The role of the SCA2 trinucleotide repeat expansion in 89 autosomal dominant cerebellar ataxia families

Frequency, clinical and genetic correlates

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Summary
The spinocerebellar ataxia type 2 (SCA2) is caused by a trinucleotide (CAG) expansion in the coding region of the ataxin 2 gene on chromosome 12q. 89 families with autosomal dominant cerebellar ataxia (ADCA) types I, II and III, and 47 isolated cases with idiopathic late onset cerebellar ataxia (ILOCA), were analysed for this mutation. The identification of the SCA2 mutation in 31 out of 38 families with the ADCA I phenotype, but in none of those with ADCA II, ADCA III or ILOCA confirms the specificity of this mutation. A clinical comparison of the ADCA I patients with the three known mutations (SCA1, -2 or -3) highlights significant differences between the groups; SCA2 patients tended to have a longer disease duration, a higher frequency of slow saccades and depressed tendon reflexes. However, these neurological signs were also seen in an ADCA I family in which the SCA2 mutation was not identified, illustrating the importance of a direct genetic test. The SCA2 families were from different geographical and ethnic backgrounds. However, haplotype analysis failed to show evidence of a founder mutation, even in families from the same geographical origin. The range of normal alleles varied from 17 to 30 CAG repeats and from 35 to 51 repeats for the pathological alleles. Similar to the other diseases caused by unstable trinucleotide repeats, a significant inverse correlation has been found between the number of repeats and age of onset, and there is a significantly higher paternal instability of repeat length on transmission to offspring. The SCA2 mutation is the most frequent amongst ADCA I patients, accounting for 40%, compared with SCA1 and SCA3 which account for 35% and 15%, respectively.

Keywords: autosomal dominant cerebellar ataxia; SCA2; trinucleotide repeat

Abbreviations: ADCA = autosomal dominant cerebellar ataxia; dNTP = deoxynucleotidetriphosphate; ILOCA = idiopathic late onset cerebellar ataxia; MJD = Machado–Joseph disease; PCR = polymerase chain reaction; SCA = spinocerebellar ataxia

Introduction
The autosomal dominant cerebellar ataxias (ADCAs) are a clinically and genetically complex group of neurodegenerative disorders. ADCA type I (ADCA I) is characterized by a progressive cerebellar ataxia and is variably associated with other extracerebellar neurological features such as ophthalmoplegia, optic atrophy, peripheral neuropathy, and pyramidal and extrapyramidal signs. The presence and severity of these signs are dependent on the duration of the disease (Harding, 1982, 1993). Mild or moderate dementia may occur, but it is usually not a prominent early feature. ADCA II is clinically distinguished from the ADCA I by the presence of pigmentary macular dystrophy (Enevoldsen et al., 1994), whereas ADCA III is a relatively 'pure' cerebellar syndrome and generally starts at a later age.

ADCA I is the most common of the ADCAs and, to date, four loci have been identified as causing this phenotype. The spinocerebellar ataxia type 1 (SCA1) locus has been localized to chromosome 6p (Morton et al., 1980), SCA2 to...
chromosome 12q (Gispert et al., 1993), SCA3/Machado–Joseph disease (MJD) to chromosome 14q (Stevanin et al., 1994; Takiyama et al., 1994) and SCA4 to chromosome 16 (Flanigan et al., 1996). The genes for SCA1 and MJD/SCA3 have been identified and the causative mutation is an expanded (CAG) trinucleotide repeat (Orr et al., 1993; Kawaguchi et al., 1994). This type of mutation had already been described for Huntington’s disease, Kennedy’s disease and Dentato-rubro-pallido-luysian atrophy (Huntington’s Disease Collaborative Reasearch Group, 1993; Koide et al., 1994; Nagafuchi et al., 1994). SCA1 and SCA3 show an inverse correlation between the number of repeats and the age of onset; also noted is a greater instability during paternal transmission (Orr et al., 1993; Jodice et al., 1994; Kawaguchi et al., 1994). For SCA1 a more rapid progression of the disease has been correlated with a higher number of CAG repeats (Jodice et al., 1994). The SCA3/MJD and SCA1 families have a widespread geographical distribution and cases have been described in all major ethnic groups (Giunti et al., 1995; Silveira et al., 1996).

The identification of SCA1 and SCA3 mutations permitted a detailed genotype–phenotype correlation (Giunti et al., 1995) and even using this retrospective approach there was no sign which reliably distinguished the two diseases. In this comparison, 20 ADCA I patients were identified from 12 families which were negative for the known mutations and too small for linkage analysis. The patients in this group were clinically distinct from the SCA1 and SCA3 subjects in that their tendon reflexes were depressed or absent, and saccades tended to be slow. These features were similar to those shown in a large family of Italian origin which had shown linkage to the SCA2 locus (Giunti et al., 1995) and also reminiscent of the SCA2 Cuban family (Orozco Diaz et al., 1990; Gispert et al., 1993). On this basis we postulated that it was likely that a large proportion of these subjects would have the SCA2 mutation.

A monoclonal antibody directed against a TATA binding protein has been used to detect protein containing an abnormal stretch of polyglutamine. The presence of these pathological proteins has been detected in lymphoblastoid cell lines in cases of Huntington’s disease, SCA1 and SCA3, and also in ADCA patients identified as SCA2 by linkage analysis (Trottier et al., 1995). Recently, three groups have confirmed, using different techniques, that the SCA2 mutation is an expanded (CAG) trinucleotide repeat (Orr et al., 1993; Kawaguchi et al., 1994). This type of mutation had already been described for Huntington’s disease, Kennedy’s disease and Dentato-rubro-pallido-luysian atrophy (Huntington’s Disease Collaborative Reasearch Group, 1993; Koide et al., 1994; Nagafuchi et al., 1994). SCA2A and SCA2B (Pulst et al., 1996) in two laboratories using slightly different methods.

Patients and methods

Patients

We have identified 77 families with the clinical phenotype of ADCA I. Of these, 27 have been shown to carry the expansion in the SCA1 gene and a further 12 were SCA3 positive (Giunti et al., 1994, 1995). Analysis of the SCA2 mutation was carried out in the remaining 38 ADCA I families. In four families, linkage analysis had previously shown segregation with SCA2 markers on chromosome 12q. We also screened 14 ADCA II and 37 ADCA III subjects, as previously described (Giunti et al., 1994), and 47 ILOCA patients with no family history (Harding, 1981). Informed consent was obtained from all patients and at-risk subjects for blood samples. The study was approved by The Joint Ethical and Medical Committee of The National Hospital for Neurology and Neurosurgery, London.

Laboratory methods

DNA was extracted from lymphocytes by standard methods. The SCA2 mutation was identified using the polymerase chain reaction (PCR) using oligonucleotide primers SCA2A (fluorescently labelled) and SCA2B (Pulst et al., 1996) in a total volume of 10 µl. Amplification was carried out for 30 cycles with denaturation at 95°C for 5 min, denaturation, annealing, and extension were carried out at 94°C (30 s), 57°C (30 s) and 72°C (30 s) for 27 cycles. The PCR products were checked on 3.2% agarose gels before analysis on an ABI 377 DNA sequencer using GENSCAN software (ABI).

In both laboratories, the same positive control sample was used with genotype of 22 and 38 repeats as determined by sequencing. Haplotypes were constructed using Genethon primers for microsatellites D12S1328, D12S1329 and D12S1332, as described by Sabbadini et al. (1995).
For statistical analysis a comparison of means for the number of CAG repeats was performed with Student’s $t$ test and the frequencies analysed using the $\chi^2$ test. The comparison of median age at onset were analysed with the Kruskal–Wallis test of variance, and the clinical features was analysed using the $\chi^2$ test with Yates’ correction for $2 \times 2$ tables (SCA2 versus SCA1 versus SCA3). A correlation analysis of CAG repeat number and age of onset was also performed.

**Results**

Screening for the SCA2 (CAG)$_n$ ($n$-repeat) expansion in 38 ADCA I families, in whom SCA1 and SCA3 mutations had been excluded, showed the presence of an expanded allele in 31 families. The SCA2 mutation was not detected in any of the other ADCA II ($n = 14$), ADCA III ($n = 37$) or ILOCA ($n = 47$) patients, confirming that the phenotype associated with the SCA2 mutation is strictly within the spectrum of ADCA I.

**Distribution and structure of repeat unit**

The repeat-size distribution for the larger allele observed in 69 patients and nine asymptomatic at-risk subjects is shown in Fig. 1A.

The average repeat size on the 78 expanded chromosomes was 41.6 ± 3.2 (range 35–51). No difference was found in average repeat size between males ($n = 38$) and females ($n = 43$).

Figure 1B shows the distribution of repeat size in 184 independent normal chromosomes from random control subjects, spouses from SCA2 families and normal chromosomes of affected subjects. As previously reported (Cancel et al., 1997; Geschwind et al., 1997) the (CAG)$_{22}$ was the most frequent allele (83%) within the normal range. In our series the range of normal alleles varied from 17 to 30 repeats. The internal structure of the repeat was analysed, by sequencing, in one affected subject with a 22/38 genotype. The normal allele has a structure (CAG)$_8$ (CAA)$_1$ (CAG)$_4$ (CAA)$_1$ (CAG)$_8$, whereas a pure CAG sequence was seen in the expanded allele.

**Sex of the transmitting parents**

The size distributions of repeat size of expanded alleles transmitted by the mothers ($n = 38$) and fathers ($n = 32$) were not significantly different (data not shown). However, analysis of the repeat instability in 15 mother–child and 11 father–child pairs, as shown in Fig. 2, demonstrates a clear paternal effect, with the largest increases in repeat size observed in paternal transmissions; all stable transmissions were from mothers. On grouping the data into three classes of change in repeat size during transmission (−1 to +1, 2 to 3 and ≥4) a statistically significant difference between the sexes could be seen [$\chi^2(2) = +7.2, P = 0.02$].

Interestingly, the largest numbers of CAG repeats (50 and 51) and the smallest (35) were transmitted from the father.

**Correlation between repeat number and age at onset**

The relationship between the age at disease onset, for 66 patients in whom the data could be ascertained, and the number of CAG triplets are shown in Fig. 3. The correlation between the two variables is highly significant ($P < 0.0001$). Noteworthy is the extremely late onset (at age 74 and 78 years) in two subjects with 35 and 36 repeats, respectively, and the juvenile onset (9 and 14 years) in two subjects with 51 and 50 repeats, respectively. There was a great variation in age of onset for intermediate repeat sizes (Fig. 3; $r = -0.735$; slope = −3). These data show that the size of the pathological repeat contributes 54% of the variance of the age at onset.
Table 1 shows the three marker haplotypes of eight chromosomes with the SCA2 mutation in five families of Italian origin, and of 39 independent normal chromosomes obtained from spouses from the families and normal chromosomes of affected subjects. The D12S1328 and the D12S1329 markers define a SCA2 gene-containing region of 3 cM (Allotey et al., 1995), while the D12S1332 is located within the above interval ~400 kp centromeric to the gene (Pulst et al., 1996). As shown in Table 1, the SCA2 mutation is carried by five different haplotypes, four of which are also present on normal chromosomes. A common origin for these haplotypes cannot be excluded. However, haplotype 3–2–1 would require complex and unlikely crossover events in order to be related to the others and therefore suggests an independent origin for this SCA2 mutation.

Clinical features of SCA1, SCA2, SCA3 and ADCAI with unknown mutation

The clinical features of 53 SCA2 patients together with 60 SCA1 and 22 SCA3 patients are reported in Table 2. There were no significant differences in the age at onset in these three groups, in contrast to the median disease duration, where SCA1 patients show a significantly shorter disease duration compared with the SCA3 and SCA2 patients. All SCA1 and SCA2 patients showed cerebellar ataxia, whereas four SCA3 patients presented with extrapyramidal features (two brothers) or with facial fasciculations (the other two).

Ophthalmoplegia (either nuclear or supranuclear) did not differentiate between genotypes in any of the three groups. Staring eyes, which were formerly thought to be a hallmark of SCA3/MJD, were also seen in all three conditions. However, slow saccades were seen much more frequently in SCA2 than in SCA1 and SCA3 patients. Interestingly, nystagmus was present at the onset of SCA2, but tended to disappear with progression of the disease and as the slow saccades emerged.
The second clinical sign that also differentiates SCA2 was the presence of depressed or absent reflexes. Conversely, pyramidal signs were much more frequently found in SCA1 and SCA3 patients. The depression of tendon reflexes usually begins in the upper limb and progresses to the legs. An EMG study performed in eight of our SCA2 patients confirmed these observations and demonstrated an axonal neuropathy with reduced sensory action potentials and signs of denervation, including fasciculations. Sensory examination revealed a degree of variability, large fibre sensory loss was generally seen, but thermalgesia with a profound small fibre loss was seen in two families.

Myokymia or facial fasciculation was less frequent in SCA1 than in SCA2 and SCA3, while limb fasciculation was usually a later sign of the disease in all three disorders. In a large SCA2 family perioral and periorbicular fasciculation were an early sign of the disease and in one patient only minimal cerebellar ataxia was present, even after 10 years.

Although cognitive impairment appears to be more frequent in the SCA2 patients this did not reach statistical significance \( (P = 0.08) \). However, of the seven SCA2 patients showing signs of dementia, five belonged to one family of Italian origin, while the other two were the index cases of two families from the UK and Denmark. The mean age of onset of ataxia in these seven patients was 33 ± 11.4 years (range 13–50 years) and the dementia became noticeable after an average disease duration of 22.6 ± 7 years (range 13–29 years).

In a further family, a 9-year-old patient, with the largest observed number of CAG repeats (51), presented with clumsiness, dizziness, difficulty in learning new tasks and a need for special schooling. At examination, 4 years after disease onset, there was truncal ataxia with dysarthria, positional tremor of the head, very slow saccadic eye movements, absence of tendon reflexes and cognitive impairment. The phenotype appeared to be aggressive with rapid progression and an early involvement of other extracerebellar systems. The patient belongs to a family which showed very striking anticipation. However, dementia was not found in two of the other affected relatives, even after 15 and 19 years of disease. These individuals had far fewer CAG repeats, i.e. 38 and 40, respectively.

No statistical difference was found comparing the frequency of extrapyramidal features in SCA1, SCA2 and SCA3. Chorea and dystonia, especially torticollis and positional tremor, were more common in SCA1 and SCA2 while parkinsonian features were present in some SCA3 patients. Extrapyramidal signs were a feature of longer disease duration in SCA2 patients and were independent of the number of CAG repeats.

Detailed clinical features from the seven ADCA I families negative for SCA1, -2 and -3 were only available for four

### Table 1 Haplotype data from five families of Italian origin

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>S1328</th>
<th>S1332</th>
<th>S1329</th>
<th>SCA2 chromosome</th>
<th>Normal chromosomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>14</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Others</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>17</td>
</tr>
</tbody>
</table>

### Table 2 Comparison of clinical features of ADCA I patients: SCA2 versus SCA1 versus SCA3

<table>
<thead>
<tr>
<th></th>
<th>SCA1</th>
<th>SCA2</th>
<th>SCA3</th>
<th>( \chi^2 )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cases</td>
<td>60</td>
<td>53</td>
<td>22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of families</td>
<td>14</td>
<td>28</td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median age at onset in years (range)</td>
<td>38 (13–52)</td>
<td>38 (9–65)</td>
<td>35 (9–60)</td>
<td>–</td>
<td>0.1*</td>
</tr>
<tr>
<td>Median disease duration in years (range)</td>
<td>7 (1–18)</td>
<td>10 (1–30)</td>
<td>8 (1–21)</td>
<td>–</td>
<td>0.02*</td>
</tr>
<tr>
<td>Cerebellar ataxia</td>
<td>60</td>
<td>53</td>
<td>18</td>
<td>10.4</td>
<td>0.005</td>
</tr>
<tr>
<td>Ophthalmoplegia</td>
<td>40</td>
<td>29</td>
<td>11</td>
<td>2.59</td>
<td>0.2</td>
</tr>
<tr>
<td>Staring gaze</td>
<td>10</td>
<td>11</td>
<td>3</td>
<td>0.63</td>
<td>0.7</td>
</tr>
<tr>
<td>Slow saccades</td>
<td>6</td>
<td>30</td>
<td>0</td>
<td>40.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Optic atrophy</td>
<td>6</td>
<td>4</td>
<td>0</td>
<td>2.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Extrapyramidal features</td>
<td>7</td>
<td>6</td>
<td>6</td>
<td>3.95</td>
<td>0.1</td>
</tr>
<tr>
<td>Fasciculations: (face, tongue or limbs)</td>
<td>14</td>
<td>31</td>
<td>13</td>
<td>16.4</td>
<td>0.0003</td>
</tr>
<tr>
<td>Increased reflexes</td>
<td>46</td>
<td>9</td>
<td>13</td>
<td>40.9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Depressed or absent reflexes</td>
<td>4</td>
<td>43</td>
<td>4</td>
<td>70.6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Sensory loss</td>
<td>20</td>
<td>21</td>
<td>4</td>
<td>3.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Dementia</td>
<td>4</td>
<td>7†</td>
<td>0</td>
<td>4.86</td>
<td>0.08</td>
</tr>
</tbody>
</table>

*a*Kruskal–Wallis test of variance. †Five of these cases were from one family.
patients belonging to three families, two British and one Chinese, and were described previously (Giunti et al., 1995). The mean age of onset was 28.75 years and mean disease duration was 11.25 years. Interestingly, the index case of the Chinese family resembled SCA2 with adult-onset progressive cerebellar ataxia, ophthalmoplegia, slow saccades, reduced tendon reflexes and large fibre sensory loss in the lower limbs.

**Discussion**

Expanded SCA2 alleles were found only among ADCA I (n = 31 out of 38) families and not in the ADCA II (n = 14) or ADCA III (n = 37) kindreds. Over 90% of our ADCA I families now have a molecular explanation for their ataxia, a proportion far larger than that reported in a recent study (Geschwind et al., 1997). This discrepancy may reflect differences in ascertainment, clinical assignment or in different genetic frequencies between countries. The latter hypothesis is the most likely as the frequencies of SCA1 and SCA3 are already known to vary markedly between countries even within western Europe (Giunti et al., 1995; Ranum et al., 1995; Durr et al., 1996; Silveira et al., 1996; Riess et al., 1997). SCA1 and SCA2 are found with equal frequency in UK and Italy, and have a much higher frequency in these countries than that reported for France or Portugal. Conversely, the SCA3 mutation was far more common in Germany, France and Portugal than in the UK or Italy; in the present study SCA3 was found in one out of 19 UK families and none out of 30 Italian families. The absence of SCA3 in the Italian population is further supported by other studies of a total of 34 ADCA families, none of which was found to carry the SCA3 mutation (Ranum et al., 1995; Filla et al., 1996).

Although, broadly speaking, the clinical characteristics amongst the families classified as ADCA I are similar, following the identification of SCA2, it is now clear there are some differences. Depressed tendon reflexes and slow saccades are much more commonly seen in SCA2 than in SCA1 or SCA3. It is noteworthy that the brunt of the neuropathy is borne by the upper limbs, and that the lower limbs are affected only later in the course of the disease. Wadia and Swami (1971) described Indian patients with this disease in the 1970s and electrophysiological investigation revealed a predominantly axonal neuropathy, also preferentially affecting the arms first (Wadia, 1991).

Of the 13 ADCA families assessed clinically (by P.G. and the late Professor A. E. Harding) and shown not to have either the SCA1 or SCA3 mutation in London in 1995, 10 families have now been identified as being SCA2 as previously predicted (Giunti et al., 1995). The negative result for the SCA2 mutation in the index case with a phenotype characterized by slow saccades and early decreased tendon

### Table 3 Origin of ADCA I families with SCA1, SCA3, SCA2 and unknown mutations

<table>
<thead>
<tr>
<th>Country</th>
<th>SCA1</th>
<th>SCA3</th>
<th>SCA2</th>
<th>Not SCA1, -2 or -3</th>
</tr>
</thead>
<tbody>
<tr>
<td>UK</td>
<td>7</td>
<td>1</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>India</td>
<td>1</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bangladesh</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pakistan</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>West Indies</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Italy</td>
<td>15</td>
<td>0</td>
<td>11</td>
<td>4</td>
</tr>
<tr>
<td>France</td>
<td>0</td>
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<td>0</td>
<td>0</td>
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<td>Poland</td>
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<td>Denmark</td>
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<td>0</td>
</tr>
<tr>
<td>Brazil</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ghana</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>China</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Ethiopia</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Other origins</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>27</td>
<td>12</td>
<td>31</td>
<td>7</td>
</tr>
</tbody>
</table>
reflexes, belonging to one of the three families in which the mutations remain unknown, was somewhat unexpected. Therefore the two clinical ‘hallmarks’ of SCA2 are not exclusive, and at least one other SCA mutation can produce a similar phenotype.

We identified three families in which dementia clustered with the ataxia and a fourth in which a patient with cognitive impairment also had a very early onset of the disease. Families with a similar phenotype have also been described by Durr et al. (1995) and Geschwind et al. (1997). This clustering in some families suggests that other intra-familial factors may be influencing the phenotype. The presence of mental retardation in a subject with a very early onset and the largest number of CAG repeats, and dementia in individuals with later onset, raises the possibility that the number of CAG repeats can lead to different forms of cognitive impairment. It will be interesting to determine whether these apparently unrelated families, with different phenotypes in terms of cognitive involvement, share a common haplotype.

Mutation analysis showed that the lower limit for disease alleles is (CAG)35, which is lower than described in some series (Cancel et al., 1997), but similar to that found by Geschwind et al. (1997). The upper limit of the distribution of normal alleles was (CAG)39 in our study, but Geschwind et al. (1997) reported one normal chromosome with 31 repeats. The presence of intermediate alleles of 32–34 repeats has been reported by Riess et al. (1997), but it is unclear whether the authors considered them to be responsible for the ataxic phenotype. Analysis of repeat instability in the parental transmissions showed a clear paternal effect. The number of affected subjects with later onset, raises the possibility that the number of CAG repeats can lead to different forms of cognitive impairment. It will be interesting to determine whether these apparently unrelated families, with different phenotypes in terms of cognitive involvement, share a common haplotype.

Mutation analysis showed that the lower limit for disease alleles is (CAG)35, which is lower than described in some series (Cancel et al., 1997), but similar to that found by Geschwind et al. (1997). The upper limit of the distribution of normal alleles was (CAG)39 in our study, but Geschwind et al. (1997) reported one normal chromosome with 31 repeats. The presence of intermediate alleles of 32–34 repeats has been reported by Riess et al. (1997), but it is unclear whether the authors considered them to be responsible for the ataxic phenotype. Analysis of repeat instability in the parental transmissions showed a clear paternal effect. The number of affected subjects with later onset, raises the possibility that the number of CAG repeats can lead to different forms of cognitive impairment. It will be interesting to determine whether these apparently unrelated families, with different phenotypes in terms of cognitive involvement, share a common haplotype.

The size of the largest (≥46) and smallest (≤37) expanded allele is the major determinant of the age of onset. For the allele between 38 and 45, a larger variability of age at onset has been shown, and other genetic or environmental factors could play an important role. The number of affected subjects with a SCA2 allele between 37 and 46 CAG repeats was the majority in our series accounting for 85% of cases. Therefore the correlation, although highly statistically significant, showed a gentler slope than other reports (Cancel et al., 1997; Geschwind et al., 1997). The present data increase the range of age at onset to 78 years and this was in a patient with 35 CAG repeats. Age at onset of >70 years has been reported for Huntington’s disease patients (Novelletto et al., 1994), whereas with SCA1 and SCA3, the age at disease onset rarely exceeds 60 years. This appears to be of importance when one is considering the lower limit of ‘abnormal’ expansions and, in particular, intermediate alleles.

The internal structure of the abnormal SCA2 alleles confirms that they do not have the CAA interruptions which are found in all but the very small non-expanded alleles (Imbert et al., 1996; Pulst et al., 1996). A similar structure has been reported for SCA1 (Chung et al., 1993; Orr et al., 1993). However, in the case of SCA1 the CAG sequence is interrupted by CAT, which encodes histidine, whereas CAA encodes glutamine. Quan et al. (1995) and C. Jodice (personal communication) have observed a large SCA1 allele interrupted by four histidine residues in two non-ADCA subjects, thus indicating that it is the length of the unbroken polyglutamine tract that determines disease status. However, although it is well known that reliable evaluation of the internal structure is important in SCA1, further data on the expanded range and the associated phenotypes in SCA2 are required.

This study identified a wider size distribution of normal SCA2 alleles compared with that reported previously (Imbert et al., 1996; Pulst et al., 1996). The difference between normal and expanded alleles was only five repeats and will probably become even less in the future, as has been the case for SCA1 and Huntington’s disease.

Overall, it appears that SCA2 is a frequent cause of ataxia among ADCA I pedigrees in both the UK and Italy and although there are clinical clues pointing to the diagnosis of SCA2, a genetic test is required to achieve a definitive diagnosis. This mutation shares some other features with the other CAG repeat disorders, including an inverse correlation between repeat length and age at disease onset, paternal transmission effects and a wide range of ages at onset.

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References


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