Novel Mutation of *SACS* Gene in a Spanish Family With Autosomal Recessive Spastic Ataxia

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Abstract: **Autosomal recessive spastic ataxia of Charlevoix–Saguenay (ARSACS) is an inherited neurodegenerative disorder characterized by early-onset, spastic ataxia and peripheral neuropathy. It was originally described in an inbred population of Quebec and later in some other countries. We report a new missense *SACS* mutation (7848C>T) in a Spanish family whose phenotype is similar to that of the previously described ARSACS patients. 7848C>T is the first *SACS* mutation reported in Spain confirming worldwide distribution of the disease.** © 2005 Movement Disorder Society

Key words: ARSACS; spastic ataxia; *SACS*

A form of early-onset autosomal recessive spastic ataxia has been described with high prevalence in the Charlevoix–Saguenay area in Quebec (ARSACS; MIM 270550). It is a neurodegenerative disorder characterized by early-onset spastic ataxia, dysarthria, nystagmus, distal muscle wasting, sensory–motor neuropathy, and superior vermis atrophy.[1] The gene responsible for ARSACS (SACS) maps to chromosome 13q11. *SACS* consists of a single huge exon and encodes the sacsin protein.[2] After gene mapping and identification in the Charlevoix–Saguenay ataxic patients, 1 Tunisian and 2 Turkish families have been linked to ARSACS locus and mutations in the *SACS* gene have been found in Italian, Turkish, Tunisian, and Japanese families.[3–9] We report a new missense mutation in the first Spanish ARSACS family.

PATIENTS AND METHODS

Clinical Study

We describe 2 patients (brother and sister) born from healthy parents. Their father died at 66 years of age from lung cancer. There was a distant consanguinity between the parents, both from the same village in the province of Murcia. The family members, including the mother and two healthy siblings, underwent thorough neurologic evaluation.

Molecular Study

After informed consent, genomic DNA was extracted from peripheral blood leukocytes from 5 family members. No father's sample was available for the study. Linkage analysis was performed using polymorphic markers spanning the *SACS* gene (D13S292, D13S787, D13S232, D13S1275) to determine whether the disease trait was associated to the ARSACS locus. The patients, homozygotes for all tested markers, were considered in possible linkage to ARSACS and were screened for mutations. The unique *SACS* exon was divided in 15 overlapping fragments. PCR products were directly sequenced on an automated sequencer (ABI 3100; Applied Biosystems, Foster City, CA). When sequence analysis revealed a missense mutation, 100 Spanish controls were analyzed by denaturing high-performance liquid chromatography (dHPLC) to rule out a polymorphism.

RESULTS

Case Reports

Patient 1 (proband) is a 37-year-old man with progressive gait imbalance since infancy. Since age 36, he needed a cane for walking. He denied having diplopia, visual loss, dysphagia, tremor, or sphincter disturbances.

Neurologic examination at age 37 years showed spastic ataxia, mild limb and truncal ataxia, slurred and scanning speech, and gaze-evoked nystagmus. Tendon reflexes were normal in the upper limbs and brisk in the lower limbs, with bilateral ankle clonus and Babinski signs. Spasticity, mild distal weakness, and reduced vibration sense in the lower extremities were present. There was atrophy of the intrinsic distal muscle of the hand, foot deformity including hammertoes, and pes cavus. Funduscopy did not reveal hypermyelinated retinal fibers. No cognitive defect was evident, and he performed well in high school. Mag-

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netic resonance imaging (MRI) scan revealed cerebellar atrophy, especially in the upper vermis, and spinal cord atrophy. Motor (39 m/sec in the elbow–wrist segment; lower limit, 55) and sensory (27 m/sec in the digit III–wrist segment; lower limit, 53 m/sec) conduction velocities were decreased at the median nerve; and motor (6.8 mV, lower limit 9.0) and sensory (0.6 μV; lower limit 6.5) action potentials were reduced at the wrist. Complex motor potential at extensor retinaculum (peroneal nerve) and sensory potential at external malleolus (sural nerve) were not recordable.

Patient 2 is a 26-year-old woman, sister of Patient 1. Onset of the disease also occurred in infancy with gait instability. Neurologic examination at age 26 revealed spasticity in her lower extremities with brisk tendon reflexes and Babinski signs. She had difficulty in smooth ocular pursuit, gaze-evoked nystagmus, and slurred and scanning speech. Her foot deformities were similar to those of Patient 1. Hypermyelinated retinal fibers were not found. At clinical examination, no evidence of mental retardation was noted in both patients. She performed well at high school and currently is studying at university and working as an administrator. Brain MRI showed cerebellar and spinal cord atrophy (Fig. 1). Motor (42 m/sec in the elbow–wrist segment) conduction velocity was decreased at the median nerve, motor potential (3.9 mV) was reduced at wrist, and sensory action potential was not recordable at wrist. Complex motor potential at extensor retinaculum was not recordable. Electromyography of tibialis anterior muscle showed in both patients reduction of motor units and the presence of large and polyphasic potentials, with no spontaneous activity.

Molecular Study

A novel homozygous missense mutation in the *SACS* gene (C-to-T transition at nt7848) was identified in the two patients (Fig. 2A). 7848C>T results in the substitution of arginine for cysteine at amino acid residue 2556 (R2556C). This substitution was found in the heterozygous state in the unaffected mother but not in the healthy siblings. The mutation was not found in 200 Spanish control chromosomes. Arginine 2556 in *SACS* is conserved in different species (Fig. 2B).

DISCUSSION

ARSACS, originally identified as a disease specific to the population of the Charlevoix–Saguenay area in Quebec, has been described in Tunisia, Turkey, Italy, and Japan in the past 2 years.[3–9] We report the first ARSACS family in Spain. A Spanish case with an ARSACS-like phenotype already has been described. However, neither spasticity nor signs of polyneuropathy were found and no molecular study has been reported up to now.[10] The ARSACS phenotype seems to be clinically uniform and comprises early-onset (12–18 months), progressive ataxia combined with spasticity, extensor plantar reflexes, reduced vibration sense, and peripheral neuropathy.[11] Reflexes are brisk at the lower limbs except for ankle reflexes, which became gradually weaker and usually were abolished after age 25.[10] The prominent my-

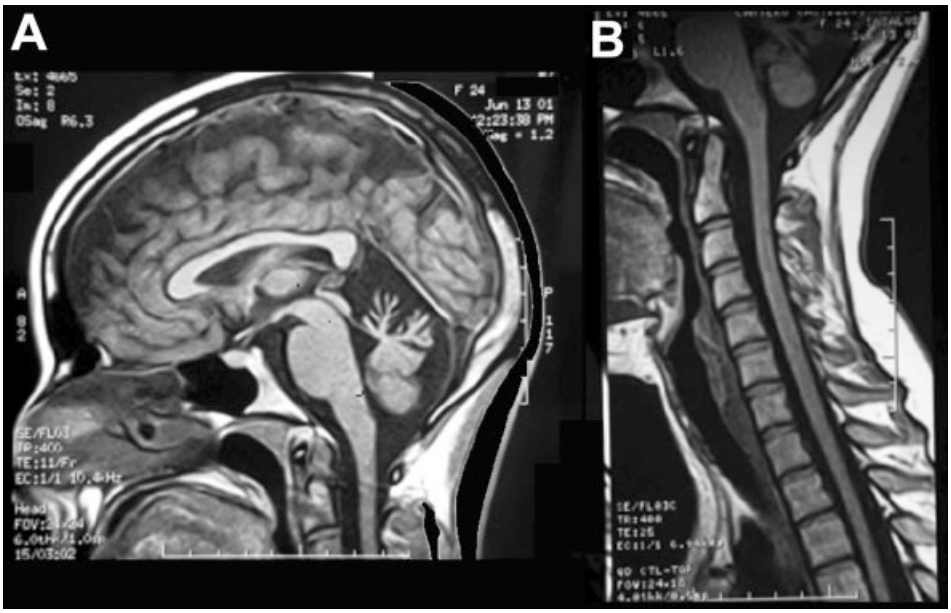

FIG. 1. Patient 2, T1-weighted sagittal magnetic resonance imaging scan showing cerebellar (**A**) and spinal atrophy (**B**).

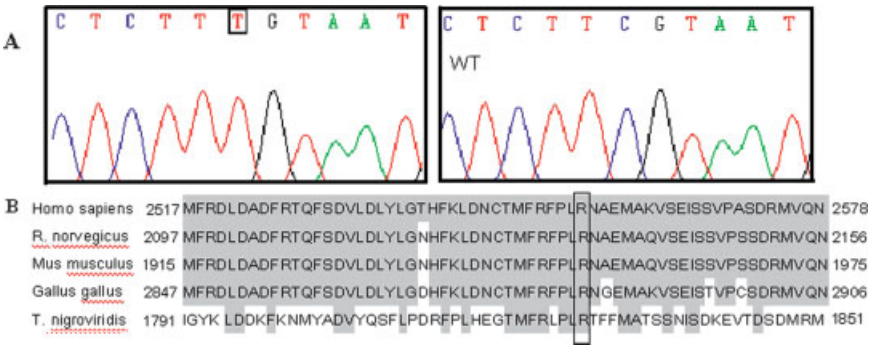

FIG. 2. A: Electropherogram of the novel homozygous missense mutation (C-to-T transition at nt7848) in *SACS* and the wild-type sequence (WT). The nucleotide position is based on the transcript GenBank accession no. NM_014363 starting at the first ATG codon. **B:** Alignments among species of 60 human saxin amino acids showing arginine 2556 conservation (GeneBank accession num.: NP055178 AAF31263, XP_224256.2, AAF31263.1, XP_417138.1, and CAG02848.1).

elinated retinal nerve fibers are a constant feature of Quebec patients but appear to be absent in patients identified in other countries.[11] Mental retardation has been sometimes reported.[4,5,8] The phenotype of our patients closely matches that of the Quebec patients, apart from persistence of ankle reflexes after age 25 and absence of prominent myelinated retinal nerve fibers, confirming that this sign is rare outside Quebec. Electrophysiological studies demonstrated an axonal sensorimotor neuropathy with associated demyelination.

A founder effect has been demonstrated in Charlevoix–Saguenay region by the identification of a homozygous single base deletion (6594delT) in *SACS* in the majority of the patients. However, in a few compound heterozygous patients, the 6594delT is associated with a nonsense mutation (5254C>T).[2] Eight truncating saxin protein and six missense mutations have been identified in 13 families from Tunisia (n = 4), Italy (n = 3), Japan (n = 2), and Turkey (n = 4).[3–9] Murcia, the region of the patients, is in the North of Spain and not in the Mediterranean basin, where most of the new mutations have been identified.

Sacsin has been tentatively grouped in the chaperonin family of proteins, because it contains a "DnaJ" motif.[2] The novel 7848C>T missense mutation does not fall in the "Dna J" motif and does not change the secondary protein structure predicted by the PROF program,[12] although it leads to a classic phenotype. We consider 7848C>T responsible of ARSACS for the following reasons: 7848C>T cosegregates with the disease in the family with an autosomal recessive transmission; the substitution was not found in 200 Spanish normal control chromosomes; arginine 2556 in *SACS* is conserved in different species; and our patients' phenotype is typical of ARSACS.

In conclusion, 7848C>T is the first mutation detected in a Spanish family, confirming the worldwide presence of ARSACS and suggesting that missense mutations are more common than previously described. The typical ARSACS phenotype of our patients confirms a uniform clinical presentation of the disease. The Genbank accession number is NM_014363, protein NP_055178.

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LEGENDS TO THE VIDEO

Segment 1. (Proband, J.C.C., Patient 1). This video segment successively shows the following signs: ataxo-spastic gait, pes cavus and hammertoes, atrophy of intrinsic hand muscles, enhanced knee jerks, extensor plantar responses, appendicular ataxia (finger-nose and heel-to-knee-to-toe tests), nystagmus, and saccadic pursuit consisting of saccadic intrusions.

Segment 2. (Case A.C.C., Patient 2). Note the following signs: ataxo-spastic gait, pes cavus and hammertoes, enhanced knee jerks, and appendicular ataxia.

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Essential Tremor Centralized Brain Repository: Diagnostic Validity and Clinical Characteristics of a Highly Selected Group of Essential Tremor Cases

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Abstract: **We studied essential tremor (ET) cases enrolled in the Essential Tremor Centralized Brain Repository to (1) assess the validity of their diagnoses and (2) characterize the clinical features in a group of highly selected cases who might reflect a far end of the disease spectrum. Our over-arching goal was to provide a perspective of ET that complements that derived from population-based and clinic-based studies. Based on a history and videotaped examination, 94 of 100 ET cases had their diagnoses confirmed; most of the remainder had Parkinson's disease. When compared with ET cases ascertained through populations and clinics, a large proportion had been prescribed medication for tremor (87.2%), had a family history of tremor (88.3%), had rest tremor (33.0%), or had neck tremor (60.6%). One patient had facial tremor, which has not been reported previously. As has been reported once before, a large proportion wore hearing aids (26.9% of the 67 participants age ≥ 70). In summary, diagnostic validity was high. In terms of their clinical characteristics, the high proportion of cases with severe tremor and varied disease manifestations (neck tremor, rest tremor) make these cases a valuable resource in pathological studies; the high proportion with familial tremor would provide an enriched sample for genetic studies.** © 2005 Movement Disorder Society

Key words: essential tremor; epidemiology; clinical characteristics; brain bank

Essential tremor (ET) cases from throughout the United States are being recruited prospectively as potential brain donors to the Essential Tremor Centralized Brain Repository (ETCBR) at Columbia University. These cases were recruited through the International Essential Tremor Foundation (IETF).[1] These cases are highly selected both because they were ascertained through a disease-specific organization and because they self-referred to the ETCBR.

As clinical research becomes more complex and collaborative and as investigators begin to recruit patients using a variety of novel mechanisms, questions will arise about the suitability of the cases they are selecting for enrollment in their studies. First, what is the diagnostic validity of their sample and how many cases will have other diagnoses? Second, what are the clinical characteristics of highly selected case groups? Cases seen in treatment settings often have more severe forms of the illness than those seen in the population[2–7]; highly selected case groups may reflect an even farther end of the disease spectrum. In this sense, they may be an enriched source of cases for some studies.

Our aims were to study a group of ET cases self-referred to the ETCBR to: (1) assess the validity of their diagnoses and to determine what alternative diagnoses (e.g., Parkinson's disease [PD]) they might have, and (2) characterize the clinical features in a group of highly selected cases who might reflect a far end of the disease spectrum. Our over-arching goal was to provide a perspective of ET that complements that derived from population- and clinic-based studies.

PATIENTS AND METHODS

ET cases were recruited through the IETF. Cases were recruited by advertising in the IETF quarterly newsletter, which is mailed to several thousand ET patients who live throughout all of the states of the United States. The

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