

CLINICAL AND MOLECULAR CHARACTERISTICS OF A BRAZILIAN FAMILY WITH SPINOCEREBELLAR ATAXIA TYPE 1

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ABSTRACT - The spinocerebellar ataxias (SCAs) are a clinically and genetically heterogeneous group of late onset neurodegenerative disorders. To date, seven different genes causing autosomal dominant SCA have been mapped: *SCA1*, *SCA2*, *Machado-Joseph disease (MJD)*/*SCA3*, *SCA4*, *SCA5*, *SCA7* and *dentatorubropallidoluysian atrophy (DRPLA)*. Expansions of an unstable trinucleotide CAG repeat cause three of these disorders: *SCA1*, *MJD/SCA3* and *DRPLA*. We studied one Brazilian family segregating an autosomal dominant type of SCA. A total of ten individuals were examined and tested for the presence of the *SCA1*, *MJD* and *DRPLA* mutations. Three individuals, one male and two females, were considered affected based on neurological examination; ages at onset were: 32, 36 and 41 years. The first complaint in all three patients was gait ataxia which progressed slowly over the years. Six individuals showed one allele containing an expanded CAG repeat in the *SCA1* gene. The mean size of the expanded allele was 48.2 CAG units. Instability of the expanded CAG tract was seen in the two transmissions that were observed in this family. In both occasions there was a contraction of the CAG tract. Our study demonstrates that *SCA1* occurs in the Brazilian population. In addition, our results stress the importance of molecular studies in the confirmation of diagnosis and for pre-symptomatic testing in SCAs.

KEY WORDS: neurodegenerative disease, spinocerebellar ataxia, trinucleotide expansion.

Características clínicas e moleculares de uma família brasileira com ataxia espinocerebelar tipo 1

RESUMO - As ataxias espinocerebelares (AECs) fazem parte de um grupo de doenças neurodegenerativas que apresentam grande heterogeneidade clínica e genética. Existem até o momento sete genes mapeados responsáveis pelas AECs de transmissão autossômica dominante: *SCA1*, *SCA2*, *doença de Machado-Joseph (DMJ)* ou *SCA3*, *SCA4*, *SCA5*, *SCA7* e *atrofia dentatorubropalidoluisiana (ADRPL)*. Uma expansão de um trinucleotídeo CAG foi identificada como a mutação responsável na *SCA1*, *DMJ* e *ADRPL*. Estudamos uma família brasileira com uma forma autossômica dominante de AEC. Dez indivíduos foram examinados e amostras de sangue foram colhidas para os estudos moleculares das mutações causadoras da *SCA1*, *DMJ* e *ADRPL*. Três membros da família foram considerados clinicamente afetados, um indivíduo do sexo masculino e dois do sexo feminino. A idade de início dos sintomas foi 32, 36 e 41 anos. Ataxia da marcha, lentamente progressiva, foi a primeira manifestação da doença nos três pacientes. Em seis indivíduos os estudos moleculares mostraram um alelo com expansão da sequência CAG contida no gene *SCA1*. O tamanho médio do alelo CAG expandido foi 48,2 unidades. O alelo *SCA1* expandido apresentou instabilidade nas duas transmissões observadas, nas quais ocorreram contrações de uma e de seis unidades CAG. O nosso estudo mostra que a *SCA1* ocorre na população brasileira. Além disso, os nossos resultados reforçam a importância dos estudos moleculares na confirmação diagnóstica e no diagnóstico pré-sintomático de pacientes com AEC.

PALAVRAS-CHAVE: doença neurodegenerativa, ataxia espinocerebelar, expansão de trinucleotídeo.

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The spinocerebellar ataxias (SCAs) are a heterogeneous group of adult onset neurodegenerative disorders characterized clinically by progressive cerebellar ataxia with variable associated features including ophthalmoplegia, dysarthria, dysphagia, pyramidal and extrapyramidal signs. Genetically, these disorders can be divided into: autosomal recessive, autosomal dominant, and isolated cases. To date, seven different loci causing autosomal dominant SCA are mapped: the *SCA1* locus on chromosome 6p^{12,39}, the *SCA2* locus on chromosome 12q^{7,16,21}, the *Machado-Joseph disease (MJD)/SCA3* locus on chromosome 14q^{30,33,36,38}, the *SCA4* locus on chromosome 16q⁶, the *SCA5* locus on the centromeric region of chromosome 11²⁵, the *SCA7* locus on chromosome 3p^{2,9} and the *dentatorubropallidoluysian atrophy (DRPLA)* locus on chromosome 12p¹⁵. However, there are families that do not map to any of these locations^{17,37}. The mutations causing three of these disorders, *SCA1*²², *MJD/SCA3*^{3,14,19} and *DRPLA*¹⁵, have been identified. In all three instances affected individuals have an expansion of a trinucleotide CAG repeat in the coding region of the disease genes.

The identification of these three mutations responsible for SCA allows us to recognize families that segregate *SCA1*, *MJD* or *DRPLA*, thus providing means for accurate classification and diagnosis of these disorders. We undertook this study in order to determine whether a mutation in one of the SCA genes identified (*SCA1*, *MJD* and *DRPLA*) was responsible for the disease in a Brazilian family with autosomal dominant SCA.

SUBJECTS

A total of ten individuals were examined and bloods were collected for molecular studies. All individuals in this family were enrolled in a genetic counseling program prior to the study and received supportive counseling throughout the clinical evaluation and molecular testing.

METHODS

DNA isolation

Genomic DNA was isolated from peripheral blood leukocytes using standard manual techniques²⁸.

PCR analysis

The published primer sequences: Rep1 and Rep2²², MJD52 and MJD25¹⁴, and B37 CAG repeat primer sequences¹⁵ were used for detection of the *SCA1*, *MJD*, and *DRPLA* mutations, respectively. Polymerase chain reaction (PCR) was carried out in a total volume of 12.5µl, with 100ng of genomic DNA; 1µM of each primer; 200µM of dGTP, dCTP, dTTP and dATP; 1 unit of Taq polymerase and 2% formamide. Samples were processed through 30 to 32 cycles of denaturation, annealing, and elongation at different temperatures, as described previously³². PCR products were separated in 6% polyacrylamide gels. Gels were transferred into Hybond N+ nylon membranes and hybridized with a α -³²P 3'-end labelled (CAG)₁₅ probe. Allele sizes were determined by comparing migration relative to an M13 sequencing ladder. Patients previously identified with the *SCA1*, *MJD* and *DRPLA* mutations were used as positive controls in all analyses.

As previously reported for the *SCA1* gene, normal alleles have a size range of 6 to 39 CAG repeats, while affected alleles have 40 to 81 CAG repeats^{4,8,13,20,23}. The normal *DRPLA* allele ranges from 8 to 25 CAGs, while in affected *DRPLA* alleles 54 to 68 CAGs are found¹⁵. In normal individuals the *MJD* gene contains 12 to 37 CAG repeats, while in affected patients the repeat number ranges from 62 to 84^{3,19}.

RESULTS

This Brazilian family is probably of Italian origin; however Portuguese background cannot be completely excluded. Of the ten family members examined there were three clinically affected individuals, one male and two females. The Figure shows the reduced pedigree of the family and the Table summarizes the demographic and molecular information obtained in the study.

Individual II-1 began to develop gait ataxia at age 41, neurological examination after 5 years of disease onset showed moderate gait ataxia, incoordination in upper limbs, globally increased deep tendon reflexes and pyramidal signs. A CT scan at the time of examination revealed cerebellar atrophy. Individual II-4 began to have gait ataxia at age 32. The disease has progressed slowly and

Table. Demographic data and molecular characteristics of ten individuals studied in the Brazilian family segregating the SCA1 mutation.

Pedigree Number	Age at Examination (years)	Age at Onset (years)	Normal Allele (CAG units)	Expanded Allele (CAG units)
II-1	46	41	31	50
II-4	36	32	30	51
II-5	37	36	30	48
III-1	23	-	32	49
II-2	24	-	30	47
III-8	26	-	32	44
II-3	43	-	31/32	-
III-2	19	-	31/33	-
II-6	47	-	31/32	-
II-7	40	-	31/32	-

Note: Double entries for normal alleles indicate the sizes of the two normal alleles found. A dash (-) in the expanded allele column indicates that no abnormal allele was found.

after 4 years of disease evolution he presented mild gait ataxia, incoordination in upper limbs and increased knee jerk. A CT scan was considered normal. Individual II-5 started symptoms about one year before examination at age 36 and showed only mild gait ataxia. Other clinical findings including cognitive impairment, optic atrophy, ophthalmoplegia, dysphagia, dystonia and Parkinsonian features were not seen in this family. All remaining individuals examined were clinically normal.

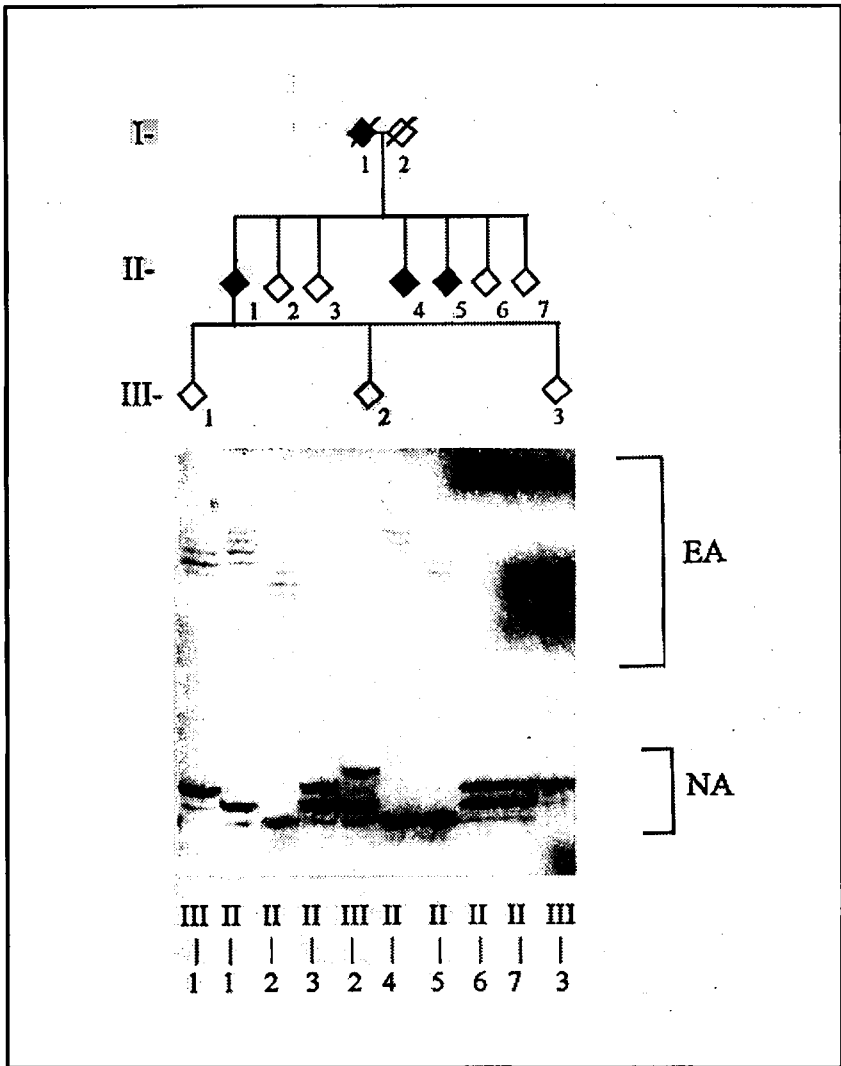
Six individuals had an abnormal CAG repeat in the *SCA1* gene (Figure), three males and three females. The number of repeats in the expanded allele varied from 44 to 51 CAG units, with a mean of 48.2. Mild instability of the CAG tract was seen in the two transmissions that could be documented in this family. In both occasions there was a contraction of the CAG tract, of one and six CAG units. By contrast, the normal alleles were transmitted in a mendelian fashion and varied from 30 to 33 CAG repeats (Figure).

The appearance of the normal and the expanded CAG repeats in the *SCA1* gene varied markedly. All the normal alleles had a single strong band distinctively seen in the autoradiographs, whereas the expanded alleles showed several bands (Figure).

The ten individuals genotyped in this family had CAG repeats of normal size in the *MJD* and *DRPLA* genes (data not shown).

DISCUSSION

The classification of the autosomal dominant SCAs has been difficult due to variability and overlapping in clinical characteristics and pathological presentation⁵. It is not uncommon to find affected individuals, in the same family, with a wide variety of symptoms¹⁰. It is now generally accepted that the controversies involving diagnosis and classification of this group of disorders will only be solved when the molecular aspects are clarified²⁶. With the cloning of the *SCA1*, *MJD* and *DRPLA* genes and the characterization of the respective mutations an accurate diagnosis can now be performed, even in small families or single affected individuals. This will result in a better estimate of the prevalence of these disorders, as well as provide more detailed clinical, pathological and molecular information that will improve our understanding of these group of diseases.



Top panel: Reduced pedigree of the Brazilian family carrying the SCA1 mutation. Blackened symbols represent clinically affected individuals. Diagonal lines indicate deceased individuals. Bottom panel: Analysis of PCR products containing the expanded CAG repeat at the SCA1 locus. Genomic DNA was amplified using primers Rep 1 and Rep 2. PCR products were analyzed on 6% polyacrylamide gels. Normal alleles (NA) had sizes varying from 30 to 33 CAG units and expanded alleles (EA) varied from 44 to 51 CAGs.

We³² and others²⁴ have determined the frequency of the three different SCAs for which direct molecular diagnosis is available: *SCA1*, *MJD* and *DRPLA*. Families of different geographic and ethnic origins have been reported with the *SCA1* mutation; however, *SCA1* seems to occur more frequently in certain ethnic groups such as Italians and Eastern (EuropeansRanum et al.²⁴, Silveira et al.³², and Lopes-Cendes unpublished results). In Southern Italy a cluster of *SCA1* families has been described sharing the same haplotype for markers closely linked to the *SCA1* locus, which suggests

a common origin of these families¹². Therefore, it is not surprising to find SCA1 families in Brazil particularly in regions of strong Italian immigration. The family described in this paper is most likely of Italian background.

Clinical variability is usually present in SCA1, even within the same family. There has been no description of any clinical feature that is specific for SCA1 patients^{8,20,34}. Symptoms usually begin in the third and fourth decade of life and are characterized by gait ataxia, dysarthria, and ophthalmoplegia⁸. Limb ataxia is typically less severe than gait ataxia. In the early stages, eye movements appear to be full and the saccadic velocities are relatively preserved³⁴. The disorder gradually progresses and patients become bedridden after 10 to 20 years of disease evolution. In the later stages of the disease, distal areflexia occurs, and dysphagia develops leading to frequent choking spells and aspiration pneumonia. Some degree of increased tone as well as dystonic movement may occur late in the course of the disease. Dementia has not been observed in genetically proven SCA1 families; although mild cognitive decline may occur in the advanced stages of the disease³⁴. Infrequent signs include optic atrophy and Parkinsonian features^{8,20}. One of the largest SCA1 families studied, the Schut kindred, shows remarkable heterogeneity in disease presentation, with cases starting in the first or second decades of life and progressing rapidly, as well as patients with onset after the fourth decade presenting a mild disease²⁹. The Brazilian SCA1 family reported here is relatively small and does not permit extensive clinical correlations; however it seems that the disease presentation has been very similar in the three clinically affected individuals, and with no specific associated features such as: slow eye movements, extrapyramidal features, peripheral signs, retinal degeneration and myoclonus, which are more frequently found in SCA2, MJD, SCA4, SCA7 and DRPLA patients, respectively^{2,6,15,16,18,25,26}. Small families with patients presenting a short evolution of the disease, such as the Brazilian SCA1 pedigree, represent a very difficult problem for the clinical differential diagnosis. We believe that diagnostic questions in SCA patients can only be solved with molecular testing.

The observation that in one single family, the disease has a tendency for a progressive earlier onset with increased severity in younger generations has intrigued researchers in the field of SCAs for a long time. This phenomenon, called anticipation, is observed in other neurodegenerative disorders, such as: Huntington disease, Kennedy disease, MJD and DRPLA³⁵. All of which are caused by an expansion of a trinucleotide CAG. It has been observed that translation of the mutant protein actually occurs and that the CAG stretch codes for a polyglutamine tract at the protein level^{11,31}. These observations suggest a toxic gain of function by the proteins containing an expanded glutamine tract^{11,27}. In addition, the observed inverse correlation between the repeat size and age at onset of the disease and the tendency of an overall increase in the CAG repeat size in successive generations^{13,23}, indicate that this toxic effect is proportional to the length of the polyglutamine tract. Therefore, offering a possible molecular explanation for the phenomenon of anticipation observed in these neurodegenerative disorders.

The molecular characteristics of the expanded CAG repeat found in this family are similar to those reported in other SCA1 families^{8,13,20,22}. We observed mild instability during transmission of the expanded CAG tract, suggesting gametic mosaicism. In addition, the observation of multiple bands for the expanded allele, that were seen in the autoradiographs, indicates the presence of somatic mosaicism, with different cells containing different lengths of the CAG repeat.

In SCA1 families an inverse correlation between the size of the CAG repeat and the age at onset of the disease has been observed^{13,20,22,23}; however this correlation is not perfect, only about 66% of variability in age at onset can be attributed to the length of the CAG repeat in these SCA1 patients. This suggests that there are factors other than the repeat size involved in the determination of the disease phenotype. Therefore, the size of the CAG repeat is not a good predictor of age at onset in SCA1. Although the CAG tract has a tendency for expansion in successive generations^{13,23}, anticipation cannot be always observed, since contractions, as seen in the Brazilian SCA1 family, also occur.

Confirmation or exclusion of diagnosis can be accomplished in almost all pre-symptomatic or suspected cases of SCA1, specially if affected family members are also tested. The availability of such highly sensitive and specific test for a late onset disorder has raised several questions about ethic and legal aspects of molecular testing in at risk individuals¹. This emphasizes the importance of a multidisciplinary supportive counseling program that should be available for all individuals undergoing this type of molecular testing.

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