Clinical and Molecular Findings of Ataxia With Oculomotor Apraxia Type 2 in 4 Families

Mathieu Anheim, MD; Marie-Celine Fleury, MD; Jerome Franques, MD; Maria-Ceu Moreira, PhD; Jean-Pierre Delaunoy, PhD; Dominique Stoppa-Lyonnet, MD, PhD; Michel Koenig, MD, PhD; Christine Tranchant, MD

Background: Ataxia with oculomotor apraxia type 2 (AOA2) is an autosomal recessive disease caused by SETX mutations in 9q34 resulting in cerebellar ataxia in association with peripheral neuropathy, cerebellar atrophy on imaging, an elevated α-fetoprotein (AFP) serum level, and occasional oculomotor apraxia.

Objective: To describe the clinical and molecular findings of 7 patients with a clinical presentation of AOA2 and their relatives.

Design: Case report.

Setting: Projet Hospitalier de Recherche Clinique.

Patients: Seven patients with AOA2 and their family members.

Intervention: Linkage analysis and direct sequencing of all exons of SETX were performed in all patients. Magnetic resonance imaging and electroneuromyography were performed and the patients’ AFP serum levels were tested.

Results: We identified 7 patients with AOA2 from 4 unrelated families. Three novel SETX mutations were found. The clinical picture of the patients reported is fairly homogeneous and in accordance with the classic AOA2 presentation: onset from 13 to 18 years of progressive cerebellar ataxia and areflexia. Oculomotor apraxia was detected in 1 patient. Predominant axonal neuropathy and a diffuse cerebellar atrophy were found in the 4 patients tested. All patients had elevated AFP serum levels and 5 of 8 nonsymptomatic heterozygous relatives had moderately increased AFP serum levels as well.

Conclusions: Ataxia with oculomotor apraxia type 2 is a homogeneous form of cerebellar ataxia with occasional oculomotor apraxia. Most nonsymptomatic heterozygous carriers present with increased AFP serum levels.

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REPORT OF CASES

We identified 7 patients with clinical features of AOA2 from 4 different families and found 3 novel SETX mutations. The clinical and biological features of these 7 patients are summarized in the Table and the pedigrees are shown in Figure 1. Ocular movements were recorded by videoneuromyography.

FAMILIES

Family 1

Patients II-1, II-2, II-4, and II-5, 4 of 7 children born in Algeria from a consanguineous marriage, were identified as having AOA2. Their parents and sisters were asymptomatic at ages 69 (I-1), 55 (I-2), 22 (II-3), 16 (II-6), and 12 (II-7) years.

Family 2

Family 2 originated from eastern France and had no documented consanguinity. Patient II-1 in this family was found to have AOA2.
The mother of family 2 was clinically evaluated at age 63 years and showed no sign of neuromuscular disorder.

Family 3

Family 3 also originated from eastern France and had no documented consanguinity. Examination of patient II-4 at 36 years revealed severe isolated cerebellar ataxia of his 4 limbs. He had a moderately increased serum creatine kinase level at 429 IU/L (normal level \(/ H11021\) 250 IU/L). One year later, a routine blood examination revealed a dramatic increase of creatine kinase level at 20 000 IU/L. Electromyography revealed severe axonal sensory-motor neuropathy but no myogenic abnormalities. Results from a histological examination of the patient’s deltoid muscle were normal and creatine kinase levels returned to normal. The patient’s mother was clinically evaluated at age 65 years and showed no sign of neuromuscular disorder.

Family 4

This family originated from Turkey. The parents share ancient consanguinity. Patient II-1 was identified as having AOA2.

GENETIC ANALYSIS

The first 17 exons of SETX in families 2 through 4 were initially analyzed for the first 17 exons of SETX (respectively, families F88, F90, and Tur1\(^{11}\)), but no mutations (or only 1) were found. Microsatellite marker analysis (Figure 1) was performed as previously reported.\(^{6}\)

MUTATION ANALYSIS

Frataxin GAA expansion and ATM gene mutations were examined using routine tests.\(^{7,8}\) We sequenced all exons of SETX from both the forward and reverse strands after purification of the polymerase chain reaction (PCR) products, as reported.\(^{1}\)

### Table. Clinical and Biological Features of Patients With Ataxia With Oculomotor Apraxia Type 2

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patient</th>
<th>Patient</th>
<th>Patient</th>
<th>Patient</th>
<th>Patient</th>
<th>Patient</th>
<th>Patient</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>II-1</td>
<td>II-2</td>
<td>II-4</td>
<td>II-5</td>
<td>II-1</td>
<td>II-4</td>
<td>II-1</td>
</tr>
<tr>
<td>Family</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Sex</td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>F</td>
</tr>
<tr>
<td>Age at onset, y</td>
<td>13</td>
<td>15</td>
<td>16</td>
<td>18</td>
<td>16</td>
<td>17</td>
<td>18</td>
</tr>
<tr>
<td>Initial symptom</td>
<td>Gait ataxia</td>
<td>Gait ataxia</td>
<td>Gait ataxia</td>
<td>Gait ataxia</td>
<td>Gait ataxia</td>
<td>Gait ataxia</td>
<td>Gait ataxia</td>
</tr>
<tr>
<td>Disease duration, y</td>
<td>14</td>
<td>13</td>
<td>4</td>
<td>&lt; 1</td>
<td>13</td>
<td>19</td>
<td>8</td>
</tr>
<tr>
<td>Disability</td>
<td>Walking with unilateral help</td>
<td>Walking with bilateral help</td>
<td>Cannot run</td>
<td>None</td>
<td>Walking with bilateral help</td>
<td>Needs wheelchair</td>
<td>Walking with unilateral help</td>
</tr>
<tr>
<td>Motor deficit</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Severe motor deficit in 4 limbs</td>
</tr>
<tr>
<td>Tendon reflexes of lower limbs</td>
<td>Abolished</td>
<td>Abolished</td>
<td>Abolished</td>
<td>Abolished</td>
<td>Abolished</td>
<td>Abolished</td>
<td>Abolished</td>
</tr>
<tr>
<td>Pyramidal signs</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Bilateral Babinski sign</td>
<td>–</td>
</tr>
<tr>
<td>Sensory loss</td>
<td>–</td>
<td>–</td>
<td>Mild loss of vibration in lower limbs</td>
<td>–</td>
<td>–</td>
<td>Mild loss of vibration in lower limbs</td>
<td>–</td>
</tr>
<tr>
<td>Oculomotor apraxia</td>
<td>–</td>
<td>Mild apraxia</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Dystonia/chorea</td>
<td>–</td>
<td>Mild dystonia in upper limbs</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Others clinical signs</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Severe amyotrophy and fasciculations of the 4 limbs</td>
</tr>
<tr>
<td>AFP level, µg/L(^{a})</td>
<td>105</td>
<td>41.2</td>
<td>26.6</td>
<td>25.2</td>
<td>32.6</td>
<td>20.6</td>
<td>31.9</td>
</tr>
<tr>
<td>Total cholesterol level</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
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<tr>
<td>Albuminemia</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Creatine kinase level, IU/L</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>429</td>
<td>N</td>
</tr>
<tr>
<td>EMG result</td>
<td>Mild sensory-motor AN</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>Mild sensory-motor AN</td>
<td>Mild sensory-motor AN and diffuse denervation</td>
<td>Mild sensory-motor AN</td>
</tr>
<tr>
<td>Cerebral MRI</td>
<td>Cerbellar atrophy</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>Cerbellar atrophy</td>
<td>Cerbellar atrophy</td>
<td>Cerbellar atrophy</td>
</tr>
</tbody>
</table>

Abbreviations: AFP, α-fetoprotein; AN, axonal neuropathy; EMG, electromyography; MRI, magnetic resonance imaging; N, normal; NT, not tested; and −, sign not observed.

SI conversion factor: To convert AFP to nanograms per milliliter, multiply by 1.0.

\(^{a}\)Normal level, less than 7 µg/L.
No frataxin or ATM mutations were found. We found SETX mutations in the 7 patients with AOA2 (Figure 1).

FAMILIES

Family 1

Patients II-1, II-2, II-4, and II-5 were homozygous for mutation 1027G>T in exon 7, which led to the nonsense variation E343X (glutamate 343 to stop codon). Patients I-1, I-2, II-3, and II-7 were heterozygous for this mutation; no mutation was found for patient II-6.

Family 2

The heterozygous mutation 5264delC in exon 8 leading to a frameshift mutation after Asn1784 was initially identified in patient II-1 after sequencing exons 1 to 17. The remaining 7 exons have since been sequenced and all 24 exons were again sequenced twice, but no other point mutation was found. Neither exon deletion nor duplication was found using quantitative PCR of SETX exons 5, 6, 7, 8, 17, 18, 23, and 24. We analyzed the SETX messenger RNA in fibroblasts of patient II-2 and in 6 controls by quantitative real-time reverse transcriptase-PCR. The 5264delC allele was reduced to 10% of the normal level, indicating that nonsense-mediated RNA decay operates on this allele, while the allele for which we did not find a mutation was expressed at about 31% of the normal level, suggesting that expression or stability was also mildly affected.

Family 3

Patient II-4 was homozygous for the mutation 7331G>A in exon 24 leading to the missense change R2444H (arginine 2444 to histidine). This mutation was not found in the 288 independent control samples.

Family 4

Patient II-1 was homozygous for the mutation 6625A>T in exon 18 leading to the nonsense variation K2209X (lysine 2209 to stop codon).

SERUM AFP ANALYSIS

Serum AFP level was investigated in all patients with AOA2 and in 11 relatives (Figure 1). Serum AFP levels were elevated in all patients, with values ranging from 20.6 to 105.3 µg/L (normal range, 0-7 µg/L [to convert to nanograms per milliliter, multiply by 1.0]). Unexpectedly, AFP levels were also elevated in 5 of 8 carrier relatives (range, 8.8-19.4 µg/L), while they were within the lower normal range in the 3 noncarrier relatives (range, 1.3-3.2 µg/L).

Non-Friedreich autosomal recessive cerebellar ataxia is a heterogeneous group of neurodegenerative disorders. Aicard et al. first isolated a clinical phenotype associated with early-onset progressive cerebellar AOA, occasional dystonia or choreoathetosis, and exclusion of ataxiatalegiectasia diagnosis. Three subgroups were later identified: one linked to mutations of MRE11 (11q21), one linked to the aprataxin gene (APTX) mutations in 9p13 (AOA1), and a third group variously called ataxiatalegiectasia–like or ataxia with peripheral neuropathy, without ATM, MRE11, or APTX mutations. Bossart

(FLANNING PRIMERT SEQUENCES AND PCR CONDITIONS ARE AVAILABLE ON REQUEST.) WE TESTED FOR THE MISSENSE MUTATION USING FLANKING PRIMERS (SPANNING AT LEAST 2 EXONS) OF THE SETX GENE. THIS MUTATION WAS FOUND IN PATIENT II-2 AND IN 6 CONTROLS BY QUANTITATIVE REAL-TIME REVERSE TRANSCRIPTASE-PCR. THE 5264DEL C ALLELE WAS REDUCED TO 10% OF THE NORMAL LEVEL, INDICATING THAT NONSENSE-MEDIATED RNA DECAY OPERATES ON THIS ALLELE, WHILE THE ALLELE FOR WHICH WE DID NOT FIND A MUTATION WAS EXPRESSED AT ABOUT 31% OF THE NORMAL LEVEL, SUGGESTING THAT EXPRESSION OR STABILITY WAS ALSO MILDLY AFFECTED.

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et al\(^6\) and Németh et al\(^{10}\) linked the third group by homozygosity mapping to 9q34 and described a new entity named AOA2. Ataxia with oculomotor apraxia type 2 is now considered one of the most frequent (\(\approx 8\%\)) non-Friedreich autosomal recessive cerebellar ataxias.\(^2\)

The main features of AOA2 are onset between 10 and 25 years, cerebellar ataxia, axonal sensory-motor neuropathy, elevation of AFP level (sometimes at the upper range of the normal level at the earlier stages but consistently found if testing is repeated during the course of the disease), cerebellar atrophy on magnetic resonance imaging, and occasional oculomotor apraxia. Other clinical symptoms may be present, including pyramidal signs, abnormal movements (dystonia, chorea), mild cognitive impairment, and early menopause.

The major ways in which AOA2 differs from AOA1 are that AOA2 has a later age at onset, a variable presence of oculomotor apraxia, a less severe neuropathy (which is the cause of the functional impairment observed in AOA1), and increased AFP level. In AOA1, hypercholesterolemia and hypoalbuminemia are more common. Differential diagnosis with ataxia-telangiectasia may be more difficult, because of the functional impairment observed in AOA1), and a less severe neuropathy (which is the cause of the functional impairment observed in AOA1). Magnetic resonance imaging was performed in 4 families in this condition. Pyramidal signs were found in 2 predicted heterozygous carriers of this mutation. The existence of a second mutation, possibly located in the 2 predicted heterozygous carriers of this mutation, the father and the unaffected brother of the patient, while noncarriers have AFP levels at the lower normal range. Patient II-4 of family 3 presents, in addition to the clinical picture of AOA2, an unusual severe lower motor neuron impairment. Although study of motor evoked potentials revealed that duration of the silent period decreased with time, as observed in some confirmed cases of amyotrophic lateral sclerosis, no pyramidal sign has been detected in this patient until now.

Figure 2. A. Position of mutations on senataxin. N1754fs indicates frameshift mutation after N1754 (5264delC mutation); K2209X, lysine 2209 to stop codon; R2444H, arginine 2444 to histidine. Positions of domains and helicase motifs are indicated. B. Conservation of the C-terminal part of the SETX helicase domain among eukaryotes. hs, gg, xt, and dr indicate vertebrates; dp and dm, invertebrates; os and at, plants; and sp1, sp2, nc, sc, eg, and dh, fungi. The position of motif VI of the helicase domain is shown by the line beneath it and position of R2444 amino-acid change of family 3 is indicated by an \(\#\) on top of the conserved R residue.

Our 7 patients illustrate the relative homogeneity of the AOA2 phenotype but also the heterogeneity of SETX mutations. Each family has a private mutation. Consanguinity was documented in only 2 families, but homozygosity for the mutation was found in 3 families. Other mutations of SETX have been associated with an autosomal dominant form of amyotrophic lateral sclerosis (ALS4) clinically characterized by juvenile onset (age < 21 years), distal muscle weakness, and atrophy associated with pyramidal signs and an electrophysiological pattern of chronic pure motor neuropathy with partial preservation of sensation-reinnervation.\(^{11}\) Only 3 SETX mutations have been associated with ALS4: L389S, R2136H, and T31. It appears that all 3 are missense mutations, with a possible gain of function that could explain the dominant inheritance. On the other hand, the AOA2 mutations most likely cause a loss of function because most are nonsense or frameshift mutations. No patient with both ALS4 and AOA2 has been reported. The affected patient from family 2 most likely has AOA2 rather than ALS4, given her clinical presentation and elevated serum AFP level, and the absence of a clinical sign from her mother, aged 63 years, who carries the same frameshift loss of function mutation. The existence of a second mutation, possibly noncoding, not identified in family 2 is also supported by serum AFP levels at the upper normal range in the 2 predicted heterozygous carriers of this mutation, the father and the unaffected brother of the patient, while noncarriers have AFP levels at the lower normal range. Patient II-4 of family 3 presents, in addition to the clinical picture of AOA2, an unusual severe lower motor neuron impairment. Although study of motor evoked potentials revealed that duration of the silent period decreased with time, as observed in some confirmed cases of amyotrophic lateral sclerosis, no pyramidal sign has been detected in this patient until now.
The peculiarity of the clinical picture of this patient could be linked to the nature and location of the R2444H mutation (Figure 2A). It is a missense mutation that affects the last conserved residue of the SETX protein (senataxin) and occurs in the C-terminal domain of the protein (Figure 2B). The C-terminal domain is the most conserved domain of this protein and corresponds to the helicase domain, the loss of function of which causes abnormal processing of RNA, as demonstrated in the yeast ortholog Sen1p.\textsuperscript{12-13} A better understanding of the role of senataxin is needed before correlating different clinical presentations with different types of mutation.

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Correspondence: Marie-Celine Fleury, MD, Département de Neurologie, Hôpital Civil, 1 Place de l'Hôpital, 67091 Strasbourg, France (marie-celine.fleury@chru-strasbourg.fr).


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REFERENCES