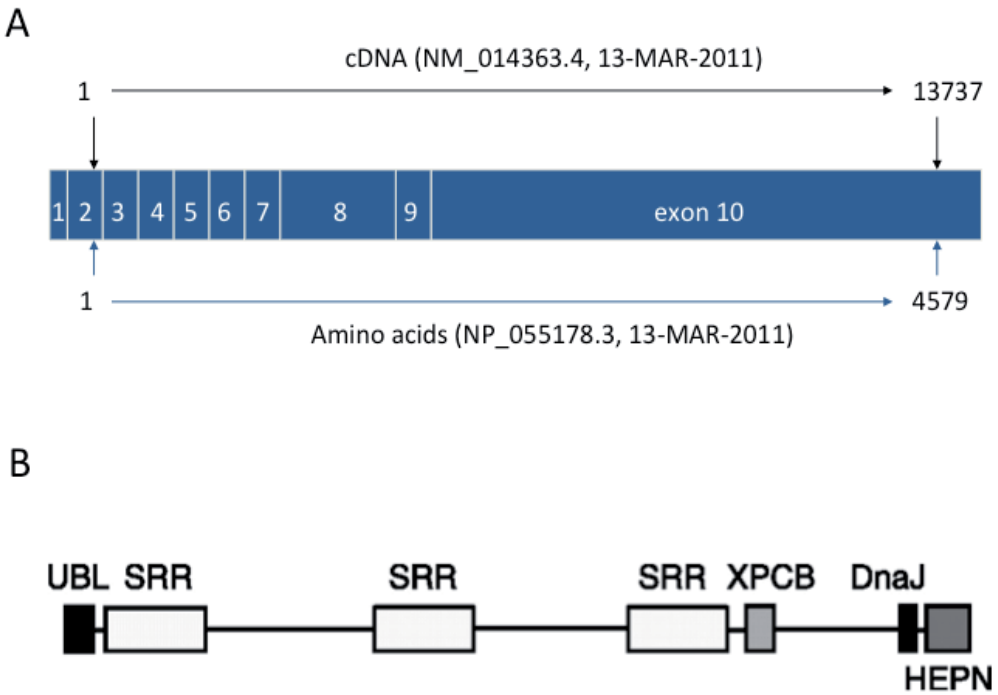

Fig. 2. Primary structure of the *SACS* gene (A) and domain organization of the sacsin protein (B). UBL: ubiquitin-like domain; SRR: sacsin repeating region; XPCB: XPC-binding domain; DnaJ: DnaJ motif (adopted from (Kozlov et al., 2011)).

Seventy-four mutations have been reported as pathological ones in the *SACS* gene (Baets et al., 2010). After publication of that report, we verified the table and found seven additional mutations in exons 6 and 10 of the *SACS* gene in Japanese patients (Table 1) (Komure et al., 2006; Tsugawa et al., 2009; Haga et al., 2011; Miyatake et al., 2011). As far as we know, at least 81 mutations have been found worldwide to date. Most of the mutations are predicted to generate truncated sacsin proteins, and are located in the largest exon, 10. Seventeen mutations were found in exons 4, 6, 7, 8 and 9. On copy number variation (CNV) analysis, an intragenic *SACS* deletion of exons 3-5 was identified (Baets et al., 2010). Two types of

large deletions of the whole *SACS* gene and adjacent genes have been reported (Breckpot et al., 2008; Terracciano et al., 2009; McMillan et al., 2009).

3.2 Normal sacsin function

Sacsin is highly expressed in neurons, especially in cerebral corticospinal neurons and cerebellar Purkinje cells (Parfitt et al., 2009). The subcellular localization of sacsin in a cultured neuroblastoma-derived cell line was predominantly cytoplasmic and overlapped with that of mitochondrial protein heat shock protein (HSP) 60 (Parfitt et al., 2009). Because sacsin contains a functional J-domain (DnaJ motif), it has been proposed to act as a co-chaperone of the HSP70 chaperone system (Parfitt et al., 2009). The N-terminal segment of sacsin containing the ubiquitin-like (UbL) domain and the first sacsin repeating region (SRR) exhibits molecular chaperone activity and ATP-hydrolyzing activity (Anderson, Siller, and Barral, 2010, 2011). The UbL domain can interact with the 20 S proteasomal subunit (Parfitt et al., 2009). The HSP70 chaperone machinery is an important component of the cellular response to aggregation prone mutant proteins, and the UbL domain protein is a part of the quality control machinery that regulates protein aggregation. Overall, the main function of sacsin, like other molecular chaperones, is probably to prevent protein misfolding and aggregation. Sacsin prevents polyglutamine-expanded ataxin-1 toxicity (Parfitt et al., 2009). The XPCB domain interacts with ataxin-3, which is involved in spinocerebellar ataxia type 3 (Kamionka and Feigon, 2004). HEPN may stabilize nucleotide binding in complexes formed with the DnaJ domain (Grynberg, Erlandsen, and Godzik, 2003). Recently, the structure and function of the HEPN domain were determined, it being shown that it dimerizes and has a high affinity binding site for GTP, but it does not have GTPase activity (Kozlov et al., 2011).

3.3 Pathogenesis of ARSACS

Although the molecular mechanism underlying ARSACS remains unclear, the autosomal recessive transmission and truncating nature of most *SACS* mutations suggest the loss of sacsin function might cause development of this disease. Several functional alterations of sacsin proteins have been reported. An aspartate to tyrosine mutation (D168Y), located in the first SSR domain, abrogates its ATP-hydrolyzing activity (Anderson, Siller, and Barral, 2010). An asparagine to aspartate mutation (N4549D) in the HEPN domain of the sacsin protein disrupts dimerization and correct protein folding (Kozlov et al., 2011). Premature termination of other mutations and loss of the HEPN domain might lead to ARSACS disease.

4. Clinical phenotypes

4.1 Original Quebec phenotype

ARSACS is clinically characterized by early-onset spastic ataxia, axonal and demyelinating neuropathy, and hypermyelination of retinal nerve fibers (Bouchard et al., 1978; Bouchard, 1991). Unsteadiness of gait is usually the initial symptom. None of the patients ever walk normally, but walking is not delayed in most cases (rarely

beyond 18 months of age) (Bouchard et al., 1978; Bouchard, 1991). The disease progression becomes most obvious in the late teens or early twenties, and the mean age for patients becoming wheelchair-bound is 41 years in Quebec patients (Bouchard, 1991). Concerning Quebec patients, ataxia, dysarthria, nystagmus, Babinski sign, hyperreflexia, spasticity, and retinal striations are noted in all of them. Distal amyotrophy of the feet is present in all patients after 20 years old (Bouchard, 1991), and pes cavus is noted in most Quebec patients. With these clinical features, ARSACS is clinically homogeneous in Quebec patients. Bouchard described progressive signs and early non-progressive ones of ARSACS in a review of this disease (Bouchard, 1991). The progressive signs are mostly spastic ataxia of the four limbs, slurred and dysrhythmic speech, discrete to severe distal amyotrophy, and absent ankle jerks after 25 years of age. The early non-progressive signs are increased deep tendon reflexes, a bilateral abnormal plantar response, marked saccadic alteration of smooth pursuit, and prominent myelinated fibers radiating from the disc on the retina.

4.2 Non-Quebec atypical phenotypes

The mean ages at onset are 5.4 and 4.5 years old in Japanese and Tunisian patients, respectively (Takiyama, 2007), while the ages at onset range from one to 1.5 years old in Quebec ones (Bouchard, 1991). Thus, the age at onset in these non-Quebec patients seems to be later than that in Quebec patients. According to a recent report from Belgium, the disease onset was over 20 years in five patients and as late as 40 years in one patient (Baets et al., 2010).

Although ataxia is noted in all non-Quebec ARSACS patients, one report stated that the cerebellar features were very mild in two patients (Baets et al., 2010). Other core clinical features of ARSACS, i.e., dysarthria, nystagmus, distal amyotrophy, Babinski sign, hyperreflexia, and pes cavus, are noted in most non-Quebec patients, which are similar to in Quebec patients.

Non-Quebec patients, however, show some atypical features in comparison with Quebec ones. First, although spasticity is a core clinical feature of ARSACS, we found that two patients in a Japanese family with ARSACS lacked spasticity in the legs and showed areflexia or hyporeflexia (Shimazaki et al., 2005). In Quebec and Tunisian patients, spasticity becomes progressively worse during the disease course and is prevalent in older patients, and tendon reflexes remain preserved throughout the disease, except for ankle jerks (Bouchard et al., 1978; El Euch-Fayache et al., 2003). Since we did not observe the two above patients in their childhood, we were not able to determine whether or not their spasticity had decreased during the disease course or had been absent from the onset (Shimazaki et al., 2005). The cases without spasticity, this depended on the fact that the neuromuscular manifestations were severe enough to diminish muscle tone and masked spasticity, and the planter responses were extensor, demonstrating that pyramidal tract sign was present. Recently, however, we observed another ARSACS patient whose spasticity had disappeared, probably due to the progressive peripheral nerve degeneration in the disease course of 29 years (Shimazaki et al., 2007). Thus, we should recognize that there is a rare ARSACS phenotype without spasticity, and the SACS gene should be analyzed even in

cases of early-onset cerebellar ataxia without spasticity. After that, several reports on spasticity-lacking ARSACS patients were published (Hara et al., 2007; Baets et al., 2010; Miyatake et al., 2011).

Second, intellectual impairment is sometimes found in non-Quebec patients. The verbal IQ was 58, 88, 100, and 66 (mean: 78.00) in the four Japanese patients we examined (Takiyama, 2006). Similarly, mental retardation and dementia have been found in Italy (Criscuolo et al., 2004), Japan (Hara et al., 2005), and Turkey (Richter et al., 2004). Meanwhile, the verbal IQ (mean: 92.67) of Quebec patients is considered to be within normal limits, and a number of ARSACS patients have completed the secondary and university levels of education (Bouchard, 1991). Thus, intellectual impairment seems to be variable in ARSACS. Since the mode of inheritance of ARSACS is autosomal recessive, genes other than *SACS* might influence the intellectual impairment.

Defects in conjugate pursuit ocular movements, decreased or absent vibration sense in the toes, hand deformities, and urinary or fecal incontinence are rather frequently noted in Quebec (Bouchard, 1991) and non-Quebec patients (El Euch-Fayache et al., 2003; Takiyama, 2006). Cardiovascular evaluation revealed mitral valve prolapse in a majority of Quebec patients examined (Bouchard, 1991).

Very recently, the disease initially presented with symptoms mainly orienting toward peripheral neuropathy in several patients, and there was one patient who did not exhibit any clinical or electrophysiologic signs of peripheral neuropathy (Baets et al., 2010).

4.3 Ophthalmologic findings

Although increased visibility of myelinated retinal nerve fibers is a hallmark of ARSACS in Quebec patients (Bouchard, 1991), there have been a considerable number of non-Quebec patients without this characteristic sign for ARSACS (El Euch-Fayache et al., 2003; Grieco et al., 2004; Criscuolo et al., 2004; Richter et al., 2004; Hara et al., 2005; Yamamoto et al., 2005; Ouyang et al., 2006; Okawa et al., 2006; Yamamoto et al., 2006; Hara et al., 2007; Baets et al., 2010). Thus, retinal hypermyelination is a variable feature in non-Quebec patients. It is, however, very useful for suspecting a diagnosis of ARSACS, especially in an unusual phenotype without spasticity (Shimazaki et al., 2005). In a case without retinal hypermyelination, ARSACS resembles conditions referred to as early onset cerebellar ataxia with retained tendon reflexes (EOCA) (Chio et al., 1993), Friedreich ataxia with retained reflexes (De Castro et al., 1999), and several clinical descriptions of hereditary spastic paraplegia such as SPG7 (Wilkinson et al., 2004), SPG21 (Simpson et al., 2003), SPG27 (Meijer et al., 2004), and SPG30 (Klebe et al., 2006).

Recently, Desserre et al. have reported that the retinal nerve fiber layer was thickened, as shown using optical coherence tomography (OCT), and that the retina did not show hypermyelinated areas on funduscopy (Desserre et al., 2011). Likewise, Vingolo et al. reported that four patients with ARSACS showed myelinated fibers on funduscopy, and also increased thickness of the retina on OCT, which is a finding not characteristic of persistent myelination of the retina (Vingolo et al., 2011). It is possible that persistent myelination of the retina, a general common finding, was present in those patients without bearing relation to the disease.

5. Neuroradiology

5.1 Brain MRI findings

Some reports have mentioned that characteristic MRI findings in ARSACS are superior cerebellar vermian atrophy and cervical spinal cord atrophy (Bouchard et al., 1998; Takiyama, 2007). Recently, MRI of five patients in Quebec revealed linear hypointensity in T2- and Fluid-Attenuated Inversion Recovery (FLAIR) images of the pons (Martin et al., 2007). Thereafter, the same findings have been reported in only one patient in each of the Netherlands (Van Damme et al., 2009), France (Anheim et al., 2010), and Italy (Terracciano et al., 2010).

We recruited six ARSACS patients with *SACS* mutations in four Japanese families. Brain MRI was performed in all six patients. Brain MRI in the six patients showed superior cerebellar vermian atrophy. In addition, not only pontine linear hypointensity but also middle cerebellar peduncle hypointensity was observed in T2-weighted and FLAIR images (Shimazaki et al., in press) (Table 2). These areas showed isointensity in T1-weighted images. Figure 3 shows representative brain MRI findings in patients 3 (A) and 2 (B). In patient 3, T2*-weighted images were obtained showing no abnormal findings in the pons and middle cerebellar peduncles (Figure 3, A-1).

We found the characteristic MRI findings in the six Japanese ARSACS patients, who all exhibited linear hypointensity in the pons, and a hypointense area in the middle cerebellar peduncles in T2-weighted and FLAIR images (Shimazaki et al., in press). A middle cerebellar peduncle hypointense area has not previously been reported in ARSACS patients, although pontine linear hypointensity was originally reported in five Quebec patients (Martin et al., 2007). Furthermore, as far as we know, hypointensity in these portions has not been described as a MRI finding in spinocerebellar ataxias and hereditary spastic paraplegias.

We thought these hypointense areas were close to the location of the pontocerebellar fibers. We could find low intensity in the middle cerebellar peduncle (MCP) because the pontocerebellar fiber runs from the middle pons to the cerebellum through the MCP. We can detect low MCP intensity in an Italian case (Terracciano et al., 2010). We think an abnormal MCP signal is not specific for Japanese patients.

The hypointensity in the pons and middle cerebellar peduncle might be specific findings for ARSACS cases even in non-Quebec ones with clinical heterogeneity (Shimazaki et al., 2012). Thus, pontine and middle cerebellar peduncle T2 hypointensity detectable on MRI should prompt us to perform *SACS* gene analysis even in such atypical early-onset cerebellar ataxia cases.

5.2 Spinal MRI findings

In Quebec cases, the cervical cord is small and flat (Bouchard, 1991). In our cases, upper cervical cord and medulla oblongata atrophy was not observed in three of the six patients for whom cervical MRI was performed (Shimazaki et al., 2012). Upper cervical atrophy is not a constant feature of ARSACS. In thirteen Belgium cases, cervical spinal cord atrophy was observed on MRI in only one case (Baets et al., 2010).

Case	1	2	3	4	5	6
Age at exam, gender	47, F	43, M	46, M	37, M	33, M	57, F
Age at onset	6	6	3	8	7	3
Cerebellar ataxia	++	+	++	+	++	++
Hyperreflexia	+	+	+	-	-	-
Leg spasticity	+	+	+	-	-	-
Babinski sign	+	+	+	+	+	+
Retinal myelinated fibers	+	+	+	+	+	+
Peripheral neuropathy	+	+	+	+	+	+
Mental impairment	NE	+(WAIS-R)	+(WAIS-R)	NE	+(WAIS-R)	+(MMSE)
Amino acid substitutions	W3248R	W3248R	W3248R	F1054S	F1054S	D1996fsX1999
Superior cerebellar vermian atrophy	+	+	+	+	+	+
Pontine linear hypointensity (T2)	+	+	+	+	+	+
MCP hypointensity (T2)	+	+	+	+	+	+
Medulla oblongata atrophy	-	-	+	+	+	-
Upper cervical cord atrophy	-	-	+	+	+	-
Superior cerebellar CBF decrease	+-	NE	+-	+	+	+

Table 2. Clinical, genetic and MRI findings in the ARSACS patients. NE: not examined; MCP: Middle cerebellar peduncle; CBF: cerebral blood flow in SPECT.

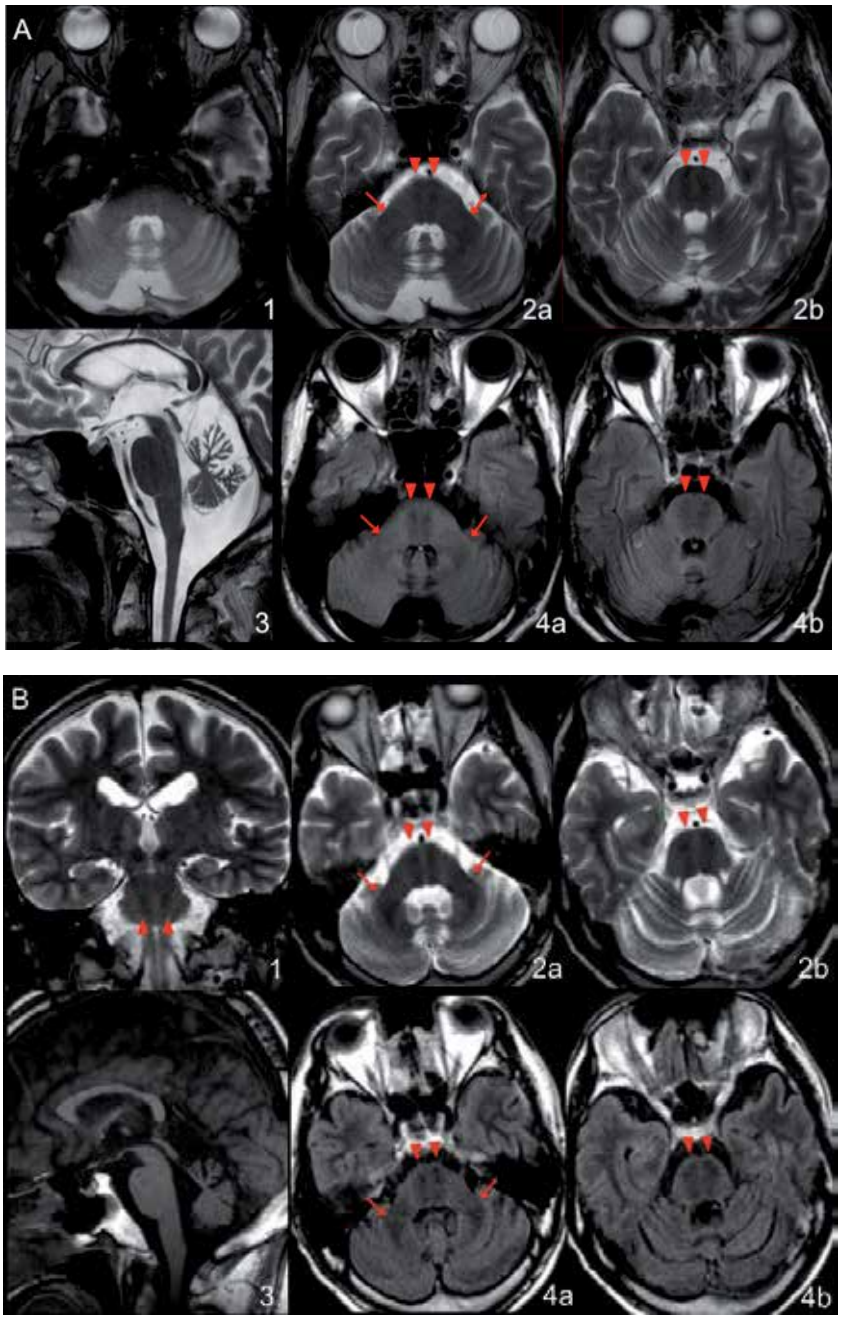

Fig. 3. Representative brain MRI findings in two ARSACS patients. T2-weighted and FLAIR images of patient 3 (A-2a, 2b, 4a, and 4b) and patient 2 (B-1, 2a, 2b, 4a, and 4b) showed hypointensity in the pons (arrowheads) and bilateral middle cerebellar peduncles (arrows). Sagittal sections (A-3 and B-3) revealed superior cerebellar vermian atrophy in all patients. A T2* image of patient 3 (A-1) disclosed no remarkable low intensity in the pontine tegmentum or cerebellar peduncles (Shimazaki et al., 2012).

5.3 SPECT findings

Single photon emission computed tomography (SPECT) of the brain with three-dimensional stereotactic surface projection (3D-SSP) analysis for five cases showed decreased blood flow in the upper cerebellar vermis in three cases (Shimazaki et al., 2007; Shimazaki, Nakano, and Takiyama, 2008) (Figure 4). Meanwhile, early onset cerebellar ataxia with retained tendon reflexes (EOCA) and Friedreich's ataxia often show a reduction in the parietotemporal cortex blood flow as well as cerebellar hypoperfusion (De Michele et al., 1998), this being a different feature from in ARSACS.

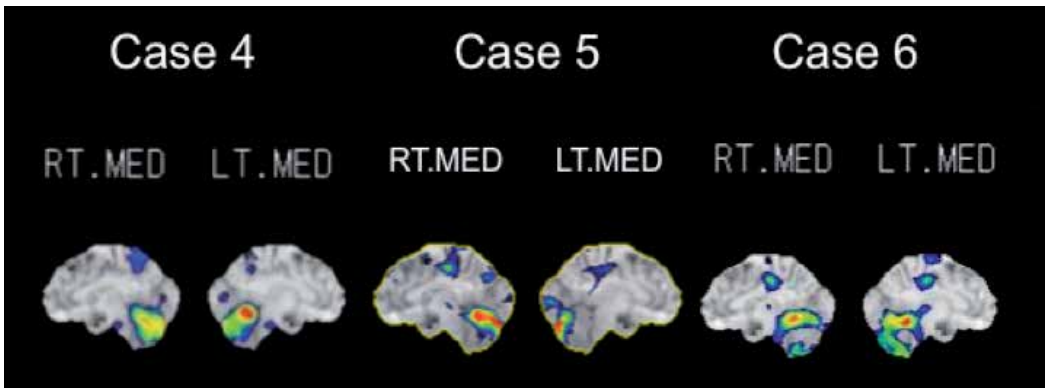

Fig. 4. ^{123}I-IMP SPECT with three-dimensional stereotactic surface projection (3D-SSP) analyses of cases 4, 5, and 6.

The results showed decreased blood flow in the superior cerebellar vermis and cerebellar hemisphere.

6. Neuropathology

6.1 Postmortem autopsy and comparison with the characteristic brain MRI findings

The pathological findings in ARSACS patients have only been reported in a 21-year-old man (Bouchard, 1991) and a 59-year-old man (Richter et al., 1996). The former report of a young man described no findings regarding pontocerebellar fibers, but mentioned a small corticospinal tract and normal pontine nuclei in the pons (Bouchard, 1991). A T2* image of patient 3 disclosed no remarkable low intensity in the pontine tegmentum or cerebellar peduncles. These findings suggest that T2 hypointensity in the pons and cerebellar peduncles is not reflected by iron deposition in these portions. However, a second autopsy on the 59-year-old man revealed the presence of swollen thalamic and cerebellar cortical neurons (Bouchard et al., 1998). Most of these neurons had dense, lipofuscin-like granules within their lysosomes, and the authors suggested that ARSACS may be a lysosomal storage disease (Richter et al., 1996). In neuronal ceroid lipofuscinosis, MRI can often reveal hypointensity of the thalamus and putamen in T2-weighted images, which may reflect the storage of lipofuscin and the increase in the viscosity in these neurons (Autti, Joensuu, and Aberg, 2007). Therefore, the linear hypointensity of the pons that was found among ARSACS patients can also be explained by possible storage of lipofuscin-like materials. Further pathological study is needed to disclose the origin of the T2 hypointensity.

6.2 Nerve and muscle biopsy

Sural nerve biopsy revealed severe axonal degeneration and loss of large myelinated fibers (Peyronnard, Charron, and Barbeau, 1979; Bouchard, 1991; El Euch-Fayache et al., 2003; Takiyama, 2006). These findings in Quebec and non-Quebec patients are consistent with an axonal neuropathy associated with demyelinating features.

Muscle biopsy disclosed typical and obvious neurogenic atrophy, i.e., grouped atrophy in the studied patients (Bouchard, 1991; El Euch-Fayache et al., 2003).

7. Neurophysiology

7.1 Peripheral nerve conduction studies

The peripheral nerve conduction study in Quebec patients revealed an axonal neuropathy with absent sensory action potentials and low motor conduction velocity (Peyronnard, Charron, and Barbeau, 1979; Bouchard, 1991). We have presented the peripheral nerve conduction data for cases 4 and 5 (Shimazaki et al., 2005). In each patient, the motor nerve conduction velocity was mildly reduced in the ulnar and median nerves, and moderately in the posterior tibial nerves. Each compound muscle action potential (CMAP) was markedly decreased. In case 5 (patient 1 of (Shimazaki et al., 2005)), a CMAP was not evoked in the common peroneal nerves. No sensory nerve action potential was evoked in any of the extremities. These data indicate not only a severe to moderate axonal neuropathy but dysmyelinating neuropathy complicated by secondary axonal degeneration as in Quebec and Tunisian patients (Peyronnard, Charron, and Barbeau, 1979; El Euch-Fayache et al., 2003).

7.2 Motor and sensory evoked potentials

Central pathway conduction studies including ones on somatosensory evoked potentials, brainstem auditory evoked potentials, and pattern reversal visual evoked potentials were performed in 67 Quebec patients (De Lean, Mathieu, and Bouchard, 1989). The findings that showed marked delays revealed widespread processes of demyelination in the primary sensory neurons as well as in the central nervous system (Bouchard, 1991). The central sensory and motor pathways were markedly impaired that could be attributed to myelinopathies, and there were high incidences of asymptomatic auditory and visual pathway involvement (Bouchard, 1991).

Electronystagmography showed horizontal gaze nystagmus in all Quebec patients with marked impairment of smooth ocular pursuit and optokinetic nystagmus, and defective fixation suppression of caloric nystagmus (Dionne et al., 1979). Recently, both the masseter and blink reflexes were found to be abnormal in two ARSACS patients (Garcia et al., 2008), whereas the masseter reflex was preserved but a bilateral delay of the late response of the blink reflex was observed in Friedreich' ataxia patients.

8. Therapy and management

Spasticity, the main feature of ARSACS during childhood, is rather mild in most patients. In the teens, however, the spasticity increases in the lower limbs and patients often present a

spastic gait with evolving pes cavus (Bouchard et al., 2007). Physical therapy and use of oral medications such as baclofen to control spasticity in the early phase of the disease may prevent tendon shortening and joint contractures. When spasticity becomes significant, intrathecal baclofen may be considered. The most effective surgical procedures were triple arthrodesis with percutaneous lengthening of the Achilles tendon, and adductor and psoas tenotomies combined with neurectomy of the obturator nerve for perineal hygiene in a retrospective study of 26 patients who received surgical orthopaedic treatment (Bouchard and Langlois, 1999).

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9

Neurochemistry and Neuropharmacology of the Cerebellar Ataxias

José Gazulla[1], Cristina Andrea Hermoso-Contreras[2] and María Tintoré[3]
[1]Department of Neurology, Hospital Universitario Miguel Servet, Zaragoza,
[2]School of Medicine, University of Zaragoza, Zaragoza,
[3]Nucleic Acid Chemistry Group, Chemistry and Molecular Pharmacology Programme, Institute for Research in Biomedicine of Barcelona, Barcelona, Spain

1. Introduction

The aim of this work has been to review the neurochemical alterations described in the cerebellar ataxias, and to enumerate the attempts made at their pharmacological treatment. As will be shown, little use has been made of the neurochemical information available, and the therapeutic trials have been far from successful.

The predominant (though not exclusive) reference to degenerative ataxias is due to the fact that the specificity of the affected cell populations should allow anticipation of more or less specific neurochemical alterations. This information could be used to look for therapeutic strategies, given the absence of curative treatments for the majority of ataxic disorders. This review covers only the pharmacologic attempts performed to treat ataxic symptoms, and is not exhaustive in terms of nosology, genetics or congenital errors of metabolism. The neurochemical basis of some non-degenerative ataxias that demonstrate favourable responses to pharmacological treatment are also reviewed. An outline of the physiological neurotransmission in the cerebellum opens this chapter (Table 1).

2. Neurotransmission in the cerebellum

The cerebellum is made up of four pairs of nuclei located in the deep white matter that covers the fourth ventricle, and is surrounded by a superficial layer of grey matter. The cerebellar cortex has a very uniform cellular structure and great cell density.

In the cortex of the cerebellum, there are several types of inhibitory interneurons that utilize γ-aminobutyric acid (GABA) as neurotransmitter. These are Golgi cells (that coexpress GABA with glycine), stellate cells, basket cells and Lugaro cells.

Purkinje cells are also GABAergic; they are the only ones whose axons exit the cortex of the cerebellum, projecting towards the cerebellar and vestibular nuclei. They use taurine as an osmotic regulator.

The excitatory amino acid glutamate is used in the cerebellar cortex by granule cells and unipolar brush cells. The axons of the granule cells constitute the parallel fibres of the molecular layer.

Most of the afferent fibres of the cerebellum are excitatory and use glutamate as main neurotransmitter. The climbing fibres that leave the contralateral inferior olive and synapse with the Purkinje cell dendrites are mostly glutamatergic, in addition to using aspartate and homocysteic acid. The mossy fibres are more numerous and originate in a number of areas, such as the pontine nuclei, reticular formation, spinal cord, deep cerebellar nuclei (as collaterals to the nuclear axons) and unipolar brush cells. They reach the dendrites of the granule cells in the so called glomerular structures. The great majority of mossy fibres use glutamate; a small proportion, acetylcholine (afferents from the vestibular nuclei and others from the cerebellar nuclei) and peptides such as enkephalins, cholecystokinin, corticotrophin, or calcitonin gene related peptide (CGRP). Part of the climbing and mossy fibres which originate in precerebellar structures, emit a collateral ramification that reaches the deep cerebellar nuclei on their trajectory toward the cortex. The efferent nuclear fibres are excitatory, with the exception of those destined for the inferior olives, which have an inhibitory function.

In addition to the mossy and climbing fibres, there is a group of beaded fibres that use monoamines as neurotransmitters, and reach the three layers of the cerebellar cortex.

Glutamate	Mossy fibers Climbing fibers Granule cells Parallel fibers Unipolar brush cells
GABA	Golgi cells Stellate cells Basket cells Lugaro cells Purkinje cells
Glycine	Golgi cells (coexpressed with GABA)
Noradrenaline	Origin in locus ceruleus
Serotonin	Origin in reticular formation
Acetylcholine	Origin in vestibular nuclei
Histamine	Origin in hipothalamus

Table 1. Neurotransmitters in the cerebellum (references 1-7).

A contingent of noradrenergic fibres stems from the locus ceruleus, and there seems to be a group of dopaminergic afferents of indeterminate origin. Serotonergic fibres originate at the paramedian and lateral reticular nuclei, the periolivary reticular formation and the lateral tegmental region; it has not been possible to demonstrate connections between the raphe nuclei and the cerebellar cortex. Some histaminergic fibres reach the cerebellar cortex from the hypothalamus.

Nitric oxide (NO) is a non-synaptic neurotransmitter present in the cerebellar cortex, mostly generated in the soma and parallel fibres of the granule cells. This substance spreads through the cell membranes and acts on glial cells and some neurons, stimulating the synthesis of cyclic guanosine-monophosphate. Basket and unipolar brush cells also synthesise NO, although not so Purkinje cells (1-7).

In conclusion, neurotransmission in the cerebellum implicates the amino acids glutamate and GABA, which establish an equilibrium between excitatory and inhibitory phenomena (Table 1).

Figures of the anatomy of the cerebellum and its connections, and of the neurochemical organization of the cerebellar cortex may be found the works of Colin et al (5), and Ottersen et al (1).

3. Neurochemistry and pharmacological therapy of the cerebellar ataxias

The abundance of neurotransmitters in the cerebellum complicates the task of determining which among them are implicated in disease pathogenesis. In addition, neurochemical data about many diseases is fragmentary. This section reviews the available neurochemical information (Table 2) and attempts at pharmacological treatment (Table 3) of the following conditions:

1. Cortical cerebellar atrophies
2. Atrophies of the cerebellar cortex and afferent fibres from the brainstem (olivopontocerebellar atrophies, OPCA).
3. Spinocerebellar atrophies.
4. Degenerations of the dentate nucleus and efferent tracts of the cerebellum.
5. Episodic ataxias.

4. Cortical cerebellar atrophies

The cortical cerebellar atrophy (CCA) of idiopathic etiology constitutes a relatively straightforward neurochemical model: the loss of Purkinje cells in the cerebellar vermis (8) causes a selective decrease of the concentration of GABA in the dentate nuclei (9) and cerebrospinal fluid (CSF) (10-13), with no reduction in that of glutamate (9), homovanillic acid (HVA), 5-hydroxiindolacetic acid (5-HIAA), or the noradrenergic metabolite 3-methoxy-4-hydroxyphenylglycol (MHPG) (14). Reduced consumption of glucose in the cerebellum has been determined by positron emission tomography (PET) (15). This condition presents as a late-onset, pure cerebellar syndrome (8). Autosomal dominant spinocerebellar ataxias (SCA) that exhibit a progressive and isolated cerebellar syndrome include SCA 5, 6, 11, 15, 22, 26 and 30.

Cortical cerebellar atrophy	Decreased content of GABA in the dentate nuclei and CSF.
Oivopontocerebellar atrophy	Decreased levels of GABA and glutamate in the cerebellar cortex, and of GABA in the dentate nuclei. Decreased concentration of dopamine and HVA in putamen, caudate and nucleus accumbens. CSF: decreased levels of GABA and glutamate
Friedreich's ataxia	Decreased glutamate concentration in the grey substance and dorsal columns in the lumbar spinal cord. Low glutamate and GABA concentrations in the cerebellar cortex.
Machado-Joseph disease	Decreased HVA in CSF.
Dentatorubral-pallidoluysian atrophy	Decreased GABA and substance P in globus pallidus and substantia nigra, and of choline-acetyltransferase in putamen and caudate nucleus. Reduced GABA in CSF.
Episodic ataxia type 6	Defective glutamate uptake

Table 2. Neurochemistry of the cerebellar ataxias.

Cortical cerebellar atrophy	Anticholinesterase drugs: physostigmine (13,53) Serotonergic drugs: L-5-hydroxytryptophan (38-41), buspirone (43-47), tandospirone (48) Serotonergic antagonists: ondansetron (49) Peptides: TRH (51,52) GABAergic drugs: gabapentin (25), pregabalin (31) NMDA agonists: D-cicloserine (54) Carbonic anhidrase inhibitors: acetazolamide (55,56) Piracetam (32,33)
Oivopontocerebellar atrophy	Anticholinesterase drugs: physostigmine (53,94) Serotonergic drugs: L-5-hydroxytryptophan (40,91), buspirone (46) Dopaminergic drugs: amantadine (89) Peptides: TRH (52) Cholinergic drugs: lecithin (95), L-acetylcarnitine (99) GABAergic drugs: vigabatrin (90), gabapentin (103), zolpidem (101) Glucocorticoid drugs: betamethasone (105) Glutamatergic drugs: ramified amino acids (100) Riluzole (102)
Friedreich's ataxia	Cholinergic drugs: L-acetylcarnitine (99) Serotonergic drugs: L-5-hydroxytryptophan (40,91) Tandospirone (48) Dopaminergic drugs: amantadine (89,115) GABAergic drugs: vigabatrin (116) Peptides: TRH (52) Iron chelators: deferiprone (133) Antioxidant agents: idebenone (118-121,123, 126,127) Erythropoietin (131, 132)
Machado-Joseph disease	Tetrahydrobiopterin (140) Trimethoprim-sulfametoxazole (141,145) Serotonergic drugs: buspirone (92), fluoxetin (120), tandospirone (147, 48) Antiepileptic drugs: lamotrigine (146) Antiarrhythmic drugs: mexiletine (148) Riluzole (102)
Episodic ataxia type 1	Acetazolamide, phenytoin (156)
Episodic ataxia type 2	Acetazolamide (161)
Episodic ataxia type 3	Acetazolamide (164)
Episodic ataxia type 4	Dimenhydrinate (166)
Episodic ataxia type 5	Acetazolamide (169)

Table 3. Pharmacological therapy of the cerebellar ataxias. Boliographic references are in brackets.

A deficiency of GABA in the cerebellum may lead to cerebellar ataxia, as suggested by abnormal GABAergic neurotransmission in the presence of antibodies directed against the enzyme glutamic decarboxylase (GAD) (16), and the coexistence of ataxia with the aforementioned antibodies (17-19). Anti-GAD antibodies are present in juvenile neuronal ceroid-lipofuscinosis, a disorder that may associate ataxia (20), and a selective vulnerability of GABAergic neurons has been found in other lysosomal disorders (21). Besides, an amelioration of ataxia was achieved with the use of GABAergic drugs in a case of adult GM2 gangliosidosis (22), and administration of gabapentin improved motor coordination in potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 1 (HCN1) knockout mice, which exhibit a decreased content of GABA in the cerebellum (23).

The pharmacological trials in CCA are reviewed in the following section.

An open-label trial of gabapentin reported a substantial clinical improvement, and statistically significant differences in the scores of some items selected from the International Cerebellar Ataxia Rating Scale (ICARS) (24). Ten patients were initially given a single dose of 400 mg of gabapentin, followed by doses between 900 and 1600 mg per day during four weeks. Every patient experienced an improvement in ataxia, and in three, gait became normal (25). Gabapentin interacts with the α2-δ subunit of the P/Q type voltage-dependent calcium channels (VDCC) (26), stimulates GABAergic neurotransmission by presynaptic mechanisms (27) and increases the concentration of GABA in the brain of healthy adults (28). More recently, gabapentin treatment decreased ICARS scores by more than 10% in 11 patients with SCA 6 (caused by an abnormal expansion in *CACNA1A*, 19p13, that encodes the α1A subunit of the P/Q-type VDCC), indicating that the drug could be beneficial in this disease (29).

Pregabalin, a molecule closely related to gabapentin, improved the scores in the Scale for the Assessment and Rating of Ataxia (SARA) (30) in a single blind, placebo controlled trial that included two patients with CCA (31).

A patient with cortical cerebellar ataxia was administered piracetam in a single-blind trial. Piracetam (a derivative of GABA that binds to H^3–glutamate sites) improved tandem gait and gait ataxia in a dose of 60 g per day, and the authors concluded that this drug might have an anti-ataxic effect when used in high doses (32). Subsequently, 60 g per day of piracetam was given to a group of two patients with hereditary CCA, and six with other hereditary ataxias (excluding Friedreich ataxia, FRDA), in an open-label trial. The reduction obtained in the mean total score of ICARS (from 39.4±17, to 30.9±14.9), and in that of the posture and gait item, reached statistical significance (33).

Chan-Palay et al induced ataxia in animals through thiamine deprivation, and found a loss of serotonergic fibres in the nervous system (34). As a consequence, the authors suggested that a deficiency of serotonin might constitute the neurochemical basis for ataxia in humans (35). Anyway, neither a deficiency of serotonin nor atrophy of structures that could cause serotonergic denervation have been demonstrated in humans with CCA. The modulating effect of serotonin on GABAergic neurotransmission could explain some of the results reported below (36,37).

In two studies on the serotonergic precursor L-5-hydroxytryptophan, improved stance and speech were obtained in patients with degenerative and secondary ataxias, CCA among

them (38,39). However, in a double-blind placebo crossover study of 13 patients with CCA, seven with OPCA, and 19 with FRDA, no improvement in ataxia was observed (40), although the inclusion of different diseases in the mentioned trials prevented a clear assessment of the effect of L-5-hydroxytryptophan on CCA. In addition, this drug was administered to six patients with CCA in an open-label study, without finding changes in the amplitude of voluntary movement or in the latency of electromyographic activity in antagonist muscles, showing that L-5-hydroxytryptophan was not an effective therapeutic agent for CCA (41).

The drug buspirone stimulates the serotonergic 5-HT1A receptor. It is currently used as an anxiolytic (42), so this effect must be ruled out in its assessment as a treatment for CCA (43-47). Trouillas et al studied the effect of buspirone on CCA in an open-label (42) and in two placebo-controlled studies (44,45). They defined their results as "a progressive modulation, rather than a radical transformation of ataxic symptoms" (43,45), referring to the limited and delayed improvement achieved. Lou et al (46) used buspirone in an open-label study in 14 patients with CCA and six with OPCA; the drug was administered in accordance with the severity of the ataxia. The authors found that buspirone was effective in cases of mild or moderate ataxia, though they did not individualize its effect on any of the two disorders. Andrade-Filho et al (47) noted improvement in 11 patients with CCA, with the addition of buspirone to other anti-ataxic and antiepileptic drugs. However, the methodology employed in this work did not make clear the aetiology of the ataxias, nor did it measure accurately the effectiveness of the drug.

The serotonergic agonist tandospirone was given during four weeks to 5 patients with SCA 6, 5 with SCA 1, 6 with SCA 2, 14 with Machado Joseph disease (MJD), and 9 with multisystem atrophy. This was an open-label, non blinded trial, and obtained reductions in the ICARS scores of the SCA 6 (p 0.043) and MJD (p 0.005) subgroups that reached statistical significance. It must be remarked, however, that the two tables in this article mentioned different values for the pre-treatment mean ICARS score of the cerebellar-multisystem atrophy subgroup, that the discussion incorporated results not specified in the corresponding section, and that the value of probability ($p<0.0001$) for the reduction of ICARS scores after treatment with tandospirone for the entire group, was out of proportion with the results of p for every subgroup of patients (48).

A double-blind, placebo controlled study of the serotonergic antagonist ondansetron showed worsening of the knee-heel manoeuvre in 15 patients with CCA (49).

Thyrotropin-releasing hormone (TRH) increases noradrenaline turnover, facilitates cholinergic transmission, and adjusts GABAergic neurotransmission (50). Although its intravenous administration had no effect on one patient with familial CCA (51), a study of patients with CCA, OPCA and FRDA showed an amelioration of postural instability (52). Obviously, the risk of hyperthyroidism prevents the prolonged use of this potentially beneficial agent.

The use of the anti-cholinesterase drug physostigmine in two double-blind, placebo controlled studies in patients with CCA, obtained no improvement in ataxia. The authors of both articles concluded that physostigmine was not effective in the treatment of this disease (13,53).

The amino-acid D-cycloserine, a partial agonist of the N-metil-D-aspartate (NMDA) glutamate receptor, was used in a placebo controlled trial in two patients with CCA, two with SCA 6 (53), 10 patients with multisystem atrophy and one with degenerative spinocerebellar ataxia. Mild improvements were found in some items of ICARS, and it was suggested that activation of NMDA receptors could lead to symptomatic improvement in spinocerebellar ataxia (54).

Finally, the use of acetazolamide in three patients with SCA 6 was found to have no effect on ataxia (55). Nevertheless, an open-label study of 9 patients with SCA 6 treated with 500 mg per day of acetazolamide, achieved a statistically significant improvement in ICARS scores and in the results of posturographic analysis (56).

Some forms of CCA have a non-degenerative etiology. Chronic abuse of ethanol may cause loss of neurons with GABA-A receptors, especially in the Purkinje cell layer, and vermian atrophy. Abstention from alcohol has been proposed to halt progression of ataxia (57).

Cerebellar paraneoplastic degeneration is a remote consequence of cancer. It is characterised histologically by loss of Purkinje cells and the presence of perivascular and leptomeningeal inflammatory infiltrates (58). An autoimmune cause is invoked by the presence of antibodies directed against epitopes common to the tumour and: 1) Purkinje cells (Yo, Tr) (59,60), 2) Hu and Ri nuclear proteins (60), 3) Tr dendritic protein (61), 4) P/Q-type VDCC (62,63), and 5) mGluR1 type glutamate metabotropic receptors (64). The latter are capable of altering both the acute and plastic response of Purkinje cells, causing cerebellar dysfunction (64). Antineoplastic treatment is recommended, or immunotherapy in its defect (60).

5. Olivopontocerebellar atrophies

The olivopontocerebellar atrophies comprise a heterogeneous group of disorders (degenerative diseases, prionopathies, hereditary errors of metabolism and mitochondrial encephalopathies) whose histological substrate is: 1) loss of neurons in the inferior olive and ventral portion of the pons; 2) loss of mossy and climbing fibres, and 3) atrophy of the cerebellar cortex (65). There is depletion of Purkinje and granule cells in the cerebellar cortex, especially in the hemispheres (8). This expresses clinically a global cerebellar syndrome, accompanied by additional neurological signs. It may be sporadic or familial; familial cases are associated with a greater frequency of medullar signs (with the exception of spasticity), dystonia and oculomotor abnormalities (65). Autosomal dominant spinocerebellar ataxias in which OPCA constitutes the pathological or radiological substrate are SCA 1, 2, 7, 12 and 13 (66).

A fourth part of sporadic OPCA cases develop multisystem atrophy (which associates parkinsonism and autonomic failure) (66,67). Analysis of pathological material has shown immunoreactive inclusions to alpha-synuclein in oligodendrocytes (68) and neurons (69) in this disease. However, this is not the case with SCA1 or SCA2 (disorders caused by expansion of CAG triplets in 6p22.3 and 12q24.13), in which olivopontocerebellar atrophy constitutes the pathological basis (66). The frequency of associated lesions (locus coeruleus, red nucleus, substantia nigra, dentate, hypoglossal and dorsal motor nuclei, nucleus ambiguus, etc) with those described, blurs the nosological limits of OPCA (70).

Neurochemical studies in OPCA have demonstrated an important decrease of GABA content in the dentate nuclei (9,71,72) and cerebellar cortex (71).

The content of glutamate in the cerebellum varied between an important reduction and normality, in different sources (9,71,72). Kanazawa et al established correlation in brains with OPCA, between: 1) the content of glutamate in the anterior vermis, and the density of granule cells; 2) the concentration of glutamate in the posterior vermis and the cerebellar hemispheres, and the cellular density of the inferior olive; 3) the content of GABA in the dentate nuclei, and the density of Purkinje cells (9).

In an autoradiographic receptor study, Albin and Gilman found a statistically significant reduction in the density of GABA, benzodiazepine (BZD) and glutamate receptors in the cerebellar cortex of OPCA brains, compatible with loss of granule and Purkinje cells (73). A PET study found diminished flumazenil binding in the brainstem and cerebellum, confirming the deficiency of GABA observed in OPCA (74).

A study of a patient with sporadic OPCA found IgM antibodies directed against the glutamate receptor subunit GluR2. Antibodies were demonstrated on Purkinje cells, basal portion of the pons and inferior olive, by immunohistochemical methods. The antibodies were shown to be able to depolarise neurons *in vitro*, a fact that pointed to excitotoxicity of autoimmune origin in the genesis of the disease (75).

A low activity of the enzyme glutamate dehydrogenase was previously considered a biochemical hallmark of OPCA (76), although later studies demonstrated a lack of specificity of this metabolic alteration (77,78).

PET studies have shown decreases in dopamine and HVA levels in the striatum in familial (79) and sporadic (80) OPCA. The density of dopamine D2 receptors was normal in the putamen and caudate nuclei in one parkinsonian patient who exhibited OPCA at autopsy, demonstrating the possibility of presynaptic parkinsonism in this disease (81).

A reduced acetylcholinesterase activity and a low density of muscarinic receptors in the cerebellar cortex were found in familial OPCA, suggesting that cholinergic denervation was a major neurochemical anomaly in this variant (82,83). Nevertheless, choline-acetyltransferase activity in mossy fibres (1,3) was greater in familial OPCA than in control cases (82), disproving the previously mentioned proposal.

In CSF, in addition to a low content of GABA (9-11), a low glutamate level was found in sporadic OPCA (11), as well as low levels of HVA, thiamine and MHPG in hereditary OPCA (84-86), with those of tryptophan and 5-HIAA in normal ranges (85).

In addition, a decrease in the levels of pontine and cerebellar N-acetylaspartate (reflecting neuronal loss), was found by high field proton magnetic resonance spectroscopy (^{1}H MRS) in patients with SCA 2 and cerebellar multisystem atrophy. An increase in myoinositol, that points to involvement of glial cells, was also found in multisystem atrophy (87).

To summarise, deficiencies of GABA, glutamate, dopamine and possibly noradrenaline, are present in the nervous system of OPCA patients, although no deficiencies of serotonin or acetylcholine have been documented (79,85).

In an ataxia-telangiectasia (AT) brain with cerebellar, inferior olive and dentate nuclei atrophy, the contents of GABA and glutamate in the cerebellar cortex, and of GABA in the dentate nuclei, were lower than those in controls (88). These neurochemical findings were similar to those in hereditary OPCA (71), and demonstrate that the neurochemical abnormalities of the ataxias are independent of the underlying condition.

The neurochemical complexity of OPCA makes successful pharmacological therapy difficult. As outlined below, a large number of clinical trials have been done, in an attempt to find a remedy.

A double-blind placebo controlled study using amantadine hydrochloride in 30 patients with OPCA without akinesia, obtained improvements in simple and movement reaction times in response to visual and auditory stimuli, that reached statistical significance. The beneficial results were attributed, either to a dopaminergic effect of the drug, or to blockade of NMDA receptors, an effect similar to that exercised by memantine (89).

In a group of 14 patients (one with sporadic OPCA, four with familial OPCA and nine with FRDA), a double-blind comparative trial of vigabatrin (an irreversible inhibitor of GABA-transaminase) with placebo, yielded no apparent benefit (90).

A previously mentioned trial (40) did not find improvement in ataxia with L5-hydroxytryptophan in a group that included seven patients with OPCA. This conclusion was shared by Currier et al, using the same drug in a group that included three patients with OPCA (91).

A group of 20 patients (5 with SCA 2, 2 with SCA 3, 4 with FRDA, and the remaining with other degenerative ataxias) was given buspirone at doses of 60 mg per day, in a double-blind, placebo-controlled, cross-over trial; buspirone was not superior to placebo in the amelioration of ataxia (92). The potential effects of oestrogen on neuroprotection, and of buspirone on ataxia, were combined in an open-label study with 18 OPCA patients. The participants were allocated either to buspirone, 15 mg/day, or to buspirone and oestrogen, 0.625 mg/day. No statistically significant differences were found in ICARS scores, compared with baseline, in any group, although a trend of improvement in gait speed and knee-tibia test was observed in the first one, suggesting that oestrogen was not beneficial in cerebellar dysfunction (93). The work of Lou et al, using buspirone in seven patients with OPCA and 14 with CCA, has been detailed earlier (46).

In another previously mentioned study, the administration of physostigmine to 10 patients with OPCA and nine with CCA gave no apparent benefit (53), although this drug was found to have a favourable effect when used in a heterogeneous group that included three cases of OPCA (94).

The administration of the cholinergic precursor lecithin to 11 patients with OPCA induced a clinical worsening coincident with elevated plasma choline levels (95). Results obtained with choline chloride (96) and physostigmine, led Harding (97) and Manyam (13) to conclude that cholinergic drugs were not effective to treat cerebellar ataxias, probably because no deficit in cholinergic neurotransmission has been confirmed in these diseases (50). In spite of this, a double-blind, placebo controlled analysis of the cholinomimetic agent L-acetylcarnitine obtained a mild improvement in the coordination items of ICARS, in a group of 14 patients with sporadic and hereditary OPCA (98), and in another group of 11 patients with FRDA (99).

Based on the hypothesis that stimulation of glutamate metabolism could favour its neurotransmission in the cerebellum, and so prevent excitotoxic damage, Mori et al gave branched amino-acids to a group of 16 patients (five with sporadic OPCA, and 11 with SCA6 and SCA7) in a double-blind crossover study. They used doses of 1.5g, 3g, 6g and

placebo (100). Starting with an ICARS score average of 42.44 ± 16.60, reductions of 2.92 ± 3.35 were obtained with a 1.5g dose, and of 4.31 ± 4.57 with a 3g dose. These modest results were nevertheless statistically significant, though the effect on patients with OPCA could not be individualized.

The favourable effect of TRH in a group of patients with several types of ataxia (including 12 with OPCA) has been referred to already (52).

In four out of five patients with SCA 2, an improvement of ataxia and intention tremor was observed after administration of zolpidem in single doses of 10 mg. In one patient, a SPECT scan verified normalization of a previously diminished Tc^{99}exametazime binding. The drug's beneficial effect was attributed to reversion of a phenomenon of diaschisis (101).

In a randomized, double-blind, placebo-controlled trial, 40 patients (4 with SCA 2, 6 with multisystem atrophy, 8 with FRDA, and others with degenerative and acquired ataxias) were assigned to riluzole (100 mg/day) or placebo, during 8 weeks. The number of patients with a 5-point drop in ICARS compared to baseline (primary endpoint of the study) was significantly higher in the riluzole group after 4 and 8 weeks of treatment, with a mean change of – 7.05 [± 4.96] points in the total score, versus 0.16 [± 2.65] with placebo (102).

Gabapentin was found to improve gait in a patient with sporadic OPCA, and dysarthria and oscillopsia in another (103). Duhigg described an unexpected regression of ataxia in a patient with OPCA that received 30 mg/day of propranolol (104).

Finally, inhaled betamethasone led to improvement in the ataxia of a patient with infantile AT (105), whilst pregabalin in combination with tiagabine ameliorated ataxia in a patient with adult-onset AT (106).

6. Spinocerebellar atrophies

The most frequent and severe spinocerebellar atrophy is Friedreich's ataxia. FRDA has autosomal recessive inheritance, and an early onset. It is associated with scoliosis, pes cavus, cardiomyopathy, dysarthria, deep tendon areflexia, loss of vibration sense and extensor plantar responses (107). The lesions are located mainly in the spinal cord, where macroscopic atrophy, loss of fibres in the dorsal columns, dorsal and ventral spinocerebellar bundles, and direct and crossed corticospinal tracts, are present. Neuronal loss is found in the gracilis and cuneatus nuclei, Clarke's dorsal nuclei and in the dorsal root ganglia. The dorsal roots are atrophic, and there is depletion of myelinated fibres in the sensory nerves. Neuronal depopulation and loss of iron in the dentate nuclei, as well as atrophy of the superior cerebellar peduncles are also found, while the cerebellar cortex is preserved (8,97,108). Hypertrophic changes are present in the heart, with increased connective tissue and loss of cardiomyocytes (108).

The genetic anomaly in FRDA is an abnormal expansion of a GAA triplet in the first intron of the *FXN* gene on chromosome 9q13, that inhibits the transcription of the mitochondrial protein frataxin. Its deficiency interferes with the synthesis of iron-sulphur complexes, and with iron transport. These cause an accumulation of reactive iron in the mitochondria, interfere with oxidative phosphorylation and allow the formation of toxic oxygen radicals (109).

Neurochemical studies in FRDA have demonstrated low concentrations of glutamate and glycine in the grey matter of the lumbar cord and of glutamate in the dorsal columns, which reflect the loss of corticospinal and sensory glutamatergic fibres (110,111). There was also a reduction in the concentrations of glutamate and GABA in the vermis and the cerebellar hemispheres (112).

HVA and 5-HIIA CSF levels were reduced in patients with FRDA (85); this was not the case with CSF levels of GABA and homocarnosine (113), nor with the density of BZD receptors in the brain (114).

Pharmacological therapy has only achieved partially favourable results in FRDA. As previously mentioned, the results of trials with L-hydroxytryptophan (40,94), physostigmine (53), TRH (52), vigabatrin (91), riluzole (102) and buspirone (92), in groups that included patients with several types of ataxia, did not permit individualization of the effect of these drugs on FRDA.

Botez et al did not find improvement in ataxia when treating a group of 27 patients with FRDA with amantadine hydrochloride (90). The same result was reported by Filla et al, in a double-blind cross-over trial using amantadine hydrochloride in 12 patients with FRDA (115). No benefit was obtained, either, in an open-label assay of vigabatrin in nine patients with FRDA (116).

Idebenone (a government-supported drug for treatment of FRDA in Canada, among other countries) is a synthetic analogue of coenzyme Q10 with powerful antioxidant properties, whose effectiveness on the ataxia and cardiomyopthy of FRDA is currently being investigated.

A positive effect of idebenone on the cardiomyopathy of FRDA reported in a preliminary trial (117) was confirmed in a randomized placebo-controlled trial with 29 patients, in which a reduction of the thickness of the interventricular septum and posterior wall of the left ventricle, that reached statistical significance, was evidenced by echocardiography (118). Another study found that six (among eight) patients with FRDA exhibited an important reduction of cardiac hypertrophy (119), although no improvement in ataxia was noticed in any of these trials.

In a study with an examination period that ranged from 6 to 84 months, Ribat et al observed that ataxia and cardiac ejection fraction deteriorated in 88 patients with FRDA while receiving 5 mg/kg per day of idebenone (in spite of finding decreased cardiac hypertrophy by echocardiography), as well as in 16 non-treated patients (120). An increase in interventricular septum and left posterior wall thickness was observed in patients without previous myocardiopathy, who received 5 mg/kg per day of idebenone. The authors concluded that idebenone did not prevent the development of myocardiopathy, although no worsening was found in patients with known cardiac disease (121).

The phase 3 Idebenone Effects on Neurological ICARS Assessments (IONIA) study randomized 70 ambulatory FRDA patients aged 8 to 18, with ICARS scores between 10 and 54, to placebo and idebenone at doses of 10-20, and 30-54 mg/kg per day. No improvement in left ventricular hypertrophy or cardiac function could be demonstrated over a six month period (122).

Artuch et al (123) reported a statistically significant amelioration in cerebellar function, compared with baseline evaluation, in paediatric patients with FRDA receiving idebenone.

Recently, emphasis has been placed on the use of high doses of idebenone in an effort to improve ataxia in FRDA (124,125); accordingly, a randomized, double-blind, placebo-controlled phase 2 six-month trial (National Institutes of Health Collaboration with Santhera in Ataxia [NICOSIA]) of this drug at doses of 5, 15 and 45 mg/kg per day, was performed on 48 ambulatory FRDA patients aged between 8 and 18, with ICARS scores between 10 and 54. Increasing doses of idebenone were associated with reductions in ICARS scores in a dose-dependent manner, even though overall statistically significant differences were not obtained; thus concluding that high doses of idebenone might be necessary to attain beneficial effects on neurological function (126).

In contrast, the "neurological" arm of the IONIA trial achieved a minimal mean reduction in ICARS scores, which did not reach statistical significance when compared to placebo (127).

The drug mitoquinone (an antioxidant derived from idebenone), which is active in the mitochondrion though not so in the cytosol, is expected to be an effective therapeutic agent in FRDA (128)

A double-blind study of 5-hydroxytryptophan and placebo in 19 patients with FRDA (129), and of an open-label study of amantadine in 16 (130), only gave slightly positive results. A similar benefit was obtained in a previously mentioned study that used L-acetylcarnitine in 11 patients with FRDA (100).

It was demonstrated recently that human recombinant erythropoietin (rhuEPO) increased frataxin in lymphocytes from patients with FRDA, in vitro; this effect was independent from the EPO receptor (131). Thus, a persistent and significant increase in frataxin levels was found in peripheral blood lymphocytes of seven (among 10) patients with FRDA who received 5.000 units of rhuEPO subcutaneously, three times a week during 8 weeks; reductions in the urinary oxidative stress marker 8-hydroxi-2'-deoxyguanosine excretion, and in SARA scores, were also found (132). The same favourable results (that reached statistical significance) were replicated in a study involving 8 patients with FRDA, who received 2.000 units of rhuEPO three times a week during six months; unfortunately, the design of the trial could not rule out a placebo effect of the drug (133).

More specific therapeutic approaches for FRDA are under investigation, such as the histone deacetylase inhibitors, which impair abnormal DNA transcription in FRDA; peroxisome proliferator-activated receptor gamma agonists, that enhance cell antioxidant activity and frataxin levels; deferiprone (a mitochondrion-specific iron chelator) reduced iron content in the dentate nuclei (as measured by MRI), and improved neuropathy and gait ataxia in the youngest patients among 9 adolescents with FRDA (134); gene-based strategies, as the use of viral vectors that express frataxin, which corrected sensitivity to oxidative stress in FRDA fibroblasts (128,135); and finally, pluripotent stem cells induced from FRDA fibroblasts were able to differentiate into neurons and cardiomyocytes (136).

An isolated deficiency of vitamin E, caused by mutations in the gene that encodes the alpha-tocopherol transfer protein in 8q13, can present with an identical phenotype to FRDA. The neurological manifestations stabilise or may partially revert with administration of vitamin E (137).

7. Degenerations of the dentate nucleus and efferent tracts of the cerebellum

This section deals about about Machado-Joseph disease and dentatorubral-pallidoluysian atrophy (DRPLA).

MJD, also designated SCA3, is caused by an unstable expansion of a CAG triplet in the *ataxin 3* gene in14q32.1, and exhibits dominant transmission (138). The lesions are found in the dentate nuclei and superior cerebellar peduncles, and respect the cerebellar cortex, striatum, inferior olive and corticospinal tracts. The pontine nuclei are sometimes affected. The dorsal columns, spinocerebellar tracts and Clarke's dorsal nuclei degenerate in the spinal cord (110). Associated lesions may be present in the anterior horns, oculomotor and subthalamic nuclei, substantia nigra, medial longitudinal fascicle, and peripheral nerves. Among the manifestations of MJD, ataxia is related to lesions in the dentate or pontine nuclei; oculomotor disorders, to those in the brainstem; and parkinsonism, to those in the substantia nigra. The frequent spasticity cannot be explained by the aforementioned findings (138).

Neurochemical abnormalities in MJD consist of a reduced CSF concentration of HVA, even in cases without apparent parkinsonism (85,139). Concentrations of 5-HIAA and MHPG were reduced in CSF in one patient with MJD (136), although these changes were not found in every instance (85,139).

Attempts at pharmacological therapy in MJD are outlined below.

Based on the finding that trimethoprim increased the concentration of tetrahydrobiopterin (THB) in CSF in MJD, Sakai et al administered 1 mg/kg of THB and placebo to five patients for 10 day periods, in a crossover scheme. They reported a statistically significant improvement in the performance of some timed tests of motor function, though deglutition and tendon hypereflexia were not modified (140).

A double-blind, placebo-controlled, crossover trial of trimethoprim-sulfamethoxazole (TS) in 20 patients with SCA3, employed: 1) a clinical scale of ataxia and other non-cerebellar symptoms; 2) posturographic analysis; 3) the Schoppe motor performance test; and 4) achromatic and colour discrimination visual sensitivity tests. After six months of TS administration, none of the patients showed improvement in any of the enumerated tests. No differences were noted in sub-group analysis according to age, sex, duration of illness, phenotype, age at onset, or number of CAG triplets (141). These categorical results contrast with the more favourable outcomes obtained in a study that included eight patients with MJD (142), and with three other reports of individual patients (143-145) that received TS. The reason for the differing results could lie in the absence of molecular diagnosis in the latter studies, or in other methodological differences (141).

An open-label study on the use of the antidepressant drug fluoxetine involved doses of 20 mg per day given to 13 patients with MJD. In spite of a statistically significant improvement according to the Montgomery-Asberg depression rating scale, the EDSS and UPDRS scales showed no differences in motor function. The study concluded that serotonergic stimulation was not effective in the treatment of MJD (146).

Buspirone, at a dose of 60 mg per day, did not improve ataxia in a group of 20 patients that included 4 with SCA 3 (92).

Another open-label study used 10 to 30 mg per day doses of tandospirone. Seven out of 10 patients with MJD had their ICARS scores slightly improved, with additional mitigation of symptoms potentially caused by 5-HT1 receptor dysfunction (insomnia, anorexia, depression and cold lower extremities). The authors concluded that MJD manifested symptoms derived from these receptors, and recommended further tests with tandospirone in this disease (147). An open-label trial of tandospirone in 39 patients (14 with MJD among them) has already been commented on (48).

The antiarrhythmic drug mexiletine was shown to alleviate muscle cramps in MJD, without improving ataxia (148).

Liu et al gave 50 mg/day of lamotrigine to six patients with MJD, and observed improvement in one leg stance and tandem gait. They proposed that this beneficial effect could be due to enhanced expression of ataxin 3, induced by the drug (149).

Dentatorubral-pallidoluysian atrophy is a dominantly transmitted illness caused by an abnormal expansion of a CAG triplet in the atrophyn gene, in 12p13.31, that codifies polyglutamine sequences of abnormal length that exert a toxic action (as in other diseases caused by expansion of CAG triplets) (150). An important neuronal loss in the dentate and red nuclei is found. Less intense degeneration of the subthalamic nuclei and external part of the globus pallidus is also present, while the cerebellar cortex is preserved. Some studies have described spinal cord lesions identical to FRDA in DRPLA, in addition to those described (151); demyelinization in the superior cerebellar peduncles and efferent tracts of the pallidum has been noted, as well. These lesions may be asymmetric (152). Polyglutamine nuclear inclusions have been found in neurons and oligodendrocytes (153).

The clinical manifestations of DRPLA are heterogeneous. Cerebellar ataxia and dementia are considered cardinal signs, accompanied by progressive myoclonic epilepsy in cases with onset before the age of 20, or choreoathetosis and psychiatric symptoms when onset occurs later. It has been determined that there is an inverse correlation between the number of CAG triplets and age at onset of the disease. The differential diagnosis includes Huntington's disease due to the possible association of chorea and dementia (150).

The neurochemical alterations in DRPLA are centred on a reduction of GABA and substance P in the globus pallidus and substantia nigra, and reduced choline-acetyltransferase activity in the caudate and putamen, in spite of preservation of the small striatal neurons; this result points to cell hypofunction as its cause (154). In CSF, the concentration of GABA was found to be very low in five cases of DRPLA, whilst levels of HVA and 5-HIAA were normal (151).

Recently, an accumulation of 8-hydroxi-2'-deoxyguanosine and 8-hydroxyguanosine, and a reduction of immunoreactivity to Cu/Zn superoxide dismutase, were found in the lentiform and dentate nuclei of DRPLA brains, suggesting the possibility that oxidative stress might play a part in the genesis of this disease (155).

No clinical assay dedicated to the treatment of ataxia caused by DRPLA has been performed to date.

8. Episodic ataxias

Episodic ataxias are transmitted by autosomal dominant inheritance, and are amenable to drug treatment.

Episodic ataxia type 1 (EA1), also known as episodic ataxia with myokymia, has its onset in infancy or early adolescence, and associates interictal myokymia in the face and limbs (identified by electromyography) with brief episodes of unsteadiness, tremor and dysarthria. The attacks are brought about by voluntary movement or startle, and may occur many times every day. They can be prevented with acetazolamide or phenytoin. EA1 is caused by mutations in the *KCNA1* gene in 12p13, which encodes the voltage- dependent potassium channel KCNA1, widely expressed in the cerebellum and peripheral nerve (156-159). It has been demonstrated that the mutated channels increase cellular excitability, and prevent physiological repolarization (160).

Episodic ataxia type 2 (EA2) is caused by mutations in *CACNA1A*, that give rise to truncated α1A subunits (161). Electrophysiological characterisation of the abnormal proteins has demonstrated reduced channel conductance, causing an abnormally low calcium ingress, with the consequent cell damage (162,163).

EA2 appears in infancy and is associated with crises of ataxia, vertigo and nausea that last hours or days and are precipitated by emotional stress, fatigue or ingestion of coffee or ethanol. Interictal nystagmus, permanent ataxia and atrophy of the cerebellar vermis may coexist. Diagnosis may be difficult, as EA2 may be confused with anxiety or paroxysmal vertigo. The ataxic episodes respond to prophylaxis with acetazolamide (156,161).

Episodic ataxia type 3 (EA3) appears between the age of one year, and forty. It is associated with ataxia, vertigo and tinnitus, frequently headache, diplopia and blurred vision; interictal myokymia is also present. It may be distinguished from EA1 by the presence of vertigo and tinnitus, and from EA2 by the absence of interictal nystagmus and the short duration of the attacks, which are prevented by acetazolamide (164). The responsible gene is located in 1q42 (165).

Episodic ataxia type 4 (EA4), or vestibulocerebellar ataxia, was described by Farmer and Mustian in 1963 and is characterised by vertigo, diplopia, and mild or moderate ataxia that lasts from a few minutes to several weeks. It appears at an average age of 23 years (166). Defects have been found in smooth ocular pursuit and suppression of the vestibulo-ocular reflex, in addition to gaze-evoked nystagmus (167). Some patients develop progressive ataxia (166). EA4 responds to prophylaxis with dimenhydrinate (166) and is genetically distinct from SCA1, 2, 3, 4, 5, EA1, EA2 and DRPLA (168).

Episodic ataxia type 5 (EA5) is caused by a point mutation in *CACNB4* (2q22-q23), that causes a change of one amino-acid (C104F) in the β4 subunit of the VDCC. It was described in patients with French-Canadian ancestry, and its clinical symptoms (ataxia and vertigo) and duration are similar to EA2; there is interictal nystagmus and it responds to prophylaxis with acetazolamide. The main difference is a later age of onset (169).

Episodic ataxia type-6 (EA6) was described in a ten year-old child that exhibited transitory episodes of ataxia and dysarthria in addition to epilepsy, migraine and alternating hemiplegia. A heterozygote mutation was identified in *SLC1A3* (5p13), the gene that encodes the excitatory amino-acid transporter 1 (EAAT1, GLAST1), pointing to abnormal reuptake of synaptic glutamate as the causing factor of the neurological syndrome (170).

9. Conclusions

As may be deduced from the exposed data, pharmacological trials of cerebellar ataxias have been flawed by a number of factors, like the recruitment of very scarce numbers of patients, the predominance of clinical assays which include patients with more than one disease, the lack of an ataxia rating scale of generalized use and that of quantitative means of measuring ataxic symptoms, the absence of standard doses of the drugs under investigation, and probably the most important, the usual lack of application of the available pathophysiological data to the trials performed to date.

The basic neurochemical anomaly in idiopathic CCA consists in a lowering of the cerebellar content of GABA. In OPCA, deficits of glutamate, dopamine, and probably, noradrenaline, are present as well. Glutamate is essentially the deficient neurotransmitter in FRDA. A deficiency of serotonin has not been demonstrated conclusively in degenerative ataxias. The neurotransmitter abnormalities of MJD and DRPLA have not been well defined yet. Thus, it seems obvious that the neurochemical complexity of these disorders is one of the reasons for the lack of effective treatments.

Some tests have shown that the drugs gabapentin, pregabalin and tiagabine are effective in ataxias that associate a predominant deficiency of GABA in the cerebellum, like CCA and OPCA. Presumably, the more selective the deficit of GABA, the more effective the GABAergic substitution.

Agents capable of restoring the physiological action of glutamate (associated with neuroprotective molecules to prevent excitotoxic phenomena) could be useful in disorders like OPCA and FRDA. Conversely, the usefulness of the peptide TRH is conditioned by the risk of hyperthyroidism. Idebenone and other agents used to treat FRDA have to prove their effectiveness on ataxia, in a definite manner. The lack of effectiveness of physostigmine and choline chloride discards them as therapeutic agents for CCA and OPCA. The use of serotonergic agents in the cerebellar ataxias must be considered controversial at least, due to insufficient neurochemical evidence, and that of riluzole should be investigated in depth, as it could benefit patients with multisystem atrophy.

Given the severity of many of the ataxias considered in this work, treatable causes, such as vitamin E deficiency, should be ruled out when faced with phenotypes similar to FRDA. In a similar way, therapeutic trials with acetazolamide should be undertaken in cases with uncertain diagnoses, with the aim of recognising ataxias that respond to this drug.

Research aimed at identifying effective drugs to treat the cerebellar ataxias should, ideally, look for agents able to neutralize the causes of these diseases. However, as this is not possible in most cases, neurochemical evidence might provide useful clues in the search for therapeutic remedies (171,172). The study of animal and experimental models of disease, the use of precise methods for the measurement of ataxia (clinical semi-quantitative scales, quantitative movement analysis, etc) and the recruitment of homogenous study populations (22), are all highly recommended. In this way, the currently exiguous therapeutic panorama of the cerebellar ataxias could be amplified until etiological remedies are found.

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The purpose of this book has been to depict as many biochemical, genetic and molecular advances as possible, in the vast field of the spinocerebellar ataxias.

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