Prominent Sensorimotor Neuropathy Due to SACS Mutations Revealed by Whole-Exome Sequencing

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Objective: To determine the genetic basis of an unexplained multisystem neurological disorder affecting 2 siblings.

Design: Case reports and whole-exome DNA sequencing.

Setting: Neurogenetics clinic, Institute of Genetic Medicine, Newcastle upon Tyne, England.

Patients: Two adult siblings with a sensorimotor neuropathy, ataxia, and spasticity.

Main Outcome Measures: Clinical, neurophysiological, imaging, and genetic data.

Results: Novel compound heterozygous frameshift mutations were detected in the SACS gene of both siblings, predicted to drastically truncate the sacsin protein.

Conclusions: Whole-exome sequencing rapidly defined the genetic cause of the disorder, expanding the clinical phenotype associated with SACS mutations to include a severe sensorimotor neuropathy.


Although originally described only in the Canadian Quebequois,1 autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) has been observed in Europe, North Africa, and Japan.2 Typically due to a founder effect in a specific region, affected individuals are usually homozygous for 1 of fewer than 30 mutations in SACS (RefSeq NM_014363.4),1,2 which codes for the 520-kDa molecular chaperone protein sacsin that is thought to be involved in intraneuronal protein folding.3 The disorder typically presents in early childhood with lower limb spasticity and a gait ataxia.2 Herein, we describe 2 nonconsanguineous siblings with a prominent sensorimotor neuropathy who presented with mild spasticity and ataxia in adulthood. Conventional investigations failed to identify the cause, which was revealed by whole-exome sequencing.

REPORT OF CASES

CASE 1

A male patient was assessed for learning difficulties at age 7 years. His gait disturbance began at age 19 years with progressive unsteadiness due to mild ataxia. Re- assessed at age 29 years, he had a spastic paraplegia, saccade dysmetria, atrophy, distal weakness, finger-flexion contractures, and pes cavus (Figure 1A and B and Figure 2). Tendon reflexes were absent and plantar responses were extensor. He had a cerebellar dysarthria, dysmetria, and dysdiadochokinesia. Progressive distal weakness led to wheelchair dependence at age 35 years. Neurocognitive assessments at ages 7, 14, and 19 years showed a nonprogressive deficit (verbal performance score was 83, 13th centile; full-scale IQ was 83, 13th centile). Results of a complete blood cell count, radiography, thyroid function tests, and electrophoresis as well as levels of blood glucose, serum creatine kinase, vitamin E, very long-chain fatty acids, phytanic acid, α-fetoprotein, and serum immunoglobulins were normal. Magnetic resonance imaging of the brain revealed cerebellar atrophy (Figure 1C). Neurophysiological examination showed a large-fiber sensorimotor axonal-demyelinating neuropathy (Table). Electrocardiographic findings were normal. Genetic tests for Friedreich ataxia, spinocerebellar ataxias 1, 2, 3, 6, 7, and 17, DRLPA, POLG, EBF-2, and mi-
tochondrial DNA m.3243G were negative. Muscle biopsy showed normal mitochondrial histochemistry and respiratory chain complex activities, with no evidence of mitochondrial DNA deletions.

CASE 2

The sister of case 1 developed poor coordination and urinary urgency at age 26 years, when she had normal

**Figure 1.** The index case (II-1) showed distal amyotrophy as well as clawed hands (A) and pes cavus (B), and sagittal T2-weighted brain magnetic resonance imaging showed atrophy of the cerebellar vermis (C). D, Whole-exome coverage and read depth are shown for patients II-1 and II-2. bp indicates base pairs. E, Confirmatory Sanger sequencing of the SACS gene (RefSeq NM_014363.4) showed c.2076delG, p.Thr692Thr fs*713 and c.3965_3966delAC, p.Gly1322Val fs*1343 mutations in individuals II-1 and II-2 as well as c.3965_3966delAC in the unaffected mother I-2, confirming that the novel mutations are in trans. No DNA was available from the father.
Eye movements, cerebellar dysarthria, brisk reflexes, and extensor plantar responses. At age 30 years, she experienced recurrent blackouts, jerky ocular pursuits, and saccade dysmetria and had mild distal weakness (Figure 2). Neurophysiological examination (Table) revealed a mixed demyelinating-axonal neuropathy. Findings on electrocardiographic, electroencephalographic, and autonomic function studies were normal, as were the results of 24-hour blood pressure monitoring. Additional blood tests included leukocyte enzyme studies, copper, ceruloplasmin, acylcarnitines, cholesterol, organic acids, and amino acids, and the results were normal. The blood lactate concentration was normal. Lumbar puncture results were normal, with a cerebrospinal fluid protein level of 0.033 g/dL. To convert to grams per liter, multiply by 10.0. Brain magnetic resonance imaging revealed generalized atrophy, most markedly affecting the cerebellum. Muscle biopsy revealed type 1 fiber clustering, normal respiratory chain complex activities, and no evidence of mitochondrial DNA deletions.

There were no other siblings. Both parents were neurologically healthy at age 70 years. There was no consanguinity.

### Whole-Exome Analysis

Whole-exome sequencing was performed on both siblings. Genomic DNA was fragmented to 150 to 200 base pairs (bp) by Adaptive Focused Acoustics (Covaris), end paired, adenylated, and ligated to adapters. Exonic sequences were enriched using SureSelect Target Enrichment with the SureSelect Human All Exon 38Mb kit (Agilent). The captured fragments were purified and sequenced on an Illumina HiSeq2000 platform using 90-bp paired-end reads. Bioinformatic analysis was performed using an in-house algorithm based on published tools. The sequence was aligned to the human reference genome (UCSC hg19) using Burrows-Wheeler Aligner. The aligned sequence files were reformatted using SAMtools. Single-base variants were identified using VarScan and indels were identified using Dindel. The raw lists of variants were filtered to include variants within the Agilent Sequence Capture target regions (±500 bp) and exclude common variants with a minor allele frequency greater than 0.01 that were present in the dbSNP135 database, the 1000 Genomes database (February 2012 data release), and 94 unrelated in-house exomes. Rare and novel variants that were shared between the 2 patients were identified; from these, homozygous and compound heterozygous variants that fit the recessive disease model were found. Protein-altering and/or putative “disease-causing” mutations were identified using MutationTaster. Novel variants were confirmed by Sanger sequencing, allowing segregation analysis.

### Results

The 2 siblings shared 3146 novel variants (eTable, http://www.archneurol.com) after the exclusion of non-dbSNP135 variants found to be shared in a panel of 94 in-house disease controls. Of the 3146 variants, 240 were predicted to be disease causing but only 3 genes con-
tained at least 2 likely pathogenic variants (eTable). Of these, only SACS has previously been associated with a phenotype resembling the family described here. SACS contained 2 novel deletions confirmed on sanger sequencing (c.2076delG, p.Thr692Thr fs*713 and c.3965_3966delAC, p.Gly1322Val fs*1343) present in both affected siblings (Figure 1D) and 1 heterozygous mutation in the unaffected mother (c.3965_3966delAC), making the 2 patients likely compound heterozygotes. The mutations were not present in 94 in-house exomes, 250 population-matched control chromosomes, or data from the 1000 Genomes Project.

The 2 novel SACS mutations are highly likely to be pathogenic: c.2076delG results in a premature stop codon and a truncated protein lacking 3887 amino acids; likewise, c.3965_3966delAC results in a truncated protein lacking 3257 amino acids. On complementary chromosomes, both are close to previously described pathogenic mutations upstream of both the HEPN and the DnaJ domains, which play essential roles in protein translation, folding, translocation, and degradation.1 3

The index patient was first noted to have pyramidal signs at age 19 years, and his sister presented at age 26 years. This supports the suggestion that non-Quebec patients present at a later age, implying a genotype-phenotype relationship for different SACS mutations.9 Phenotypic variability is further exemplified by the lack of retinal hypermyelination in this family (Figure 2), which is a hallmark of ARSACS in Quebec patients.2

Although a neuropathy has been described in ARSACS,10,11 this was not the dominant phenotype in the published cases, unlike the siblings described here. Our family therefore demonstrates the importance of considering SACS in complicated Charcot-Marie-Tooth disease. As in other cases, neurophysiological examination showed a mixed axonal-demyelinating picture,4 and no mutations were identified in known Charcot-Marie-Tooth disease genes despite adequate coverage. Lastly, our findings add weight to previous suggestions of a neurocognitive dimension in ARSACS,2 although mild learning difficulties are common and this could simply be a coincidence.

Our findings show that ARSACS, which is generally considered extremely rare, of childhood onset, and often found within consanguineous families, should be considered in adults presenting with a spinocerebellar syndrome in which the prominent peripheral neuropathy may mask the pyramidal signs. Consecutively sequencing candidate genes is both expensive and time-consuming. Our findings also show that whole-exome analysis has the capacity to diagnose rare neurogenetic disorders at great speed.

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Online-Only Material: The eTable is available at http://www.archneurol.com.

REFERENCES