

# Molecular characteristics of Machado-Joseph disease mutation in 25 newly described Brazilian families

Isclia Lopes-Cendes<sup>1</sup>\*, Hélio G.A. Teive<sup>2</sup>, Francisco Cardoso<sup>3</sup>, Erika M. Viana<sup>3</sup>,  
Maria E. Calcagnotto<sup>4</sup>, Jaderson C. da Costa<sup>4</sup>, Paulo C. Trevisol-Bittencourt<sup>5</sup>,  
Jayme A. Maciel<sup>6</sup>, Marylene Rousseau<sup>1</sup>, André S. Santos<sup>7</sup>,  
Abelardo Q.C. Araújo<sup>8</sup> and G.A. Rouleau<sup>1</sup>

## ABSTRACT

Machado-Joseph disease (MJD) is a form of autosomal dominant spinocerebellar ataxia first described in North-American patients originating from the Portuguese islands of the Azores. Clinically this disorder is characterized by late onset progressive ataxia with associated features, such as: ophthalmoplegia, pyramidal and extrapyramidal signs and distal muscular atrophies. The causative mutation is an expansion of a CAG repeat in the coding region of the *MJD1* gene.

We have identified 25 unrelated families segregating the *MJD* mutation during a large collaborative study of spinocerebellar ataxias in Brazil. In the present study a total of 62 family members were genotyped for the CAG repeat in the *MJD1* gene, as well as 63 non-MJD individuals (126 normal chromosomes), used as normal controls. We observed a wide gap between the size range of the normal and expanded CAG repeats: the normal allele had from 12 to 33 CAGs (mean = 23 CAGs), whereas the expanded alleles ranged from 66 to 78 CAGs (mean = 71.5 CAGs). There were no differences in CAG tract length according to gender of affected individuals or transmitting parent. We observed a significant negative correlation between age at onset of the disease and length of the CAG tract in the expanded allele ( $r = -0.6$ ,  $P = 0.00006$ ); however, the size of the expanded CAG repeat could explain only about 40% of the variability in age at onset ( $r^2 = 0.4$ ).

There was instability of the expanded CAG tract during transmission from parent to offspring, both expansions and contractions were observed; however, there was an overall tendency for expansion, with a mean increase of +2.4 CAGs. The tendency for expansion appeared to be greater in paternal (mean increase of +3.5 CAGs) than in maternal transmissions (mean increase of +1.3 CAGs). Anticipation was observed in all transmissions in which ages at onset for parent and offspring were known; however, anticipation was not always associated with an increase in the expanded CAG repeat length. Our results indicate that the molecular diagnosis of MJD can be confirmed or excluded in all suspected individuals, since alleles of intermediary size were not observed.

## INTRODUCTION

Machado-Joseph disease (MJD) is an adult-onset neurodegenerative disorder with autosomal

<sup>1</sup> Centre for Research in Neuroscience and the Montreal General Hospital Research Institute, McGill University, Montreal, Quebec, Canada. E-mail: isclia@turing.unicamp.br. Send correspondence to I.L.-C. \*Present address: Departamento de Genética Médica, Faculdade de Ciências Médicas (FCM), Universidade Estadual de Campinas (UNICAMP), Cidade Universitária Zeferino Vaz, Caixa Postal 6111, Distrito de Barão Geraldo, 13083-970 Campinas, SP, Brasil. Tel. (019) 788-8210, Fax (019) 239-3114.

<sup>2</sup> Serviço de Neurologia, Universidade Federal do Paraná, Curitiba, PR, Brasil.

<sup>3</sup> Departamento de Neurologia, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brasil.

<sup>4</sup> Departamento de Neurologia, Hospital Universitário São Lucas, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, RS, Brasil.

<sup>5</sup> Serviço de Neurologia, Hospital Universitário da Universidade Federal de Santa Catarina, Florianópolis, SC, Brasil.

<sup>6</sup> Departamento de Neurologia, Faculdade de Ciências Médicas, Universidade Estadual de Campinas, Campinas, SP, Brasil.

<sup>7</sup> Hospital de Caridade, Florianópolis, SC, Brasil.

<sup>8</sup> Departamento de Neurologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brasil.

dominant inheritance. It was first reported in North-American families of Portuguese-Azorean descent (Nakano *et al.*, 1972; Rosenberg *et al.*, 1976; Romanul *et al.*, 1977; Coutinho and Andrade, 1978), but it is now shown to occur in patients from many different ethnic backgrounds (Cancel *et al.*, 1995; Ranum *et al.*, 1995; Schöls *et al.*, 1995; Brunt *et al.*, 1996; Burt *et al.*, 1996; Lopes-Cendes *et al.*, 1996a; Silveira *et al.*, 1996; Zhou *et al.*, 1997). Clinically, MJD is characterized by progressive cerebellar ataxia, with associated features such as external ophthalmoplegia, pyramidal signs, dystonic postures, distal muscular atrophies, eyelid retraction, faciolingual fasciculations and no mental impairment (Coutinho, 1994). The different clinical expressions of MJD resulted in its classification into three clinical subtypes (Lima and Coutinho, 1980): type 1 shows an early onset, a rapid course, and patients have marked pyramidal and extrapyramidal signs in addition to the common features of ataxia and ophthalmoplegia; type 2, the most common subtype, is a more benign form with clinical manifestations limited to cerebellar ataxia, pyramidal signs and ophthalmoplegia, and type 3 shows a late onset and a very slow progression of the disease, which is characterized mainly by peripheral signs in addition to the features of type 2.

The MJD locus, also known as SCA3, was first mapped to chromosome (ch) 14q in Japanese pedigrees (Takiyama *et al.*, 1993) and subsequently found to assign to the same chromosomal region in Portuguese (Sequeiros *et al.*, 1994), Brazilian (Twist *et al.*, 1995) and French families (Stevanin *et al.*, 1994). Recently, the expansion of a trinucleotide CAG repeat located in the coding region of the MJD1 gene was identified as the causative mutation (Kawaguchi *et al.*, 1994). Expansions of CAG repeats have been associated with several neurodegenerative disorders, including Huntington disease, Kennedy disease, and five types of autosomal dominant spinocerebellar ataxia (SCA), including MJD (Sutherland and Richards, 1995; Zoghbi, 1996; Zhuchenko *et al.*, 1997). In all of these disorders affected individuals carry abnormally expanded and unstable CAG tracts in the coding region of the disease genes.

We have studied 62 individuals belonging to 25 unrelated Brazilian families segregating the MJD mutation in order to: a) compare the size range of the CAG repeat in the MJD and control populations, b) investigate the relationship between the size of the expanded CAG allele and age at onset of the disease, and c) analyze 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

This study was performed using DNA samples from 62 family members, including 49 clinically affected individuals, belonging to 25 unrelated families. Families were ascertained in six states from the southern and southeastern regions of Brazil: Rio Grande do Sul, Paraná, Santa Catarina, São Paulo, Minas Gerais and Bahia. The clinical features of one of these families were reported previously (Teive *et al.*, 1991). The 25 families were identified as segregating the MJD mutation during a large collaborative project involving the screening for mutations in Brazilian families with different types of SCA (Lopes-Cendes *et al.*, in press). Of the 25 families, 22 were of Portuguese background, two were of Italian origin and one had a mixed Portuguese and African ancestry. None of the families could trace their ancestors back to the Portuguese Islands of the Azores. 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 we used 37 normal chs identified in the MJD families, as well as 126 normal chs identified in 63 non-MJD Brazilian individuals.

## METHODS

Genomic DNA was isolated from peripheral lymphocytes by standard methods (Sambrook *et al.*, 1989). The CAG-containing fragment of the MJD gene was amplified by PCR using primers MJD52 and MJD25 (Kawaguchi *et al.*, 1994). PCR was performed in a final volume of 12.5  $\mu$ l, containing 100 ng of genomic DNA, 10 mM Tris-HCl (pH 8.8), 1.5 mM MgCl<sub>2</sub>, 50 mM KCl, 2% formamide, 250  $\mu$ M each dCTP, dGTP and dTTP, 25  $\mu$ M ATP, 1.5  $\mu$ Ci <sup>35</sup>S  $\alpha$ -dATP, 100 ng of each primer, 1 unit 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 (AS), PCR products were analyzed on 6% polyacrylamide gels in parallel with an M13 sequencing ladder (-40 primer) and were visualized by autoradiography. Allele sizes were determined by comparison with the M13 sequence and were converted into CAG unit number (N), using the formula  $N = (AS - 121) / 3$ , assuming that the variation in size of the PCR product occurred within the repetitive CAG stretch (Maciel *et al.*, 1995).

Linear regression techniques were used to determine the association between repeat number in the MJD or normal alleles and age at onset. Differences in

size of the CAG repeat among groups were assessed by analysis of variance and *post hoc* tests (Tukey HSD).

## RESULTS

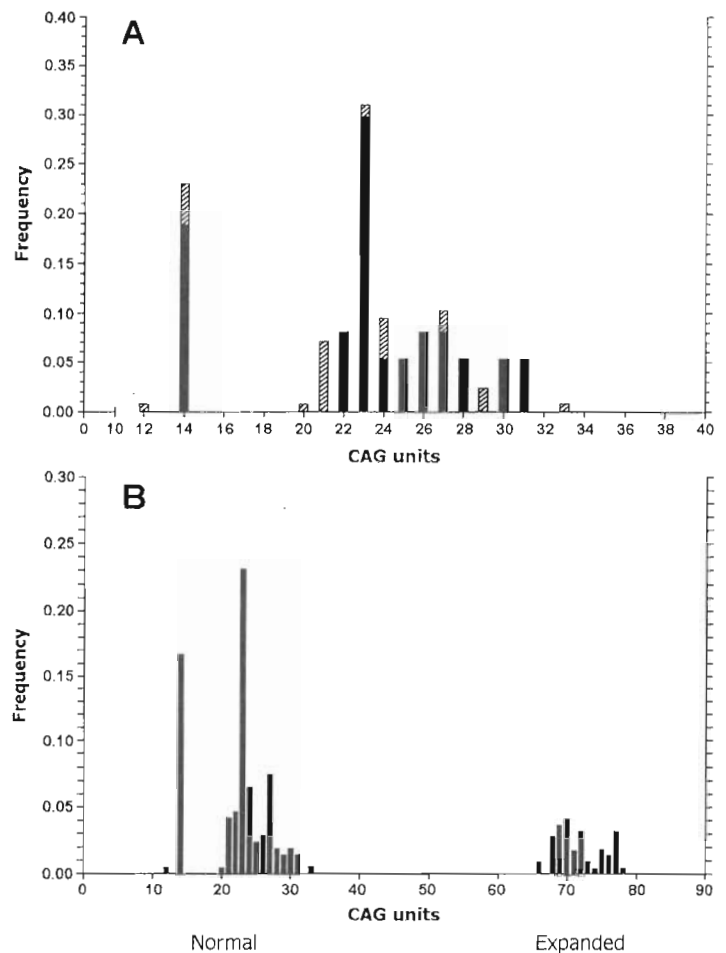
We identified 37 normal chs containing alleles from 14 to 31 CAGs (mean = 23 CAGs) in the 25 MJD families. Among the 126 normal chs identified in 63 unrelated non-MJD individuals we found CAG alleles ranging from 12 to 33 CAGs (mean = 23 CAGs). Most individuals (91.3%) from the control group were heterozygous for the normal CAG repeat. No significant difference was found in the distribution of the normal alleles in MJD and non-MJD families ( $P = 0.2$ , Figure 1A).

We found expanded alleles in 49 patients, as well as in five individuals at risk of developing the disease. The size of the expanded alleles varied from 66 to 78 CAGs (mean = 71.5 CAGs) (Figure 1B). There were no significant differences in the size of the expanded alleles inherited paternally vs. maternally (73 CAGs for paternal and 71 CAGs for maternal transmission,  $P = 0.3$ ), as well as between affected males and females (71 CAGs for male and 73 CAGs for female patients,  $P = 0.3$ ).

Age at onset was known for 34 of the 49 affected individuals studied. It varied from 15 to 53 years (mean = 34 years). There were no differences between mean age at onset due to gender of transmitting parent (30 years for paternal and 35 years for maternal transmission,  $P = 0.6$ ) or between affected males and females (35 years for males and 34 years for females,  $P = 0.9$ ).

The relationship between the CAG repeat length in the MJD allele of affected individuals and age at onset of the disease is shown in Figure 2A. A correlation coefficient of -0.6 was obtained ( $r^2 = 0.4$ ,  $P = 0.00006$ ), assuming a linear relationship between age at onset and repeat length in MJD chs. This indicates a tendency for age at onset to decrease as the CAG repeat length increases. No significant correlation was found between the normal CAG allele and age at onset ( $r = -0.05$ ,  $r^2 = 0.003$ ,  $P = 0.8$ ) as shown in Figure 2B.

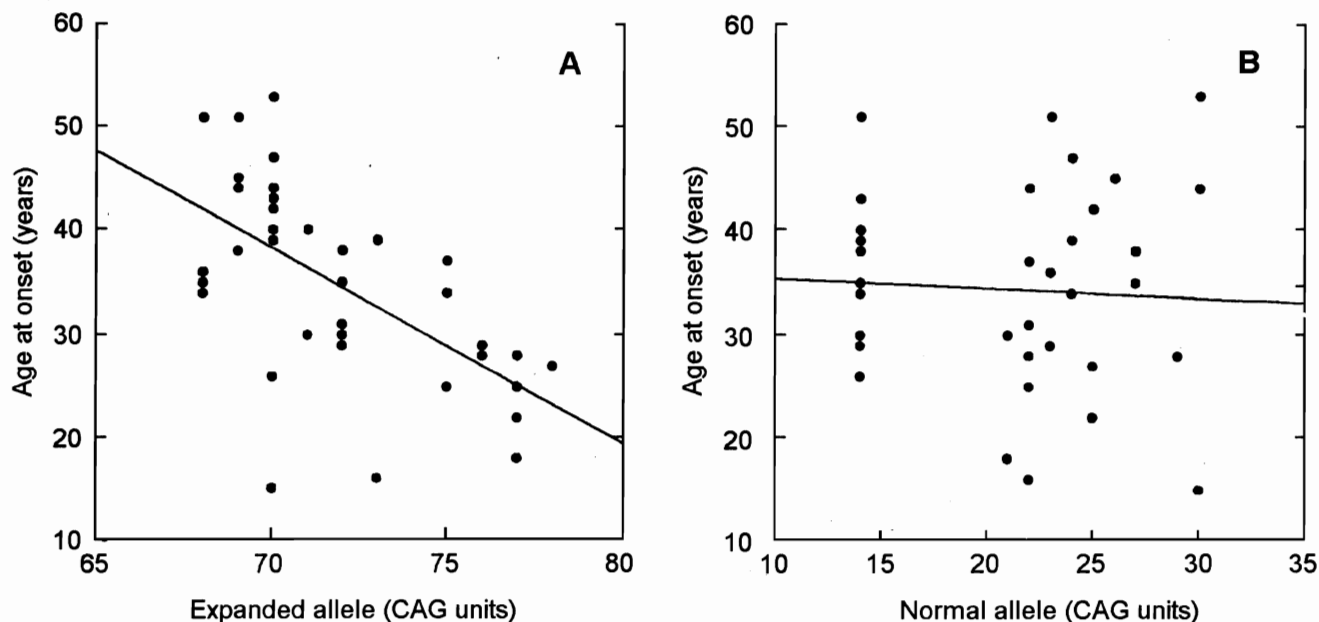
We observed instability of the expanded CAG repeat during transmission from parent to offspring. Expansions as well as contractions of the expanded CAG repeat length occurred in our families (Figure 3). We documented eight transmissions of the expanded CAG allele, four maternal and four paternal. There was an overall tendency for increase in the expanded CAG repeat length, with a mean increase of +2.4 CAGs. This tendency was greater for paternal vs. maternal trans-



**Figure 1** - Frequency distribution of CAG repeat sizes in controls and MJD individuals. **A)** Distribution of normal alleles; black bars show the distribution for 37 normal alleles found in 25 unrelated MJD families, hatched bars show the distribution for 126 normal alleles found in 63 unrelated non-MJD individuals. There was no significant difference in the distribution of the normal alleles in the two groups ( $P = 0.2$ ). **B)** Overall distribution of 163 normal CAG alleles and 54 expanded alleles found in 25 unrelated Brazilian MJD families.

missions (mean increase of +3.5 CAGs in paternal and +1.3 CAGs in maternal transmissions); however, this difference was not statistically significant ( $P = 0.5$ ). Expansions of the CAG tract occurred 3/4 times in paternal and 1/2 times in maternal transmissions. In four transmissions, ages at onset for parent and offspring were known (two paternal and two maternal), and anticipation occurred in all four instances; however, an increase in expanded CAG repeat length was observed in only three of these transmissions (Table I). Normal CAG alleles were always stable during transmission (Figure 3).

Normal and expanded alleles had a different appearance in the autoradiographs. The normal alleles had a single strong band distinctively seen in the X-ray films, whereas the expanded alleles showed several bands, suggesting the presence of cells with different lengths of the expanded CAG tract (Figure 3).



**Figure 2** - A) Correlation of CAG repeat length in the *MJD* chromosomes of 34 affected individuals with age at onset of the disease (Pearson correlation coefficient ( $r$ ) = -0.6,  $r^2$  = 0.4,  $P$  = 0.00006). B) Absence of correlation between age at onset and CAG repeat length in the normal chromosomes of 34 affected individuals ( $r$  = -0.05,  $r^2$  = 0.003 and  $P$  = 0.8).

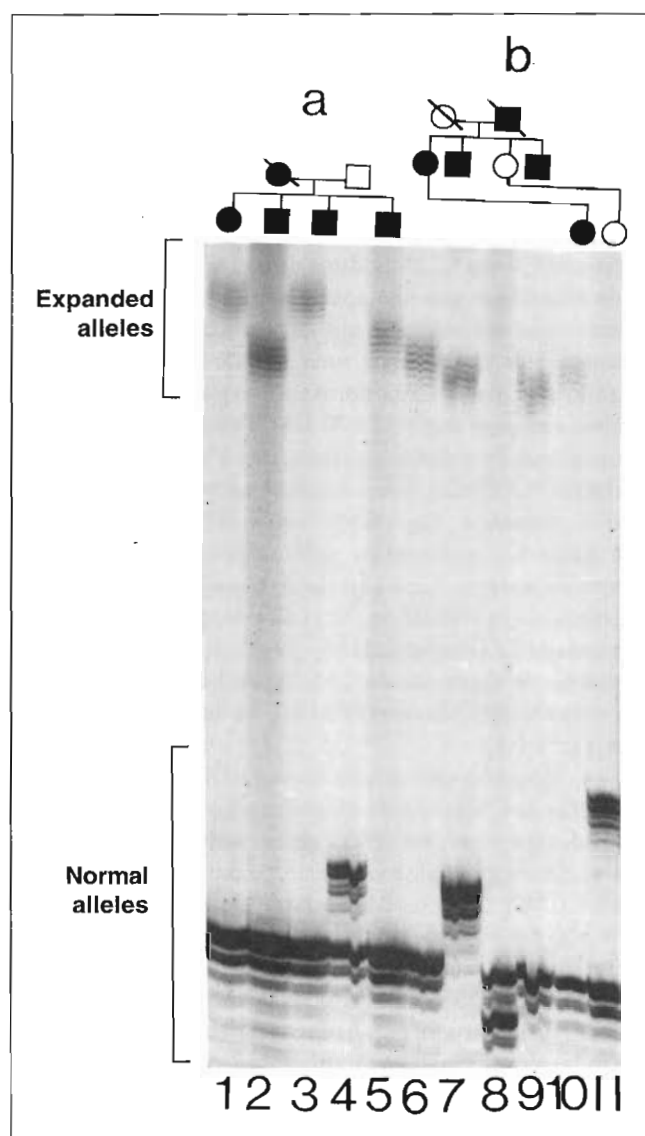
## DISCUSSION

Over the past six years a new class of mutations, dynamic mutations, has been identified (Richards and Sutherland, 1992; Sutherland and Richards, 1995). These mutations are characterized by expansions of different types of unstable trinucleotide repeats. They have been divided into three main classes according to the type of trinucleotide involved, their location within the disease gene and their possible mechanism of action in determining disease (Strachan and Read, 1996): a) large scale expansions of very unstable (CGG) $_n$  which are associated with fragile sites, as in the fragile X syndromes (FRAXA and FRAXE). In these syndromes it is likely that the massive expansion of the CGG repeat causes loss of function by disrupting nearby genes (Richards and Sutherland, 1992); b) large expansions of CTG repeats located in the 3'untranslated region of a protein kinase gene (*DMPK*), causing myotonic dystrophy (MD). The molecular pathology of MD is not known; however, no other type of mutation has been found in MD patients, suggesting a specific gain of function caused by the expansion of the CTG repeat (Caskey et al., 1992) and c) moderately expanded CAG

repeats within coding regions, encoding expanded polyglutamine tracts at the protein level. The exact mechanism by which expansions of CAG repeats cause disease is not understood; however, evidence points to a mechanism involving gain of function (Ross, 1995). To date, expansions of trinucleotide CAG repeats have been found in neurodegenerative disorders such as Huntington disease (Huntington's Disease Collaborative Research Group, 1993), Kennedy disease (LaSpada et al., 1991) and five types of autosomal dominant SCA: spinocerebellar ataxia type 1 (SCA1) (Orr et al., 1993), spinocerebellar ataxia type 2 (SCA2) (Imbert et al., 1996; Pulst et al., 1996; Sampei et al., 1996), Machado-Joseph disease or spinocerebellar ataxia type 3 (MJD/SCA3) (Kawaguchi et al., 1994), spinocerebellar ataxia type 6 (SCA6) (Zhuchenko et al., 1997) and a related disorder dentatorubropallidoluysian atrophy (DRPLA) that can be present as SCA in late onset cases (Koide et al., 1994; Nagafuchi et al., 1994). More recently, a GAA expansion in the first intron of a novel gene ( $\chi 25$ ) was identified as the causative mutation in 94% of Friedreich ataxia (FA) patients (Campuzano et al., 1996). Point mutations have also been identified in a few FA patients, suggesting that the disease is probably caused by loss of function.

**Table I** - Relationship between instability of the expanded CAG repeat in the *MJD1* gene and anticipation during transmission from parent to offspring.

	Transmission 1 (paternal)	Transmission 2 (paternal)	Transmission 3 (maternal)	Transmission 4 (maternal)
Anticipation	29 years	29 years	13 years	5 years
Variation of the CAG tract	+7 CAG units	+4 CAG units	+8 CAG units	-3 CAG units



**Figure 3** - Pattern of segregation for the normal and expanded CAG alleles in two MJD sibships. *Top panel (a and b)*: Pedigrees of two small MJD sibships. Black symbols represent clinically affected individuals and diagonal lines indicate deceased individuals. *Bottom panel*: Analysis of PCR products containing normal and expanded CAG repeats at the *MJD1* gene. Genomic DNA, extracted from peripheral blood leukocytes, was amplified using primers *MJD52* and *MJD25* (Kawaguchi *et al.*, 1994). PCR products were analyzed on 6% polyacrylamide gels. Normal alleles had sizes varying from 21 to 31 CAG units and expanded alleles varied from 68 to 78 CAGs. In pedigree **a**, variability in size of the expanded CAG allele can be seen. The same expanded allele, which was transmitted from the deceased mother, had different lengths in the offspring (*lane 1*: 77 CAGs, *lane 2*: 68 CAGs, *lane 3*: 78 CAGs and *lane 5*: 72 CAGs); whereas Mendelian segregation can be observed for the normal allele, the offspring inherited the normal allele with 23 CAGs from the unaffected father (*lane 4*: normal alleles = 23 and 27 CAGs). In pedigree **b**, differences in the length of the expanded allele in the same sibship are also seen (*lane 6*: 71 CAGs, *lane 7*: 68 CAGs and *lane 9*: 68 CAGs). A maternal transmission showing a contraction of the expanded allele can be observed (*lane 6*: 71 CAGs to *lane 10*: 70 CAGs). By contrast, there is a stable segregation of the normal allele (23 CAGs) from the unaffected female in *lane 8* (normal alleles = 21 and 23 CAGs) to her unaffected daughter in *lane 11* (normal alleles = 23 and 31 CAGs).

We (Silveira *et al.*, 1996) and others (Ranum *et al.*, 1995; Schöls *et al.*, 1995) have established that the *MJD* mutation is the most frequent cause of autosomal dominant SCA worldwide, including the Brazilian SCA patients (Lopes-Cendes *et al.*, in press). In Brazilian patients, as in other ethnic groups (Cancel *et al.*, 1995; Maciel *et al.*, 1995; Schöls *et al.*, 1995) there is a wide gap between the normal and expanded allele sizes, thus allowing a clear differentiation between normal and disease alleles, making it possible to confirm or exclude the molecular diagnosis of MJD in small families or in single affected individuals. In addition, the high heterozygosity observed for the normal CAG allele in the Brazilian population (91.3%) reduces the probability of finding individuals that are homozygous for the normal CAG allele. Individuals homozygous for the CAG allele in the normal range may be difficult to differentiate from failure to amplify an expanded allele during the PCR testing.

We have shown that there is a significant inverse correlation between the length of the expanded CAG allele and age at onset of the disease; however, only part of the variability in age at onset can be explained by the length of the expanded CAG tract (about 40%). Thus, the majority of the variation in age at onset is determined by other, still unidentified, factors. Therefore, a prediction of age at onset of the disease based on the length of the expanded CAG allele is not accurate. In our series, individuals with an expanded allele of 72 CAGs (the average size found in our study) had ages at onset varying from 29 to 38 years.

Anticipation is defined as a progressive earlier onset of a disease in successive generations, usually associated with increased severity (Bell, 1942; Penrose, 1948). Anticipation has been observed in most disorders associated with dynamic mutations (Caskey *et al.*, 1992; Richards and Sutherland, 1992). However, in the CAG repeat disorders the effect of anticipation is less marked, possibly due to the mild instability of the expanded allele observed in these disorders (Ross, 1995; Zoghbi, 1996). Anticipation occurred in all transmissions that were documented in this study; nonetheless, this was associated with both increases and decreases in the size of the expanded CAG repeat. Many factors, such as an increased awareness of the disease in the family, and difficulty in recalling age at onset in the older generation may introduce bias in the evaluation of anticipation (Penrose, 1948). We observed an overall tendency for further increase in size of the expanded CAG repeat over successive generations (+2.4 CAGs); however, anticipation did not always correlate with expansions in individual transmissions. The greater overall increase in size of the expanded repeat in males (+3.5

CAGs) than in females (+1.3 CAGs) was observed previously in MJD (Maciel *et al.*, 1995) and in other CAG related disorders such as Huntington disease (Andrew *et al.*, 1993) and SCA1 (Chung *et al.*, 1993).

The multiple bands shown by the expanded alleles in the autoradiographs (Figure 3) suggest the presence of somatic mosaicism, with different cells containing different lengths of the expanded CAG allele. Somatic mosaicism of the expanded CAG repeat has been described in MJD, but no relationship was found between the size of the expanded CAG repeat in different parts of the central nervous system and site of neurodegeneration (Lopes-Cendes *et al.*, 1996b).

In conclusion, although some of the parameters examined here have been previously studied in other populations (Cancel *et al.*, 1995; Maciel *et al.*, 1995; Schöls *et al.*, 1995), and molecular studies were conducted in Brazilian MJD patients (Stevanin *et al.*, 1995; Iughetti *et al.*, 1996), we believe that the present study provides further information that can be used to guide the molecular testing of Brazilian MJD patients, as well as the genetic counseling of patients and family members. The molecular diagnosis, based on the determination of the length of the CAG repeat in the *MJD1* gene, is highly sensitive and specific. However, the length of the expanded CAG allele should not be used to predict age at onset or severity of clinical presentation, since genetic and/or environmental factors, yet to be identified, are responsible for most of the clinical variability seen in these patients.

## ACKNOWLEDGMENTS

The authors would like to thank the family members who participated in this study. This work was supported by: the joint Program FRSQ-ACAF (Fonds de la Recherche en Santé du Québec and Association Canadienne de l'Ataxie de Friedreich), the NIH (grant NS 31687) and the Network of Centres of Excellence (Canadian Genetic Disease Network). G.A.R. is supported by the Medical Research Council of Canada and the Fonds de la Recherche en Santé du Québec.

Publication supported by FAPESP.

## RESUMO

A doença de Machado-Joseph (MJD) é uma forma de ataxia espinocerebelar (AEC) de herança autossômica dominante, que foi descrita inicialmente em pacientes norte-americanos provenientes das ilhas portuguesas dos Açores. Clinicamente essa doença é caracterizada por uma ataxia cerebelar progressiva, de início tardio e com algumas características associadas tais como: oftalmoplegia, sinais pirami-

dais e extrapiramidais e amiotrofias. A mutação responsável é uma expansão de trinucleotídeos CAG localizada na região codificadora do gene *MJD1*.

Como parte de um estudo colaborativo sobre AEC no Brasil, nós identificamos 25 famílias, não aparentadas, segregando a mutação *MJD*. Nesse artigo nós relatamos as características moleculares do trinucleotídeo CAG presente no gene *MJD1* em 62 indivíduos dessas famílias com MJD e em 63 indivíduos que não apresentam a mutação *MJD* (126 cromossomos normais) e que foram considerados como controles. Nós observamos uma grande diferença entre o tamanho dos alelos CAG normais e expandidos. Os alelos normais variaram entre 12 e 33 CAGs (média de 23 CAGs), enquanto que os alelos expandidos tiveram de 66 a 78 CAGs (média de 71.5 CAGs). Não encontramos nenhuma diferença entre o tamanho dos alelos expandidos em pacientes masculinos e femininos ou entre alelos transmitidos via paterna ou materna. Uma correlação negativa significativa foi observada entre a idade de início da doença e o tamanho do segmento de CAG expandido ( $r = -0,6$ ,  $P = 0,00006$ ); no entanto o tamanho do segmento de CAG expandido foi responsável por somente 40% da variabilidade na idade de início da doença ( $r^2 = 0,4$ ).

Nós observamos também instabilidade do segmento expandido de CAG durante a transmissão de pais para filhos. Expansões e contrações foram observadas; contudo, houve uma tendência geral para expansão, com um aumento médio de +2,4 CAGs. Essa tendência para expansão pareceu ser maior nas transmissões paternas (aumento médio de +3,5 CAGs) que nas maternas (aumento médio de +1,3 CAGs). Antecipação foi observada em todas as transmissões nas quais as idades de início para pais e filhos eram conhecidas, porém, nem todas as antecipações foram acompanhadas de aumento no tamanho do segmento de CAG expandido.

Em conclusão, os nossos resultados mostram que o diagnóstico molecular da mutação responsável pela MJD pode ser estabelecido em todos os indivíduos que apresentam a doença, bem como excluído naqueles portadores de outras formas de AEC, já que não encontramos alelos de tamanho intermediário que poderiam dificultar a interpretação do teste molecular. Porém, o tamanho do segmento de CAG expandido não deve ser usado para prever a idade de início da doença, já que apenas 40% da variabilidade na idade de início pode ser explicada pelo tamanho do segmento de CAG expandido.

## REFERENCES

- Andrew, S.E., Goldberg, P.Y., Kremer, B., Telenius, H., Theilmann, J., Adam, S., Starr, E., Squitieri, F., Lin, B., Kalchman, M.A., Graham, R.K. and Hayden, M.R. (1993). The relationship between trinucleotide (CAG) repeat length and clinical features of Huntington's disease. *Nat. Genet.* 4: 398-403.
- Bell, J. (1942). On the age at death in hereditary muscular dystrophy with some observations bearing on the question of antedating. *Ann. Eugen.* 11: 272-289.

- Brunt, E.R.P., Verschuuren, C.C., Mensink, A.J., Stolte, I. and Scheffer, H. (1996). CAG repeat extension correlates with age at onset but does not explain anticipation in Dutch SCA3/MJD family. *Neurology* 46: 197 (Abstract).
- Burt, T., Currie, B., Kilburn, C., Lethlean, A.K., Dempsey, K., Blair, I., Cohen, A. and Nicholson, G. (1996). Machado-Joseph disease in east Arnhem Land, Australia: chromosome 14q32.1 expanded repeat confirmed in four families. *Neurology* 46: 1118-1122.
- Campuzano, V., Montermini, L., Moltò, M.D., Pianese, L., Cossée, M., Cavalcanti, F., Monros, E., Rodius, F., Duclos, F., Monticelli, A., Zara, F., Cañizares, J., Koutnikova, H., Bidichandani, S.I., Gellera, C., Brice, A., Trouillas, P., de Michele, G., Filla, A., De Frutos, R., Palau, F., Patel, P.I., Di Donato, S., Mandel, J.-L., Cocozza, S., Koenig, M. and Pandolfo, M. (1996). Friedreich's ataxia: autosomal recessive disease caused by an intronic GAA triplet repeat expansion. *Science* 271: 1423-1427.
- Cancel, G., Abbas, N., Stevanin, G., Durr, A., Chneiweiss, H., Néri, C., Duyckaerts, C., Penet, C., Cann, H.M., Agid, Y. and Brice, A. (1995). Marked phenotypic heterogeneity associated with expansion of a CAG repeat sequence at the spinocerebellar ataxia 3/Machado-Joseph disease locus. *Am. J. Hum. Genet.* 57: 809-816.
- Caskey, T.C., Pizzuti, A., Fu, Y.-H., Fenwick Jr., R.G. and Nelson, D.-L. (1992). Triplet repeat mutations in Human disease. *Science* 256: 784-788.
- Chung, M.Y., Ranum, L.P.W., Duvick, L.A., Servadio, A., Zoghbi, H.Y. and Orr, H.T. (1993). Evidence for a mechanism predisposing to intergenerational CAG repeat instability in spinocerebellar ataxia type 1. *Nat. Genet.* 5: 254-258.
- Coutinho, P. (1994). *Doença de Machado-Joseph: Estudo Clínico, Patológico e Epidemiológico de uma Doença Neurológica de Origem Portuguesa*. Grande Prémio BIAL de Medicina 1992. Tipografia Nunes Ltda., Porto, Portugal.
- Coutinho, P. and Andrade, C. (1978). Autosomal dominant system degeneration in Portuguese families of the Azores Islands. *Neurology* 28: 703-709.
- Huntington's Disease Collaborative Research Group, The. (1993). A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. *Cell* 72: 971-983.
- Imbert, G., Saudou, F., Yvert, G., Dvys, D., Trottier, Y., Garnier, J.-M., Weber, C., Mandel, J.-L., Cancel, G., Abbas, N., Dürr, A., Didierjan, O., Stevanin, G., Agid, Y. and Brice, A. (1996). Cloning of the gene for spinocerebellar ataxia 2 reveals a locus with high sensitivity to expanded CAG/glutamine repeats. *Nat. Genet.* 14: 285-291.
- Iughetti, P., Zatz, M., Passos-Bueno, M.R. and Marie, S.K. (1996). Different origins of mutations at the Machado-Joseph locus (MJD1). *J. Med. Genet.* 33: 439-440.
- Kawaguchi, Y., Okamoto, T., Taniwaki, M., Aizawa, M., Inoue, M., Katayama, S., Kawakami, H., Nakamura, S., Nishimura, M., Akiguchi, I., Kimura, J., Narumiya, S. and Kakizuka, A. (1994). CAG expansions in a novel gene for Machado-Joseph disease at chromosome 14q32.1. *Nat. Genet.* 8: 221-228.
- Koide, R., Ikeuchi, T., Onodera, O., Tanaka, H., Igarashi, S., Endo, K., Takahashi, H., Kondo, R., Ishikawa, A., Hayashi, T., Saito, M., Tomoda, A., Miike, T., Naito, H., Ikuta, F. and Tsuji, S. (1994). Unstable expansion of CAG repeat in hereditary dentatorubral-pallidolusian atrophy (DRPLA). *Nat. Genet.* 6: 9-13.
- LaSpada, A.R., Wilson, E.M., Lubahn, D.B., Harding, A.E. and Fischbeck, K.H. (1991). Androgen receptor gene mutation in X-linked spinal and bulbar muscular atrophy. *Nature* 352: 77-79.
- Lima, L. and Coutinho, P. (1980). Clinical criteria for diagnosis of Machado-Joseph disease: report of a non-Azorean Portuguese family. *Neurology* 30: 319-322.
- Lopes-Cendes, I., Silveira, I., Maciel, P., Gaspar, C., Radvany, J., Chitayat, D., Babul, R., Stewart, J., Dolliver, M., Robitaille, Y., Rouleau, G. and Sequeiros, J. (1996a). Limits of clinical assessment in the accurate diagnosis of Machado-Joseph disease. *Arch. Neurol.* 53: 1168-1174.
- Lopes-Cendes, I., Maciel, P., Kish, S., Garpar, C., Silveira, I., Sequeiros, J. and Rouleau, G.A. (1996b). Somatic mosaicism of the CAG repeat length in post-mortem CNS specimens of spinocerebellar ataxia type 1 (SCA 1) and Machado-Joseph disease (MJD) patients. *Ann. Neurol.* 40: 199-206.
- Lopes-Cendes, I., Teive, H.G.A., Calcagnotto, M.A., Costa, J.C., Cardoso, F., Maciel, J.A., Radvany, J., Arruda, W.O., Trevisol-Bittencourt, P.C., Rosa Neto, P., Silveira, I., Steiner, C.E., Pinto-Júnior, W., Santos, A.S., Correa Neto, Y., Werneck, L.C., Araújo, A.Q.C., Carakushansky, G., Mello, L.R., Jardim, L.B. and Rouleau, G.A. Frequency of the different mutations causing spinocerebellar ataxia (SCA1, SCA2, MJD/SCA3 and DRPLA) in a large group of Brazilian patients. *Arq. Neuropsiquiatr.* (in press).
- Maciel, P., Gaspar, C., DeStefano, A., Silveira, I., Coutinho, P., Radvany, J., Dawson, D.M., Sudarsky, L., Guimaraes, J., Loureiro, J.E.L., Nazareth, M.M., Corwin, L.I., Lopes-Cendes, I., Rooke, K., Rosenberg, R., MacLeod, P., Farrer, L.A., Sequeiros, J. and Rouleau, G.A. (1995). Correlation between CAG repeat length and clinical features in Machado-Joseph disease. *Am. J. Hum. Genet.* 57: 54-61.
- Nagafuchi, S., Yanagisawa, H., Sato, K., Shirayama, T., Ohsaki, E., Bundo, M., Takeda, T., Tadokoro, K., Kondo, I., Murayama, N., Tanaka, Y., Kikushima, H., Umino, K., Kurosawa, H., Furukawa, T., Nihei, K., Inoue, T., Sano, A., Komure, O., Takahashi, M., Yoshizawa, T., Kanazawa, I. and Yamada, M. (1994). Dentatorubral and pallidolusian atrophy expansion of an unstable CAG trinucleotide on chromosome 12p. *Nat. Genet.* 6: 14-18.
- Nakano, K.K., Dawson, D.M. and Spence, A. (1972). Machado disease: a hereditary ataxia in Portuguese immigrants to Massachusetts. *Neurology* 22: 49-55.
- Orr, H.T., Chung, M., Banfi, S., Kwiatkowski, T.-J., Servadio, A., Beaudet, A.L., McCall, A.E., Duvick, L.A., Ranum, L.P.W. and Zoghbi, H.Y. (1993). Expansion of an unstable trinucleotide CAG repeat in spinocerebellar ataxia type 1. *Nat. Genet.* 4: 221-226.
- Penrose, L.S.C. (1948). The problem of anticipation in pedigrees of dystrophia myotonica. *Ann. Eugen.* 14: 125-132.
- Pulst, S.-M., Nechiporuk, A., Nechiporuk, T., Gispert, S., Chen, X.-N., Lopes-Cendes, I., Pearlman, S., Starkman, S., Orozco-Diaz, G., Lunkes, A., DeJong, P., Rouleau, G.A., Auburger, G., Korenberg, J.R., Figueroa, C. and Sahba, S. (1996). Moderate expansion of a normally

- biallelic trinucleotide repeat in spinocerebellar ataxia type 2. *Nat. Genet.* 14: 269-276.
- Ranum, L.P.W., Lundgren, J.K., Schut, L.J., Ahrens, M.J., Perlman, J.A., Bird, T.D., Gomez, C. and Orr, H.Y. (1995). Spinocerebellar ataxia type 1 and Machado-Joseph disease: incidence of CAG expansions among adult-onset ataxia patients from 311 families with dominant, recessive or sporadic ataxia. *Am. J. Hum. Genet.* 57: 603-608.
- Richards, R.I. and Sutherland, G.R. (1992). Dynamic mutations: a new class of mutations causing human disease. *Cell* 70: 709-712.
- Romanul, F.C.A., Fowler, H.L., Radvany, J., Feldman, R.G. and Feingold, M. (1977). Azorean disease of the nervous system. *N. Engl. J. Med.* 226: 1505-1508.
- Rosenberg, R.N., Nyhan, W.L. and Bay, C. (1976). Autosomal dominant striatonigral degeneration: A clinical, pathological, and biochemical study of a new genetic disorder. *Trans. Am. Neurol. Assoc.* 101: 1-3.
- Ross, C.A. (1995). When more is less: pathogenesis of glutamine repeat neurodegenerative diseases. *Neuron* 15: 493-496.
- Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, New York.
- Sampei, K., Takano, H., Igarashi, S., Sato, T., Oyake, M., Sasaki, H., Wakisaka, A., Tashiro, K., Ishida, Y., Ikeuchi, T., Koide, R., Saito, M., Sato, A., Tanaka, T., Hanyu, S., Takiyama, Y., Nishizawa, M., Shimizu, N., Nomura, Y., Segawa, M., Iwabuchi, K., Eguchi, I., Tanaka, H., Takahashi, H. and Tsuji, S. (1996). Identification of the spinocerebellar ataxia type 2 gene using a direct identification of repeat expansion and cloning technique, DIRECT. *Nat. Genet.* 14: 277-284.
- Schöls, L., Vieira-Saecker, A.M.M., Schöls, S., Przuntek, H., Epplen, J.T. and Riess, O. (1995). Trinucleotide expansion within the *MJD1* gene presents clinically as spinocerebellar ataxia and occurs most frequently in German SCA patients. *Hum. Mol. Genet.* 4: 1001-1005.
- Sequeiros, J. and Coutinho, P. (1981). Genetic aspects of Machado-Joseph disease. *Brotéria-Genét.* 2: 137-147.
- Sequeiros, J., Silveira, I., Maciel, P., Coutinho, P., Manaia, A., Garpar, C., Burlet, P., Loureiro, L., Guimarães, J., Tanaka, H., Takiyama, Y., Sakamoto, H., Nishizawa, M., Nomura, Y., Segawa, M., Tsuji, S., Melki, J. and Munnich, A. (1994). Genetic linkage studies of Machado-Joseph disease with chromosome 14q STRPs in 16 Portuguese-Azorean kindreds. *Genomics* 21: 645-648.
- Silveira, I., Lopes-Cendes, I., Kish, S., Maciel, P., Gaspar, C., Coutinho, P., Botez, M.I., Teive, H., Arruda, W., Steiner, C.E., Pinto-Junior, W., Maciel, J.A., Jain, S., Sack, G., Andermann, E., Sudarsky, L., Rosenberg, R., MacLeod, P., Chitayat, D., Babul, R., Sequeiros, J. and Rouleau, G.A. (1996). Frequency of spinocerebellar ataxia type 1, dentatorubropallidolusian atrophy and Machado-Joseph disease mutations in a large group of spinocerebellar ataxia patients. *Neurology* 46: 214-218.
- Stevanin, G., Le Guern, E., Ravisé, N., Chneiweiss, H., Dürr, A., Cancel, G., Vignal, A., Boch, A.-L., Ruberg, M., Penet, C., Pothin, Y., Lagroua, I., Haguenu, M., Rancurel, M., Weissenbach, J., Agid, Y. and Brice, A. (1994). A third locus for autosomal dominant cerebellar ataxia type 1 maps to chromosome 14q24.3-qter: evidence for the existence of a fourth locus. *Am. J. Hum. Genet.* 54: 11-20.
- Stevanin, G., Cassa, E., Cancel, G., Abbas, N., Dürr, A., Jardim, E., Agid, Y., Sousa, P.S. and Brice, A. (1995). Characterization of the unstable expanded CAG repeat in the *MJD1* gene in four Brazilian families of Portuguese descent with Machado-Joseph disease. *J. Med. Genet.* 32: 827-830.
- Strachan, T. and Read, A.P. (1996). Molecular pathology. In: *Human Molecular Genetics* (Strachan, T. and Read, A.P., eds.). Wiley-Liss, New York, pp. 401-426.
- Sutherland, G.R. and Richards, R.I. (1995). Simple tandem DNA repeats and human genetic disease. *Proc. Natl. Acad. Sci. USA* 92: 3636-3641.
- Takiyama, Y., Nishizawa, M., Tanaka, H., Kawashima, S., Sakamoto, H., Karube, Y., Shimazaki, H., Soutome, M., Endo, K., Ohta, S., Kagawa, Y., Kanazawa, I., Mizuno, Y., Yoshida, M., Yuasa, T., Horikawa, Y., Oyanagi, K., Nagai, H., Kondo, T., Inuzuka, T., Onodera, O. and Tsuji, S. (1993). The gene for Machado-Joseph disease maps to human chromosome 14q. *Nat. Genet.* 4: 300-304.
- Teive, H.A.G., Arruda, W.O. and Trevisol-Bittencourt, P.C. (1991). Doença de Machado Joseph. *Arq. Neuropsiquiatr.* 49: 172-179.
- Twist, E.C., Casaubon, L.K., Rutledge, M.H., Farrer, L.A., MacLeod, P.M., Radvany, J., Rosenberg, R.N. and Rouleau, G.A. (1995). Machado Joseph disease maps to the same region of chromosome 14 as the spinocerebellar ataxia type 3 locus. *Am. J. Med. Genet.* 32: 25-31.
- Zhou, Y.X., Takiyama, Y., Igarashi, S., Li, Y.F., Zhou, B.Y., Giu, D.C., Endo, K., Tanaka, H., Chen, Z.H., Zhou, L.S., Fan, M.Z., Yang, B.X., Weissenbach, J., Wang, G.X. and Tsuji, S. (1997). Machado-Joseph disease in four Chinese pedigrees: molecular analysis of 15 patients including two juvenile cases and clinical correlation. *Neurology* 48: 482-485.
- Zhuchenko, O., Bailey, J., Bonnen, P., Ashizawa, T., Stockton, D.W., Amos, C., Dobyns, W.B., Subramony, S.H., Zoghbi, H.Y. and Lee, C.C. (1997). Autosomal dominant cerebellar ataxia (SCA6) associated with small polyglutamine expansions in the  $\alpha_{1A}$ -voltage-dependent calcium channel. *Nat. Genet.* 15: 62-69.
- Zoghbi, H.Y. (1996). The expanding world of ataxins. *Nat. Genet.* 14: 237-238.

(Received April 1, 1997)