Trinucleotide Repeats in 202 Families With Ataxia

A Small Expanded \((\text{CAG})_n\) Allele at the SCA17 Locus

I. Silveira, PhD; C. Miranda, MSc; L. Guimarães, BSc; M.-C. Moreira, MSc; I. Alonso, BSc; P. Mendonça, BSc; A. Ferro, BSc; J. Pinto-Basto, MD; J. Coelho, BSc; F. Ferreirinha, BSc; J. Poirier, BSc; E. Parreira, MD; J. Vale, MD, PhD; C. Januário, MD; C. Barbot, MD; A. Tuna, MD; J. Barros, MD; R. Koide, MD; S. Tsuji, MD, PhD; S. E. Holmes, PhD; R. L. Margolis, MD; L. Jardim, MD, PhD; M. Pandolfo, MD; P. Coutinho, MD, PhD; J. Sequeiros, MD, PhD

Background: Ten neurodegenerative disorders characterized by spinocerebellar ataxia (SCA) are known to be caused by trinucleotide repeat (TNR) expansions. However, in some instances the molecular diagnosis is considered indeterminate because of the overlap between normal and affected allele ranges. In addition, the mechanism that generates expanded alleles is not completely understood.

Objective: To examine the clinical and molecular characteristics of a large group of Portuguese and Brazilian families with ataxia to improve knowledge of the molecular diagnosis of SCA.

Patients and Methods: We have (1) assessed repeat sizes at all known TNR loci implicated in SCA; (2) determined frequency distributions of normal alleles and expansions; and (3) looked at genotype-phenotype correlations in 202 unrelated Portuguese and Brazilian patients with SCA. Molecular analysis of TNR expansions was performed using polymerase chain reaction amplification.

Results: Patients from 110 unrelated families with SCA showed TNR expansions at 1 of the loci studied. Dominantly transmitted cases had \((\text{CAG})_n\) expansions at the Machado-Joseph disease gene \((\text{MJD})\) (63%), at SCA2 (3%), the gene for dentatorubropallidoluysian atrophy \((\text{DRPLA})\) (2%), SCA6 (1%), or SCA7 (1%) loci, or \((\text{CTG})_n\) expansions at the SCA8 (2%) gene, whereas \((\text{GAA})_n\) expansions in the Friedreich ataxia gene \((\text{FRDA})\) were found in 64% of families with recessive ataxia. Isolated patients also had TNR expansions at the MJD1 (6%), SCA8 (6%), or FRDA (8%) genes; in addition, an expanded allele at the TATA-binding protein gene \((\text{TBP})\), with 43 CAGs, was present in a patient with ataxia and mental deterioration. Associations between frequencies of SCA2 and SCA6 and a frequency of large normal alleles were found in Portuguese and Brazilian individuals, respectively. Interestingly, no association between the frequencies of DRPLA and large normal alleles was found in the Portuguese group.

Conclusions: Our results show that (1) a significant number of isolated cases of ataxia are due to TNR expansions; (2) expanded DRPLA alleles in Portuguese families may have evolved from an ancestral haplotype; and (3) small \((\text{CAG})_n\) expansions at the TBP gene may cause SCA17.

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SUBJECTS, MATERIALS, AND METHODS

SUBJECTS

Individuals with SCAs who were referred to UnIGENe, Instituto de Biologia Molecular e Celular (Porto, Portugal) between 1997 and 2000 were included on a consecutive basis. These individuals were referred to this laboratory for the molecular diagnosis of SCAs. Dominant inheritance was assumed based on the presence of at least 1 affected family member in 2 or more successive generations. Recessive transmission was presumed when the patient had a history of consanguinity or siblings affected without (aged) affected parents. An isolated occurrence was assumed in the absence of a family history. We studied 202 unrelated families: 145 lived in Portugal, and 57 in Brazil. Dominant inheritance was apparent in 106 families, whereas recessive transmission was suspected in 33 Portuguese families; 63 individuals had isolated cases of SCA.

METHODS

Peripheral blood was collected from patients and their relatives after written informed consent was obtained. Genomic DNA was isolated from peripheral blood leukocytes using standard techniques.17

Molecular analysis of the CAG and CTG repeat loci were performed by polymerase chain reaction (PCR) amplification using the published primer sequences.14,16,18 The PCR was carried out with 1 mM of each primer, 200 μM of deoxynucleotides, 1.0 mM of magnesium chloride, 10 mM of Tris (pH 9.0), 50 mM of potassium chloride, 1 U of Taq polymerase, and 2% formamide, in a final volume of 25 μL. Polymerase chain reaction products were analyzed on 6% polyacrylamide gels. Allele sizes were determined by comparing migration relative to an M13 sequencing ladder. DNA sequencing was performed to accurately assess repeat size and the presence of interruptions. Sequencing reactions were performed using a Thermosequenase DNA sequencing kit (USB, Cleveland, Ohio) with 5 μL of amplified DNA. Both the CTA and CTG repeats on the SCA8 gene are polymorphic, and PCR assay determines the combined size of the 2 repeats.

The analysis of the intronic (GAA), on the FRDA gene was performed by PCR using primers 2500F12 and 104FGAA21 for expanded alleles, and primers GAA-R and GAA-F for normal alleles, following the conditions described.22 Expanded allele sizes were analyzed by comparing migration relative to molecular weight standards. Normal sizes were assessed by analysis of fluorescent-labeled PCR products in an automated DNA sequencer (model 4200; Li-COR, Lincoln, Neb) using 5.5% Long Ranger gels (FMC Bioproducts, Rockland, Me).

STATISTICAL ANALYSES

Possible differences between Portuguese and Brazilian groups in normal repeat frequency distributions were assessed using 2 nonparametric tests: the Kolmogorov-Smirnov 2-sample test and the Mann-Whitney U test. Allele frequencies at each locus were estimated by the gene count method, and heterozygosity (H) was calculated as

\[ H = 1 - \sum_{i=1}^{n} X_i^2, \]

where \( X_i \) is the estimated frequency of the \( i \)th allele at the locus. Statistical analyses of differences in estimates of heterozygosity between Portuguese and Brazilian individuals, as well as differences in the frequency of large normal alleles for each locus, were performed with the Fisher exact test.

RESULTS

TNR DISTRIBUTIONS IN NORMAL ALLELES

The frequency distributions of normal alleles in the dominant SCAs caused by translated TNR expansions in the Brazilian group were not significantly different from those in the Portuguese group (\( P > 0.05 \) using the Mann-Whitney U test and Kolmogorov-Smirnov 2-sample test) (Figure 1). In the case of the SCA2 locus, the normal (CAG)\( n \) alleles ranged in size from 17 to 31 repeats in the Portuguese group and from 19 to 24 repeats in the Brazilian group; the allele with 22 repeats was the most frequent in both groups. No significant differences were found between the 2 groups at the most recently identified SCA8, SCA12, and SCA17/TBP ataxia loci (Figure 1). Interestingly, no SCA8 alleles smaller than 18 repeats and larger than 35 repeats in the Brazilian patients, or larger than 30 repeats in the Brazilian patients, were observed. A third class of extremely large normal alleles at the SCA8 locus, varying in size from 40 to 91 CTG repeats,23 was not found in either group; this might be due to the small sample size. At the SCA12 locus, small normal alleles were closely distributed around an allele with 10 CAGs, which represented more than 65% of all normal alleles in each group; the other class of normal alleles comprised CAG repeat sizes of 12 to 18 units. A large normal SCA12 allele containing 28 CAG repeats was also observed in the Portuguese group. The recently described SCA17/TBP ataxia locus presented a distribution of normal allele sizes varying from 29 to 40 CAGs in
both groups, with distributions peaking at the allele with 38 CAG units. At the FRDA gene, normal alleles can be subdivided into 2 classes depending on their GAA repeat length: short normal alleles, with 5 to 10 GAA triplets, and long normal alleles, with 12 to 60 GAA triplets. At this locus, long normal alleles represented 18% and 22% of Portuguese and Brazilian individuals, respectively (Figure 2A). Although the Brazilian group had a slightly larger proportion of long normal alleles, this difference was not significant.

The most polymorphic loci were DRPLA, MJD1, SCA1, SCA8, SCA17/TBP, and SCA6, whereas SCA2, SCA7, and SCA12 were the least polymorphic ones (Table 1).

Figure 1. Distribution of triplet repeats in normal alleles at SCA1, SCA2, MJD1, SCA6, SCA7, DRPLA, SCA8, SCA12, and SCA17/TBP loci in studied individuals of Portuguese (black bars) and Brazilian (hatched bars) origin. Vertical axes represent allele frequency, and horizontal axes represent number of repeat units.

SCA indicates spinocerebellar ataxia; MJD, Machado-Joseph disease; DRPLA, dentatorubropallidoluysian atrophy; and TBP, TATA-binding protein. SCA6 and SCA12 refer to the loci for the respective diseases, not to the genes themselves.

Analysis of loci involved in the SCAs showed that 110 unrelated patients (55%) had ataxia due to a TNR expansion. Analysis of loci with translated (CAG)n tracts showed that 78 unrelated patients with ataxia had 1 allele in the expanded range. Expansions at the MJD1 locus were found in 114 individuals, 102 affected and 12 asymptomatic, from 67 families that had ataxia with dominant inheritance (63%), as well as in 4 isolated cases (6%). Six individuals (5 affected) from 3 families with dominant ataxia (3%) had an expanded allele at the SCA2 gene. An expanded allele at the DRPLA locus was found in 5

FREQUENCY OF TNR EXPANSIONS

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patients and 1 possibly affected individual, from 2 kindreds with dominant ataxia (2%). One patient from a family that had ataxia with dominant inheritance (1%) had an expanded allele at the SCA6 locus. Three patients from 1 kindred with dominant ataxia (1%) had 1 expanded allele at the SCA7 gene. Analysis of the untranslated (CTG)n at the SCA8 gene showed that 4 patients from 2 unrelated families with dominant ataxia (2%), as well as 4 isolated cases (6%), exhibited 1 expanded allele. Expansions of the intronic (GAA)n at the FRDA gene (Figure 2B) were found in both alleles in 31 patients from 21 families with recessively inherited ataxia (64%) and in 5 isolated cases (8%).

FREQUENCY OF LARGE NORMAL ALLELES

Evidence from several populations has suggested that the disease prevalence of many of these SCAs may be associated with the presence of large normal alleles at the respective loci. To study the frequency of TNR expansions in our 2 groups relative to the frequency of normal alleles of larger size at the various loci, we used the criteria of Takano et al. (Table 3). Large normal alleles at the SCA1 (>34 repeats) and SCA6 (>13 repeats) loci were significantly more frequent in Brazilian individuals than in the Portuguese individuals. Normal alleles in the upper tail of the SCA2 distribution (>22 repeats) appeared to be overrepresented in the Portuguese group, but the P value obtained was of borderline statistical significance.

GENETIC AND CLINICAL FEATURES OF PATIENTS

The clinical phenotypes were very heterogeneous. All of our affected individuals showed clinical symptoms of cerebellar ataxia, with or without other associated features. Epilepsy was present in patients with DRPLA from 1 family, only cognitive impairment was observed in some pa-
patients with DRPLA and SCA8, and visual impairment was seen in affected individuals with SCA7. Two of the 3 families with SCA2 had been thought to have MJD. The families with SCA6, SCA7, and SCA8 and 1 of the 2 kindreds with DRPLA did not have a previous clinical diagnosis of these disorders. Altogether, 13 patients with no family history tested positive for a TNR expansion: 4 isolated patients had a mutation for MJD (2 of them having a clinical diagnosis of possible MJD), 3 of the 5 isolated patients had a mutation for SCA8 (1 of these disorders), and the (CTG)n expansion at the SCA8 gene was present in 4 isolated cases.

### SCA2 ALLELE WITH 32 CAG REPEATS

The analysis of the SCA2 gene in our patients disclosed an allele with 32 CAGs in a 64-year-old patient with ataxia. Sequence analysis of this allele showed an interrupted repeat configuration of (CAG)3(CAA)3(CAG)9, encoding 43 glutamines. This patient began experiencing symptoms of gait ataxia at age 52 years. During the last 5 years, he exhibited progressive mental deterioration and dementia. The family history indicated that his deceased mother presumably had the same disease. There are no other patients in this family. The normal allele range previously described for TBP was 25 to 42 CAG repeats.

### SMALL EXPANDED ALLELE AT THE SCA17/TBP LOCUS

Besides the mutations described previously, the analysis of the CAG repeat at the TBP gene showed an expanded allele with 43 units in a 64-year-old patient with ataxia. Sequence analysis of this allele showed an interrupted repeat configuration of (CAG)3(CAA)3(CAG)9, CAACAGCAA(CAG)3, encoding 43 glutamines. This patient began experiencing symptoms of gait ataxia at age 52 years. During the last 5 years, he exhibited progressive mental deterioration and dementia. The family history indicated that his deceased mother presumably had the same disease. There are no other patients in this family. The normal allele range previously described for TBP was 25 to 42 CAG repeats.

### DOMINANT ATAXIA AND FRDA

The analysis of TNR sizes at the loci implicated in SCA in a large group of Portuguese and Brazilian patients allowed the identification of an expansion in more than half of them, including a significant number without a family history. This study showed that DRPLA disease, frequent in Asian populations but rare in European individuals, was present in the Portuguese families. On the other hand, no expanded alleles were seen at the SCA12 locus, confirming previous impressions that they are very rare. (GAA)n expansions at the FRDA gene were also frequent among our recessive Portuguese families with ataxia (64%). The genetic basis of approximately 40% of families with ataxia remains unknown.

In most cases of SCAs, phenotypic features are helpful indicators of the underlying genotype. Dementia and seizures associated with symptoms of cerebellar ataxia are characteristic features of DRPLA; however, in 1 of our families with the DRPLA mutation, the only patient available had symptoms of cerebellar ataxia alone.
ISOLATED CASES OF TNR EXPANSIONS

We identified the molecular basis in 13 patients with ataxia who had no family history. Eight (13%) did not have a clinical diagnosis of a specific SCA type. A recent study has also shown that 19% of cases of apparently idiopathic ataxia were due to TNR expansions. Previous findings have implicated a de novo expansion of 63 CAG repeats at the TBP gene in a 14-year-old Japanese patient, confined to a wheelchair at age 13 years with symptoms of ataxia and intellectual deterioration. Exchanges in the SCA17/TBP gene have also been found in 4 Japanese families with SCA17. Moreira, Alonso, Ferro, and Ferreirinha and Laboratório de Genética Médica, Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto (Dr Sequeiros, Mr Miranda, and Mss Guimarães, Moreira, Alonso, Ferro, and Ferreirinha), Serviço de Neuropediatría, Hospital Maria Pia (Dr Barbot), and Serviço de Neurologia, Hospital Geral de Santo António (Drs Tuna and Barros), Porto, Serviço de Neurólogo, Hospital Fernando Fonseca, Amadora (Dr Parreira), Serviço de Neurologia, Hospital Egas Moniz, Lisboa (Dr Vale), Serviço de Neurologia, Hospital Universidade Coimbra, Coimbra (Dr Januário), and Serviço de Neurologia, Hospital São Sebastião, Feira (Dr Coutinho), Portugal; Center Hospitalier de l'Université de Montréal, Hôpital Notre-Dame, Montréal, Quebec (Dr Pandolfo, Ms Miranda, and Ms Poirier); Serviço de Genética Médica, Hospital Clínicas, Porto Alegre, Brazil (Dr Jardim); Department of Neurology, Brain Research Institute, Niigata University, Niigata, Japan (Drs Tsuji and Koide); and Johns Hopkins University School of Medicine, Baltimore, Md (Drs Holmes and Margolis).

Author contributions: Study concept and design (Drs Silveira, Januário, Margolis, Koide, Tsuji, and Holmes and Ms Guimarães); acquisition of data (Drs Pinto-Basto, Vale, Parreira, Januário, Barbot, Tuna, Barros, Holmes, Margolis, Jardim, Pandolfo, Coutinho, and Sequeiros, Messrs Miranda, Moreira, Mendonça, and Coelho, and Mss Moreira, Ferro, Ferreirinha, and Poirier); analysis and interpretation of data (Drs Barbot, Koide, Pandolfo, Tsuji, and Sequeiros, Mr Miranda, and Ms Poirier); drafting of the manuscript (Drs Silveira, Januário, Margolis, Koide, Tsuji, and Holmes and Ms Guimarães); critical revision of the manuscript for important intellectual content (Drs Pinto-Basto, Parreira, Vale, Januário, Barbot, Tuna, Barros, Jardim, Coutinho, and Sequeiros, Messrs Mendonça and Coelho, and Mss Moreira, Alonso, Ferro, Ferreirinha, and Poirier); statistical expertise (Ms Guimarães); obtained funding (Drs Barbot, Pandolfo, and Sequeiros); administrative, technical, and material support (Drs Pinto-

SMALL CAG EXPANSION AT THE SCA17/TBP GENE

The normal CAG size range described for the TBP gene (in 2525 chromosomes belonging to individuals of European, Asian, African American, and Hispanic origin) was 25 to 42 repeat units. Previous findings have implicated a de novo expansion of 63 CAG repeats at the TBP gene in a 14-year-old Japanese patient, confined to a wheelchair at age 13 years with symptoms of ataxia and intellectual deterioration. Exchanges in the SCA17/TBP gene have also been found in 4 Japanese families with SCA17. Moreira, Alonso, Ferro, and Ferreirinha and Laboratório de Genética Médica, Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto (Dr Sequeiros, Mr Miranda, and Mss Guimarães, Moreira, Alonso, Ferro, and Ferreirinha), Serviço de Neuropediatría, Hospital Maria Pia (Dr Barbot), and Serviço de Neurologia, Hospital Geral de Santo António (Drs Tuna and Barros), Porto, Serviço de Neurólogo, Hospital Fernando Fonseca, Amadora (Dr Parreira), Serviço de Neurologia, Hospital Egas Moniz, Lisboa (Dr Vale), Serviço de Neurologia, Hospital Universidade Coimbra, Coimbra (Dr Januário), and Serviço de Neurologia, Hospital São Sebastião, Feira (Dr Coutinho), Portugal; Center Hospitalier de l'Université de Montréal, Hôpital Notre-Dame, Montréal, Quebec (Dr Pandolfo, Ms Miranda, and Ms Poirier); Serviço de Genética Médica, Hospital Clínicas, Porto Alegre, Brazil (Dr Jardim); Department of Neurology, Brain Research Institute, Niigata University, Niigata, Japan (Drs Tsuji and Koide); and Johns Hopkins University School of Medicine, Baltimore, Md (Drs Holmes and Margolis).

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Basto, Vale, Januario, Tuna, Barros, Koide, Tsuji, Holmes, Margolis, Pandolfo, and Sequieros, Messrs Miranda, Moreira, Mendonça, and Coelho, and Ms. Silveira; Porto, Portugal (e-mail: isilveir@ibmc.up.pt). UnIGENe, IBMC, Rua do Campo Alegre 823, 4150-180, PRAXIS Programme, FCT. and Ferreirinha are the recipients of scholarships from the PRAXIS Programme, FCT.

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Corresponding author and reprints: Isabel Silveira, UniGENe, IBMC, Rua do Campo Alegre 823, 4150-180, Porto, Portugal (e-mail: isilveir@ibmc.up.pt).

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