**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. Reassessed at age 29 years, he had a spastic paraplegia, saccade dysmetria, ataxia, and spasticity. Conventional investigations failed to identify the cause, which was revealed by whole-exome sequencing.
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
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 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.archneur.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-
showed a mixed axonal-demyelinating picture,2 and no neurocognitive dimension in ARSACS,2 although mild mutations were identified in known Charcot-Marie-Tooth disease genes despite adequate coverage. Lastly, our findings also add weight to previous suggestions of ARSACS,10,11 this was not the dominant phenotype in the published cases, unlike the siblings described here. Our focus was on finding the cause of this family’s phenotype, 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,3 and no mutations were identified in known Charcot-Marie-Tooth disease genes despite adequate coverage. Lastly, our findings also 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.

Accepted for Publication: April 18, 2012.
Published Online: July 2, 2012. doi:10.1001/archneurol.2012.1472

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Financial Disclosure: None reported.

Funding/Support: Dr Yu-Wai-Man is a Medical Research Council Clinician Scientist. Dr Pickering-Brown is a Medical Research Council Senior Nonclinical Fellow. Dr Horvath is supported by the Medical Research Council. Dr Chinnery is a Wellcome Trust Senior Fellow in Clinical Science and a National Institute for Health Research Senior Investigator and receives funding from the Medical Research Council, the UK Parkinson’s Disease Society, and the National Institute for Health Research Biomedical Research Centre for Ageing and Age-Related Disease award to the Newcastle upon Tyne Foundation Hospitals National Health Service Trust.

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