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FREQUENCY OF THE DIFFERENT MUTATIONS CAUSING SPINOCEREBELLAR ATAXIA (*SCA1, SCA2, MJD/SCA3* AND *DRPLA*) IN A LARGE GROUP OF BRAZILIAN PATIENTS

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ABSTRACT - Spinocerebellar ataxia type 1 (SCA1), spinocerebellar ataxia type 2 (SCA2) and Machado-Joseph disease or spinocerebellar ataxia type 3 (MJD/SCA3) are three distinctive forms of autosomal dominant spinocerebellar ataxia (SCA) caused by expansions of an unstable CAG repeat localized in the coding region of the causative genes. Another related disease, dentatorubropallidoluysian atrophy (DRPLA) is also caused by an unstable triplet repeat and can present as SCA in late onset patients. We investigated the frequency of the *SCA1*, *SCA2*, *MJD/SCA3* and *DRPLA* mutations in 328 Brazilian patients with SCA, belonging to 90 unrelated families with various patterns of inheritance and originating in different geographic regions of Brazil. We found mutations in 35 families (39%), 32 of them with a clear autosomal dominant inheritance. The frequency of the *SCA1* mutation was 3% of all patients; and 6 % in the dominantly inherited SCAs. We identified the *SCA2* mutation in 6% of all families and in 9% of the families with autosomal dominant inheritance. The *MJD/SCA3* mutation was detected in 30 % of all patients; and in the 44% of the dominantly inherited cases. We found no *DRPLA* mutation. In addition, we observed variability in the frequency of the different mutations according to geographic origin of the patients, which is probably related to the distinct colonization of different parts of Brazil. These results suggest that SCA may be occasionally caused by the *SCA1* and *SCA2* mutations in the Brazilian population, and that the *MJD/SCA3* mutation is the most common cause of dominantly inherited SCA in Brazil.

KEY WORDS: neurodegenerative disease, spinocerebellar ataxia type 1, spinocerebellar ataxia type 2, spinocerebellar ataxia type 3, Machado-Joseph disease, dentatorubropallidoluysian atrophy, trinucleotide repeat expansion.

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Frequência das mutações que causam ataxia espinocerebelar (*SCA1*, *SCA2*, *MJD/SCA3* e *DRPLA*) em um grupo numeroso de pacientes brasileiros

RESUMO - Ataxia espinocerebelar tipo 1 (SCA1), ataxia espinocerebelar tipo 2 (SCA2) e doença de Machado-Joseph ou ataxia espinocerebelar tipo 3 (MJD/SCA3) são três formas de ataxia espinocerebelar (SCA) que apresentam herança genética autossômica dominante. Nessas três doenças foi encontrada uma expansão instável de trinucleotídeo CAG localizada na região codificadora dos genes responsáveis pelas três doenças. Portanto, para SCA1, SCA2 e MJD/SCA3 o diagnóstico molecular é agora possível. A atrofia dentatorubropalidoluisiana (DRPLA) é também causada pela expansão de trinucleotídeos CAG e pode por vezes se apresentar como uma *SCA*. Nós investigamos a frequência das mutações responsáveis por *SCA1*, *SCA2*, *MJD/SCA3* e *DRPLA* em um grupo de 328 pacientes brasileiros com SCA pertencentes a 90 famílias não aparentadas. Esses pacientes apresentavam padrões diferentes de herança genética e eram provenientes de várias regiões do Brasil. Nós identificamos mutações em 35 famílias, 32 das quais com herança claramente autossômica dominante. A frequência da mutação *SCA1* foi de 3% no grupo total de pacientes, e 6% nos pacientes com herança autossômica dominante. Nós encontramos a mutação *SCA2* em 6% de todas as famílias e em 9% das famílias com herança autossômica dominante. A mutação *MJD/SCA3* foi encontrada em 30% de todos os pacientes, e em 44% quando consideramos somente os pacientes com herança autossômica dominante. Nenhuma mutação *DRPLA* foi encontrada. Nós observamos também variabilidade na frequência das diferentes mutações em pacientes provenientes de diferentes regiões geográficas, o que provavelmente se correlaciona com os padrões distintos de colonização do Brasil. Nossos resultados sugerem que os casos de SCA no Brasil podem ser causados ocasionalmente pela mutação *SCA1* e *SCA2*, mas que a causa mais frequente de SCA de herança autossômica dominante no Brasil é a mutação *MJD*/SCA3.

PALAVRAS-CHAVE: doença neurodegenerativa, ataxia espinocerebelar tipo 1, ataxia espinocerebelar tipo 2, ataxia espinocerebelar tipo 3, doença de Machado-Joseph, atrofia dentatorubropalidoluisiana, expansão de trinucleotídeo CAG.

The spinocerebellar ataxias (SCAs) represent a wide spectrum of degenerative disorders of the central nervous system. Clinically this group of diseases is characterized by cerebellar dysfunction manifested by gait and limb ataxia, incoordination and dysarthria[17,19]. Inheritance is variable and the prevalence of the autosomal dominant cases is about 1 per 100,000 population[18,57]. Early onset cases (before the second decade of life) are usually autosomal recessive, whereas adult onset patients commonly have autosomal dominant inheritance[19]. To date, two loci for autosomal recessive SCA have been mapped: Friedreich ataxia on chromosome (ch) 9q[9], and ataxia with vitamin E deficiency on ch 8q[3]. There are a total of eight loci for autosomal dominant SCAs described: spinocerebellar ataxia type 1 (*SCA1*) on ch 6p[23,24,67,70], spinocerebellar ataxia type 2 (*SCA2*) on ch 12q[2,14,29,39], Machado-Joseph disease (*MJD*) or spinocerebellar ataxia type 3 (*SCA3*) on ch 14q[55,59,61,64], spinocerebellar ataxia type 4 (*SCA4*) on ch 16q[12], spinocerebellar ataxia type 5 (*SCA5*) on the centromeric region of ch 11[47], spinocerebellar ataxia type 6 (*SCA6*) on ch 19p[69], spinocerebellar ataxia type 7 (*SCA7*) on ch 3p[4,16] and a related disorder, dentatorubropallidoluysian atrophy (*DRPLA*) on ch 12p[36]. However, there are families that do not map to any of these locations[30,63].

A total of seven genes, two for the autosomal recessive[8,41] and five for the autosomal dominant forms (*SCA1*, *SCA2*, *MJD/SCA3*, *SCA6* and *DRPLA*)[21,25,26,36,40,43,52,69], have been identified. All the genes identified in the autosomal dominant forms have polymorphic CAG trinucleotide repeats that are expanded and unstable in affected individuals. The same type of dynamic mutation is also found in other neurodegenerative disorders, such as Huntington disease[20] and Kennedy disease[27]. The identification of five mutations responsible for autosomal dominant SCA allows us to recognize families that segregate *SCA1*, *SCA2*, *MJD/SCA3*, *SCA6* or *DRPLA*, thus providing us with means for accurate classification and diagnosis of these disorders in small families or single individuals.

We undertook the present study in order to determine the frequency of the *SCA1*, *SCA2*, *MJD/SCA3* and *DRPLA* mutations in a large group of Brazilian SCA patients from various geographic regions and showing different modes of inheritance. This strategy will allow for a better understanding of the disease presentation and characterization of the clinical picture in each of the different types

of SCA. In addition, one might be able to answer questions regarding the clinical criteria for differential diagnosis, which ultimately will improve the classification of this group of disorders.

SUBJECTS

We studied a total of 328 individuals belonging to 90 unrelated Brazilian families from various geographic regions affected with different types of SCA. In 48 of these families, only one affected individual per family was examined. In the remaining 42 families, an average of 6.7 individuals per family were examined. The largest family included in this study had 41 family members examined. Patients were recruited in eleven different clinics (eight neurology clinics and three genetic services). All patients with progressive cerebellar ataxia seen at these clinics and who agreed to participate in the study were enrolled. Most patients were recruited between April 1994 and February 1997; however there were three families enrolled prior to 1994.

Progressive ataxia was the main clinical finding in all patients. Associated features such as abnormal eye movements and pyramidal signs were present in the majority of patients. A few cases with dementia, extrapyramidal signs and peripheral neuropathy were also found. One patient showed pigmentary retinal degeneration. Ages at onset varied from seven to 58 years.

METHODS

To determine the frequency of the different mutations, at least one affected individual of each family was genotyped for the CAG repeat in the *SCA1*, *SCA2*, *MJD/SCA3* and *DRPLA* genes. If any of these mutations were found, all available family members were genotyped. Overall, 269 individuals were genotyped in this study.

Genomic DNA was isolated from peripheral blood leukocytes and lymphoblastoid cell lines transformed by Epstein-Barr virus following standard techniques[1,51].

The published primer sequences: Rep 1 and Rep 2[40], SCA2 A and SCA2 B[43], MJD 52 and MJD 25[25], and B 37 CAG repeat[26] were used for detection of the *SCA1*, *SCA2*, *MJD/SCA3*, 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 previously[25,26,40,43,56]. PCR products were separated in 6% polyacrylamide gels. Gels were transferred into Hybond N+ nylon membranes and hybridized with a ^{32}P 3'-end labeled $(CAG)_{15}$ probe. Allele sizes were determined by comparing migration relative to an M13 sequencing ladder. Patients previously identified with the *SCA1*, *SCA2*, *MJD/SCA3* and *DRPLA* mutations were used as positive controls in all analyses. The determination of the size of normal and expanded alleles was based on previous reports[15,25,26,33,34,36,40,43,46].

RESULTS

Of the 90 families studied, 54 showed a clear autosomal dominant inheritance (60%). The parents of 36 patients did not have the disease at the time of examination; however, there were several families in which parents were deceased at a young age or accurate information on the parents was not available. Sixteen of these patients (18%) had another sibling with a similar disease and were considered to have autosomal recessive inheritance. The remaining 20 patients (22%) had no family history of a similar disease and were considered sporadic cases.

Most of our patients originated in the southern and southeastern regions of Brazil. Patients were ascertained in seven different Brazilian States: Rio Grande do Sul (RS), Santa Catarina (SC), Paraná (PR), São Paulo (SP), Minas Gerais (MG), Rio de Janeiro (RJ) and Bahia (BA).Table 1 shows the distribution of patients according to geographic origin.

We found mutations in patients belonging to 35 families (39%). In 32 of these families inheritance was clearly autosomal dominant. The overall frequency of the different mutations is shown in Table 2 and the distribution of the positive cases by geographic region is given in Table 3.

Table 1. Geographic distribution and inheritance pattern of the 90 unrelated Brazilian SCA families studied.

	RS	SC	PR	SP	MG	RJ	BA
Autosomal Dominant (n=54)	8	14	15	8	7	1	1
Autosomal Recessive (n=16)	3	1	9	1	2	0	0
Sporadic (n=20)	4	1	13	0	2	0	0
Total (n=90)	**15**	**16**	37	9	11	1	1

RS, Rio Grande do Sul; **SC**, Santa Catarina; **PR**, Paraná; **SP**, São Paulo; **MG**, Minas Gerais; **RJ**, Rio de Janeiro; **BA**, Bahia.

Table 2. Frequency of the SCA1, SCA2, MJD/SCA3 *and* DRPLA *mutations determined in a group of 90 unrelated Brazilian SCA families.*

Mutation	Overall (n=90)	Autosomal dominant cases (n=54)
SCA1	3 (3%)	3 (6%)
SCA2	5 (6%)	5 (9%)
MJD/SCA3	27 (30%)	24 (44%)
DRPLA	**0**	**0**
Total	35 (39%)	32 (59%)

Total, total number of mutations identified.

Table 3. Geographic distribution of the 35 SCA families in which mutations were identified.

Mutation	RS	SC	PR	SP	MG	RJ	BA
SCA1	0	0	1(7%)	1 (13%)	1(14%)	0	0
SCA2	2 (25%)	0	1(7%)	0	2 (29%)	0	0
MJD/SCA3	4* (38%)	10 (71%)	5* (27%)	3 (38%)	4* (43%)	0	1
n	8	14	15	8	7	1	1

Numbers in brackets indicate the percentage of autosomal dominant cases with the different mutations found in each State. An asterisk indicate that there were three patients with the MJD/SCA3 mutation (one in **RS**, one in **PR** and one in **MG**) in whom autosomal dominant inheritance could not be confirmed.
n, total number of autosomal dominant SCA families genotyped in each State; **RS**, Rio Grande do Sul; **SC**, Santa Catarina; **PR**, Paraná; **SP**, São Paulo; **MG**, Minas Gerais; **RJ**, Rio de Janeiro; **BA**, Bahia.

We found three families with the *SCA1* mutation (Fig 1), the frequency of the *SCA1* mutation was 3% of all patients, and 6% of the autosomal dominant cases. We found the *SCA2* mutation (Fig 2) in five families, which makes 6% of the overall group, and 9% of the autosomal dominant cases. The *MJD/SCA3* mutation (Fig 3) was present in 27 families, consisting of 30% of all patients. In three families with the *MJD/SCA3* mutation, parents were believed to be unaffected (but were not clinically examined or genotyped) or died at a young age; in two of these families another sibling also had the disease. Therefore, in only 24 of the 27 families with the *MJD/SCA3* mutation the inheritance was confirmed to be autosomal dominant. The frequency of the *MJD/SCA3* mutation among autosomal dominant patients was 44%. We found no *DRPLA* mutation.

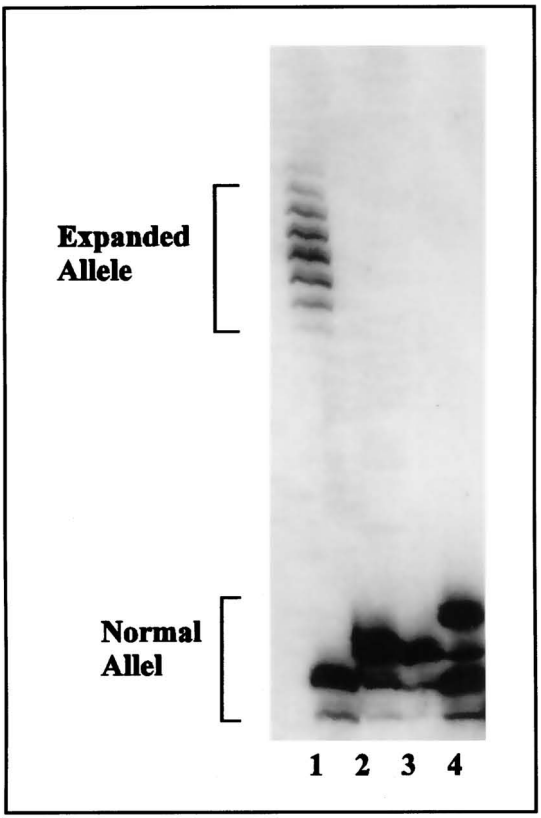

Figure 1. Analysis of PCR products containing the CAG repeat in the SCA1 gene. Genomic DNA extracted from peripheral blood leukocytes was amplified using primers Rep1 and Rep 2[40]. PCR products were analyzed on 6% denaturing polyacrylamide gels.
Lane 1: Individual with the SCA1 mutation, normal allele has 31 CAGs and expanded allele has 46 CAG repeats. Lanes 2 and 3: Two individuals not carrying the SCA1 mutation, in both lanes one normal allele with 32 repeats is seen suggesting that these two individuals are homozygous for the normal CAG repeat in the SCA1 gene.
Lane 4: Individual not carrying the SCA1 mutation and showing two normal alleles of 31 and 33 CAGs.
Note that the appearance of the normal and expanded alleles varies markedly, all normal alleles have a single strong band distinctively seen in the autoradiograph, whereas the expanded allele shows several bands indicating the presence of cells with different lengths of the expanded CAG repeat in the SCA1 gene.

The frequency of the different mutations varied according to the geographic origin of the patients (Table 3). The three families with *SCA1* were from PR, SP and MG. Of the five families with *SCA2*, two were from RS, one from PR and two from MG. The *MJD/SCA3* was found in all States from which more than one family was examined, and the frequencies varied from 71% of the autosomal dominant patients genotyped in SC to 27% of the autosomal dominant cases tested in PR.

The ethnic origin of the families with different types of mutations also varied, two of the three families with the *SCA1* mutation were of Italian background and the third was from Portuguese origin. All the 5 families with the *SCA2* mutation were of Portuguese ancestry. Most of the families with the *MJD/SCA3* mutation were of Portuguese ancestry; however, there were two families of Italian background and one family had a mixed Portuguese and African ancestry. Only one family with the *MJD/SCA3* mutation and Portuguese descent could trace their origins to the Azorean islands[45].

Twenty-eight of the 90 families studied had a clinical diagnosis proposed prior to molecular testing: 25 were presumed to be MJD and three suspected DRPLA cases. Of the 25 families with the clinical diagnosis of MJD, molecular testing was able to confirm the presence of the *MJD/SCA3* mutation in 19 families. One presumed MJD family had the *SCA1* mutation and a second family had the *SCA2* mutation. The remaining four families with the clinical diagnosis of MJD did not have any of the mutations tested. None of the three families with suspected DRPLA had mutations identified.

DISCUSSION

Since the first description of the autosomal dominant forms of SCA by Pierre Marie in 1893[35], the classification has been controversial[17,19]. This is mainly due to the variety of symptoms observed and to the inter- and intrafamilial variability in age of onset, as well as to diverse neuropathological and biochemical findings[19]. Over the past 100 years, the presence of such clinical variability made

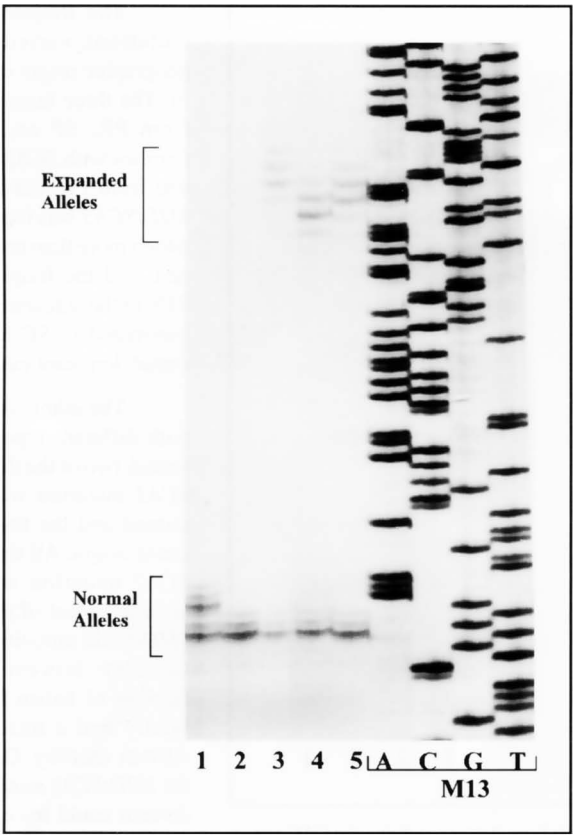

Figure 2. Analysis of PCR products containing the CAG repeat in the SCA2 gene. Genomic DNA extracted from peripheral blood leukocytes was amplified using primers SCA2 A and SCA2 B[43]. PCR products were analyzed on 6% denaturing polyacrylamide gels.
Lane 1: Individual without the SCA2 mutation, two normal alleles (22 and 23 CAGs) are seen.
Lane 2: Individual without the SCA2 mutation, only one allele of 22 CAGs is seen suggesting that this individual is homozygous for the normal CAG allele in the SCA2 gene.
Lanes 3 to 5: Three individuals with the SCA2 mutation, normal alleles have 22 CAGs, expanded alleles have 43, 41 and 42 CAGs.
M13: sequencing ladder used for allele size determination.
Expanded alleles show multiple bands in the autoradiograph suggesting the presence of somatic mosaicism.

the elaboration of a consensus classification for the SCAs virtually impossible. The cloning of the *SCA1*, *SCA2*, *MJD/SCA3*, *SCA6* and *DRPLA* genes and the determination of the mutations has made it possible to confirm previously suspected diagnosis and eventually improve our understanding of these disorders.

The first SCA1 family reported in the literature was a large US kindred of Russian background, the Schut kindred[54]. The *SCA1* locus was first mapped to ch 6p in 1974 in a small Japanese family[67]. This first report was followed by the description of several other families linked to the same location[23,24,70]. The *SCA1* gene was cloned in 1993 and the mutation identified as an expansion of a

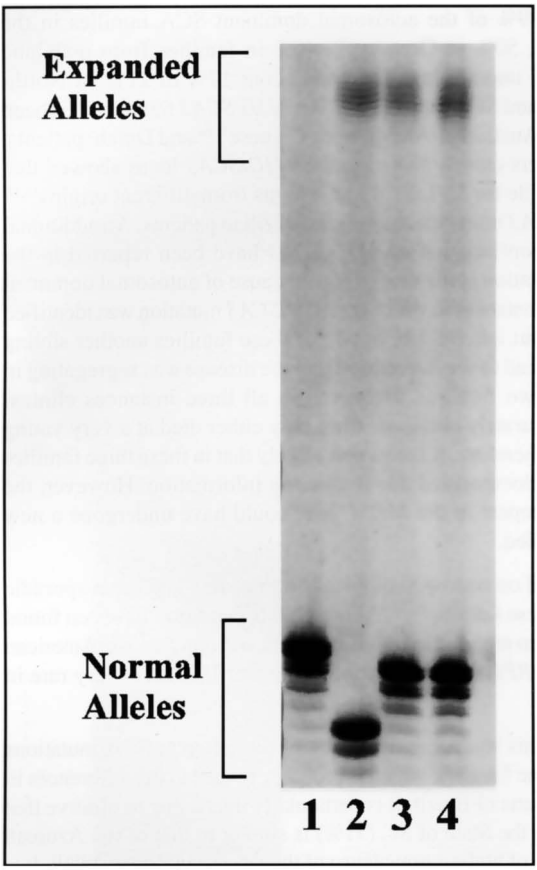

Figure 3. Analysis of PCR products containing the CAG repeat in the MJD1 gene. Genomic DNA extracted from peripheral blood leukocytes was amplified using primers MJD 25 and MJD 52[25]. PCR products were analyzed on 6% denaturing polyacrylamide gels.
Lane 1: Individual without the MJD/SCA3 mutation, only one allele of 27 CAGs is seen which suggests that this individual is homozygous for the normal CAG allele in the MJD1 gene.
Lanes 2 to 4: Three individuals with the MJD/SCA3 mutation, normal alleles range from 23 to 26 CAGs and expanded alleles have 77 CAG repeats.
Expanded alleles show multiple bands in the autoradiograph suggesting the presence of somatic mosaicism.

CAG repeat[40]. Many families with the *SCA1* mutation have been reported worldwide[15,32,46,48,56]; however, *SCA1* is most common in Italy[24], the United Kingdom[15] and eastern Europe[48] (Lopes-Cendes et al., unpublished data). We found the *SCA1* mutation in three Brazilian families with autosomal dominant SCA, one of which had been previously reported[32]. Two of the families identified in this study were of Italian ancestry, confirming the previous observations that *SCA1* is frequent in patients of Italian descent. The frequency of the *SCA1* mutation in North-American autosomal dominant SCA families is between 3 to 10 %[47,56], in our Brazilian families we found a frequency of 6%.

The *SCA2* locus was first mapped in a cluster of Cuban families showing a founder effect[14]. Soon after the first report this locus was confirmed as present in families of different ethnic origins, such as: Austrian[29], French-Canadian[29], Italian[39] and Tunisian[2]. The *SCA2* gene has been recently cloned and the causative mutation shown to be an expansion of a CAG repeat[21,43,52]. Preliminary data show that the frequency of the *SCA2* mutation in autosomal dominant North-American SCA families is about 9%[33]. In the present study we report the first five Brazilian families segregating the *SCA2* mutation, giving a frequency of 9% of the Brazilian families with autosomal dominant inheritance studied.

MJD was originally described as three different clinical entities in North-American patients originating from the Portuguese islands of the Azores[10,38,49,50]. In Brazil, the first report of a family with the clinical diagnosis of MJD was presented in an abstract form in 1984[28], and two additional families were presented in 1988[44]. The first Brazilian family with the clinical diagnosis of MJD was documented in a full paper format in 1991[62]. The two families reported by Radvany et al. in 1988[44,45] and the family published by Teive et al. in 1991[62] were confirmed to have the *MJD/SCA3* mutation[31,34] and have been included in the present study. The *MJD/SCA3* locus was first mapped to ch 14q in Japanese families[61] and subsequently found to map to the same location in families from Portuguese[55,64] and French origin[59]. With the identification of the *MJD/SCA3* mutation in 1994[25] it was determined that the CAG expansion in the *MJD1* gene is the most frequent cause of autosomal dominant SCA worldwide[48,56]. The *MJD/*

SCA3 mutation is responsible for about 89% of the autosomal dominant SCA families in the Azores[56](Silveira et al., unpublished data), 50% in Germany[53], 40% in families from mainland Portugal[56](Silveira et al. and Maciel et al., unpublished data) and about 17% to 21% in North-American non-Portuguese autosomal dominant SCA patients[48,56]. The *MJD/SCA3* mutation has been also described in other populations, such as Australian-Aborigines[7], Chinese[31,68] and Dutch[5] patients. More recently, haplotype analysis of markers closely linked to the *MJD/SCA3* locus showed that there are different mutation events responsible for *MJD/SCA3* in patients from different origins[13,22]. We have found 27 families with the *MJD/SCA3* mutation among our Brazilian patients. An additional 14 Brazilian families with the molecular confirmation of *MJD/SCA3* have been reported in the literature[22,60]. Therefore, the *MJD/SCA3* mutation is the most frequent cause of autosomal dominant SCA in Brazil. In our study, there were three instances in which the *MJD/SCA3* mutation was identified in families with no clear autosomal dominant inheritance. In two of these families another sibling was also found to be affected, which could lead to the impression that the disease was segregating in an autosomal recessive fashion in these two families. However, in all three instances clinical information on the parents could not be accurately obtained, since they either died at a very young age or were not available for examination. Therefore, it seems more likely that in these three families autosomal dominant transmission was not documented due to missing information. However, the possibility that in these patients the CAG repeat in the *MJD1* gene could have undergone a new mutation event cannot be completely excluded.

DRPLA was initially described based on neuropathological findings in a late onset sporadic patient[66], and later reported in several Japanese families[37,58]. Although this condition has been found mainly in Japan, the *DRPLA* mutation has been reported in four European[65] and one African-American family[6]. In our study, we did not find any *DRPLA* mutation confirming that *DRPLA* is very rare in the non-Japanese population.

In the Brazilian patients included in this study, the frequency of the different SCA mutations varied according to the geographic origin of the families, which is probably related to the differences in ethnic background of patients from various parts of Brazil. It is particularly interesting to observe that the frequency of the *MJD/SCA3* mutation in the State of SC (71%) is similar to that of the Azorean Islands (89%)[56], which is believed to be place of highest prevalence of the disease in the world[10,11]. It is well known that SC received a large contingent of immigrants from the Azorean islands[42,45].

Only two clinical diagnosis were attributed to some of the patients prior to molecular testing: MJD and DRPLA. These two entities are believed to have a distinctive clinical presentation that permits to establish their differential diagnosis[10,11,37,58]. The main clinical characteristics that led to the clinical diagnosis of MJD were: a) the presence of extrapyramidal features, specially dystonic posturing and b) the staring aspect of the eyes associated with retraction of the eyelids and leading to the characteristic appearance of "bulging eyes". Although very typical of MJD, only a small percentage of MJD patients present this two clinical findings. Among a large series of Portuguese MJD patients examined by Coutinho[11] less than one third had dystonic posturing and only one fourth had "bulging eyes". It has been demonstrated that the clinical diagnosis of MJD may be missed in patients coming from small families, patients from ethnic origins not usually associated with MJD, patients in the initial stages of the disease (less than 5 years of disease evolution), mild cases and patients not showing the more typical characteristics of the disease[31]. Therefore, molecular confirmation is always necessary for the diagnosis of MJD.

In conclusion, molecular diagnosis was possible in about 60% of the autosomal dominant SCA families in Brazil. The *MJD/SCA3* mutation was the most frequent cause of autosomal dominant SCA in Brazilian patients. It is important to note that not all Azorean or Portuguese SCA families have the *MJD/SCA3* mutation. As mentioned above, the frequency of the *MJD/SCA3* mutation in autosomal dominant families from the Azores is 89%[56] and from mainland Portugal is 40%[56]. Therefore, molecular testing is important for confirmation of diagnosis in all families with SCA

regardless of geographic or ethnic origin. However, it is important to emphasize that these new molecular diagnostic techniques for late onset disorders for which no treatment has been developed should be used only when a multidisciplinary, counseling and supportive group, is available to deal with questions from patients and family members.

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