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SCA28, a novel form of autosomal dominant cerebellar ataxia on chromosome 18p11.22–q11.2

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We describe a four-generation Italian family with a novel form of juvenile-onset, slowly progressive, autosomal dominant cerebellar ataxia. Eleven affected family members have been evaluated. The mean age at onset was 19.5 years with no evidence of anticipation. The first symptoms were invariably unbalanced standing and mild gait incoordination. Gaze-evoked nystagmus was prominent at onset, while patients with longer disease duration developed slow saccades, ophthalmoparesis and, often, ptosis. Deep tendon reflexes in lower limbs were increased in 80% of the cases. Genetic analysis excluded the presence of pathological repeat expansions in spinocerebellar ataxia (*SCA*) types 1–3, 6–8, 10, 12 and 17, and *DRPLA* genes. Linkage exclusion tests showed no evidence of association with other known *SCA* loci. A genome-wide screen analysis identified linkage with chromosome 18 markers. A maximum two-point limit of determination score of 4.20 was found for marker D18S53. Haplotype analysis refined a critical region of 7.9 Mb between markers D18S1418 and D18S1104. This new *SCA* locus on 18p11.22–q11.2 has been designated *SCA28*. Candidate genes within the critical interval are currently screened for mutations.

Keywords: spinocerebellar ataxia; SCA; oculomotor function; autosomal dominant cerebellar ataxia; linkage analysis

Abbreviations: ADCA = autosomal dominant cerebellar ataxia; DRPLA = dentato-rubral-pallido-luysian atrophy; LOD = log of the odds; SCA = spinocerebellar ataxia; SCA1 = spinocerebellar ataxia type 1

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Introduction

Autosomal dominant cerebellar ataxias (ADCAs) are a clinically and genetically heterogeneous group of neurodegenerative disorders primarily characterized by imbalance, progressive gait and limb ataxia, and dysarthria (Harding, 1982). The clinical phenotype appears often complicated by the presence of additional neurological signs, which are highly variable among and within families. Since the identification of the first gene responsible for spinocerebellar ataxia type 1 (SCA1) in 1993 (Orr *et al.*, 1993), an increasing number of genes and chromosomal loci have been characterized, demonstrating large genetic heterogeneity in these hereditary disorders (Schöls *et al.*, 2004; Taroni and Di Donato, 2004).

At present, 25 distinct genetic forms of spinocerebellar ataxias (SCAs) are known: SCA1–8, SCA10–23, SCA25–26 and SCA27/FGF14 (Knight *et al.*, 2004; Schöls *et al.*, 2004; Taroni and Di Donato, 2004; Ishikawa *et al.*, 2005; Yu *et al.*, 2005; www.gene.ucl.ac.uk/hugo) (Table 1). The genetic form designated as dentato-rubral-pallido-luysian atrophy (DRPLA) is also commonly classified within this group of disorders. The disease gene has been identified in 12 SCAs and in DRPLA. In SCAs 1, 2, 3, 6, 7 and 17 subtypes and in DRPLA the molecular mutation is a trinucleotide CAG repeat expansion within the coding region of the corresponding gene (Schöls *et al.*, 2004; Taroni and Di Donato, 2004).

The prevalence of SCAs has been estimated to be ~3 in 100 000, being the relative frequency of specific genotypes variable in different geographical areas and in populations of different ethnic origins (Schöls *et al.*, 2004; Brusco *et al.*,

Table 1 Genetic classification of SCAs*

Disease	Locus	Gene (mutations)	Prevalence
SCA1	6p23	*Ataxin-1* (CAG exp)	10% worldwide; common in South Africa (41%), Italy, India, Germany
SCA2	12q24	*Ataxin-2* (CAG exp)	15–20% worldwide; common in US, India, Italy
SCA3	14q24.3–q31	*Ataxin-3* (CAG exp)	Commonest worldwide 20–50% of SCA; 85% in Portugal/Brazil
SCA4	16q22.1	*Puratrophin-1* (5′-UTR 1 nt substitution)	Scandinavian (1), German (1) and Japanese (6) families
SCA5	11p11–q11	?	US and German families
SCA6	19p13	*CACNA1A* (CAG exp)	13–15% worldwide; common in Germany, US, Japan, Netherlands
SCA7	3p21.1–p12	*Ataxin-7* (CAG exp)	3–5% worldwide; common in South Africa, US, Netherlands
SCA8	13q21	Unknown (3′-UTR CTG exp)	3% worldwide; reduced penetrance
SCA9	Not assigned	–	–
SCA10	22q13	*Ataxin-10* (intronic ATTCT repeat exp)	Mexican and Brazilian families
SCA11	15q14–q21.3	?	One British family
SCA12	5q31–q33	*PPP2R2B* (5′-UTR CAG exp)	German (1) and Indian (6) families
SCA13	19q13.3–q13.4	?	One French family
SCA14	19q13.4–qter	*PRKCG* (missense mutations)	Japanese, French, English-Dutch families; reduced penetrance
SCA15	3p24.2–pter	?	Australian (1) and Japanese (1) families
SCA16	8q22.1–q24.1	?	One Japanese family
SCA17	6q27	*TBP* (CAG exp)	~50 families (Japan, Italy, Germany, France)
SCA18	7q22–q32	?	One Irish family
SCA19	1p21–q21	?	One Dutch family
SCA20	11p13–q11	?	One Anglo-Celtic family
SCA21	7p21.3–p15.1	?	One French family
SCA22	1p21–q23	?	One Chinese family
SCA23	20p13–12.3	?	One Dutch family
SCA24	Reserved	–	–
SCA25	2p15–21	?	One French family
SCA26	19p13.3	?	One Norwegian family
SCA27	13q34	*FGF14* (missense mutations)	Dutch (1) and German (1) families
DRPLA	12p13.31	*Atrophin-1* (CAG exp)	3% worldwide; common in Japan

*References: Schöls *et al.*, 2004; Taroni and Di Donato, 2004; Ishikawa *et al.*, 2005; Yu *et al.*, 2005; www.neuro.wustl.edu/neuromuscular/ataxia/

2004). In addition, population screening for the currently known *SCA* gene mutations demonstrates that ~30–60% of the clinically identified ADCA families remain genetically unassigned, indicating further genetic heterogeneity (Schöls *et al.*, 2004). We recently demonstrated that 45% of the Italian families are associated with *SCA1* and *SCA2* genotypes, a small percentage of cases are caused by expansions in *SCA3*, *SCA6*, *SCA7* and *SCA17* and *DRPLA* genes, whereas ~40% of the families are negative for mutations in the known *SCA* genes (Brusco *et al.*, 2004). From this group, we have characterized a four-generation Italian family with an inherited form of slowly progressive cerebellar ataxia. Genome-wide linkage studies allowed mapping of the disease locus on chromosome 18p11.22–q11.2. This new locus has been assigned the *SCA28* symbol by the Human Genome Nomenclature Committee (www.gene.ucl.ac.uk/hugo).

We report here the clinical features of the affected subjects and the results of genetic studies.

Patients and methods

Patients

The pedigree of the family (MI-A091) is shown in Fig. 1. In the past years, we have collected clinical information and blood samples from the proband III-1 and other family members in generation II. More recently, all the available affected subjects (11 patients) have been clinically evaluated. Four affected patients (III-1; III-7; III-14 and IV-4) were admitted at the Istituto Neurologico Carlo Besta for more extensive clinical evaluation including brain MRI, electrophysiology, electro-oculographic examination and muscle biopsy. Clinical information and blood samples from additional subjects of generation III and IV were collected for linkage studies. A total of 26 subjects (both affected and healthy individuals, and spouses) have been included in the study. Clinical and genetic investigations have been performed after obtaining informed consent from all participants or the parents of participating minors.

Analysis of known *SCA* genes and repeat expansion detection (RED) analysis

PCR analysis of the *SCA1–3*, *6–8*, *10*, *12* and *17*, and *DRPLA* gene expansions, and the RED analysis were performed as described previously (Brusco *et al.*, 2004).

Genotyping and linkage analysis

Linkage exclusion was carried out using an affected-only approach on nine patients (II-2, II-5, II-6, II-7, III-1, III-7, III-8, III-14 and III-16) and three spouses (II-1, II-4 and II-8) (Fig. 1). Genotyping was performed using 3–13 microsatellite markers for each of the following *SCA* loci (primer sequences are available on the Genome

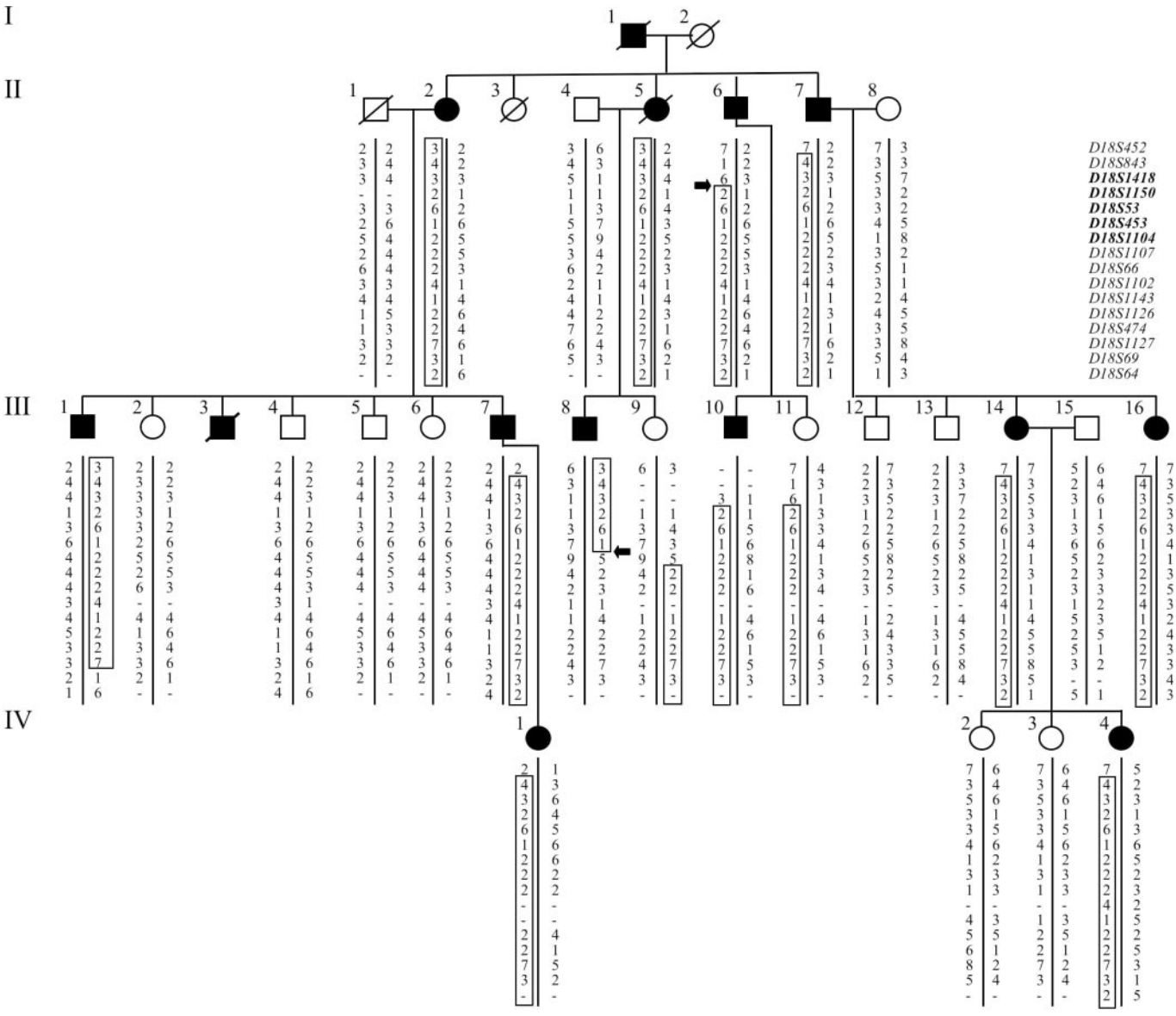

Fig. 1 Pedigree of the family MI-A091 with autosomal dominant spinocerebellar ataxia. Filled symbols indicate affected subjects. Open symbols indicate unaffected spouses and presently asymptomatic family members. Deceased subjects are marked by a diagonal line. Haplotype analysis is shown for 16 non-consecutive markers on chromosome 18. The haplotype assumed to carry the disease allele is boxed, and markers corresponding to the minimal linked region are in bold.

Database website, www.gdb.org): *SCA4*, *SCA5/20*, *SCA11*, *SCA13-16*, *SCA18*, *SCA19/22*, *SCA21*, *SCA25* and *SCA27*.

To localize the disease gene, we performed a genome-wide scan using 383 fluorescent-labelled microsatellite markers from the ABI-PRISM Linkage Mapping Set MD-10 version 2.5 (Applied Biosystems, Foster City, CA) spaced at intervals of ~10 cM on autosomes. Chromosome X was excluded because a male-to-male transmission was present. In addition to the subjects used for linkage exclusion, three patients (III-10, IV-1 and IV-4), one spouse (III-15) and one healthy relative (III-6) were included in the analysis. Polymerase chain reactions (PCRs) were performed as recommended by the supplier on GeneAmp 9700 or 2700 PCR machines (Applied Biosystems). Amplified fragments labelled with VIC, FAM or NED were pooled in 27 different panels, added to a mix of highly deionized formamide (10 μl) and LIZ-500 fluorescent marker (0.05 μl), denatured for 1 min at 95°C and loaded on an ABI-Prism 3730 automatic sequencer (Applied Biosystems). Data were collected and analysed using the Genemapper software (ver3.0; Applied Biosystems). Eleven additional polymorphic markers (D18S843, D18S1418, D18S1150, D18S453, D18S1104, D18S1107, D18S66, D18S1143, D18S1126, D18S1127 and D18S69, indicated in bold in Table 4) were analysed to narrow the positive candidate region on chromosome 18. Relative positions were derived from the human genome draft sequence and the Marshfield map (www.ncbi.nlm.nih.gov).

Pairwise and multipoint log of the odds (LOD) scores were calculated using the MLINK and SIMWALK2 programs (Lathrop *et al.*, 1984; Sobel *et al.*, 1996). The disease was considered to be autosomal dominant with a frequency of 0.001%. Recombination fractions were assumed equal for men and women, and were

converted to map distances using the Kosambi mapping function. Multipoint analysis was performed with the sex-averaged recombination fractions and the order of loci as calculated on Marshfield map. Final linkage data were calculated using 12 patients, 4 spouses and 10 healthy relatives (Fig. 1). Age-dependent penetrance was taken into account by assigning healthy subjects to one of two liability classes determined from the age at onset curve of the family [25–35 years (80%), 36–50 years (90%)]. Allele frequencies at each microsatellite marker were assumed to be equal.

Results

Clinical features

As shown in Fig. 1 the disease was probably transmitted through individual I-1, originating from Southern Italy, who was reported to have gait and speech difficulties. This subject died at the age of 74 of heart failure. The disease was inherited by four of his five children, three of whom are still alive and have been examined by us. The original index case was subject III-1, who was first evaluated at our Institute at the age of 39 years. He suffered from disequilibrium and gait difficulties since the age of 20 years. Neurological examination showed: nystagmus in lateral and vertical gaze, mild ophthalmoparesis in the horizontal plane, dysarthria, mild gait and limb ataxia, increased deep tendon reflexes at the four limbs, bilateral ankle clonus and Babinski sign. Muscle tone, sensation and mental status were normal. IQ was 83. Routine blood tests and cerebrospinal fluid (CSF) examination were normal. The patient was subsequently re-evaluated at 42 and 54 years of age. At the last examination, after 34 years of disease, the patient showed a moderate progression of the gait ataxia and was still able to walk independently. Neurological examination revealed severe ophthalmoparesis in the horizontal and vertical planes, very slow saccades and bilateral palpebral ptosis, wide-based ataxic gait and moderate limb dysmetria. Tendon reflexes were still hyperactive but there was no spasticity.

The other affected members of the family had similar clinical histories and phenotypes. Two patients had died: subject II-5 deceased at age 73 of gastric tumour, while subject III-3 died of acute myocardial infarction at the age of 45. The disease course was slowly progressive with most of the patients remaining ambulant in their late sixties. Patients II-2, II-7 and II-5 required a walking support at the age of 72, 75 and 70, respectively, whereas patient II-6 was confined to a wheelchair at 73 years of age. The clinical features of the 11 affected subjects are summarized in Table 2. The mean age at onset was 19.5 years (range 12–36). Ascertained ages at onset in generation II were 20 and 36 years; ages at onset in generation III ranged from 16 to 20 years, whereas in the two affected subjects of generation IV the onset was at 12 and 16 years. These data do not suggest overt anticipation.

The first symptoms were invariably imbalance in standing and mild gait and limb incoordination. Disease duration ranged from 1 to 58 years. No cognitive impairment was observed at clinical examinations. Increased patellar tendon

Table 2 Clinical features of affected individuals of family MI-A091 with autosomal dominant SCA

	II-2	II-6	II-7	III-1	III-7	III-8	III-10	III-14	III-16	IV-1	IV-4
Sex	F	M	M	M	M	M	M	F	F	F	F
Age at onset	36	nd	20	20	17	16	19	19	20	12	16
Age at exam	76	74	78	54	41	55	51	48	45	13	18
Walking	Unilateral support	Wheel chair	Unilateral support	Independent	Independent	Independent	Independent	Independent	Independent	Independent	Independent
Gait ataxia	+++	+++	+++	++	++	++	++	++	++	+	+
Dysarthria	+++	++	++	++	++	++	+	+	+	–	–
Limb ataxia	++	++	++	++	++	++	++	++	++	Kinetic tremor	+
Nystagmus	–	–	–	–	++	–	++	+++	–	+	++
Slow saccades	+++	++	++	+++	+	++	–	–	nd	–	–
Ophthalmoparesis	+++	++	+++	+++	+	++	–	+	++	–	–
Ptosis	++	nd	+	+	–	+	–	+ Unilateral	+	–	–
Reflexes (lower limbs)	Increased	nd	Increased	Increased	Normal	Increased	Increased	Increased	Increased	Increased	Increased
Increased muscle tone (lower limbs)	+	nd	+	–	–	–	+	–	nd	–	–
Babinski sign	nd	–	nd	+	–	–	–	+	nd	+	+

Clinical signs are graded as follows: +++ = severe; ++ = moderate; + = mild; (–) = absent. nd = not determined.

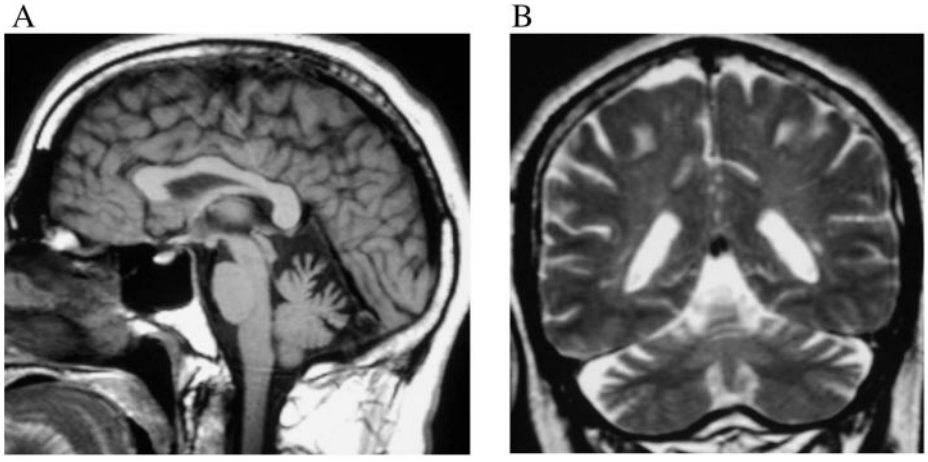

Fig. 2 Brain MRI 1.5T of patient III-1. Panel (**A**) Midline sagittal T1-weighted image showing cerebellar atrophy, particularly evident in the superior vermis. The supratentorial compartment and the brainstem are normal. Panel (**B**) Coronal T2-weighted image showing atrophy of the cerebellar hemispheres with enlarged cortical sulci.

reflexes were found in 9 out of 10 patients, increased muscle tone in lower limbs in 3 out of 9 and Babinski sign in 4 out of 8. There was no clinical evidence of sensory involvement. In 5 out of 11 patients a mild-to-severe horizontal gaze-evoked nystagmus was present, 6 out of 10 had slow saccades and 8 out of 11 had mild-to-severe ophthalmoparesis. In 6 out of 10 subjects palpebral ptosis was observed.

Patients III-1, III-7, III-14 and IV-4 underwent electrophysiological, electro-oculographic and brain imaging studies. Nerve conduction studies, visual, auditory, somatosensory and motor evoked potentials were normal in all cases. Brain MRI showed cerebellar atrophy (Fig. 2).

At electro-oculographic evaluation, hypometric saccades were found in patients III-1, III-7 and III-14, and hypermetric saccades in patient IV-4. Reduced gain in smooth horizontal pursuit was detected in the three patients (range 0.5–0.6; frequency 0.2 Hz), and saccadic break-down of pursuit was present in patients III-14 and IV-4. Optokinetic gain was reduced in patients III-14 and IV-4 (0.3 and 0.7, at drum velocity 20°/s), while it was abolished in patient III-1 and III-7.

Muscle biopsies, performed in patients III-1, III-7, III-14 and IV-4, showed no ragged-red fibres. Biochemical assays on muscle homogenates demonstrated normal activities of the respiratory chain enzymes (complexes I-V), and Southern blot analysis excluded the presence of mitochondrial DNA deletions (data not shown).

Genetic tests and linkage analysis

SCA1–3, *SCA6–8*, *SCA10*, *SCA12*, *SCA17* and *DRPLA* gene repeat expansions were excluded, and RED analysis did not reveal CAG/CTG expansions >40 repeats. In addition, there was no evidence of linkage of this family to most of known mapped *SCA* loci (*SCA4*, *SCA5/20*, *SCA11*, *SCA13-16*, *SCA18*, *SCA19/22*, *SCA21*, *SCA25* and *SCA27*) (Table 3).

We used a genome-wide scan to map the new locus. The most suggestive evidence of linkage was obtained at two distinct non-consecutive markers on chromosome 18, D18S53 and D18S474. To confirm linkage in one of the two regions and for fine mapping, we tested 11 additional polymorphic markers. Pairwise and multipoint linkage analyses confirmed the presence of two regions, one on the short arm and one on the long arm of chromosome 18 (Fig. 3 and Table 4). The highest LOD scores were found in the first region between markers D18S1418 and D18S1104 with a maximum of $Z = 4.77$ at marker D18S453 in multipoint analysis. LOD scores in the second region, spanning markers D18S1102 and D18S69, were below the threshold of $Z = 3.3$ both in two-point and multipoint tests (Table 4).

Haplotype reconstruction (Fig. 1) showed that a single haplotype segregated with the disease in the family. Two key recombination events, centromeric to D18S1418 in patient II-6 and to D18S1104 in patient III-8, defined the minimal in-linkage interval between D18S1418 and D18S1104 as a 7.9 Mb tract. The region is located on cytogenetic bands 18p11.22–q11.2. This new locus has been assigned the *SCA28* symbol by the Human Nomenclature Committee (www.gene.ucl.ac.uk/hugo).

Discussion

We studied an Italian family with hereditary cerebellar ataxia characterized by juvenile onset, slow disease progression, eye movement abnormalities and, in some cases, pyramidal signs. The transmission of the trait was compatible with autosomal dominant inheritance. The phenotype observed in the affected family members was homogeneous as regards neurological symptoms and disease progression. The clinical features of this family are not fully distinctive in comparison with other SCA subtypes. However, the association of very slowly progressive cerebellar ataxia, ophthalmoparesis, increased deep tendon reflexes and Babinski sign is peculiar. The neurological condition observed in this family combines a few clinical features characteristic of ADCA type I as

Table 3 Two-point and multipoint LOD score values for markers spanning the 13 different *SCA* loci analysed

Marker	Two-point LOD score at θ =							Multipoint
	cM or Mb	0.00	0.01	0.05	0.1	0.2	0.3	LOD score
SCA4 (~3 cM)								
D16S398	0	−7.89	−1.92	−0.65	−0.21	0.06	0.06	−7.16
D16S397	0.7†	−3.84	−0.86	−0.24	−0.04	0.07	0.06	−4.73
D16S421	1.3†	−4.29	−1.30	−0.63	−0.37	−0.14	−0.05	−4.49
SCA5/20 (~26.7 Mb)								
D11S905	0	−21.6	−7.3	−3.9	−2.5	−1.2	−0.5	−17.6
D11S1920	14.1†	−4.48	−1.48	−0.78	−0.49	−0.22	−0.09	−6.04
D11S4191	18.9†	−8.65	−2.66	−1.32	−0.78	−0.32	−0.11	−9.13
D11S1258	26.2†	−8.80	−2.80	−1.44	−0.89	−0.39	−0.15	−9.20
D11S987	26.7†	−8.30	2.30	−1.00	−0.50	−0.10	0.03	−9.14
SCA11 (~7.6 cM)								
D15S968	0	−12.3	−2.08	−0.79	−0.32	0.01	0.09	−12.2
D15S123	5.4	−3.89	−0.91	−0.29	−0.10	0.01	0.01	−6.94
D15S978	5.4	−8.62	−2.63	−1.29	−0.75	−0.29	−0.10	−8.55
SCA13 (~8 cM)								
D19S412	0	−4.10	−1.11	−0.46	−0.23	−0.06	−0.01	−6.32
D19S902	2.6	−13.2	−5.72	−3.01	−1.90	−0.89	−0.40	−12.6
D19S866	7.4	−4.10	−1.11	−0.48	−0.26	−0.10	−0.04	−5.79
SCA14 (~10.2 cM)								
D19S601	0	−4.40	−1.40	−0.72	−0.44	−0.19	−0.08	−5.28
D19S921	4.5	−4.24	−1.25	−0.60	−0.35	−0.15	−0.06	−4.56
D19S927	6.5	−8.80	−2.80	−1.44	−0.89	−0.39	−0.15	−4.52
D19S926	11.4	−12.9	−5.59	−2.88	−1.77	−0.77	−0.30	−12.4
SCA15 (~4 cM)								
D3S3050	0	−12.2	−4.08	−2.07	−1.28	−0.59	−0.27	−12.1
D3S1560	4.5	−12.8	−4.50	−2.44	−1.59	−0.78	−0.37	−12.1
SCA16 (~37.6 cM)								
D8S1779	0	−4.10	−1.11	−0.48	−0.26	−0.11	−0.05	−4.97
D8S1802	5.2	−4.25	−1.26	−0.59	−0.34	−0.12	−0.04	−2.12
D8S1799	10.1	−8.80	−2.80	−1.44	−0.89	−0.39	−0.15	−8.66
D8S1774	14.4	−8.75	−2.76	−1.41	−0.86	−0.37	−0.14	−8.46
SCA18 (~14 cM)								
D7S523	0	−8.00	−2.29	−0.99	−0.50	−0.14	−0.02	−10.1
D7S486	1.1†	−8.06	−2.09	−1.00	−0.41	−0.16	−0.10	−8.09
D7S530	17.5†	−3.55	−2.09	−0.86	−0.45	−0.18	−0.09	−4.40
SCA19/22 (~35 cM)								
D1S2841	0	−17.3	−5.78	−3.06	−1.95	−0.93	−0.43	−16.7
D1S207	7.2	−17.6	−5.61	−2.91	−1.81	−0.85	−0.38	−16.7
G15725	22.9	−12.4	−4.03	−2.04	−1.26	−0.60	−0.29	−12.0
D1S2808	25.4	−7.83	−0.77	−0.16	0.02	0.09	0.06	−7.40
UT5210	27.7	−7.32	−1.95	−0.68	−0.25	0.01	0.01	−7.12
D1S1631	30.4	−8.33	−2.94	−1.57	−1.00	−0.50	−0.25	−7.32
D1S495	30.4	−8.27	−2.88	−1.52	−0.96	−0.47	−0.24	−7.33
D1S2651	35.8	−8.54	−2.55	−1.22	−0.70	−0.27	−0.09	−8.88
D1S534	45.4	−12.4	−2.33	−1.05	−0.58	0.24	−0.11	−13.2
cen								
D1S498	49.4	−12.3	−3.98	−1.99	−1.23	−0.60	−0.31	−12.0
D1S484	63.2	0.68	0.67	0.60	0.52	0.37	0.22	−1.51
D1S2878	71.4	−12.4	−2.00	−0.73	−0.29	−0.01	0.05	−12.2
D1S196	75.0	− 8.19	−3.72	−1.73	−0.94	−0.29	−0.03	−8.95
SCA21 (~24 cM)								
D7S513	0	−8.75	−2.76	−1.40	−0.86	−0.37	−0.14	−8.58
D7S664	3.1	−8.45	−2.55	−1.22	−0.70	−0.27	−0.09	−8.60
D7S2557	12.5	−8.80	−2.80	−1.44	−0.89	−0.39	−0.15	−11.0
D7S503	13.0	−8.49	−2.50	−1.18	−0.66	−0.25	−0.08	−8.95
D7S493	20.6	−8.52	−2.53	−1.20	−0.69	−0.26	−0.09	−8.30
D7S2525	24.3	−8.58	−2.59	−1.26	−0.73	−0.29	−0.10	−8.27
SCA25 (~12.6 cM)								
D2S2240	0	−12.6	−5.12	−2.45	−1.39	−0.48	−0.11	−13.6
D2S1248	4.5†	−12.9	−3.92	−1.93	−1.15	−0.50	−0.20	−14.4
D2S2736	13.9†	−8.96	−2.96	−1.56	−0.97	−0.44	−0.18	−10.6
SCA27								
D13S159	0	−12.5	−5.48	−2.80	−1.72	−0.79	−0.35	−13.0
D13S158	5.4	−3.74	−0.86	−0.24	−0.04	0.07	0.06	−6.13
D13S173	16.0	−13.2	−4.21	−2.18	−1.37	−0.65	−0.31	−13.6

cen = centromeric region. Marker distances are expressed in cM (Marshfield map) whenever available, from the first tested marker; †Marker distances are in Mb (SCA4, SCA5, SCA18 and SCA25 loci) from the first tested marker.

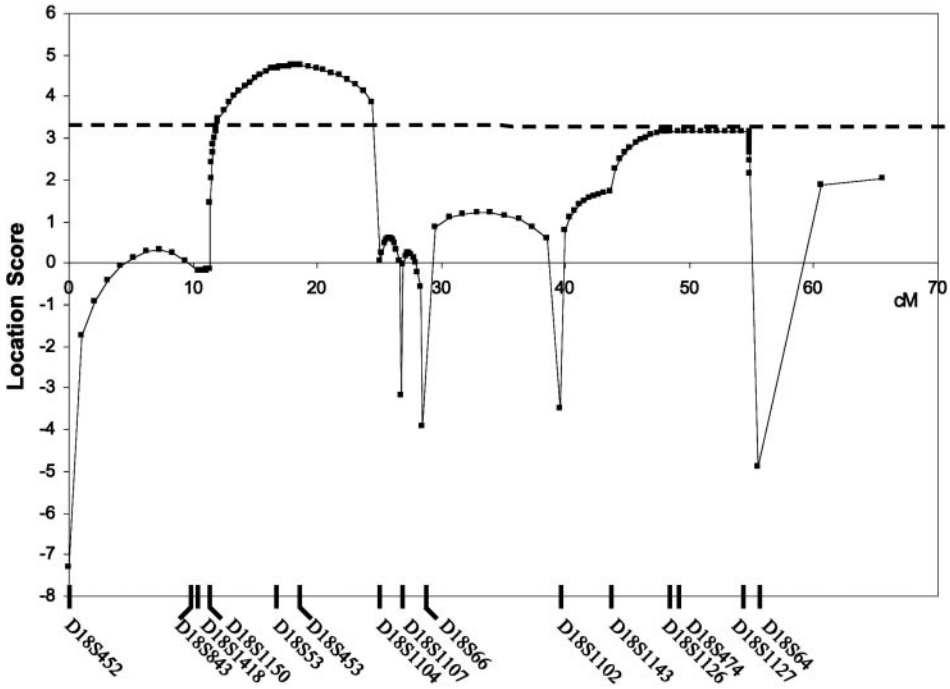

Fig. 3 Multipoint analysis of 16 markers on chromosome 18 (list in Table 4). Distance in cM is indicated on the abscissa, whereas ordinate reports the LOD score values. Hyphened line indicates the threshold of $Z = 3.3$.

Table 4 Two-point and multipoint LOD scores between the disease locus and chromosome 18 markers

Marker	Two-point LOD score at θ =						Multipoint LOD score
	0.00	0.01	0.05	0.1	0.2	0.3	
D18S452	−17.2	−5.15	−2.34	−1.17	−0.21	0.09	−6.32
D18S843	−1.60	1.38	1.93	2.00	1.73	1.21	−0.19
D18S1418	−6.90	−1.48	−0.22	0.21	0.45	0.41	−0.16
D18S1150	3.81	3.77	3.57	3.28	2.56	1.72	3.48
D18S53	4.20	4.15	3.93	3.60	2.82	1.90	4.67
D18S453	3.67	3.63	3.44	3.16	2.46	1.63	4.77
cen							
D18S1104	−0.71	2.24	2.74	2.73	2.28	1.57	−2.91
D18S1107	2.17	2.16	2.06	1.89	1.46	0.94	−3.18
D18S66	−1.73	1.26	1.89	2.01	1.78	1.26	−3.09
D18S1102	−3.30	−0.41	0.17	0.33	0.33	0.22	−2.79
D18S1143	0.73	0.77	0.87	0.91	0.80	0.54	1.71
D18S1126	3..00	2.99	2.91	2.72	2.18	1.50	3.17
D18S474	2.25	2.27	2.28	2.19	1.81	1.23	3.17
D18S1127	2.55	2.56	2.55	2.45	2.02	1.39	3.16
D18S69	−2.84	0.18	0.91	1.16	1.15	0.83	−4.98
D18S64	−3.01	−0.04	0.52	0.63	0.54	0.33	−2.74

cen = centromeric region. Markers are located on Marshfield map except D18S1418 that lies at 9.5 Mb from the 18p telomere. For multipoint analysis we assumed 1 Mb = 1 cM. Markers in bold are not included in the genome-wide ABI-PRISM Linkage Mapping Set MD-10 (see Patients and methods).

described by Harding (1982), with an unusually slow disease course. ADCA type I is characterized by the association of cerebellar ataxia, supranuclear ophthalmoplegia, pyramidal or extrapyramidal signs, mild dementia and peripheral neuropathy. In addition, it has been estimated that the mean time to wheelchair confinement in patients with ADCA type I is ~17 years of disease duration (Klockgether *et al.*, 1998). In our patients, the disease course before overt disability was much longer.

An interesting and consistent finding in our family was the presence of eye movement abnormalities. Two distinct clinical patterns were observed: in patients with short disease duration, persistent gaze-evoked nystagmus was the prevalent finding, while in patients with more than 20 years of disease

duration, slow saccades, ophthalmoparesis and ptosis were also observed. A similar progression of oculomotor alterations has been described in other *SCA* genotypes; for example, in SCA1 patients, possibly as the result of brainstem degeneration (Schöls L *et al.*, 1995; Klostermann *et al.*, 1997; Bürk *et al.*, 1999). However, in SCA1 and SCA2 patients, brainstem involvement is usually supported by the MRI finding of pontine atrophy, whereas in our affected patients neuroimaging did not clearly demonstrate brainstem abnormalities. We have also considered the possibility that the neurological phenotype in this family might be due to one of the nuclear-encoded disease genes that affects mitochondrial DNA stability and causes ophthalmoparesis and ataxia transmitted in an autosomal dominant fashion (Zeviani and Di Donato, 2004). This hypothesis was ruled out by normal morphological, biochemical and molecular findings in muscle biopsies from four patients.

Genetic analysis excluded the presence of known *SCA* gene mutations, and linkage exclusion tests showed no evidence of linkage to most of the known *SCA* loci, suggesting a genetically distinct form of SCA. A genome-wide linkage analysis allowed us to map a new locus on chromosome region 18p11.22–q11.2, designated *SCA28*, thus confirming that the disorder observed in this family represents a novel form of autosomal dominant SCA. The disease locus spans a 7.9 Mb region on chromosome 18, a chromosome that has never been associated with hereditary cerebellar ataxias.

Since anticipation was not present in this family and RED analysis did not reveal expanded CTG/CAG $\geq$40 repeats, we speculate that repeat expansions are an unlikely cause of the disease. A common disease haplotype was found in all affected members but also in a 47-year-old woman (III-11) who had no symptoms of cerebellar ataxia. This subject was neurologically evaluated and found to present horizontal gaze-evoked nystagmus with no gait or limb ataxia. These findings can be explained by reduced penetrance or expressivity, or a late onset of the disease in this subject.

Approximately 65 known genes map in the critical interval (Human Genome Assembly NCBI35.1) including one disease-gene (*MC2R*) that encodes the melanocortin-2 receptor and is mutated in the autosomal recessive glucocorticoid deficiency type 1 (*GCCD1*, OMIM 202200). Since none of the genes in the critical region exhibits obvious similarity with any of the currently known ADCA-causing genes, a candidate gene approach based on expression in disease-affected tissues is being used (Tiffin *et al.*, 2005). According to the GNF Affy HG U95 expression dataset (Hide *et al.*, 2003; Ensembl EnsMart Genome Browser, http://www.ensembl.org/Homo_sapiens/martview), 12 genes in the region are significantly expressed in the CNS including cerebellum and their sequence analysis is currently underway.

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