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

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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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TAXIA WITH OCULOMOTOR apraxia type 2 (AOA2) is one of the most recently described non-Friedreich autosomal recessive cerebellar ataxias and is genetically defined by mutations in SETX (OMIM *608465) at 9q34.1 It was first reported in Pakistani and Japanese families and has been subsequently reported in families from Western countries. The typical presentation is early-onset progressive cerebellar ataxia (age < 25 years), oculomotor apraxia, sensory-motor axonal neuropathy, and elevation of serum α-fetoprotein (AFP) level.2 Only a few cases with identified SETX mutations have been reported.3-5

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 videoculography.

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 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. Her serum creatine kinase level was normal.

### 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 \( \text{SETX} \) in families 2 through 4 were initially analyzed for the first 17 exons of \( \text{SETX} \) (respectively, families F88, F90, and Tur11), but no mutations (or only 1) were found. Microsatellite marker analysis (Figure 1) was performed as previously reported.

#### MUTATION ANALYSIS

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

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### 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>Family</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>Sex</td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>F</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>17</td>
<td>19</td>
<td>8</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 bilateral 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>−</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>Mild dystonia in lower limbs</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>429</td>
<td>N</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>Cerebellar atrophy</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>Cerebellar atrophy</td>
<td>Cerebellar atrophy</td>
<td>Cerebellar atrophy</td>
</tr>
</tbody>
</table>

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

\(^a\)Normal level, less than 7 µg/L.

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

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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, I-3, and I-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). 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).

COMMENT

Non-Friedreich autosomal recessive cerebellar ataxia is a heterogeneous group of neurodegenerative disorders. Aicardi et al. first isolated a clinical phenotype associated with early-onset progressive cerebellar AOA, occasional dystonia or choreoathetosis, and exclusion of ataxia-telangiectasia 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 ataxia-telangiectasia–like or ataxia with peripheral neuropathy, without ATM, MRE11, or APTX mutations. Bomont
et al9 and Németh et al10 linked the third group by homoygosity mapping to 9q34 and described a new entity named AOA2. Ataxia with oculomotor apraxia type 2 is now considered one of the most frequent (< 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 AFP is increased in both this disease and AOA2. Presence of telangiectasia and an earlier age at onset (age < 5 years) point to ataxia-telangiectasia, the prognosis of which is often more severe. No chromosomal abnormalities, immunodeficiency, or neoplasia, which are frequent in ataxia-telangiectasia, have been described in AOA2.

The clinical picture of the patients with AOA2 reported in this study is fairly homogenous and in accordance with the classic AOA2 presentation: age at onset ranging from 13 to 18 years, progressive cerebellar ataxia of the 4 limbs, and areflexia indicative of the neuropathy. Electromyography, performed in 4 patients, confirmed the existence of a predominant axonal neuropathy. Magnetic resonance imaging was performed in 4 patients (1 in each family) and demonstrated a diffuse cerebellar atrophy. As already described, oculomotor apraxia was rare, found in only 1 patient. Indeed, oculomotor apraxia was found in only 23% of previously reported cases.2,5 However, great differences between series (0%-56% of cases) emphasize the difficulties of recognizing such oculomotor disorders; absence of this sign should not delay genetic study in AOA2. Mild dystonia has been found in 2 patients; abnormal movements seem to be rare (found in only 4 of 21 reported families) in this condition. Pyramidal signs were found in 1 of our 7 patients and were noticed in only 3 previously described patients.

α-Fetoprotein serum level was elevated for all patients. No correlation could be established between severity of the disease and the level of serum AFP. Remarkably, AFP level was found to be increased in 5 of 8 nonsymptomatic heterozygous participants. α-Fetoprotein level was also higher in the other 3 heterozygous carriers (including the youngest carrier sister of family 1 at 8 years) than in the 3 noncarrier relatives. These results suggest that increased AFP level in relatives indicates asymptomatic carrier status. Nevertheless, the correlation between carrier status and AFP level should be further evaluated, as it was investigated in only one other study and was normal.4

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. 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 R 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 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 sensory reinnervation.11 Only 3 SETX mutations have been associated with ALS4: L389S, R2136H, and T31I. 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.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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