Late-onset sacsinopathy diagnosed by exome sequencing and comparative genomic hybridisation

Angela Pyle¹, Helen Griffin¹, Jennifer Duff¹, Simon Zwolinski¹, Tania Smertenko¹, Patrick Yu-Wai-Man¹, Mauro Santibanez-Koref¹, Rita Horvath¹, and Patrick F. Chinnery¹
¹Wellcome Centre for Mitochondrial Research, Institute of Genetic Medicine, Newcastle upon Tyne, UK
²Northern Genetics Service, Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, UK

Abstract

Objective—The molecular diagnosis of adult-onset autosomal recessive cerebellar ataxias remains challenging because of genetic heterogeneity. However, recently developed molecular genetic techniques will potentially revolutionise the diagnostic approach. Here we set out to define the genetic basis of the ataxia in two brothers with no molecular diagnosis.

Methods—Clinical evaluation followed by whole exome second generation sequencing and comparative genomic hybridisation.

Results—Whole exome sequencing identified a hemizygous novel spastic ataxia of Charlevoix-Saguenay (SACS) stop-codon mutation in both brothers (c.13048G>T, p.E4350X) which was present in the mother, but not the father. Comparative genomic hybridisation revealed a 0.7Mb deletion on chromosome 13q12.12 in both brothers, which included SACS and was heterozygous in the asymptomatic father.

Conclusions—The milder phenotype, caused by a whole gene deletion and a stop codon mutation in SACS, indicates a loss-of-function mechanism in late-onset autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS), and illustrates the importance of chromosomal rearrangements in the investigation of adult-onset ataxia.

Keywords
ARSACS; ataxia; whole exome sequencing; comparative genomic hybridisation
INTRODUCTION

The precise diagnosis of adult-onset ataxia remains challenging. The clinical phenotype and imaging findings are often non-specific, and after excluding common toxic, inflammatory, neoplastic, degenerative, and metabolic causes, ~60% of patients fall into the “idiopathic” category. Routine genetic testing is normal in the majority of adult onset cases, leaving a long differential diagnosis of rare metabolic and genetic diseases that cannot be easily diagnosed. Second generation sequencing techniques have the potential to circumvent this problem, using either custom-made panels incorporating known ataxia genes, or through a non-targeted approach sequencing the whole exome (WES) or genome. However, neither approach is comprehensive, in part due the inherent difficulty of detecting DNA repeat-expansions and copy-number variations using massively parallel sequencing. Here we show that combining WES and comparative genomic hybridisation can reveal the genetic aetiology of adult-onset autosomal recessive ataxia, expanding the phenotype, and casting light on the underlying molecular mechanisms.

PATIENTS

II-1: Now 46 years-of-age, he presented in teenage years with clumsiness and a poor sporting performance. By age 20, he had developed a gait ataxia, slurred speech, and urinary urgency, but no bulbar symptoms. He required sticks to walk by age 30, but remains ambulant. On examination he had a scoliosis, a spastic-ataxic gait and a cerebellar dysarthria. He had bilateral optic atrophy, with hypometric saccades, and there was gaze-evoked nystagmus. He had a brisk jaw-jerk, but cranial nerves were otherwise normal. Limb examination revealed symmetric distal amyotrophy and pes cavus. There was proximal leg weakness in a pyramidal distribution, and global weakness distally. Tendon reflexes were brisk with spreading, but ankle reflexes were absent. There was reduced proprioception in the feet. There was dysmetria and dysdiadochokinesis.

II-2: Now 43 years-of-age, he was born after a prolonged labour, followed by an apnoeic spell shortly after delivery. He walked late at 18 months of age, and was given a diagnosis of “mild cerebral palsy”. He was never good at sport, but noticed a deterioration in his gait in his late teens, followed by a slurring dysarthria. On examination he had a spastic-ataxic gait, and a cerebellar dysarthria. Fundoscopy revealed bilateral optic atrophy. Ocular pursuit movements were jerky, and there was gaze evoked nystagmus in all directions. His jaw jerk was brisk, but the remaining cranial nerve examination was normal. Limb examination revealed distal amyotrophy and pes cavus, proximal spastic increased tone, but flaccid ankles. He had mild weakness in both legs, pathologically brisk tendon reflexes, but absent ankle jerks. Plantar responses were flexor. There was dysmetria and dysdiadochokinesis.

The parents are currently in their seventh decade, are unrelated, and have no neurological symptoms or signs. A 44 year-old sister is asymptomatic.

Clinical investigations were normal, including a full blood count and film, routine biochemistry, long chain fatty acids, phytanic acid, vitamin E levels, and leukocyte
enzymes. Electrocardiography and echocardiography were normal. Brain MRI in II-2 showed marked cerebellar vermian atrophy, moderate diffuse cortical atrophy, a normal brainstem, but reduced spinal cord diameter (Fig.1a). Peripheral neurophysiology revealed longstanding chronic partial denervation. Sensory and motor responses could not be elicited, consistent with a generalised axonal neuropathy. Somatosensory evoked potentials were unrecordable, presumed secondary to the peripheral lesion. A muscle biopsy in II-2 revealed neurogenic features including angulated fibres, small group atrophy, type II fibre predominance, and fibre type grouping. Muscle respiratory chain studies and ubiquinone levels were normal (238 pmol.mg, normal >140).

MOLECULAR GENETICS

Genetic testing was negative for spinocerebellar ataxias (SCA) 1,2,3,6,7,17; FXN, DRPLA, POLG, ABHD12, and MTATP genes. There were no mtDNA deletions in skeletal muscle. Whole exome sequencing in the older sibling followed enrichment with the Agilent SureSelect Human All Exon 38Mb kit on an Illumina HiSeq 2000 Analyser. Bioinformatic analysis was performed using an in-house algorithm (see supplementary methods). The mean per CCDS (Consensus Coding Sequence) base coverage was 52-fold and 79% of CCDS bases were covered at a minimum sequence read depth of 5-fold. Variant filtering led to a final list of one apparently homozygous and 3 compound heterozygous potential protein altering and disease causing variants in the following genes: SACS, DNAH8, CDC27, LRRC8A (Table 1). Comparison of the mean per exon sequence depth of the older sibling versus a control exome revealed a region of low coverage depth on chromosome 13 (Fig. 1b). Sanger sequencing showed that the SACS variant (c.13048G>T, p.E4350X) appeared homozygous in the affected offspring, was heterozygous in the mother, but not present in the father who appeared to be homozygous wild-type (Fig.1c). c.13048G>T was not present in 258 control chromosomes, nor 238 in-house exomes from disease controls. CGH array analysis on the affected sibs revealed an abnormal heterozygous karyotype in both brothers: (arr 13q12.12[23,772,658-24,489,253]x1) (Fig.1d), corresponding to the region of low coverage on the exome sequence, and consistent with a 0.7Mb deletion on the long arm of chromosome 13q12.12. This deletion incorporated two genes previously associated with disease (SGCG, SACS), and three not previously associated with disease (TNFRSF19, MIPEP, Q10TNF9B), and was also detected in the unaffected father.

DISCUSSION

The ataxia in this family is likely due to compound heterozygous genetic defects in SACS. Exome sequencing identified c.13048G>T, which is predicted to introduce a premature stop-codon (p.E4350X) in the sacsin protein. CGH analysis showed that this allele was hemizygous in the affected siblings, indicating that they were only able to produce a drastically shortened form of sacsin. The truncated sacsin lacks 229 amino acids which form a major component of both the DnaJ molecular chaperone domain, and the HEPN Nucleotide-binding domain, which are critically involved in protein translation, folding, translocation and degradation. Nonsense mutations in a similar position have been described in other patients with autosomal recessive ataxia, and the detection of the same
deletion in the asymptomatic father supports functional data indicating a haploinsufficiency mechanism, where disease is onset by two dysfunctional alleles.

One advantage of the whole exome capture approach is the identification of likely pathogenic mutations in genes not characteristically associated with the presenting phenotype, as illustrated by the family we describe here. Sacsinopathy was first described in Canadian pedigrees with autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSCACS),\(^3\) and typically presents in early childhood.\(^5\) Although adult-onset cases have been described, they are uncommon, and SACS would not normally be considered in this context. With hindsight, our patients did have non-specific symptoms which suggest a childhood onset, but neither progressed significantly until early adult life, which was when they presented to the neurology clinic. This mirrors early clinical observations in the Quebecois.\(^6\) Although no clear genotype-phenotype relationship has been demonstrated to date, it is conceivable that nonsense mutations affecting the terminal region of this large protein are responsible for the milder phenotype that we have observed in this family.

Secondly, although rarely considered in adult neurogenetic practice, chromosomal rearrangements are emerging as an important cause of late-onset neurological disease, and may account for upto 3% of SACS mutations.\(^7\);\(^8\) As shown here, regions of low exome coverage and apparent homozygosity provide a clue to a large genomic deletion, which may lie below the resolution of cellular karyotype analysis, but confident resolution requires CGH analysis. The combined exome-CGH approach led to the molecular diagnosis in this family, because of the high index of clinical suspicion after acquiring the first set of exome data.

In addition to the identification of new genetic causes for inherited neurological disease, second generation sequencing will also play a key role in re-defining the phenotype of “classical” neurogenetic disorders, broadening the differential diagnosis of a particular phenotype.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

**Acknowledgments**

PFC is an Honorary Consultant Neurologist at Newcastle upon Tyne Foundation Hospitals NHS Trust, is a Wellcome Trust Senior Fellow in Clinical Science (084988/Z/08/Z), and a UK National Institute for Health Research (NIHR) Senior Investigator. PFC receives additional support from the Wellcome Trust Centre for Mitochondrial Research (096919/Z/11/Z), the Medical Research Council (UK) Centre for Translational Muscle Disease research, the Association Française contre les Myopathies, and EU FP7 TIRCON, and the NIHR Newcastle Biomedical Research Centre based at Newcastle upon Tyne Hospitals NHS Foundation Trust and Newcastle University. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health. RH is supported by the MRC (UK) and the European Research Council (ERC).

**REFERENCES**


Figure 1.
(a) Sagittal TR2 brain MRI in II-2 showing generalised cerebral atrophy and marked atrophy of the cerebellar vermis. (b) Ratio of mean per exon sequence depth for II-1 against a control exome in a region of chromosome 13. The orange bar represents exons from positions 23,777,834-24,471,125 where the coverage ratio drops more than a standard deviation below the mean ratio for chromosome 13 and is confirmed cytogenetically as a deletion. (c) Confirmatory Sanger sequencing of the SACS gene (RefSeq NM_014363.4) showing the apparently homozygous c.13048G>T, p.E4350X mutation in the two affected brothers (II-1 & II-2). The mother I-2 is heterozygous for this mutation, but the father (I-1) has a wild-type sequence. (d) Comparative array hybridisation in II-1. Red bar represents the 0.7Mb deletion on chromosome 13 in this patient between base pairs 23,772,659-24,489,253 (Genome Build GRCh37, UCSC Assembly hg19) as identified by array CGH using an ISCA 8x60k platform (v 2.0). This deletion was confirmed by a TCAG FISH probe made from BAC RP11-88F2 (shown in purple). The deletion involves five known OMIM genes including SACS (dark green are known to be disease-causing).
Figure 2.
**Table 1**

<table>
<thead>
<tr>
<th>Exome coverage and genetic variants detected in the index case</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of Genes with Rare Variants</strong></td>
</tr>
<tr>
<td><strong>Patient ID</strong></td>
</tr>
<tr>
<td>II-1</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

---

(a) Variant numbers following analysis pipeline of BWA (Sequence Aligner); Varscan v2.2 (SV calling); Dindel v1.01 (Indel calling). Single nucleotide variants (SNV) – Varscan parameters minimum total coverage ≥5-fold, minimum variant coverage ≥3-fold, minimum quality > 10; Insertion/deletion variants (Indels) – Dindel output filter minimum variant coverage ≥4. Variants with position within Agilent 38Mb targets +/- 500bp, seen on both (Forward & Reverse) strands and (SNVs only) variant allele frequency ≥24%.

(b) Common variants (MAF>0.01) that match dbSNP135 and/or 1000 Genomes (Feb 2012 release).

(c) Rare (MAF<0.01) or novel variants with exclusion of common variants in a panel of 238 unrelated in-house exomes.