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Molecular and clinical correlations in autosomal dominant cerebellar ataxia with progressive macular dystrophy (*SCA7*)

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Spinocerebellar ataxia 7 (SCA7) is caused by the expansion of an unstable CAG repeat in the first exon of the *SCA7* gene. We have analyzed the SCA7 mutation in 19 families and one isolated case of various geographical origins, presenting with autosomal dominant cerebellar ataxia with progressive macular dystrophy. The SCA7 CAG repeat was expanded in 77 patients and in 11 at-risk individuals, with alleles containing from 37 to 130 repeats, demonstrating that SCA7 is genetically homogeneous. Repeats on normal alleles contained from 7 to 35 CAGs. There was a strong negative correlation ($r = -0.84$) between the age at onset and the size of the CAG repeat expansion in SCA7 patients. Larger expansions were associated with earlier onset, a more severe and rapid clinical course, and a higher frequency of decreased vision, ophthalmoplegia, extensor plantar response and scoliosis. The frequency of other clinical signs such as dysphagia and sphincter disturbances increased with disease duration. The mutation was highly unstable during transmission, with a mean increase of 10 ± 16 CAG repeats, which was significantly greater in paternal (15 ± 20) than in maternal (5 ± 5) transmissions. This correlated well with the marked anticipation (19 ± 13 years) observed in the families. Gonadal mosaicism, observed in the sperm of a patient, was particularly important, with expanded alleles ranging from 42 to >155 CAG repeats. The degree of instability during transmission, resulting mostly in expansions, is greater than in the seven other neurodegenerative disorders caused by polyglutamine expansions.

INTRODUCTION

The autosomal dominant cerebellar ataxias (ADCAs) are a complex group of hereditary neurodegenerative disorders, characterized by cerebellar ataxia that may be associated with ophthalmoplegia, loss of vision, dysarthria, pyramidal and extrapyramidal signs, deep sensory loss or dementia. Classification of the ADCAs was greatly simplified by the recognition of three clinical subtypes designated ADCA I–III (1,2). To date, six loci implicated in ADCA I or III have been mapped (3), and four of the corresponding genes have been identified: spinocerebellar ataxia 1 (SCA1) (4), SCA2 (5–7), SCA3/MJD (Machado–Joseph disease) (8) and, recently, SCA6 (9).

ADCA II (1,2), however, is a clinically and genetically distinct entity, in which progressive loss of photoreceptors and bipolar cells results in progressive macular dystrophy and ultimately in blindness (10–12). The gene for ADCA II (*SCA7*) has been mapped to chromosome 3 in families of different geographical origins (13–18). Very recently, we identified the *SCA7* gene in five French families (19) and have established that ADCA II is the eighth disease, in addition to spinal and bulbar muscular atrophy (SBMA), Huntington's disease (HD), dentatorubral-pallidoluysian atrophy (DRPLA), SCA1, SCA2, SCA3/MJD and SCA6, caused by the expansion of a trinucleotide CAG repeat in the coding region of the responsible gene (20). The *SCA7* gene encodes a nuclear protein of unknown function, containing 892 amino acids.

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We now report molecular features and phenotype/genotype correlations in 19 families and one isolated case, of various geographical origins, with the SCA7 mutation.

RESULTS

The SCA7 mutation was detected in 19 families with autosomal dominant cerebellar ataxia and decreased visual acuity, including 77 affected and 11 at-risk individuals. Eleven families were North African (six Moroccan, four Algerian and one Tunisian), seven from France including one with Belgian ancestry, and one from Israel of Arabic origin. In addition, the SCA7 mutation was found in an Algerian patient with cerebellar ataxia and decreased visual acuity with no known family history. His mother, examined at age 48, was still apparently asymptomatic and his father died at age 45.

Distribution of normal and pathological alleles

The size of 154 normal chromosomes from 77 controls ranged from 7 to 35 CAG repeats with a major allele of 10 CAG repeats (75%). There were no alleles with between 19 and 35 repeats. The heterozygosity rate (41%) was intermediate between that observed in SCA1 and SCA3/MJD (80% or above) and in SCA2 (24%) (7). The largest normal alleles were found in a 58-year-old asymptomatic at-risk man and in an unaffected spouse, aged 53. The latter transmitted her 35 CAG repeat allele, without size variation, to three of her offspring, two of whom also carried expanded alleles of 47 and 51 CAG repeats and presented with ADCA II at age 29 and 32 years, respectively. These ages at onset are in the range of those observed in patients with expanded alleles of the same size suggesting that the large normal allele with 35 repeats does not influence the age at onset. The third child with alleles carrying 9 and 35 CAG repeats is still unaffected at age 18.

The number of CAG repeats in the expanded alleles of 77 affected individuals and 11 at-risk carriers ranged from 37 to 130 with a mean of 51 ± 13 and a median of 47.5. The sex of the patient or the sex of the affected parent had no effect on the size of the expansion.

Symptoms and age at onset

The mean age at onset, assessed in 71 patients (29 ± 16 years; range 1–70), was not significantly different in males (27 ± 18 years) and females (31 ± 14 years), and was not affected by the sex of the transmitting parent. Forty-seven of the 71 patients presented with both cerebellar ataxia and maculopathy. In 21 patients, the first sign was cerebellar ataxia followed by decreased visual acuity. Vision failed first in 15 patients and both signs occurred at the same time in 11 cases. In patients who presented initially with cerebellar ataxia, visual acuity was sometimes retained as long as 45 years (mean 8 ± 9 years). In the reverse situation, however, the latency between the decrease in visual acuity and the onset of cerebellar symptoms did not exceed 9 years (mean 5 ± 2 years). Nineteen patients presented with cerebellar ataxia, but without visual symptoms after a mean duration of 7 ± 5 years (range 1–23 years), and their mean age at onset (38 ± 17 years) was significantly older than in patients with both signs (26 ± 15 years, $P < 0.01$). At the time of examination, no patients presented with an isolated decrease in visual acuity. The mean age at onset of cerebellar signs, 30 ± 16 years (median 27), was similar to that of decreased visual acuity, 29 ± 17 years (median 25).

There was a significant negative correlation between the size of the CAG repeat expansion and the age at onset that was more exponential ($r = –0.84$, $P < 0.0001$) than linear ($r = –0.64$, $P < 0.001$) (Fig. 1). The correlation did not depend on the sex of the patient or of the affected parent. In the range of 40–65 CAG repeats, there was a significant linear inverse correlation between the repeat number and age at onset ($r = –0.78$, slope = –1.9 years per CAG repeat, $P < 0.0001$). The size of the normal allele did not influence the age at onset.

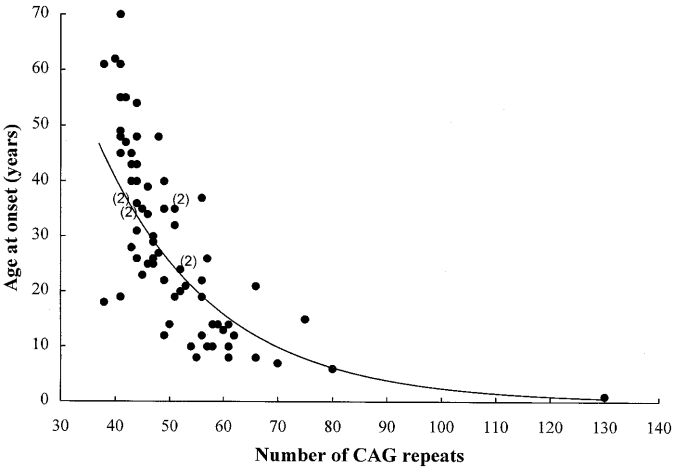

Figure 1. Correlation between the age at onset and the number of CAG repeats in expanded alleles from 77 SCA7 patients. Values in parentheses indicate the number of patients. The coefficient was calculated for an exponential regression ($r = –0.84$).

The initial symptom, like the age at onset, varied with the size of the CAG repeat. The mean size of the expanded CAG repeat was significantly larger in patients who began with visual failure (51 ± 7 CAG repeats, $n = 15$) than in those with cerebellar onset (47 ± 6, $n = 40$, $P < 0.05$). The largest expansions were found in patients with both symptoms at onset (mean 68 ± 23 CAG repeats, $n = 11$, $P < 0.0001$).

Clinical features

All patients had cerebellar ataxia. It was associated with decreased visual acuity in 83% (Table 1). Fundoscopy performed in 48 of these patients, showed pigmentary macular degeneration in 27, optic atrophy in 9 or both in 12. Almost all patients (78%) had increased reflexes, associated in 41% with extensor plantar reflexes and in 52% with spasticity of the lower limbs (Table 1). Viscous eye movements or slow saccades were frequently observed (88%), as was ophthalmoplegia (53%), but nystagmus (18%) was rare. Hearing was impaired in 24% of the patients. Extrapyramidal signs (18%), as well as postural tremor (19%), were also seen. Disease duration was significantly longer in patients with swallowing difficulties (13 ± 9 versus 7 ± 6) and sphincter disturbances (13 ± 10 versus 8 ± 6) than in those without ($P < 0.01$). In patients with similar mean disease durations, the number of CAG repeats were significantly longer in those with decreased visual acuity (53 ± 14 versus 43 ± 3 years, $P < 0.001$), extensor plantar reflexes (54 ± 16 versus 48 ± 9 years, $P < 0.05$) and ophthalmoplegia (52 ± 9 versus 47 ± 8 years, $P < 0.01$). This was confirmed when patients were divided into two groups according to the size of the expansion (Table 2): in patients with longer expansions the age at onset and the age at which they could no longer walk unaided were significantly earlier than in those with smaller expansions, and decreased visual

acuity, ophthalmoplegia, extensor plantar response and scoliosis were significantly more frequent.

Nine cerebral magnetic resonance images showed various degrees of olivo-ponto-cerebellar atrophy, with additional cortical atrophy in three. Electrophysiological studies of conduction velocities were normal in nine patients and electromyograms showed signs of denervation in two out of nine. In all tested patients, visual evoked potentials (n = 11) and brainstem evoked auditory potentials (n = 9) were abnormal.

Table 1. Frequency of clinical signs in 71 patients from 20 SCA7 families

Measurement	Value
Number of patients (families)	71 (20)
Men:women	39:32
Mean age at examination (years, n = 66)	38.9 ± 18.4 (2–85)
Mean age at onset of cerebellar ataxia (years, n = 64)	30.3 ± 15.9 (1–70)
Mean age at onset of decreased visual acuity (years, n = 47)	28.9 ± 17.5 (1–69)
Mean disease duration until death (years, n = 10)	13.8 ± 7.5 (3–25)
Clinical sign	**Frequency**
Cerebellar gait ataxia	100%
Cerebellar limb ataxia	95%
Cerebellar dysarthria	98%
Decreased visual acuity	83%
Slow eye movements or decreased saccadic velocity	88%
Hyper reflexia in the lower limbs	78%
Decreased vibration sense	62%
Swallowing difficulties	58%
Ophthalmoplegia	53%
Extensor plantar reflexes	52%
Sphincter disturbances	50%
Lower limb spasticity	41%
Lower limb wasting	25%
Decreased hearing	24%
Postural tremor	19%
Parkinsonian symptoms	18%
Facial myokymia	16%
Mental deterioration	11%
Scoliosis	11%

Table 2. Clinical differences as a function of the number of CAG repeats in SCA7 patients

	Number of CAG repeats		
	CAG < 49	CAG ≥ 49	*P*-value
Patients	38	39	
Mean age at onset (years)	40 ± 13	18 ± 11	<0.0001
Mean disease duration (years)	11 ± 9	8 ± 7	NS
Mean age at which no longer walk unaided (years)	58 ± 9	26 ± 14	<0.001
Decreased visual acuity (%)	67	97	<0.001
Ophthalmoplegia (%)	41	68	<0.05
Extensor plantar response (%)	36	70	<0.001

NS, not significant.

Transmission and anticipation

A change in the size of the expanded allele during transmission was observed in 40 parent–child pairs, with a mean increase of

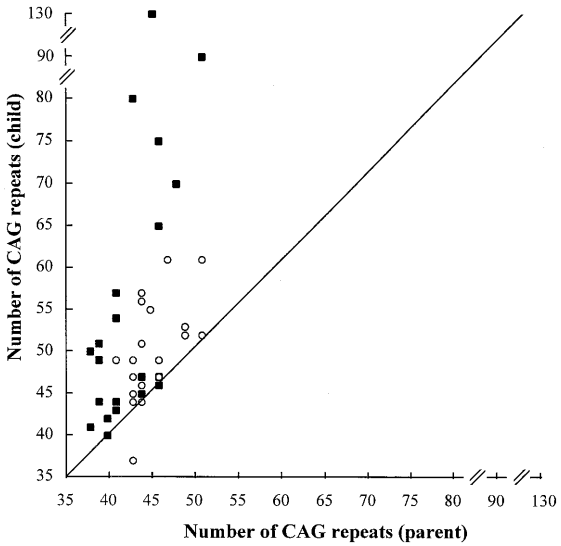

Figure 2. Instability of the SCA7 repeat in 40 parent/child transmissions. Black squares indicate paternal transmissions, open circles indicate maternal transmissions. Note the absence of decreases in CAG repeat length in paternal transmissions and the greater instability.

10 ± 16 CAG repeats (median 4.5). Instability was significantly greater during male (n = 21, mean +15 ± 20 CAG repeats, median 10) than during female transmissions (n = 19, mean +5 ± 5, median 3.5, P < 0.05) (Fig. 2). Paternal transmissions were associated with a larger distribution of changes (range 0 to +85 CAG repeats) than in maternal transmissions (range –6 to +14). Notably, the only contraction of six CAG repeats occurred during a mother to son transmission; the six largest expansions ranging from +19 to +85 CAG repeats were all paternally transmitted. The mean anticipation, assessed in 38 parent–offspring pairs, was 19 ± 13 years (range –10 to +47 years), but the sex of the transmitting parent had no significant effect (fathers: n = 15, 22 ± 16 years; mothers: n = 23, 17 ± 10 years). There was a significant correlation between the number of CAG repeats in paternal expanded alleles and the transmission of larger CAG repeats. The mean length of the expanded allele was 46.5 repeats (n = 6) in fathers who transmitted +20 CAG repeats or more to their offspring, and 41.1 (n = 15) in fathers who transmitted alleles containing <20 additional CAG repeats (P < 0.01). The largest paternal expanded alleles are therefore prone to greater instability during transmission, with marked bias toward increases.

There was a significant negative correlation between the number of CAG repeats and disease duration until death (r = –0.7, P < 0.05) (Fig. 3). The increase in the number of CAG repeats in successive generations is therefore associated with both an earlier age at onset and a more severe course of the disease.

Somatic and gonadal mosaicism

Direct evidence of somatic and gonadal mosaicism was obtained by Genescan analysis of blood and sperm DNA from a 34-year-old male patient (Fig. 4) who transmitted his expanded 45 CAG repeat allele to his son, with an additional 85 CAG repeats, resulting in striking anticipation. The electrophoretic pattern of expanded alleles in blood DNA showed a major peak surrounded by several peaks of larger or smaller molecular weight, indicating significant mosaicism, that was not observed

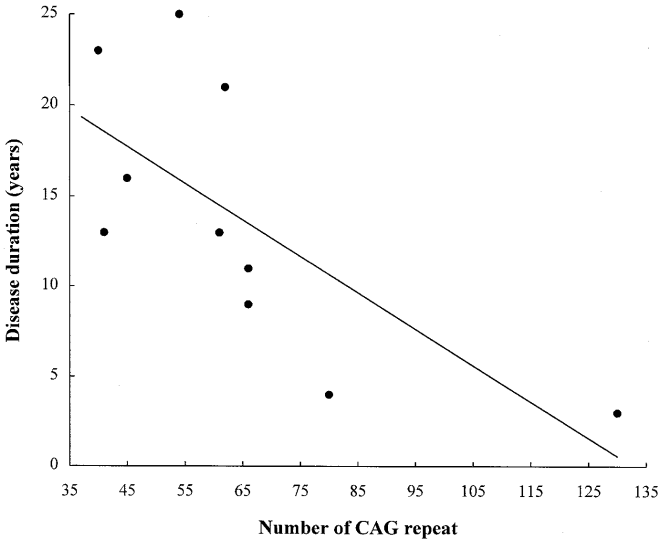

Figure 3. Correlation between the number of CAG repeats and disease duration until death. The larger the repeat the shorter the duration ($r = -0.7$, $P < 0.05$).

in DNA from lymphoblastoid cell lines (not shown). Gonadal mosaicism was markedly greater than somatic mosaicism. Few alleles were smaller but many were larger than the major peak observed in blood (45 CAG repeats). The major peak in sperm contained 49 CAG repeats, but a continuous distribution of alleles ranging from 42 to >155 CAG repeats, larger than the 130 CAG repeats transmitted by this patient to his son, was visible.

DISCUSSION

An expanded CAG repeat at the SCA7 locus was detected in the 19 families and one isolated case with ADCA II included in this study, originating from France, Belgium, Morocco, Algeria, Tunisia and Israel. This confirms that ADCA II is likely to be genetically homogeneous, as suggested by linkage analysis in families of different origins (13–18), and from molecular analysis of the SCA7 CAG repeat in five French families (19). The observation of a clinically isolated case with the expansion suggests that molecular analysis is useful for diagnosis in patients with typical features of ADCA II even without family histories. Since DNA from the parents was not available, it is not possible to determine if this isolated case occurred by *de novo* mutation.

The size distribution of normal alleles (7–35, $n = 154$) was larger than previously reported (19), reducing the difference between the normal and the pathological range (37–130, $n = 77$) to only two CAG repeats. This very small gap between the normal and the pathological range is similar to that observed in SCA2 (6,21), SCA1 or HD, and is much smaller than in SCA3/MJD or DRPLA (22). This is particularly important for molecular diagnosis. In addition, a $(CGG)_5$ repeat is also located 41 bp upstream of the CAG repeat in the first exon of the SCA7 gene (19), and lies in the PCR product generated during mutation analysis. A CGG repeat polymorphism is also observed in HD (23,24), in addition to the pathogenic CAG repeat, which led to inaccurate estimates of the HD CAG expansion in initial surveys using primers that flanked both repeats (25–28). Re-evaluation of the size of expansions in HD using primers flanking only the CAG expansion revealed that the normal and pathological ranges did not overlap (24). This type of polymorphism has not been detected in the seven normal and two expanded SCA7 alleles sequenced so far, but cannot be excluded. Unfortunately, due to the high G/C content of the DNA sequence between the CGG and the CAG repeats, primers that flank only the SCA7 CAG repeat have not been successfully designed.

The clinical features of the 71 analyzed patients were similar to those reported in previous studies (11,13,19,29). In addition to the presence of progressive macular degeneration, which is characteristic of ADCA II, hypoacousia, which is absent or very rare in other ADCAs, was present in 24% of SCA7 patients. Increased reflexes and slow eye movements were found in >75%

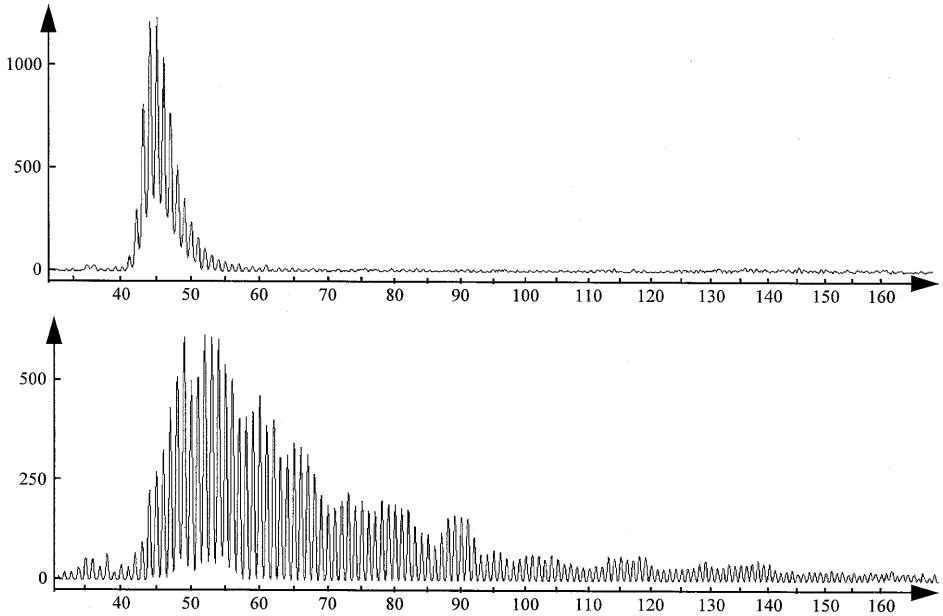

Figure 4. Comparison of CAG repeat expansions in blood and sperm DNA of an SCA7 patient. Electrophoretic profiles of PCR-amplified CAG repeat expansions in blood (top) and sperm (bottom) DNA obtained with Genescan software (Perkin-Elmer). Horizontal axis, number of CAG repeats; vertical axis, intensity of fluorescence (arbitrary units).

of the patients. Although these signs can be found in other types of ADCA, their frequencies are higher in SCA7 and reflect a more homogeneous clinical picture (21,30–33).

There was a strong negative correlation between the size of the CAG repeat and the age at onset ($r = -0.84$, $P < 0.0001$ for an exponential model), which indicates that 71% of the variability in the age at onset is determined by the size of the expanded allele. No influence of the normal allele was detected in our series. Clinical presentation varied according to the size of the CAG repeat and/or disease duration. Larger expansions were associated with earlier age at onset, shorter duration until death and higher frequency of decreased vision, ophthalmoplegia, extensor plantar response and scoliosis, whereas swallowing difficulties and sphincter disturbances increased in frequency with disease duration. The size of the normal allele did not influence the age at onset. The phenotype of a given SCA7 patient is therefore influenced by both the size of the CAG repeat expansion and the duration of the disease.

The expanded allele is unstable during transmissions, with a mean increase of 10 ± 16 CAG repeats. According to the calculated slope of –1.9 years/CAG repeat for a linear regression between 40 and 65 CAG repeats, this correlates well with the mean anticipation of 19 ± 13 years observed in the families. The instability of the expanded allele is significantly greater in paternal (15 ± 20 repeats) than in maternal (5 ± 5 repeats) transmissions. Among the 40 observed parent/child transmissions, only one, of maternal origin, showed decrease in the number of CAG repeats. In contrast, the six largest increases were paternally transmitted. This is reminiscent of DRPLA, in which the repeat length always increases during paternal transmissions (32,33). As in HD (34,35), large paternal expanded alleles are associated with greater increases (+20 or more) of CAG repeat number in transmitted alleles. Gonadal mosaicism is, however, greater in SCA7 than in the other analyzed neurodegenerative diseases associated with a translated CAG repeat. Increases of up to 85 CAG repeats have been observed in paternal transmissions. In sperm DNA from an SCA7 patient, most alleles were larger than the major allele in blood (45 CAG repeats). Alleles with 155 or more repeats were detected. Consistent with this, no decreases in the CAG repeat number were observed during paternal transmissions. The progressive increase in size of the CAG repeat in succeeding generations is reflected by a marked anticipation affecting both age at onset and disease severity evaluated by duration until death. Although the instability is greater in paternal transmissions, there is no significant influence of the parental sex on anticipation.

In conclusion, this analysis of 19 families and one isolated case confirms that ADCA II is genetically homogeneous and caused by a CAG repeat expansion in the *SCA7* gene. Clinically decreased visual acuity and, although less frequently, hearing loss distinguish ADCA II from other ADCAs. As in SCA2 (21), SCA3/MJD (31) or DRPLA (32,33), both the size of the expansion and disease duration influence the clinical phenotype, accounting in part for the observed inter-individual clinical variability. Although most SCA7 alleles carry CAG repeat expansions in the same size range as in SCA1, SCA2 or HD, the SCA7 mutation is more unstable during transmission, resulting in striking anticipation. This suggests that additional factors *in cis* or *in trans*, that affect stability at these loci, are likely to be involved.

Screening of families as well as isolated cases for the SCA7 mutation can now be performed by routine methods. However, because of the very small gap between the normal and pathological ranges of repeat number, to the presence of a $(CGG)_5$ repeat close to the CAG repeat, that might also be polymorphic and result in inaccurate estimates of the CAG expansion size, and to possibly incomplete penetrance (11,13), the results should be interpreted with caution, especially when small expanded alleles are detected in asymptomatic at-risk individuals.

MATERIAL AND METHODS

Patients

Seventy-six patients and 65 at-risk individuals from 19 families with clinical suspicion of ADCA type II, as well as one isolated case with cerebellar ataxia and decreased visual acuity, were screened for the SCA7 mutation. Five French families were previously analyzed (19). Detailed clinical data was available for 71 patients. Blood samples were obtained from all consenting family members and high molecular weight DNA was extracted. Sperm DNA was extracted according to Duyao *et al.* (26).

Genotyping

Genotypes and sequencing of the *SCA7* CAG expansion were performed by PCR amplification followed by gel electrophoresis, as described elsewhere (19). Mosaicism was analyzed in PCR products, amplified under the same conditions with primer 4U1024 fluorescently labeled, and resolved on an ABI-Prism 377 automated fluorescent DNA sequencer and analyzed with the Genescan software version 2.0.2 (Perkin-Elmer).

Statistical analysis

Means were compared with ANOVA and non-parametric tests, frequencies with the χ^2 test or the Yates corrected χ^2 test, when necessary.

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