Clinical, ophthalmological, imaging and genetic features in Brazilian patients with ARSACS

Characterization of ARSACS phenotype in Brazil

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ABSTRACT

BACKGROUND: Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is an important form of inherited ataxia with a varied clinical spectrum. Detailed studies of phenotype and genotype are necessary to improve diagnosis and elucidate this disorder pathogenesis. OBJECTIVE AND METHODS: To investigate the clinical phenotype, retinal architecture, neuroimaging features and genetic profile of Brazilian patients with ARSACS, we performed neurological and ophthalmological evaluation in thirteen Brazilian patients with molecularly confirmed ARSACS, and examined their mutation profiles. Optical coherence tomography protocol (OCT) consisted in peripapillary retinal nerve fiber layer (RNFL) measurement and qualitative analysis of perifoveal scans. Neuroimaging protocol accessed the frequency of atrophy in cerebellum, corpus callosum and parietal lobe, brainstem signal abnormalities, and posterior fossa arachnoid cysts. We reviewed the literature to delineate the ARSACS phenotype in the largest series worldwide. RESULTS: All patients had ataxia and spasticity, and 11/13 had peripheral neuropathy. Macular microcysts were present in two patients. Peripapillary striations, dentate appearance of inner retina and papillomacular fold were found in eleven cases. All individuals exhibited thickening of RNFL in OCT. The most frequent radiological signs were cerebellar atrophy (13/13), biparietal atrophy (12/13), and linear pontine hypointensities (13/13). Genetic analysis revealed 14 different SACS variants, of which two are novel. CONCLUSION: Macular microcysts, inner retina dentate appearance and papillomacular fold are novel retinal imaging signs of ARSACS. Ophthalmological and neuroimaging changes are common findings in Brazilian patients. The core clinical features of ARSACS are ataxia, spasticity and peripheral neuropathy with onset predominantly in the first decade of life.

INTRODUCTION

Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is a neurodegenerative disorder described in patients from Quebec exhibiting the very early onset triad of ataxia, spasticity and mixed sensorimotor peripheral neuropathy, combined with increased visibility of retinal nerve fibers [1,2]. Cerebellar atrophy and linear pontine hypointensities on T2 and T2-FLAIR weighted images are characteristic radiological signs [3–5], and optical coherence tomography (OCT) demonstrated thickened retinal nerve fiber layer (RNFL) [6–9].

Two founder mutations in the *SACS* gene, which encodes the protein sacsin, were linked to ARSACS in Quebec [10]. Since then, the identification of over two hundred distinct mutations have confirmed the occurrence of ARSACS in several regions around the globe [4,5,9,11–14].

ARSACS phenotype is more variable than originally recognized, with atypical cases showing adult-onset [12], prominent cognitive dysfunction [11], hearing loss [12,14], and lack of ataxia, spasticity or peripheral neuropathy [5]. Additionally, retinal abnormalities are less common outside Canada [4,5,11,12]. Such variability can make ARSACS difficult to diagnose. As exome sequencing becomes more available in clinical practice, an increasing number of variants of unknown significance will complicate this task. Therefore, reporting novel mutations and characterizing ARSACS phenotypes is important to improve diagnosis of this ever-expanding spectrum disorder and understand its pathogenesis.

We present thirteen Brazilian ARSACS patients, and describe the results of a comprehensive clinical, ophthalmological, radiological and genetic evaluation, comparing them to the largest reported series.

METHODS

Subjects and clinical examination

We enrolled thirteen consecutive cases of ataxia with biallelic *SACS* mutations, evaluated from 2010 up to 2017 at the Ataxia Unit, Department of Neurology and Neurosurgery of São Paulo Hospital, Universidade Federal de São Paulo, in São Paulo, Brazil. Patients underwent detailed neurological examination and ataxia severity was quantified using the Scale for the Assessment and Rating of Ataxia (SARA).

Standard protocol approvals, registrations, and patient consents

This study received approval by the Ethics Committee of the Federal University of São Paulo (reference number 61538516.9.0000.5505) and complied with the Declaration of Helsinki. We obtained written informed consent from all patients or from their parents when appropriate.

Ophthalmologic evaluation

An experienced ophthalmologist performed anterior biomicroscopy, intraocular pressure measurement and fundoscopy, and obtained color fundus photographs (TRC-50IX fundus camera, Topcon, Tokyo, Japan) and red-free fundus images (Heidelberg Retinal Angiograph 2, Heidelberg Engineering) of each eye. The OCT protocol (Spectralis®, Heidelberg Engineering) measured RNFL thickness in 12-degree circular B-scans encircling the optic disc (peripapillary RNFL, pRNFL). The OCT device measured RNFL thickness in superior, inferior, nasal, and temporal quadrants and calculated the average pRNFL thickness. Perifoveal OCT comprising 25 single horizontal sections of the macula was obtained and used for qualitative analysis of retinal architecture (by JMFS). The quality of OCT images was determined based on Spectralis blue quality bar, whose score ranges from 0 (poor quality) to

40 (excellent quality). Only images scoring 20 or higher were analyzed. Scans presenting artifacts and missing parts were discarded and repeated.

Neuroimaging

All patients underwent brain magnetic resonance imaging (MRI). The imaging protocol included 3D sagittal T1-TFE, axial T2-TSE, axial and coronal FLAIR sequences, and qualitative analysis by visual inspection. An experienced neuroradiologist evaluated each scan to determine the presence of cerebellar atrophy, pontine linear hypointensities, lateral pontine hyperintense signal, middle cerebellar peduncle thickening, posterior fossa arachnoid cyst, thinning of the corpus callosum and parietal lobe atrophy.

Genetic Analysis

Genetic analysis was undertaken with genomic DNA extracted from peripheral blood lymphocytes using standard procedures. Molecular analysis was performed by next generation sequencing (NGS), using either an Illumina TruSeq Custom Amplicon panel or a Nextera Rapid Capture Expanded Exome (Illumina, San Diego, CA USA). Library preparation followed manufacturer's protocol using either MiSeq reagent kit version 3 for amplicons designed to cover *SACS* exons with 99% coverage or the Nextera DNA Library Preparation kit, designed for exome sequencing. NGS was conducted in different Illumina platforms (MiSeq, HiSeq2500 or HiSeq4000; San Diego, CA USA). Analysis was undertaken using company's in-house software. *SACS* variants are annotated according to reference sequence NM_014363.4 and protein sequence NP_055178.3. Common *SACS* polymorphisms were filtered out. Novel or rare *SACS* variants either not present or with a low frequency in dbSNP132, gnomAD, or 1000 Genomes databases were analyzed *in silico* using multiple pipelines (www.mutationtaster.org, PolyPhen2, SIFT, PhyloP, PhastCons, GERP, PWM-SpliceSiteFinder-like, MaxEntScan, NNSplice, Human Splicing Finder) to evaluate pathogenicity.

Review of phenotype in other populations

We performed a comprehensive search in MEDLINE database to determine ARSACS phenotype in the largest series published from 1978 to 2017. The following search strategy was employed: "sacsin" OR "Charlevoix" OR "Saguenay" OR "Charlevoix-Saguenay" OR "ARSACS" AND "ataxia". We included articles written in English, Spanish and French containing clinical, ophthalmological or radiological data of non-Canadian individual patients with genetically confirmed ARSACS. The studied variables were: number of families, number of patients, age at examination, age at onset, presence of ataxia, dysarthria, nystagmus, pyramidal signs, Babinski sign, spasticity, peripheral neuropathy, distal amyotrophy, pes cavus, retinal striations in fundoscopy and RNFL thickening in OCT. We recorded other findings as well. Peripheral neuropathy was classified as axonal, demyelinating or axonal with demyelinating component.

RESULTS

Patient's outcome

Thirteen patients (10 females) from nine nuclear families, with ages ranging from 16 to 57 years, were included in this investigation. In twelve cases, clinical criteria for *SACS* gene sequencing were ataxia and lower limbs spasticity with onset before the age of 25 years. One woman presenting symptoms after the age of 25 was tested after molecular diagnosis of ARSACS in her sister (patient 4.2). Consanguinity was present in families 4, 7 and 9. The first symptom was abnormal gait in all patients. Symptoms appeared in the first year of life in seven patients. Three cases had onset after the first decade of life, starting at 11, 12 and 44 years. Cerebellar ataxia (truncal and appendicular, with SARA score ranging from 9 to 30 points) and lower limb spasticity were universally present. Eleven individuals had peripheral neuropathy, one had only pes cavus and another decreased vibration sense. Variable abnormal eye movements were recognized, including saccadic pursuit (13/13), horizontal (12/13) and

vertical gaze-evoked nystagmus (5/13), square-wave jerks (2/13), and hypermetric (8/13) and hypometric saccades (1/13). Seven individuals reported dysphagia, four had constipation and six informed muscle cramps. Other features, each seen in one patient, included mild upper limb dystonia, urinary dysfunction, and epilepsy.

All individuals with previous electrophysiological studies had mixed type sensorimotor polyneuropathy. Table 1 summarizes clinical and genetic data. Comparative data regarding ARSACS phenotypes among different populations is shown in Supplementary Table 1.

Ophthalmological features

One patient (case 4.1) exhibited bilateral cataracts precluding retinal examinations. The remaining 12 individuals underwent the full ophthalmological protocol. None of them had visual complains. Visual acuity was 20/30 or better and intraocular pressure measurement was below 19 cmH2O in all eyes. Peripapillary retinal striations were present bilaterally in 11 patients. Case 2 had high myopia (-6 diopters), myopic fundus and no retinal striations. OCT confirmed increased average pRNFL thickness in all eyes of 12 patients, ranging from 138 micrometers to 231 micrometers. In all individuals except for case 2, perifoveal OCT imaging showed a dentate appearance of inner retinal layers in both eyes, including inner nuclear layer (INL), outer plexiform layer and outer nuclear layer. Patients 1.2 and 1.3 exhibited macular microcysts in OCT B-scans. A fold in the papillomacular region was present bilaterally in color and red-free fundus photography in all cases, except for case 2. Table 2 and Figure 1 present the results of ophthalmological evaluation.

Neuroimaging features

Brain MRI showed cerebellar atrophy in all cases, exclusively in the vermis in four. The others had a vermal-predominant, pancerebellar atrophy. Seven individuals displayed

posterior fossa arachnoid cysts, and 10 exhibited T2 hyperintense signal in the lateral pons and/or middle cerebellar peduncle and middle cerebellar peduncle thickening. All patients had linear hypointensities in T2 and T2-FLAIR images in the pons. Thinning of the mid-posterior portion of the corpus callosum occurred in 10 individuals and biparietal atrophy in 12 patients (Table 3 and Figure 2). Supplementary Table 2 provides comparative data regarding the frequency of radiological features in ARSACS among different populations.

Genetic testing

Molecular analysis by NGS revealed 14 different *SACS* variants, as shown in Table 4. Two of these are novel. The identified variants included 4 frameshift, 6 missense, 2 nonsense, 1 inframe deletion, and 1 splice-site mutation, in homozygosity in 3 families and in compound heterozygosity in the remaining 6.

Review of ARSACS cases in other populations

Search in MEDLINE database resulted in 177 articles, of which 64 contained phenotype description. Researchers reported 279 non-Canadian ARSACS patients from 1978 to 2017. Mean age at onset was within the first decade of life in all series, except for the Belgian. The most consistent findings were ataxia, spasticity and peripheral neuropathy. The frequency of retinal striations was less than 50% in most series, except for the Spanish and the Dutch. Supplementary Table 1 provides detailed data.

DISCUSSION

This study expands the range of retinal architecture abnormalities of ARSACS, confirms the frequency of radiological signs, and adds two novel variants to the literature, demonstrating that the majority of Brazilian patients have early-onset spastic ataxia and axonal-demyelinating peripheral neuropathy, similar to French-Canadians subjects [1,2]. This phenotype occurs in most non-Canadian cases reported, regardless of ethnic origin [4,5,9,11–

14, Supplementary Table 1]. The majority, including ten out of the thirteen described herein, have gait disturbance as the first symptom and onset in the first decade of life [4,5,11–13]. Disease onset after the age of 25, seen in one of our patients, was reported in Italian, Belgian, Turkish and German families [4,5,12]. Higher mean age of onset in recent series likely reflects the recognition of ARSACS occurrence in adulthood and the extension of *SACS* sequencing to older adults [9,12]

Over 50% of our patients reported swallowing difficulties. This aligns with a study involving eleven ARSACS cases, in which dysphagia was ubiquitous [15]. Therefore dysphagia can be an important issue in ARSACS, despite formerly considered uncommon or mild [4,13,16]. Bowel and bladder symptoms were also significant in this series, occurring in 30%. These are not well studied in ARSACS, but urinary urge-incontinence is commonly reported [1,4,5,13]. Constipation seemingly correlates with long disease duration [16].

One single patient (case 1.1) had mild dystonia, while her sister had seizures. Dystonia was also uncommon among 23 Dutch patients, occurring in only three individuals [13]. The connection between epilepsy and ARSACS is not clear, but seizures may occur in up to 15% of Canadian patients [16,17]. Electrophysiological studies showed axonal-demyelinating sensorimotor neuropathy in six patients. Thus, peripheral neuropathy in ARSACS is associated with demyelinating features, distinguishing it from the axonal pattern of other recessive ataxias, such as Friedrich's ataxia [2].

In this series, only one case lacked the typical peripapillary retinal striations. A concomitant high myopic status, in which increased ocular globe size provided more space for the RNFL to spread, could explain their absence. Retinal striations were more visible on red-free fundus images than on ophthalmoscopy. pRNFL thickening in OCT was universal, with average thickness ranging from 138 to 231 micrometers, above the 95th percentile of Spectralis® normative data. This suggests that, irrespective of mutation type, retinal

abnormalities are common in Brazilian ARSACS patients and more frequent in this ethnic group than in others [5,11,12].

Increased RNFL thickness and retinal striations are very unusual findings in clinical practice, and should prompt genetic investigation for ARSACS when associated with appropriate clinical features [7–9]. OCT is likely more accurate in identifying retinal abnormalities than routine fundoscopy in ARSACS [7,9]. A recent study measured pRNFL thickness and performed genetic investigation in 79 British patients with undetermined ataxia. All those diagnosed with ARSACS had pRNFL thickening, indicating this is a hallmark of this disorder [9]. Remarkably, our results confirm this in a different ethnic group.

Previous studies have demonstrated thickening in pRNFL and ganglion cell layer (GCL) in ARSACS, and proposed increased nerve fiber and cell body density (hyperplasia) [6,7] or axonal edema [8] as underlying causes. We report distinct structural abnormalities that may provide new insights: macular microcysts, a dentate appearance of inner retina and a papillomacular fold. Folds in a surface result from compressive, tensile or shearing stress. We hypothesize the redundant neural tissue of abnormally thick RNFL and GCL wrinkles to accommodate to a limited retinal area, forming the papillomacular fold, and compresses the underlying retina to produce smaller folds in the inner nuclear, outer plexiform and outer nuclear layers, which have a dentate appearance in horizontal sections [18,19]. The excess of neural tissue in RNFL and GCL could result from insufficient programmed cell death of ganglion cells, an important step in retinal development [20]. This conception suggests that hyperplasia contributes to retinal thickening and supports a neurodevelopmental pathogenesis theory in ARSACS [21].

Neurodegeneration may also contribute to retinal thickening in ARSACS by inducing edema in cell bodies and axons. Post-mortem studies have shown swollen thalamic and cerebellar cortical neurons [22], supporting this assumption. Sacsin appears to regulate

mitochondrial dynamics. In sacsin knock-out mice, mitochondrial dysfunction and neurofilament aggregation occur, and dendrites and axons of Purkinje cells increase in volume before cell-death [23,24].

Macular microcysts, present in two patients (cases 1.2 and 1.3), located predominantly in the INL and were visible in two or more adjacent scans, fulfilling proposed diagnostic criteria [25]. These siblings also had similar abnormalities in the ganglion cell layer. Macular microcysts occur in the INL in optic neuropathy of various causes, including inflammatory diseases, ischemia, compression and hereditary conditions. Cystoid changes in these disorders possibly result, at least in part, from neurodegeneration [26], and thus macular microcysts could constitute evidence of neurodegeneration in the ARSACS retina. However, the microcysts detected are unusual because: (i) they also occur in the GCL; and (ii) in contrary to other conditions, visual function is preserved (normal microperimetry, and best visual acuity 20/20 in both eyes of cases 1.2 and 1.3). Further studies are necessary to confirm macular microcysts occurrence in ARSACS and determine their significance.

Brazilian patients with ARSACS have the same neuroimaging abnormalities originally described in Canadians. Irrespective of the genotype, cerebellar atrophy and linear pontine T2-hypointensities were ubiquitous, akin to previous investigations [3,4]. These are the most consistent radiological signs of ARSACS, occurring in 92.8% and 71% of patients [3–5, Supplementary Table 2]. This study recorded a high frequency of posterior fossa arachnoid cysts, bilateral parietal lobe atrophy and thinning of the mid-posterior body of the corpus callosum, as previously demonstrated [5]. Our findings indicate that specific radiological abnormalities are common in ARSACS, reinforcing their diagnostic value.

The ARSACS differential diagnosis includes many neurogenetic disorders. Friedreich's ataxia (FA) is a chief consideration. Patients with late-onset FA exhibit peripheral neuropathy and may display cerebellar atrophy, brisk reflexes and spasticity.

However, OCT in FA does not show RNFL thickening and pontine signal abnormalities are absent. Ataxia, cerebellar atrophy and peripheral neuropathy are also signs of the ataxias with oculomotor apraxia types 1 and 2 and ataxia-telangiectasia, but the lack of pyramidal signs and presence of oculomotor apraxia distinguish them from ARSACS [27]. Moreover, complicated hereditary spastic paraplegias (SPGs) are typically autosomal recessive and often present ataxia, as observed in SPG7. Neuroimaging discloses corpus callosum and cerebellar atrophy in some forms, such as SPG11 and SPG15 [28], but unlike ARSACS, SPGs do not exhibit RNFL thickening. Other rarer causes of ataxia could mimic ARSACS, including spastic ataxia types 1-5, ataxia with vitamin E deficiency, abetalipoproteinemia and Refsum's disease [27]. Regarding retinal findings, one concern is to exclude papilledema and optic neuritis. A detailed history and a swollen optic nerve head differentiate these conditions from ARSACS [9,27]. The authors of the original series compared ARSACS fundus to the acute phase of Leber's hereditary optic neuropathy (LHON) [1]. RNFL thickening may occur in LHON [29] and some patients have ataxia, peripheral neuropathy or pyramidal signs [30], but visual loss and optic atrophy develop in late stages of LHON, while visual function is usually preserved in ARSACS.

We identified 14 *SACS* variants, 2 of them novel, in our series. Of these, 7 are pathogenic and predicted to cause protein loss of function, leading, if translated, to premature stop codon or disruption of natural splice site. Four of the 7 remaining variants (6 missense and 1 in-frame deletion) are likely pathogenic, as their prevalence in affected individuals is significantly increased if compared to the prevalence in normal controls. The other three missense mutations are VUS; one is on ClinVar and the others have not been published. They affected residues of varying degrees of conservation, and 2 are predicted to be have an impact on sacsin by several computational algorithms (SIFT, PolyPhen, Mutation Taster, Provean and/or splice site prediction programs). Considering their rarity in population databanks

(GnomAD; Table 4) and/or co-existing variants previously reported as likely pathogenic, they are potentially recessive pathogenic mutations, and notably occurred in individuals with clinical presentation and OCT appearances highly suggestive of ARSACS.

Ideally, for diagnostic purposes, Sanger sequencing would confirm the genetic findings and parental testing would ratify the phase of the alleles, but unfortunately, these samples were unavailable. Another limitation of our work is that we selected patients with typical clinical features (ataxia and spasticity with onset before the age of 25), while wider criteria for SACS sequencing may reveal a broader disease spectrum [5]. Phenotypic differences between different genotypes were not remarkable, and surprisingly siblings of family 4 had disease onset at the ages of 2 and 44 years.

In conclusion, our description of Brazilian patients with ARSACS and review of the largest series show that the main clinical features of this disease are ataxia, spasticity and peripheral neuropathy with onset in the first decade of life, regardless of ethnic origin. We add 2 novel mutations to the literature, which may cause this phenotype. The frequency of specific infra- and supra-tentorial neuroimaging abnormalities reinforces that MRI is an important diagnostic tool in ARSACS. This series demonstrates retinal changes are frequent in this condition, and confirms OCT could guide the molecular investigation of inherited ataxias. Moreover, we expanded the spectrum of retinal architecture abnormalities of ARSACS and the understanding of its pathogenesis.

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Legend of the figure 1: Retinal findings in ARSACS. A. Retinography of the left eye of case 1.2 disclosing a papillomacular fold (arrowheads) and increased visibility of retinal nerve fibers (arrows). B. Red-free fundus image of the same eye depicting augmented demarcation of retinal nerve fibers (arrows) and a darker area in the papillomacular area due to a fold (arrowheads). C. Horizontal B-scan from OCT obtained from the same eye shown in A/B, demonstrating dentate appearance of inner retinal layers (arrowheads) and perifoveal inner retina microcysts in inner nuclear layer (thick arrow) and ganglion cell layer (thin arrow). D. Adjacent scan confirms microcysts in inner nuclear layer (arrow). E. Dentate appearance in detail, seen in inner nuclear layer (long arrow), outer plexiform layer (short arrow) and outer nuclear layer (asterisk).

Legend of the Figure 2: Radiological findings. A. Sagital T1 scan demonstrating atrophy of upper cerebellar vermis (short white arrow), posterior fossa arachnoid cyst (asterisk), thinning of the corpus callosum (long white arrow) and cervical spinal cord atrophy (black arrow) in case 5. B. Axial T2 scan disclosing linear pontine hypointensities (arrow) and lateral pontine hyperintensities (star) in case 2. C. Axial T1 scan showing bilateral parietal atrophy in case 2 (arrows).