Human Molecular Genetics, 1997, Vol. 6, No. 5 709–715

Molecular and clinical correlations in spinocerebellar ataxia 2: a study of 32 families

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Received December 9, 1996; Revised and Accepted February 19, 1997

Spinocerebellar ataxia 2 (SCA2) is caused by the expansion of an unstable CAG repeat encoding a polyglutamine tract. One hundred and eighty four index patients with autosomal dominant cerebellar ataxia type I were screened for this mutation. We found expansion in 109 patients from 30 families of different geographical origins (15%) and in two isolated cases with no known family histories (2%). The SCA2 chromosomes contained from 34 to 57 repeats and consisted of a pure stretch of CAG, whereas all tested normal chromosomes (14–31 repeats), except one with 14 repeats, were interrupted by 1–3 repeats of CAA. As in other diseases caused by unstable mutations, a strong negative correlation was observed between the age at onset and the size of the CAG repeat ($r = -0.81$). The frequency of several clinical signs such as myoclonus, dystonia and myokymia increased with the number of CAG repeats whereas the frequency of others was related to disease duration. The CAG repeat was highly unstable during transmission with variations ranging from –8 to +12, and a mean increase of +2.2, but there was no significant difference according to the parental sex. This instability was confirmed by the high degree of gonadal mosaicism observed in sperm DNA of one patient.

INTRODUCTION

Autosomal dominant cerebellar ataxias (ADCAs) are a clinically heterogeneous group of neurodegenerative disorders. Type I ADCA, characterized by the variable association of cerebellar ataxia with supranuclear ophthalmoplegia, optic atrophy, extrapyramidal signs, dementia and amyotrophy (1,2), is genetically heterogeneous. Five different loci have been mapped: SCA1 on 6p (3), SCA2 on 12q (4), SCA3/MJD (Machado–Joseph disease) on 14q (5,6), SCA4 on 16q (7) and SCA5 on 11cen (8). SCA1 (9) and SCA3/MJD (10) were the first to be identified. The mutation is the expansion of an unstable CAG repeat encoding a polyglutamine tract. Recently, Trottier *et al.* (11), using a monoclonal antibody which specifically recognizes proteins with polyglutamine expansions, detected an abnormal 150 kDa protein in SCA2 patients, suggesting that this disorder was caused by translated CAG expansions. Very recently, three independent groups (12–14) identified the SCA2 gene which carries an expanded CAG repeat in its coding sequence in patients, confirming this hypothesis. The gene encodes a 1312 amino acid protein of unknown function (12,14).

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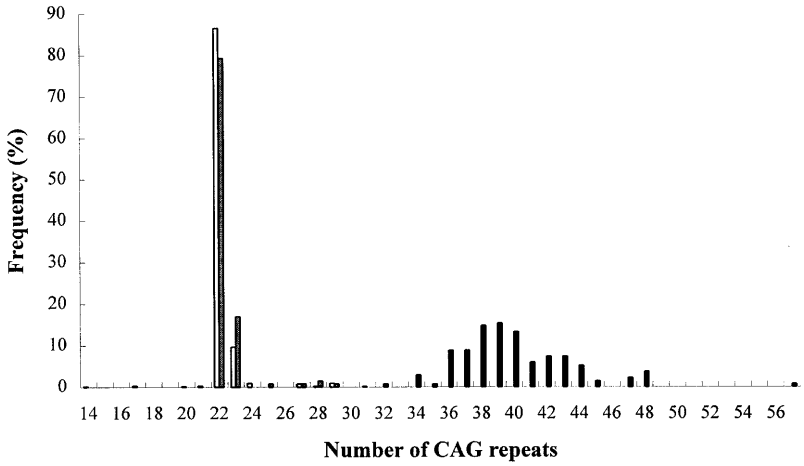

Figure 1. Distribution of CAG repeats on 135 pathological chromosomes from 32 families with the SCA2 mutation and on 642 normal chromosomes. White bars, controls; grey bars, normal; black bars, pathological chromosomes from patients.

In this study, we have determined the frequency of the SCA2 mutation in type I ADCA families and have analyzed the molecular and clinical features of this mutation.

RESULTS

Frequency of the SCA2 mutation

The SCA2 mutation was detected in 30 out of 184 families with ADCA type I (15%). Eight of these families were previously shown or suspected to be linked to the SCA2 locus (15–19) and nine were identified as SCA2 by screening with the monoclonal antibody 1C2 (11,20) which specifically recognizes proteins with expanded polyglutamines. An expanded CAG repeat at the SCA2 locus was also found in two isolated cases with no known family histories (2%), but this could not be confirmed since the parents were deceased. The ages at death of the parents were 70 and 80 years for one isolated case, and 38 and 70 for the other. The SCA2 families were of different origins: 13 were French, six North African (four Moroccan, one Tunisian, one Algerian), four West Indian (three from Martinique, one from Guadeloupe), four German and the other five from Portugal, Serbia, Guyana, Jordan and China.

Distribution of the CAG repeats in the SCA2 gene

Analysis of 642 normal chromosomes revealed the presence of two major alleles with 22 (86.4%) and 23 (9.7%) CAG repeats (Fig. 1). Despite low heterozygosity (24%) compared to SCA1 and SCA3/MJD (80% and above) (12), normal SCA2 alleles carried from 14 to 31 CAG repeats. All the normal (n = 20) alleles that were sequenced were interrupted by 1–3 CAA repeats, except the smallest (14 repeats), that was a pure CAG repeat.

The number of CAG repeats in the expanded alleles of 111 affected individuals and 24 at-risk carriers ranged from 34 to 57 repeats with a median of 39 (Fig. 1). Notably, alleles with 34 and 35 repeats were found only in asymptomatic carriers, with ages at examination from 29 to 56 years. The two isolated cases carried expanded alleles of 37 and 39 CAG repeats, respectively. An expanded allele with 40 CAG repeats, carried by an affected father, underwent contraction during transmission to his still unaffected 19 year old son (Fig. 2). Because of the young age of this at-risk individual, it was not possible to determine whether

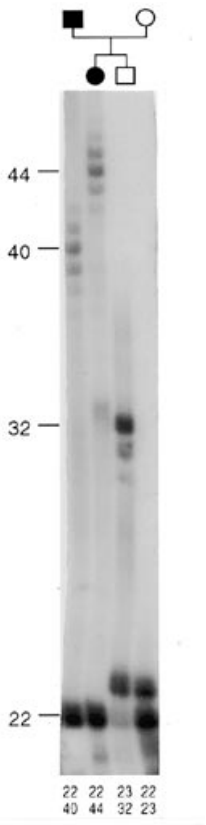

Figure 2. Contraction of a 40 CAG expanded allele to 32 CAG repeats during paternal transmission. The number of repeats on both alleles is indicated for each individual below the corresponding lane.

this represents reversion to the normal range, or whether this 32 CAG repeat is the smallest pathological allele. The 32 CAG repeat allele, as well as 11 independent expanded alleles, were sequenced. All consisted of pure CAG repeats.

CAG repeat length and age at onset

The mean age at onset of 110 patients was 35 ± 14 years (range 8–67). There was a significant negative correlation between the

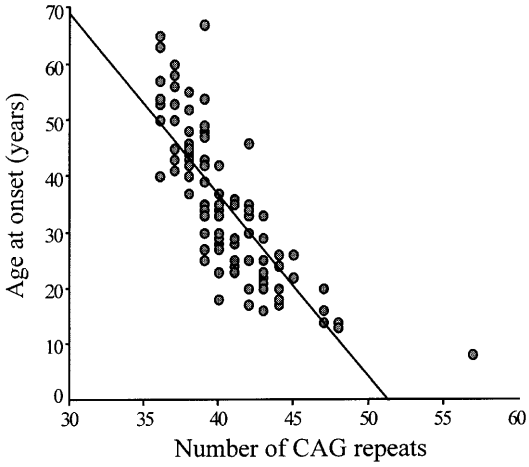

Figure 3. Correlation between age at onset (years) and CAG repeat number on the expanded allele (r= –0.81).

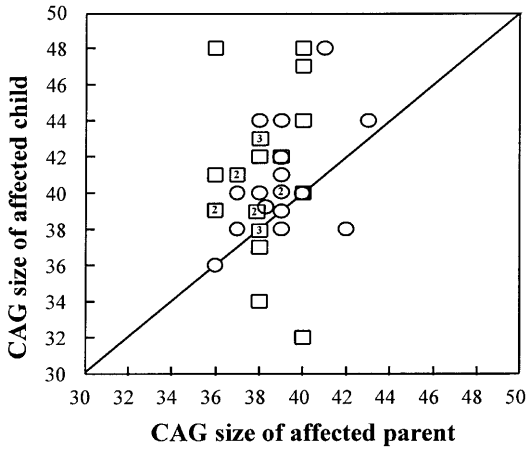

Figure 4. Variation in the number of CAG repeats in the expanded allele in 40 parent–child couples. Maternal transmissions are represented by circles and paternal transmissions by squares. Values in the symbol indicate the number of parent–child couples. The diagonal line represents transmissions with no size variation between parent and child.

size of the CAG repeat and the age at onset (r = –0.81, slope = –3.24 years per CAG repeat, P<0.001) (Fig. 3). This indicates that the size of the pathological allele accounts for 66% of the variability of the age at onset. There was no difference according to the sex of the patient or of the transmitting parent. The size of the normal allele did not influence the age at onset of the disease (r = –0.07; P = 0.44).

Transmission of the CAG repeat expansion

There was no difference between the mean ages at onset of offspring of affected mothers (34 ± 14; n = 48) and affected fathers (37 ± 14; n = 62). The mean length of maternally transmitted CAG repeats (41 ± 4; n = 52) was similar to that of paternal transmissions (41 ± 3; n = 55). The chromosome with the expanded CAG repeat was transmitted with a mean increase of 2.2 ± 4 repeats (n = 40) ranging from –8 to +12 (Fig. 4). There was no significant difference between paternal and maternal transmissions expressed in absolute or relative values. In 17 maternal transmissions, the mean variation was +1.6 ± 3 (median +1) and ranged from –4 to +7. The paternal transmissions (n = 23) showed a larger distribution, ranging from –8 to +12, with a mean variation of +2.7 ± 4 (median +3) that was not significantly different from the maternal transmissions.

In 26 parent–offspring pairs, for whom the age at onset and the size of CAG expansion were known, the mean observed anticipation was 20 years and the difference in CAG repeat number +3.7. This was significantly different from the expected 12 year anticipation, determined from the slope of the regression line for a 3.7 increase in repeat number (paired t test; P = 0.0004), indicating an observation bias in the assessment of age at onset in parent–offspring pairs.

There was no correlation between CAG repeat length and instability in the transmitting parent or the tendency to expansion (Fig. 4). The mean length of the CAG repeat was 38.3 in parents who transmitted +1 CAG repeat or more, and 38.8 in parents who transmitted CAG repeats of the same length or smaller. Therefore, larger CAG repeats did not predispose to instability or to the transmission of larger CAG repeats.

In 19 sibships with at least two siblings, in which the number of CAG repeats was determined in all siblings, the proportion of carriers was similar in offspring of paternal (16/31, 0.5) and maternal (12/20, 0.6) transmitters (P = 0.41), indicating that there is no meiotic distortion due to the sex of the transmitting parent.

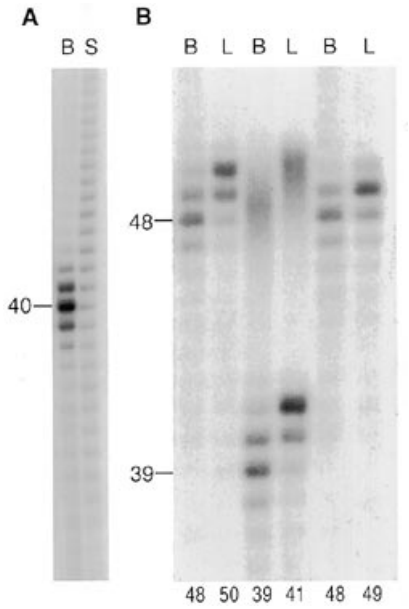

Figure 5. (**A**) PCR-amplified CAG repeats in blood (B) and sperm (S) DNA from the same SCA2 patient. (**B**) PCR-amplified CAG repeats in blood (B) and lymphoblast (L) DNA from three patients with the SCA2 mutation. The number of repeats in the normal and expanded alleles is indicated below each lane.

Gonadal and somatic mosaicism

Direct evidence of gonadal mosaicism was obtained by PCR amplification of sperm DNA from a 36 year old male patient whose blood DNA showed a major band for the expanded allele corresponding to 40 repeats (Fig. 5A). The number of repeats was markedly more variable in sperm than in blood. The increased variability in sperm included smaller, as well as larger, alleles.

Table 1. Clinical presentation in 111 patients from 32 SCA2 families

Number of patients (families)	111 (32)
Mean age at onset (years) (*n* = 110)	35.1 ± 14 (8–67)
Mean age at examination (years) (*n* = 109)	47 ± 16 (16–82)
Women:Men	49:62
Cerebellar gait ataxia	98%
Cerebellar limb ataxia	97%
Cerebellar dysarthria	92%
Decreased vibration sense in the lower limbs	62%
Slow eye movements	56%
Lower limb decreased or abolished reflexes	55%
Supranuclear ophthalmoplegia	52%
Swallow difficulties	49%
Urinary symptoms	39%
Facial myokymia	34%
Saccadic eye poursuit	28%
Fasciculations	25%
Extensor plantar response	22%
Postural tremor	21%
Amyotrophy in the lower limbs	20%
Proximal weakness in the lower limbs	14%
Mental deterioration (only memory loss)	14% (4%)
Lower limb reflexes brisk	10%
Horizontal nystagmus	10%
Dystonia	9%
Optic atrophy	6%

As in SCA1 and SCA3/MJD, when DNA extracted from blood of patients was amplified by PCR with the primers defining the CAG repeat sequence at the SCA2 locus, the expanded alleles consisted of a major band and several larger bands, whereas the normal allele appeared as a single band. These additional bands were absent or reduced in number when DNA from lymphoblastoid cell lines from patients were amplified by PCR (Fig. 5A). In addition, the expanded allele in lymphoblastoid cell lines differed by one to two repeats from the major allele in blood, supporting the hypothesis of somatic mosaicism in blood and suggesting a clonal origin of lymphoblasts.

Clinical features

Almost all patients had cerebellar ataxia (98%) which was associated with decreased vibration sense in 62%, decreased or abolished reflexes in lower limbs in 55%, extensor plantar response in 22% (Table 1). Oculomotor disturbances were often present, with slow eye movements in 56%, supranuclear ophthalmoplegia in 52%, saccadic eye pursuit in 28% and nystagmus in 10%. Facial myokymia (34%) and fasciculations (25%) are also observed in SCA2 patients.

The frequency of several clinical signs associated with the cerebellar ataxia, such as dysphagia, ophthalmoplegia, extrapyramidal signs, sphincter disturbances, mental impairment, dysarthria and axonal neuropathy, were correlated with disease duration (Table 2). The mean disease durations of patients who presented these clinical signs and those that did not, were significantly different. The size of the CAG repeat was significantly larger in patients with dystonia, myoclonus or myokymia than those without (Table 2). Both the size of the CAG repeat and disease duration influenced the frequency of decreased or absent reflexes in the lower limbs, decreased vibration sense, amyotrophy in the lower limbs, slow eye movements and fasciculations.

Table 2. Clinical signs dependent on the size of the CAG repeat or/and disease duration

Clinical sign	Mean CAG repeat length			Mean disease duration (years)		
	Absent (N)	Present (N)	*P*	Absent (N)	Present (N)	*P*
Dysarthria	39.5 ± 3 (8)	40.6 ± 4 (97)	ns	4.8 ± 4 (8)	12.6 ± 8 (97)	<0.01
Decreased or absent reflexes in the lower limbs	39.8 ± 3 (59)	41.6 ± 4 (45)	<0.01	8.9 ± 7 (59)	15.8 ± 7 (45)	<0.0001
Amyotrophy in the lower limbs	40.0 ± 3 (80)	42.4 ± 5 (21)	<0.01	11.2 ± 8 (80)	15.4 ± 8 (21)	<0.05
Fasciculations	40.0 ± 3 (74)	41.8 ± 4 (25)	<0.05	10.6 ± 8 (74)	17.0 ± 7 (25)	<0.0005
Decreased vibration sense	41.1 ± 3 (35)	39.8 ± 3 (62)	<0.05	8.9 ± 8 (35)	13.7 ± 8 (62)	<0.01
Distal hypoesthesia (touch, prink)	40.5 ± 3 (86)	38.8 ± 1 (8)	ns	10.9 ± 7 (86)	19.5 ± 8 (8)	<0.005
Slow eye movements or viscosity	39.5 ± 3 (41)	41.2 ± 4 (53)	<0.05	8.8 ± 7 (41)	13.8 ± 7 (53)	<0.01
Dystonia	40.3 ± 3 (95)	42.9 ± 4 (9)	<0.05	12.0 ± 8 (95)	12.4 ± 9 (9)	ns
Parkinsonian syndrome	40.6 ± 4 (89)	39.9 ± 2 (16)	ns	11.2 ± 8 (89)	16.2 ± 9 (16)	<0.05
Myoclonus	40.2 ± 3 (99)	47.7 ± 7 (4)	<0.0001	12.2 ± 8 (99)	11.3 ± 7 (4)	ns
Myokymia	40.0 ± 3 (61)	41.7 ± 4 (26)	<0.05	11.4 ± 8 (61)	13.7 ± 9 (26)	ns
Memory loss or dementia	40.2 ± 3 (84)	40.9 ± 3 (18)	ns	10.8 ± 7 (84)	17.3 ± 9 (18)	<0.005
Swallowing difficulties	40.1 ± 3 (50)	40.9 ± 4 (49)	ns	9.3 ± 7 (50)	15.3 ± 8 (49)	<0.0005
Sphincter disturbances	40.5 ± 3 (53)	40.5 ± 4 (47)	ns	8.9 ± 6 (53)	15.9 ± 8 (47)	<0.0001

DISCUSSION

In this study, an expanded CAG repeat at the SCA2 locus was found in 15% of ADCA type I families of various geographical origin including Europe, North Africa, Middle-East, China and South America. In this population, the SCA2 mutation was as frequent as SCA1 (14%), but less than SCA3/MJD (34%). It is not yet possible to determine whether the relative frequency of SCA2 is similar in all countries, but it is of interest that this mutation was detected in Portugal, where the SCA3/MJD mutation accounts for most of the families with ADCA (21). The SCA2 mutation was also detected in two of 90 patients with isolated olivopontocerebellar atrophy, none of whom carried the SCA1 or SCA3/MJD mutation. Since the DNA of the parents of these patients with no family history could not be analyzed, it could not be determined whether these were *de novo* mutations.

Unlike SCA1 and SCA3/MJD, the size distribution of the normal SCA2 allele showed little polymorphism, the 22 repeat allele accounting for 86.4%. The range (14–31) was, however, larger than previously reported (12–14). Sequence analysis revealed that, as in SCA1, the normal SCA2 repeats are interrupted, whereas expanded alleles contain pure CAG repeats. Unlike SCA1, however, where the CAG repeats are interrupted by CAT (histidine) codons (22), normal SCA2 alleles are interrupted by CAA encoding glutamine as CAG. The observation of a pure repeat of 14 CAGs in a normal SCA2 allele is also reminiscent of SCA1 in which only the smallest alleles are uninterrupted (22).

There is a difference of only three CAG repeats between the normal (14–31) and expanded (34–57) alleles, that is much smaller than in SCA3/MJD (23,24). In this study, the smallest expanded allele detected in an affected subject contained 36 repeats. The 35 repeat allele is probably also pathological, since Sanpei *et al.* (14) reported a patient with a 35 CAG repeat allele. The status of the 32 CAG repeat carried by an unaffected 19 year old remains unclear. It results from contraction of a paternally transmitted allele with 40 repeats (Fig. 2) and may constitute a regression to the normal range or may be pathological. The expected age at onset, given an expansion of this size would be over 60. However, even if the expansion is not pathological in this individual, the 32 CAG repeat allele might be unstable and undergo expansion in following generations, since it contains no interruption.

Expanded alleles were unstable during most transmissions (33 out of 40), with variations ranging from –8 to +12. Although there was a tendency for variation to be larger in paternal (+2.7 repeats) than in maternal transmissions (+1.6 repeats), the mean variation was similar. The observation of a large range of expansions in sperm DNA is consistent with the instability observed during father–child transmission. Gonadal mosaicism in sperm is more marked in SCA2 than in SCA3/MJD (25) and may explain why expanded alleles are more unstable during transmission in SCA2 than in SCA3/MJD. However, expansions of more than 20 CAG repeats, observed in SCA1, DRPLA or HD (9,26–28), have not been found in SCA2.

As in other disorders caused by expansion of a CAG repeat in the coding region of the gene, anticipation was present in families with the SCA2 mutation. The mean anticipation of 20 years in 26 parent–child couples, in which both were affected and sampled, is larger than the 12 year anticipation expected from the observed mean increase in CAG repeat number of 3.7. The selection of the parent–child couples introduces a bias in the calculation of anticipation, this is much less pronounced, however, than in SCA3/MJD (23,29). The expected anticipation, when the mean increase in CAG repeat number (2.2) is calculated from all the transmissions in the present study, is close to 7 years.

The negative correlation between the age at onset and the number of CAG repeats on the mutated allele was very strong ($r = -0.81$, $P<0.0001$). The size of the expanded allele, therefore, accounted for 66% of the variability in age at onset. Similar results were found in SCA1 (9,30) and in SCA3/MJD (23) whereas in HD, the size of the expanded allele accounted for only 50% of this variability (27). Clinical presentation, characterized by inter-individual variability, was influenced by both the size of the CAG repeat and the disease duration: the frequency of dystonia, myoclonus and myokymia increased with the size of the CAG repeat; that of ophthalmoplegia, extrapyramidal signs, sphincter and swallowing disturbances, mental impairment, dysarthria and axonal neuropathy increased with disease duration; other signs, such as decreased or absent reflexes in the lower limbs, decreased vibration sense, amyotrophy, slow eye movements and fasciculations was determined by both. In SCA3/MJD (23) and DRPLA (31), clinical presentation also varied with the number of CAG repeats. In SCA3/MJD as in SCA2, the frequency of decreased vibration sense was negatively related to the size of the repeat. However, the frequency of decreased or absent reflexes increased with the size of the CAG repeat in SCA2 patients, whereas it decreased in SCA3/MJD (23).

The clinical features of the 111 SCA2 patients were similar to those reported in previous series (15,17,19,32–34) and confirm group differences between SCA2 and SCA1 or SCA3/MJD patients (23). Decreased or absent reflexes in the lower limbs, oculomotor disturbances and amyotrophy were more frequent in SCA2 than in SCA1 and SCA3/MJD, as were postural tremor and memory impairment. These signs do not guarantee the diagnosis of SCA2 in individual patients, but do indicate the first mutation that should be searched in families where these signs are frequent. Major neuropathological features distinguish SCA2 with brainstem and cerebellar atrophy, from SCA1, with inferior olive and Purkinje cell degeneration, and SCA3, with internal pallidum and intermediolateral column lesions.

In conclusion, we have found that the SCA2 mutation is as frequent as SCA1 in this series of ADCA type I families. Notably, two isolated cases with no family history were found to carry the SCA2 expansion. Isolated cases should therefore be screened for this mutation. Age at onset and clinical signs associated with cerebellar ataxia are highly variable but are partly accounted for by both the number of CAG repeats and the duration of the disease. As in other disorders caused by polyglutamine expansions, the expanded CAG repeat is unstable. However, the instability in SCA2 appears intermediate between that observed in SCA3/MJD and SCA1, and does not appear to be related to the size of the transmitted allele. This supports the hypothesis that different *cis* or *trans* factors regulate instability at these loci (35). Finally, the pathological threshold of the CAG expansion is lower, and the curve relating CAG repeat number to age at onset is steeper than in other diseases caused by translated CAG expansions. The SCA2 protein seems, therefore, to be very sensitive to polyglutamine or, contrary to the proposed effect of SCA3/MJD protein (36), exert no protection against a putative polyglutamine toxicity.

MATERIALS AND METHODS

Patients

One hundred and eighty four index patients with ADCA type I and 90 isolated cases with olivopontocerebellar atrophy were screened for the SCA2 mutation. All consenting family members were examined using a standardized procedure. Blood samples were obtained and used for extraction of high-molecular weight DNA, and for establishment of lymphoblastoid cell lines, permitting comparison of PCR analyses from both sources. Sperm DNA was prepared according to the method of Duyao *et al.* (37).

Genotyping

Genotypes at the SCA2 locus were determined as previously described (13). The number of CAG repeats was determined by gel electrophoresis in comparison with normal and expanded alleles that had been sequenced.

Statistical analysis

Comparisons of means were performed with non-parametric tests and Student's *t* test, and comparisons of frequencies with the χ^2 and Yates corrected χ^2 tests. Mean values are given with the standard deviations. Pearson's *R* and regression coefficients were calculated to evaluate the correlation between age at onset and length of CAG repeat. The percentage of the variability of the age at onset due to the size of the pathological allele was estimated with the square of Pearson's *R* coefficient.

ACKNOWLEDGEMENTS

We are grateful to the families for participating. We thank Dr M. Ruberg for critical reading of the manuscript; Profs M. Ben Hamida, M. Clanet and Drs R. Bellance, D. Smadja, G.-G. Buisson, M. Abele and M. Serdaru for referring their patients; Y. Pothin, A. Camuzat, J. Bou and C. Penet for technical assistance. This work was supported by grants from Association Française contre les Myopathies, Groupement d'Etudes et de Recherches sur les Génomes, Verum Foundation, ADRMGNP, Biomed Concerted action (#708039 CEE) and Secrétariat d'Etat à la Recherche Scientifique de Tunisie.

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