Am. J. Hum. Genet. 57:54–61, 1995

Correlation between CAG Repeat Length and Clinical Features in Machado-Joseph Disease

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Summary

Machado-Joseph disease (MJD) is associated with the expansion of a CAG trinucleotide repeat in a novel gene on 14q32.1. We confirmed the presence of this expansion in 156 MJD patients from 33 families of different geographic origins: 15 Portuguese Azorean, 2 Brazilian, and 16 North American of Portuguese Azorean descent. Normal chromosomes contain between 12 and 37 CAG repeats in the MJD gene, whereas MJD gene carriers have alleles within the expanded range of 62–84 CAG units. The distribution of expanded alleles and the gap between normal and expanded allele sizes is either inconsistent with a premutation hypothesis or most (if not all) of the alleles we studied descend from a common ancestor. There is a strong correlation between the expanded repeat size and the age at onset of the disease as well as the clinical presentation. There is mild instability of the CAG tract length with transmission of the expanded alleles; both increase and decrease in size between parents and progeny occur, with larger variations in male than in female transmissions. Together, these effects can partly explain the variability of age at onset and of phenotypic features in MJD; however, other modifying factors must exist.

Introduction

Machado-Joseph disease (MJD) is an autosomal dominant spinocerebellar degeneration characterized by a wide range of clinical manifestations, including ataxia, progressive external ophthalmoplegia, pyramidal and extra pyramidal signs, dystonia with rigidity, and distal muscular atrophies (Coutinho et al. 1977; Lima and Coutinho 1980). Typically, patients will become confined to a wheelchair and will later be bedridden; the median survival time after onset is 20 years. Disease manifestations usually start during adulthood, with a mean age at onset of 37.4 years (SD 14.1), but the distribution of age at onset is very wide, ranging from 5 to 73 years (Sequeiros and Coutinho 1993).

Pathologically, the disease is characterized by degeneration of the spinocerebellar, dentate, pontine, and vestibular nuclei as well as extra pyramidal structures, such as the substantia nigra, locus coeruleus, and the pallidoluysian complex. In addition, there is neuronal loss in motor cranial nerve nuclei, anterior horn cells, and posterior root ganglia. Structures such as the cerebral and cerebellar cortex and inferior olives are spared (Rosenberg et al. 1976; Coutinho and Andrade 1978; Coutinho et al. 1982; Sakai et al. 1983; Kanda et al. 1989).

Detailed clinical investigation of the variability in MJD led to its classification into three subphenotypes, occasionally present in the same family: type 1 patients, with earlier onset (mean 24.3 years) and marked pyramidal and extrapyramidal signs, in addition to the common features of cerebellar ataxia and ophthalmoplegia, as well as a rapid and more severe clinical course; type 2 patients have a mean age at onset of 40.5 years and show mainly cerebellar ataxia and ophthalmoplegia; type 3 patients show a later onset (mean 46.8 years), have marked peripheral signs, weakness, and amyotrophy, along with cerebellar ataxia and ophthalmoplegia. Type 2 may be transitional, since all patients start as a type 2; some later evolve into either type 1 or type 3, while others remain as a type 2 all their lives.

The disease locus was mapped to chromosome 14q32.1 in Japanese families (Takiyama et al. 1993),

Received February 13, 1995; accepted for publication April 6, 1995.

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0002-9297/95/5701-0007$02.00

and linkage was confirmed in families of Portuguese Azorean origin as well as in Portuguese American and Brazilian pedigrees (Sequeiros et al. 1994; Twist et al. 1995). The gene was recently identified (Kawaguchi et al. 1994) and shown to contain a CAG repeat motif in the 5′ region of the coding sequence, which is selectively expanded in MJD patients. Therefore, MJD is the most recent addition to the growing number of neurodegenerative disorders for which the causative mutation is the unstable expansion of a CAG repeat.

Correlation of repeat length with age at onset of the disease has been demonstrated in Huntington disease (HD) (Andrew et al. 1993; Duyao et al. 1993; Snell et al. 1993), dentatorubropallidoluysian atrophy (DRPLA) (Koide et al. 1994), spinocerebellar ataxia type 1 (SCA1) (Jodice et al. 1994; Ranum et al. 1994), and X-linked spinal and muscular atrophy or Kennedy disease (La Spada et al. 1992). Yet, the correlation of repeat size with age at onset is not perfect, and some clinical features do not correlate with repeat length, suggesting that factors other than repeat size may influence the clinical presentation in these disorders (Andrew et al. 1993; Ranum et al. 1994).

We have studied 33 families of different geographic origins diagnosed as MJD, in order to confirm the presence of the CAG repeat expansion on 14q32.1, to compare the size range of this repeat in MJD and control populations, and to investigate the relationship between the size of the expanded allele and the clinical presentation. Furthermore, we analyzed the instability of the repeat size in its transmission from parent to progeny, in an attempt to determine the molecular basis for the previously described effect of anticipation in this disease (Sequeiros and Coutinho 1981).

Subjects and Methods

Subjects

This study was performed using DNA samples from 212 members of 33 MJD families (Coutinho and Andrade 1978; Sudarsky et al. 1992; Radvany et al. 1993) of different geographical origins: 15 Portuguese Azorean, 2 Brazilian, and 16 North American . All families had traceable Portuguese ancestry; 23 of these families previously had been used for linkage studies with 14q32.1 markers (Sequeiros et al. 1994; Twist et al. 1995). The diagnosis of MJD was determined by clinical examination by an experienced neurologist using established diagnostic criteria (Lima and Coutinho 1980). Ages at onset were based on information provided by the patient and/or a close relative. For determination of the frequency of the normal alleles, a total of 83 normal controls were used, including a group of unrelated unaffected spouses from MJD families and a group of controls from different ethnic origins. Since both groups had similar distributions of allele sizes, the data were pooled and the controls were considered a single group.

Methods

Genomic DNA was isolated from peripheral lymphocytes by standard methods (Sambrook et al. 1989) or from lymphoblastoid cell lines established by transformation with Epstein-Barr virus (Anderson and Gusella 1984). The CAG-containing fragment of the MJD gene was amplified by PCR using primers MJD52 and MJD25a (a slight modification of MJD25, of sequence ATCCATGTGCAAAGGCCAGCC) (Kawaguchi et al. 1994). PCR was performed in a final volume of 20 µl, containing 40 ng of genomic DNA; 10 mM *Tris*-HCl (pH 8.8); 1.5 mM $MgCl_2$; 50 mM KCl; 0.1% Triton X-100; 10% dimethylsulfoxide; 250 µM each dCTP, dGTP, and dTTP; 25 µM dATP; 1.5 µCi 35S alpha-dATP; 125 ng each primer; and 3 units of Taq polymerase (Perkin Elmer). The DNA was denatured at 94°C for 5 min; then 32 cycles at 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min were performed, followed by a final extension at 72°C for 5 min.

For determination of allele sizes, PCR products were analyzed on denaturing 5% polyacrilamide gels in parallel with an M13 sequencing ladder (−40 primer) and were visualized by autoradiography. Allele sizes (S) were determined by comparison with the sequence and were converted to CAG unit numbers (N), using the formula $N = (S - 121)/3$, assuming that the variation in size of the PCR product occurred within the repetitive CAG stretch.

Linear regression techniques were used to determine the association between repeat number in the MJD or normal allele and age at onset, association between variation in repeat number during transmission and anticipation, and the association between variation in repeat number during transmission and initial size of the allele (Neter et al. 1985). Differences in repeat number between the three clinical subtypes were assessed by analysis of variance and Tukey's multiple comparison of means test (Neter et al. 1985). Chi-square and Fisher's exact tests were used to determine whether variation in repeat number among parent and offspring, expressed as raw data or as a trichotomous variable (expansion, contraction, and stable transmission), is associated with sex of transmitting parent or affection status (affected or asymptomatic) of MJD allele carriers. All analyses were carried out using procedures of the Statistical Analysis System version 6 (1989).

Results

Distribution of Repeat Length in Normal and MJD Chromosomes

Figure 1 shows the frequencies of different CAG repeat lengths in 186 normal and 156 MJD chromosomes.

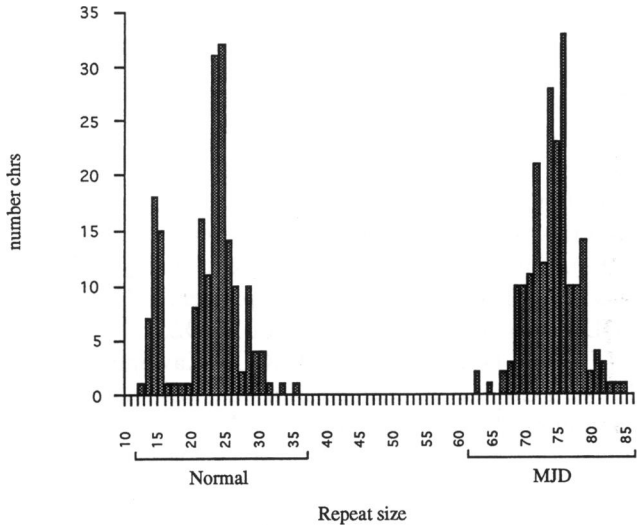

Figure 1 Distribution of CAG repeat sizes in unaffected control individuals and in MJD alleles from affected individuals.

The normal chromosomes have alleles containing from 12 to 37 repeats, whereas affected individuals have at least one expanded allele, with sizes varying between 66 and 84. Expanded alleles of 62 and 64 repeats were found in two asymptomatic carriers, of ages 38 and 45 years, respectively. Thus, there was a wide gap between the size ranges of normal and MJD alleles, and no intermediate alleles (37–62 repeats) were found in the control population. There was no significant difference between mean allele size for paternally (72.8 ± 3.4) and maternally (73.1 ± 4.3) inherited alleles ($P = .51$).

Family Studies with the MJD Repeat

An expanded allele was found in 156/165 individuals considered to be affected. Among the nine cases having two normal alleles and no expansion, five did not show the disease haplotype in previous studies with markers on 14q32.1 and haplotype data for four patients were not available. On retrospective review of clinical records, two of the nine cases were found not to be affected, and two were found to have had diagnostic difficulties (incipient signs, other diseases as possible confounding factors). In the remaining five cases, however, no explanation was found, and the results may be due to sample mix-ups, misdiagnosis, or clerical errors (Andrew et al. 1994).

Trinucleotide Repeat Length and Clinical Features

The relationship between the trinucleotide repeat length in the MJD allele of affected individuals and age at onset of the disease is shown in figure 2. A correlation coefficient of −.68 was obtained ($P = .0001$), assuming a linear relationship between age at onset and repeat length for the MJD chromosome. This suggests a tendency for age at onset to decrease as the CAG repeat length increases. No significant correlation was found between the normal allele repeat length and age at onset ($r = .006$; $P = .95$).

Data were available on 10 cases of juvenile onset of MJD (between 10 and 20 years of age). In this group, the CAG repeat size ranged from 73 to 84, with a mean of 80.4 ± 2.5 repeats. This is significantly different from the adult onset mean of 72.6 ± 3.3 ($P = .0001$). Of these patients, 7 (70%) inherited the expanded allele from the mother and 3 (30%) from the father, but, given the small sample, the apparent excess of female transmission in juvenile onset cases when compared to the rest of the population is not significant ($P = .13$, χ^2 test).

When repeat sizes of patients of different clinical subphenotypes were compared, a significant difference was observed between subtype 1 and the other two subtypes, with a tendency for increased severity with larger repeat size ($P < .05$). Type 1 patients tend to have larger alleles (mean 76.2 ± 5.08, $N = 11$), whereas milder forms (types 2 and 3) tend to show smaller sizes (mean 73.0 ± 3.64, $N = 88$, and 72.2 ± 2.97, $N = 31$, respectively). This relationship between repeat size and subtype may reflect the fact that age at onset, which is correlated with repeat size, is tightly associated with the clinical classification of subtype. Since type 2 may be a transitional form and the group of type 2 patients could include some individuals that will later become either type 1 or 3, the data were reanalyzed using only patients who have continued to have a type 2 disease presentation for ⩾10 years. We obtained the same results as described above.

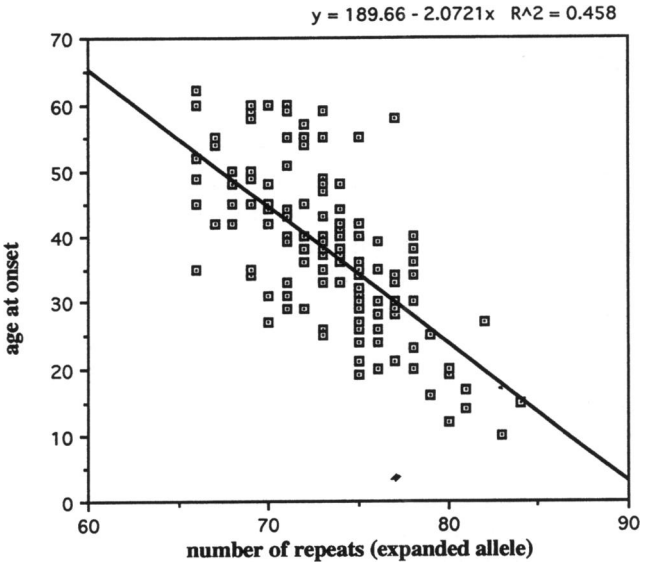

Figure 2 Correlation of CAG repeat length in the MJD chromosomes of 156 affected individuals with age at onset of disease. A squared linear correlation coefficient of $r^2 = .458$ was obtained.

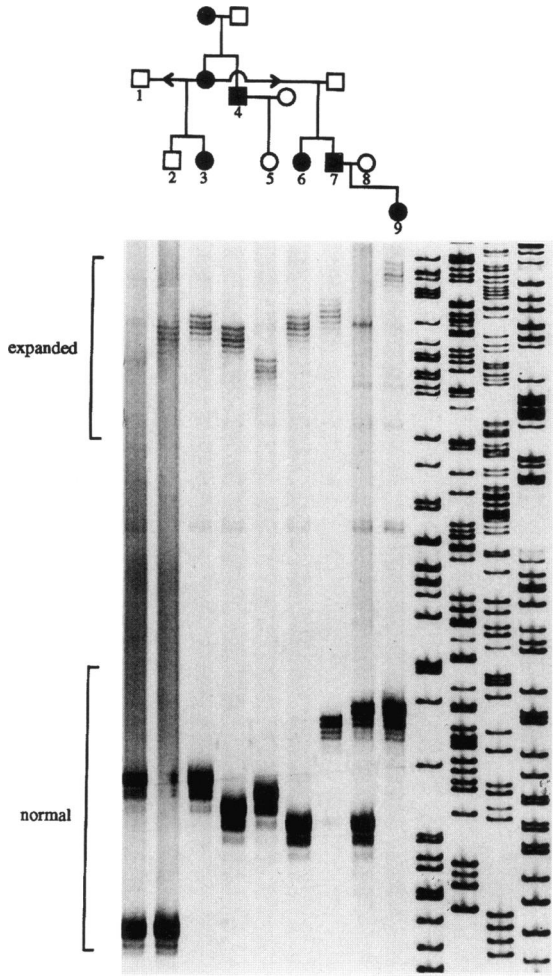

Figure 3 PCR analysis of the CAG repeat in the MJD gene in a branch of family N3. The four tracks to the right contain M13 sequence, which was used to determine the sizes of alleles.

Instability of CAG Repeat Length

Instability of the CAG repeat number during transmission from parent to offspring has been observed for HD, SCA1, spinal bulbular muscular atrophy, and DRPLA. The instability of the CAG repeat size in transmission of the MJD allele is illustrated in figure 3. Expansion, as well as contraction, of repeat tract length occurred in this family.

Within our cohort group, there were 58 individuals who had an expanded allele and for whom data on the expanded allele in the affected parent was available. Of these 58 pairs, 24 of the children were diagnosed as affected and the remaining 34 are currently at risk and presumed to be presymptomatic carriers. Figure 4*A* shows the distribution of variation in repeat number during transmission. In 41 (55%) of these transmissions, there was a variation in the size of the repeat, with 32 (78%) increasing in size and 9 (22%) contracting. The mean variation was +0.84 repeats, with a range from −5 to 9. The mean amplitude of variation (absolute value) was 1.6 repeat units for each transmission. Each variation of absolute value >4 occurred in transmission from unique affected parents, from two families, B1 and N3. These two families contribute 30% and 8.6% of the pairs in this analysis, respectively. Comparing the distribution of variation in repeat size by disease status (fig. 4*B*), we observed that affected individuals tend to have inherited expansions, while the asymptomatic carriers tend to have inherited contractions. This difference in distribution is significant (P = .0002, Fisher's exact test).

The distribution of instability of repeat size by sex of

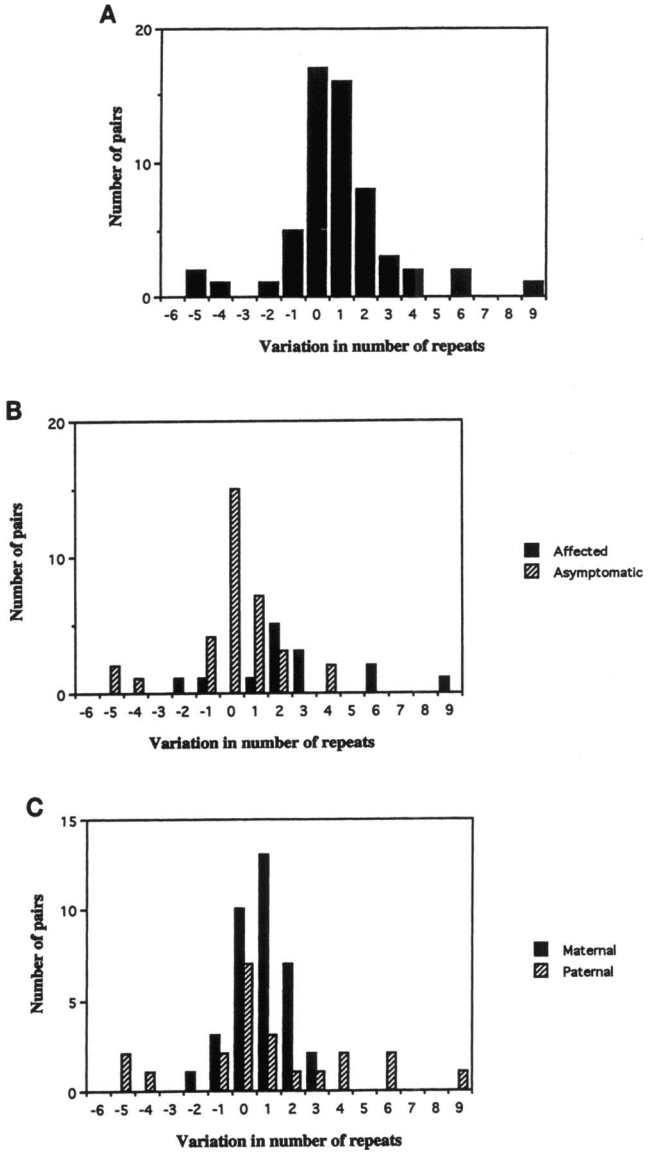

Figure 4 Distribution of the variation in repeat number during transmission of the MJD allele from parent to progeny for 58 parent/child pairs *(A)* for all transmissions, *(B)* for affected and asymptomatic carrier offspring, and *(C)* by sex of parent.

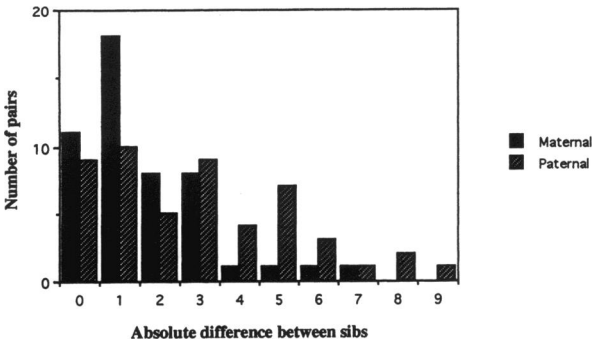

Figure 5 Distribution of absolute difference in size of repeat between sib pairs by sex of transmitting parent.

transmitting parent is shown in figure 4C. The observations at either tail of the distribution are due to alleles inherited from different affected fathers, suggesting that paternally inherited alleles are more volatile than maternal alleles. Although, when the instability data was classified as contractions, stable inheritance, or expansions we observed no significant difference in frequency of each classification for male or female meioses ($P = .393$), the mean amplitude of variation was significantly different between male (2.4 ± 2.6) and female transmissions (1.0 ± 0.86) ($P = .026$). Interestingly, the four juvenile cases for which we typed parent and child corresponded to expansions of the CAG repeat from the transmitting parent, with increases of 9, 6, 5, and 2 repeats.

The number of repeats in the transmitting parent does not correlate with the absolute value of the change in repeat during transmission ($r = -.0013$; $P = .99$). In other words, there is no effect of the initial size of allele on its stability.

Sib pairs provide another opportunity to examine instability of the CAG repeat. In our cohort there were 53 sibships containing two or more individuals carrying the expanded allele (affected and at risk). The majority of sibships consisted of a pair of individuals; however, there was 1 sibship of five, 3 sibships of four, and 13 sibships consisting of three persons with the MJD allele. Figure 5 shows the distribution of the absolute difference between sibs by sex of affected parent. There is a significant difference in mean absolute difference for paternal (2.9 ± 2.4, $N = 51$) and maternal (1.6 ± 1.6, $N = 49$) transmissions ($P = .002$). This supports the evidence presented earlier indicating paternal transmissions may be less stable than maternal transmissions. Among 52 sib pairs in which both sibs were affected, there was a marginal correlation between difference in age at onset between sib pairs and difference in repeat number ($r = .027$; $P = .07$). For nine sib pairs with identical repeat number, difference in age at onset ranged from 0 to 12 years. In contrast, a sib pair with a difference in repeat number of 9 had only a 6-year difference in age at onset.

Anticipation data were available on 22 of the affected parent-child pairs for which molecular data were also available. Anticipation is shown plotted against variation in repeat size, in figure 6. There was no significant correlation between change in repeat number between parent and offspring and anticipation ($r = .10$; $P = .66$).

Discussion

The CAG repeat within the coding region of the MJD gene was found to be highly polymorphic, both in the normal size range (12–37 repeat units) and in the expanded MJD size range (62–84 repeat units). There is a bimodal distribution of the normal alleles, with peaks at 14 and 24 repeats. Frequencies of normal alleles were not significantly different in the control populations we studied—spouses of Azorean, Brazilian, and Portuguese American patients and other controls of multiple ethnic origins (data not shown).

No overlap was found between the ranges of normal and MJD repeat size. Unlike other disorders caused by an expansion of a CAG repeat, there was a wide gap between the normal and MJD allele sizes, and no intermediate alleles were found in our control population. This may be just a matter of sample size, and the study of additional families may later change these figures. However, if this observation is confirmed in a sufficiently large control population, it will greatly facilitate presymptomatic testing for MJD, since the existence of overlap between the normal and disease size ranges was one of the difficulties encountered in HD (Andrew et al. 1993; Snell et al. 1993) and SCA1 (Goldfarb et al. 1994), although in SCA1 the distinction between normal (interrupted CAG repeat) and the disease (pure CAG repeat) intermediate size alleles can be made using a

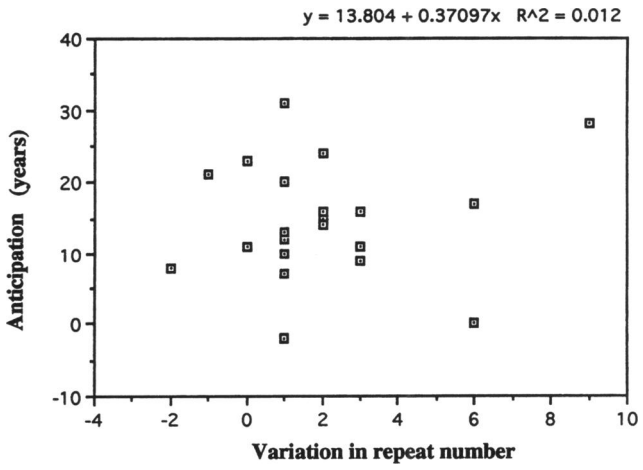

Figure 6 Correlation between variation in size of repeat and anticipation for 23 affected parent-child pairs. A correlation coefficient of $r = .045$ ($P = .83$) was found.

restriction polymorphism (Chung et al. 1993). The clustering of expanded repeat sizes is also suggestive of a unique ancient founder mutation rather than multiple expansions from the intermediate "premutation" size range (Barceló et al. 1993; Goldberg et al. 1993; Rubisztein et al. 1994). This question can be addressed by detailed haplotype analysis. The mean variation in size of +0.84 repeats on transmission with each generation suggests that, although both contractions and expansions of variable magnitude occur, the general tendency is toward a slow increase in expanded repeat size in the population.

The mild instability of the expanded CAG repeat provides a molecular basis to explain this disease's great variability in age at onset and clinical presentation. The highly significant correlation found in this study between expanded repeat size in the MJD gene with age at onset supports the influence of the CAG tract length in the determination of the disease presentation and progression. The repeat size accounts for ~50% of the variation in the age at onset (r^2 = .485); other factors—genetic, environmental, or both—therefore influence the natural history of the disorder. Similar patterns have been reported for other diseases caused by the same mutational mechanism (La Spada et al. 1992; Andrew et al. 1993; Duyao et al. 1993; Snell et al. 1993; Jodice et al. 1994; Ranum et al. 1994). However, the exact contribution of the repeat size to the of age at onset may not be precise, since several difficulties may affect the correlation between repeat size and age at onset. First, the age at onset of disease is never a precise figure, because the information given by the patients or their relatives may be inaccurate; namely, awareness of the disease in the family facilitates detection of first symptoms, creating a bias toward earlier onset in younger generations. On the other hand, the determination of the exact repeat size may also be inaccurate, because of the presence of multiple bands in the expanded allele, as seen in figure 4. Finally, the size of the expanded CAG repeat in lymphocytes can be different from the size in cells of involved structures that would be the actual determinants of the development of disease. This type of mosaicism has been described for HD, DRPLA, and SCA1 (Aoki et al. 1994; Chong et al. 1994; Telenius et al. 1994). Further studies are necessary to determine whether the phenomenon of somatic mosaicism occurs in MJD and whether repeat size in cells other than lymphocytes correlates more closely with age at onset of the disease.

The observed correlation between disease subphenotypes and repeat expansion is not surprising. Our results of this analysis and of juvenile onset cases suggest that the greater CAG expansions result in a more severe phenotype, which is qualitatively different from latter onset forms. This may represent cell-specific variable susceptibility, perhaps age dependent, of the target structures in the CNS.

The distribution of expanded alleles in the MJD population appears symmetric. This is in contrast to the skewed distribution observed for the expanded allele in HD and SCA1. In both HD and SCA1, the largest alleles are associated with paternal transmission. In HD, severe instability of the allele during selected paternal transmission with unusually large expansions of up to 41 repeat units may explain the skewed distribution. Although our studies of variation in repeat size between both parent-offspring and sib pairs suggest increased volatility of paternal transmissions, large expansions have not been observed in MJD; the largest observed expansion was 9 repeats. This suggests that the mechanism of expansion may be different for these two diseases or may indicate the existence of genetic or environmental factors influencing some paternal transmissions in HD but not in MJD. Length of uninterrupted CAG repeat sequence is one factor that has been demonstrated to affect stability of the allele during transmission in fragile X syndrome (Eichler et al. 1994) and SCA1 (Chung et al. 1993). For SCA1, a case of an affected individual who had an apparently "normal" size allele consisting of an uninterrupted repeat has been reported (Goldfarb et al. 1994). It is known that a polymorphism CAG/CAA exists within the CAG repeat tract in the MJD gene (Kawaguchi et al. 1994). Further studies are required to determine whether this interruption of the CAG tract has an effect on stability during transmission and on clinical presentation of disease.

In contrast with myotonic dystrophy (Harley et al. 1993; Lavedan et al. 1993), there is no increased instability in the trinucleotide repeat with larger size of repeat in the transmitting parent. This suggests a different mechanism may be involved in instability during transmission for these two diseases.

The absence of association between variation in repeat number and anticipation, a finding also observed in HD (Andrew et al. 1993), remains unexplained. For MJD and other late onset trinucleotide diseases, it is important to consider that the data used to examine the relationship between anticipation and change in repeat size are subject to ascertainment bias. In retrospective studies like ours, one is likely to have large anticipation (i.e., the age at onset of the children will be much earlier than that of the parents) in order to obtain blood from affected offspring while the affected parent is still alive. In our analysis, mean age at onset in children of the 23 pairs was significantly lower than the age at onset of the entire population of affected individuals. Ascertainment bias is also suggested by comparison of variation in allele size during transmission between the affected and presymptomatic offspring, as shown in figure 4*B*. A correlation between anticipation and variation in allele size may exist but be masked by the nonrandom selection of the data used in the analysis.

In conclusion, our results validate the use of a direct

analysis of the CAG expansion in the MJD gene in the diagnosis of this disease. The significant correlation between CAG expansion size and disease severity/age at onset may also be of clinical value, though further studies will be required to clarify other genetic or environmental factors involved in the determination of the clinical presentation in MJD patients.

Acknowledgments

We would like to thank the families for their willingness to participate in this study, all the clinicians in the International Joseph Disease Foundation who provided clinical information for the study, Drs. B. White and J. Holden for their assistance. This project is supported by grants from the National Institutes of Health (NS 31687), the Fonds de Recherche en Santé du Québec, the Network of Centres of Excellence (Canadian Genetic Diseases Network) and the European Community Program STRIDE. P.M., C.G., and I.S. are recipients of a scholarship by the Junta Nacional de Investigaçã Científica e Tecnológica, Portugal.

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