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Molecular and Clinical Study of 18 Families with ADCA Type II: Evidence for Genetic Heterogeneity and De Novo Mutation

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Summary

The *SCA7* mutation has been found in 54 patients and 7 at-risk subjects from 17 families who have autosomal dominant cerebellar ataxia (ADCA) II with progressive pigmentary maculopathy. In one isolated case, haplotype reconstruction through three generations confirmed a de novo mutation owing to paternal meiotic instability. Different disease-associated haplotypes segregated among the *SCA7*-positive kindreds, which indicated a multiple origin of the mutation. One family with the clinical phenotype of ADCA type II did not have the CAG expansion that indicated locus heterogeneity. The distribution of the repeat size in 944 independent normal chromosomes from controls, unaffected at-risk subjects, and one affected individual fell into two ranges. The majority of the alleles were in the first range of 7–19 CAG repeats. A second range could be identified with 28–35 repeats, and we provide evidence that these repeats represent intermediate alleles that are prone to further expansion. The repeat size of the pathological allele, the widest reported for all CAG-repeat disorders, ranged from 37 to ~220. The repeat size showed significant negative correlation with both age at onset and age at death. Analysis of the clinical features in the patients with *SCA7* confirmed that the most frequently associated features are pigmentary maculopathy, pyramidal tract involvement, and slow saccades. The subjects with <49 repeats tended to have a less complicated neurological phenotype and a longer disease duration, whereas the converse applied to subjects with ⩾49 repeats. The degree of instability during meiotic transmission was greater than in all other CAG-repeat disorders and was particularly striking in paternal transmission, in which a median increase in repeat size of 6 and an interquartile range of 12 were observed, versus a median increase of 3 and interquartile range of 3.5 in maternal transmission.

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Introduction

Autosomal dominant cerebellar ataxias (ADCAs) constitute a group of neurodegenerative disorders that can be classified on clinical grounds into three major categories (Harding 1993). In ADCA type I, cerebellar ataxia is variably associated with other neurological features, including involvement of the central and/or peripheral nervous system. Four genes have been identified via molecular studies—*SCA1* (MIM 164400), *SCA2* (MIM 183090), *SCA3* (MIM 109150), and *SCA4* (MIM 600223)—on chromosomes 6, 12, 14, and 16, respectively (Orr et al. 1993; Kawaguchi et al. 1994; Flanigan et al. 1996; Imbert et al. 1996; Pulst et al. 1996; Sanpei et al. 1996). Although there are some group differences between phenotypes, as highlighted by Burke et al. (1997) and Giunti et al. (1998), it is difficult to make a definitive clinical diagnosis in a single patient, and genetic testing is required. ADCA type III is characterized by a pure cerebellar syndrome, is genetically heterogeneous, and includes *SCA5* (MIM 600224) on chromosome 11 (Ranum et al. 1994) and *SCA6* (MIM 183086) on chromosome 19 (Zhuchenko et al. 1997).

ADCA type II is distinguished clinically by the presence of a pigmentary maculopathy (Harding 1982, 1993) and by the variability in the mode of presentation and striking anticipation (Enevoldson et al. 1994; Benomar et al. 1994, 1995; David et al. 1996). ADCA type II has been linked to the *SCA7* (MIM 164500) locus on chromosome 3p in all previous reports of families with this phenotype (Benomar et al. 1994; Gouw et al. 1995; Holmberg et al. 1995; David et al. 1996; Jöbsis et al. 1997). It has hitherto been thought to be genetically homogeneous. The *SCA7* gene has recently been identified (David et al. 1997; Del Favero et al. 1998; Koob et al. 1998), and the mutation has been confirmed to be a CAG expansion in the coding region similar to that in seven other diseases: spinal-bulbar muscular atrophy (SBMA), Huntington disease (HD), dentatorubropallidoluysian atrophy (DRPLA), and spinocerebellar ataxia (SCA) 1, 2, 3 (Machado-Joseph disease), and 6. Here we analyze the *SCA7* gene in 7 families with ADCA type I, 18 families with ADCA type II, 26 families with ADCA type III, 56 patients with idiopathic late-onset

cerebellar ataxia (ILOCA), and 1 subject with early-onset idiopathic cerebellar ataxia and maculopathy without a family history.

Material and Methods

Family Data

Eighteen families with ADCA type II who had been classified according to the criteria suggested by Enevoldson et al. (1994) were analyzed for the *SCA7* mutation. These families have different ethnic origins: 1 Brazilian, 1 Italian, 1 Indian, 1 Filipino, 1 South African, and 13 British. Seven families with ADCA type I, 26 families with ADCA type III, 56 patients with ILOCA, and 1 patient with early-onset maculopathy and cerebellar ataxia were also screened. It was confirmed that no patient had the *SCA1, SCA2, SCA3,* or *SCA6* mutation.

Genotyping

DNA was extracted from lymphocytes by standard methods. The *SCA7* mutation was identified by use of PCR with oligonucleotide primers 4.1–1,024 (fluorescently labeled) and 4.1–716 (David et al. 1997).

PCR was performed in a final volume of 50 μl containing 100 ng of DNA in 1.5 m*M* $MgCl_2$, 60 m*M* KCl, 200 μM of each dNTP, 10% of dimethyl sulfoxide, 12 pmol of each primer, and 1.25 U of *Taq* polymerase. After an initial denaturation at 95°C for 5 min, denaturation, annealing, and extension were done at 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s for 27 cycles and one final extension for 2 min. The PCR products were checked on 3.2% agarose gels before analysis on an ABI 373 and 377 DNA sequencer by means of GENESCAN software (ABI).

Haplotype reconstruction was possible in 11 families by use of six microsatellite markers flanking the *SCA7* gene and spanning 8 cM (David et al. 1996; Kröls et al. 1997): D3S3566–3698-1600- $(CAG)_n$-1287-3635-3644. Microsatellite markers were amplified and analyzed as described elsewhere by use of the same allele numbering (David et al. 1996).

For statistical analysis, means were compared with Student's *t* test or *F* test for mean distribution. Results are mean ± SD, unless otherwise indicated. Correlation analysis was done for CAG-repeat number with age at onset, age at death, and disease duration.

Results

The analysis of the *SCA7* CAG expansion showed that 17 of 18 families with ADCA type II carried the novel triplet mutation, but none of the 7 families with ADCA type I, 26 families with ADCA type III, or 56 subjects with ILOCA carried it. We identified a de novo mutation in a subject with no family history who presented with maculopathy at age 14 years (family VN, fig. 1).

Distribution of Normal, Intermediate, and Pathological Alleles

The distribution of the *SCA7* CAG repeats in our series of 944 independent chromosomes from random controls, from spouses of family members with ADCA type II, and the normal chromosomes from affected subjects is shown in figure 2. Two distinct allele ranges are evident. The first, 7–19 repeats, contains the majority of the alleles found in normal controls and unaffected at-risk subjects. The most common allele, with 10 repeats, accounted for 72% of these normal alleles and was found in a homozygous state in 55% of normal controls, in agreement with a previous report (David et al. 1998). Alleles in the second range of 28–35 CAG repeats were found in one affected individual (genotype 29/53 repeats), in seven unaffected at-risk relatives, and in one spouse from a total of three families. These at-risk subjects, whose mean age was 58.3 ± 17.4 years (range, 39–85), shared the same haplotype with their affected relatives. Therefore, we propose that this group represents intermediate alleles with a propensity for pathological expansion, as is clearly shown in family VN (fig. 1) (Stevanin et al. 1998), whereas the first group, with alleles in the range of 7–19 repeats, represents the true normal range. Repeat-size distribution for 55 affected subjects and the 7 at-risk carriers is shown in figure 3. These pathological alleles had a range of 37 to ~220 repeats (median, 48; interquartile range, 7.75), the largest of which were 180 and ~220 repeats and were transmitted by affected fathers with 39 and 54 repeats, respectively.

Alleles with such a large number of CAG repeats have not been reported in other CAG-repeat diseases (SBMA, HD, DRPLA, SCA 1, SCA 2, or SCA 3).

There was no significant difference in the distribution of the expanded repeats between male ($n = 32$) and female ($n = 30$) subjects.

Correlation with the Number of CAG Repeats and Clinical Parameters

There was a significant inverse correlation with the age at onset of 45 patients and the number of CAG repeats ($r = 0.74$; $P < .0001$), as shown in figure 4. We did not include in the linear regression analysis the two large alleles of 180 and 220 repeats with age at onset of 2 years and 1 year, respectively, since these skewed the slope of the line disproportionately. Statistical significance was also found when the age at onset of the maculopathy and the age at onset of the ataxia were correlated independently with the size of the repeat

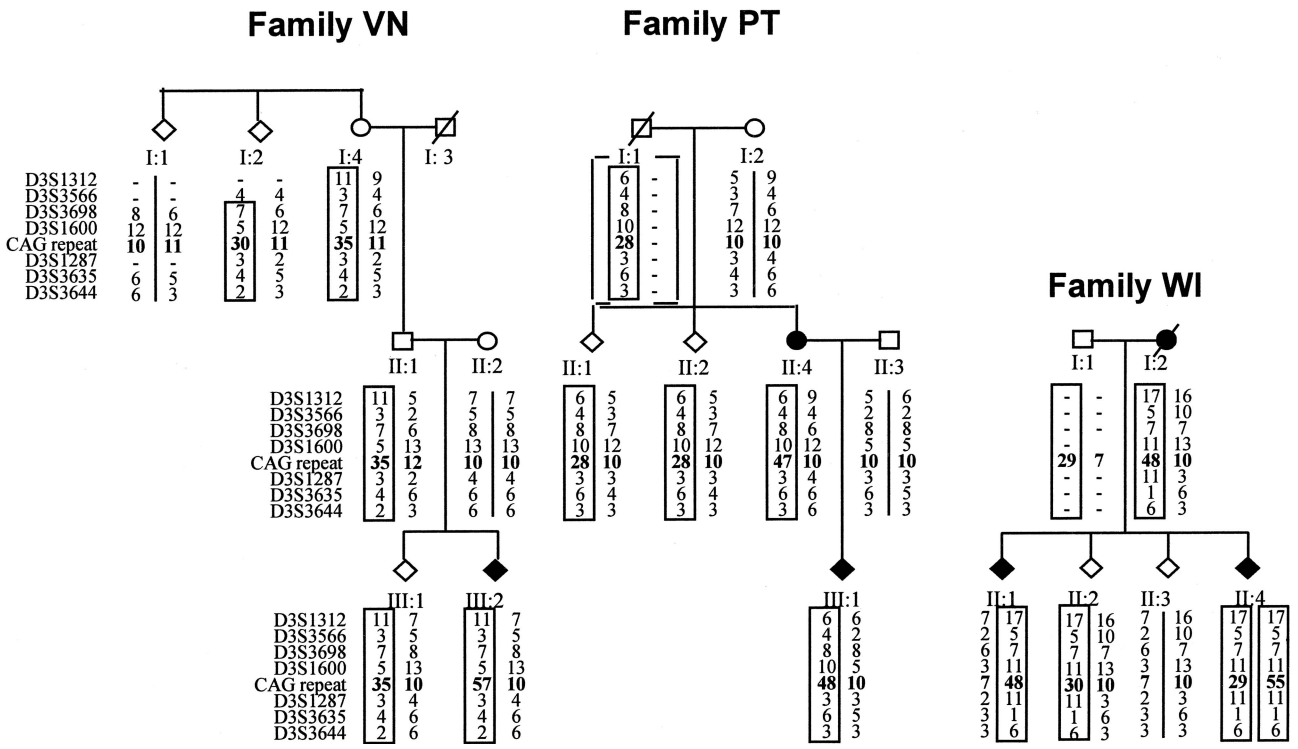

Figure 1 Pedigrees of VN, PT, and WI families. Solid symbols denote affected status. The pedigrees have been altered by changing birth order and by substituting diamond symbols for some individuals of both sexes. Haplotypes for chromosome 3p markers are shown. Boxed haplotypes cosegregate with the expanded and intermediate CAG repeats. Boldface numerals denote number of CAG repeats. Dashes are used when alleles could not be typed or deduced.

($P = .0006$ and $P < .0001$, respectively). Age at death and disease duration at death in eight patients also showed highly significant inverse correlation with CAG-repeat size ($P = .0064$ and $P = .004$, respectively).

Sex of the Transmitting Parent

The change in size of the pathological allele in 31 parent–child transmissions is shown in figure 5. The overall median was 4 and interquartile range was 9 in 31 parent-child pairs, whereas the median and interquartile range were 6 and 12 in 17 father-child pairs versus 3 and 3.5 in 14 mother-child pairs, respectively. This difference was statistically significant (F test, $P < .0001$). Increases in size >10 repeats, although less common, occurred more frequently during paternal transmissions.

There was no difference in the mean repeat number between alleles that underwent expansion during transmission by ≤10 (44 ± 6.1; $n = 23$) and >10 repeats (43.4 ± 5.5; $n = 8$).

In family VN, an intermediate allele with 35 repeats was stable during maternal transmission but underwent pathological expansion to 57 repeats when transmitted by the unaffected father. In contrast, in the WI pedigree, only mild instability of the intermediate allele was observed, with 29 repeats in the unaffected father and 30 repeats in one of his unaffected offspring. Haplotype reconstruction confirmed the parental origin of these repeats.

Clinical Features of Subjects with an Intermediate Allele

When subjects II-1 and II-2 in family PT, both with 28 repeats (fig. 1), were examined, neither ataxia nor maculopathy was found. In family VN, a 57-year-old subject (II-1) and a 39-year-old subject (III-1), both with intermediate alleles of 35 repeats, underwent extensive ophthalmologic examination, including Ishihara plates, blue-yellow plates, fluorescein angiography of the retina, and ERG, the results of which were normal. An 85-year-old relative (I-4) with the same number of CAG repeats had nonprogressive visual impairment but no other neurological signs. The ophthalmologic examination identified a maculopathy that could not be distinguished from senile maculopathy, a finding quite common in elderly subjects. Moreover, in our experience, patients who have *SCA7* with 49 repeats present with cerebellar ataxia and dysarthria rather than maculopathy, but we cannot

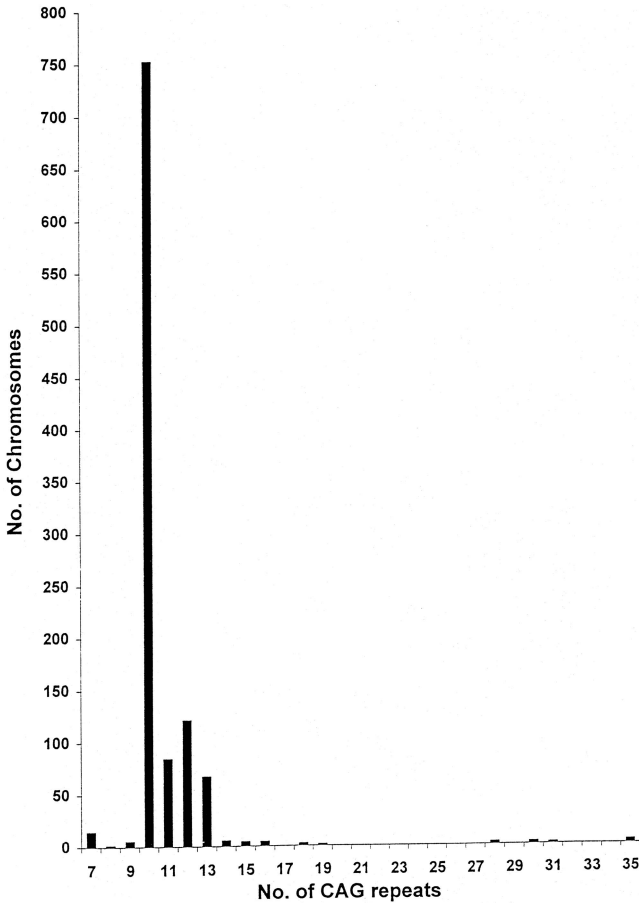

Figure 2 Distribution of CAG-repeat numbers in 1,054 independent normal chromosomes from control subjects, spouses, and affected subjects. Two ranges are evident: the first, 7–19, represents the true normal range, and the second, 28–35, represents intermediate alleles (see text).

exclude the possibility that the maculopathy was a very late presenting sign of a *SCA7* phenotype.

The WI family comes from Jamaica, and, although the parents are of different ethnic origins (Indian and Afro-Caribbean), they share the same disease-associated haplotype. The father has 29 repeats and is still unaffected at the age of 65 years.

Clinical Features of Patients with ADCA Type II

For 47 subjects, the median age at onset was 32 years (range, 1–76). The median age at death and disease duration at death in 8 patients were 48.5 years (range, 3–82) and 20.5 years (range, 2–28), respectively. Disease duration leading to an inability to walk was 10.9 years (range, 3–21) ($n = 25$).

For 36 of the *SCA7*-positive patients from 13 families, it was possible to obtain detailed clinical information. The mean time interval in 32 patients between age at onset of ataxia and age at onset of maculopathy, or vice versa, was 4.3 ± 11.6 years (range, 0–22). There was a time interval of ⩾10 years between these two presenting features in 6 patients, 3 of whom presented with maculopathy at a mean age of 32.6 ± 11.1 years; the other 3 patients presented with ataxia at a mean age of 44.3 ± 14.7 years.

The median disease duration (age at examination minus age at onset) was 15.3 years in 21 subjects with <49 repeats and 10.9 years in 15 patients with ⩾49 repeats. This difference was statistically significant ($P = .02$). The length of the CAG-repeat expansion also appeared to influence the mode of presentation. In 19 subjects in whom the onset of maculopathy preceded the onset of ataxia, the mean number of repeats on the pathological allele was significantly more at 53.5 ± 6.8, compared with 45.6 ± 3.7 repeats in 15 subjects in whom ataxia was the presenting clinical feature ($P = .0003$). In 11 patients in whom the presentation of ataxia and maculopathy were coincident, the mean number of repeats was 48 ± 5.5. The two subjects with very long repeats (180 and 220) were not included in this analysis, because the clinical information was insufficient to assign the sign(s) at onset. The sex of the transmitting parent did not influence the presenting feature of the disease (data not shown).

Table 1 compares the clinical features, separated according to repeat size, of 36 patients from 13 *SCA7* expansion–positive families with those of the 3 patients belonging to the family clinically assessed as ADCA type II but lacking an abnormal *SCA7* expansion. At the time of examination, all the *SCA7*-positive patients showed cerebellar ataxia, whereas maculopathy was identified in 32 of 36 patients. Pyramidal signs with hyperreflexia were present in 33 patients. Nuclear ophthalmoplegia was present in 11 patients, 10 of whom were from the same Brazilian family; supranuclear ophthalmoplegia was present in 25 patients and slow saccades in 20 patients. Subjects with ⩾49 repeats had a more complicated disease phenotype.

The phenotype of the *SCA7*-negative family did not show striking differences from that of the 13 *SCA7*-positive families. The median age at onset and disease duration were similar in both *SCA7*-positive and -negative patients. All three patients had both ataxia and maculopathy, and there was evidence of involvement of the pyramidal system with hyperreflexia, extensor plantar reflexes, and/or spasticity. None of the *SCA7*-negative patients showed ophthalmoplegia or slow saccades.

Overall, lower motor neuron signs and extrapyramidal signs, as detailed in table 1, were associated with a higher number of repeats, as were sphincter disturbance and sensory loss.The neuroimaging of eight *SCA7*-positive and two *SCA7*-negative patients showed cerebellar atrophy and a variable degree of brain-stem atrophy.

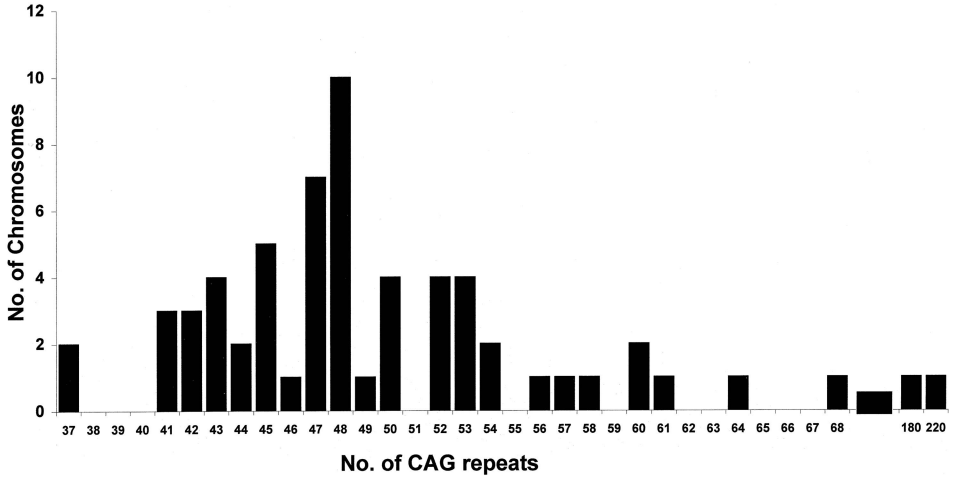

Figure 3 Distribution of repeat numbers of 55 chromosomes from patients and 7 from at-risk subjects

Reconstruction of Haplotypes Segregating with the Disease; Evidence for Multiple Founders

Three different disease-associated haplotypes were observed among five British families and one North American family with markers D3S1600 and D3S1287 (table 2), which flank the *SCA7* gene (Del Favero et al. 1998). This probably reflects the multiple origin of the *SCA7* mutation in this Anglo-Saxon population. In five of six of these kindreds, allele 4 of D3S1287 segregated with the pathological allele.

The South African and Indian haplotypes were different from those in the British families, suggesting that different founder mutations are likely to have occurred. Furthermore, the two Italian kindreds from the same area in central Italy had haplotypes that were divergent telomeric and centromeric to the *SCA7* locus except for allele 3 of D3S1287, although this allele is frequent in European controls (0.42%). These data again suggest the possibility of multiple founders.

Discussion

These data have shown that an expanded CAG repeat within the *SCA7* gene segregated with the disease in 17 of 18 families with a clinical diagnosis of ADCA type II and in one patient with no family history of the disease.

There was a significant inverse correlation between the age at onset and the number of CAG repeats, which was still observed when the age at onset of ataxia or maculopathy was correlated independently. We did not include the two largest alleles of 180 and ~220 repeats in the correlation analysis, because of the small numbers of these rare large alleles. However, an exponential plot to fit these additional data resulted in an r value of 0.9

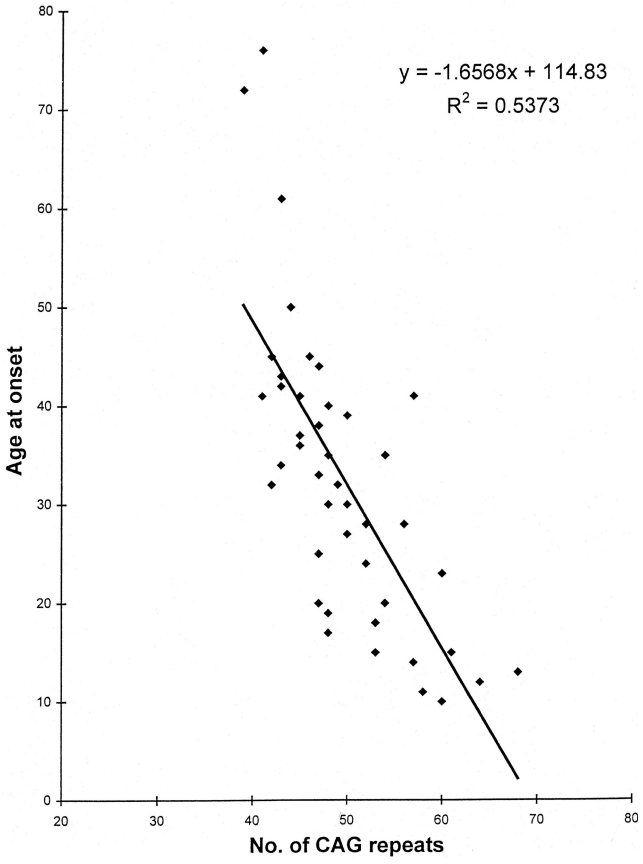

Figure 4 Correlation between the number of *SCA7* CAG repeats and age at onset in years. The linear regression line has been applied without including the two largest alleles, as these skew the slope disproportionately.

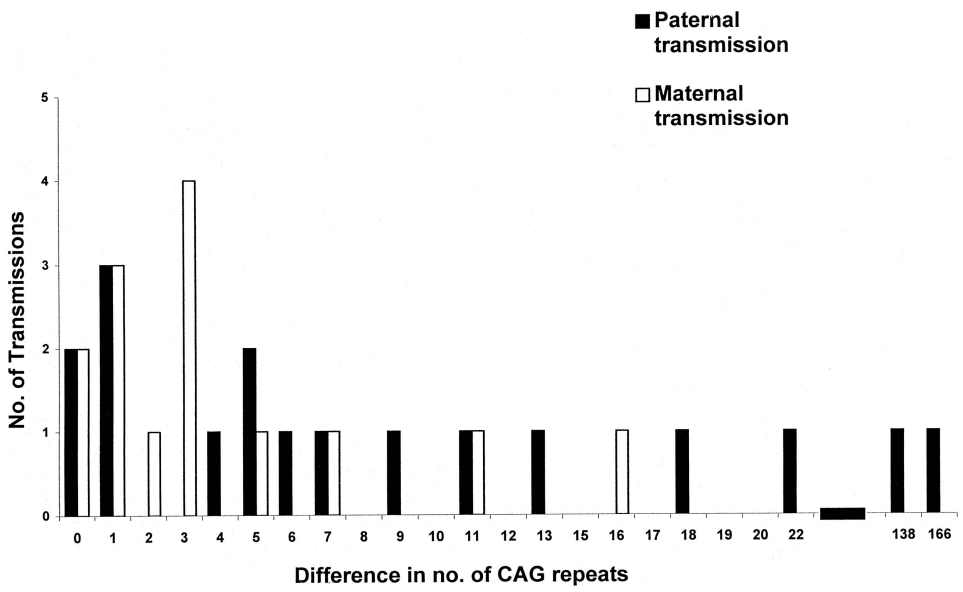

Figure 5 Difference in CAG-repeat instability between 14 mother-child pairs and 17 father-child pairs. Only positive values, representing an increase in CAG repeats during transmission, are shown for both sexes. The largest increases occur during paternal transmission.

($P < .0001$). There was also a significant correlation with age at death and disease duration until death. Therefore, higher numbers of CAG repeats are associated with an earlier age at onset and a more rapid progression of the disease, leading to an early death.

ADCA type II has a variable phenotype. Patients with <49 CAG repeats tend to have a rather pure cerebellar ataxia for many years and a more benign progression of the disease. In contrast, patients with ⩾49 repeats tend to show a phenotype characterized by cerebellar ataxia complicated with other neurological signs, with a shorter disease duration. Macular degeneration remains the most common of the associated clinical features and was present in 32 of our 36 patients. The absence of the *SCA7* expansion in our 7 families with ADCA I and 26 families with ADCA III confirmed this clinical exclusivity.

The comparison of our clinical results with those of others (David et al. 1998; Johansson et al. 1998) showed no substantial differences. Hypoacusis was a frequent associated symptom in the patients with SCA 7 reported by David et al. (1998) but was not found in our patients or in the patients reported by Johansson et al. (1998). David et al. 1998 did not report whether hypoacusis was present in patients belonging to a single family or was found in unrelated affected subjects. This sign may be a variable associated symptom, analogous to the presence of dementia in only some families with SCA 2, and may not be influenced by the number of CAG repeats (Schöls et al. 1997; Giunti et al. 1998).

The range of normal *SCA7* alleles was 7–19 in 939 independent chromosomes, and we identified an intermediate range of 28–35 repeats among seven asymptomatic relatives and one affected individual in three families with SCA 7. Evidence for multiple founders was observed after haplotype reconstruction.

The smallest pathological alleles had 37 repeats, similar to the number of repeats found in affected subjects with SBMA (37), HD (35), SCA 1 (42), and SCA 2 (33). All these diseases share a common feature in that only a few repeats define the difference between normal and pathological alleles. Rubinsztein et al. (1996) found that some subjects with HD who have an intermediate number of CAG repeats (i.e., 36–39) may be unaffected at a late age, whereas others with 36 CAG repeats are affected. This study concluded that incomplete penetrance occurred in HD, contrary to previous clinical observations. Although ADCA type II is a rarer condition, incomplete penetrance has been suggested on the basis of pedigree analysis (Enevoldson et al. 1994; Benomar et al. 1995; David et al. 1996; Kröls et al. 1997). This might be explained by these intermediate alleles that are not in themselves pathological but can be unstable during transmissions and give rise to new mutations.

The high degree of meiotic instability that we observed, along with the finding of the *SCA7* mutation in a patient with the typical phenotype of ADCA type II but with no family history, raised the possibility of frequent de novo mutations, perhaps caused by the expansion of an intermediate allele. This prompted us to screen 56 patients with ILOCA for the *SCA7* mutation, and all were negative. ADCA type II was linked to the short arm of chromosome 3 in all previously reported families despite different ethnic origins, suggesting genetic ho-

Table 1

Clinical Features of ADCA Type II Patients, Divided According to Repeat Number and Presence of the *SCA7* Pathological Expansion

Clinical Feature	*SCA7* Positive			*SCA7* Negative
	<49 Repeats (n = 20)	≥49 Repeats (n = 16)	All (n = 36)	(n = 3)
Median age at onset (range) [years]:				
Ataxia	40.5 (19–72)	22 (1–54)	32.5 (1–72)	47 (30–53)
Maculopathy	37 (17–73)	19 (1–56)	32.5 (1–73)	53 (14–54)
Median disease duration (range) [years]	11 (2–29)	9.5 (2–23)	7 (2–29)	7 (5–19)
Mean interval between onset of ataxia and of maculopathy, or vice versa (range) [years]	5.9 (0–22)	3.8 (0–17)	4.8 (0–22)	7 (0–16)
Cerebellar ataxia[a]	20 (100)	16 (100)	36 (100)	3
Maculopathy	16 (80)	16 (100)	32 (89)	3
Supranuclear ophthalmoplegia	14 (70)	11 (69)	25 (69)	0
Nuclear ophthalmoplegia	3 (15)	8 (50)	11 (31)	0
Staring gaze	0 (0)	1 (6)	1 (3)	0
Slow saccades	11 (55)	9 (56)	20 (55)	0
Optic atrophy	0 (0)	3 (19)	3 (9)	0
Dysphagia	8 (40)	12 (75)	20 (55)	1
Dysarthria	20 (100)	16 (100)	36 (100)	3
Extrapyramidal features[b]:	0 (0)	4 (25)	4 (11)	0
Facial dystonia	0 (0)	1 (6)	1 (3)	0
Dystonia in arms or legs	0 (0)	2 (13)	2 (6)	0
Chorea	0 (0)	2 (13)	2 (6)	0
Rigidity	0 (0)	2 (13)	2 (6)	0
Bradykinesia	0 (0)	0 (0)	0 (0)	0
Hypomimia	0 (0)	1 (6)	1 (3)	0
Fasciculations of face, tongue, or limbs	3 (15)	6 (38)	9 (25)	0
Increased reflexes	18 (90)	15 (94)	33 (92)	2
Babinski	4 (20)	7 (44)	11 (31)	1
Sensory loss	0 (0)	1 (6)	1 (3)	0
Dementia	0 (0)	0 (0)	0 (0)	0

[a] Values are number (%) of patients except for *SCA7*-negative patients, for whom only numbers are given.

[b] Total numbers of patients with extrapyramidal features are given, but some patients had more than one extrapyramidal sign.

mogeneity (Benomar et al. 1995; Gouw et al. 1995; David et al. 1996; Holmberg et al. 1995; Jöbsis et al. 1997). Additional evidence came from pathological and clinical classifications, which have distinguished this disease as a separate entity since its first description by Froment et al. (1937). Here we report the first family with ADCA type II without a pathological expansion at the *SCA7* locus. To exclude an error in sample handling, we obtained repeat samples from the affected members of the family.

No striking difference between the phenotypes of the *SCA7*-positive and *SCA7*-negative patients was observed, although no statistical analysis could be performed because of the small sample number of the latter group. However, neither ophthalmoplegia nor slow saccades was observed in any of the three *SCA7*-negative subjects, despite evidence of brain-stem atrophy on the computed tomographic scan in two subjects. Although an allelic disorder is a possibility, this has been reported in only one other CAG-repeat disease, in which point mutations in sites on the *SCA6* gene independent of the CAG-repeat domain can lead to episodic ataxia type 2 or familial hemiplegic migraine (Jodice et al. 1997; Ophoff et al. 1997). However, haplotype analysis demonstrates no common alleles among the affected subjects of the expansion-negative family at the *SCA7* locus (data not shown), and we may therefore exclude this hypothesis. We conclude that ADCA type II is genetically heterogeneous, in common with ADCA type I and ADCA type III.

We provide evidence that a reservoir of intermediate alleles has a tendency to undergo pathological expansion, particularly in paternal transmission (fig. 1). In family PT, the haplotype segregating with the pathological expansion is the same as that reconstructed in the two members with 28 repeats, II-1 and II-2. This haplotype was derived from the father, who died at the age of 75 years without showing either cerebellar ataxia or impairment of vision. Since DNA from this individual was not available, we cannot exclude homozygosity for

Table 2

Haplotypes Segregating in 11 *SCA7* Families in Which Haplotype Reconstruction Was Possible

Origin	*D3S3566*	*D3S3698*	*D3S1600*	*SCA7*	*D3S1287*	*D3S3635*	*D3S3644*
India	5	7	11		11	1	6
South Africa	2	6	13		2/10	3	3
United States	4	7/8	13/5		4	6	6
United Kingdom	4	7	5		4	6	6
United Kingdom	4	7	5		4	6	6
United Kingdom	4	7	5		4	6/4	6
United Kingdom	4	6	13		4	6	2
United Kingdom	4	6	13		3/4	6	3/6
Italy	4	8	10		3	6	3
Italy	3	7	5		3	4	2
Philippines	1/3	6	5/13		3	4	6

the six markers spanning the 8 cM in the *SCA7* region. However, the absence of 19 repeats in 939 independent normal chromosomes in this study and in other reports (David et al. 1998; Del Favero et al. 1998; Johansson et al. 1998) makes the hypothesis of two intermediate alleles undergoing expansion unlikely. A similar event occurred in the family VN; in the first generation, two relatives had the same haplotype, segregating with 30 and 35 repeats, respectively. In this case, we were unable to identify the sex of the transmitting carrier. Both parents died in their 80s without signs of the disease. During maternal transmission, no change in the number of CAG repeats was seen. However, the couple's son (II-1) transmitted an unchanged allele to one offspring and a 22-repeat increase in the expansion (from 35 to 57) to the other child, leading to disease onset at the age of 14 years in the latter (fig. 1).

David et al. (1998) showed that the gonadal mosaicism in SCA 7 is strikingly greater than in blood with respect to other CAG-repeat diseases. This finding could explain not only the marked anticipation but also the great variability in age at onset in siblings. This complicates genetic counseling for presymptomatic and prenatal testing, especially in male transmissions.

The *SCA7* mutation has the widest range of CAG repeats (from 37 to 220) reported to date in comparison with all the other CAG disorders, in which alleles with 90 repeats are uncommon (Sathasivam et al. 1997). The differences in the normal and disease-causing ranges among these differing disorders must lie, in part, in the ability of the rest of the protein to "tolerate" these variable polyglutamine lengths.

Greater paternal instability during transmission is a common finding in HD, SCA 1, SCA 2, SCA 3, and DRPLA. The present data show the greatest consistent increase in repeat size during meiosis thus far reported, with a mean increase of 23.9 repeats during paternal transmissions versus a mean of 4 repeats during maternal transmission. A modest decrease of two CAG repeats has been reported in one Swedish family (Johansson et al. 1998). It may be that the instability is associated with a specific intragenic polymorphism similar to that seen in SCA 3 (Takiyama et al. 1997), for which meiotic segregation distortion in favor of the expanded alleles has been proposed (Ikeuchi et al. 1996).

In summary, this study showed that the majority of families with ADCA type II and one affected subject with no family history had the *SCA7* expansion. Haplotype reconstruction highlights that different ancestral mutations are involved among families with SCA 7. The number of CAG repeats influences the phenotype by affecting age at onset, disease duration, and the presenting feature. The *SCA7* mutation shows a greater instability than that in other CAG-repeat diseases, which may result in striking anticipation. Affected children may die before reproduction, and the finding of de novo expansion is in keeping with the perpetuation of this disease. Specific intragenic polymorphism may play a role in determining meiotic instability.

Genetic diagnosis will increase the possibility to define a diagnosis in patients without a family history. The small gap, only a 2-repeat difference, between intermediate alleles and the pathological allele is similar to that found in HD. We therefore suggest that genetic counseling of subjects at risk for SCA 7 should follow the same guidelines as those for HD.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/omim (for *SCA1* [MIM 164400], *SCA2* [MIM 183090], *SCA3* [MIM 109150], *SCA4* [MIM 600223], *SCA5* [MIM 600224], *SCA6* [MIM 183086], and *SCA7* [MIM 164500]).

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