

CASE REPORT

Novel compound heterozygous mutation in SACS gene leads to a milder autosomal recessive spastic ataxia of Charlevoix-Saguenay, ARSACS, in a Finnish family

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Key Clinical Message

Autosomal recessive spastic ataxia of Charlevoix-Saguenay is a rare disorder outside Quebec causing childhood-onset cerebellar ataxia, peripheral neuropathy, and pyramidal tract signs. A Finnish family with milder form of ARSACS was found to harbor three mutations, p.E1100K, p.N1489S, and p.M1359T, in SACS gene. The mutations segregated with the disease.

Funding Information

No sources of funding were declared for this study.

Keywords

Autosomal recessive, SACS gene, spastic ataxia.

Received: 7 March 2016; Revised: 31 August 2016; Accepted: 20 September 2016

Clinical Case Reports 2016; 4(12): 1151–1156

doi: 10.1002/ccr3.722

Introduction

ARSACS is a recessive neurodegenerative disease causing childhood-onset cerebellar ataxia, peripheral neuropathy, and pyramidal tract signs [1]. It was first described in the Charlevoix-Saguenay-Lac-Saint-Jean region of Quebec where the estimation of frequency of carriers is 1 of 22 [2]. The disease-causing gene, SACS, encodes a large protein saccin that is expressed in brain motor systems and in various other tissues [3]. More than 100 mutations in SACS have been identified worldwide [4–6]. The protein's function is still largely unknown, although its role in the regulation of mitochondrial physiology has been proposed [7, 8]. Recent functional and molecular analyses of mitochondrial activity in fibroblasts obtained from ARSACS patients suggested involvement of oxidative stress and mitochondrial dysfunction in the pathogenesis of the disease [9, 10].

The first symptoms are typically balance problems before the age of 5 years in most cases, followed by spasticity and axonal-demyelinating sensorimotor peripheral neuropathy in their teens. The progression is slow; patients become wheelchair-bound approximately in their 4th decade [11]. Additional features include hypermyelinated retinal fibers, urge incontinence, and erectile dysfunction. Cerebellar vermis atrophy and linear hypointensities in the pons are typical early findings [12, 13]. Not all patients display the classic triad; occasional atypical and late-onset forms of ARSACS have been reported, and recently, SACS mutations have been identified in a few patients with nonprogressive congenital ataxia [9, 14].

We report here the first Finnish ARSACS family with compound heterozygous mutation in SACS that causes the classic triad phenotype, although with later onset and slower progression than in most reported cases.

Methods

The patients

A Finnish family with four affected siblings was examined and followed up for more than 30 years. The clinical symptoms were classified as spastic ataxia, although two brothers were first diagnosed with Charcot–Marie–Tooth (CMT) disease due to distal weakness and electrophysiology findings. Two other siblings and the parents were healthy (Fig. 1A). The patients were regularly examined by neurologists and investigated by nerve conduction velocities (NCV), electromyogram (EMG), brain imaging and the proband also by sural nerve biopsy and two muscle biopsies. Two patients underwent ophthalmic examinations. None of the patients had intellectual problems, visual symptoms, or epilepsy.

Genetic evaluation

DNA was extracted from leukocytes by standard methods. The DNA sample of the proband was used for enrichment of a sequencing library by NimbleGen SeqCap EZ Human Exome v2.0. Paired-end sequencing (100 bp) was performed using Illumina HiSeq 2000 sequencer with a sequencing depth of 30X. Sequence reads were aligned to the human reference genome (UCSC hg19) using the Burrows–Wheeler aligner [15]. Variant calling was made with Genome Analysis Toolkit. The data were visualized with Integrative Genomics Viewer [16]. From the

sequencing data to further analysis were selected all the exons and exon–intron borders of the following genes known to be associated with recessive ataxia: *FXN*, *TTPA*, *C10orf72*, *APTX*, *SETX*, *SYNE1*, *ADCK3*, *TDPI*, *SIL1*, *POLG*, *ATM*, *MRE11A*, *SACS*, *PHYH*, and *PEX7*. DNA samples of the other siblings (three affected, two unaffected) were used to study the segregation of the variants detected in the proband by Sanger sequencing.

The study was approved by the IRB of Tampere University Hospital. All participants provided appropriate consent.

Results

Family description

The proband (II:2), a 63-year-old female, presented with progressive ataxic gait starting at age 25 (Table 1). She had, however, experienced mild problems with balance since her teens. The first examination at age 30 found mild dysarthria, mild horizontal nystagmus, and slight intention tremor in the limbs. Achilles and brachioradialis reflexes were absent, but Babinski sign was present. Distal muscles in the lower limbs were atrophic, and gait was spastic ataxic. Spasticity, ataxia, and problems with balance steadily progressed and led to wheelchair confinement at age 43.

Electrophysiological studies showed decreased sensory and motor NCVs. At age 30, muscle and sural nerve biopsies were performed with normal histology. Five years later, a new muscle biopsy showed mild neurogenic

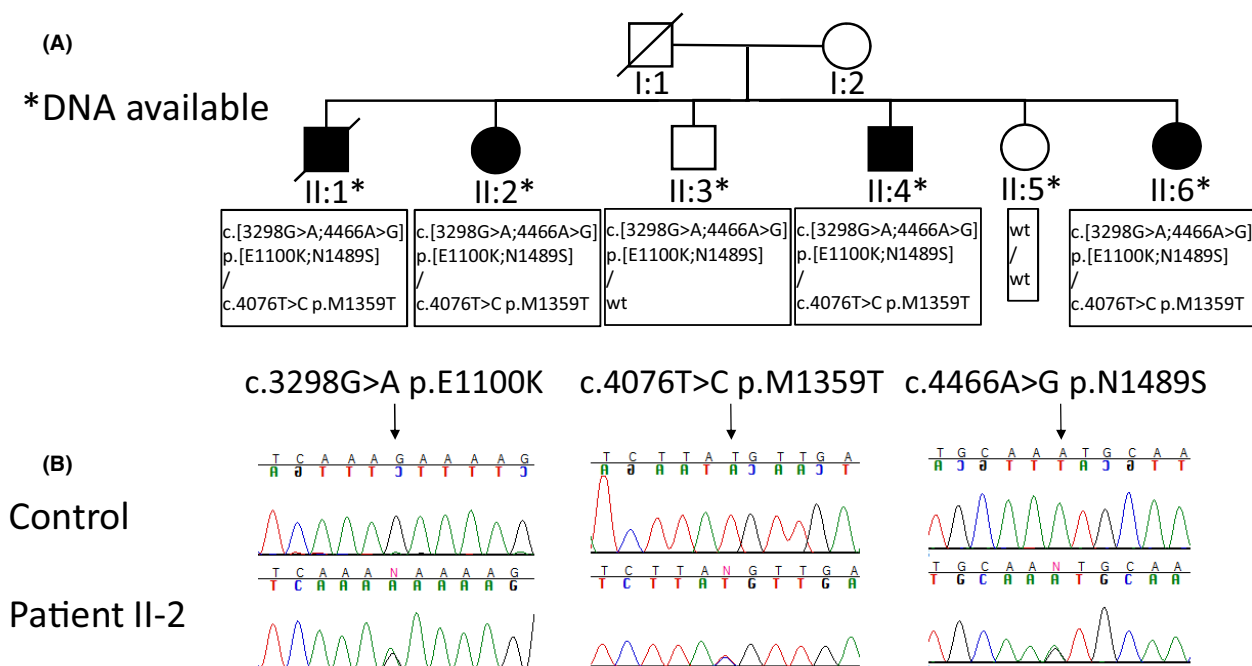


Figure 1. Pedigree of the family (A) and electropherograms from Sanger sequencing (B).

Table 1. Clinical characteristics of the family.

Pat	Age/sex	Age of onset	Clinical symptoms at presentation	Cerebellar ataxia	Spasticity	Polyneuropathy	Muscle weakness/atrophy	Ambulation	Brain CT/MRI
II:1	65*/M	10	Lower limb weakness and spasticity	Yes	Yes, marked	Sensorimotor demyelinating	Marked spastic paraparesis and atrophy in all limbs	WCB since age 39	CT: Cortical atrophy in cerebrum, cerebellum, brain stem
II:2	63/F	(15) 25	Poor balance since teens; ataxic gait since age 25	Yes. Gait, UL and LL I.a.	Yes, LL	Sensorimotor demyelinating	Distal lower legs, hands	WCB since age 45	CT: normal at age 35
II:4	59/M	6	Distal lower limb weakness	Yes. Gait, LL, speech	Yes, LL severe	Sensorimotor demyelinating	LL weakness	WCB since age 33	MRI: Cortical atrophy
II:6	54/F	18	Distal lower limb weakness, ataxic gait	Yes. LL: marked UL: moderate	Yes	Sensorimotor demyelinating	Marked weakness in LL	Walker, WCh when needed since age 52	MRI: Cortical atrophy in cerebrum, cerebellum

*Age at death; UL, upper limbs; LL, lower limbs; WCB, wheelchair bound; WCh, wheelchair.

findings: scattered angular and atrophic fibers, and fiber-type grouping. Genetic analyses at that time excluded CMT1A and spinocerebellar ataxia SCA1.

Her brother (II:1) had been more thoroughly investigated at age 26 due to muscle weakness and problems with balance starting at school age. On electrophysiology, NCVs were decreased leading to a diagnosis of CMT. However, spasticity soon became evident, as did progressive ataxia of all limbs. He became wheelchair-bound at age 39 and, at that time, marked spastic paraparesis and atrophy in all limbs were noted. He died 25 years later of pneumonia. Patient II:4 experienced lower limb weakness and balance problems since age 6. He was later diagnosed with spinocerebellar ataxia and polyneuropathy. At age 36, he had normal strength in the upper limbs but generalized weakness in the lower limb muscles. Gait was ataxic with unsteady balance; he became wheelchair-dependent at age 33. At the most recent examination (age 59), the strength and coordination in the upper limbs were still within normal range, although lower limbs, eye movements, and speech were severely ataxic. Spasticity was marked in the lower limbs. The youngest of the siblings (II:6) presented with distal lower limb weakness and ataxic gait at the age of 18. The progression of the disease has been slow; at age 54, there were marked weakness in the lower limbs and moderate ataxia in the upper limbs. Severe spasticity in the lower limbs led to intrathecal baclofen treatment, but she is still ambulant with a walker and uses a wheelchair occasionally.

In addition, all affected siblings had dysarthric speech, saccadic eye movement and nystagmus, pes cavus, and hammertoes. Distal areflexia was evident in two patients,

all reflexes were absent in one patient, and Babinski sign present in two and a loss of vibration sense in three. One patient had symptoms indicative of neurogenic bladder. Ophthalmic examination revealed thickening of the retinal nerve fiber layer in one patient, and the other had normal findings (Fig. 2A).

The proband had normal findings on brain imaging at age 35. She did not undergo further imaging studies. In patient II:6, MRI was performed at age 43 (Fig. 2B), showing cerebellar atrophy that had slightly progressed in five years. Cortical atrophy was present in frontal and parietal lobes as well as typical linear hypo-intensities in the pons. More advanced changes were evident in II:4 at age 59 (Fig. 2C) and in II:1 at age 41.

Molecular genetics

Exome sequencing of patient II:2 revealed three rare heterozygous mutations in SACS. All three mutations were confirmed by Sanger sequencing (Fig. 1B). Of the mutations, c4466A>G p.N1489S has a low frequency (0.0046) in normal population, whereas the other two are novel. Sanger sequencing analysis of the other siblings indicated that mutation combination c.[3298G>A;4466A>G]; [4076T>C] p.[E1100K;N1489S]; [M1359T] segregated with the disease (Fig. 1A).

Discussion

ARSACS is increasingly recognized worldwide and considered to be one of the most frequent types of spastic ataxia after Friedreich ataxia and ataxia telangiectasia [5, 9].

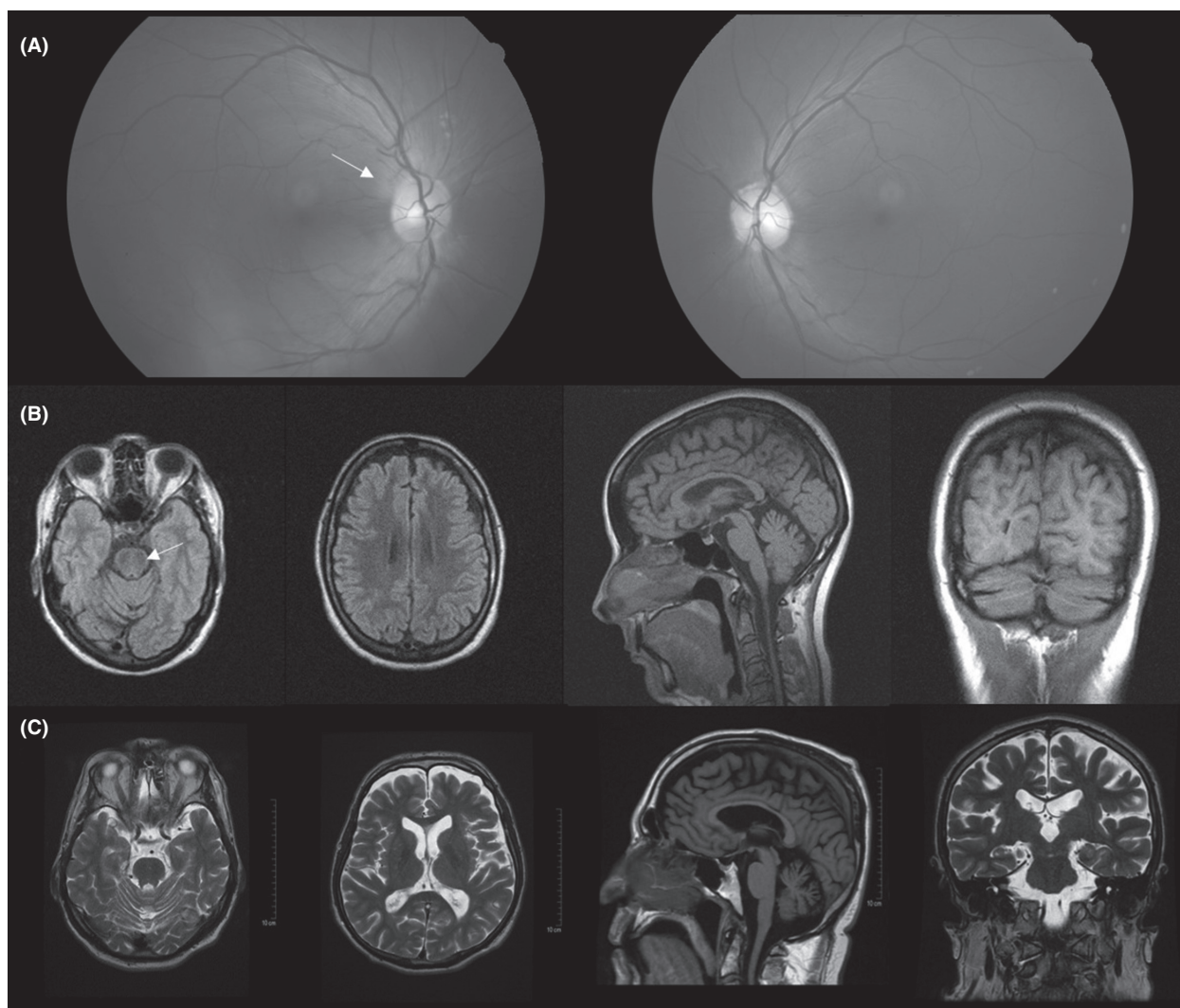


Figure 2. Imaging findings of the patients. Funduscopy findings of patient II:4 (A). There is thickening of the myelinated layers of retina (arrow). Patient II:2 underwent brain imaging at age 43 (B). There is cerebellar atrophy, and it has slightly progressed since the last imaging five years previously (not shown). Cortical atrophy is seen in frontal and parietal lobes and linear hypo-intensity in the pons (arrow). Brain MRI of patient II:4 (C) shows more advanced cortical atrophy of cerebrum and cerebellum.

Nevertheless, our family is the first ARSACS family identified in Finland.

The patients harboring two founder mutations identified in Quebec have manifested with the uniform presentation of unsteadiness and ataxia in early childhood, followed by spasticity during childhood and neuropathy during the teens [1–3]. In other populations with different pathogenic mutations, the phenotype is still quite constant, although occasional later onset and atypical forms have been identified [4–9, 14]. The age of onset and the presenting symptoms as well as the rate of progression varied in our family. Problems with gait started in adolescence in two siblings, whereas another two had lower limb weakness since early school years, leading to

the initial diagnosis of CMT. The final diagnosis based on clinical symptoms and findings was therefore challenging and, in fact, early on one clinical geneticist dismissed ARSACS as a possible cause of the family's disease in his consultation.

Retinal nerve fiber layer hypermyelination and cerebellar vermis atrophy as well as hypo-intensities in the pons are considered the early hallmarks of the disease, and together with typical clinical features, they could help in reaching accurate diagnosis early [12, 13, 17]. In one sibling, there was a finding of hypermyelination in retina, and in another, hypo-intensities were present in the pons in addition to cerebellar atrophy, thus completing the clinical phenotype.

We identified three mutations in SACS segregating in the family. None of these were previously reported in ARSACS, but based on segregation analysis, the novel p.M1359T mutation is a disease-causing allele. Two other mutations p.E1100K and p.N1489S are on the same allele, and it cannot be concluded which one is disease causing, but the allele as such is recessively pathogenic. According to the frequency in normal population and mutation prediction program, MutationTaster [18], p.E1100K is more likely pathogenic, but without further functional studies, this cannot be determined. Of the amino acids mutated in our patients (p.E1100, p.M1359, and p.N1489), p.N1489 is located in the second SRR domain of saccin protein, whereas the other two are located outside the described domains. At least two missense mutations, p.D168Y and p.R2703C, known to be located in SRR domain have been identified to cause spastic ataxia of Charlevoix-Saguenay disease phenotype [4, 19]. Of these, p.D168Y has been reported to abrogate the ability of the saccin protein to hydrolyze ATP [20]. However, because it is uncertain whether p.N1489S is pathogenic or not, its impact on protein function is difficult to predict.

The classic triad, cerebellar ataxia, peripheral neuropathy, and pyramidal tract signs, was evident in our family and compatible with ARSACS, although disease onset was later and progression was slower than in most reported cases. Many of the previously reported ARSACS mutations have been nonsense or frameshifts which may be a hint of genotype–phenotype aspects regarding severity, although no clear genotype–phenotype correlations have been found [6, 9]. Anyway, it is important to recognize the typical symptoms and to complement the triad with the more specific findings in the pons and retina to reach early and accurate diagnosis.

Acknowledgments

There are no acknowledgements to be reported.

Conflict of Interest

None declared.

References

- Bouchard, J.-P., A. Barbeau, R. Bouchard, and R. W. Bouchard. 1978. Autosomal recessive spastic ataxia of Charlevoix-Saguenay. *Can. J. Neurol. Sci.* 5:61–69.
- De Braekeleer, M., F. Giasson, J. Mathieu, M. Roy, J.-P. Bouchard, and K. Morgan. 1993. Genetic epidemiology of autosomal recessive spastic ataxia of Charlevoix-Saguenay in northeastern Quebec. *Genet. Epidemiol.* 10:17–25.
- Engert, J. C., P. Bérubé, J. Mercier, C. Doré, P. Lepage, B. Ge, et al. 2000. ARSACS, a spastic ataxia common in northeastern Québec, is caused by mutations in a new gene encoding an 11.5-kb ORF. *Nat. Genet.* 24:120–125.
- Vermeer, S., R. P. Meijer, B. J. Pijl, J. Timmermans, J. R. M. Cruysberg, M. M. Bos, et al. 2008. ARSACS in the Dutch population: a frequent cause of early-onset cerebellar ataxia. *Neurogenetics* 9:207–214.
- Synofzik, M., A. Soehn, J. Gburek-Augustat, J. Schicks, K. N. Karle, R. Schüle, et al. 2013. Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS): expanding the genetic, clinical and imaging spectrum. *Orphanet. J. Rare Diseases* 8:41.
- Bouhlal, Y., R. Amouri, G. El Euch-Fayeche, and F. Hentati. 2011. Autosomal recessive spastic ataxia of Charlevoix-Saguenay: an overview. *Parkinsonism Relat. Disord.* 17:418–422.
- Larivière, R., R. Gaudet, B. J. Gentil, M. Girard, T. C. Conte, S. Minotti, et al. 2015. Sacs knockout mice present pathophysiological defects underlying autosomal recessive spastic ataxia of Charlevoix-Saguenay. *Hum. Mol. Genet.* 24:727–739.
- Girard, M., R. Larivière, D. A. Parfitt, E. C. Deane, R. Gaudet, N. Nossova, et al. 2012. Mitochondrial dysfunction and Purkinje cell loss in autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS). *Proc. Natl Acad. Sci. USA* 109:1661–1666.
- Pilliod, J., S. Moutton, J. Lavie, E. Maurat, C. Hubert, N. Bellance, et al. 2015. New practical definitions for the diagnosis of autosomal recessive spastic ataxia of Charlevoix-Saguenay. *Ann. Neurol.* 78:871–886.
- Crisuolo, C., C. Procaccini, M. C. Meschini, A. Cianflone, R. Carbone, S. Doccini, et al. 2015. Powerhouse failure and oxidative damage in autosomal recessive spastic ataxia of Charlevoix-Saguenay. *J. Neurol.* 262:2755–2763.
- Duquette, A., B. Brais, J.-P. Bouchard, and J. Mathieu. 2013. Clinical presentation and early evolution of spastic ataxia of Charlevoix-Saguenay. *Mov. Disord.* 28:2011–2014.
- Martin, M. H., J. P. Bouchard, M. Sylvain, O. St-Onge, and S. Truchon. 2007. Autosomal recessive spastic ataxia of Charlevoix-Saguenay: a report of MR imaging in 5 patients. *AJNR Am. J. Neuroradiol.* 8:1606–1608.
- Leavitt, J. A., W. Singer, W. L. Brown, J. S. Pulido, and M. C. Brodsky. 2014. Retinal and pontine striations: neurodiagnostic signs of autosomal recessive spastic ataxia of Charlevoix-Saguenay. *J. Neuroophthalmol.* 34:369–371.
- Pyle, A., H. Griffin, J. Duff, S. Bennett, S. Zwolinski, T. Smertenko, et al. 2013. Late-onset saccinopathy diagnosed by exome sequencing and comparative genomic hybridization. *J. Neurogenet.* 27:176–182.
- Li, H., and R. Durbin. 2009. Fast and accurate short read alignment with Burrows-Wheeler Transform. *Bioinformatics* 25:1754–1760.

16. Robinson, J. T., H. Thorvaldsdóttir, W. Winckler, M. Guttman, E. S. Lander, G. Getz, et al. 2011. Integrative genomics viewer. *Nat. Biotechnol.* 29:24–26.
17. Yu-Wai-Man, P., A. Pyle, H. Griffin, M. Santibanez-Korev, R. Horvath, and P. F. Chinnery. 2014. Abnormal retinal thickening is a common feature among patients with ARSACS-related phenotypes. *Br. J. Ophthalmol.* 98:711–713.
18. Schwarz, J. M., D. N. Cooper, M. Schuelke, and D. Seelow. 2014. MutationTaster2: mutation prediction for the deep-sequencing age. *Nat. Methods* 11:361–362.
19. Criscuolo, C., F. Saccà, G. De Michele, P. Mancini, O. Combarros, J. Infante, et al. 2005. Novel mutation of SACS gene in a Spanish family with autosomal recessive spastic ataxia. *Mov. Disord.* 20:1358–1361.
20. Anderson, J. F., E. Siller, and J. M. Barral. 2010. The sascin repeating region (SRR): a novel Hsp90-related supra-domain associated with neurodegeneration. *J. Mol. Biol.* 400:665–674.