The trinucleotide repeat expansion on chromosome 6p (SCA1) in autosomal dominant cerebellar ataxias

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Summary

Affected members of 73 families with a variety of autosomal dominant late onset cerebellar ataxias (ADCAs) were investigated for the trinucleotide (CAG) repeat expansion which is found in pedigrees exhibiting linkage to the SCA1 locus on chromosome 6. Most of the families were too small for linkage analysis. The mutation was only found in ADCA type I, in 19 out of 38 such kindreds investigated (50%). It was slightly more common in Italian (59%) than British (50%) families, and was also found in Malaysian, Bangladeshi and Jamaican kindreds. Overall, ADCA type I patients with the

expansion had a lower incidence of hyporeflexia and facial fasciculation than those without. The trinucleotide expansion was not found in eight families with ADCA and maculopathy or 24 kindreds with a pure type of ADCA, confirming that these syndromes are genetically distinct. It was also not detected in 12 patients with sporadic degenerative ataxias. DNA analysis for the SCA1 mutation is useful diagnostically in single patients or small families, and can be used for presymptomatic testing where appropriate.

Key words: dominant ataxia; trinucleotide repeat

Introduction

The autosomal dominant late onset cerebellar ataxias (ADCAs) are clinically and genetically heterogeneous. Family studies suggest that, compared with other syndromes which are genetically distinct, there is little clinical evidence of heterogeneity in families with ADCA associated with variable combinations of supranuclear ophthalmoplegia, optic atrophy, mild dementia, peripheral neuropathy or extrapyramidal dysfunction (Harding, 1982). There is, nevertheless, evidence for locus heterogeneity within this broad group (ADCA type I). Linkage studies have shown that the disease gene maps to the short arm of chromosome 6 in several large families (Zoghbi *et al.*, 1988, 1993; Jodice *et al.*, 1993; Kwiatkowski *et al.*, 1993), and there is a locus on chromosome 12 in Cuban pedigrees (Gispert *et al.*, 1993). It is difficult to distinguish these clinically, and the same applies to families with Machado–Joseph disease, a syndrome initially reported in individuals of Azorean/Portuguese descent (Coutinho and Andrade, 1978). The gene locus for Machado–Joseph disease has been mapped to chromosome 14q in both Japanese and

Portuguese families (Takiyami *et al.*, 1993). However, an ADCA gene has also been mapped to this locus in three French families not considered to have Machado–Joseph disease on clinical grounds (Stevanin *et al.*, 1994).

Autosomal dominant late onset cerebellar ataxia and pigmentary macular dystrophy (ADCA II) is clinically and genetically distinct from ADCA type I (Harding, 1982; Enevoldson *et al.*, 1994). No positive linkage data have been reported in this disorder, although the disease locus is not linked to the HLA locus on chromosome 6p (Kumar *et al.*, 1986). The pathological findings in ADCA types I and II are usually those of olivopontocerebellar atrophy, but nearly always with abnormalities elsewhere, including the basal ganglia, spinal cord and peripheral nerves. There is also a later onset (usually over 50 years) relatively 'pure' cerebellar syndrome in which ocular or extrapyramidal features do not occur (ADCA type III), with autopsy findings of cerebellar cortical atrophy with loss of cells in the dentate and/or olivary nuclei (Hoffman *et al.*, 1971). No linkage studies have been

Table 1 Clinical classification and results of SCA1 mutation analysis

Disease category	Number of families with mutation/total number of families (%)			
	British	Italian	Other origins	Total
ADCA I	6/12 (50)	10/17 (59)	3/9 (33)	19/38 (50)
ADCA II	0/6	0/1	0/1	0/8
ADCA III	0/21	0/1	0/2	0/24
Other dominant ataxias				
With dementia/myoclonus	0/1	—	—	0/1
With severe dementia	0/1	—	—	0/1
Intermittent ataxia	0/1	—	—	0/1
'Idiopathic' late onset cerebellar ataxia (cases)	0/9	0/1	0/2	0/12

reported in this disorder, apart from exclusion of linkage to 6p in a family with rather earlier onset and a fairly indolent course (Frontali *et al.*, 1992).

Recently, the mutation in the ADCA gene on chromosome 6p (at the locus termed SCA1) has been identified (Chung *et al.*, 1993; Orr *et al.*, 1993). It is an unstable expanded trinucleotide (CAG) repeat sequence, with similar characteristics to the Huntington's disease mutation (Huntington's Disease Collaborative Group 1993). There is an inverse correlation between repeat length and age of onset of the disease. This discovery makes it possible to determine if affected members of families too small for linkage analysis have the SCA1 mutation. The results of such a study in 73 families with various forms of ADCA and 12 patients with late onset degenerative ataxias who had no history of affected relatives are reported in this paper.

Patients and methods

Patients

DNA was analysed from at least one member of 73 families with a variety of dominant ataxias (Table 1). Families were considered to have ADCA type I if the age of onset was <50 years in the majority of affected family members, and at least one patient per family had features such as supranuclear ophthalmoplegia, optic atrophy, peripheral neuropathy, dementia and fasciculations of the limbs and/or face during their illness. Also included in this group is one family where the only patient examined had a pure cerebellar syndrome at an early stage of the disease (<5 years duration), as is common in ADCA type I (Harding, 1982). Inclusion in this category was warranted by consistently early onset in the family (<45 years) and the presence of cerebellar and brainstem atrophy seen on imaging studies, suggesting a pathological substrate of olivopontocerebellar atrophy rather than cerebello-olivary atrophy which is usually seen in ADCA type III. Three of the 12 British families with ADCA type I were in the study of Harding (1982), including families 1 and 2; one of these (family 2) was known to have a locus on chromosome 6 on the basis of linkage analysis (Shrimpton *et al.*, 1993). Of the 17 Italian families with ADCA type I,

seven were known to have a locus on chromosome 6p (Conighi *et al.*, 1984; Spadaro *et al.*, 1992; Jodice *et al.*, 1993), and in two this had been excluded (unpublished data).

Type II ADCA was defined by the consistent finding of maculopathy in affected family members (Harding, 1982; Enevoldson *et al.*, 1994). Type III ADCA was defined as a pure cerebellar syndrome, occasionally with distal loss of vibration sense or pyramidal signs in the legs, but without the additional features seen in ADCA type I referred to above, after disease durations >5 years in all affected family members. This syndrome is probably heterogeneous. In 11 families, the age of onset was consistently late, over 40 in all patients and 50 or over in all but three. In the other 13 (one Italian; Frontali *et al.*, 1992), onset was earlier, ranging between the second to fourth decade of life. In all these 13 families the disease course was only slowly progressive, with patients not losing the ability to walk until 30 or 40 years after onset. Three families with rare or less well characterized dominant ataxias were also studied, of which details are given in Table 1.

Fifty-six of the families were studied clinically in the UK by P.G. or A.E.H. They originated from the UK (42), India, Pakistan or Bangladesh (three), the West Indies (three), Italy (two), Malaysia (one), the Philippines (one), Brazil (one), China (one) Denmark (one) and Belgium (one). The 17 families studied in Italy (all Italian) were assessed clinically by M.S. or P.G. We also investigated 12 patients (11 in the UK, one in Italy) with 'idiopathic' late onset cerebellar ataxia (Harding, 1981), in whom there was no history of affected relatives. Nine of these were British, one Afro-Caribbean, one Indian and one Italian.

For clinical comparisons, age of onset data were compared using the Mann-Whitney test, and the presence or absence of clinical features using χ^2 analysis of raw data with Yates' correction.

DNA analysis

In London, analysis of the trinucleotide repeat in the SCA1 gene was performed in UK-based patients and 44 healthy

Table 2 Comparison of clinical features of ADCA I families with and without the SCA1 mutation

	SCA1	Not SCA1
Median age at onset (years) in all patients (range)	34 (17–53) (<i>n</i> = 103)	34 (9–54) (<i>n</i> = 69)
	Percentage of cases*	
Supranuclear ophthalmoplegia	63	67
Staring gaze	5	10
Dysphagia	41	46
Cognitive impairment	8	10
Extrapyramidal features	14	4
Fasciculation		
Face/tongue	21	48 ($\chi^2 = 4.5$, $P = 0.03$)
Limbs	20	24
Increased reflexes	67	31
Depressed/absent reflexes	15	42 ($\chi^2 = 8.2$, $P = 0.016$)
Sensory loss	37	36

*From available clinical data on 46 SCA1 patients and 30 non-SCA1 patients.

control subjects, using a modification of the method described (Orr *et al.*, 1993). The polymerase chain reaction (PCR) was carried out in a 25 μ l reaction volume using 100 ng DNA, 1.5 mM MgCl₂, 50 mM KCl, 10 mM Tris, pH 8, 100 μ M dATP, 10 μ M dCTP, 100 μ M dTTP, 100 μ M dGTP, 10 pmol each primer, 0.4 μ Ci [α -³²P]dCTP and 2.5 units Taq polymerase. Primers were as described (Orr *et al.*, 1993). Initial DNA denaturation at 94°C for 3 min was followed by 25 cycles of 30 s each at 94, 57 and 72°C, respectively, and a final elongation step of 10 min at 72°C. Products of the PCR were fractionated on 6% denaturing acrylamide gels. Allele sizes were estimated using an M13 sequencing ladder, which we consider gives an accuracy of +2 repeat units; the SCA1 mutation was defined by the presence of >42 repeats in one allele (Orr *et al.*, 1993). The patients based in Italy were investigated in Rome using the method reported by Orr *et al.* (1993).

Results

The classification of the families is shown in Table 1, as is the number of kindreds with the SCA1 mutation. Ten of 17 families originating from Italy with ADCA type I (59%) had the mutation, compared with six of 12 families of British origin (50%), and three of nine other kindreds. The last were Malaysian, Jamaican and Bangladeshi; other ADCA type I families from India, China, the West Indies, Brazil, Belgium and Denmark did not have the SCA1 mutation. Not surprisingly, all of the eight families who had shown linkage to chromosome 6p markers had the SCA1 expansion and this was not present in the two ADCA type I families in which linkage to this region had been excluded. The characteristics of the SCA1 mutation in these families have been reported elsewhere (Jodice *et al.*, 1994), confirming the relationship between repeat length, age of onset and severity

and more marked instability during paternal transmission (Orr *et al.*, 1993).

A comparison of the clinical features of ADCA type I in patients with and without the SCA1 expansion is given in Table 2. The median and ranges of age at onset of symptoms were very similar. Overall, the non-SCA1 patients had a higher incidence of depressed or absent, as opposed to increased, reflexes. Reflex loss increases with disease duration in ADCA type I (Harding, 1982), but there was a higher proportion of non-SCA1 cases with hyporeflexia (four out of 11 compared with three out of 31 SCA1, $\chi^2 = 10.7$, $P = 0.004$) in patients with disease durations of 7 years or less. In longer duration cases there was no significant difference between the two groups in terms of incidence of hyporeflexia (four out of 15 SCA1, seven out of 17 non-SCA1). The other significant finding was that more non-SCA1 patients had facial or lingual fasciculation. This was not related to disease duration (data not shown).

None of the seven families with ADCA type II (ataxia and macular dystrophy), or the 24 with the 'pure' ADCA type III, had the SCA1 mutation, and the same applied to those with less common dominant disorders, including a family with intermittent ataxia, and the 12 patients without affected relatives.

Discussion

This study confirms the previous clinical observations which separated the pure type of ADCA (type III) and that with maculopathy (type II) from the more common and variable multisystem syndrome ADCA type I (Harding, 1982). The SCA1 expansion was only observed in patients with ADCA type I, in half of the families originating from the UK and slightly more of those of Italian origin. This may reflect a degree of ascertainment bias in the Italian families as these were collected initially for chromosome 6 linkage studies,

and certainly one was known to be 6p-linked in 1984 (Conighi *et al.*, 1984). Also, four of the SCA1 families are from Calabria and probably reflect a founder effect in this part of Italy. The British families were ascertained solely by systematic DNA storage from all families with ADCA seen by A.E.H. over a 7 year period, and a more recent intensive collection of British families with any type of dominant ataxia. In the French population, six of the 10 families for whom linkage analysis information is available showed evidence for a locus on chromosome 6 (Khati *et al.*, 1993), a similar proportion to that observed in our European patients. The presence of the SCA1 mutation in Malaysian, Jamaican and Bangladeshi kindreds suggests that this genetic defect is not confined to Caucasian populations, and may well have multiple origins in different racial groups, as is the case in Huntington's disease. SCA1 has previously been described in Black American and Cape coloured (South African) families (Zoghbi *et al.*, 1988; Bryer *et al.*, 1992). However, SCA1 as defined by linkage analysis has been reported most commonly in families of European origin (Zoghbi *et al.*, 1993), and other types of ADCA may be more frequent in certain populations. This clearly applies to Azorean or Portuguese families, and possibly the Japanese (Takiyama *et al.*, 1993; Zoghbi *et al.*, 1993).

As in the French study (Khati *et al.*, 1993), in which 6p-linked ADCA was indistinguishable from non-6p-linked ADCA, we could identify relatively few clinical differences between ADCA type I patients with the SCA1 mutation and those without. The latter tend to exhibit depressed or absent tendon reflexes, as opposed to hyperreflexia, in the first 7 years of the disease. The higher incidence of facial fasciculation in the non-SCA1 families is of interest as this has been reported frequently in patients with Machado-Joseph disease (Coutinho and Andrade, 1978) and in the Cuban families with a disease locus on chromosome 12 (Orozco Diaz *et al.*, 1990). It should be stressed that these were group differences derived from a relatively large number of patients and do not serve to distinguish SCA1 from other types of ADCA type I in individual patients or families. Our non-SCA1 families are likely to be genetically heterogeneous and presumably have disease genes on chromosomes 12, 14 or elsewhere (Stevanin *et al.*, 1994). It seems highly probable that all the mutations underlying ADCA type I, and ADCA type II which exhibits striking anticipation and a paternal effect (Enevoldson *et al.*, 1994), will be unstable trinucleotide repeats.

Most of our families with the expansion were too small for linkage analysis, and direct mutation detection is clearly very useful in clinical practice in making a diagnosis of SCA1 in such kindreds. If applicable, this analysis can be used for presymptomatic diagnosis, and we have already performed this in two at risk subjects, using the same pre- and post-test counselling protocols as in Huntington's disease families (World Federation of Neurology Research Group on Huntington's disease, 1989).

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