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Expanded CAG repeats in Swedish spinocerebellar ataxia type 7 (SCA7) patients: effect of CAG repeat length on the clinical manifestation

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Spinocerebellar ataxia 7 (SCA7) is a neurodegenerative disorder characterized by degeneration of the cerebellum, brainstem and retina. The gene responsible for SCA7, located on chromosome 3p, recently was cloned and shown to contain a CAG repeat in the coding region of the gene, that is expanded in SCA7 patients of French origin. We examined the SCA7 repeat region in four Swedish SCA7 families as well as in 57 healthy controls. All Swedish SCA7 patients exhibited expanded CAG repeats with a strong negative correlation between repeat size and age of onset. The repeat length in SCA7 patients ranged from 40 to >200 repeats. The largest expansion was observed in a juvenile case with an age of onset of 3 months, and represents the longest polyglutamine stretch ever reported. In patients with 59 repeats or more, visual impairment was the most common initial symptom observed, while ataxia predominates in patients with <59 repeats. Two of the Swedish SCA7 families analysed in this study were shown to be related genealogically. The other two SCA7 families could not be traced back to a common ancestor. All four families shared the same allele on the disease chromosome at a locus closely linked to SCA7, suggesting the possibility of a founder effect in the Swedish population.

INTRODUCTION

SCA7 is a neurodegenerative disorder that belongs to the clinically and genetically heterogeneous group of autosomal dominant cerebellar ataxias, ADCAs. The ADCAs have been subdivided into three groups denoted ADCA types I–III. SCA7 belongs to the ADCA type II subtype where the ataxia is associated with retinal degeneration. ADCA type II seems to be genetically very homogeneous, and in all ADCA type II families analysed the gene responsible has been localized to the same region on chromosome 3p, regardless of ethnic background (1–3). The gene for spinocerebellar ataxia (SCA) 7 recently was cloned and shown to contain a $(CAG)_n$ repeat in the coding region of the gene that is expanded in SCA7 patients (4). To date, eight disorders caused by expanded CAG repeats have been identified and include, besides SCA7, four other ADCAs: SCA1, 2, 3 and 6, as well as spinal and bulbar muscular atrophy (SBMA), Huntington's disease (HD) and dentatorubral pallidoluysian atrophy (DRPLA) (5–15). The function of the genes responsible for these disorders are in most cases unknown, and there are no significant sequence homologies between the different genes outside of the CAG repeat region. The subcellular localization of the genes also differs and for SCA7 has been shown to be nuclear. The mechanism underlying the polyglutamine disorders is not known, but a common dominant gain of function, causing neuron- and disease-specific cell death, has been proposed.

All the CAG disorders identified so far have been shown to be associated with genetic anticipation, where an earlier age of onset and a more severe progression of the disease is observed in successive generations. The age of onset has, in all cases, been shown to be correlated negatively with the size of the $(CAG)_n$ expansion, and the anticipation seen is due to the tendency of the repeats to expand during parent to child transmission (5–15). In all these disorders except SCA6, the threshold for the pathological allele is similar and of the order of 37–40 CAG repeats.

Here we describe the expansion of the SCA7 CAG repeat region in patients from four Swedish ADCA type II families and the effect of the repeat length on the clinical manifestation.

RESULTS

Analysis of the SCA7 CAG repeat region in Swedish ADCA type II patients and healthy controls

The SCA7 CAG repeat region was amplified in 16 diagnosed patients from four Swedish ADCA type II families as well as from three asymptomatic individuals known to carry the disease haplotype (1) and compared with healthy relatives and 57 unrelated healthy controls.

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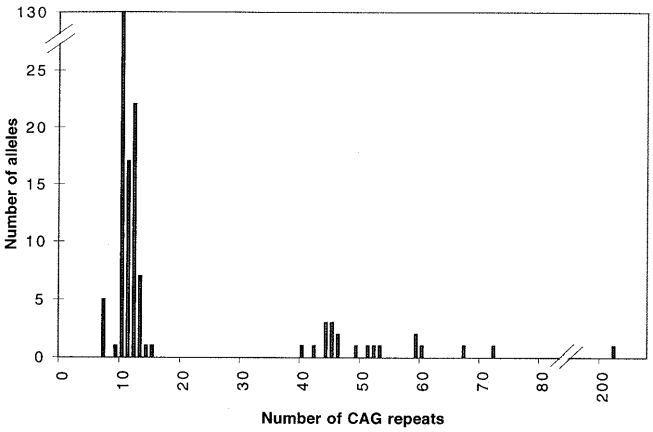

Figure 1. Distribution of SCA7 alleles of different sizes in 16 patients and healthy relatives from four Swedish SCA7 families and 57 unrelated healthy controls.

The control individuals showed allele sizes ranging from seven to 15 repeats. Ten CAG repeats was the most common allele observed, constituting 63% of the alleles in the normal population (Fig. 1), and 34 individuals (60%) were heterozygous at the SCA7 repeat locus.

In contrast, all the ADCA type II patients analysed and the three asymptomatic carriers with the disease haplotype carried expanded CAG repeats with disease alleles ranging from 40 up to >200 repeats (Fig. 2). The largest repeat, due to limitations in gel resolution, could not be determined more precisely, but is in the range of 230–300 repeat units (Fig. 2). The three at-risk individuals, all below the age of 45 years, showed repeat lengths of 42, 40 and 44 respectively (Fig. 2). For two juvenile cases (age of onset 1 and 5 years), where only DNA from paraffin-embedded tissue exists, the SCA7 CAG repeat region failed to amplify.

No obvious sex bias could be seen in the change of the size of CAG repeats in the 10 parent to child transmissions observed in the four Swedish families (Fig. 4, Table 1). The change in CAG size during parent to child transmission ranged from –2 to >200. It is of note that none of the maternal transmissions resulted in a decrease in CAG size, while this was the case in two out of the four parternal transmissions analysed. The largest expansion observed, from 49 to >200, repeats was paternally transmitted (Fig. 2).

Correlation between CAG repeat length and clinical manifestation

Clinical information on the Swedish SCA7 patients included in this study is summarized in Table 1. In contrast to the French study (4), no sphincter disturbances were observed.

In the 16 Swedish SCA7 patients studied, the age of onset was correlated negatively to the number of CAG repeats (Fig. 3). The correlation between the number of CAG repeats and the age of onset was plotted and calculated assuming a linear relationship for the two parameters ($R = -0.94$, $P<0.001$; Fig. 3). Patient A IV:16 was excluded from this calculation due to difficulties in determination of the exact repeat size of the expanded allele.

Initial symptoms in patients with 44–46 repeats occurred at ages 38–46 years, in patients with 49–60 repeats at 17–41 years and in patients with 67 repeats or more at 0–12 years of age. Ataxia preceded subjective visual impairment as the initial

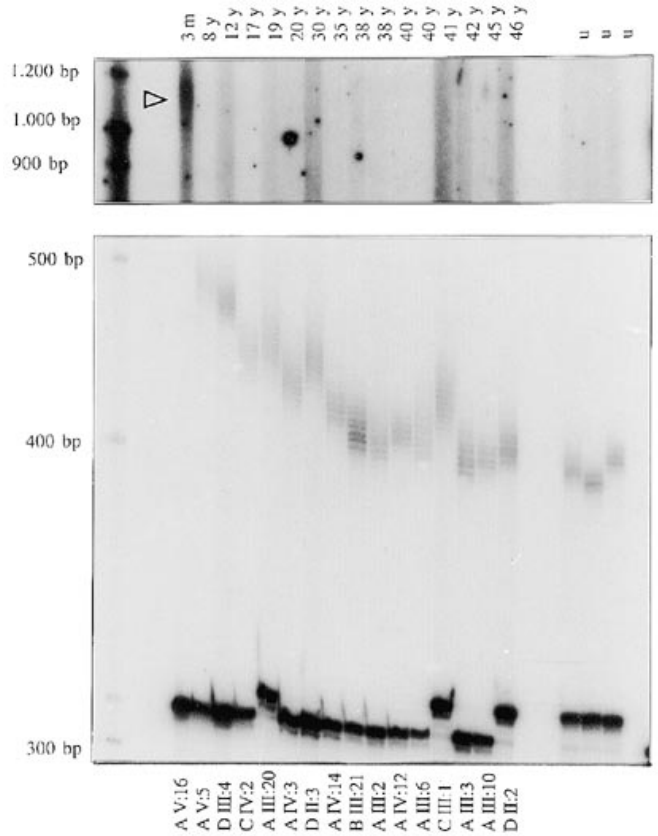

Figure 2. Autoradiogram showing the SCA7 CAG repeat region amplified in 16 Swedish SCA7 patients and three asymptomatic individuals. The gel was loaded, from left to right, in decreasing order of the patients' age at onset of disease. The three carriers, all exhibiting CAG expansions, are shown on the right (u = unaffected). The size marker shown on the left is the 100 bp ladder (N.E. Biolabs). Individual identifications are shown below the picture and their respective age at onset above (m = months, y = years, see also Table 1 and Fig. 4). In order to visualize readily the very large expansion of >200 repeats in individual A IV:16, the same gel was re-exposed for a prolonged period of time.

symptom in all but one case with <59 repeats, while four out of five cases with 59 repeats or more started with visual impairment (case A V:16 not included). Furthermore, patients' with longer repeats had more severe disease and life was substantially shortened, with childhood death in the two patients carrying the largest repeats (72 and >200 repeat units).

The most extreme case, A V:16, a boy with >200 CAG repeats in the SCA7 gene, was born without complications but showed a poor weight gain. Patent ductus arteriosus was discovered and the ductus was closed successfully. The boy had breathing difficulties, a metabolic acidosis and continued to gain weight poorly. The patient died at 7 months of age.

Haplotype analysis and genealogical investigations

In order to determine if the SCA7 mutation in the different Swedish SCA7 families analysed in this study is due to a common founder mutation, genealogical and haplotype analyses were performed. Families A and B could by genealogical analysis be traced back to a common ancestor 5–8 generations back (Fig. 4), born in the mid-17th century. Families C and D could not, however, be genealogically connected to any of the other SCA7 families.

Table 1. Clinical features in 20 patients, 11 males and nine females, from four Swedish families with SCA7

Symptom/sign	Family and no. in pedigree																			
	Family A											Family B		Family C			Family D			
	AIII:2	III:3	III:6	III:10	III:20	IV:3	IV:12	IV:14	V:4	V:5	V:16[a]	**B**III:21	IV:33	**C**III:1	IV:1	IV:2	**D**II:2	II:3	III:3	III:4
Ataxia[b]	38	42	40	45	25	29	40	35	1	8		38	4	41	7	19	46	30	12	13
Visual impairment[b]	43	49	44	50	19	20	40	37	1	10		–	5	41	7	17	47	30	11	12
Dysarthria[b]	67	50	48	50	48	30	41	36	1.8	10		43	5	46	9	27	?	30	12	16
Hyperreflexia	+	+	+	–	+	+	–	+		?		+	+	+	–	+	?	–	+	–
Spasticity	+	+	+	?	+	+	–	–		–		–	–	–	+	+	?	+	+	–
Babinski's sign	–	+	–	–	+	–	–	–		–		–	–	+	+	–	?	+	+	–
Supranuclear ophtalmoplegia	+	–	+	–	–	–	–	–		+		–	–	–	–	–	–	+	–	+
Nystagmus	–	–	–	–	–	–	+	–		–		–	–	–	–	–	–	–	+	–
Dysphagia	–	–	–	–	+	+	–	–		+		–	+	–	+	–	–	–	+	–
Muscle atrophy	–	–	+	–	–	+	–	–		+		+	+	–	+	–	–	+	+	–
Sensory impairment	+	+	+	–	+	–	–	–		–		–	–	–	–	–	–	–	–	–
Cognitive dysfunction	–	–	–	–	+	–	–	–		–		–	+	–	+	–	–	+	–	–
Age wheelchair	60	62	62	50	39	–	–	–		–		–	–	–	11	28	–	37	15	–
Age at exam	72	68	67	60	49	35	43	38	2	11	7m	48	7	50	9–15	28	52	43	19	14
Disease duration[c]	34	26	27	15	30	15	3	3	1	3	4m	10	3	9	8	11	6	13	8	2
Age at death	–	71	–	–	–	–	–	–	2	13	7m	–	7	–	15	–	–	45	19	–
No. of CAG repeats	45	44	46	44	60	53	46	49	n.e.	72	>200	45	n.e.	52	n.e.	59	45	59	n.e.	67

[a]Clinical information in the text.
[b]Age (years) at onset of ataxia, visual impairment and dysarthria.
[c]At time of examination.
m, months; ?, presence or absence of the sign/symptom not specified in available information; n.e., not examined.

Haplotype analysis was done using 10 microsatellite markers covering a 12 cM region harbouring the SCA7 locus. This analysis confirmed the relationship between families A and B, with a common disease haplotype between markers *D3S1300* and *D3S1285*, spanning the whole 12 cM region tested. However, haplotype data from family A telomerically restricts the SCA7 locus to an 8 cM interval between the markers *D3S3566* and *D3S1285* (Fig. 4). In contrast, families C and D had only two markers, *D3S1287* and *D3S3635*, in common with families A and B. For the marker *D3S1287*, allele 3 was observed on all the disease chromosomes in all four families examined, while only one of the normal chromosomes harboured allele 3 at this locus (Fig. 4). Families C and D also shared a common haplotype centromeric of the marker *D3S1287*.

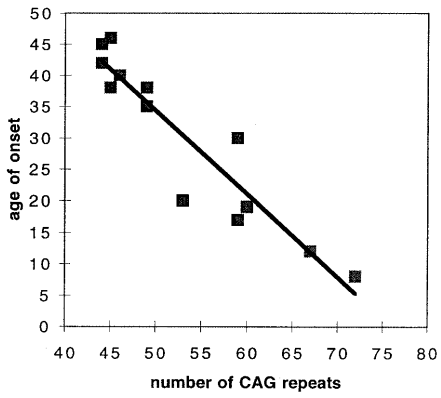

Figure 3. Negative correlation between the number of CAG repeats and age at onset of disease in 15 Swedish SCA7 patients. The fitted linear regression line has a coefficient of $R = -0.94$, $P < 0.001$.

DISCUSSION

In this study, we have analysed the SCA7 CAG repeat region in four Swedish SCA7 families and in 57 neurologically unaffected controls. The study reveals a highly polymorphic CAG expansion on the disease chromosome of all SCA7 patients tested, in contrast to a relatively stable normal allele in all unaffected family members and healthy controls. The 10 CAG allele represents 60% of the alleles in the normal Swedish population and less instability is observed in the SCA7 locus of the normal population than has been observed for most other polyglutamine disorders (5,13–14,16–17). All Swedish SCA7 patients and three asymptomatic individuals with the disease haplotype were shown to carry CAG expansions with great variability in repeat size on their disease chromosomes. The repeat size varied from 40 to >200 repeats. The size difference between the normal and mutated repeat range is as much as 28 repeats in the Swedish population, and no intermediate alleles were observed. The largest SCA7 expansion (>200 repeats) exceeds the largest expansion reported so far, including a juvenile case of HD with 180 repeats (18).

Our data show a strong negative correlation between the number of CAG repeats and the age at onset and disease severity, including death. SCA7 has been reported to show a more pronounced anticipation when paternally transmitted (4). This is less obvious in the Swedish SCA7 families, where one paternal transmission resulted in an unchanged repeat size and in two paternal transmissions small contractions were observed (44–40 and 42 repeats respectively). These three individuals are still asymptomatic but, assuming a linear correlation between CAG repeat size and age at onset of disease, the carriers have an expected age at onset of 42–47 years and are likely to develop the disease in the near future. However, the most extreme anticipation

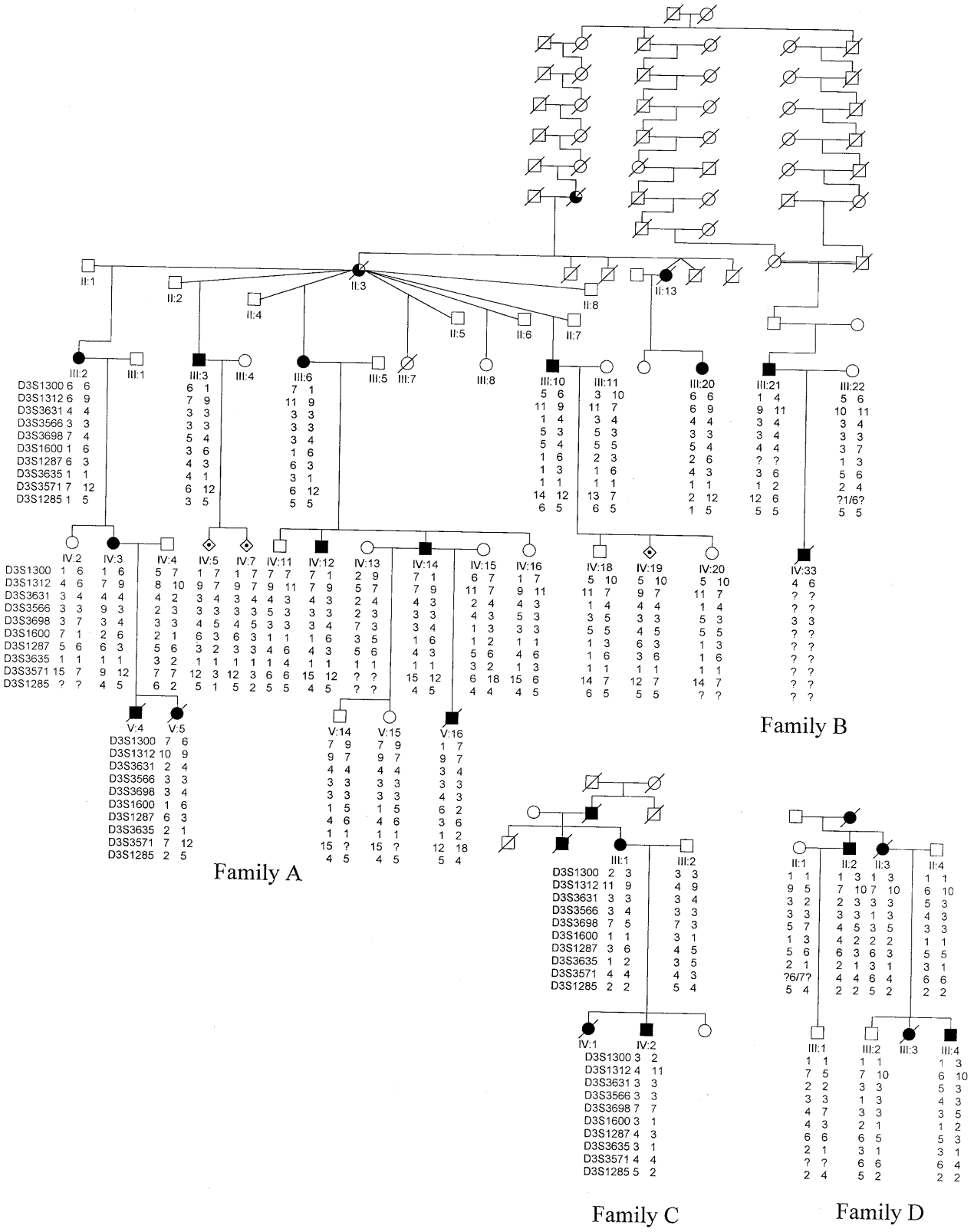

Figure 4. Pedigrees of four Swedish SCA7 families, A–D. Circles indicate females, squares indicate males, filled symbols represent affected individuals and unfilled symbols unaffected individuals. Microsatellite markers are shown in order from the telomere to the centromere, with *D3S1300* as the most telomeric marker.

observed, from 51 to >200 repeats, is also paternally transmitted. The fact that the CAG repeat in this case has expanded to more than four times its original size from one generation to the next suggests high repeat instability during spermatogenesis.

The cardinal symptoms of SCA7, cerebellar ataxia and visual impairment, were present in all but one case, BIII:21, who has no decrease in visual acuity 13 years after onset of the disease although the retina shows slight pigmentary mottling of the type seen in SCA7. Visual impairment was the initial symptom in all patients but one (A V:5) with ≥59 repeats. Patient A V:5 started with ataxia 2 years before visual impairment was noted; however, at the same time as ataxia developed, a slight supranuclear ophthalmoplegia for ascending vertical movements was observed.

Even if the onset of overt ataxia could be defined in all cases, several patients reported a history of clumsiness or a feeling of clumsiness since childhood although they were able to participate in ordinary physical school activities. Thus, 'subclinical' impairment of coordination due to the disease process may precede clinical onset of SCA7.

By genealogical studies, we have been able to show that two of the four families analysed in this study have a common ancestor that dates back to the mid-17th century. This is 5–8 generations before the appearance of any individual with documented symptoms of SCA7 and suggests that a pre-mutation exists in these families. It is possible that an increase over the observed, relatively stable, range of normal alleles (7–15 repeats) makes the locus more unstable and the carriers at risk for a further increase in the repeat to a pathological size. Alternatively, they may carry a polymorphism or mutation outside of the CAG repeat region that predisposes this allele to further expansion, as has been proposed for Machado–Joseph disease in which an intragenic CGG/GGG polymorphism 3′ of the CAG repeat seems to effect the stability of the repeat (19,20). The haplotypes carrying a CGG on the expanded allele and a GGG on the normal allele were reported to show significantly larger repeat instability than individuals homozygous for either the CGG or the GGG polymorphism, suggesting an inter-allelic interaction involved in the repeat instability (19).

Previous reports have mapped the SCA7 locus to the 12 cM region between markers *D3S1300* and *D3S1285* by recombinations (21) and, more recently, to a 5 cM region between markers *D3S1600* and *D3S3635* on yeast artificial chromosomes (YACs) (4). Taken together with these data, our haplotype data suggests that all Swedish SCA7 patients may indeed have a common ancestor, as they all share the same allele for the marker *D3S1287* (immediately flanked by *D31600* and *D3S3635*) on their disease chromosomes. Since it might be difficult genealogically to establish kinship further back than that which has already been done in these families, a polymorphism within or immediately next to the SCA7 gene that enables linkage disequilibrium studies would be needed to test this hypothesis.

MATERIALS AND METHODS

Family and control material

This study presents clinical information on 20 SCA7 patients from four Swedish ADCA type II families, families A–D. The study includes DNA from 16 affected patients as well as three at-risk carriers with the disease haplotype (1) and 34 healthy siblings of affected individuals or non-affected spouses/parents. Family A is a five generation kindred previously described, descending from a couple born in the late-19th century in the province of Västerbotten in the northern part of Sweden (22). Family B descends from the same geographical area as family A, while families C and D both originate from different parts of the province of Dalarna in the middle part of Sweden. Diagnosis of SCA7 as well as non-carrier state was ascertained by clinical examination by a neurologist (L.F.) and an ophthalmologist (O.S.) in 12 of the patients and two of the at-risk carriers. The clinical diagnosis in the remaining eight cases (including five deceased patients) was based on information from medical records which included assessments by neurologists or neuropaediatricians. All cases had a family history compatible with autosomal dominant inheritance, with at least two family members in different generations with progressive cerebellar ataxia and pigmentary retinopathy which caused visual impairment in all but one case. The patients age at onset of ataxia, visual impairment and dysarthria are difficult to evaluate in many cases with SCA7 where the impairment has a gradual onset and progression. We defined the age at onset of a symptom as the age at which the patient (from direct interview or from available medical records) first had noticed the symptom, irrespective of whether medical contact was made or not at that time.

The normal range of CAG repeats was examined by analysis of 57 neurologically healthy individuals from northern Sweden.

Analysis of SCA7 $(CAG)_n$ repeat region

DNA was extracted from peripheral blood, histopathological material and cell lines by standard procedures (23). The repeat region was amplified by PCR using primers 4U1024 (5′-TGTTACATTGTAGGAGCGGAA-3′) and 4U716 (5′-CACGACTGTCCCAGCATCACTT-3′) (4). One primer was end-labelled using [γ-^{32}P]dATP (Amersham, 3000 Ci/mM) and T4 polynucleotide kinase. Amplifications were performed in 5 µl reactions using 0.2 mM end-labelled and unlabelled primer respectively, 1× buffer for *Taq* polymerase (Boehringer Mannheim), 0.1 mM dNTP, 10% dimethylsulfoxide and 0.1 U of *Taq* polymerase (Boehringer Mannheim). Cycling conditions were as follows: 95°C for 4 min, 57°C for 1 min, 72°C for 1 min (1 cycle), 95°C for 1 min, 57°C for 1 min, 72°C for 1 min (35 cycles) and finally 95°C for 1 min, 57°C for 1 min, 72°C for 5 min (1 cycle). Five µl of formamide dye mix were added to the samples before denaturing at 95°C for 4 min. Two µl of the product was separated on a 6% polyacrylamide–urea gel. Fragments were detected by autoradiography.

Haplotype analysis

Polymorphic microsatellite regions were amplified by PCR using primer pairs from the Genethon map (24), obtained from the Nordic Microsatellite Marker Bank, Uppsala, Sweden, or from Research Genetics. The primers were end-labelled with [γ^{32}P]dATP (Amersham, 3000 Ci/mM) using polynucleotide kinase, subjected to PCR amplification and analysed as previously described (25).

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