**Phenotypic Features and Genetic Findings in Sacsin-Related Autosomal Recessive Ataxia in Tunisia**

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**Background:** Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is a clinically homogeneous disorder reported in Quebec caused by mutations in the SACS gene (chromosome 13q12). Recently, we identified a Tunisian kindred demonstrating linkage to the ARSACS locus.

**Objective:** To report clinical, neurophysiological, and nerve biopsy findings in patients with autosomal recessive cerebellar ataxia related to the SACS gene in Tunisia.

**Patients and Methods:** Genetic linkage analysis of patients with early-onset autosomal recessive cerebellar ataxia allowed the identification of 4 families from which 18 patients demonstrated linkage to the ARSACS locus. The patients were evaluated according to the International Cooperative Ataxia Rating Scale. Peripheral nerve conduction, sensory evoked potentials, and nerve biopsy were performed in most patients.

**Results:** The mean age at onset was 4.5 years. The clinical phenotype was stereotyped and associated with a progressive cerebellar syndrome, a pyramidal syndrome with brisk knee reflexes, and Babinski sign and absent ankle reflexes. The course of the disease varied among patients. Sensory evoked potentials showed severe posterior column involvement. Peripheral nerve investigations demonstrated axonal and demyelinating neuropathy. Four mutations, 2 missense and 2 nonsense, were found.

**Conclusion:** In Tunisia, autosomal recessive cerebellar ataxia related to the SACS gene demonstrated a homogeneous phenotype and heterogeneous allelic mutations.

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members were examined, identifying 53 secondary patients. Pedigrees were documented, and blood samples were collected from consenting family members, including the 73 affected patients.

CLINICAL EVALUATION

The following clinical factors were specified for each patient: evaluation of the cerebellar syndrome according to the International Cooperative Ataxia Rating Scale (score range, 0-100),6 the tendon reflex status in the 4 limbs, evaluation of the pyramidal syndrome by the degree of spastic gait, the degree of amyotrophy in all 4 limbs, deep sensory disturbances (proprioception and vibratory sense), the presence of joint deformities, and funduscopic examination findings.

LINKAGE ANALYSIS

After obtaining informed consent, genomic DNA was collected from 170 individuals belonging to 20 families for linkage analysis. Analysis was carried out using microsatellite markers on 13q11-12, including D13S232, D13S1275, and D13S292. Four families comprising 18 patients (13 male and 5 female) demonstrating linkage to the ARSACS locus were selected (Figure 1).

MUTATION ANALYSIS

Forty-seven primer sets were used to amplify the entire open reading frame of sacsin. After 32 cycles of polymerase chain reaction amplification, the products were separated by electrophoresis on 1.2% agarose gels and purified using the QIAquick gel extraction kit (QIAGEN, Valencia, Calif). The polymerase chain reaction products were sequenced using the Big Dye Deoxy Terminator Cycle Sequencing Kit (Perkin-Elmer, Norwalk, Conn) on the ABI 377 automated sequencer or the Beckman Coulter CEQ 2000 XL DNA analysis system (Beckman Coulter Inc, Fullerton, Calif). Single-strand conformational polymorphism analysis was used to determine segregation in the 4 pedigrees and to

Figure 1. Haplotype analysis. A, Family 1. B, Family 2. C, Family 3. D, Family 4. Black symbols indicate affected individual; white, unaffected individual; circle, female; square, male; D13S1275, D13S232, and D13S292, microsatellite markers; and double horizontal bars, individuals are consanguineous.
establish the absence of the 4 reported mutations in 100 control chromosomes.

LABORATORY INVESTIGATIONS

The various findings of peripheral nerve conduction, sensory evoked potentials, and visual-evoked potentials were recorded in most patients using routine techniques. Visual evoked potentials were measured by monocular stimulation with an alternating black-and-white checkerboard pattern. The amplitude and the latency of the P100 wave were recorded. A superficial peroneal nerve biopsy was carried out in 5 patients and studied according to the techniques generally used. Routine laboratory tests, including blood vitamin E level, cerebrospinal fluid analysis, electrocardiography, echocardiography, and cerebral computed tomographic scan, were carried out in most patients.

RESULTS

CLINICAL DATA

The clinical data are summarized in Table 1. The mean±SD age at onset was 4.5±3.3 years (range, 1-14 years). The mean±SD age at the last examination was 32.6±11.5 years (range, 16-55 years). The cerebellar syndrome had been present in all patients since disease onset and involved movement and gait coordination. However, this syndrome remained moderately severe throughout the course of the illness. Cerebellar dysarthria was present in 14 patients (mean±SD disease duration, 31.2±8.4 years) and was absent in 4 patients (patients 4, 5, 10, and 12) (mean±SD disease duration,
17.3±5.1 years), suggesting that the occurrence of cerebellar dysarthria was related to the duration of the disease. Nystagmus was constant. The pyramidal syndrome was mild in the youngest patients (patients 12 and 15 of family 3). Spasticity became progressively worse during the disease and was prevalent in older patients, making it difficult to evaluate the severity of the cerebellar syndrome. Sensory disturbances were constant and involved vibratory and segmentary position sense. Ankle reflexes were absent in all of the patients independent of the age at onset or the duration of the disease. Knee jerks were brisk in all patients. In the upper limbs, the tendon reflexes remained brisk throughout the disease. Mild hand and peroneal wasting was found in 12 patients, all of them older than 24 years. Pes cavus was noted in 8 patients from the 5 families and hammer toes were seen in 8 patients. Scoliosis was observed in 2 patients. Micturition urgency was reported by more than 65% of the patients. Fundi showed prominent retinal myelinated fibers converging toward the optic disc in 2 patients (patient 12 from family 3 and patient 18 from family 4). The clinical syndrome was gradually progressive, with 4 patients becoming wheelchair bound at around 30 years of age (patient 8, 31 years; patient 16, 29 years; and patients 17 and 18, 30 years).

**PERIPHERAL NERVE CONDUCTION STUDY**

Peripheral nerve conduction data are reported in Table 2. Median nerve conduction studies were performed in 10 patients and showed a significant decrease in nerve conduction velocity, which was less than 38 meters per second in 8 patients (mean±SD, 34.6±4.5 m/s). A notable decrease in peroneal motor nerve conduction velocity was found in 7 of 9 patients and was totally absent in the other 2. Median and sural sensory nerve action potentials were absent or had low amplitudes. Sensory nerve conduction velocities were also reduced. These data support the presence of a severe to moderate axonal neuropathy associated with some demyelinating features.

**NERVE BIOPSY**

Nerve biopsy showed a variable degree of reduction of large myelinated fibers associated with remarkable regenerating axonal clusters and rare demyelinating aspects, seen as thin myelinated sheaths and some sparse onion bulbs. The degree of nerve biopsy abnormalities was not related to the age of the patients (Figure 2). Morphometric data showed a moderate decrease in the density of large myelinated fibers, with a normal overall myelinated fiber density (Table 3). The relative increase in the number of small myelinated fibers in patients compared with control subjects was probably related to axonal sprouting. These data are consistent with an axonal neuropathy associated with mild demyelinating features.

**MUSCLE BIOPSY**

Muscle biopsy showed typical and obvious neurogenic atrophy in the studied patients (data not shown).

**SOMatosensory evoked potentials**

Sensory evoked potentials were obtained in 5 patients (Table 4). No cortical response was obtained in 3 patients. In 1 patient, cerebral waves were detectable only after median stimulation and showed a delayed response. In the fifth patient, the sensory evoked potentials were normal. These observations suggest a variable and frequently severe involvement of the lemniscal pathway.

**VISUAL evoked potentials**

Visual evoked potentials were measured in 5 patients and demonstrated a delayed P100 wave latency (mean±SD, 119±16 milliseconds [range, 102-146 milliseconds]) in all cases, although the amplitude remained normal.


**Table 4. Sensory Evoked Potentials**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>N13 Wave</th>
<th>N13 Wave</th>
<th>N20 Wave</th>
<th>N20 Wave</th>
<th>N13 and N20 Wave</th>
<th>Posterior Tibial</th>
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<tr>
<td></td>
<td>Latency</td>
<td>Amplitude</td>
<td>Latency</td>
<td>Amplitude</td>
<td>Conduction Time</td>
<td>Latency</td>
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<td>...</td>
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<td>...</td>
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<tr>
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<td>5.3</td>
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</table>

Abbreviation: Nm, not measurable.
*Ellipses mean impossible to calculate.

(mean±SD, 5.73±2.69 µV [range, 2-15 µV]), suggesting an optic neuropathy.

**OTHER LABORATORY DATA**

Cerebrospinal fluid analysis performed in 10 patients showed a variable protein level from 0.19 to 0.90 mg/mL (mean±SD, 0.35±0.21 mg/mL). Cerebral computed tomographic findings studied in 10 patients consistently demonstrated atrophy of the superior cerebellar vermis. Electrocardiography was carried out in all patients, and echocardiography was performed in 4 patients belonging to 3 different families; no abnormalities were observed.

**MUTATION ANALYSIS**

We sequenced DNA from affected patients and controls. Four novel mutations were found in the recessive cerebellar ataxia pedigrees (Figure 3). Family 1 showed a missense mutation (10046G→C), producing an amino acid change alanine to proline at position 3324. Family 2 had a single base deletion at position 1411 (1411delT). This deletion produces a frameshift, resulting in a premature stop codon and a truncated peptide of 456 amino acids. Family 3 had a single nucleotide insertion (1155insA), producing a frameshift and a truncated peptide of 360 amino acids. Family 4 also showed a mis-
The patients described are all of Tunisian descent. Three families (families 1, 3, and 4) originated from and are still living in the center of Tunisia in the same region as the first Tunisian family described by Mrissa et al. One family resides in Tunis. The families do not seem to be related, but it is difficult to trace their lineage for more than 3 generations. The Canadian patients descended from French ancestors. At the beginning of the 17th century, they lived in Charlevoix, close to Quebec. Later, most of them emigrated to Saguenay. It was thought that this disease was confined to Charlevoix-Saguenay until the description of relatives. At the beginning of the 17th century, they lived in Charlevoix, close to Quebec. Later, most of them emigrated to Saguenay. It was thought that this disease was confined to Charlevoix-Saguenay until the description of the Tunisian family with linkage to the same locus.5

The clinical, electrophysiological, and nerve biopsy findings of the patients described herein are similar to those described in the first Tunisian family linked to the ARSACS locus and are also comparable to those in the Canadian patients except for some minor differences. In our patients, the age at onset varied from 1 to 20 years, whereas in the Canadian patients, disease onset was reported to occur around walking age (12-18 months). In the Tunisian and Canadian patients, the cerebellar syndrome had been present since the onset of the disease and remained moderate throughout, whereas the pyramidal syndrome worsened, becoming predominant in the later stages. Nystagmus was a constant finding in all patients.

The tendon reflexes had a stereotyped profile in the Canadian patients, as they remained preserved throughout the disease, except for the ankle reflexes, which disappeared around 24 years of age. The ankle reflexes were absent early in all of our patients, whereas other tendon reflexes were brisk. Fundi consistently showed prominent retinal myelinated fibers in the Canadian patients. This sign was rarely encountered in our patients. Deep sensory disturbances were constant and predominantly involved vibratory sensation in the lower limbs of all patients. Distal muscle wasting and joint deformities were found among Tunisian and Canadian patients. Distal muscle wasting became obvious in lower limbs toward 20 years of age in Canadian patients and 24 years in our patients, with notable intrafamilial variability in the degree of muscle atrophy in the 2 patient groups. Joint deformities such as hammer toes were similar in the 2 groups. Kyphoscoliosis was rare. Our patients became wheelchair bound at about 30 years of age.

A variability in progression of the disease between siblings was also a typical feature. Bladder dysfunction was frequent, with urge incontinence being the most common manifestation reported by patients. Intelligence was normal in all patients. Diabetes was noted in 2% of Canadian patients but was not found in our patients. There was no cardiac involvement. Cerebral imaging consistently demonstrated atrophy of the superior cerebellar vermis. The neurophysiological data were similar in the Tunisian and Canadian patients. Sensory evoked potentials were markedly impaired, predominantly in the lower limbs, suggesting a severe degeneration of the posterior columns similar to data reported in Friedreich ataxia. Such abnormalities were encountered at an early stage of the disease and remained stable throughout the course. Visual evoked potentials were disturbed in most of our patients and showed a delayed and a dispersed response in Canadian patients. In most patients, there was a moderate peripheral axonal neuropathy with remarkable regenerating features and mild demyelination, as shown by results of the peripheral nerve conduction studies and nerve biopsies.

Including our results, 6 pathogenic mutations have been reported in the SACS gene. Interestingly, in the 4 pedigrees we studied, each showed a different SACS mutation. In 2 of our families, frameshift mutations were found that resulted in truncated peptides. These truncations result in loss of the Dnaj domain. The bacterial Dnaj heat shock protein has several human homologues, including Hsp40. These proteins interact with Hsp70 and are involved in chaperone-mediated protein folding. Therefore, one may speculate that sacsin also has a molecular chaperone-like function. Loss of the Dnaj domain, as predicted in families 1 and 2, may result in loss of a critical chaperone function of this protein. The normal biological function of sacsin remains to be elucidated. A gene homologous to SACS has been reported in the mouse. Transgenic knockout of the homologous mouse gene may yield important insights into the possible protein substrates of sacsin and its proposed role in protein folding. Because Tunisia is a country with a high prevalence of inbreeding, a founder effect might be expected, as reported previously for ataxia with vitamin E deficiency, for which only 1 mutation has been encountered in the Tunisian population. The presence of 4 different mutations in the SACS gene is surprising. It may be related to the heterogeneous origin of the Tunisian population (eg, Arabs, Berbers, Europeans, and Africans). The high rate of consanguinity may play a role in the appearance of such different and rare mutations. It would be interesting to look for this type of mutation in patients with hereditary autosomal recessive ataxia in Mediterranean and Arab countries, which could help to identify the origin of the different mutations observed in Tunisia.

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**Author contributions:** Study concept and design (Drs Amouri and Hentati); acquisition of data (Drs El Euch-Fayache, Lalani, Amouri, Turki, Hung, Belal, and Hentati); analysis and interpretation of data (Drs El Euch-Fayache, Lalani, Ouaichi, Hung, Siddique, and Hentati); drafting of the manuscript (Drs El Euch-Fayache, Lalani, Amouri, Turki, Hung, Belal, and Hentati); critical revision of the manuscript for important intellectual content (Drs El Euch-Fayache, Lalani, Ouaichi, Hung, Siddique, and Hentati); statistical expertise (Drs El Euch-Fayache and Hentati); obtained funding (Dr Hentati); administrative, technical, and material support (Drs Lalani, Amouri, Hung, and Hentati).
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