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Machado-Joseph Disease: from first descriptions to new perspectives

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Abstract

Machado-Joseph Disease (MJD), also known as spinocerebellar ataxia type 3 (SCA3), represents the most common form of SCA worldwide. MJD is an autosomal dominant neurodegenerative disorder of late onset, involving predominantly the cerebellar, pyramidal, extrapyramidal, motor neuron and oculomotor systems; although sharing features with other SCAs, the identification of minor, but more specific signs, facilitates its differential diagnosis. MJD presents strong phenotypic heterogeneity, which has justified the classification of patients into three main clinical types. Main pathological lesions are observed in the spinocerebellar system, as well as in the cerebellar dentate nucleus. MJD's causative mutation consists in an expansion of an unstable CAG tract in exon 10 of the *ATXN3* gene, located at 14q32.1. Haplotype-based studies have suggested that two main founder mutations may explain the present global distribution of the disease; the ancestral haplotype is of Asian origin, and has an estimated age of around 5,800 years, while the second mutational event has occurred about 1,400 years ago. The *ATXN3* gene encodes for ataxin-3, which is ubiquitously expressed in neuronal and non-neuronal tissues, and, among other functions, is thought to participate in cellular protein quality control pathways. Mutated *ATXN3* alleles consensually present about 61 to 87 CAG repeats, resulting in an expanded polyglutamine tract in ataxin-3. This altered protein gains a neurotoxic function, through yet unclear mechanisms. Clinical variability of MJD is only partially explained by the size of the CAG tract, which leaves a residual variance that should be explained by still unknown additional factors. Several genetic tests are available for MJD, and Genetic Counseling Programs have been created to better assist the affected families, namely on what concerns the possibility of pre-symptomatic testing. The main goal of this review was to bring together updated knowledge on MJD, covering several aspects from its initial descriptions and clinical presentation, through the discovery of the causative mutation, its origin and dispersion, as well as molecular genetics aspects considered essential for a better understanding of its neuropathology. Issues related with molecular testing and Genetic Counseling, as well as recent progresses and perspectives on genetic therapy, are also addressed.

Keywords: Ataxin-3, *ATXN3* gene, CAG repeats, Polyglutamine disorders, SCA3

Introduction

Spinocerebellar ataxias (SCAs) are autosomal dominant inherited ataxias, which constitute a heterogeneous group of typically late-onset, progressive, and often fatal neurodegenerative disorders, characterized by progressive cerebellar dysfunction, variably associated with other symptoms of the central and peripheral nervous systems [1-3]. Nearly 30 subtypes of SCAs have been described, and based on the nature of the underlying causative

mutations, these subtypes can be divided into three major categories: 1) "polyglutamine" ataxias, caused by CAG repeat expansions that encode a pure repeat of the amino acid glutamine in the corresponding protein; 2) non-coding repeat ataxias, caused by repeat expansions falling outside of the protein-coding region of the respective disease genes; and 3) ataxias caused by conventional mutations in specific genes (deletion, missense, nonsense, and splice site mutations) [1]. The focus of this review, Machado-Joseph disease (MJD; MIM #109150) [4], also known as spinocerebellar ataxia type 3 (SCA3) [5], belongs to the first of the above cited categories [6]. Several alternative designations have been given to this

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disorder, namely "Machado disease" [7], "nigro-spino-dentatal degeneration with nuclear ophthalmoplegia" [8], "autosomal dominant striatonigral degeneration" [9] and "Azorean disease of the nervous system" [10]. Presently, the most widely used designations are MJD and SCA3.

Epidemiology

Globally, SCAs are considered rare disorders, with prevalence estimates varying from 0.3 to 2.0 per 100,000 [11]. MJD is presently considered the most common form of SCA worldwide [12]. The availability of a molecular test has allowed a thorough identification of cases, changing the initial geographic distribution pattern of MJD, initially thought to be related with the Portuguese discoveries and currently known to be present in many ethnic backgrounds [12], with strong geographic variation.

Among SCAs, the relative frequency of MJD is higher in countries such as Brazil (69-92%) [13,14], Portugal (58-74%) [15,16], Singapore (53%) [17], China (48-49%) [18,19], the Netherlands (44%) [11], Germany (42%) [20], and Japan (28-63%) [21,22]. It is relatively less frequent in Canada (24%) [23], United States (21%) [24], Mexico (12%) [25], Australia (12%) [26], and India (5-14%) [27,28], and it is considered as relatively rare in South Africa (4%) [29] and Italy (1%) [30].

Even within each country the geographic distribution pattern of MJD is not homogeneous. Although constituting the most prevalent subtype of SCA, in Portugal, for example, MJD is relatively rare in the mainland (1/100,000) [31], with few exceptions such as a small area of the Tagus River Valley (1/1,000) [32], but highly prevalent in the Azores Islands, where the highest worldwide prevalence occurs in Flores Island (1/239) [33].

Clinical Presentation

MJD is a multisystem neurodegenerative disorder involving predominantly the cerebellar, pyramidal, extrapyramidal, motor neuron and oculomotor systems. A clinical diagnosis is suggested in individuals with progressive cerebellar ataxia and pyramidal signs, associated with a complex clinical picture extending from extrapyramidal signs to peripheral amyotrophy [34]. Minor, but more specific, features such as external progressive ophthalmoplegia (EPO), dystonia, intention fasciculation-like movements of facial and lingual muscles, as well as bulging eyes, may also be of major importance for the clinical diagnosis of MJD [34]. The mean age at onset is around 40 years, with extremes of 4 [35] and 70 years [31], and a mean survival time of 21 years (ranging from 7 to 29 years) [31,36]. Gait ataxia and diplopia are reported as first symptoms in 92.4% and 7.6% of cases, respectively [31].

MJD is characterized by a high degree of pleomorphism, not only in the variability in the age at onset, but also in the neurological signs presented by different patients as well as in the resulting degree of incapacity. The striking clinical heterogeneity characteristic of this disease is demonstrated by the history of its initial description. In fact, the observation of three families of Azorean ancestry (Machado, Thomas and Joseph), living in the United States of America, by three distinct groups of researchers, led to the initial description, during the 1970s, of three apparently independent diseases [7-9]. The subsequent identification of several Portuguese families living both in the Azores Islands and in the mainland of Portugal, within some of which were patients covering the three forms described, led to the unification of the disease. MJD was afterward considered as a single genetic entity, with variable phenotypic expression [4]. The marked clinical heterogeneity and the progressive nature of MJD rendered its clinical classification difficult. Coutinho and Andrade [4] systematized the disease phenotypes into three main clinical types. They observed that almost every patient presents with cerebellar signs and EPO, associated with pyramidal signs in variable degrees. Clinical types could, therefore, be distinguished on the basis of the presence/absence of important extrapyramidal signs, and the presence/absence of peripheral signs. Type 1 ("type Joseph") is characterized by an early onset (mean of 24.3 years) and a rapid progression of symptoms, which together with cerebellar ataxia and EPO include marked pyramidal and extrapyramidal signs (such as dystonia). Type 2 ("type Thomas") corresponds to presentations with an intermediate onset (mean of 40.5 years), cerebellar ataxia and EPO, with or without pyramidal sings. When present, the extrapyramidal and peripheral signs are tenuous. Patients with type 2 features may maintain these for long periods or evolve (5 to 10 years later) to type 1 or type 3, by the manifestation of important extrapyramidal or peripheral signs, respectively. Type 3 ("type Machado") presents a later onset (mean of 46.8 years) and is characterized by cerebellar ataxia and EPO, associated with peripheral alterations, with or without slight pyramidal and extrapyramidal signs [31]. As previously mentioned, these three clinical types can occasionally be present in the same family. Additionally, some authors consider as type 4 a rare presentation with parkinsonian features, with mild cerebellar deficits and a distal motor sensory neuropathy or amyotrophy [37]. Furthermore, Sakai and Kawakami [38] observed two siblings that presented spastic paraplegia without cerebellar ataxia and proposed the existence of a fifth type for MJD.

Pathological studies reveal, in most cases, that the brain weight of MJD patients is considerably reduced, in comparison to individuals without medical history of neurological or psychiatric diseases [39-42]. Furthermore, depigmentation of the substantia nigra, and atrophy of the cerebellum, pons, and medulla oblongata, as well as

of the cranial nerves III to XII, has been consistently observed in MJD brains [40,43-45]. Neuropathological studies typically reveal neuronal loss in the cerebellar dentate nucleus, pons, substantia nigra, thalamus, globus pallidus, anterior horn cells and Clarke's column in the spinal cord, vestibular nucleus, many cranial motor nuclei, and other brainstem nuclei [39-41,46-55]. Such studies indicate that central nervous white matter lesions are confined to the medial lemniscus, spinocerebellar tracts and dorsal columns [39,40,45,51-55]. Although the inferior olive, as well as the cerebellar cortical neurons, were thought to be typically spared [31,41,56], conflicting results have been reported [39,40,51-53,55].

Magnetic resonance imaging (MRI) has been considered a useful tool in the study and in the diagnostic process of MJD [42,57-61]. Volumetric analyses performed on MRI of MJD patients have previously demonstrated atrophy of the cerebellum, brainstem, caudate nuclei, and putamen [62]. MR spectroscopy studies have also shown abnormalities in apparently normal deep white matter [63]. A recent study [61], using MRI-Texture analysis, showed significant differences among images texture of the caudate nucleus, thalamus, and putamen between patients and a control group, showing that this could constitute a promising tool for the detection and quantification of cerebral tissue areas affected in MJD.

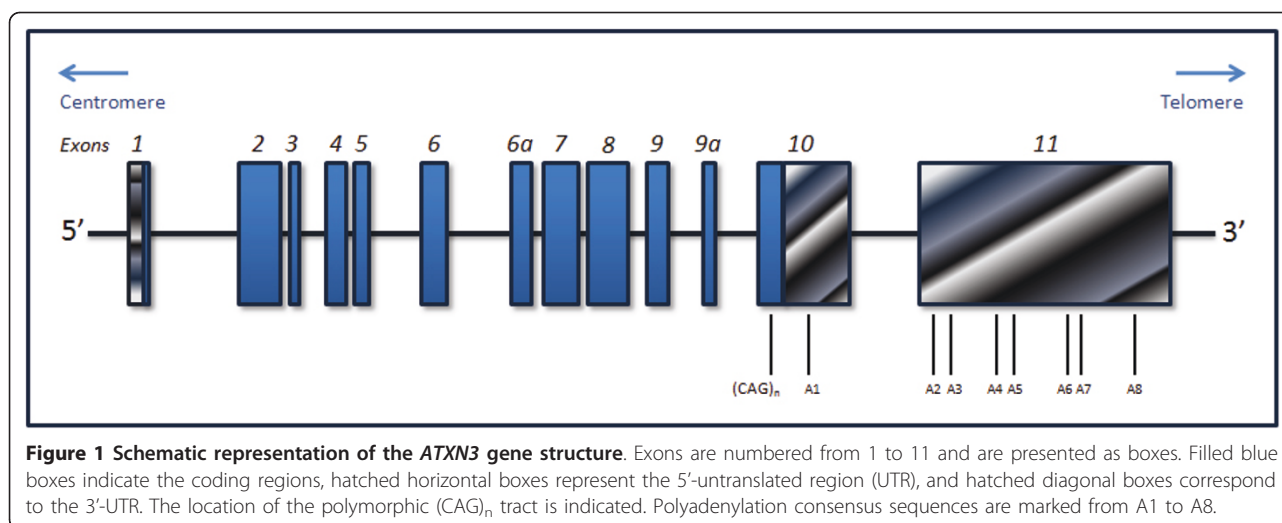## Molecular Genetics And Pathogenesis

The disease locus was first mapped to the long arm of chromosome 14 (14q24.3-q32) by Takiyama *et al.* in 1993 [64]. In 1994, Kawaguchi *et al.* [65] showed that an expansion of a CAG repeat motif at the *MJD1* gene, mapped to 14q32.1, was present in all affected individuals of a pathologically confirmed MJD family. The genomic structure of the *MJD* gene was published seven years later [66]. The gene was found to span about 48 kb and was described as containing 11 exons, with the $(CAG)_n$ tract located at the exon 10 (Figure 1). Two additional exons, 6a and 9a, were recently described (Figure 1) [67]. Currently, the official name of the gene is *ATXN3*, but other aliases, such as *MJD* and *MJD1*, are still in use.

Consensually, wild-type alleles range from 12 to 44 CAG repeats, whereas well established limits of expanded alleles comprise from 61 to 87 repeat units [32]. Intermediate size alleles are rare, but there are a few reports of disease associated alleles containing 56, 55, 54, 53, 51, and 45 CAG repeats [68-73]. On the other hand, an allele with 51 repeats was described, in a Portuguese family, apparently not associated with the disease [32]. Thus, there is the possibility that low penetrance alleles, of intermediate size, which are relatively frequent in other polyglutamine disorders, namely in Huntington's disease (HD) [74], may also occur in MJD.

The *ATXN3* gene encodes for a protein named ataxin-3, which was originally reported to be composed of 339 amino acid residues plus a variable number of glutamine repeats, with an estimated molecular weight of 40-43 kDa for normal individuals [65]. Northern blot analysis showed that the *ATXN3* mRNA is ubiquitously transcribed in neuronal and non-neuronal human tissues [66]. Moreover, such ubiquitous expression was also demonstrated, by immunohistochemical studies, at the protein level, which is expressed not only in the brain but also throughout the body, existing both in the cytoplasm and the nucleus of various cell types. However, in neurons, ataxin-3 is predominantly a cytoplasmic protein [50]. Given its ubiquitous pattern, cellular expression of the disease gene is not itself sufficient to explain selective neuronal degeneration, suggesting that other cell-specific factors are involved in the restricted neuropathology observed in MJD [50].

At least four different species of *ATXN3* transcripts with different sizes, estimated in approximately 1.4, 1.8, 4.5, and 7.5 kb, were reported by Northern blot analysis [66]. These different mRNA species are thought to result from differential splicing of, at least, exons 10 and 11 of *ATXN3* gene, and alternative polyadenylation of exon 11. From sequence analysis of cDNA clones, Ichikawa *et al.* [66] reported the existence of a minimum of five MJD gene products (MJD1a; pMJD1-1; pMJD2-1; pMJD5-1; H2). The MJD1a was first described by Kawaguchi *et al.* [65]. Three additional transcripts (pMJD1-1; pMJD2-1; pMJD5-1) that differ from the MJD1a, mainly at the C-terminal, were then reported by Goto *et al.* [75]. Finally, Ichikawa *et al.* [66] described the variant H2 as having an amino acid sequence identical to the one of pMJD1-1, except for a gap of 55 amino acids, which results from the skipping of exon 2 by alternative splicing. Additional *ATXN3* splicing variants have been deposited in databases, such as ASPicDB [76]. Recently, a large number of alternative splicing variants (n = 56) generated by four types of splicing events (exon skipping, new exons, usage of alternative 5' or 3' splice sites), occurring in a simple or combined way, were described for the *ATXN3* gene [67]. Fifty of those had not been previously described (either in the literature or in databases), and are thought to constitute new alternative splicing variants for this gene. This suggests that alternative splicing may be an important mechanism regulating ataxin-3 diversity, and clearly indicates that there are mechanisms generating variability, beyond genomic DNA.

Ataxin-3 belongs to the family of cysteine proteases. Structurally, it is composed of a globular N-terminal Josephin domain (amino acid residues 1-182 in the human protein) [77] with a papain-like fold, combined with a more flexible C-terminal tail that contains 2 or 3 ubiquitin

Figure 1 Schematic representation of the *ATXN3* gene structure. Exons are numbered from 1 to 11 and are presented as boxes. Filled blue boxes indicate the coding regions, hatched horizontal boxes represent the 5'-untranslated region (UTR), and hatched diagonal boxes correspond to the 3'-UTR. The location of the polymorphic $(CAG)_n$ tract is indicated. Polyadenylation consensus sequences are marked from A1 to A8.

interaction motifs (UIMs) and the polymorphic polyglutamine tract (polyQ tract) [78]. The Josephin domain (JD) contains highly conserved amino acids, reminiscent of the catalytic residues of a deubiquitinating cysteine protease. The catalytic pocket consists of a glutamine (Q9) and a cysteine (C14) residue located in the N-terminal part of JD, and of a histidine (H119) and an asparagine (N134) in the JD C-terminal part. The cysteine, the histidine, and the asparagine constitute the catalytic triad characteristic of cysteine proteases [79]. Although the physiologic role of ataxin-3 is still unclear, it has been proposed that the wild-type form acts as a deubiquitinating enzyme (DUB) in the ubiquitin-proteasome pathway [80,81]. Moreover, it has been established that ataxin-3 can be directly activated by ubiquitination [82]. Additionally, ataxin-3 has been described having a deneddylase activity [83]. Its involvement in transcriptional regulation has also been proposed [80,84]. Furthermore, the participation of ataxin-3 in the regulation of aggresome formation, as well as in the degradation of proteins sent from the endoplasmic reticulum has been described [85]. Taken together with its enzymatic properties, these facts suggest that ataxin-3 normally participates in protein quality control pathways in the cell [46,82]. Recently, it has been suggested that this protein may also be important for a correct cytoskeletal organization [86], as well as for muscle differentiation through the regulation of the integrin signaling transduction pathway [87]. In its mutated form, when the polyQ tract reaches the pathological threshold (about 50 glutamine residues), the protein is thought to gain a neurotoxic function that, as a consequence, leads to selective neuronal cell death through a not fully understood process [50,88].

From the recently described *ATXN3* alternative splicing variants, 20 are thought to encode distinct ataxin-3 isoforms. Although by the analysis of their domain composition, it can be predicted that some may play a protective role while others may lead to increased toxicity [67], their effective role is still unknown. It also remains unexplored if differential expression of the distinct ataxin-3 isoforms could be involved in the specificity of neuronal vulnerability. Nevertheless, it has been observed that the subcellular distribution of ataxin-3 (independently of its isoform) differs in diseased brain versus normal brain. While normally it is a predominantly cytoplasmic protein in neurons (as mentioned earlier), ataxin-3 becomes concentrated in the nucleus of neurons during disease. Moreover, in many brain regions, ataxin-3 forms intranuclear inclusions [89]. These neuronal inclusions, which are also found in other polyglutamine disorders, are heavily ubiquitinated and contain certain heat shock molecular chaperones and proteasomal subunits, suggesting that they are repositories for aberrantly folded, aggregated proteins [90]. The presence of ubiquitinated neuronal intranuclear inclusions (NIIs) has thus been recognized as a neuropathologic hallmark of these diseases, although the significance of NIIs in the pathogenesis remains a matter of controversy [45]. Relatively recent neuropathologic studies [91,92] suggest that inclusions are not directly pathogenic structures and may rather be the byproduct of neuronal efforts to wall off abnormal proteins in a nontoxic manner.

Origins And Mechanisms Of Mutation

Two large studies focus the worldwide origin of the MJD mutation [93,94]. Gaspar *et al.* [93], by haplotype analyses of three intragenic SNPs ($\underline{A}^{669}TG/\underline{G}^{669}TG$, $\underline{C}^{987}GG/\underline{G}^{987}GG$, and $TA\underline{A}^{1118}/TA\underline{C}^{1118}$), found that two (ACA and GGC), out of the four observed MJD haplotypes, were present in 94% of the MJD families. For the families of Azorean extraction, these two main haplotypes were found, presenting a distribution specific to the island of origin: ACA was observed in the families from

Flores Island, while GGC was found in the families from São Miguel Island. These results indicated that two distinct mutational events accounted for the presence of MJD in the Azorean Islands and in families of Azorean extraction, a fact previously evidenced by studies based on the genealogical reconstruction of affected families [95,96]. In Portugal mainland, both haplotypes were also found. Worldwide, 72% of the families share the ACA, further supporting the idea of few mutational events. Based on haplotype analyses, it has been suggested that two main founder mutations may explain the present global geographic distribution of MJD [93,94]. In opposition to the Portuguese/Azorean origin that was proposed at the time of the initial descriptions of the disease, an Asian origin was recently suggested by Martins *et al.* [94]. Their work, which aimed to determine the origins, age, and spread of the two main mutational events, through more extensive haplotype analyses, revealed that the worldwide spread lineage TTACAC reaches its highest diversity in Asia (Japanese population). An ancestral STR-based haplotype was identified in that population, and a postneolithic mutation with about 5,774 ± 1,116 years old was suggested. More recent introductions of this lineage are reported for North America, Germany, France, Portugal, and Brazil. A second mutational event, in the GTGGCA lineage, is thought to be more recent (about 1,416 ± 434 years old). The matter of its origin is more controversial, but its dispersion may be mainly explained by recent Portuguese emigration [94].

The existence of repeat instability has been reported for mutated MJD alleles, similarly to what has been described for the group of "polyglutamine" disorders or for the even larger group of triplet repeat disorders, in which MJD is included [97]. However, the underlying mutational process that allows for alleles in the normal range to, ultimately, expand to pathological size is not clearly understood. Lima *et al.* [98], on a study of nearly 2,000 chromosomes of the Portuguese population, found an allelic distribution biased towards the smaller alleles, not supporting, therefore, the idea that the larger alleles could constitute a reservoir from where expanded alleles could be continuously generated. Analysis of the distribution of the CAG repeat length frequency within the four most frequent wild-type lineages (defined by intragenic polymorphisms) supports the existence of a multistep mutation mechanism on the basis of the evolution of *ATXN3* alleles, either by gene conversion or DNA slippage [99].

Inheritance And Genotype-Phenotype Correlations

MJD displays an autosomal dominant pattern. Therefore, each sibling of an affected individual, or an asymptomatic carrier, has an *a priori* risk of 50% of being itself a carrier, with both genders having equal probabilities of receiving/ transmitting the mutated allele and expressing the disease. Very few cases (2%) of non-penetrance are known [100], and therefore, in the context of genetic counseling (GC), MJD is considered fully penetrant. However, given the fact that MJD penetrance displays an age-dependent pattern (table 1), the probability of being a mutation carrier, and consequently the *a posteriori* risk, diminishes with age in asymptomatic individuals, reaching approximately zero at the age of 70 years [33].

An inverse correlation is found between the size of the CAG repeat tract at the expanded alleles (and consequently the size of the polyQ tract) and the age at onset of the disease. Depending on the series of patients in study, it accounts from 50% to nearly 75% of variation in the age of appearance of the first symptoms [101,102]. A similar inverse correlation has also been described at the mRNA level [103]. Furthermore, the size of the expanded alleles has also been associated with the frequency of other clinical features, such as pseudoexophthalmos and pyramidal signs, which are more frequent in subjects with larger repeats [104]. Moreover, a gene dosage effect seems to be present in MJD, since homozygosity aggravates the clinical phenotype, with a more severe progression and an early age at onset in subjects carrying the expanded allele in both chromosomes [35,105,106]. Anticipation has been reported for MJD and other triplet repeat (TR) diseases [97,107]. Such phenomenon implicates more severe phenotypes and/or earlier ages at onset in successive generations. This can be explained by the dynamic process of mutation underlying TR diseases, which involves intergenerational instability. Normal alleles are usually transmitted to the offspring without modifications [108], while most expanded alleles are unstable upon transmission due to germinal instability, especially in male meiosis [109]. The observed tendency of expanded alleles to further increase the size of its repeat tract, in successive generations, is thought to be the genetic cause of anticipation [97].

Besides the $(CAG)_n$ tract size, familial factors that may increase the explanation of the onset variance have been described [31,110,111]. Although the influence of environmental factors cannot be excluded, the fact that variability within families is lower than the one observed between families supports the contribution of other genetic factors, namely modifier genes, to the remaining phenotypic variance. Modifier genes of the MJD phenotype have been, so far, searched using a candidate-gene approach. Jardim *et al.* [112] analyzed the polymorphic CAG repeats in other repeat loci (SCA2, SCA6 and DRPLA), and concluded that the CAG repeat length of the larger SCA2 allele (22-23 CAG repeats) is associated with the severity of fasciculations. No associations were found with the remaining phenotypic features, namely age of onset, anticipation, and clinical types. An exhaustive search for MJD

Table 1 Age-dependent risk for asymptomatic individuals with an MJD *a priori* risk of 50% (data from Bettencourt et al. [33])

Age in years	Probability of detectable gene expression	Probability of heterozygous if unaffected
10	0.02	0.50
20	0.03	0.49
30	0.22	0.44
40	0.53	0.32
50	0.80	0.17
60	0.96	0.04
70	1.00	~0

modifier genes remains difficult to perform, among other aspects, because of constrains in sample size.

Genetic Testing And Counseling

In the early stages of the disease, when minor but specific signs are missing, when the disease seems sporadic, when it is present in patients belonging to small family units, or when the ethnic or geographic background of the patient is thought to be unusual for this disease, a clinical diagnosis of MJD may not be simple to establish. The identification of MJD's causative gene allowed the direct detection of the mutation, thus enabling the molecular diagnosis of the disease [101]. Furthermore, it allowed worldwide molecular studies about MJD, leading, as previously referred, to a distribution of cases that was clearly different from the initial scenario, obtained exclusively by clinical criteria [113]. Predictive Testing (PT) also became possible for at-risk family members, providing an accurate confirmation of the carrier/non-carrier status in asymptomatic individuals. Targeted mutation analysis of the *ATXN3* gene is also used for the Prenatal Diagnosis (PND) of this disease [114]. However, since a positive result for the MJD mutation raises issues concerning the termination of the pregnancy, several psychological and ethic questions emerge. An alternative for PND, the Preimplantation Genetic Diagnosis (PGD) is also presently available [115]. Levels of adherence to these genetic tests remain to be determined at a large scale. In the Azores Islands, participation in PT was estimated as being around 21%. If, however, only the small Azorean island of Flores is considered, the adherence levels reach nearly 36% [116]. In another small community, the rural region of the Tagus Valley (Portugal mainland), adherence levels to PT program were also high (over 80%) [117]. These high adherence levels in small, isolated communities raise interesting issues, since in such populations genetic diseases can represent a source of stigmatization to the affected families [116]. Therefore, a careful intervention regarding genetic tests, adapted to each specific context, is mandatory.

There is a current lack of effective therapeutics for MJD (see "Patients Management"). Therefore, it is crucial to provide adequate GC to patients and their families, providing information concerning the nature of the disease, the current lack of disease treatment, the risk for other family members as well as the availability of molecular tests, previously mentioned. PT, PND and PGD are offered within the frame of a GC Program. As an example, the Portuguese GC Program, which was based mainly on the experience with HD, aims to provide to at-risk adults the access to the genetic information that can reduce the uncertainty about their genetic status. Another of its goals is to provide the necessary psychological support to allow the proper adaptation to the test results [118]. Candidates for the MJD PT Program have been defined as those: a) at 50% risk and wishing to receive genetic information; b) over 18 years old and capable of providing informed consent; c) with a molecularly confirmed familial history of MJD; and d) asymptomatic for the disease [118].

Teams offering GC to MJD families must provide adequate and comprehensible information concerning the genetics of MJD to the affected families. A study with Azorean MJD families, conducted prior to the application of the PT in this population [119] showed that a large percentage of individuals were unable to comprehend the notion of "pre-symptomatic carrier" and, therefore, could not quantify the objective risk of inheriting/transmitting the disease.

Analysis of the motives for undertaking the PT and of the impact of the test on the psychological well-being of those tested is of major importance for the design of effective GC programs. Leite *et al.* [120] developed a Psychological General Well-Being Schedule, to evaluate psychological well-being in persons coming for MJD pre-symptomatic testing in comparison with normal population. These authors observed that, contrarily to what was expected, individuals at-risk presented higher psychological well-being indicators than the control group. Two possible explanations were suggested by Leite *et al.*[120] to justify such results: a) the group of individuals at-risk has a defensive and denial attitude, and/or b) the group of individuals at-risk is psychologically more resilient, which may have motivated their

adhesion to pre-symptomatic testing, through their own self-selection. Gonzalez *et al.* [116], in a short-term study of the impact of PT in the Azores, found no differences in the mean scores of depression or anxiety before and one year after the PT. These authors concluded that the disclosure of the genetic status did not decrease the psychological well-being of the individuals that undertook testing. Accordingly, the study by Rolin *et al.* [121], which compared data obtained before and 3 to 6 months after the disclosure of genetic testing results, showed no significant changes in well-being and specific distress of PT applicants, both in the individuals identified as carriers and non-carriers. A similar result to what was observed in another study in Japan [122]. Furthermore, it has been shown that the anxiety levels are reduced in those who received a non-carrier result [122,123].

With the advent of pre-symptomatic testing, several laboratory difficulties emerged, and improvements in the diagnosis of MJD had to be made. The first problem was the occurrence of intermediate size alleles, for which it is still not possible to determine whether they are associated with a phenotype or not [32]. To minimize this constrain, clinical and molecular analysis, including the determination of CAG repeat length and the establishment of intragenic haplotypes, of large pedigrees of the affected families, is essential. Furthermore, the study of the healthy population, from the same region, to assess the distribution of the normal $(CAG)_n$ length in that specific population, may also be important [98]. The second problem relied on the presence of homoallelism, i.e., homozygosity for two normal alleles with exactly the same $(CAG)_n$ length (about 10% of all test results). This was solved by studying intragenic polymorphisms, which allowed the distinction of the two normal chromosomes. Furthermore, using a new Southern blot based method, the possibility of existence of an expanded allele in the presumed homoallelic individuals can also be excluded [32]. There are limitations in sizing precision of the CAG repeats due to the existence of somatic mosaicism [124], which originates differences in $(CAG)_n$ length among subpopulations of lymphocytes as well as between lymphocytes (where length is usually measured) and central nervous system cells. However, for molecular diagnosis purposes, an error of ±1 CAG repeat is considered as acceptable [32].

Patients Management And New Perspectives In Treatment
On what concerns disease treatment, effective pharmacologic approaches for the MJD treatment as well as for other SCAs are still lacking or inadequate. Symptomatic pharmacologic therapeutics are used to alleviate some of the clinical signs, namely spasticity [125,126], parkinsonism [127,128], dystonia [129,130], and muscle cramps

[131]. Several clinical trials have also been carried out. The initial double-blind, placebo-controlled, clinical trials were performed with sulfamethoxazole and trimethoprim, in a small number of MJD patients [126,132-134]. From those studies, encouraging results were obtained in terms of lessened spasticity, improvements in walker-assisted gait [132], improvements in contrast sensitivity [133], mild improvements of hyperreflexia of knee jerks and of rigospasticity of the legs [134], beneficial effects on gait and coordination [126]. However, in a larger study, also double-blind and placebo-controlled, trimethoprim-sulfamethoxazole therapy showed no significant effects [135]. The treatment of MJD patients with fluoxetine, failed to improve motor abilities [136]. On the other hand, the use of taltirelin hydrate, was shown to be effective on the ataxic speech of patients with MJD [137]. The treatment with tandospirone pointed for a reduction of ataxia and of depression levels, alleviation of insomnia and leg pain, suggesting that this is a useful drug for these symptoms in patients with MJD [138]. Another trial [139] involved the clinical response of lamotrigine (LTG) on MJD patients with early truncal ataxia and the effect of LTG on the alteration of ataxin-3 expression in the transformed MJD lymphoblastoid cells. Results from this trial indicated that LTG may have significant benefits in relief of gait disturbance in MJD patients with early ataxia, which may be related to the decreased expression of mutant ataxin-3. Notwithstanding some promising results, all these trials were carried out in a small number of patients (1 to 22 patients) and over short periods of time. Studies with a length, design and sample size to provide adequate power to detect meaningful effects should be carefully planned on the basis of underlying basic science before undergoing trials [140].

In addition to pharmacological approaches, physiotherapy may help the patients to cope with the disability associated with gait problems [141]. Physical aids, such as walkers and wheelchairs, can assist the patients in their everyday activities. Moreover, regular speech therapy evaluation for dysarthria and dysphagia as well as occupational therapy may also help patients [141].

Recent advances have been made in the field of genetic therapy. The use of small interfering RNA (siRNA) has been taken as a promising approach for treating autosomal dominant disorders. Although the mouse [142] and *Caenorhabditis elegans* [143] knockout models for ataxin-3 were viable and displayed no overt phenotype, suggesting that ataxin-3 is a non-essential protein, in both cases its importance as a DUB enzyme was confirmed. Nevertheless, there is no correspondent model in humans at our days that could support the hypothesis of ataxin-3 as a non-essential protein. Therefore, discrimination between wild-type and mutant transcripts should be an important point to be addressed in therapeutics

development, in order to preserve wild-type ataxin-3 expression and function. Strategies based on the presence of a single nucleotide polymorphism (SNP) have been proposed to ensure discrimination between wild-type and mutant transcripts [144]. After the understanding of the worldwide distribution of the MJD haplotypes [93,94], the intragenic SNP $\underline{G}^{987}GG/\underline{C}^{987}GG$ at the 3' end of the CAG tract, which variant C is present in more than 70% of the expanded alleles, seemed to bring good perspectives to the possibility of discriminating between wild-type and mutant *ATXN3* alleles. Promising results were obtained by Alves *et al.* [145], who, using siRNA assays targeting that SNP, reached therapeutic efficacy and selectivity in a rat model of MJD. However, transposing this to MJD patients would result inefficient in the case of homozygosis for the C variant, or in the absence of this variant in the expanded allele. Thus, the search for *ATXN3* transcript variation is still imperative for the application of such siRNA approaches. Recently, another strategy for allele-specific silencing of the mutant *ATXN3* mRNA was applied [146], via antisense oligomers, that discriminate between the wild-type and the expanded alleles on the basis of the $(CAG)_n$ repeat length in cell lines. Much is still needed to transpose those allele-specific silencing strategies to effective treatment of patients, but good perspectives are foreseen in the future.

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CB drafted the manuscript. ML revised critically the content of the manuscript. Both authors have read and gave their final approval of the version to be published.

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The authors declare that they have no competing interests.

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