Original Paper

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Machado-Joseph Disease: Correlation between the Clinical Features, the CAG Repeat Length and Homozygosity for the Mutation

Abstract

Machado-Joseph disease (MJD) is an autosomal dominant neurodegenerative disorder associated with the expansion of a CAG trinucleotide repeat in the MJD1 gene located on 14q32.1. We confirmed that the CAG expansion caused MJD in a Yemenite Jewish family and demonstrated that most of the clinical variation among members of this family was due to the genotype of the affected individuals. Six patients who presented with an early onset (25 years) and severe disorder were found to be homozygous for the CAG expansion. Among 5 heterozygotes for the CAG expansion older than 40 years, one had neurological symptoms from the age of 45, while the others were asymptomatic. In one of the heterozygotes, no neurological symptoms were present when last examined at the age of 66. Homozygosity for the MJD1 mutation was the main cause of variability in this large family, however, other factors clearly played a role in the expression of the gene. We could demonstrate that homozygote sibs with similar expansion in both alleles had significant differences in disease severity. Gender did not affect the clinical expression in this family.

Introduction

Machado-Joseph disease (MJD) is considered to be one of the autosomal dominant ataxias. It is a multisystem degeneration in which spinocerebellar, pyramidal, lower motor neuron, peripheral nerve, basal ganglia, extraocular-movement and autonomic functions are impaired [1]. While the majority of patients are of Portuguese ancestry originating from the Azores, MJD has been reported in other ethnic groups [1, 2]. It is well docu-mented that the clinical severity is related to the age of symptomatic onset and varies mostly between families. For instance, among Indian, Japanese, and black American families, the age of onset is relatively early (25–30 years), while in Portuguese and Italian families it is significantly later (>40 years). The penetrance is complete by the age of 46 years in Japanese patients, while a few Portuguese obligate heterozygotes are still unaffected at the age of 70–90 years [3]. This variability of age of onset and clinical presentation is also seen within families [1].

The gene for MJD was recently identified and the mutation was characterized as an unstable CAG repeat within the gene that is expanded in affected individuals. The same mutation was also observed in patients with spinal cerebellar ataxia 3 (SCA3) which, like MJD, has been classified on the basis of clinical presentation of individual patients as a form of autosomal dominant ataxia type I [4, 5]. From these studies, it appears that the MJD/SCA3 mutation is relatively frequent in all populations.

In Israel, the first occurrence of MJD was reported in a large Yemenite Jewish kindred [6]. The original 4 patients and the 3 additionally diagnosed recently show significant clinical variability. We report on the molecular studies in the Yemenite Jewish family and on the basis of the results we offer a possible explanation for the wide range of clinical severity present among the affected patients.

Patients and Methods

The family originates from a small isolated region in Yemen. Because of religious belief and geographical conditions, the community remained isolated and consanguineous marriages were frequent. The pedigree of the kindred appears in the clinical report previously published and has not been updated here, in order to protect the confidentiality of the family members [6], but the pedigree has been made available in the review procedure. For the same reason, details that could help to identify the individuals are omitted.

Four patients have been previously reported and 3 new patients have been diagnosed since then. The parents of most patients were closely related; in one branch of the family a man who is an obligate heterozygote, originated from a close village. Blood was obtained from these 7 patients and 4 asymptomatic adults. When it became evident that 6 patients are homozygotes, we also obtained blood from their spouses and their children who are obligate heterozygotes. Informed consent was obtained from each subject or his/her guardian prior to DNA analysis.

DNA was extracted by standard techniques. Amplification of the CAG repeats was done by PCR with the primers MJD52 and MJD25 as reported elsewhere [2]. The PCR products were run on 3:1 Nusieve:agarose (FMC) gel. The number of CAG repeats was determined by direct sequencing for the normal alleles [7] and the number of expanded alleles was calculated after running the fragments on a denaturing polyacrylamide gel (6%) according to Maciel et al. [8]. Silver staining was used to detect the PCR products.

Table 1. Clinical and molecular data of symptomatic living affected members in the family

Age of onset years	Gait ataxia	Dys-arthria	Ophthal-moplegia	Facial grimacing	LMN disease	CAG repeats
45	++	–	–	–	+	21:72
29	+++	++	++	+	+	64:70
37a	+	+/–	–	–	+	65:66
25	++	–	–	–	+	67:68
17	+++	+++	+++	+++	+++	65:69
30	+++	++	++	++	++	67:68
36	+++	+++	+++	+++	+++	65:69

To protect confidentiality, the sex of the affected individuals is not shown. The degree of severity of the symptoms is indicated by – when absent, and when present the progression in severity is indicated by +/–, +, ++, and +++. LMN = Lower motor neuron.

a The symptoms described in this patient are those which were present at onset.

Table 2. Asymptomatic carriers of the MJD mutation, more than 25 years old (to protect confidentiality, the sex of the affected individuals is not shown)

Genotype	Age at last examination years	CAG repeat length
Heterozygote	60	21:71
Unknown	died at 70 (alcoholism)	unknown
Heterozygote	66	19:68
Heterozygote	42	19:67
Heterozygote	40	19:65

Results

The cardinal clinical features and the results of the molecular analysis of the patients are presented in table 1. Six patients were found to be homozygotes for the MJD mutation; in all of them, the age at onset of the disease was before 40 years and their symptoms were particularly severe (table 1). One apparent exception in the table is the homozygote in whom the onset was at the age of 37, however, the symptoms reported in the table are those which were present at the first examination close to the onset of the disease. Disease progression was more rapid in the homozygotes than in the heterozygotes.

The 4 parents of the 6 homozygotes are obligate carriers. Two of them were siblings and according to the family report were affected with MJD: in one, the disease first manifested at the age of 20 years and death was at the age of 55; in the other, the first manifestations were at the age of 25 and death was at the age of 40. Both may have been homozygotes according to the age of onset and the

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disease progression; in addition, their parents were closely related and all their children who were examined carried the MJD1 mutation. Their spouses who were also obligate heterozygotes did not present any symptoms of MJD. One of them died at the age of 70 without any features of the disease and the other was living, still neurologically intact at the age of 66 years, with one normal allele and one expanded allele of 68 repeats (table 2).

All the spouses of the living homozygote patients had normal CAG repeat lengths (data not shown, range 11–29). An expanded repeat length was present in the 7 patients (table 1, range 64–72), the obligate heterozygotes' children (data not shown, range 64–71) and 4 asymptomatic adults (table 2, range 65–71) (fig. 1). When the mutation was transmitted from a parent to a child, there was no change or an increase/decrease in length up to 2 repeats. However, it was often difficult to determine the change in allele size from one generation to the next. The first reason was that the parents of the majority of the affected individuals had died prior to the study. The second reason was that since almost all the living patients were homozygotes, it was impossible to determine which of the two alleles each child received from the parent. For instance, one patient had two expanded alleles with 64 and 70 repeats; 3 of her children had a normal allele from their father and an amplified allele with 65, 65, or 72 repeats. It is probable that the alleles 65 originate from the maternal allele 64 with a 1 repeat increase and 72 from the maternal 70 with an increase of 2 repeats. However, the 72 allele may have originated from the 65 with a 7-repeat amplification.

Discussion

The family reported here originates from a relatively isolated region in Yemen. In this Jewish community, consanguineous marriages were frequent, often by preference. In addition, because of the geographical isolation, even marriages which are reported as non-consanguineous were most probably between individuals with common ancestors. Therefore, in such a community, homozygosity for a dominant mutation is not unexpected, in particular since the first symptoms of the disease appear after the age of reproduction in the heterozygotes. A similar

Fig. 1. Analysis of the MJD1 alleles in the family. **a** (CAG)$_n$ alleles separated by PAGE (n = number of alleles). **b** Typing of all family members on Nusieve:agarose gel (E = expanded, N = normal allele).
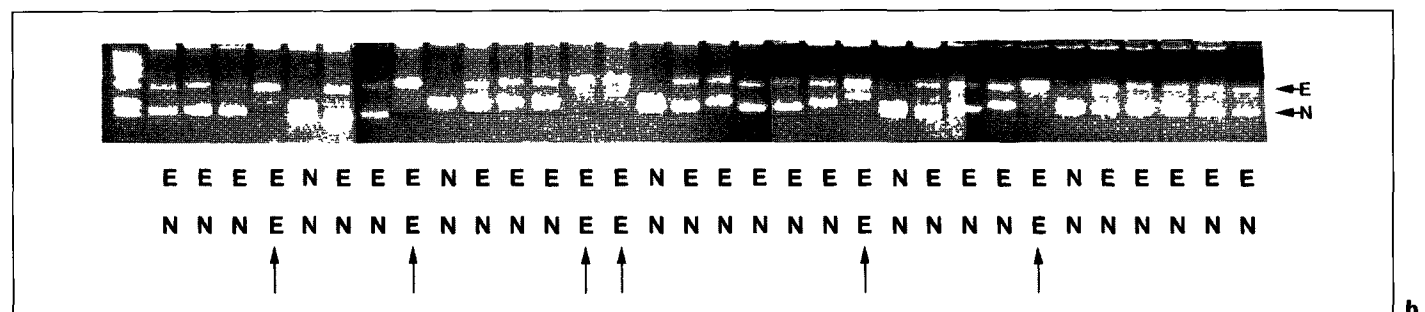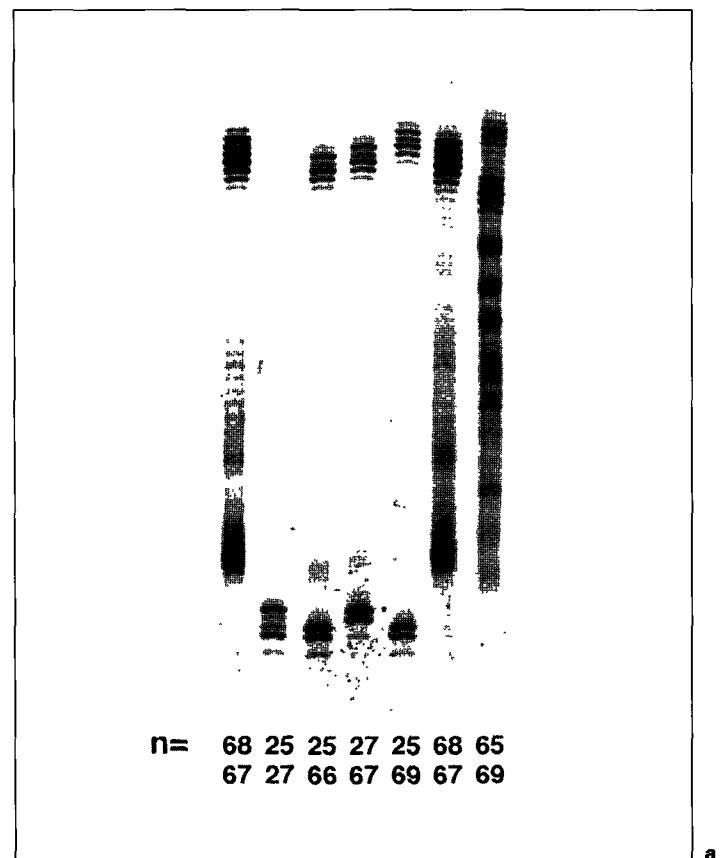
phenomenon was reported for Creutzfeld-Jakob disease, which is relatively frequent among Libyan Jews. The Jews in Libya were relatively isolated, consanguineous marriages were frequent and, indeed, a homozygote for a dominant mutation in the prion gene was found in this community [9].

Kawaguchi et al. [2] sequenced the gene responsible for MJD which is mapped to 14q32.1. In the normal individual, this gene contains a sequence of 13–36 CAG repeats. Patients with MJD show expansion of the repeat number (from 68 to 79); the greater expansions result in the most severe phenotypes. A negative correlation between the age of onset and the CAG repeat number was reported, and it was calculated that it accounts for some 50% of the variation at the age of onset [4, 5, 8, 10]. Still, the size of CAG expansions appears to be comparatively stable within a family. These findings correlate with the clinical observations that in MJD, most of the variability is interfamilial with less intrafamilial differences, even though some degree of anticipation may be observed. Another factor which was found to influence the age of onset is homozygosity for the mutation. St George-Hyslop et al. [11] reported a family originating from the Azores with late onset MJD in which a patient with severe disease and very early onset was diagnosed. Molecular analysis using markers linked to the MJD gene demonstrated that the patient was homozygous for the dominant mutation. A similar observation of a homozygote with early onset was made by Maruyama et al. [10]. They calculated that in this individual, the onset was earlier than expected by the length of the CAG repeat only.

In the family reported here, two groups of patients can be distinguished according to the age of onset. In some of the patients, the onset was relatively late in life (>40 years) and the course was relatively slow: some have not shown any symptoms up to the age of 66. In the others, the onset was early (median 25 years), the clinical course was rapidly progressive and the symptoms were more severe (table 1). All the patients with onset after the age of 40 years were heterozygous for the MJD gene mutation while those with an earlier onset were homozygotes. Within each group, some variability of the age of onset was observed. In the early onset group, in which the patients are homozygotes, the range of age of onset is from 17 to 36 years. The clinical variability could not be explained by an expansion of the CAG repeats or the sex of the affected individuals. Two homozygous siblings of the same sex were at the extremes of age of onset in this group (17 and 36 years) and had a similar expansion in both alleles (65:69); on the other hand, another pair of siblings also of the same sex with a similar expansion in both alleles (67:68) presented with almost the same age of onset (25 and 30 years). Similar findings were observed among the patients heterozygous for the mutation with a late onset. One individual had his first symptoms at the age 45, while one of his siblings was still asymptomatic at the age of 60. They both had one amplified allele with a similar CAG repeat length (72 and 71 repeats, respectively). The age of onset in heterozygotes was in the range predicted by the size of the expanded allele according to the figures published in large series of patients [4, 5, 8, 9]. However, in the homozygotes, the age of onset was always earlier than that predicted according to the largest repeat. For instance, in the homozygote with the later onset (37 years), the largest expanded allele was 66 repeats, which according to the predicted correlation would have a mean age of onset of 60 years.

From these observations and those from the literature, it may be concluded that in MJD the homozygote is more severely affected than the heterozygote. In some dominant disorders, the expression of the mutant gene in heterozygotes is similar to that of homozygotes, but often the expression in homozygotes is more severe. Huntington disease [12] and Creutzfeld-Jakob disease [9] are examples of the former, while in Waardenburg syndrome in which the mutation in PAX3 causes a loss of function [13] or in achondroplasia in which an FGFR3 mutation causes a gain of function [14], a more severe phenotype is observed in the homozygotes. From recent studies of disorders with CAG expansions like MJD, it appears that the effect of the mutation is a gain of function. In Huntington disease, spinal cerebellar ataxia type 1 and dentatorubral and pallidoluysian atrophy (DRPLA), direct demonstration of the translation of the polyglutamine tract has been reported [15]. However, the question of what function is altered in each disorder is still unanswered. It has been speculated that the accumulation of the abnormal product causes progressive damage to the nervous system, but no evidence for such accumulation has been seen. Another possibility is that the polyglutamine stretch leads to a change in protein conformation and to a pathological interaction with other proteins [16]. It may then be expected that in homozygotes of disorders with CAG expansions, the symptoms would appear earlier and be more severe. While this is indeed what is observed in MJD, and maybe also in DRPLA [17], in Huntington disease, the homozygotes are affected like the heterozygotes [12]. Only with understanding the change of function caused by these mutations in each of the specific disorders will we be able to solve this puzzle.

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