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ORIGINAL ARTICLE

Clinical and genetic variability among Bulgarian patients with autosomal recessive spastic ataxia of Charlevoix–Saguenay

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Abstract

Background: Autosomal recessive spastic ataxia ofCharlevoix–Saguenay (ARSACS) is a rare neurodegenerative disorder characterizedby early-onset cerebellar ataxia, peripheral sensorimotor neuropathy, and lowerlimb spasticity. We present clinical andgenetic data of the first Bulgarian patients diagnosed with ARSACS by wholeexome sequencing (WES).

Methods: Variant filtering was performed usinglocally established pipeline and the selected variants were analysed by Sangersequencing. All patients underwent clinical examination and testingincluding the standard rating scales for spastic paraplegia and ataxia.

Results: Five different SACS gene variants, three of which novel, have been identified inpatients from three different ethnic groups. In addition to the classicalclinical triad, brain MRI revealed cerebellar atrophy, linear pontineT2-hypointensities, and hyperintense rim lateral tothalamus combined with retinal nerve fiber layer thickening on opticcoherence tomography (OCT).

Conclusion: We expand the mutation, geographic, and phenotypic spectrum of ARSACS, adding Bulgaria to the world map of the disease, and drawing attention

Teodora Chamova and Neviana Ivanova contributed equally to the study.

to the fact that it is still misdiagnosed. We demonstrated that brain MRI and OCT are necessary clinical tests for ARSACS diagnosis, even if one of the cardinal clinical features is lacking

KEYWORDS

ARSACS, Bulgarian patients, *SACS* gene, whole-exome sequencing

1 | INTRODUCTION

Autosomal recessive spastic ataxia of Charlevoix–Saguenay (ARSACS; OMIM #270550) is a neurodegenerative disorder caused by genetic defects in the *SACS* gene (OMIM *604490) (Bouchard et al., 1978; Synofzik et al., 2013). Although initially confined to Quebec, Canada (Bouchard et al., 1978), the number of genetically confirmed ARSACS patients is gradually increasing worldwide (Baets et al., 2010; Kuchay et al., 2019; Vermeer et al., 2008), making this disorder an important cause of spastic ataxia (Ali et al., 2016; Synofzik & Németh, 2018).

ARSACS is characterized by early-onset cerebellar ataxia, lower limb spasticity, and demyelinating axonal sensorimotor peripheral neuropathy (Bouchard et al., 1978; Synofzik et al., 2013). The increasing number of reported patients has broadened the phenotypic spectrum, either by lacking one of the core features or adding some atypical symptoms, such as intellectual deficit, deafness, and generalized seizures (Ali et al., 2016; Briand et al., 2019).

We report the first Bulgarian patients, genetically confirmed with ARSACS, showing variable age at onset and clinical features. They belong to three unrelated families from three different ethnic groups in Bulgaria. The whole-exome sequencing (WES) analysis revealed five mutations in the *SACS* gene, three of which were novel.

2 | MATERIALS AND METHODS

The patients underwent clinical examination, including the Spastic Paraplegia Rating Scale (SPRS) (Schüle et al., 2006) and the Scale for the Assessment and Rating of Ataxia (SARA) (Schmitz-Hübsch et al., 2006). Nerve conduction studies (NCS), neuropsychological assessment, complete ophthalmological evaluations, optical coherence tomography (OCT), and fundus photographs were performed in all cases. The OCT protocol consisted of peripapillary retinal nerve fiber layer (RNFL) measurement and qualitative analysis of perifoveal scans (NIDEK RS-3000). All patients had 3 T non-enhanced brain MRI (Siemens MAGNETOM® Verio) with T1 thin volumetric slices, axial, sagittal and coronal T2, FLAIR, and DWI sequences.

Genomic DNA was isolated from blood samples using an automatic Chemagen™ Magnetic Separation Module I system (Perkin-Elmer, Germany). WES was carried out on the Illumina NovaSeq™ 6000 System utilizing paired-end sequencing of 350 bp fragments using the company's kit for library construction and exome enrichment. Secondary analysis followed on the Illumina DRAGEN™ Bio-IT Platform. Sequences were aligned to the human reference genome (GRCh37/HG19) using Burrows–Wheeler Aligner (BWA-MEM algorithm). A hard-filtering method using adjustable parameters was applied to select only high-confident variants. We performed advanced analysis on GoldenHelix VarSeq®. Variants were annotated based on NCBI databases RefSeq Genes 105 Interim v1 and dbSNP build 153, and then filtered using Exome Aggregation Consortium v.0.3 (http://exac.broadinstitute.org/) and Genome Aggregation Database v.3 (gnomAD), Exome Variant Server release ESP6500SI-V2 (http://evs.gs.washington.edu/EVS), and 1000 Genomes Project (http://browser.1000genomes.org). A panel of 241 genes related to hereditary ataxias or clinical conditions that include symptoms of ataxia was applied in the filter chain. To predict the possible effect of the variants, we used SIFT (http://sift.jcvi.org/), Polyphen-2 (http://genetics.bwh.harvard.edu/), PROVEAN (http://provean.jcvi.org/), Mutation Taster (http://www.mutationtaster.org/), Mutation Assessor (http://mutationassessor.org/r3/), FATHMM (http://fathmm.biocompute.org.uk/), and Human Splicing Finder (http://www.umd.be/HSF3/). CADD scores with a cutoff of 20 were integrated to determine deleteriousness. Sanger sequencing was performed to confirm selected variants and to test their segregation in the families. It was carried out on ABI PRISM® 3130xl DNA Analyzer (Applied Biosystems) using ABI PRISM® Big-Dye™ Terminator Cycle Sequencing v.3.1 Ready Reaction Kit (Thermo Fisher Scientific).

Additionally, we performed a database screening for the identified variants of the Biobank at the Molecular Medicine Centre which includes the WES data of 1048 individuals—604 patients with rare genetic disorders and 444 patients with SARS-CoV-2. All variants were classified

as pathogenic, likely pathogenic, or variants of uncertain significance according to the criteria of the American College of Medical Genetics and the Association of Molecular Pathology (ACMG/AMP), and following the recommendation for "in trans" criterion (PM3) of the Association for Clinical Genomic Science (ACGS) (Ellard et al., 2020; Richards et al., 2015).

3 | RESULTS

We identified five different genetic variants in the *SACS* gene in four patients from three unrelated families (Figure 1). Table 1 summarizes their genetic findings and clinical presentation.

3.1 | Patient 1

Patient 1 is a 37-year-old male born to a non-consanguineous couple without a family history of neurodegenerative disorders. The pregnancy was uneventful, with complicated delivery due to the umbilical cord around the neck. He had a delay in his motor milestones, as he started to walk at 1 year and 8 months with a wide-based gait. His neuropsychological development was normal. During the first decade of life, his gait ataxia progressed. In his second decade, weakness and deformities in the four limbs were noticed, more pronounced on the left side. At the age of 30 years, he underwent surgeries for Achilles tendon elongations. Since the age of 36 years, he started complaining of neuropathic pain and muscle cramps in his calves, which diminished after therapy with pregabalin.

On neurological examination a year later, he showed a spastic–ataxic–steppage gait, lower limb spasticity with brisk patellar tendon reflexes, and positive Babinski sign. On the SPRS, he scored 23/52. The patient demonstrated ataxia of stance and gait, dysmetria, and intention tremor in the four limbs. On SARA, he scored 19/40. Severe peripheral neuropathy in the four limbs, including distal muscle weakness, bilateral pes cavus and equinovarus, hammer toes, and flexion contractures in the interphalangeal joints in both hands, more pronounced in the left, was present. Cognitive functions were unaffected.

The NCS revealed demyelinating axonal sensorimotor peripheral neuropathy with absent sensory responses in all limbs, motor responses in the lower extremities, decreased amplitudes of compound motor action potentials, and prolonged distal latencies in the upper ones. Sudoscan showed normal sudomotor function.

The brain MRI showed characteristic hypointense stripes in the pons on T2 and FLAIR sequences (tigroid pattern), prominent atrophy in the superior cerebellar vermis, and mild thinning of the posterior part of the corpus callosum body, Figure 2. Additionally, a T2 hyperintense rim lateral to the thalamus was also seen.

The best corrected visual acuity of the patient was 0.7 (metric) of each eye with spherical corrections of −1.75D. Ocular movements were unaffected with spontaneous nystagmus and gaze-evoked left-, right-, and up-beating ones. Fundus examination revealed normal optic discs with clear margins. Myelinated retinal nerve fibers were not observed, but slight whitish peripapillary striations were present following the major arcades in both eyes, Figure 3a,b. The OCT of the macula demonstrated an absence of physiological foveal depression in both eyes, Figure 3c,d, and significant thickening of the global

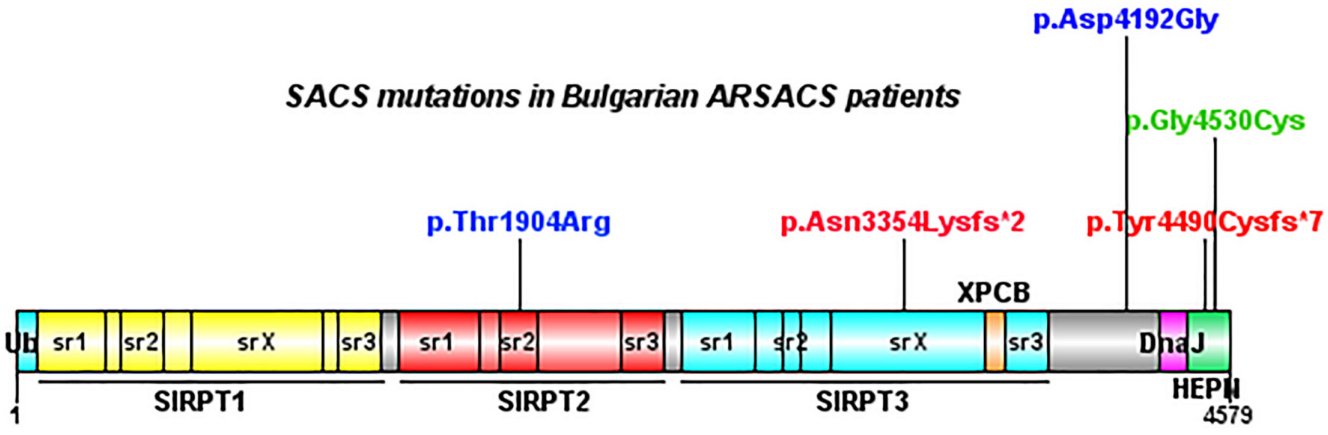

FIGURE 1 Schematic representation of the domain architecture of the Sacsin protein showing the relative position of the *SACS*-gene disease-causing variants found in Bulgarian patients with ARSACS (DOG 1.0 software). Protein domain structure: Ubl, ubiquitin-like domain; SIRPT1, SIRPT2, and SIRPT3 supradomains; srX domain; XPCB, xeroderma pigmentosum complementation group C-binding domain; DnaJ domain; HEPN domain.

TABLE 1 SACS-gene variants and the clinical presentation of the disease in Bulgarian patients with ARSACS.

Patient	SACS-gene variants		ACMG classification (criteria)	Age at examination/ onset, sex	Early motor milestones	SARA	SPRS	Independent ambulation	PN on NCS[d]	Additional features	Brain MRI findings	OCT[g]
	c.DNA	Protein										
1	c.13469_13470delAT[a] c.10062delT[a]	p.Tyr4490fs p.Asn3354fs	Pathogenic (PVS1, PM2, PM3) Pathogenic (PVS1, PM2, PM3)	37 years/ 8 years, male	Walking delay	19	23	Still possible	+	Muscle cramps and neuropathic pain	[e]	+
2	c.13588G>T	p.Gly4530Cys	Likely pathogenic (PM2, PM3, PP1, PP3)	44 years/7 years, female	Normal	na	na	Walker—at age of 35 years	+	Epilepsy and mild cognitive impairment	[e]	+
3	c.13588G>T	p.Gly4530Cys	Likely pathogenic (PM2, PM3[b], PP1, PP3)	40 years/ 15 years, male	Normal	na	na	Crutch—at age of 34 years	+	Epilepsy and mild cognitive impairment	No data	+
4	c.12575A>G c.5711C>G	p.Asp4192Gly[a] p.Thr1904Arg	VUS (PM2, PM3[c], PP3) VUS (PM2, PM3[c], PP1, PP3)	17 years/ <1 years, female	Early motor development delay	21	16	Still possible	−	Mild cognitive impairment	[f]	−

Note: Patients 2 and 3 belong to the same pedigree. Variants were annotated according to the HGVS nomenclature using reference sequence NM_014363.5, Genome Reference Consortium Human Build 37 (GRCh37).

[a]Novel variants.

[b]PM3 is moderate according to the ACGS.

[c]PM3 is supporting according to the ACGS.

[d]Polyneuropathy (PN) on NCS—demyelinating, with secondary axonal loss.

[e]Prominent cerebellar vermian atrophy, hypointense strips, and diffuse slight hyperintensity of the lateral pons when merging into the middle cerebellar peduncles.

[f]Mild cerebellar vermian atrophy, Blake's pouch cyst.

[g]Absence of physiological foveal depression in both eyes and significant thickening of the global peripapillary retinal nerve fiber layer.

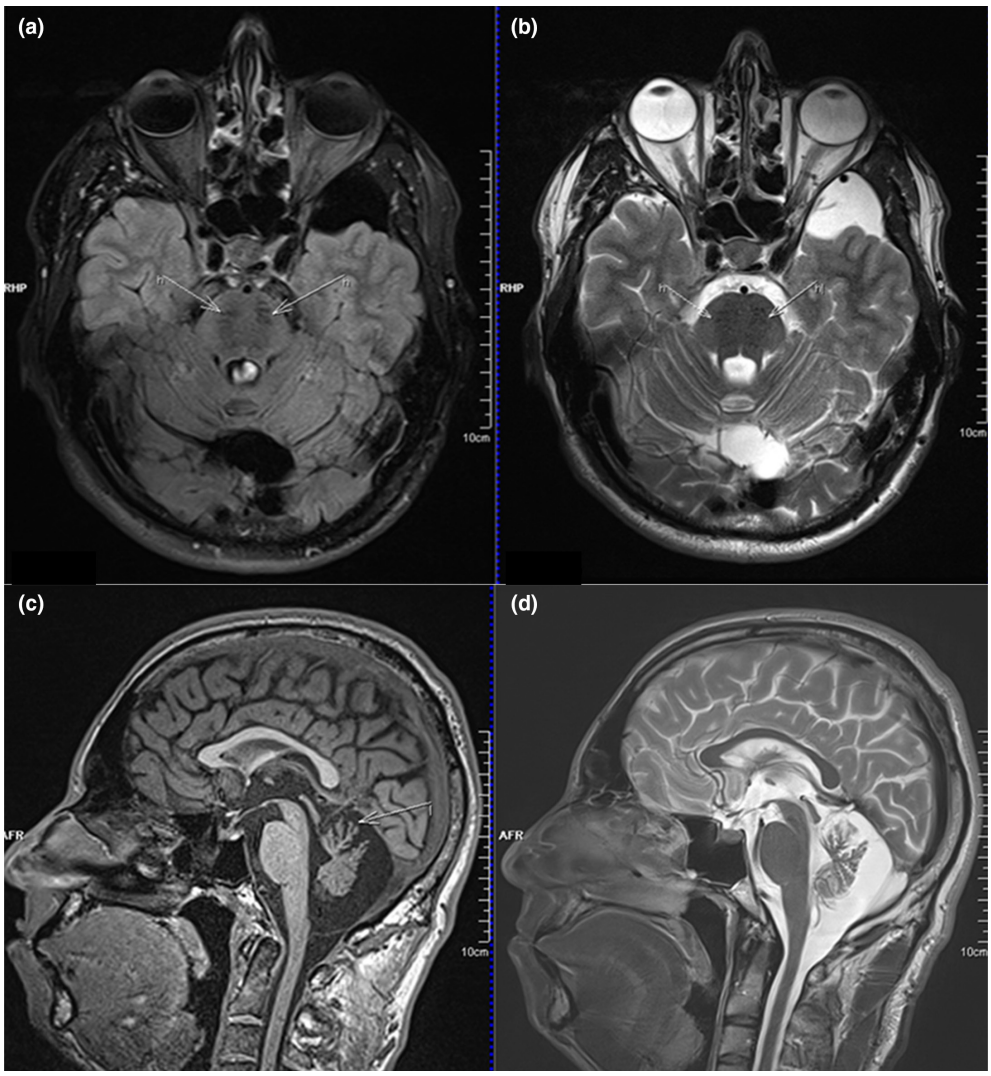

FIGURE 2 Characteristic MRI findings in the Bulgarian ARSACS Patient 1 at the age of 37 years. Axial FLAIR (a) and T2 (b) images at the upper pons level demonstrate linear hypointense striations. Sagittal T1-weighted (c) and T2-weighted (d) images show superior vermian atrophy.

peripapillary RNFL of 196 μm in the right eye and 202 μm in the left one (normal range: 96–99 μm), Figure 3e,f. Patient 1 was initially suspected to have Friedreich ataxia, which was ruled out after genetic testing.

Subsequently, WES analysis revealed two novel frameshift variants—c.10062delT/p.Asn3354fs and c.13469_13470delAT/ p.Tyr4490fs, in exon 10 of the *SACS* gene, both of them leading to premature stop-codons downstream (Table 1). Segregation analysis in the family confirmed the *trans*-position of the variants, consistent with the autosomal recessive inheritance pattern of ARSACS, Figure 4.

3.2 | Patient 2

Patient 2 is a 44-year-old female from a Roma family, with a clinical onset at the age of 7 years, when she started having generalized tonic–clonic seizures for the next 4 years. In her childhood, she was not good at sports and was considered clumsy. At the age of 18 years, due to deformities in the lower limbs, she had an orthopedic consultation with a proposal for surgical corrections. About 10 years later, her gait became unstable due to a weakness in the lower limbs, more pronounced for the left. Since the age of 35 years, she has been using a walker. At the age of 39 years, bilateral hand tremor appeared. Currently, she is free of epileptic seizures without any treatment.

On neurological examination at the age of 42 years, she showed an ataxic–steppage gait. On the SPRS, she scored 6/52. The patient demonstrated ataxia of stance and gait, dysmetria, and intention tremor for all limbs. On SARA, she scored 24/40. She had severe peripheral neuropathy, including distal muscle weakness, bilateral pes cavus and

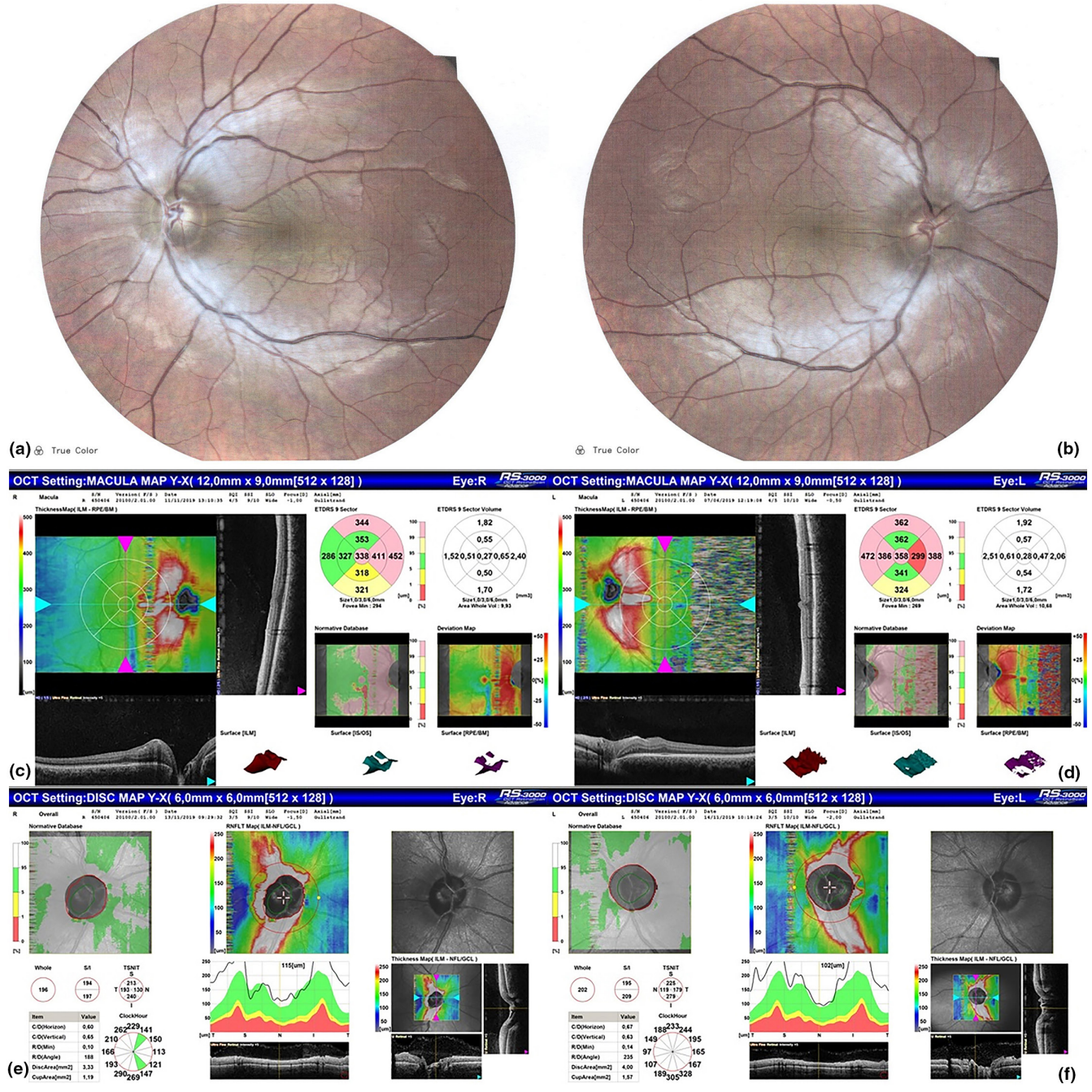

FIGURE 3 Neurophthalmological features of Patient 1. Fundus photograph of right (a) and left (b) eye. Fundus examination revealed normal optic discs with clear margins. Absent myelinated retinal nerve fibers. Slight whitish peripapillary striations following the major arcades in both eyes. Macular OCT showed no physiological foveal depression in the right and left eyes, (c and d), respectively. Significant thickening of the global peripapillary RNFL—196 μm in the right (e) and 202 μm in the left (f) eye was also present.

equinovarus, hammer toes, and flexion contractures in the interphalangeal joints in both hands. A severe impairment of the superficial and deep sensations in the lower extremities was evident. The patient had a mild cognitive impairment.

The NCS revealed demyelinating axonal sensorimotor peripheral neuropathy with absent sensory responses in the four limbs, motor responses in lower limbs, decreased amplitudes of compound motor action potentials, and prolonged distal latencies in the upper ones. Sudoscan was consistent with moderate sudomotor dysfunction.

The brain MRI showed T2 hyperintense rim lateral to thalamus, Figure 5a,b, as well as cerebellar atrophy, more prominent in the cerebellar vermis and the cranial parts of the cerebellar hemispheres, enlarged and bulky pons, thinning of the posterior part of the corpus callosum body,

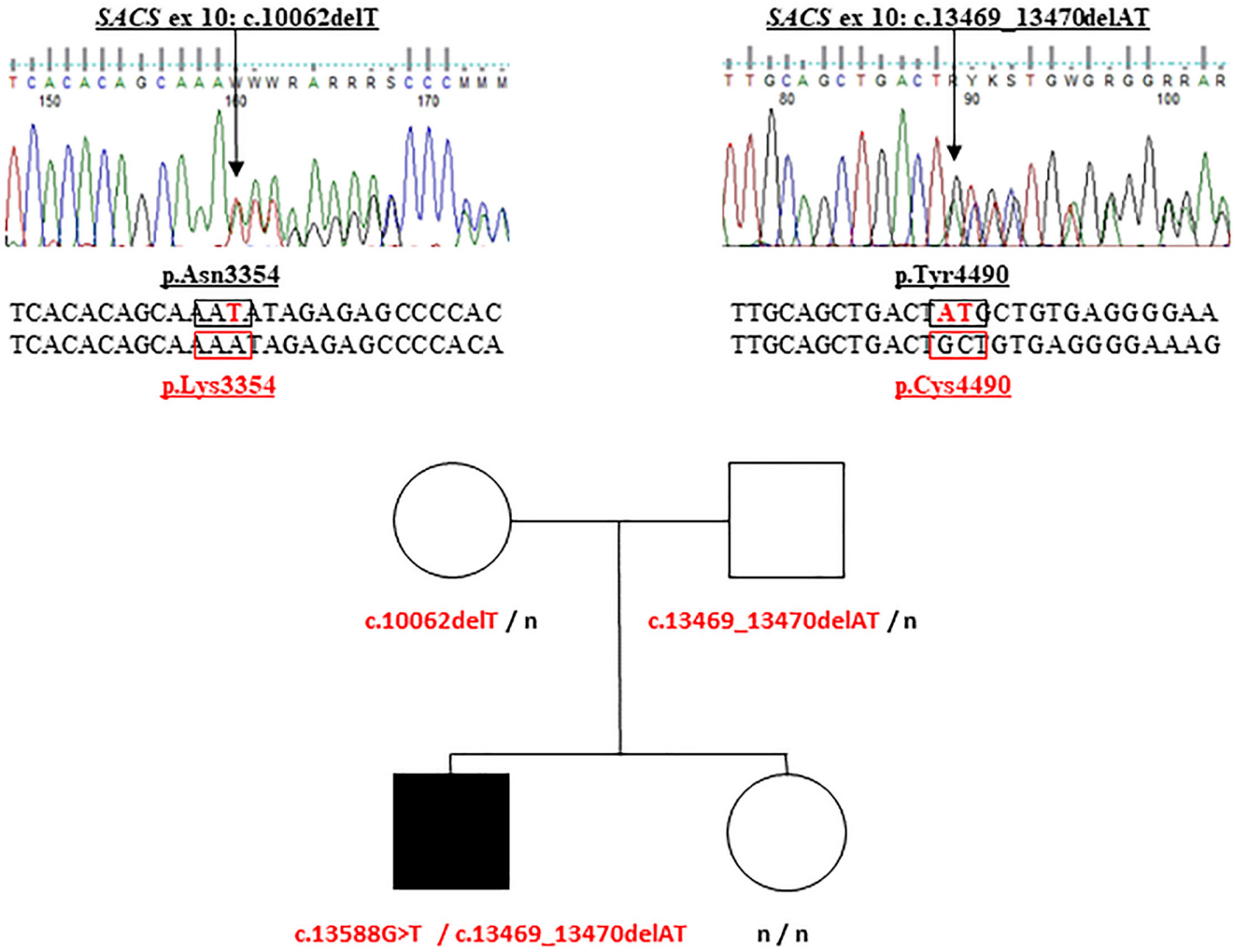

FIGURE 4 Genetic findings in Patient 1. Chromatograms showing the *SACS*-gene sequences in the regions of the deletion variants c.10062delT and c.13469_13470delAT found in Patient 1 (a). The family's pedigree shows the segregation of the disease-causing variant (b).

and the cervical spinal cord, Figure 5c,d. At the level of the pons, T2 FLAIR hypointense stripes were present.

The best corrected visual acuity was 0.9 (metric) for each eye, with spherical corrections of −0.5D. The anterior ocular segment was normal in both eyes. The bulbar movements were full, with spontaneous nystagmus and gaze-evoked left-, right-, and up-beating ones. Fundus examination revealed unaffected optic discs with clear margins. Myelinated retinal nerve fibers were not observed, but there was evidence for slight whitish peripapillary striations following the major arcades in both eyes. The OCT of the macula showed an absence of physiological foveal depression in both eyes.

3.3 | Patient 3

Patient 3 is a 40-year-old male, a brother of Patient 2 from the same Vlax Gypsy family. The clinical onset of the disease was at the age of 15 years, with an unstable gait presentation and observed deformities in the distal parts of the lower limbs. Since the age of 34 years, he has been using a crutch. At the age of 38, he had several focal motor seizures on the left side and is currently on treatment with valproic acid 500 mg twice daily.

On neurological examination at the age of 40 years, he had an ataxic–steppage gait. Ambulation was possible with aid. On the SPRS, he scored 10/52. The patient demonstrated ataxia of stance and gait, dysmetria, and intention tremor in the four limbs. On SARA, he scored 27/40. The examination revealed signs of severe peripheral neuropathy, including distal muscle weakness, bilateral pes cavus and equinovarus, hammer toes, and flexion contractures in the interphalangeal joints in both hands. The superficial and deep sensation was impaired in all extremities.

The NCS showed demyelinating axonal sensorimotor peripheral neuropathy with advanced

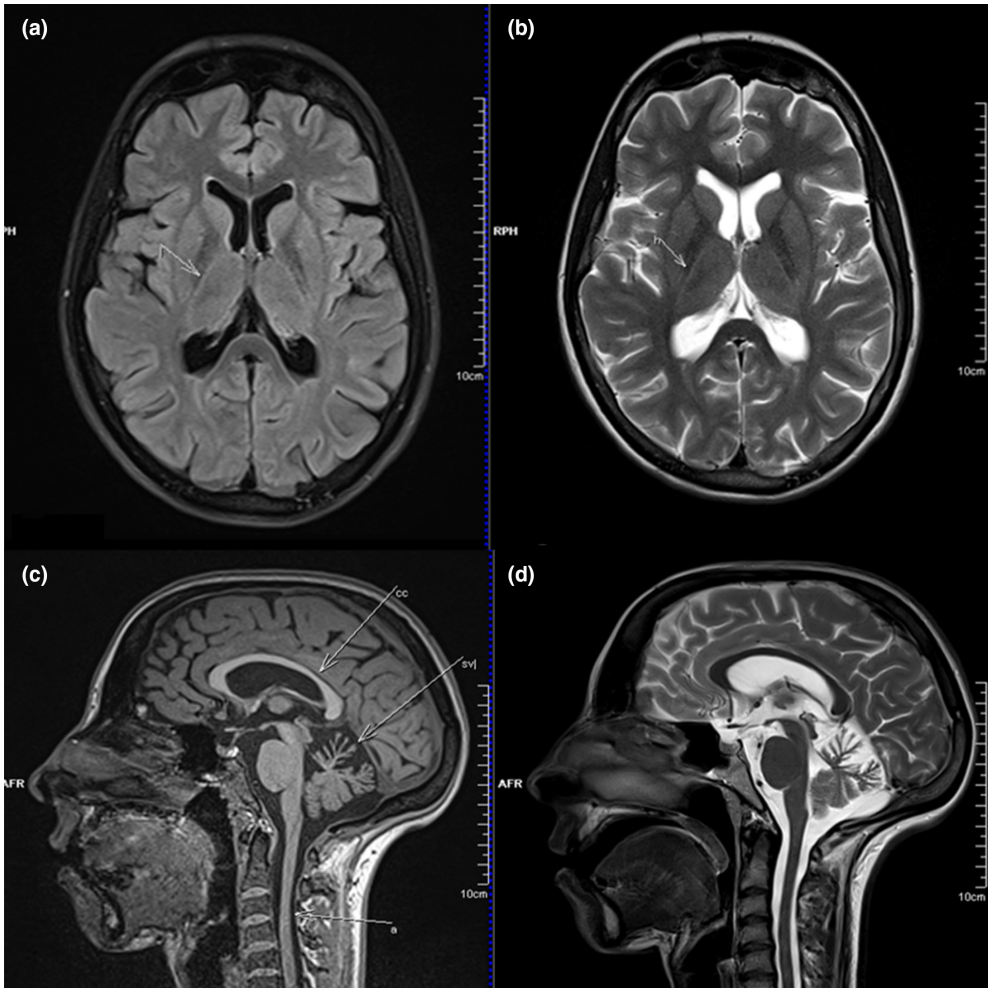

FIGURE 5 Characteristic MRI findings in the Bulgarian ARSACS Patient 2 at the age of 44 years. Axial FLAIR (a) and T2-weighted (b) images at the level of internal capsules show a hyperintense rim around the thalamus. The same pattern of superior vermian atrophy is shown on the sagittal T1- (c) and T2-weighted (d) images. In that patient, additional findings such as bulky pons, thinning of the posterior mid-body of the corpus callosum, and cervical spinal cord are seen.

sudomotor dysfunction on Sudoscan. He had mild cognitive impairment.

The best corrected visual acuity was 0.9 (metric) of each eye without refractive correction. Anterior eye segment was normal in both eyes. Ocular movements were full, with spontaneous nystagmus and gaze-evoked left-, right-, and up-beating nystagmus. Fundus examination revealed normal optic discs with clear margins. Myelinated retinal nerve fibers were not observed, but slight whitish peripapillary striations were found following the major arcades in both eyes. The OCT of the macula showed an absence of physiological foveal depression in both eyes. The last also revealed significant thickening of the global peripapillary RNFL—193 μm in the right and 137 μm in the left eye.

Patients 2 and 3 carried a known variant c.13588G>T in a homozygous state. This variant leads to amino acid substitution p.Gly4530Cys in the evolutionarily conserved region of the higher eukaryotes and prokaryotes nucleotide-binding, HEPN domain of the sacsin protein. It was predicted as damaging and deleterious by five of the six software tools we used. Segregation analysis showed that the parents are heterozygous carriers for c.13588G>T/p.Gly4530Cys (Figure 6a,b). One heterozygous carrier of this variant was identified in our WES database, in a patient with non-neurologic disorder from the Roma ethnic group.

3.4 | Patient 4

Patient 4 is a 17-year-old girl, born to a non-consanguineous couple of Bulgarian Muslims from a second uneventful pregnancy and delivery with a low weight of 2100 g. Due to prolonged hyperbilirubinemia and delayed psychomotor development with starting to walk at the age of 2 years and first words at the age of 3 years, she was initially diagnosed with cerebral palsy. This diagnosis was afterward

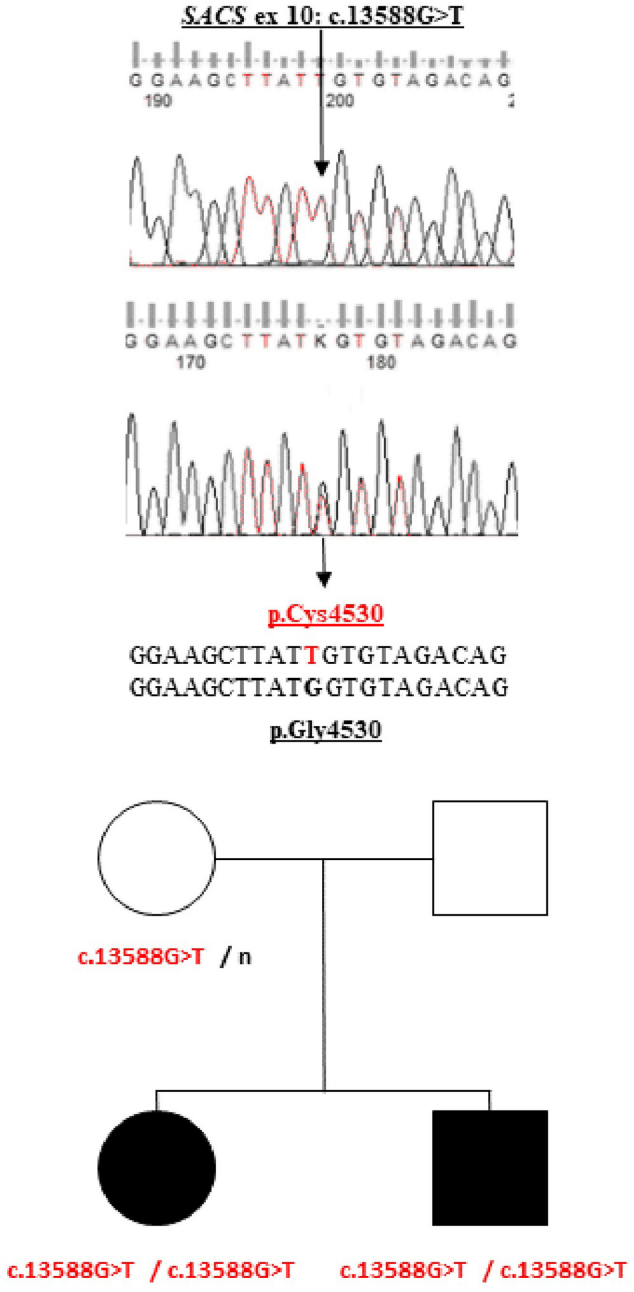

FIGURE 6 Genetic findings in family cases with ARSACS, Patients 2 (sister) and 3 (brother). The chromatograms show the *SACS*-gene sequence in the region of the homozygous variant c.13588G>T found in both subjects. The pedigree of the family presents the segregation of the disease-causing variant.

revised due to the progression of ataxia of stance and gait after the age of 5 years.

On neurological examination at the age of 17 years, she had a spastic–ataxic gait. On the SPRS, she scored 16/52. The patient demonstrated ataxia of stance and gait, dysmetria, and intention tremor in the four limbs. On SARA, she scored 21/40. Her speech was difficult to understand. No polyneuropathy or foot deformities were present.

The NCS were within normal ranges. Brain MRI was consistent with mild cerebellar atrophy and Blake cyst. The neuropsychological evaluation found mild cognitive impairment.

The best corrected visual acuity was 1.0 (metric) for each eye. On OCT, white peripapillary striations were found following the major arcades in the right eye, while RNFL had normal thickness bilaterally.

Two different missense variants have been identified in Patient 4—one novel c.12575A>G/p.Asp4192Gly affecting evolutionarily conserved amino acid close to the HEPN domain and one known variant c.5711C>G/p.Thr1904Arg (dbSNP: rs758570844) located in the *sr2* subrepeat of the second internal repeat SIRPT2 of the sacsin protein (Figure 5d). The first variant was predicted as damaging to the protein structure and function by five of the six software tools we used. However, the second variant c.5711C>G/p.Thr1904Arg lies in an evolutionary-conserved domain, only half of the programs—SIFT, Polyphen, and Mutation tester, predicted damaging effect of this variant and the rest suggested neutral. Segregation analysis in the family confirmed that those two variants are in *trans*-position (Figure 7).

4 | DISCUSSION

Although mutations of the *SACS* gene were initially identified in Quebec patients (Bouchard et al., 1978; Synofzik et al., 2013), ARSACS have been reported worldwide (Baets et al., 2010; Kuchay et al., 2019; Vermeer et al., 2008). The combination of spinocerebellar ataxia with spasticity, superior vermis atrophy, pontine linear hypointensities (Martin et al., 2007; Synofzik & Németh, 2018), and RNFL thickening should prompt the diagnosis of ARSACS (Desserre et al., 2011; Kuchay et al., 2019; Parkinson et al., 2018).

We present the first four Bulgarian ARSACS patients from three unrelated families with different ethnic origins carrying five different genetic variants. Although with considerable variability in terms of initial complaints and age at onset, in three of our patients, the phenotype showed the typical triad in combination with characteristic MRI and NCS features (Gazulla et al., 2012; Gerwig et al., 2010). The phenotype of Patient 4 was consistent with the milder atypical forms of ARSACS sometimes observed in non-Quebec cases. Notably, this patient did not show peripheral nerve involvement on neurological examinations or NCS. Currently, only two patients without overt polyneuropathy have been reported in the literature (Baets et al., 2010; Synofzik et al., 2013), but since this is our youngest patient, she has to be followed up in the future. The main differential diagnoses

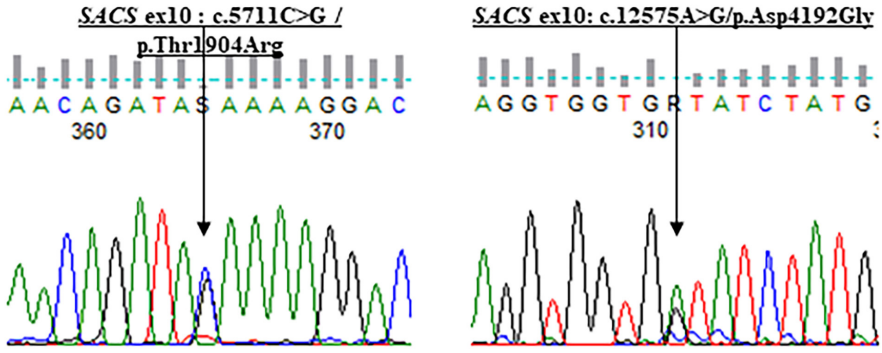

FIGURE 7 Chromatograms showing the *SACS*-gene sequences in the regions of genetic variants c.5711C>G and c.12575A>G in Patient 4.

that were previously discussed among our group were Friedreich ataxia, ataxia with isolated vitamin E deficiency (AVED), early-onset cerebellar ataxia with retained tendon reflexes (EOCA), and complicated forms of HSP (SPG5, SPG7, SPG11, SPG15, SPG20–21, SPG46), but they were subsequently excluded after WES. In Patient 4, we also ruled out potentially pathogenic variants in two other genes—*MTPAP* and *AFG3L2*, recently known to cause spastic ataxias beginning in infancy and childhood. From a clinical point of view, we observed some other features, as two of the affected had generalized and focal epileptic seizures with relatively benign course. Additionally, 9.3% of the patients in the Canadian ARSACS cohort had epilepsy, which was considered much higher than the average populational frequency (Briand et al., 2019).

Lower limb neuropathic pain and muscle cramps, present in Patient 1, were described in a high proportion of ARSACS patients, 23.3% and 58.5%, respectively (Briand et al., 2019). These symptoms are due to an axonal demyelinating pattern of peripheral nerve involvement, a core feature of the disease (Bouchard et al., 1978; Synofzik et al., 2013). Although they can start at every age, their onset was quite late in our patient, appearing for the first time in the fourth decade.

Our cases shared the characteristic key MRI findings of ARSACS, namely, the highly specific linear pontine T2-hypointensities, diffuse hyperintensity of the lateral pons when merging into the middle cerebellar peduncles and atrophy of the superior cerebellar vermis (Martin et al., 2007; Synofzik & Németh, 2018).

Thinning of the peripapillary RNFL, assessed by OCT, is a common feature of various progressive neurodegenerative disorders (Parkinson et al., 2018), but ARSACS seems to be an exception. Likewise, RNFL thickening, which has recently been proposed as a reliable diagnostic biomarker for ARSACS with a sensitivity of 100% and specificity of 99.4%, was observed in all but one of our cases (Desserre et al., 2011; Kuchay et al., 2019; Parkinson et al., 2018). The Bulgarian patients did not show retinal hypermyelination. Still, they had slight whitish peripapillary striations following the major arcades of the eyes, thus illustrating that increased demarcation of RNFL might be a specific but not obligatory finding in ARSACS (Baets et al., 2010; Synofzik et al., 2013), especially in non-Quebec ARSACS individuals (Ouyang et al., 2008).

Our patients belong to three unrelated families of different ethnic origins and carry five different genetic variants. All of them meet at least PM2, PM3, and PP3 criteria, according to the ACMG/AMP and ACSC. Three of the variants are novel and have not been reported in public databases or in literature so far, and the other two are known ultra-rare variants (PM2). Segregation analysis in all families was consistent with the autosomal recessive inheritance pattern of the ARSACS disease (PM3). Except for one, all variants were not detected in the cohort of 1048 individuals from our WES/WGS database, confirming that they are rare genetic variants. The only variant that was found in the database screening was c.13588G>T/p.Gly4530Cys was identified initially in a homozygous state in Patients 2 and 3. They come from Roma ethnic group in the Northwest of Bulgaria. The heterozygous carrier of this variant belongs to the same ethnic group but lives in the Southwest part of the country. We might speculate that variant c.13588G>T/p.Gly4530Cys is not that rare among the Bulgarian Roma and might be that other unrevealed cases of the disease exist in this sub-population.

All variants were located in the giant exon 10 of the *SACS* gene and were either truncating or affected evolutionary-conserved sequences in functionally important domains (Figure 1) (PP3). A clear-cut definition of pathogenicity was possible only in Patient 1, in whom two novel truncating variants were detected. Loss of function is a well-proven mechanism of the ARSACS disease (PV1). Expression studies in patients carrying such variants revealed a nonsense-mediated decay of the mutant transcript. Regardless of the variant type, the nascent mutant sacsin protein undergoes pre-emptive co-translational degradation, revealing a novel human disease mechanism in the *SACS* gene (Longo et al., 2010; Romano et al., 2013).

Variant c.13588G>T/p.Gly4530Cys was previously reported in a homozygous state in a patient with ARSACS (ClinVar ID: 1172523) but not in the public population database (dbSNP: rs2137547970) (PM2). In the recent study, this variant was found also in a homozygous state in two siblings (Patients 2 and 3) (PP1). According to the ACGS recommendations, the determined evidence strength level for PM3 increases to 1.0—each proband is awarded a point value of 0.5 based upon the *in trans* position of a variant of unknown significance (ClinGen Sequence Variant Interpretation Recommendation for in trans Criterion, PM3). Furthermore, the affected aminoacid p.Gly4530 falls in the functionally important HEPN domain that has a nucleotide- and anione-binding affinity. Upon dimerization, it forms a high-affinity GTP-binding site, which is necessary for the chaperone activity of the sacsin protein. It was suggested that variants in this domain interfere with the proper folding/oligomerization of the sacsin protein (Bagaria et al., 2022). Based on these data, the variant c.13588G>T/p.Gly4530Cys in the *SACS* gene could be classified as likely pathogenic (PM2, PM3, PP1, and PP3).

Genetic analyses of the *SACS*-gene variants in Patient 4 were not conclusive. The novel variant c.12575A>G/p.Asp4192Gly does not affect a particular motif but was predicted as deleterious in silico and lay in the sequence just before the HEPN domain. According to ACMG/AMP criteria, the data were not enough to evaluate its clinical significance. The other variant found in this patient, c.5711C>G/p.Thr1904Arg, is present at low frequency in the population (gnomAD, 0.00005081) and was reported as having conflicting interpretation in the ClinVar database (ID: 806988). In silico analysis also showed controversial results. These data did not allow a straightforward interpretation of its clinical significance. However, we could not exclude c.5711C>G/p.Thr1904Arg as a disease-causing variant based on the segregation analysis in the family, its evolutionary-conserved position in the sacsin protein, and the proposed structural and functional significance of the affected motif. The sr2 subdomain of the SIRPT2 motif is highly conserved across sacsin proteins in all vertebrates (Romano et al., 2013). Although the function of the sr2 sacsin subdomains has not been clarified yet, it was proposed that together with sr1 subdomains in SIRPT supradomains, they are directly involved in the ATP-driven supermolecular chaperone activities of the sacsin protein (Bagaria et al., 2022; Perna et al., 2022).

5 | CONCLUSION

In conclusion, we expand the mutation, geographic, and phenotypic spectrum of ARSACS, adding Bulgaria to the world map of the disease, and drawing attention to the fact that it is still misdiagnosed. In addition, we demonstrate that brain MRI and OCT are necessary clinical tests for ARSACS diagnosis, even if one of the cardinal clinical features is lacking.

AUTHOR CONTRIBUTIONS

TC and NI: concept and study design, data collection and interpretation, drafting, revision, and final approval of the manuscript; SC, MK, DZ, VB, KM, MG and SB: data collection and interpretation; DF: administration, manuscript preparation, and submission of the manuscript; RK and VM: study supervision, concept and study design, data interpretation, revision, and final approval of the manuscript, project managment; AJ and IT: concept and study design, study supervision, data collection and interpretation, revision and final approval of manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The datasets generated during the current study are not publicly available due to patient privacy concerns. Still, they are available from the corresponding author on reasonable request.

ETHICS STATEMENT

The Institutional Ethical Review Board of Medical University—Sofia approved this study. Written informed consent was obtained from all subjects.

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