Online journal ISSN - 1676-5680
GMR Genetics and Molecular Research

Genetic polymorphism at spinocerebellar ataxia 1 and 2 loci in Brazil

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Genet. Mol. Res. 2 (4): 360-365 (2003)
Received July 7, 2003
Accepted September 30, 2003
Published December 5, 2003

ABSTRACT. Dynamic mutation involves the expansion of a tandem arrayed DNA sequence that is polymorphic in the population. This mechanism is associated with neurological/neuromuscular disorders and the pathology depends on the extension of the repeated tract, with a specific threshold for each disease. We made a PCR-based characterization of allelic polymorphism of SCA1 and SCA2 loci in a sample of 200 pairs of chromosomes in a population in Rio de Janeiro and found 23 different alleles at the SCA1 locus, varying from 10 to 39 CAG repeats (mean 27.7 ± 3.3, mode 28) and 10 different alleles ranging from 19 to 29 CAG (mean 22.1 ± 1.0, mode 22) at the SCA2 locus. The level of heterozygosis was 53% (SCA1) and 8% (SCA2).

Key words: Spinocerebellar ataxia, Dynamic mutation, SCA1, SCA2, Genetic polymorphism

INTRODUCTION

Since the discovery of dynamic mutation in the last decade (Fu et al., 1991; Oberlé et al. 1991), several disorders have been associated with this mechanism. The molecular event underlying dynamic mutation is the amplification of a tandem-arrayed DNA sequence that expands until it reaches a pathological size, which is specific for each disorder (Cummings and Zoghbi, 2000). The expandable motif is generally a trinucleotide; however, other types of sequence have also been reported (Lalioti et al., 1997; Matsuura et al., 2000). The tract is polymorphic, both among patients and in normal individuals, and can be located within or outside the coding region of the gene.

Genetic ataxias comprise a heterogeneous group of progressive late-onset neurodegenerative disorders. Most familial cases show a monogenic-autosomal-dominant pattern of inheritance, with variable expressivity and age-dependent penetrance (Pujana et al., 1999; Orr and Zoghbi, 2000). At least nine types of spinocerebellar ataxia (SCA) have been associated with dynamic mutation and most of them are promoted by the expansion of exonic CAG repeats. There is an inverse correlation between the age of onset and the extension of the repeat-containing sequence and, although the individual is born carrying the mutant allele, the neurological consequences only become apparent after a variable period of life, during which the neuronal functions are normal (Zoghbi, 2000; Cummings and Zoghbi, 2000).

The SCA type 1 (*SCA1*) locus has been localized in the short arm of chromosome 6, at 6p22-23. This gene spans 450 kb of genomic DNA and is organized in nine exons, resulting in a 10.6-kb transcript expressed both in neuronal and non-neuronal tissues. The coding region falls within the last two exons, and the first four non-coding exons undergo alternative splicing (Ranum et al., 1991; Banfi et al., 1993, 1994; Servadio et al., 1995). The gene product, ataxin-1, contains about 800 amino acids, depending on the size of the CAG repeat, and shares no homology with other proteins, except for the presence of a polyglutamine tract, which is located in the amino half of the protein (Banfi et al., 1993; Servadio et al., 1995). The CAG repeat at *SCA1* is highly polymorphic, with an average heterozygozity of up to 80%. Normal alleles contain from 6 to 40 CAG, while mutated alleles harbor 41 to 81 repeats (Chung et al., 1993; Ranum et al., 1994; Pujana et al., 1999; Saleem et al., 2000). The occurrence of CAT interruptions in normal alleles that contain over 20 repeats is supposed to maintain the sequence stability, which is also influenced by the parental origin of the allele. The development of this disease is also dependent on the extension of the expansion, and longer alleles are associated with faster and earlier development of the disease (Chung et al., 1993; Ranum et al., 1994; Jodice et al., 1994; Burright et al., 1995). *SCA1* is found in 3-10% of the SCA occurrences (Ranum et al., 1995; Riess et al., 1997; Pujana et al., 1999).

Cloning and characterization of the *SCA2* gene was achieved simultaneously by three groups, which have described a 4.5-kb transcript that is ubiquitously expressed and encodes a protein, ataxin-2, that is found in the cytoplasm and shares no homology with any other protein, including other types of ataxin (Sanpei et al., 1996; Imbert et al., 1996; Pulst et al., 1996). Although its function is still unknown, ataxin-2 contains Sm1 and Sm2 motifs, which have been described in proteins involved in RNA splicing and protein-protein interactions. The polyglutamine tract of ataxin-2 occurs in the amino terminal portion of the coding region and the number of repeats in the normal range varies from 15 to 30 CAG, interrupted by 1 to 3 CAA (Imbert et al., 1996; Sanpei et al., 1996; Pujana et al., 1999). One striking characteristic of the trinucleotide

repeats in the normal *SCA2* alleles is the low polymorphism, as 95% of the alleles contain either 22 or 23 repeats. Disease alleles containing 32 to 59 pure repeats have been described, but the majority are in the 36-43 range (Imbert et al., 1996; Sanpei et al., 1996; Pulst et al., 1996; Zoghbi and Orr, 2000). As observed in other trinucleotide repeat diseases, the size of the expansion as well as the presence of CAA interruptions are directly related to allele stability during transmission. There have been few studies on the pattern of allelic distribution of SCA alleles in South American populations. We made a molecular characterization of *SCA1* and *SCA2* alleles in a sample of normal individuals from Rio de Janeiro, Brazil.

MATERIAL AND METHODS

Blood samples from 200 individuals (100 males and 100 females, from 18 to 60 years old) were obtained from the Blood Bank of Hospital Universitário Pedro Ernesto (HUPE/UERJ) and were made anonymous by code number. DNA was extracted from blood leukocytes according to standard procedures (Miller et al., 1988) and submitted to PCR as described elsewhere (Orr et al., 1993; Pulst et al., 1996). Reactions were performed in a final volume of 25µl with annealing steps at 62°C for *SCA1* and 66°C for *SCA2*. Amplified fragments were resolved by electrophoresis in 12% native polyacrylamide gels and visualized after silver staining. Allele sizes were estimated by correlation with molecular weight markers. Comparison between male and female allelic distributions was performed by a chi-square test with a significance of 5%. The University Ethics Committee approved the protocol of this research.

RESULTS

We identified 23 *SCA1* allele sizes in the 400 chromosomes, ranging from 10 to 39 CAG repeats, with a mean number of 27.7 ± 3.3. The level of heterozygosis was 53% and the distribution was unimodal (mode 28). Most of the alleles were in the 25- to 32-CAG interval (84%) and the least frequent alleles harbored 10, 13 and 37 repeats (0.25%). We did not detect alleles harboring 11, 12, 15, 16, 18 or 19 repeats. We found that 28-CAG alleles were more frequent in males (26.5%, n = 53) than in females (14.5%, n = 29) ($\chi^2 = 6.45$, $P < 0.05$).

Based on the analysis of *SCA2* alleles there were 10 different allele sizes, ranging from 19 to 29 repeats, with a mean of 22.1 ± 1.0 CAG. The distribution was also unimodal (mode 22), and 95% of the alleles harbored 21 to 23 CAG repeats. The least frequent allele contained 28 repeats (0.25%). We did not detect alleles containing 20 CAG. The level of heterozygosis was 8%, and no difference in allele distribution between the sexes was found.

DISCUSSION

There are few reports concerning the allelic distribution of SCA alleles in the Brazilian population. There is a considerable mixture of ethnical groups in our population's genetic background, resulting in a high level of heterogeneity (Mingroni Netto et al., 1999). We adopted the population of Rio de Janeiro to represent the Brazilian genetic pool since industrial and economic development of this region has promoted internal migrations from all regions of the country.

The allelic polymorphism at the *SCA1* locus that we observed in our sample is similar to that found in studies performed in Spain, where *SCA1* normal alleles harbored 6 to 40 trinucle-

otide repeats (Pujana et al., 1999), and India, where the range varied from 7 to 37 CAG (Saleem et al., 2000) and different from the restricted range (29 to 37 CAG) found in the Netherlands (Warrenburg et al., 2002). On the other hand, while high levels of heterozygosis were reported in the USA (80%) and Spain (72%) (Ranum et al., 1994; Pujana et al., 1999) we found only 53% heterozygous individuals. The most frequent allele in our sample contained 28 CAG repeats; the same was found in a Japanese survey (Takano et al., 1996). However, this is different from the modes (31 and 32) reported for a mixed population of Portuguese and Brazilian individuals (Silveira et al., 2002) as well as the mode reported for USA and India populations, which is 29 (Chung et al., 1993; Ranum et al., 1994; Saleem et al., 2000; Basu et al., 2000). In Spain the modal value is 31 CAG repeats (Pujana et al., 1999). The pattern of *SCA1* allele distribution found in our Brazilian sample is similar to that found in India, Japan and USA where 87-90% of the alleles harbor 25 to 32 CAG repeats (Ranum et al., 1995; Takano et al., 1996; Saleem et al., 2000). As also observed in Indian and Spanish populations, we did not detect alleles harboring 11, 12, 15, 16, 18 or 19 CAG repeats.

Molecular analysis of the *SCA2* locus revealed 10 different allele sizes, ranging from 17 to 29 CAG, as also observed in a French sample (Imbert et al., 1996). The variation in the number of CAG repeats found in Japan and India was also similar to our findings (Sanpei et al., 1996; Saleem et al., 2000), but differs from that found in Korea (Jin et al., 2001). The most frequent allele of our sample had 22 CAG, and it represented 84% of the alleles analyzed. Elevated frequencies of the 22-CAG allele were also reported in French and Japanese populations (Sanpei et al., 1996; Imbert et al., 1996). The great majority of alleles (95%) in our sample were included in a limited range of 21 to 23 repeats, as also observed in USA and Japan (Takano et al., 1996; Geschwind et al., 1997). In conclusion, we found a pattern of distribution similar to that found in other populations.

ACKNOWLEDGMENTS

We are grateful to the board of directors of the Blood Bank of HUPE (UERJ) for sending the blood samples. We thank Dr. Patricia Diniz for suggestions and critical reading of the manuscript. Research supported by CEPUERJ, CAPES (to R.S. Gestinari) and PIBIC/UERJ (to M. Campos-Jr.).

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