A novel mutation in SACS gene in a family from southern Italy

C. Criscuolo, S. Banfi, M. Orio, et al.

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A novel mutation in *SACS* gene in a family from southern Italy

C. Criscuolo, MD; S. Banfi, MD; M. Orio, MD; P. Gasparini, MD; A. Monticelli, MD; V. Scarano, MD; F.M. Santorelli, MD; A. Perretti, MD; L. Santoro, MD; G. De Michele, MD; and A. Filla, MD

Abstract—A form of autosomal recessive spastic ataxia (ARSACS) has been described in the Charlevoix and Saguenay regions of Quebec. So far a frameshift and a nonsense mutation have been identified in the *SACS* gene. The authors report a new mutation (1859insC), leading to a frameshift with a premature termination of the gene product sacsin, in two sisters from consanguineous parents. The phenotype is similar to previously described patients with ARSACS.

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Autosomal recessive spastic ataxia of Charlevoix–Saguenay (ARSACS; MIM 270550) is a neurodegenerative disorder showing pyramidal, cerebellar progressive involvement and peripheral neuropathy. More than 300 patients with ARSACS have been described in the Charlevoix–Saguenay region of northeastern Quebec, where the estimated carrier frequency is 1 of every 22 persons.[1] Main clinical features include early-onset progressive ataxia, dysarthria, spasticity, nystagmus, retinal striation, and distal amyotrophy. Imaging shows cerebellar vermis atrophy, and peripheral nerve conduction studies reveal markedly decreased amplitude of the sensory potentials and reduced motor conduction velocities. The disease is pathologically characterized by atrophy of the upper cerebellar vermis and loss of Purkinje cells.[2]

The gene responsible for ARSACS (*SACS*) maps to chromosome 13q11[3] and has a single large exon spanning 12,794 base pairs (bp),[4] the largest exon in any vertebrate organism. The *SACS* product is a predicted 3829 amino acid protein, sacsin, which probably exerts a chaperon-mediated protein-folding activity.[4] Until now, two causal mutations have been described in this gene in the French-Canadian population: g.6594 delT, resulting in a frameshift with introduction of a stop codon, and g.5254 C→T, a nonsense mutation.[4] Ninety-four percent of the disease alleles carry the g.6594 delT; 3% carry the g.5254 C→T; and the remaining 3% carry an unknown mutation always in the heterozygous state with g.6594 delT.[5] Recently, one Tunisian and two Turkish families have been linked to 13q11.[6-8] The presence and the frequency of this disease are not known in most European countries. In this study, we describe a novel single-base insertion mutation in the *SACS* gene in two affected sisters from southern Italy.

Additional material related to this article can be found on the Neurology Web site. Go to www.neurology.org and scroll down the Table of Contents for the December 23 issue to find the title link for this article.

Methods. We recruited 22 index patients with an ARSACS-like phenotype from a series of 85 patients from southern Italy with early-onset cerebellar ataxia. Consanguinity was present in six families. The inclusion criteria were sporadic or autosomal recessive progressive ataxia, corticospinal signs (at least two among brisk tendon reflexes, hypertonus, and Babinski signs), clinical (decreased or absent ankle reflexes and decreased vibration sense) or neurophysiologic signs of peripheral neuropathy, and negative molecular test for Friedreich ataxia.

After informed consent was obtained from all patients, genomic DNA was extracted from peripheral blood samples. Primer pairs were designed to screen for the two French-Canadian mutations in all patients using the web-based version of the Primer 3.0 program. PCR products, subjected to electrophoresis in 1.5% agarose gel, were then analyzed by denaturing high-performance liquid chromatography (DHPLC). DHPLC was performed on a WAVE nucleic acid fragment analysis system HSM (Transgenomic, Crewe, UK). The composition of buffer A was 0.1 mol/L triethylammonium acetate (TEAA), pH 7.0, 0.025% acetonitrile (v/v). Buffer B contained 0.1 mol/L TEAA, 25% aceto-

See also pages 10, 103, and 107

From the Departments of Neurological Sciences (Drs. Criscuolo, Orio, Scarano, Perretti, Santoro, De Michele, and Filla) and Molecular and Cellular Biology and Pathology (Dr. Monticelli), Federico II University, Naples, Italy; Department of Neurological Sciences (Dr. Santorelli), La Sapienza University, Rome, Italy; and Telethon Institute of Genetics and Medicine (Drs. Banfi and Gasparini), Naples, Italy.

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Address correspondence and reprint requests to Dr. Chiara Criscuolo, Dipartimento di Scienze Neurologiche, Università degli Studi di Napoli Federico II, Via Pansini 5, I-80131, Naples, Italy; e-mail: sky569@libero.it

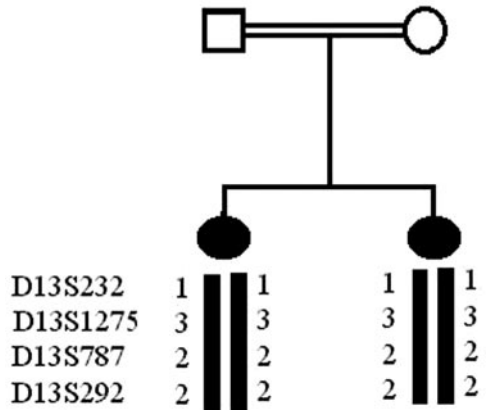

Figure 1. Pedigree and haplotypes for the autosomal recessive spastic ataxia of Charlevoix–Saguenay (ARSACS) locus.

nitrile (v/v). Analysis was carried out at a flow rate of 0.9 mL/min and buffer B gradient increase of 2% per minute. Start and end concentrations of buffer B were adjusted according to the size of the PCR product. In addition, in the patients from consanguineous marriages, linkage analysis was performed using polymorphic markers spanning the *SACS* gene (D13S292, D13S787, D13S232, D13S1275) to determine if the disease trait was associated with the AR*SACS* locus. Patients homozygous for all tested markers were considered in possible linkage to ARSACS and were screened for mutations in the entire gene. The unique *SACS* exon, because of the large size, was divided in 15 overlapping fragments of approximately 800 bp. Oligonucleotide primer pairs and PCR conditions are shown in the table available online (see table E-1 on the Neurology Web site). The PCR reaction was carried out in a total volume of 25 μL containing 100 ng of genomic DNA, 2.5 μL 10 × PCR buffer containing $MgCl_2$ (15 mmol/L), 2 μL dNTP (2.5 mmol/L), 0.5 μL each primer (20 pm/μL), and 0.3 μL "AmpliTaq Gold" (Perkin-Elmer, Foster City, CA). PCR products were purified from agarose gels by the QIAGEN gel extraction kit (Hilden, Germany) and directly sequenced, from the forward and reverse strands, on an automated sequencer (ABI 3100; Applied Biosystem, Weiterstadt, Germany) using the ABI-PRISM big-dye terminator cycle sequencing ready reaction kit (Applied Biosystem).

Peripheral nerve conduction study was performed using needle electrodes.

Results. We did not find any of the previously described mutations in our patients. Conversely, linkage analysis revealed that one patient from a consanguineous marriage was homozygous for all tested markers suggesting linkage to the ARSACS locus. The affected sister showed the same haplotype (figure 1). Sequencing of the entire gene in both sisters revealed a novel homozygous 1859insC mutation (figure 2). The screening of this new mutation in the remaining patients was negative.

Clinical and neurophysiologic features of the two patients are summarized in the table. Onset was characterized by delay in walking, followed by gait unsteadiness and tendency to fall. The patients, now aged in their 40s, need support when walking. Mental retardation and hypoacusia are present in Patient 2. Sural nerve biopsy, performed in Patient 1, showed a severe loss of large myelinated fibers (>11 μm) with almost normal total number of myelinated fibers. Neuroimaging showed cerebellar atrophy more marked in the vermis than in the hemispheres.

Discussion. We report a new mutation in the *SACS* gene in two sisters from consanguineous parents in southern Italy. The novel 1859insC mutation results in a frameshift and the introduction of an early stop codon at residue 599. The resulting predicted protein lacks 3230 amino acids. The French-

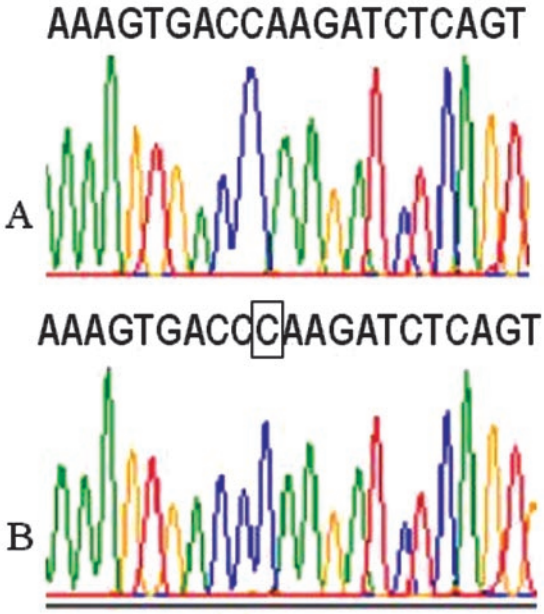

Figure 2. Electropherogram of the SACS *normal sequence (A) and the novel single-base insertion [C] mutation in a patient (B). The nucleotide position is based on the transcript GenBank accession number AF193556.*

Table *Clinical and neurophysiological findings*

Patients	1	2
Onset age/disease duration, y	2/41	2/36
Gait ataxia	++	++
Nystagmus	+	–
Dysarthria	++	++
Dysmetria	+	+
Lower limb spasticity	++	++
Increased knee jerks	++	++
Ankle jerks	–	–
Babinski signs	+	+
Lower limb amyotrophy	–	+
IQ	74	58
Median nerve		
SCV digit III-wrist, m/s	42.2	38.8
SAP at wrist, μV	0.8	0.7
MCV elbow-wrist, m/s	41.6	40.2
SSEP N13–N20, m/s	10.7	10.8

Presence and severity of features: + = mild to moderate; ++ = severe; – = absent.
SCV = sensory conduction velocity (normal lower limit 51 m/s);
SAP = sensory action potential (normal lower limit 6 μV);
MCV = motor conduction velocity (normal lower limit 54 m/s);
SSEP = somatosensory evoked potential (normal upper limit 7.4 m/s).

Canadian g.6594T and g.5254C mutations also cause the premature termination of the predicted protein. The recessive character and the truncating nature of the sacsin mutations suggest that the development of the disease is associated with a loss of function. Sacsin shows significant sequence similarity to proteins and domains involved in chaperon-mediated protein folding because it contains a "DnaJ" motif and the N-terminal domain of the Hsp90.[4] In addition, sacsin is characterized by the presence of two leucine zipper and three coiled-coil domains and seven nuclear localization signals.[4] The mutation described in this report is localized at the N-terminal region before any known domain.

The proteins paraplegin and HSPD1, involved in two forms of hereditary spastic paraplegias (SPG7 and 13), show chaperon-like activities.[9,10] The corticospinal tract involvement present in these two diseases is a prominent feature in patients with ARSACS.

There are no phenotypic differences among the patients homozygous for the g.6594delT, the compound heterozygotes g.6594delT/g.5254C→T, and g.6594delT/unknown mutation.[5] Although the new mutation leads to a shorter truncated protein, its harmful effect is not increased. Our patients had clinical and neurophysiologic findings similar to those observed in the French-Canadian group except for the presence of mental retardation in one patient. Intellectual impairment was also observed in one of the four Turkish patients linked to ARSACS locus.[7] A finer genotype and phenotype correlation may be possible when more mutations are identified.

This report confirms that ARSACS is present in populations other than those previously described and should be considered in patients with autosomal recessive cerebellar ataxia, pyramidal signs, and peripheral neuropathy. Failure to detect g.6594delT and g.5254C→T mutations in our series suggests that these two mutations may be a rare cause of ARSACS in southern Italy.

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