BMC Medical Genomics

RESEARCH

Open Access

Genetic analysis of three patients from two unrelated Chinese families with autosomal recessive spastic ataxia of Charlevoix-Saguenay

Hui Liu[1†], Ranran Li[2†], Chen Chen[1], Lin Shang[3], Ying Bai[1], Duo Chen[1], Xiangdong Kong[1] and Qianqian Li[1*]

Abstract

Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is a rare early-onset neurodegenerative disorder characterized by progressive cerebellar ataxia, spasticity, and sensorimotor peripheral neuropathy. This disorder is caused by homozygous or compound heterozygous variants in the sacsin (*SACS*) gene on chromosome 13q12.12. Three patients with ARSACS from two unrelated Chinese families were recruited for this study. Patient #1 was an 18-year-old male who had been walking unstably for 12 years. Patient #2, the younger sister of Patient #1, was a 5-year-old girl who had been walking unstably for 2 years. Patient #3 was a 19-year-old female who had been walking unstably and a tendency to fall for 17 years. For Patient #1, whole-exome sequencing (WES) identified a hemizygous variant c.8310_8313delAGAT (p.Asp2771fs4*) in *SACS* (NM_014363.6), with the father being heterozygous, the mother wild-type, and Patient #2 hemizygous, as verified by Sanger sequencing. Additional copy number variant analysis of the WES data indicated that Patient #1 had a heterozygous gross deletion of chr13q12.12 (chr13:23,808,732 – 24,890,322). Low-coverage whole-genome sequencing results revealed that Patient #2 carried a chr13q12.12 deletion (chr13:23,520,000–24,940,000). Together with Sanger sequencing results, this gross deletion was speculated to have been inherited from the mother, further explaining the hemizygous state of c.8310_8313delAGAT (p.Asp2771fs4*) in Patients #1 and #2. Through WES, Patient #3 was identified as having suspected compound heterozygous variants of c.2881 C>T (p.Arg961*) and c.6409 C>T (p.Gln2137*), inherited from the father and mother, respectively, as confirmed by Sanger sequencing. This study identified three variants in *SACS*. The c.8310_8313delAGAT (p.Asp2771fs4*) is novel, whereas c.2881 C>T (p.Arg961*) and c.6409 C>T (p.Gln2137*) have been reported previously. Moreover, this study highlights the growing trend that ARSACS has become increasingly prevalent worldwide rather than being localized to a specific region or race. As an increasing number of patients with ARSACS are diagnosed, the genetic spectrum of ARSACS will gradually broaden, providing an accurate genetic basis for prenatal diagnosis of mothers in the years ahead, if possible.

[†]Hui Liu and Ranran Li contributed equally to this work.

*Correspondence:
Qianqian Li
qianqianlizzu@163.com

Full list of author information is available at the end of the article

Keywords Autosomal recessive spastic ataxia of Charlevoix-Saguenay, Whole-exome sequencing, *SACS*, Gene mutation, Heterozygous gross deletion, Low-coverage whole-genome sequencing

Introduction

Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS, online Mendelian inheritance in man [OMIM]: #270550), a rare early-onset progressive neurodegenerative disease, was originally identified in the 1970s in a French Canadian population from Charlevoix–Saguenay in Quebec [1]. Since then, ARSACS cases outside Quebec populations, such as Italy [2], Japan [3], Spain [4], Turkey [5], and China [6], have also been reported. The clinical phenotypes of ARSACS are highly diverse owing to the increasing number of patients worldwide. ARSACS is characterized by three main clinical phenotypes, including early-onset cerebellar ataxia, spasticity, and sensorimotor peripheral neuropathy, accompanied by other atypical clinical phenotypes, for instance, weakness of limbs, skeletal abnormalities, flexion deformity of fingers, dental abnormalities, hearing loss, and mental retardation [7].

ARSACS is caused by homozygous or compound heterozygous variants in the sacsin (*SACS*, OMIM: #604490) gene mapping on chromosome 13q12.12. *SACS* consists of 10 exons. The last exon, spanning exactly 12.8 kb, is the largest exon found in vertebrates [8]. To date, more than 600 *SACS* variants have been registered as likely pathogenic or pathogenic in the public databases of ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) and OMIM (https://www.omim.org/), with most of the variants located in exon 10 [9]. The vast majority of *SACS* variants are single nucleotide variants (SNVs) or small insertions and deletions (indels), although copy number variants (CNVs) have also been reported.

Sacsin, a large multidomain protein (520 kDa) encoded by *SACS*, consists of an internal repeat region (termed *SACS* repeated region), three large internal repeats (SIRPTs 1–3), and three smaller domains, including the ubiquitin-like, J-domain, and higher eukaryotic–prokaryotic nucleotide-binding domain [10]. Sacsin is highly expressed in skin cells, skeletal muscles, and large neurons of the brain, particularly in the motor systems and cerebellar Purkinje cells [11]. However, its function has not been fully elucidated. Parfitt et al. indicated that sacsin protects against mutant ataxin-1 and recruits Hsp70 chaperone action [12].

Here, we present three patients with ARSACS from two unrelated Chinese families. Two patients from one family had a hemizygous frameshift variant in *SACS* (NM_014363.6), c.8310_8313delAGAT (p.Asp2771fs4*), probably owing to the heterozygous gross deletion of chr13q12.12. The third patient had compound heterozygous variants of c.2881 C>T (p.Arg961*) and c.6409 C>T (p.Gln2137*). To the best of our knowledge, c.8310_8313delAGAT (p.Asp2771fs4*) is novel, whereas c.2881 C>T (p.Arg961*) and c.6409 C>T (p.Gln2137*) have been reported previously.

Materials and methods

Ethics statement

Three patients with ARSACS from two unrelated Chinese families were recruited at our center. Written informed consent forms were obtained from the legal guardians or next of kin of the minors for the publication of any potentially identifiable images or data included in this study. The study was approved by the ethical committee of the First Affiliated Hospital of Zhengzhou University (Ethics No. 2022-KY-0892-001).

DNA extraction

The genomic DNA from peripheral blood was obtained using the Lab-Aid 824s Nucleic Acid (DNA) Isolation Kit (Lot#: 606001, Zeesan, Xiamen, China) in accordance with the manufacturer's instructions.

Quantitative fluorescent polymerase chain reaction (QF-PCR)

The genetic relationship was confirmed using the Goldeneye™ DNA ID System 20 A Kit (Peoplespot, Beijing, China) through QF-PCR.

Whole-exome sequencing (WES)

Whole-exome sequencing (WES) was performed using Illumina library construction and capture kits (Illumina, San Diego, CA, United States) in accordance with standard instructions and 150 bp pair-end sequencing with 300 cycles was conducted on NovaSeq 6000 (Illumina). The methods of mapping, variant calling, and variant annotation have been described in detail in our previous study [13]. After filtering, the candidate variants in *SACS* (NM_014363.6) were verified by Sanger sequencing using the primer pairs in Table 1.

Low-coverage whole-genome sequencing (WGS)

The experimental methods and data analysis of low-coverage WGS have been previously described [14, 15]. CNVs (GRCh37.p13) were analyzed and queried against the public databases, including database of genomic variants (DGV), gnomAD, DECIPHER, OMIM, UCSC, and ClinGen. Pathogenicity was assessed according to the latest guidelines outlined by the American college of medical genetics and genomics (ACMG).

Table 1 Primer sequences used for Sanger sequencing

Primer name	Primer sequence (Forward)	Primer sequence (Reverse)
SACS-c.2881 C > T-Sequencing	5′-TGCCCTGAGGAAGTTCTTGG-3′	5′-GGACAAGCACCGACCTGTAA-3′
SACS-c.6409 C > T-Sequencing	5′-TGGTTCAGCAGCCTTCAAGA-3′	5′-TGGTTCAGCAGCCTTCAAGA-3′
SACS-8310_8313delAGAT-Sequencing	5′-AATGGTCCAGAATCTTTTG-3′	5′-GAGCTGATATGACACTTTTAGA-3′

Results

Case presentation

Patient #1, the proband of Family 1, was an 18-year-old male from Zhumadian, Henan, who had been experiencing walking unstably and articulation disorders for nearly 12 years (Supplementary video S1). Moreover, the calves were pseudohypertrophic (Fig. 1C). Patient #2, a 5-year-old female and the younger sister of Patient #1, had been experiencing unstably walking for nearly 2 years (Supplementary video S2). Their learning ability, height, weight, memory, and comprehension were not abnormal compared with their peers. Both parents were normal, with no abnormal clinical manifestations and no other suspected family members with similar symptoms.

Living in a remote and under-served area, Patients #1 and #2 remained undiagnosed until March 19, 2022, when they visited the Zhengzhou Orthopedic Hospital. As outpatients, they did not undergo detailed tests such as the Romberg test, finger-to-nose test, heel-knee-shin test, and imaging examinations. Based on the clinical symptoms and family history, Professor Shang preliminarily suspected that the two patients might have hereditary ataxia. However, the genetic pathogenetic cause could not be verified because the department was not equipped to perform genetic testing. As a result, on the same day, Professor Shang recommended that the family visit our center to seek additional information about the genetic etiology. The family tree is presented in Fig. 1A.

Patient #3, the proband of Family 2, was a 19-year-old female from Nanyang, Henan. She first presented with symptoms of walking unstably, falls, and fatigue in her lower limbs 17 years before presentation. She was admitted to the neurology department of our hospital on October 3, 2022, with the chief complaint of general weakness and walking unstably for over 15 years, with symptoms worsening over the past 2 months. Born premature, she began walking at 20 months of age and has been prone to falls since then. Although the height and weight development were normal, her academic performance was poor. Both her grandparents had a history of hypertension; however, her younger brother was in good health. She is currently alert and oriented, with an average mental status. She exhibited unclear speech and articulation disorders but had normal memory and comprehension. Muscle atrophy or pseudomuscular hypertrophy was absent, and the limb muscle tone was normal. She performed poorly on various physical tests, including bilateral finger-nose test, rapid rotation test, and heel-knee-tibial tests. She also exhibited a positive Romberg's sign. The knee tendon reflex (++++) and Achilles tendon reflex (++++) were hyperactive. Brain magnetic resonance angiography (MRA) conducted on October 4, 2022, revealed the typical linear "tigroid" T2 hypointense stripes in the pons (Fig. 1J). Thoracic spine magnetic resonance imaging (MRI) on October 8, 2022, revealed a thin thoracic spinal cord (Fig. 1K). The family tree is presented in Fig. 1B.

Genetic findings

The genetic relationships between the two unrelated families in this study were confirmed by QF-PCR (Supplementary Tables S1–S3 and Supplementary Figs. S1–S3). Quality control of the WES data for Patients #1 and #3 is summarized in Supplementary Table S4. Candidate gene variants related to the clinical symptoms of Patients #1 and #3 were screened to identify potential pathogenic variants. After filtering, Patient #1 was identified with a hemizygous variant c.8310_8313delAGAT (p.Asp2771fs4*) in *SACS* (NM_014363.6) (reference allele/alternative allele, ref/alt: 0/31) (Supplementary Excel S1_rawSnp, line 11279), and Patient #3 was suspected with compound heterozygous variants of c.2881 C > T (p.Arg961*) (ref/alt: 57/47) (Supplementary Excel S2_rawSnp, line 14373) and c.6409 C > T (p.Gln2137*) (ref/alt: 35/33) (Supplementary Excel S2_rawSnp, line 14372) in *SACS* (NM_014363.6).

For Patient #1, Sanger sequencing results confirmed that the father was heterozygous, the mother was wild-type, and Patient #2 was hemizygous (Fig. 1D). For Patient #3, Sanger sequencing results confirmed that c.2881 C > T (p.Arg961*) was inherited from the father and c.6409 C > T (p.Gln2137*) from the mother (Fig. 1H and I). The pathogenicity of these three variants was assessed in accordance with the guidelines of the American College of Medical Genetics and Genomics (ACMG) and also assessed as pathogenic (Table 2).

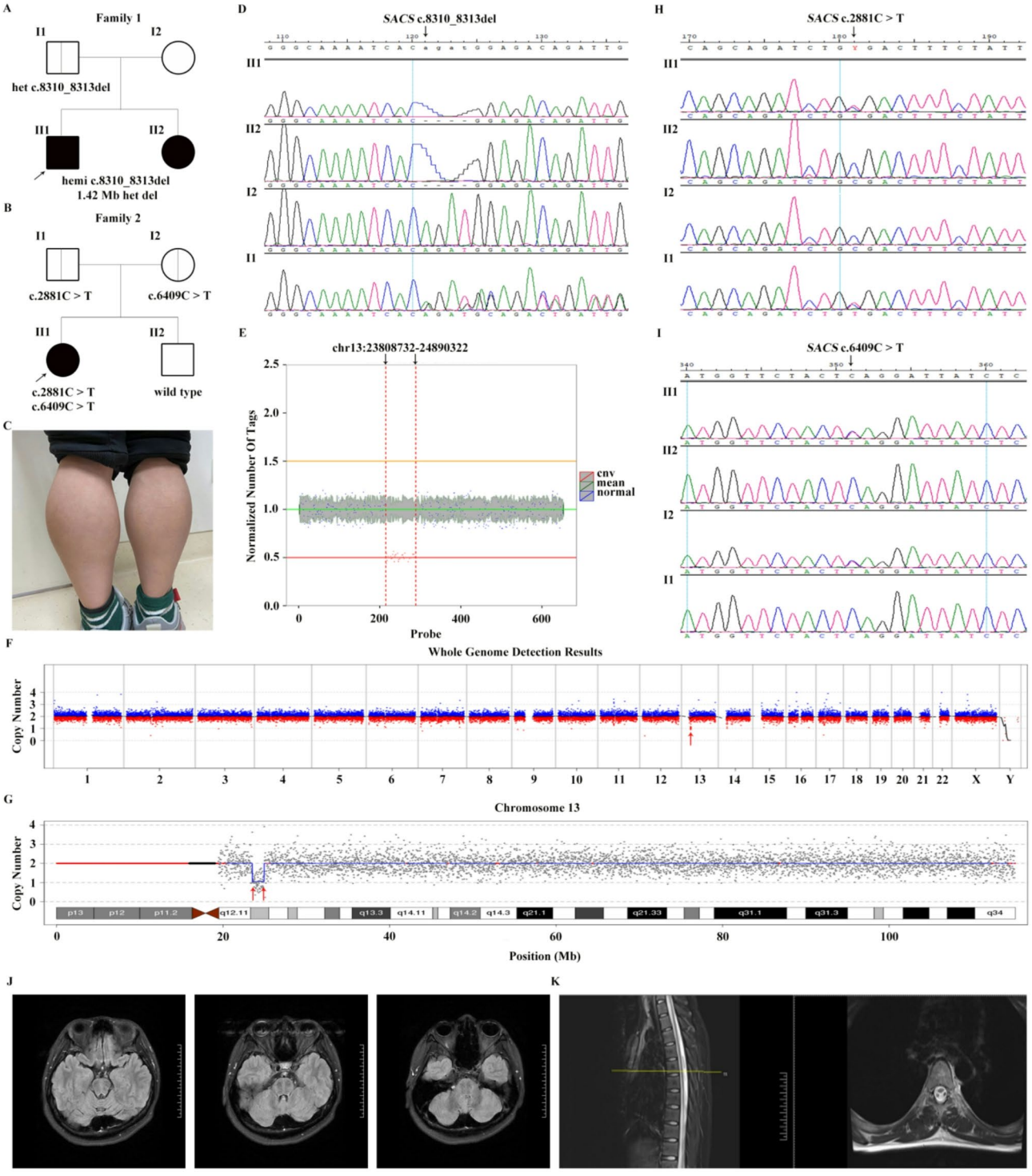

Fig. 1 (See legend on next page.)

(See figure on previous page.)

Fig. 1 Genetic analysis of three patients from two unrelated Chinese families with autosomal recessive spastic ataxia of Charlevoix-Saguenay. (**A**) The family tree of Family 1. (**B**) The family tree of Family 2. (**C**) For Patient #1, the calves were pseudohypertrophic. (**D**) Sanger sequencing results confirmed that Patients #1 (II1) and #2 (II2) were hemizygous for c.8310_8313delAGAT (p.Asp2771fs4*) in *SACS* (black arrow), the mother (I2) wild type, and the father (I1) heterozygous. (**E**) Copy number variant analysis of the whole-exome sequencing (WES) data revealed a large chromosome 13 deletion in Patient #1. *X*-axis: the position on the chromosome corresponding to the region of variation currently displayed. *Y*-axis: ratio of target sample reads per kilobase per million mapped reads (RPKM) value to the mean value of background library RPKM. (**F**) Overview of the low-coverage whole-genome sequencing (WGS) of Patient #1. The red arrow indicates the region with abnormal copy numbers in Patient #1. (**G**) Low-coverage WGS result of chromosome 13 in Patient #2. The red arrow shows the chr13q12.12 deletion. (**H**) Sanger sequencing results confirmed that Patient #3 (II1) was heterozygous for 2881 C > T (p.Arg961*) in *SACS* (black arrow), the little brother (II2) wild type, the mother (I2) wild type, and the father (I1) heterozygous. (**I**) Sanger sequencing results confirmed that Patient #3 (II1) was heterozygous for 6409 C > T (p.Gln2137*) in *SACS* (black arrow), the little brother (II2) wild type, the mother (I2) heterozygous, and the father (I1) wild type. (**J**) For Patient #3, brain magnetic resonance angiography conducted on October 4, 2022, revealed the typical linear "tigroid" T2 hypointense stripes in the pons. (**K**) For Patient #3, thoracic spine magnetic resonance imaging on October 8, 2022, revealed a thin thoracic spinal cord.

CNV analysis of the WES data

For Patient #1, Sanger sequencing results were not consistent with the expected recessive inheritance of ARSACS, which may be attributed to either a uniparental disomy or a partial heterozygous deletion of chromosome 13.

Additional CNV analysis of the WES data was conducted to investigate the cause of this non-Mendelian inheritance. The result revealed that Patient #1 carried approximately 1.082 Mb heterozygous deletion of chr13q12.12 (chr13:23808732–24890322, probes number: 73) (Fig. 1E), including seven OMIM genes: *SACS*, *C1QTNF9B*, *C1QTNF9*, *MIPEP*, *SGCG*, *SPATA13*, and *TNFRSF19*. This deletion might explain the hemizygous state of c.8310_8313delAGAT (p.Asp2771fs4*).

chr13q12.12 heterozygous deletion verified by low-coverage WGS

Since the economic conditions of this family were poor, low-coverage WGS was performed in Patient #2 to save costs and to further identify the source of the chr13q12.12 deletion revealed by CNV analysis of the WES data. The low-coverage WGS result confirmed that Patient #2 had 1.42 Mb heterozygous deletion of chr13q12.12 (chr13:23520000–24940000) (×1.051) (Fig. 1F and G). This region contains seven OMIM genes, including *SACS*, suggesting that the gross deletion in Patient #1 may have been inherited from the mother.

Discussion and conclusion

ARSACS is a rare multisystemic neurodegenerative disease characterized by progressive cerebellar ataxia, spasticity, and peripheral neuropathy. The disease typically presents from early childhood to early adulthood and is considered one of the most common types of spastic ataxia globally after Friedreich ataxia and ataxia telangiectasia [16, 17]. The first ARSACS case was identified in Quebec, Canada and was initially believed to be geographically limited [1]. With advancements in medical research and diagnostic tools, individuals with various genetic variants and clinical presentations have been being identified worldwide. In China, the number of patients diagnosed with ARSACS has been increasing [6].

The combination of early-onset cerebellar ataxia, lower-extremity spasticity, and peripheral neuropathy is common in most patients with ARSACS [16]. However, the clinical phenotypes of ARSACS vary among patients, and not all patients exhibit this triad. Some patients experience intellectual disabilities, seizures, voiding dysfunction, or hearing loss [6, 16, 18]. The presence of T2-hypointensities in the pons on radiographic imaging [19] and the evolving clinical phenotypic spectrum may aid the diagnosis of patients with unexplained ARSACS for timely clinical intervention. In this study, the brainstem slices of the MRI indicated that Patient #3 has the typical linear "tigroid" T2 hypointense stripes in the pons that is a classic ARSACS finding.

Loss-of-function variants in *SACS* result in ARSACS. The first exon of *SACS* does not encode a protein, and the remaining exons are the coding regions. Most *SACS* variants are located in the gigantic exon 10, and only a few variants are located upstream of this exon. All three variants reported in this study are located in exon10. The c.8310_8313delAGAT (p.Asp2771fs4*) is novel, whereas c.2881 C > T (p.Arg961*) and c.6409 C > T have been reported in a 39-year-old Italian patient [20] and Patient #2 with ARSACS [21], respectively. Additionally, c.2881 C > T (p.Arg961*) has been documented as a disease mutation (DM) in the human gene mutation database (HGMD) (ACC number: CM131362) and is pathogenic in ClinVar database (Variation ID: 640122, Accession: VCV000640122.8). The c.6409 C > T (p.Gln2137*) is documented as DM in HGMD (ACC number: CM105605) and is likely pathogenic in ClinVar database (Variation ID: 551526, Accession: VCV000551526.1).

Table 2 The pathogenicity of the three variants in this study according to the latest ACMG guidelines

Patient ID	Variant information	Evidence of pathogenicity	ACMG classification
Patient #1	c.8310_8313delAGAT (p.Asp2771fs4*)	PVS1 + PM2_Supporting + PP4	Pathogenic
Patient #2			
Patient #3	c.2881 C > T (p.Arg961*)	PVS1 + PM2_Supporting + PS4 + PM3 + PP4	Pathogenic
	c.6409 C > T (p.Gln2137*)	PVS1 + PM2_Supporting + PS4 + PM3 + PP4	Pathogenic

Chr13q12.12 (chr13:23520000–24940000), identified in Patient #2, contains seven OMIM genes, including *SACS*, but there is insufficient evidence for a haploid gene dosage effect. The DECIPHER database included two similar cases with overlapping deletions. Case ID (337666, 46XX, heterozygous) (chr13:22992950–24336636, 1.34 Mb) presented with iris, optic disc, and retinal colobomas. Case ID (264434, 46XY, *de novo*, heterozygous) (chr13:22992950–24336636, 1.34 Mb) showed a prominent ear helix, 2–3 toes cutaneous syndactyly, macrocephaly, and autistic behavior. The proportion of similar cases included in the DGV gold standard database was approximately 0.08% (2 out of 2,373, gssvl28916). The pathogenicity of the chr13q12.12 deletion was initially assessed as uncertain significance. However, since this region contains *SACS*, and given Sanger sequencing results, the pathogenicity was finally re-evaluated as pathogenic, which may further explain the hemizygous state of c.8310_8313delAGAT (p.Asp2771fs4*) in Patients #1 and #2.

The most previously reported variants in patients with ARSACS are either SNVs or small indels in *SACS*. To date, only a few ARSACS cases caused by the deletion of chr13q12.12 have been reported (Table 3). In 2008 and 2009, two large deletions (1.54 Mb/1.5 Mb) including six genes (*SGCG*, *SACS*, *TNFRSF19*, *MIPEP*, *SPATA13*, and *C1QTNF9*) were identified in a Belgian and two Italian patients with ARSACS with hearing loss, respectively [22, 23]. Excepted for these three patients, the others, including two patients (Patients #1 and #2), did not have hearing loss. Notably, the calves of Patient #1 showed pseudohypertrophy. To the best of our knowledge, this clinical phenotype has not been reported previously. Therefore, the calf state of Patient #1 is probably a rare clinical phenotype, which may further expand the clinical phenotype spectrum of ARSACS. In addition, although Patient #2 does not currently have an articulation disorder, regular clinical observations should be conducted to monitor when this clinical symptom may appear in the future.

One major limitation of this study was the lack of clarity regarding the specific pathogenic mechanisms of the three variants that cause ARSACS, which will be the focus of future research.

In conclusion, we described three patients with ARSACS from two unrelated Chinese families, caused by hemizygous variant c.8310_8313delAGAT (p.Asp2771fs4*) in *SACS* probably owing to an approximately 1.42 Mb heterozygous gross deletion of chr13q12.12 and compound heterozygous variants of c.2881 C > T (p.Arg961*) and c.6409 C > T (p.Gln2137*). The c.8310_8313delAGAT (p.Asp2771fs4*) is novel, whereas c.2881 C > T (p.Arg961*) and c.6409 C > T (p.Gln2137*) have been reported previously. These three variants will expand the genetic spectrum of *SACS*, aid in the early screening and diagnosis of ARSACS, provide a theoretical basis for prenatal genetic diagnosis for the mothers in these two families, and potentially help improve the efficiency of genetic diagnosis in the future, if possible. Additionally, in clinical settings, when the clinical symptoms are consistent with those of ARSACS, WES remains the preferred genetic testing method.

Table 3 Summary of ARSACS cases with deletion of chr13q12.12

Patient ID	Country	Deletion region	Length (Mb)	Involved genes	SNVs or small indels in SACS	Manifestations	MRI	Ref
Patient #1	China	chr13:23,520,000–24,940,000	1.801	*SACS*, *C1QTNF9B*, *C1QTNF9*, *MIPEP*, *SGCG*, *SPATA13*, *TNFRSF19*	c.8310_8313delAGAT (p.Asp2771fs4*)	Walking unstably, articulation disorders, pseudohypertrophic calves	-	This study
Patient #2	China	chr13:23,520,000–24,940,000	1.801	*SACS*, *C1QTNF9B*, *C1QTNF9*, *MIPEP*, *SGCG*, *SPATA13*, *TNFRSF19*	c.8310_8313delAGAT (p.Asp2771fs4*)	Walking unstably	-	This study
-	African-American	-	1.422	14 genes including *SGCG*, *SACS*, *MIPEP*	c.11824dupA (p.Met3942Asnfs*4)	Gait abnormality, gross motor delay, frequent falls, a below average IQ	Normal (3 Y), normal (5 Y)	[24]
-	China	chr13:23,539,563–24,874,926	1.33	*SGCG*, *SACS*, *TNFRSF19*, *MIPEP*, *C1QTNF9*, a part of *SPATA13*	c.11,803 C > T (p.Gln3935*)	Progressive peripheral neuropathy (CMTNS2 = 15) with horizontal gaze nystagmus, mild spastic gait	Cerebellum atrophy particularly in the vermis superior, mild atrophy of the spinal cord and linear T2 hypointensities in the basis pontis	[25]
Patient II–1	United Kingdom	Chr13:23,772,658–24,489,253	0.7	*SGCG*, *SACS*, *TNFRSF19*, *MIPEP*, *C1QTNF9*	c.13048G > T (p.Glu4350*)	Clumsiness, a poor sporting performance, gait ataxia, slurred speech, urinary urgency	-	[26]
Patient II–2	United Kingdom	Chr13:23,772,658–24,489,253	0.7	*SGCG*, *SACS*, *TNFRSF19*, *MIPEP*, *C1QTNF9*	c.13048G > T (p.Glu4350*)	Early-onset cerebellar ataxia, spastic paraparesis	Marked cerebellar vermian atrophy, moderate diffuse cortical atrophy, a normal brainstem, but reduced spinal cord diameter	[26]
-	Belgian	-	1.54	*SGCG*, *SACS*, *TNFRSF19*, *MIPEP*, *SPATA13*, *C1QTNF9*	c.10517T > C (p.Phe3506Ser)	Early-onset cerebellar ataxia, progressive spasticity, learning difficulties, hearing loss	Atrophy of the vermis superior and the superior cerebellar peduncles	[22]
Patient 1-LF	Italian	-	~ 1.5	*SGCG*, *SACS*, *TNFRSF19*, *MIPEP*, *SPATA13*, *C1QTNF9*	c.600_604 + 1delAACAGG (p.Ile202fs*6)	Early-onset cerebellar ataxia, dysarthria, distal amyotrophy of all limbs, hearing impairment	Vermian atrophy	[23]
Patient 2-MPG	Italian	-	~ 1.5	*SGCG*, *SACS*, *TNFRSF19*, *MIPEP*, *SPATA13*, *C1QTNF9*	c.6680T > C (p.Leu2374Ser)	Developmental motor delay, early-onset cerebellar ataxia, spastic paraparesis, mild intellectual decline, impaired night vision, hearing loss	Marked cerebellar atrophy, mainly affecting the vermis	[23]

Not available

Abbreviations

ARSACS	Autosomal recessive spastic ataxia of Charlevoix–Saguenay
SNV	Single-nucleotide variant
DM	Disease mutation
OMIM	Online mendelian inheritance in man
CNV	Copy number variation
WES	Whole-exome sequencing
WGS	Whole-genome sequencing

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12920-025-02151-2.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5

Acknowledgements

The authors were very grateful to the two families for participating in this study.

Author contributions

QL designed the research. HL, RL, and CC performed the experiments. LS is the first doctor to diagnose Patients #1 and #2. HL, RL, and CC analyzed the data. HL wrote the original manuscript. QL, RL, YB, DC, and XK revised the manuscript. All authors contributed to the article and approved the submitted version.

Funding

This study was supported by Henan Province Youth Health Science and Technology Innovation Talent Training Project (No. YQRC2024012), the Natural Science Foundation of Henan Province (No. 232300420278), the Program for Ph.D. Starting Research Funding from Xinxiang Medical University (No. XYBSKYZZ202143), the National Natural Science Foundation of China (No. 82000321), Key Scientific Research Projects of Higher Education Institutions in Henan Province (No. 25A310015), and the Science and Technology Research Program of Henan Province (No. 242102311096).

Data availability

The datasets used and analyzed during the current study are included in the article/Supplementary Material, further inquiries can be available from the corresponding author upon reasonable ruest.The raw data of Sanger sequence reported in this paper have been deposited in the Genome Sequence Archive (Genomics, Proteomics & Bioinformatics 2021) in National Genomics Data Center (Nucleic Acids Res 2022), China National Center for Bioinformation/Beijing Institute of Genomics, Chinese Academy of Sciences (GSA-Human: HRA011261) that are publicly accessible at https://ngdc.cncb.ac.cn/gsa-human. The Shared URL is https://bigd.big.ac.cn/gsa-human/browse/HRA011261. c.8310_8313delAGAT (p.Asp2771fs4*) in ***SACS*** has been deposited in the ClinVAR database, and the accession number is VCV003777063.1.

Declarations

Ethics approval and consent to participate

Informed consent has been provided by the participants and from the parents or legal guardians for minors. This study was approved by the Ethics Committee of the First Affiliated Hospital of Zhengzhou University (Ethics No. 2022-KY-0892-001) and conformed to the guidelines of the Declaration of Helsinki.

Consent for publication

All the participants and parents/legal guardians of minors gave written informed consent for their personal or clinical details along with any identifying images to be published in this study.

Competing interests

The authors declare no competing interests.

Author details

[1]Genetics and Prenatal Diagnosis Center, Department of Obstetrics and Gynecology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, China
[2]School of Life Science and Technology, Xinxiang Medical University, Xinxiang, China
[3]Department of Foot and Ankle Surgery, Zhengzhou Orthopedic Hospital, Zhengzhou, China

Received: 17 February 2025 / Accepted: 25 April 2025
Published online: 03 May 2025

References

1. Bouchard JP, Barbeau A, Bouchard R, Bouchard RW. Autosomal recessive spastic ataxia of Charlevoix-Saguenay. Can J Neurol Sci. 1978;5(1):61–9.
2. Scaravilli A, Negroni D, Senatore C, Ugga L, Cosottini M, Ricca I, Bender B, Traschütz A, Başak AN, Vural A, van de Warrenburg BP, Durr A, La Piana R, Timmann D, PROSPAX Consortium, Schüle R, Synofzik M, Santorelli FM, Cocozza S. MRI-ARSACS: an imaging index for autosomal recessive spastic Ataxia of Charlevoix-Saguenay (ARSACS) identification based on the multicenter PROSPAX study. Mov Disord. 2024 Jun 7.
3. Ouyang Y, Takiyama Y, Sakoe K, Shimazaki H, Ogawa T, Nagano S, Yamamoto Y, Nakano I. Sacsin-related ataxia (ARSACS): expanding the genotype upstream from the gigantic exon. Neurology. 2006;66(7):1103–4.
4. Criscuolo C, Saccà F, De Michele G, Mancini P, Combarros O, Infante J, Garcia A, Banfi S, Filla A, Berciano J. Novel mutation of SACS gene in a Spanish family with autosomal recessive spastic ataxia. Mov Disord. 2005;20(10):1358–61.
5. Çakar A, İnci M, Özdağ Acarlı AN, Çomu S, Candayan A, Battaloğlu E, Tekgül Ş, Başak AN, Durmuş H, Parman Y. Phenotypical spectrum of SACS variants: neuromuscular perspective of a complex neurodegenerative disorder. Acta Neurol Scand. 2022;145(5):619–26.
6. Wang M, Yang H, Lin Z, Li X, Liu L, Huang S, Zhao H, Zhu X, Xiao Q, Duan R, Wang J, Zuchner S, Tang B, Zhang R. The genetic and clinical spectrum in a cohort of 39 families with complex inherited peripheral neuropathies. J Neurol. 2023;270(10):4959–67.
7. Longo F, De Ritis D, Miluzio A, Fraticelli D, Baets J, Scarlato M, Santorelli FM, Biffo S, Maltecca F. Assessment of Sacsin turnover in patients with ARSACS: implications for molecular diagnosis and pathogenesis. Neurology. 2021;97(23):e2315–27.
8. Engert JC, Bérubé P, Mercier J, Doré C, Lepage P, Ge B, Bouchard JP, Mathieu J, Melançon SB, Schalling M, Lander ES, Morgan K, Hudson TJ, Richter A. ARSACS, a spastic ataxia common in Northeastern Québec, is caused by mutations in a new gene encoding an 11.5-kb ORF. Nat Genet. 2000;24(2):120–5.
9. Aly KA, Moutaoufik MT, Zilocchi M, Phanse S, Babu M. Insights into SACS pathological attributes in autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS)☆. Curr Opin Chem Biol. 2022;71:102211.
10. Feenstra I, Vissers LE, Pennings RJ, Nillessen W, Pfundt R, Kunst HP, Admiraal RJ, Veltman JA, van Ravenswaaij-Arts CM, Brunner HG, Cremers CW. Disruption of teashirt zinc finger homeobox 1 is associated with congenital aural Atresia in humans. Am J Hum Genet. 2011;89(6):813–9.
11. Del Bondio A, Longo F, De Ritis D, Spirito E, Podini P, Brais B, Bachi A, Quattrini A, Maltecca F. Restoring calcium homeostasis in purkinje cells arrests neurodegeneration and neuroinflammation in the ARSACS mouse model. JCI Insight. 2023;8(12):e163576.
12. Parfitt DA, Michael GJ, Vermeulen EG, Prodromou NV, Webb TR, Gallo JM, Cheetham ME, Nicoll WS, Blatch GL, Chapple JP. The ataxia protein Sacsin is a functional co-chaperone that protects against polyglutamine-expanded ataxin-1. Hum Mol Genet. 2009;18(9):1556–65.
13. Zhu X, Gao Z, Wang Y, Huang W, Li Q, Jiao Z, Liu N, Kong X. Utility of trio-based prenatal exome sequencing incorporating splice-site and mitochondrial genome assessment in pregnancies with fetal ultrasound anomalies: prospective cohort study. Ultrasound Obstet Gynecol. 2022;60(6):780–92.
14. Wang J, Chen L, Zhou C, Wang L, Xie H, Xiao Y, Zhu H, Hu T, Zhang Z, Zhu Q, Liu Z, Liu S, Wang H, Xu M, Ren Z, Yu F, Cram DS, Liu H. Prospective chromosome analysis of 3429 amniocentesis samples in China using copy number variation sequencing. Am J Obstet Gynecol. 2018;219(3):287.e1-287.e18.

15. Li Q, Zhu X, Wang C, Meng J, Chen D, Kong X. Identification of a rare case with Nagashima-Type palmoplantar keratoderma and 18q deletion syndrome via exome sequencing and Low-Coverage Whole-Genome sequencing. Front Genet. 2021;12:707411.
16. Synofzik M, Soehn AS, Gburek-Augustat J, Schicks J, Karle KN, Schüle R, Haack TB, Schöning M, Biskup S, Rudnik-Schöneborn S, Senderek J, Hoffmann KT, MacLeod P, Schwarz J, Bender B, Krüger S, Kreuz F, Bauer P, Schöls L. Autosomal recessive spastic ataxia of Charlevoix Saguenay (ARSACS): expanding the genetic, clinical and imaging spectrum. Orphanet J Rare Dis. 2013;8:41.
17. Pilliod J, Moutton S, Lavie J, Maurat E, Hubert C, Bellance N, Anheim M, Forlani S, Mochel F, N'Guyen K, Thauvin-Robinet C, Verny C, Milea D, Lesca G, Koenig M, Rodriguez D, Houcinat N, Van-Gils J, Durand CM, Guichet A, Barth M, Bonneau D, Convers P, Maillart E, Guyant-Marechal L, Hannequin D, Fromager G, Afenjar A, Chantot-Bastaraud S, Valence S, Charles P, Berquin P, Rooryck C, Bouron J, Brice A, Lacombe D, Rossignol R, Stevanin G, Benard G, Burglen L, Durr A, Goizet C, Coupry I. New practical definitions for the diagnosis of autosomal recessive spastic ataxia of Charlevoix-Saguenay. Ann Neurol. 2015;78(6):871–86.
18. Baets J, Deconinck T, Smets K, Goossens D, Van den Bergh P, Dahan K, Schmedding E, Santens P, Rasic VM, Van Damme P, Robberecht W, De Meirleir L, Michielsens B, Del-Favero J, Jordanova A, De Jonghe P. Mutations in SACS cause atypical and late-onset forms of ARSACS. Neurology. 2010;75(13):1181–8.
19. Gazulla J, Benavente I, Vela AC, Marín MA, Pablo LE, Tessa A, Barrena MR, Santorelli FM, Nesti C, Modrego P, Tintoré M, Berciano J. New findings in the ataxia of Charlevoix-Saguenay. J Neurol. 2012;259(5):869–78.
20. Prodi E, Grisoli M, Panzeri M, Minati L, Fattori F, Erbetta A, Uziel G, D'Arrigo S, Tessa A, Ciano C, Santorelli FM, Savoiardo M, Mariotti C. Supratentorial and Pontine MRI abnormalities characterize recessive spastic ataxia of Charlevoix-Saguenay. A comprehensive study of an Italian series. Eur J Neurol. 2013;20(1):138–46.
21. H'mida-Ben Brahim D, M'zahem A, Assoum M, Bouhlal Y, Fattori F, Anheim M, Ali-Pacha L, Ferrat F, Chaouch M, Lagier-Tourenne C, Drouot N, Thibaut C, Benhassine T, Sifi Y, Stoppa-Lyonnet D, N'Guyen K, Poujet J, Hamri A, Hentati F, Amouri R, Santorelli FM, Tazir M, Koenig M. Molecular diagnosis of known recessive ataxias by homozygosity mapping with SNP arrays. J Neurol. 2011;258(1):56–67.
22. Breckpot J, Takiyama Y, Thienpont B, Van Vooren S, Vermeesch JR, Ortibus E, Devriendt K. A novel genomic disorder: a deletion of the SACS gene leading to spastic ataxia of Charlevoix-Saguenay. Eur J Hum Genet. 2008;16(9):1050–4.
23. Terracciano A, Casali C, Grieco GS, Orteschi D, Di Giandomenico S, Seminara L, Di Fabio R, Carrozzo R, Simonati A, Stevanin G, Zollino M, Santorelli FM. An inherited large-scale rearrangement in SACS associated with spastic ataxia and hearing loss. Neurogenetics. 2009;10(2):151–5.
24. Dougherty SC, Harper A, Al Saif H, Vorona G, Haines SR. A chromosomal deletion and new frameshift mutation cause ARSACS in an African-American. Front Neurol. 2018;9:956.
25. Liu L, Li XB, Zi XH, Shen L, Hu ZM, Huang SX, Yu DL, Li HB, Xia K, Tang BS, Zhang RX. A novel hemizygous SACS mutation identified by whole exome sequencing and SNP array analysis in a Chinese ARSACS patient. J Neurol Sci. 2016;362:111–4.
26. Pyle A, Griffin H, Duff J, Bennett S, Zwolinski S, Smertenko T, Yu-Wai Man P, Santibanez-Koref M, Horvath R, Chinnery PF. Late-onset sacsinopathy diagnosed by exome sequencing and comparative genomic hybridization. J Neurogenet. 2013;27(4):176–82.

Publisher's note