Characterisation of the unstable expanded CAG repeat in the MJD1 gene in four Brazilian families of Portuguese descent with Machado-Joseph disease

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Abstract

Machado-Joseph disease (MJD) is an autosomal dominant neurodegenerative disorder which has been shown to result, in Japanese families, from the expansion of a CAG repeat in the MJD1 gene on chromosome 14q. We show that the same molecular mechanism is responsible for MJD in four large Brazilian kindreds of Portuguese descent. The behaviour of the mutation was evaluated in 28 affected and 19 asymptomatic gene carriers. The number of repeats in the expanded alleles ranged from 66 to 77 with a strong negative correlation with age at onset ($r=0.79$). A mean 1.6 repeats increase from generation to generation correlated with clinical anticipation. Instability of the CAG repeat was bidirectional, with expansions as well as contractions, and was more marked in paternal transmissions. Finally, linkage disequilibrium was complete at locus D14S280 in the four Portuguese-Brazilian kindreds and four previously reported French families with the same mutation, which suggests the existence of a common founder.

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Machado-Joseph disease (MJD) is an autosomal dominant neurological disorder characterised by cerebellar ataxia variably associated with pyramidal signs, a dystonic-rigid extrapyramidal syndrome, amyotrophy, facial-lingual fasciculations, and progressive external ophthalmoplegia.^{1,2} Originally described in Azorean families of Portuguese descent, this disease occurs in other ethnic groups as well.³⁻⁵ Because of clinical and genetic similarities to autosomal dominant cerebellar ataxia (ADCA) type I, MJD has been classified as a form of ADCA type I.⁶

In Japanese kindreds, Takiyama *et al*⁷ assigned the MJD gene to chromosome 14q24.3-q32, in a region of approximately 29 cM, which was later reduced to 18⁸ then 3 to 4 cM^{9,10} in Portuguese-Azorean, Brazilian, and Japanese families, respectively. Recently, the MJD gene was identified and found to contain an unstable CAG repeat that is expanded in affected subjects.¹¹ This gene is identical to the spinal cerebellar ataxia 3 (SCA3) gene since it has been

mapped to the same 3 cM on chromosome 14q¹² and expanded alleles in the MJD1 gene are observed in all SCA3 patients.^{12A}

We have now collected and analysed four Brazilian kindreds of Portuguese descent presenting with MJD, in one of which (GC1) linkage to the MJD locus has previously been reported.⁹ Expansion of the CAG repeat at the SCA3/MJD locus is present in all patients. The behaviour of the mutation has been analysed.

Materials and methods

SUBJECTS

One hundred and four members from four Brazilian families (GC1, GC2, GC4, and GC5), including 28 patients, were examined and blood was taken for DNA extraction. All patients fulfilled the diagnostic criteria of MJD.¹² A detailed description of these kindreds has been reported elsewhere (Cassa *et al*, in preparation). Although the same frequent Portuguese surname was found among the affected ancestors of the four kindreds, the families, whose genealogies were traced back to 1820, did not appear to be related.

Statistical analyses were performed using the non-parametric, Mann-Whitney U, and Kruskal-Wallis tests for comparison of means, the F test for comparison of variances and the Yates Corrected χ^2 for comparison of percentages and linkage disequilibrium. The age at onset of 16 dead patients was included in the anticipation calculation.

GENOTYPING

Genotypes at the MJD locus (MJD1 gene) were determined by PCR amplification as previously described,¹¹ except that an annealing temperature of 55°C was used. Four microsatellites from the SCA3/MJD region (D14S291, D14S280, AFM343vfl, and D14S81) were also amplified by PCR as previously described.¹²

Aliquots of the PCR products were then diluted 1:2 in formamide loading buffer, denatured for 10 minutes at 94°C, and run on a 6% acrylamide/7 mol/l urea gel. After blotting on nylon membrane (Hybond N+, Amersham), hybridisation was carried out as described previously¹² with 5'γP³² labelled (CA)₁₀ and (CAG)₇ oligonucleotides. Membranes were washed in 2 × SSC, 0.1% SDS for 10 minutes at room temperature and exposed for

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Figure 1 Distribution of the number of CAG repeats in normal ($n=108$) and expanded ($n=47$) alleles. White bars = controls, black bars = patients.

two to eight hours to x ray film for autoradiography. Allele sizes were determined by comparison with an M13 sequencing ladder. The allele numbering for the microsatellite markers was as in Stevanin *et al.*¹²

Results

MOLECULAR CHARACTERISATION OF THE MUTATION

All patients ($n=28$) from the four Brazilian families were heterozygous for the mutation, with a number of CAG repeats in the normal range (14 to 28) in one allele, but increased by at least 38 repeats (range 66 to 77) in the other. Nineteen out of 58 at risk subjects also carried an allele with an expanded CAG repeat. Analysis of 70 normal chromosomes from 35 unrelated Brazilian controls and of 48 normal chromosomes from the patients and at risk carriers showed the existence of 15 different alleles ranging from 14 to 35 CAG repeats. Their distribution was not significantly different from that observed in Japanese¹³ and French controls.^{12A} Two major alleles, containing 14 and 23 repeats with a frequency of

23% and 35%, respectively, were detected. As shown in fig 1, there was a clear cut difference between the normal and expanded alleles.

BEHAVIOUR OF THE MUTATION

The normal allele was always transmitted with the same number of repeats. The size of the pathological allele varied in most of the transmissions (fig 2) with a tendency to increase in successive generations (table). The mean increase per generation was 1.6 repeats when generation III, in which only one person was tested, was excluded ($p<0.05$).

Repeat size increased in seven and decreased in four out of 15 parent-child pairs, resulting in a mean increase of +0.2 repeat. Mean alteration in repeat size was similar whether maternally (+0.4, $n=5$) or paternally (+0.1, $n=10$) transmitted (fig 2). However, the range of variation (fig 2) was larger in paternal (variance=4.7) than in maternal (variance=0.3) transmissions ($p<0.05$). Furthermore, analysis of variance of 11 children from five sibships who received the mutation from their mother compared to 21 children from 10 sibships who received the mutated allele from their father, showed a greater variance when the disease was paternally (3.1) than maternally (0.3) transmitted ($p<0.05$).

The mean number of CAG repeats was similar in men ($n=26$, 72.7 (SD 3.0) repeats) and women ($n=21$, 71.7 (SD 2.3) repeats).

CLINICAL CORRELATIONS

The mean age at onset was 45 (SD13) (range 25–72) years and decreased in successive generations: GII, 72 ($n=1$); GIII, 53 (SD12) ($n=10$); GIV, 47 (SD 12) ($n=25$), and GV, 32 (SD 5) ($n=10$) ($p<0.01$). In the 22 parent-child pairs for which the ages at onset were

Figure 2 Variation of the number of repeats in the expanded allele in 15 parent-child couples. Paternal and maternal transmissions are represented by hatched and white bars, respectively.

Number of CAG repeats in the expanded allele in successive generations

Generation	No of CAG repeats (range)	No of subjects
III	66	1
IV	71.2 (SD 2.7) (66–76)	18
V	73.0 (SD 2.3) (68–77)	24
VI	74.3 (SD 1.3) (73–76)	4

known, a mean anticipation of 8.2 (SD 10.8) (range –24 to +14) years was observed ($p < 0.05$). Anticipation was more marked, although not significantly, in maternal (10.6 (SD 8.6) years, $n = 10$) than in paternal (6.2 (SD 12.4) years, $n = 12$) transmissions. Evidence for anticipation in parent-offspring pairs remained statistically significant ($p < 0.05$), even if asymptomatic carriers were considered to have onset at the same age as the affected parent in order to minimise the bias introduced by the inability to observe late onset patients in the youngest generation.

A good correlation ($r = 0.79$) between the age at onset of symptoms and the number of repeats in the expanded allele was shown (fig 3), although there was wide variation among individual subjects. For example, age at onset varied from 25 to 55 years in patients with 72 CAG repeats. This result indicates that accurate predictions of age at onset cannot be made for unaffected mutation carriers.

Age at onset in women ($n = 22$) and men ($n = 24$) did not diverge significantly (49.5 (SD 12.4) and 41.8 (SD 13.3) years, $p > 0.05$). Furthermore, in five sibships (three pairs and two triplets) of different sexes, both the mean number of CAG repeats (73.0 (SD 3.5) and 73.2 (SD 1.8)) and the mean age at onset (34.8 (SD 9.5) and 38.8 (SD 10.9) years) were similar for men ($n = 6$) and women ($n = 6$). There was no difference in age at onset between these five mixed sibships and five sibships of the same sex (8.6 (SD 6.8) and 8.8 (SD 7.8) years, respectively; $p > 0.05$).

Figure 3 Correlation between age at onset (years) and the number of repeats of CAG in the mutated allele ($r = 0.79$). Squares and hatched circles represent subjects who received the mutation from their father and their mother, respectively.

LINKAGE DISEQUILIBRIUM

Haplotypes segregating in the four families were reconstituted for four markers spanning 3 cM on chromosome 14q: D14S291/D14S280–2cM-AFM343vfl-lcM-D14S81.^{9,12} Markers D14S280 and AFM343vfl were determined as the centromeric and the telomeric markers, respectively.^{9,14} All patients and at risk carriers in the four families shared the same haplotype from D14S291 to D14S81 (D14S291: 1; D14S280: 5; AFM343vfl: 2; D14S81: 0). Furthermore, the alleles at D14S291 (0.26), AFM343vfl (0.22), and D14S81 (0.05) segregating with the disease were infrequent in the Brazilian population. Haplotype 1–5–2–0 was not present in 20 Brazilian and 60 French control chromosomes. Allele 5 at D14S280 also segregated with the disease in four French SCA3 families.^{9,12,15} Although this allele is frequent in both French (0.52) and Brazilian (0.58) populations, significant linkage disequilibrium was detected when data from both groups were combined ($p < 0.05$).

Discussion

This study shows that the expansion of a CAG repeat sequence at the SCA3/MJD locus is involved in the pathogenesis of MJD in Brazilian families of Portuguese descent, as well as in Japanese kindreds.

By the analysis of 28 patients and 19 unaffected expansion carriers, we show that MJD shares the following features with four other neurodegenerative disorders, caused by expansions of CAG repeat^{16–21}: (1) distinct distributions of the number of repeats on normal and mutated alleles; (2) strong negative correlation between the number of repeats in the expanded allele and the age at onset of the disease; (3) instability of the expanded allele, particularly during paternal transmission; (4) evidence for anticipation in age at onset of the disease. Although the number of CAG repeats in the expanded allele in the Brazilian families was clearly different from the normal allele, was narrow in range (66–77), and negatively correlated with age at onset, it only accounted for 62% of the variance of this parameter, and therefore does not allow accurate prediction of the age at onset in asymptomatic gene carriers. The mutation was unstable in the four kindreds, since a mean increase in repeat number from generation to generation of 1.6 was observed, although when parent-child pairs were analysed, the repeat expanded ($n = 7$) or did not change ($n = 4$) or even decreased ($n = 4$) in individual cases. The mean increase, however, explains, at least in part, the mean anticipation of 13.3 years observed in successive generations and the mean anticipation of 8.2 years in 22 parent-offspring pairs.

In other disorders with unstable repeat expansions such as Huntington's disease (HD), spinocerebellar ataxia 1 (SCA1), and dentatorubral and pallidolusian atrophy (DRPLA), instability and tendency towards expansion was more marked in male than in female transmissions.^{19,21–25} Maruyama *et al*¹³ have recently reported greater instability of the

CAG repeat in paternal transmissions, but they only observed expansions. Surprisingly, in the Brazilian families, larger increases, but also larger decreases, were transmitted by the fathers, which led to a mean increase of 0.1 repeat in father-child couples. This value is close to that obtained for maternal transmissions (+0.4) for which the variance was smaller, although the number of transmissions was also smaller. Despite a greater paternal instability, no evidence of effect of paternal sex on the mean increase in CAG repeat numbers in parent-child couples could be found, because of the bidirectional instability during paternal transmission. The sex of the transmitting parent did not affect anticipation, which was in good agreement with the study of Barbeau *et al.*²⁶ This may be because of the absence of early onset cases (<20 years) in this series, since large expansions, mostly of paternal origin, resulting in young onset in the children have been reported for HD, SCA1, and DRPLA.^{18 19 21 24} The gender of the patients also had no effect on repeat size or anticipation in contrast to the report of Kawakami *et al.*²⁷

Finally, MJD/SCA3 segregated with the same haplotype in all the Brazilian kindreds studied, suggesting a common founder. The haplotype segregating with the disease carried allele 5 at the D14S280 locus in the four Portuguese-Brazilian families as well as in four French SCA3 linked kindreds with no known Portuguese ancestry.^{12 15} Thus, since the same mutation is involved in MJD and SCA3 families,^{12A} and linkage disequilibrium with D14S280 is significant, it is tempting to extend the founder effect to French families as well. However, different or recurrent mutations occurring in a predisposing haplotype could also account for the linkage disequilibrium as in HD.^{28 29} Allele 5 of D14S280 was not associated with repeat lengths in the upper limit of the normal range in controls. This result does not support, but cannot rule out, the hypothesis of a predisposing haplotype for SCA3/MJD. Sasaki *et al.*¹⁰ have observed linkage disequilibrium with D14S81 but not with D14S280 in 24 Japanese families, but it has not been proven that the MJD/SCA3 expansion is responsible for the disease in all of their families. Further studies in families of different ethnic origins with proven SCA3/MJD mutations are needed to answer this question definitively.

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