Ataxia with oculomotor apraxia type 2

A clinical, pathologic, and genetic study

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Abstract—*Background:* Ataxia with oculomotor apraxia type 2 (AOA2) is characterized by onset between age 10 and 22 years, cerebellar atrophy, peripheral neuropathy, oculomotor apraxia (OMA), and elevated serum alpha-fetoprotein (AFP) levels. Recessive mutations in *SETX* have been described in AOA2 patients. *Objective:* To describe the clinical features of AOA2 and to identify the *SETX* mutations in 10 patients from four Italian families. *Methods:* The patients underwent clinical examination, routine laboratory tests, nerve conduction studies, sural nerve biopsy, and brain MRI. All were screened for *SETX* mutations. *Results:* All the patients had cerebellar features, including limb and truncal ataxia, and slurred speech. OMA was observed in two patients, extrapyramidal symptoms in two, and mental impairment in three. High serum AFP levels, motor and sensory axonal neuropathy, and marked cerebellar atrophy on MRI were detected in all the patients who underwent these examinations. Sural nerve biopsy revealed a severe depletion of large myelinated fibers in one patient, and both large and small myelinated fibers in another. Postmortem findings are also reported in one of the patients. Four different homozygous *SETX* mutations were found (a large-scale deletion, a missense change, a single-base deletion, and a splice-site mutation). *Conclusions:* The clinical phenotype of oculomotor apraxia type 2 is fairly homogeneous, showing only subtle intrafamilial variability. OMA is an inconstant finding. The identification of new mutations expands the array of *SETX* variants, and the finding of a missense change outside the helicase domain suggests the existence of at least one more functional region in the N-terminus of senataxin.

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Ataxia with oculomotor apraxia type 2 (AOA2, OMIM 606002) was first described in a Japanese[1] and a Pakistani family.[2] Reported clinical features include onset between the ages of 10 and 22 years, elevated serum alpha-fetoprotein (AFP) levels, peripheral neuropathy, and cerebellar atrophy on MRI.[3] Subtle cognitive changes, consistent with executive dysfunction, have previously been reported,[3] but mental retardation or dementia have not. Other clinical features include extrapyramidal signs (choreiform movements, dystonia, and tremor) and elevated serum creatine kinase (CK) levels. Neuropathologic features of AOA2 have not been reported. Recessive mutations in *SETX* on chromosome 9q34 have been found in AOA2.[4] Dominant mutations in the same gene occur in a form of juvenile amyotrophic sclerosis (ALS4).[5] *SETX* encodes senataxin, a 2677-amino acid protein of still unknown function.[4]

We report the clinical and molecular findings in 10 patients from four Italian AOA2 families carrying novel *SETX* mutations, and postmortem brain examination of one of these patients.

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Methods. *Clinical study.* The pedigrees of the four families are shown in figure 1. Families 1 and 2 were from southern Italy, Family 3 from central Italy, and Family 4 from a mountain region in northern Italy populated by the descendants of a small number of German settlers who have inhabited these isolated Alpine valleys for generations. The patients of Families 1 and 2 were from a group of 35 selected patients with recessive or sporadic progressive ataxia and peripheral neuropathy. They were screened for *SETX* mutations in exon 10. The members of Families 3 and 4 were referred by L.C. from the Italian ataxia-telangiectasia (AT) registry, and had normal levels of ATM protein. They were screened for all *SETX* exons.

Detailed medical histories were obtained, and all 10 patients underwent complete neurologic examinations and routine laboratory tests, including vitamin E and serum CK. Serum AFP was

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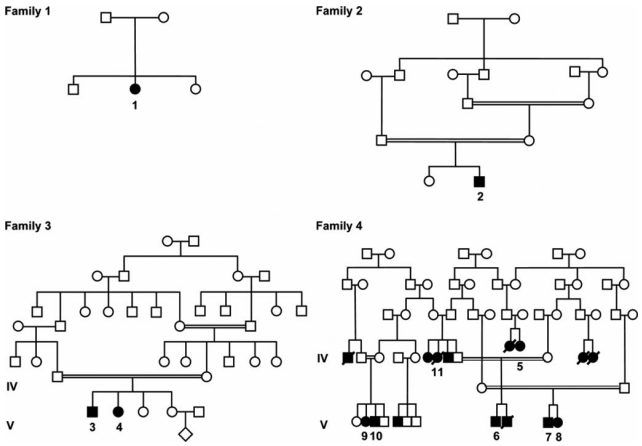

Figure 1. Pedigree of the Italian families with AOA2.

measured in six patients. Eight patients underwent brain MRI. Peripheral nerve conduction studies were carried out in eight patients, and sural nerve biopsies in two. A postmortem examination was performed in Patient 11.

Molecular study. After written informed consent was obtained from all the patients, genomic DNA was extracted from peripheral blood. The coding sequence and the intron-exon junctions of *SETX* (GenBank accession number: NM_015046.4) were amplified by PCR using primers listed in table E-1 (on the *Neurology* Web site at www.neurology.org). PCR-amplified products were agarose gel-purified, and directly sequenced. A duplex PCR using primer pairs encompassing exons 15–24 and 16–17 was designed to search for the large-scale deletion in Family 3. A PCR-RFLP strategy, employing *Rsa*I, was adopted to assess the segregation of the mutation in Family 4 (table E-2). To verify the effects of the deletion and the donor splice-site mutations, an RT-PCR was carried out on polyA$^+$ RNA purified from skin fibroblasts (Patient 4) or immortalized lymphoblastoid cell lines (Patient 9) using the 1st Strand cDNA Synthesis Kit (Roche, Germany).

Results. *Clinical features.* The clinical findings are summarized in the table. The mean age at disease onset ± SD was 20.3 ± 8.1 year (range 3 to 30). The initial symptom was gait ataxia in all cases. Although the disease progression was generally slow, no patient was able to walk independently at the time of evaluation. Mean time to wheelchair ± SD was 15.8 ± 7.4 years. All the patients had cerebellar features, including limb and truncal ataxia, and slurred speech. Gaze nystagmus was present in eight subjects. Oculomotor apraxia (OMA)—or rather, eye-head dissociation during voluntary lateral head movements—was present in two patients. The presence and the severity of this feature varied in different clinical examinations. All the patients presented swallowing difficulties, clinical and neurophysiologic features suggesting peripheral neuropathy, and skeletal and foot deformities. Vibration and position senses were impaired in all but one. Three patients presented mild mental decline (onset at around age 50 years), but formal neuropsychological tests were not performed. Extrapyramidal symptoms, including choreiform head movements and truncal dystonia in one patient, and

Table *Clinical and molecular features of AOA2 Italian patients*

Family	1	2	3	3	4	4	4	4	4	4
Patient	1	2	3	4	5	6	7	8	9	10
Parental consanguinity	No	Yes	Yes	Yes	Possible	Possible	Possible	Possible	Yes	Yes
Sex/age at onset, y/age at examination, y	F/15/27	M/3/32	M/15/40	F/19/38	F/25/67	M/18/66	M/30/54	F/30/58	F/25/60	M/23/49
Duration until wheelchair, y	17	21	17	17	28	15	5	5	23	10
Sign at onset	CA	CA	CA	CA	CA	CA	CA	CA	CA	CA
Gait ataxia	++	++	++	++	++	++	++	++	++	++
Dysarthria	+	+	+	+	+	+	+	+	+	+
Nystagmus	+	+	+	+	+	+	+	–	–	+
Dysphagia	+	+	+	+	+	+	+	+	+	+
Ocular motor apraxia	+	+	–	–	–	–	–	–	–	–
Areflexia	++	++	++	++	++	++	++	++	++	++
LL weakness	+	+	+	+	++	++	++	++	++	++
UL-LL distal wasting	+	++	++	++	++	++	++	++	++	++
Impaired position/ vibration sense	+	+	+	+	+	+	+	+	+	+
Cognitive decline	–	?	–	–	+	+	+	–	–	–
Skeletal deformities	+	+	+	+	+	+	+	+	+	+
Foot deformity	+	+	+	+	+	+	+	+	+	+
Cerebellar atrophy at MRI	+	+	+	+	NP	NP	+	+	+	+
Axonal neuropathy	+	+	+	+	NP	NP	+	+	+	+
Increased serum AFP levels	+	+	+	+	NP	NP	NP	NP	+	+
Homozygous *SETX* mutation	3466delG	1406A>G	c.6106+3393_ c.7101–22 del20648bp/ ins 25bp	c.6106+3393_ c.7101–22 del20648bp/ ins25bp	c.7199+5G>A	c.7199+5G>A	c.7199+5G>A	c.7199+5G>A	c.7199+5G>A	c.7199+5G>A
Mutation effects	fs1157X	H469R	Eight exons deleted	Eight exons deleted	Exon 25 skipped	Exon 25 skipped	Exon 25 skipped	Exon 25 skipped	Exon 25 skipped	Exon 25 skipped

See text for mutations details. Numbering of the mutation refers to the first ATG codon of the mRNA as nucleotide +1–3. Presence and severity of features: + = mild to moderate; ++ = severe; – = absent.

NP = not performed; LL = lower limbs; UL = upper limbs; AFP = alpha-fetoprotein; CA = cerebellar ataxia.

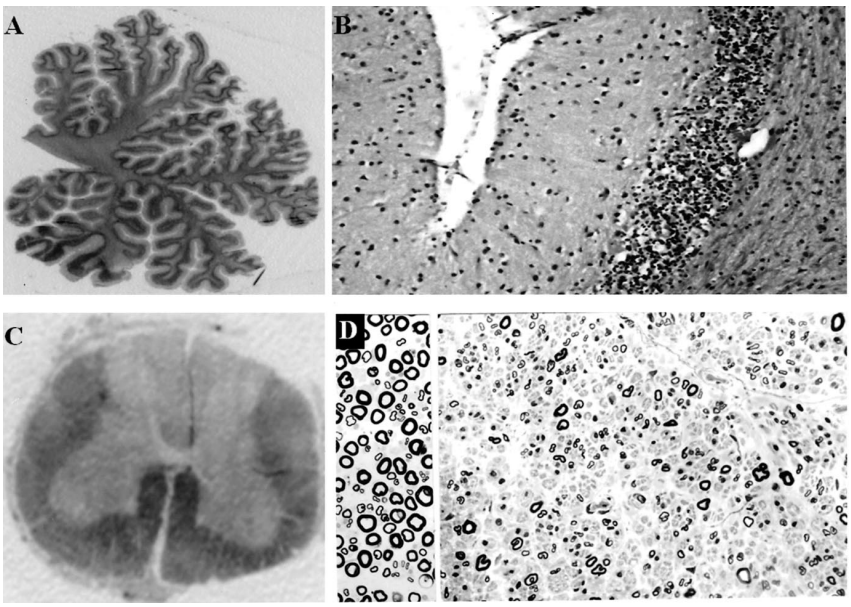

Figure 2. (A–C) Pathology findings in Patient 11. (A) Cerebellar atrophy appears more evident at vermis and anterior lobe level. (B) Marked loss of Purkinje cells. (C) Demyelination of gracilis and cuneate funiculi. (D) Nerve biopsy of Patient 2 showing severe loss of large myelinated fibers.

head tremor in another, rapidly disappeared as the disease progressed. Routine laboratory findings, including serum CK, cholesterolemia, albuminemia, and vitamin E levels, were normal. Raised serum AFP levels, motor and sensory axonal neuropathy, and marked cerebellar atrophy on brain MRI were detected in all the patients, who underwent these examinations. Sural nerve biopsy revealed a severe loss of large myelinated fibers in Patient 1 (figure 2D), and both small and large myelinated fibers in Patient 2.

Pathologic findings. Postmortem brain examination was performed in Patient 11, who died of heart failure at age 79 years. The overall size of the brain, which weighed only 850 g after fixation, appeared considerably reduced. All brain structures appeared small; in particular, we observed atrophy of the cerebellar folia, and a marked widening of the sulci. The cerebellar atrophy was most evident at the level of the vermis and the anterior lobe (figure 2A). The brainstem and spinal cord were slightly reduced in size, but otherwise unremarkable. The substantia nigra appeared normally pigmented. Atheromatous plaques were present in all the arteries of the circle of Willis. Histologically, the cortical neurons were normal in both number and shape. The cerebellar cortex showed a marked loss of Purkinje cells, and mild fibrous gliosis, which was more severe in the vermis than in the hemispheres (figure 2B). Neither inclusion bodies nor torpedoes were found. The neurons of the dentate nuclei were slightly reduced in number. Chromatolysis of the oculomotor and raphe nuclei was observed in the brainstem. The inferior and accessory olives appeared relatively spared. Severe demyelination of the gracilis and cuneatus funiculi, and degeneration of the Clarke's columns with gliosis, were observed in the spinal cord (figure 2C).

Molecular analyses. We identified four new mutations in *SETX* (figure 3). In Family 1, a homozygous single-base deletion, c.3466delG, was identified, predictively producing a frameshift after amino acid residue 1155 with a premature stop codon at 1157 (fs1157X). In Family 2, a homozygous c.1406A>G mutation was found, resulting in the substitution of arginine for histidine at residue 469 (H469R). This substitution was not found in 200 Italian control chromosomes. In Family 3, we identified a homozygous large-scale rearrangement about 20 kb in size (c.6106 + 3393_c.7101-22del20648bp/ins25bp). The mutation deleted the sequences of exons 16–23, and was flanked by an 8-bp tandem repeat (5′-GTAAATTT-3′). In cultured skin fibroblasts, the large-scale rearrangement produced two alternative transcripts (figure E-1b), both predictively resulting in premature protein truncation at residue 2040. In Family 4, we detected a homozygous c.7199 + 5G>A mutation in the conserved donor splice site of intron 25. Analysis of the cDNA from lymphoblastoid cells showed that the splice-site mutation caused skipping of exon 25 (figure E-1d) and a predicted protein cropped of 10% of its C-terminus sequence. Cosegregation between the different mutations and the disease was confirmed in Families 3 (figure E-1a) and 4 (figure E-1c) but could not be verified in Family 2 because the patient's relatives were not available for testing.

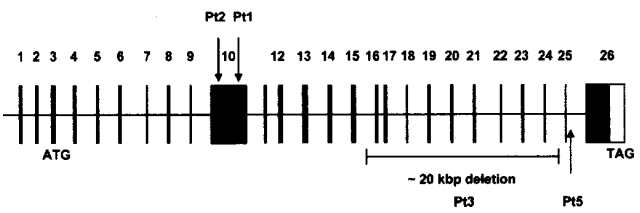

Figure 3. Localization of the four new mutations in SETX.

Discussion. Autosomal recessive cerebellar ataxias (ARCAs) are heterogeneous diseases. It is now possible to identify a group of ARCAs with confirmed or highly likely DNA repair defects.[6] As regards the neurologic phenotype, these diseases are commonly characterized by progressive ataxia and peripheral neuropathy, usually associated with OMA.[7] Two major subgroups can be identified according to impairment of double and single strand repair. AT and AT-like disorder both fall into the first category, in

which chromosomal instability, sensitivity to ionizing radiations, and susceptibility to cancer (AT) are associated features.[8-10] The second category includes ataxia with oculomotor apraxia type 1 (AOA1), spinocerebellar ataxia with neuropathy 1 (SCAN1), and possibly AOA2.[4,11-14] AOA2 shares several clinical features with AOA1, including gait ataxia, cerebellar atrophy, and sensorimotor neuropathy, but can be distinguished by a later age at onset, the presence of high or mildly elevated serum AFP levels, and a better functional prognosis. Additional clinical features such as OMA and choreoathetosis are less common.

This report describes a series of Italian AOA2 patients with proven genetic defects in *SETX*. Only two such series have been reported to date: one included six families from different Mediterranean areas and from the West Indies, in which the disease was linked to the 9q34 locus[3]; the other was a single cluster of 10 French-Canadian families with ataxia and neuropathy (known as the Quebec cluster).[15] Mean age at onset in our series was slightly higher than in these studies (20.3 years vs 15.1 and 14.8 years).[3,15] Although it is included in the acronym, OMA is not mandatory in AOA2[3,15]: it was found in 20% of our patients, in 56% of the multiethnic cohort,[3] and in none of the Quebec cluster.[15] Whereas three of the Italian patients presented cognitive impairment, and executive dysfunction was described in the multiethnic study,[3] the French-Canadian patients had normal intelligence.[15] Similarly, extrapyramidal features do not appear to be constant in AOA2: only one patient in our study presented choreic or dystonic movements—which later disappeared—whereas about 40% of the patients in the multiethnic study[3] had persistent chorea throughout the course of the disease. Conversely, none of the Quebec patients presented movement disorders. As in previous studies, we observed a fairly homogeneous inter- and intrafamilial phenotype. The pattern of disease progression varied in Family 4: Patients 7 and 8 were confined to a wheelchair 5 years after disease onset, whereas the progression was slower in the other family members (range 10 to 28 years). Unlike AT, AOA2 does not seem to be associated with an increased susceptibility to cancer, although two of the French-Canadian patients died of cancer.[15]

Taken together, the pathologic data reported here in one Italian patient, and the clinical presentation described both in this new series and in the previous AOA2 families,[3,15] suggest that senataxin plays a crucial role in cerebellar, anterior horn, and dorsal root neuron survival. In addition, the fact that two patients in our study entered menopause in early adulthood, and that early-onset menopause was also reported in the previous multiethnic study,[3] suggests that senataxin might also be involved in germ line cell survival. However, the function of senataxin is poorly understood. Truncations (observed in Families 1, 3, and 4) and missense changes (Family 2) result in a similar phenotype, suggesting that both mutations cause disease through a loss-of-function mechanism. Senataxin's carboxy-terminus shares similarities with the superfamily 1 of helicases. The missense H469R identified in Family 2 falls outside this domain, and thus raises the possibility that there is at least one other regulatory region in the N-terminus of senataxin. Given the extensive homologies with the fungal Sen1p proteins,[4] which have RNA and DNA helicase activity, and are involved in splicing and termination of tRNA and small nuclear RNA, the possible role of senataxin in the spliceosome machinery warrants further investigation.[16]

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