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# Acetazolamide-responsive episodic ataxia in an Italian family refines gene mapping on chromosome 19p13

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## Summary

*Episodic ataxia type 2 is an autosomal dominant disorder with attacks of vertigo and ataxia which respond to acetazolamide treatment. The gene, distinct from the KCNA1 responsible for episodic ataxia type 1, has been mapped on chromosome 19p13 in a 11–12 cM region. A large Italian kindred affected with acetazolamide-responsive episodic ataxia is reported, with onset in adulthood, a strong vestibular component during attacks and a high frequency of cerebellar vermis degeneration. The genetic analysis (i) showed strong linkage between the disease and the 19p13 microsatellite markers in a region which widely overlaps that previously reported and (ii) set a new distal boundary of the gene-containing region. Combining present and previous mapping data, the gene of episodic etaxia type 2 is most probably located in an interval ~1.5 Mb between markers D19S221 and D19S226.*

**Keywords**: episodic ataxia type 2; vestibulocerebellar paroxysmal ataxia; autosomal dominant cerebellar ataxias; chromosome 19p; cerebellar vermis atrophy

**Abbreviations**: BAEPs = brainstem auditory evoked potentials; CMAP = compound motor action potentials; EA = episodic ataxia; MCV = Motor conduction velocity; PCR = polymerase chain reaction; SAP = sensory action potentials; SCV = sensory conduction velocity; SEPs = somatosensory evoked potentials

## Introduction

Autosomal dominant episodic cerebellar ataxias are characterized by recurrent episodes of ataxia of variable duration. They are clinically and genetically heterogeneous disorders. In episodic ataxia type 1 (EA1), due to mutations at the KCNA1 gene on chromosome 12p13 (Browne *et al*., 1994; Litt *et al*., 1994), patients experience brief episodes of ataxia and dysarthria, often accompanied by neuromyotonia or myokymia, and show, interictally, spontaneous myokymia arising from peripheral nerves (Gancher and Nutt, 1986). In episodic ataxia type 2 (EA2), also known as acetazolamide-responsive paroxysmal cerebellar ataxia, or vestibulocerebellar ataxia, affected individuals have longer episodes, lasting from minutes to days, characterized by attacks of vertigo, visual disturbances, dysarthria and ataxia, which respond to acetazolamide treatment. Interictally, nystagmus and other mild cerebellar signs are present (Gancher and Nutt, 1986; Baloh and Winder, 1991; Bain *et al*., 1992). Vermian atrophy has been found at MRI (Vighetto *et al*., 1988). In some cases, the disease may progress to a severe persistent cerebellar ataxia (Gancher and Nutt, 1986).

EA2 shows highly variable features, such as age at onset, frequency and duration of attacks, triggering stimuli and constellation of signs during attacks (Moon and Koller, 1991). In some cases episodes appear to have a stronger vestibular component being triggered by quick head movements or postural changes and accompanied by vertigo, nausea and vomiting. In others, episodes are characterized by a diffuse cerebellar syndrome triggered mainly by physical and

emotional stress (Farmer and Mustian, 1963; White, 1969; Donat and Auger, 1979; Gancher and Nutt, 1986; Friedman and Hollman, 1987; von Brederlow *et al*., 1995). The gene responsible for EA2 has recently been mapped to chromosome 19p13 (Kramer *et al*., 1995; Teh *et al*., 1995; Vahedi *et al*., 1995; von Brederlow *et al*., 1995) in a region of ~11–12 cM with a distal boundary at locus D19S413 (Kramer *et al*., 1995) and a proximal boundary at locus D19S226 (Kramer *et al*., 1995; Teh *et al*., 1995; von Brederlow *et al*., 1995).

We identified a large Italian kindred with acetazolamide-responsive episodes of ataxia with late onset, a marked vestibular component and a high frequency of cerebellar anomalies at MRI. The genetic study of the family showed strong evidence of linkage to markers of the 19p13 region, confirming its homogeneity with previous EA2 families, and it provided a further refinement of the EA2 gene mapping.

## Subjects and methods

The family (Fig. 1) is of Italian origin (Campania) with evidence of the disease over three generations. Fifty-two family members over 18 years of age were neurologically examined by at least two of the authors. Interictal cerebellar signs were scored according to the rating scale of Klockgether *et al.* (1990) with the addition of a score for oculomotor findings based on the disease evolution observed in the present family (normal = 0, saccadic pursuit and reduction of optokinetic response = 1, nystagmus in the extreme lateral/vertical gaze = 2, nystagmus in intermediate lateral/vertical gaze = 3). Sixteen subjects underwent MRI, performed on a 0.5 Tesla superconductive magnet (General Electric, Fairfield, Conn., USA) with $T_1$ (TR = 650, TE = 25) and $T_2$ (TR = 650, TE = 25) spin-echo pulse sequences. $T_1$-weighted images of the posterior fossa were acquired on the axial and sagittal plane at a slice thickness of 5 mm, and $T_2$-weighted images of the whole brain on the axial plane at a slice thickness of 10 mm. Vermian atrophy was diagnosed when a reduction of the vermis image against the cisterna magna was present in the sagittal plane, and atrophy of cerebellar hemispheres on the basis of foliation visible on the axial plane. The degree of atrophy was qualitatively subdivided as follows: normal = 0; mild upper vermis atrophy = 1; mild upper and lower vermis atrophy = 2; moderate upper and lower vermis atrophy = 3; severe vermian atrophy and moderate hemisphere atrophy = 4; severe diffuse cerebellar atrophy = 5.

Neurophysiological examination was conducted using a Medelec Sapphire 2ME apparatus (Surrey, UK). Motor conduction velocity (MCV), compound motor action potentials (CMAPs), sensory conduction velocity (SCV), sensory action potentials (SAP), somatosensory evoked potentials (SEPs) and brainstem auditory evoked potentials (BAEPs) were obtained according to methods reported by Kimura (1989).

Fifty-two members and nine spouses married into the family gave blood samples upon informed consent. DNA was extracted according to standard procedures, and typed, by polymerase chain reaction (PCR) amplification, for CHLC (Cooperative Human Linkage Centre) and Généthon microsatellite markers spanning a 19p13 interval of ~21 cM: tel-D19S391-D19S413-D19S204-D19S394-D19S221-D19S226-D19S179-D19S410-D19S215-cen. The map order was obtained by comparing CHLC and Généthon genetic maps (*see* also von Brederlow *et al*. 1995) as well as the chromosome 19 physical map (Ashworth *et al*., 1995), placing D19S179 proximal to D19S226.

PCR was performed according to methods already described (Sabbadini *et al*., 1995). Two-point and multipoint linkage analyses were performed using the LINKAGE package (Lathrop *et al*., 1984) and, assuming four classes of age-dependent penetrances (i.e. 0.167, 0.417, 0.750 and 0.950 for ages $\leqslant$24, 25–34, 35–44 and $\geqslant$45 years, respectively), derived from the cumulative distribution of age at disease onset in the family.

Alleles were reduced according to Ott (1978). Allele frequencies for CHLC markers (D19S391, D19S204, S19S394, D19S179) were those observed in 70 independent chromosomes from the present and other Italian families (data not shown). For the remaining markers, belonging to the Généthon set, the frequencies were those reported in GDB (The Genome Data Base), although different allele names were used in Fig. 1. The frequency of the disease allele was set at 0.0001.

## Results

### *Case reports and clinical examination*

Patient IV-24, the index case, was first seen at 48 years of age because of a progressive cerebellar syndrome with onset at the age of 42 years and a family history of balance impairment. At examination he showed ataxic gait, marked cerebellar nystagmus, dysmetria, limb tremor and dysarthria. Electrophysiological examination (BAEP, SAP, SEP, SCV, MCV, CMAP) was normal. At MRI (Fig. 2) a cerebellar atrophy, mainly of vermis, was evident without truncal involvement. The patient had had short vertigo episodes during the 9-year period preceding the impairment of balance.

Patient IV-2 had episodes of dizziness since the age of 35 years, lasting a few seconds and triggered by postural changes such as raising himself from bed. The episodes became more frequent (several times per day) over the next few years and were accompanied by true vertigo and inability to stand up without support. These episodes were followed by difficulties in speaking and walking lasting several minutes. Five years after the onset he noted a progressive gait imbalance which limited his daily activities, as well as dysarthria. An MRI performed when he was aged 57 years showed a vermian cerebellar atrophy. He was seen for the first time by our group at the age of 60 years, and presented marked cerebellar deficits (*see* Table 1). Neurophysiological (BAEP, SAP, SEP, SCV, MCV, CMAP) and audiovestibular examinations were

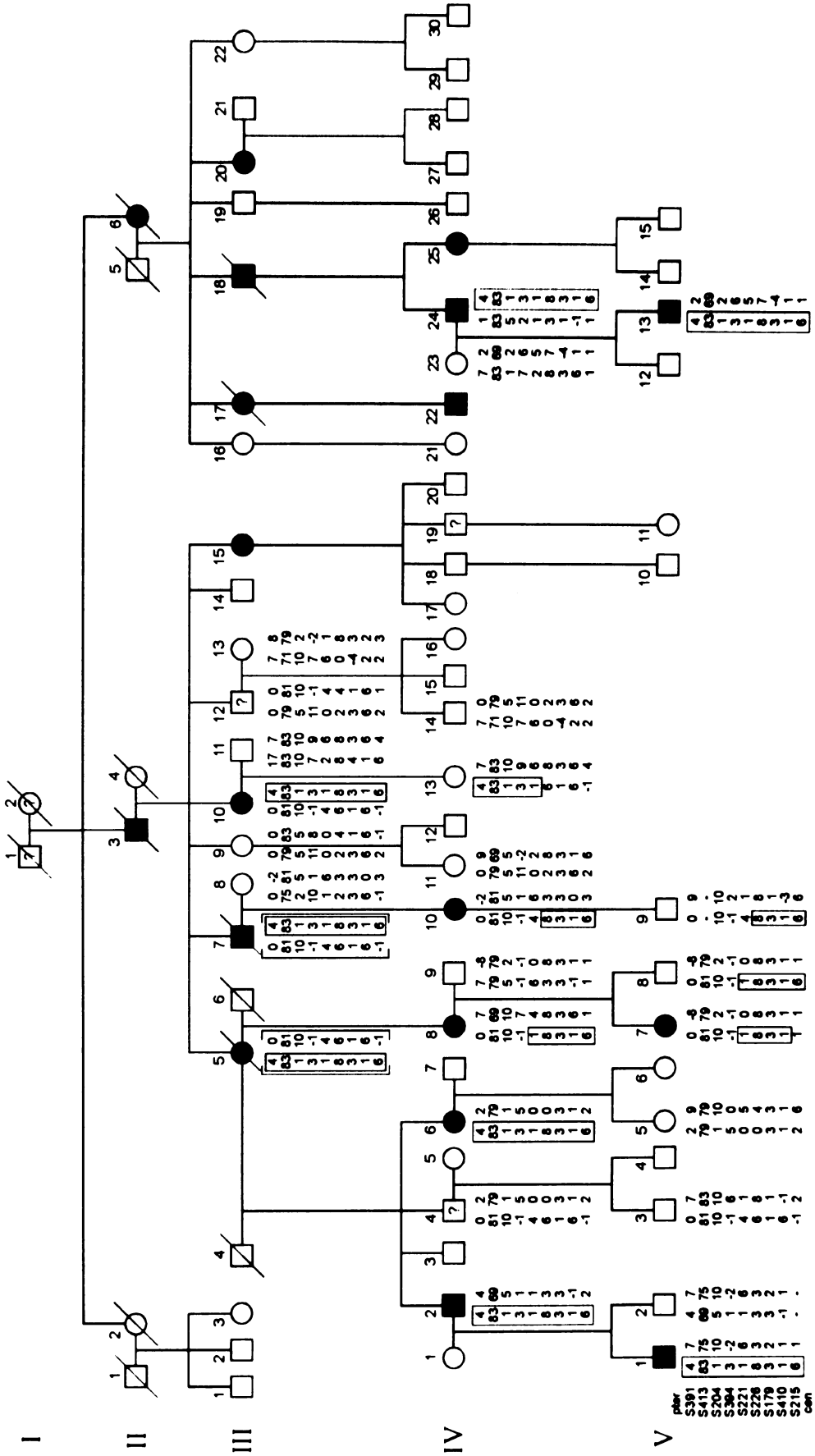


**Fig. 1** Pedigree of the EA2 family. Filled symbols indicate affected individuals and open symbols unaffected ones. Question marks within symbols indicate undefined phenotype (*see* text). Nine-marker haplotypes are reported only when relevant to the reconstruction of recombinant chromosomes. Inferred haplotypes are in brackets. Haplotypes segregating with the disease are boxed.

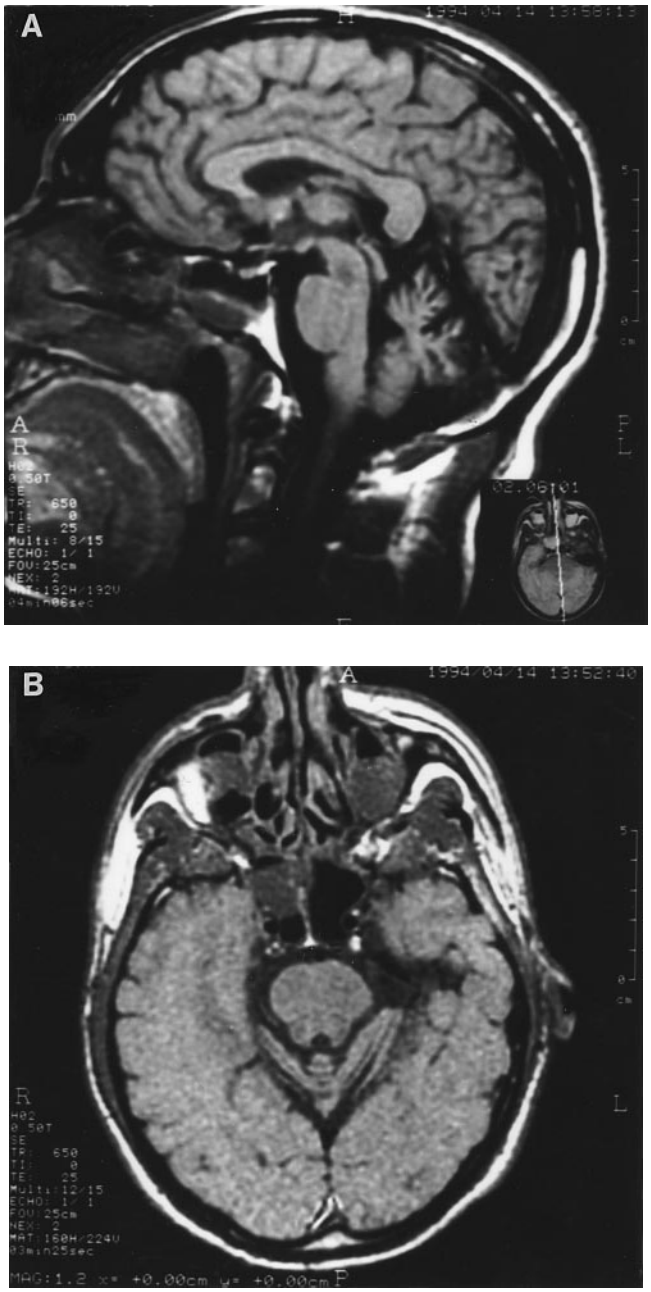


**Fig. 2** Brain MRI of the proband. (**A**) Midsagittal $T_1$-weighted image shows atrophy of the cerebellar vermis. (**B**) Coronal $T_1$-weighted image shows an enlargement of cerebellar sulci.

normal. A new MRI scan performed 3 years after the previous one showed that atrophy had extended to the cerebellar hemispheres. Treatment with 250 mg/day acetazolamide completely resolved the daily episodes and only slightly improved the cerebellar signs.

Overall, out of the 52 examined, 13 family members were found to have a clear history of repeated vertigo episodes, always beginning in adulthood (Table 1). Age at onset seemed to show a tendency toward anticipation along generations. The attacks, as reported by patients, were mostly triggered by postural changes, except for three subjects who could not identify the stimuli. They were characterized by vertigo, oscillopsia and cerebellar signs limited to an inability to stand up or walk and dysarthria. No inaccuracy or incoordination in upper limb movement was reported. The attack observed by one of the authors in Patient V-1 was mainly characterized by vertigo and nystagmus without other symptoms. Eleven of the 13 patients had interictal ataxia of various degrees and the two patients with shorter disease duration had interictal nystagmus and a mild tremor only. No impairment of other neurological systems was present. All the 11 patients undergoing MRI (Table 1) had vermian with or without hemisphere cerebellar atrophy. Six had migraine without aura.

A few family members showed atypical neurological signs. Subject III-12, aged 70 years, reported only one episode of vertigo lasting a few days, but had no interictal signs. Subject IV-4, aged 57 years, with a history of alcohol abuse, presented with uncertain gait, intentional tremor and a diffuse cerebral atrophy at MRI. Subject IV-19, aged 44 years, with a history of migraine, reported several episodes of dizziness but had no definite interictal signs. These three subjects were considered as having an undefined phenotype.

The effect of acetazolamide treatment could be reliably evaluated in seven patients only (Table 2), since the others had a very poor compliance with drug-taking. In all of them the treatment was highly effective in reducing the frequency and duration of episodes, while showing only a mild improvement of the permanent cerebellar signs.

## *Genetic study*

The result of two-point linkage analyses between the disease and nine microsatellite markers of the 19p13 region are reported in Table 3. Highly positive lod scores were obtained with markers proximal to D19S221. When subjects with undefined phenotypes were considerd as affected, somewhat lower, but still highly positive lod scores were obtained (e.g. D19S226 maxlod = 2.65, theta 0.11). Multipoint linkage analysis with the disease locus against a fixed map D19S413-cM6-D19S221-cM12-D19S215 provided 910:1 odds in favour of a disease gene location proximal, rather than distal, to D19S221 (Fig. 3).

At haplotype inspection three relevant crossovers were observed (Fig. 1). Patient IV-10 carried a disease chromosome recombining with the normal paternal haplotype distal to D19S226. The recombinant chromosome was passed on to her 28-year-old healthy son, whose residual risk of manifesting the disease later on is ~0.60. A new blood sample and a neurological rexamination of Subject IV-10 confirmed previous findings. This crossover sets a new distal boundary for the EA2 gene-containing region at D19S221, which maps 6 cM proximal to D19S413 (distal boundary set by Kramer *et al*., 1995) and 8 cM proximal to D19S391 (distal boundary set by von Brederlow *et al*., 1995). Further support to a location proximal to D19S221 comes from the complementary crossover in Subject IV-13 who carries the normal chromosome recombined with the distal part of the disease-associated

**Table 1** *Features of the disease in 13 family members*

| | Subjects | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | III-10 | III-15 | III-20 | IV-2 | IV-6 | IV-8 | IV-10 | IV-22 | IV-24 | IV-25 | V-1 | V-7 | V-13 |
| Age (years) | | | | | | | | | | | | | |
| at examination | 58 | 74 | 65 | 60 | 54 | 45 | 58 | 50 | 50 | 48 | 35 | 25 | 23 |
| at onset of episodes | 52 | 50 | 44 | 35 | 48 | 30 | 35 | 42 | 39 | 30 | 31 | 23 | 20 |
| at onset permanent ataxia | 56 | 70 | 50 | 40 | 44 | 41 | 56 | 45 | 42 | 45 | 35 | - | - |
| Attacks, as reported by patients | | | | | | | | | | | | | |
| Frequency | W | W | W | D | D | D | D | W | W | D | D | W | W |
| Duration | Brief | Brief | 1–2′ | Brief | 2–5′ | 1–2′ | 1′–2 h | 1–2′ | 1–2′ | 1–2′ | 1–2′ | Brief | Brief |
| Precipitating factors: | | | | | | | | | | | | | |
| postural change | + | + | − | + | + | + | + | + | − | + | + | + | − |
| emotional/physical stress | − | − | − | − | − | − | + | − | − | − | − | − | − |
| alcohol | − | − | − | − | − | − | − | − | − | − | − | − | − |
| Vertigo | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Nausea or vomiting | − | − | − | − | + | − | − | − | − | − | − | − | − |
| Tinnitus | − | + | + | − | + | − | − | − | − | − | − | − | − |
| Oscillopsia | + | + | + | + | + | + | − | + | + | + | + | + | + |
| Diplopia | − | − | − | − | + | − | + | − | − | − | − | − | − |
| Gait ataxia | − | − | + | + | + | − | + | + | + | + | − | − | − |
| Upper limb ataxia | − | − | − | − | − | − | − | − | − | − | − | − | − |
| Dysarthria | + | + | − | + | + | + | + | + | + | + | − | − | − |
| Interictal signs | | | | | | | | | | | | | |
| Oculomotor findings | 2 | 2 | 3 | 3 | 3 | 3 | 2 | 3 | 3 | 3 | 2 | 2 | 2 |
| Intention tremor | 2 | 1 | 4 | 4 | 1 | 3 | 2 | 3 | 3 | 2 | 2 | 1 | 1 |
| Dysarthria | 1 | 2 | 4 | 3 | 1 | 3 | 2 | 3 | 4 | 3 | 1 | 0 | 0 |
| Dysdiadochokinesis | 2 | 2 | 4 | 4 | 2 | 3 | 2 | 3 | 4 | 3 | 1 | 0 | 0 |
| Upper limb ataxia | 1 | 2 | 3 | 3 | 2 | 2 | 2 | 3 | 3 | 2 | 1 | 0 | 0 |
| Lower limb ataxia | 2 | 3 | 4 | 3 | 1 | 2 | 1 | 3 | 4 | 1 | 1 | 0 | 0 |
| Ataxia of stance | 1 | 2 | 3 | 3 | 2 | 3 | 1 | 3 | 3 | 1 | 1 | 0 | 0 |
| Ataxia of gait | 1 | 2 | 3 | 3 | 1 | 2 | 1 | 3 | 4 | 2 | 1 | 0 | 0 |
| Total score: | 12 | 16 | 28 | 26 | 13 | 21 | 13 | 24 | 28 | 17 | 10 | 3 | 3 |
| Cerebellar atrophy (MRI) | 2 | 3 | 4 | 4 | NP | 3 | 3 | NP | 4 | 3 | 2 | 1 | 1 |

D = daily; W = weekly; h = hours (′ = minutes); + = present; − = absent; NP = not performed. *See* text for scoring system.

**Table 2** *Evaluation of acetazolamide in seven patients, comparing the ictal and interictal signs prior to and during treatment*

| | Subjects | | | | | | |
|---|---|---|---|---|---|---|---|
| | III-20 | IV-2 | IV-8 | IV-10 | IV-24 | IV-25 | V-1 |
| Attacks before/during acetazolamide treatment (250–500 mg/day) | | | | | | | |
| Duration | 1-2′/brief | Brief/none | 1–2′/brief | 1′–2 h/brief | 1–2′/none | 1–2′/none | 1–2′/none |
| Frequency | W/M | D/none | D/M | D/M | W/none | D/none | D/none |
| Cerebellar signs | | | | | | | |
| Oculomotor findings | 3/3 | 3/3 | 3/3 | 2/2 | 3/3 | 3/2 | 2/2 |
| Intention tremor | 4/2 | 4/2 | 3/2 | 2/1 | 3/3 | 2/1 | 2/1 |
| Dysarthria | 4/3 | 3/2 | 3/1 | 2/1 | 4/2 | 3/1 | 1/0 |
| Dysdiadochokinesis | 4/2 | 4/2 | 3/1 | 2/1 | 4/2 | 3/1 | 1/0 |
| Upper limb ataxia | 3/2 | 3/2 | 2/1 | 2/2 | 3/3 | 2/1 | 1/0 |
| Lower limb ataxia | 4/3 | 3/3 | 2/1 | 1/1 | 4/4 | 1/0 | 1/0 |
| Ataxia of stance | 3/2 | 3/2 | 3/1 | 1/0 | 3/3 | 1/0 | 1/0 |
| Ataxia of gait | 3/2 | 3/3 | 2/1 | 1/1 | 4/4 | 2/1 | 1/0 |
| Total score | 28/19 | 26/19 | 21/11 | 13/9 | 28/24 | 17/7 | 10/3 |

D = daily, W = weekly, M = monthly, h = hours (′ = minutes). For scoring system *see* text.

haplotype including D19S221. This subject has no sign of the disease at the age of 38 years and her residual risk of having the disease is ~0.25. Finally, patient IV-8 carried a disease chromosome recombining with the normal maternal haplotype distal to D19S221, which is passed on to the affected daughter V-7 with the normal maternal allele at

**Table 3** *Pairwise linkage analyses between the disease and each 19p13 marker*

| Locus | θ | | | | | | $Z_{max}$ | θ at $Z_{max}$ |
|---|---|---|---|---|---|---|---|---|
| | 0.00 | 0.05 | 0.10 | 0.20 | 0.30 | 0.40 | | |
| D19S391 | –∞ | 1.95 | 2.35 | 2.17 | 1.51 | 0.62 | 2.39 | 0.12 |
| D19S413 | –∞ | 0.40 | 0.89 | 0.94 | 0.61 | 0.26 | 0.99 | 0.15 |
| D19S204 | –∞ | 0.76 | 1.31 | 1.42 | 1.03 | 0.44 | 1.46 | 0.16 |
| D19S394 | –∞ | 1.02 | 1.26 | 1.12 | 0.73 | 0.29 | 1.27 | 0.12 |
| D19S221 | –∞ | 2.99 | 2.79 | 2.05 | 1.19 | 0.39 | 2.99 | 0.04 |
| D19S226 | 5.00 | 4.54 | 4.05 | 3.01 | 1.87 | 0.71 | 5.00 | 0.00 |
| D19S179 | 2.43 | 2.24 | 2.04 | 1.55 | 0.98 | 0.40 | 2.43 | 0.00 |
| D19S410 | 3.34 | 2.96 | 2.62 | 1.96 | 1.25 | 0.50 | 3.34 | 0.00 |
| D19S215 | –∞ | 4.82 | 4.54 | 3.60 | 2.43 | 1.12 | 4.84 | 0.04 |

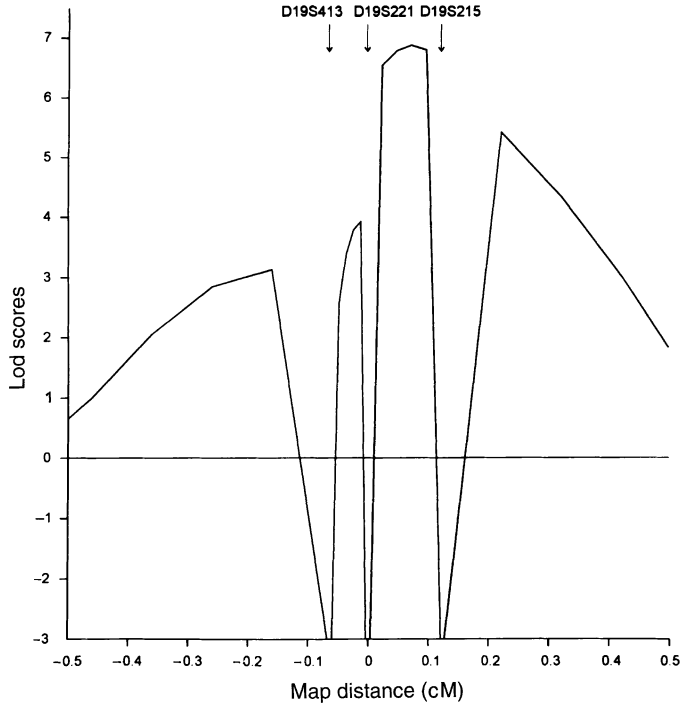


**Fig. 3** Four-point linkage analysis of EA2 locus against a fixed map D19S413-D19S221-D19S215 (cM).

locus D19S215. This crossover sets the proximal limit of the candidate interval in our family, but does not refine the previous proximal boundary at D19S226 (Kramer *et al*., 1995; Teh *et al*., 1995; von Brederlow *et al*., 1995). It is notable that the three subjects with undefined phenotypes are not carrying the disease haplotype. In fact, their atypical features could be ascribed to different causes such as anxiety or migraine equivalents (IV-19), alcoholism (IV-4) and an occasional vestibular impairment (III-12).

## Discussion

A large Italian family with an autosomal dominant acetazolamide-responsive episodic ataxia linked to 19p13 markers is reported. Patients showed repeated attacks of vertigo and unsteadiness lasting from minutes to hours, and, interictally, cerebellar signs of variable severity (from nystagmus only to severe permanent ataxia) and vermian with or without hemispheric atrophy. None of the family members showed myokymia. Migraine was a common complaint among patients but none of them had features of familial hemiplegic migraine.

Compared with families segregating for the EA2 gene on chromosome 19p13 reported in the literature (Kramer *et al*., 1995; Teh *et al*., 1995; Vahedi *et al*., 1995; von Brederlow *et al*., 1995), the present family showed attacks with a more definite vestibular component, being triggered by quick movements or postural changes and characterized by vertigo and oscillopsia with cerebellar signs limited to dysarthria and gait ataxia. Acetazolamide-responsive episodic ataxia displays a wide variability of signs and symptoms during attacks (Moon and Koller, 1991). Some authors distinguish two different types of EA2: a vestibulocerebellar one, with episodes similar to those in the present family, and a ‘diffuse cerebellar syndrome’, in which both trunk and limb ataxia are present during the episodes while vertigo is infrequent (Farmer and Mustian, 1963; Baloh and Winder, 1991; von Brederlow *et al*., 1995). The latter form is more similar to that found in previous families with linkage to the 19p markers. However, Donat and Auger (1979) reported both types of attacks in the same family, indicating that they have to be considered as a variable expression of the same gene mutation. In the present family with the vestibulocerebellar type, the linkage of the disease to 19p13 markers further supports the hypothesis that the two types are due to the same gene.

Another distinctive feature of this family is the onset of the disease in middle/late adulthood, while the families on which the disease gene was previously mapped had an onset in infancy or adolescence (Kramer *et al.*, 1995; von Brederlow *et al.*, 1995). However, a late age of onset is also reported by Farmer and Mustian (1963) and Farris *et al.* (1986).

A final aspect worth considering in these patients is the high frequency of MRI anomalies. No neuroimaging study as extended as the present one was performed in the families previously reported. Atrophy of cerebellar vermis has been documented in three patients with episodic ataxia and interictal nystagmus by Vighetto *et al.* (1988). However, patients with normal MRI or CT scans are also reported

occasionally (Zasorin *et al.*, 1983; Baloh and Winder, 1991; Vahedi *et al.*, 1995). The atrophy of cerebellar vermis, therefore, appears to be a variable feature of the disease, but our findings seem to indicate that it is more frequent than previously thought, assuming identical criteria for the radiological diagnosis.

The genetic study mapped the disease locus in our family on chromosome 19p13 between markers D19S221 and D19S215. This interval widely overlaps that reported by Kramer *et al.* (1995), Teh *et al.* (1995) and von Brederlow *et al*. (1995), D19S221 being 6–8 cM proximal to the distal boundary (D19S391/D19413) previously reported, and D19S215 6 cM proximal to the previous proximal boundary (D19S226). Therefore, D19S221 constitutes the new distal limit of the EA2 gene-containing region. By combining the present data with that reported by Kramer *et al*. (1995), Teh *et al*. (1995) and von Brederlow *et al*. (1995), the most probable location of the EA2 gene is in the D19S221–D19S226 interval. Although the estimated distance between these two markers is genetically ~6 cM, their physical distance appears to be relatively small. Ashworth *et al*. (1995), in their chromosome 19 physical map, provide an estimated distance of ~1.5 Mb between the two markers. The discrepancy between the genetic and physical estimates could be explained by hypothesizing a relatively high recombination frequency in this interval. The present location is still compatible with the hypothesis of an allelism between the EA2 and familial hemiplegic migraine genes (Vahedi *et al*., 1995), the latter being localized in a 11 cM interval between D19S413 and D19S226 which includes the above region (Joutel *et al*., 1994).

During the editorial processing of this paper Ophoff *et al.* (1996) reported the characterization of the $Ca^{2+}$ gene CACNL1A4 on a 19p13 cosmid contig and the discovery of missense mutations in five familial hemiplegic migraine and premature stop mutations in two EA2 families. The gene was found in the interval D19S221–D19S226 as indicated in the present paper. Although no clinical details are provided by Ophoff *et al*. (1996), the EA2 patients screened for CACNL1A4 mutations included those reported by von Brederlow *et al.* (1995), i.e. with an EA2 type characterized by early onset and attacks with a diffuse cerebellar syndrome. However, in the present family the 13 patients consistently showed the clinical features of the vestibulo-cerebellar type of EA2 with late onset and frequent progression towards a permanent cerebellar degeneration. The identical gene location in the two EA2 types suggests the hypothesis that a mutation different from those found by Ophoff *et al*. (1996), possibly affecting different protein domains, is responsible for the unusual phenotype in the present family.

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**References**

Ashworth LK, Batzer MA, Brandriff B, Branscomb E, de Jong P, Garcia E, et al. An integrated metric physical map of human chromosome 19. Nat Genet 1995; 11: 422–7.

Bain PG, O’Brien MD, Keevil SF, Porter DA. Familial periodic cerebellar ataxia: a problem of cerebellar intracellular pH homeostasis. Ann Neurol 1992; 31: 147–54.

Baloh RW, Winder A. Acetazolamide-responsive vestibulocerebellar syndrome: clinical and oculographic features. Neurology 1991; 41: 429–33.

Browne DL, Gancher ST, Nutt JG, Brunt ERP, Smith EA, Kramer P, et al. Episodic ataxia/myokymia syndrome is associated with point mutations in the human potassium channel gene, KCNA1 [see comments]. Nat Genet 1994; 8: 136–40. Comment in: Nat Genet 1994; 8: 111–2.

Donat JR, Auger R. Familial periodic ataxia. Arch Neurol 1979; 36: 568–9.

Farmer TW, Mustian VM. Vestibulocerebellar ataxia. A newly defined hereditary syndrome with periodic manifestations. Arch Neurol 1963; 8: 471–80.

Farris BK, Smith JL, Ayyar DR. Neuroopthalmologic findings in vestibulocerebellar ataxia. Arch Neurol 1986; 43: 1050–3.

Friedman JH, Hollmann PA. Acetazolamide responsive hereditary paroxysmal ataxia. [Review]. Mov Disord 1987; 2: 67–72.

Gancher ST, Nutt JG. Autosomal dominant episodic ataxia: a heterogeneous syndrome. Mov Disord 1986; 1: 239–53.

Joutel A, Ducros A, Vahedi K, Labauge P, Delrieu O, Pinsard N, et al. Genetic heterogeneity of familial hemiplegic migraine. Am J Hum Genet 1994; 55: 1166–72.

Kimura J. Electrodiagnosis in diseases of nerve and muscle. Principles and practice. 2nd ed. Philadelphia, F.A.Davis, 1989.

Klockgether T, Schroth G, Diener H-C, Dichgans J. Idiopathic cerebellar ataxia of late onset: natural history and MRI morphology. J Neurol Neurosurg Psychiatry 1990; 53: 297–305.

Kramer PL, Yue Q, Gancher ST, Nutt JG, Baloh R, Smith E, et al. A locus for the nystagmus-associated form of episodic ataxia maps to an 11-cM region on chromosome 19p [letter]. Am J Hum Genet 1995; 57: 182–5.

Lathrop GM, Lalouel J-M, Julier C, Ott J. Strategies for multilocus linkage analysis in humans. Proc Natl Acad Sci USA 1984; 81: 3443–6.

Litt M, Kramer P, Browne D, Gancher S, Brunt ERP, Root D, et al. A gene for episodic ataxia/myokymia maps to chromosome 12p13. Am J Hum Genet 1994; 55: 702–9.

Moon SL, Koller WC. Hereditary periodic ataxias. In: Vinken PJ, Bruyn, Klawans HL, editors. Handbook of clinical neurology, Vol 60. Amsterdam: Elsevier, 1991: 433–43.

Ophoff RA, Terwindt GM, Vergouwe MN, van Eijk R, Oefner PJ,

Hoffman SMG, et al. Familial hemiplegic migraine and episodic ataxia Type-2 are caused by mutations in the $Ca^{2+}$ channel gene CACNL1A4. Cell 1996; 87: 543–52.

Ott J. A simple scheme for the analysis of HLA linkages in pedigrees Ann Hum Genet 1978; 42: 255–7.

Sabbadini G, Francia A, Calandriello L, Di Biasi C, Trasimeni G, Gualdi GF et al. Cerebral autosomal dominant arteriopathy with subcortical infarcts and leucoencephalopathy (CADASIL). Clinical, neuroimaging, pathological and genetic study of a large Italian family. Brain 1995; 118: 207–15.

Teh, BT, Silburn P, Lindblad K, Betz R, Boyle R, Schalling M, et al. Familial periodic cerebellar ataxia without myokymia maps to a 19-cM region on 19p13. Am J Hum Genet 1995; 56: 1443–9.

Vahedi K, Joutel A, Van Bogaert P, Ducros A, Maciazeck J, Bach JF, et al. A gene for hereditary paroxysmal cerebellar ataxia maps to chromosome 19p [see comments]. Ann Neurol 1995; 37: 289–93. Comment in: Ann Neurol 1995; 37: 285–7.

Vighetto A, Froment J-C, Trillet M, Aimard G. Magnetic resonance imaging in familial paroxysmal ataxia. Arch Neurol 1988; 45: 547–9.

von Brederlow B, Hahn AF, Koopman WJ, Ebers GC, Bulman DE. Mapping the gene for acetazolamide responsive hereditary paroxysmal cerebellar ataxia to chromosome 19p. Hum Mol Genet 1995; 4: 279–84.

White JC. Familial periodic nystagmus, vertigo, and ataxia. Arch Neurol 1969; 20: 276–80.

Zasorin NL, Baloh RW, Myers LB. Acetazolamide-responsive episodic ataxia syndrome. Neurology 1983; 33: 1212–4.

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