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Spinocerebellar ataxia type 2 in southern Italy: a clinical and molecular study of 30 families

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Abstract Autosomal dominant cerebellar ataxia type I is the most common form of dominant ataxia. A genetic heterogeneity has been identified with five different loci (SCA1, 2, 3, 4, and 6). A pathological expansion of a CAG sequence has been identified in SCA1, 2, 3, and 6. We performed molecular analysis in 51 families with autosomal dominant cerebellar ataxia type I, mainly originating from southern Italy and Sicily. Thirty families carry an expanded CAG sequence within SCA2 gene. The mean number of repeats was 39.9 ± 3.3 in 85 expanded alleles, with a range of 34–52. The number of triplets was inversely correlated with age at onset and explained 76% of the variance. The best fit was obtained with an exponential relationship between variables. Expanded alleles were unstable when transmitted from parents to offspring. Expansions were more common than contractions, accounting for 59% of the total meioses and for 80% of the father-child transmissions. The mean intergenerational variation was 1.9 repeats (range –3 to +15) with higher values for male transmissions. Bulbar and autonomic signs were related to disease duration, pyramidal signs to CAG size, cerebellar features and peripheral neuropathy to both. Among the remaining 21 families, three carried the SCA1 and one the SCA6 mutation. This study suggests that SCA2 is the prevalent mutation in southern Italy.

Key words SCA2 · Autosomal dominant cerebellar ataxia · CAG expansion · Intergenerational instability · Anticipation

Introduction

Autosomal dominant cerebellar ataxia (ADCA) type I is the most common form of dominant ataxia, with a prevalence of 0.3–1.2 per 100,000 [14, 20]. Onset usually occurs in the third or fourth decade. Anticipation, associated with a more severe phenotype, is common. The clinical picture is characterized by gait ataxia, dysarthria, dysmetria, corticospinal signs, mild cognitive impairment, dysphagia, sphincter disturbances, and decreased vibration and position sense. Slow saccades and supranuclear ophthalmoplegia are frequent in several studies [5, 8, 11]. Genetic heterogeneity with five different loci (SCA1, 2, 3, 4, and 6) has been identified in ADCA type I [5, 26].

Orr et al. [19] showed that the genetic defect of SCA1 is an expanded cytosine-adenine-guanine (CAG) trinucleotide repeat within the coding sequence of the affected alleles. The same mutational mechanism is responsible for SCA3/Machado-Joseph disease (MJD) [13], SCA2 [12, 21, 24], and SCA6 [26]. A pathological expanded CAG repeat is also responsible for SCA7, which is characterized by retinal degeneration (ADCA type II) [4] and for dentatorubropallidoluysian atrophy (DRPLA) [15, 18]. Therefore it is now possible to analyze single patients

by direct mutation analysis and to establish the occurrence of the various mutations in the population.

ADCA type I is characterized by high variability of age at onset, clinical picture, and pathological findings, even within families. Therefore it is difficult to establish that different genotypes result in different phenotypes. Our study and those of others have suggested that some clinical findings are more frequently associated with a given genotype [7, 23]. Patients from SCA5 and, to a lesser extent, SCA6 families show mainly cerebellar involvement and might be classified as ADCA type III, according to Harding [10].

We present a study of the CAG expansion within SCA1, 2, 3, 6 and DRPLA genes in 51 ADCA type I families mainly from southern Italy and Sicily. We also report the molecular and clinical data on a large series of patients carrying the SCA2 mutation, which was the most common identified cause of ADCA type I in our study group.

Patients and methods

Patient ascertainment

The Department of Neurology of the Federico II University in Naples is a referral center for inherited ataxias for patients mainly from central and southern Italy and Sicily. All the patients included in the study were observed personally. The diagnosis of ADCA type I was made according to the following criteria: autosomal dominant inheritance, onset after 20 years of age in the majority of affected individuals in a given family, progressive unremitting cerebellar ataxia variably associated with supranuclear ophthalmoplegia, cognitive impairment, corticospinal signs, extrapyramidal features, peripheral neuropathy [11]. No family with retinal degeneration was included. Onset was defined as the date at which the patients or the relatives noticed the first appearance of symptoms.

Molecular analysis

A blood sample was taken in 50 mM EDTA and genomic DNA was prepared according to previously described procedures [17]. The polymerase chain reaction (PCR) was used to analyze CAG repeats of SCA1, SCA2, SCA3, SCA6, and DRPLA. We used Rep1-Rep2 [19], SCA2A-SCA2B [21], MJD52-MJD70 [13], S-5-F1/S-5-R1 [26], and CTG-B37 [18] as oligonucleotide primers to amplify the region surrounding an expanded CAG repeat. Amplified products were separated in 6% polyacrylamide gel and analyzed by silver staining (Silver Sequence DNA sequencing system, Promega). All sizes were determined by comparing migration relative to a known DNA sequence (plasmid pUC18).

Statistical analysis

Means and percentages were compared by *t* test and χ^2 test. Correlations were studied by Pearson's correlation coefficient. The best fit of the correlation between onset age and CAG repeat size was obtained by an exponential model. The regression equation was: $y = \exp(-ax) \times A$, where y gives the age at onset in years and x the number of CAG repeats.

Results

Of the 51 families affected by ADCA type I, 5 originated from central Italy, 38 from southern Italy, and 8 from Sicily. A total of 72 patients and 13 at-risk carriers from 30 families showed an expanded repeat sequence at the SCA2 locus, 10 patients from 3 families at the SCA1 locus and 2 patients from a single family at the SCA6 locus. No patient carried a CAG expansion at the SCA3/MJD or DRPLA loci.

We studied the 85 normal and 85 expanded alleles from the 30 SCA2 families. Of the normal alleles 95% had 22 CAG repeats, and 5% 23 repeats. In the expanded alleles the average number of CAG repeats was 39.3 ± 3.3 (median 39, range 34–52; Fig. 1). Two individuals carried 34 CAG repeats. One was affected with disease onset at 57 years, and the other was asymptomatic at the age of 41.

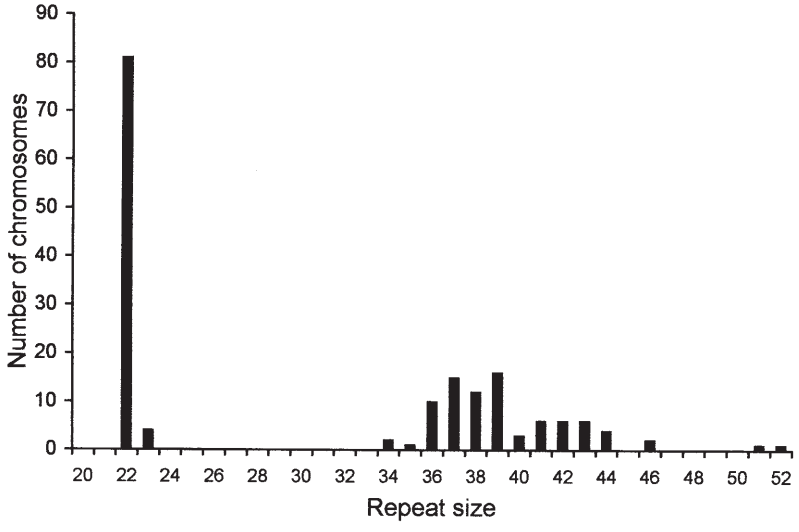

Fig. 1 Distribution of CAG repeat sizes in normal and expanded alleles from 85 expansion carriers

Of the remaining 12 asymptomatic individuals, whose expansion ranged from 36 to 44 CAG repeats, two individuals aged 41 (39 repeats) and 52 years (36 repeats) were above the mean age at onset.

The age at onset was reliably reported in 65 patients. The average was 36.7 ± 14.5 years (range 10–80). There was no significant difference between mean age at onset of offspring of affected fathers (35.1 ± 14.1 years, $n = 28$) and affected mothers (37.5 ± 15.8, $n = 33$). The mean allele size of paternally (39.8 ± 3.6 repeats; $n = 39$) and of maternally (39.1 ± 3.1, $n = 39$) transmitted alleles was similar. Seventeen patients (26%) had a juvenile-onset (10–25 years); nine inherited the expanded allele from the father, and eight from the mother. Among juvenile onset patients the CAG repeat size ranged from 39 to 52 with a mean of 43.5 ± 3.6, significantly higher than that of the remaining patients (38.3 ± 2.0, $P < 0.001$).

Age at onset and repeat length were inversely correlated ($r_p = –0.81$; $P < 0.001$). The best fit was obtained with exponential relationship between variables. The regression equation was: age at onset = exp (–0.1150 × number of CAG repeats) × 3203, mean square residual = 57.6, $R^2 = 0.76$ (Fig. 2).

CAG repeat lengths were known in 27 parent-child pairs. Sixteen of the children were diagnosed as affected and the remaining are currently at-risk. In 19 (70%) of these transmissions there was a variation in the repeat size, with 16 (59%) increasing in size and 3 (11%) contracting. Delta was defined as the difference in size between child and parent repeat. The mean delta was +1.9 ± 3.6 (range –3 to +15). In 15 father-child pairs 12 CAG repeats expanded, 2 contracted, and 1 showed no variation; in 12 mother-child pairs 5 expanded, 1 contracted, and 6 showed no variation ($P < 0.05$). In the father-child pairs mean delta was +3.2 ± 4.4 (range –3 to +15) and in mother-child pairs 0.3 ± 0.6 (range –1 to +1; $P < 0.05$).

The mean anticipation in 33 parent-child pairs was 15.0 ± 14.1 years (range –13 to +40). Mean anticipation did not

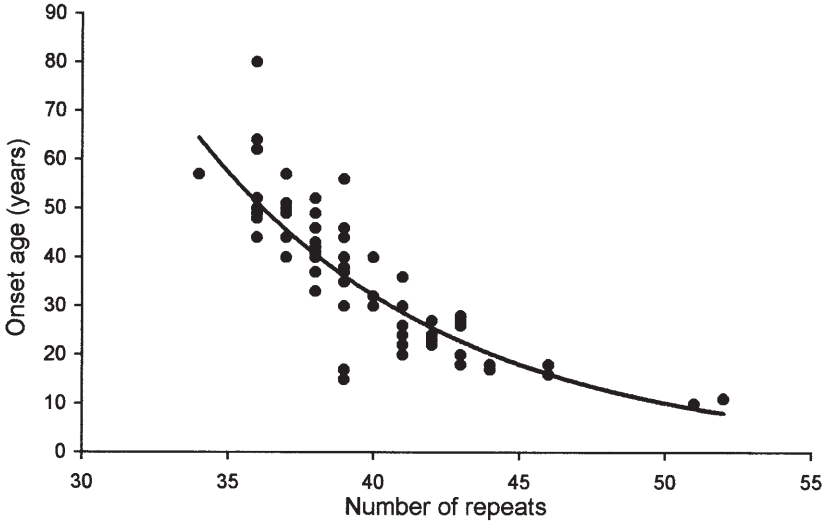

Fig. 2 Correlation of CAG repeat length and age at onset in 65 SCA2 patients. *Line*, the best fit, which is given by the exponential equation: onset age = exp (–0.1150 × number of CAG repeats) × 3203

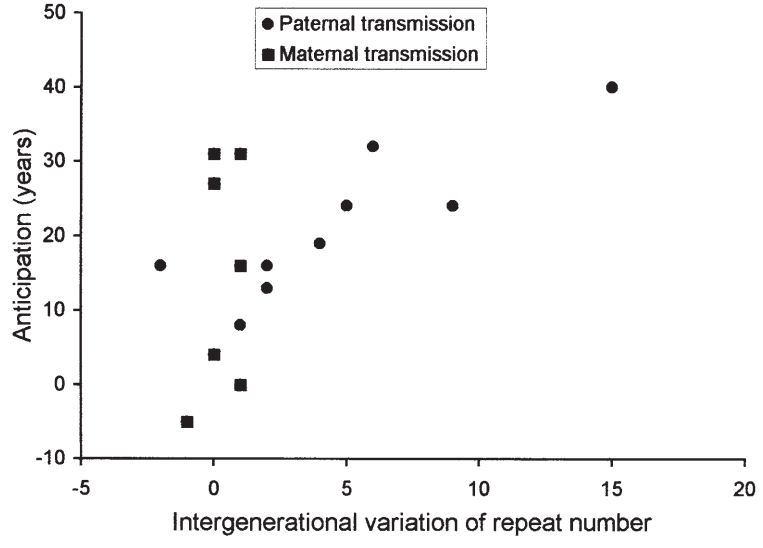

Fig. 3 Correlation between intergenerational variation in the size of the CAG repeat and anticipation of onset age in 16 parent-child pairs

differ in 18 father-child pairs (18.8 ± 13.4) and in 15 mother-child pairs (10.4 ± 13.7 years; $P < 0.1$). In 16 parent-child pairs in which both CAG repeats and age at onset were available, a correlation was found between anticipation and delta ($r_p = 0.59$; $P < 0.05$), and it was due to the contribution of the nine father-child pairs ($r_p = 0.86$; $P < 0.01$). The correlation was absent in the seven mother-child pairs (Fig. 3).

Cerebellar signs (gait ataxia, dysarthria and intention tremor) were constant and frequently associated with abnormal eye movements (slow saccades and gaze paralysis) and clinical signs of peripheral neuropathy (decreased/absent ankle and knee jerks associated with decreased vibration sense). Signs of corticospinal impairment (Babinski sign and increased tone) affected about a one-fourth of the patients. Other not uncommon signs were head tremor, perioral fasciculations, and cramps. Dysphagia and sphincter disturbances were prominent in late stages of the disease. Cognitive impairment, slight distal amyotrophy, weakness, and dystonia were observed in few patients (Table 1).

In addition, we compared mean disease duration and mean CAG repeat size in patients with and without the signs. Patients with dysmetria, weak/absent knee and ankle jerks, dysphagia, sphincter disturbances had longer disease duration than those without these signs. Patients with dysarthria, dysmetria, weak/absent knee jerks, Babinski sign, cramps, head and intention tremor differed from those without these signs for larger CAG expansions.

Table 1 Clinical presentation and effect of disease duration and CAG repeat size on occurrence of clinical findings in 65 SCA2 patients; we compared mean disease duration and CAG repeat size in patients with and without the feature

Mean age at onset (years)	36.7 ± 14.5
Mean age at the study (years)	49.2 ± 15.6
Mean CAG repeat size	39.6 ± 3.4
Gait ataxia	100%
Dysarthria	90%***
Nystagmus	5%
Dysmetria	88%**, ****
Decreased/absent knee jerks	39%*, ****
Decreased/absent ankle jerks	64%**
Amyotrophy	10%
Decreased vibration sense	59%
Increased tone	23%
Weakness	9%
Babinski sign	27%****
Slow saccades	86%
Gaze palsy	50%
Dysphagia	41%**
Sphincter disturbances	55%**
Cognitive impairment	15%
Cramps	36%****
Intention tremor	48%***
Head tremor	35%****
Perioral fasciculations	27%
Dystonia	12%

* $P \leq 0.05$, disease duration;
** $P \leq 0.01$, disease duration;
*** $P \leq 0.05$, CAG repeat size;
**** $P \leq 0.01$, CAG repeat size

Discussion

A CAG expansion within the SCA2 gene was present in 59% of the ADCA type I families in our series. This is the highest occurrence reported in the literature. Two recent studies reported values of 15% in 184 families [2] and 13% in 47 families [9] from ethnically heterogeneous populations. The occurrence of SCA1 and SCA3 was 14% and 34% [2] in the former and 6%, 23% in the latter study [9]. We found the SCA1 mutation in 6% of the families. The SCA3 mutation was absent and has never been reported in Italian families. One family carried the SCA6 mutation. We previously excluded linkage to SCA 4 and SCA5 loci in the two largest families [8]. Therefore unknown mutations may have been present in 33% of our families.

Our study confirms that normal alleles show little polymorphism within the SCA2 gene, the 22 CAG repeat allele being the most common (95%) [2, 9, 24]. The number of CAG repeats of the affected alleles ranged from 34 to 52 and varied from 35 to 59 in other studies [2, 9, 24, 25].

We found the largest expansion (52 repeats) in a 20-year-old man with onset at 11 years. One 67-year-old patient with onset at 57 carried a 34 repeat expansion. Geschwind observed 34 repeats in a 50-year-old asymptomatic woman whose affected daughter carried 49 repeats (personal communication). Thirty-four triplets is the lowest pathogenic allele reported in a CAG disorder, excluding SCA6, in which the expansion might interfere with the normal function of the Purkinje cell calcium channel. This finding suggests a higher sensitivity to the toxic effect of the expanded polyglutamine tract in SCA2.

There was a strong inverse correlation between CAG repeat size and age at onset in agreement with previous studies [2, 9, 12, 24]. The best fit was obtained by an exponential curve, as previously shown by Pulst et al. [21] and 76% of the variability of onset age was explained by the CAG repeat length. Increments in repeat size had a greater effect on age at onset for shorter than larger alleles. For instance, the expected anticipation was 5.5 years for a change from 36 to 37 CAG repeats but 1.4 years for a change from 48 to 49.

The CAG repeat was unstable during transmission from parents to offspring, with variation ranging from –3 to +15 and a mean increase of +1.9. Intergenerational instability is usually found in triplet disorders. In CAG disorders expansions are more frequent than contractions. The expansion/contraction ratio was 1.9 in SCA1 [3], 3.7 in MJD/SCA3 [16], and 5.4 in the present study. We also observed an effect of the sex of the transmitting

parent, since delta and its variability range were larger in paternal transmission. This sex bias is similar to that reported in the other CAG disorders [1].

Intergenerational instability of the CAG repeats offers a molecular explanation for anticipation. In 16 parent-child pairs the mean CAG repeat increase was 2.75 repeats, and mean observed anticipation was 18.5 years. Expected anticipation was calculated in each parent-child pair from the value of delta on the basis of the exponential equation reported above. The mean expected anticipation was 8.7 years. The difference between expected and observed anticipation is probably due to observational bias [22].

We also confirm our previous suggestion that the SCA2 phenotype is characterized by supranuclear ophthalmoplegia and peripheral neuropathy [7, 8]. We did not find the high occurrence of chorea and dystonia reported by Geschwind et al. [9]. Occurrence of clinical signs is affected by both disease duration and number of CAG repeats. Bulbar and autonomic signs may be related to disease duration, pyramidal signs to CAG size, and cerebellar impairment and peripheral neuropathy to both.

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