The Cerebellum (2025) 24:24
https://doi.org/10.1007/s12311-024-01777-9

REVIEW

ARSACS: Clinical Features, Pathophysiology and iPS-Derived Models

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Accepted: 17 December 2024

Abstract
Autosomal-recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is an early-onset neurodegenerative disease caused by mutations in the *SACS* gene. The first two mutations were identified in French Canadian populations 20 years ago. The disease is now known as one of the most frequent recessive ataxias worldwide. Prominent features include cerebellar ataxia, pyramidal spasticity, and neuropathy. Neuropathological findings revealed cerebellar atrophy of the superior cerebellar vermis and the anterior vermis associated with Purkinje cell death, pyramidal degeneration, cortical atrophy, loss of motor neurons, and demyelinating neuropathy. No effective therapy is available for ARSACS patients but, in the last two decades, there have been significant advances in our understanding of the disease. New approaches in ARSACS, such as the reprogramming of induced pluripotent stem cells derived from patients, open exciting perspectives of discoveries. Several research questions are now emerging. Here, we review the clinical features of ARSACS as well as the cerebellar aspects of the disease, with an emphasis on recent fields of investigation.

Keywords ARSACS · *SACS* · Sacsin · Mutation · Purkinje Cells · Cerebellum

Introduction

Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) (OMIM #270550) was originally described in 1978 in the inhabitants of the Charlevoix-Saguenay region in Quebec, Eastern Canada [1]. Accounting for about 5% of all autosomal-recessive cerebellar ataxias (ARCAs) [2], ARSACS is one of the most frequent ARCAs worldwide, after Friedreich ataxia (FRDA) and ataxia telangiectasia (A-T) [3–5]. To date, cases of ARSACS have been reported in more than 20 countries [6–18]. Because of a founder effect, one in 22 Charlevoix-Saguenay residents carries a mutation in one copy of the *SACS* gene [1, 2, 19], which was found to cause the disease [20–22]. The founder mutation was probably brought to this region by French immigrants four centuries ago [19].

From a neurological perspective, ARSACS generally combines pyramidal, cerebellar, and neuropathic features that exhibit a high level of variability among individuals in terms of clinical presentation, severity, and progression [2]. The clinical spectrum ranges from the core phenotype, consisting of an early-onset progressive spastic ataxia with sensorimotor polyneuropathy, to atypical phenotypes. The utilization of brain imaging techniques, such as MRI, has played a crucial role in pinpointing the specific anatomical source of ARSACS as the degeneration of the Purkinje cells located in the cerebellum. [23–25]. Since the discovery of the causal *SACS* gene, more than 200 mutations have been reported [20–22]. Because of the relatively large clinical spectrum observed, the relationship between phenotype and genotype in ARSACS needs further consideration.

Pathogenic mutations result in a loss of function of the encoded protein sacsin, a large 520-kDa cytoplasmic protein that possesses chaperone activity [26, 27]. However, the causal link between the mutations and the neuronal loss – particularly the extensive loss of Purkinje cells – remains unclear despite investigations using *SACS*

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Published online: 03 January 2025

knockout SH-SY5Y neuroblastoma cells as well as patients' skin fibroblasts [28, 29]. A *Sacs* knockout mouse model was instrumental in discovering the association between the loss of sacsin function and a disruption in the mitochondrial fission process [28]. Additionally, in a study by Larivière et al., expression of the p.R272C missense mutation in the *SACS* gene in a knock-in mouse model resulted in a significant reduction of sacsin protein levels in the brain of up to 21% compared to wildtype. The $Sacs^{R272C}$ mouse model develops an ataxia phenotype similar to that observed in the *Sacs* knockout model, which suggests that even a partial loss of sacsin function can lead to the manifestation of disease symptoms. Therefore, this study supports the notion that for certain *SACS* mutations, a partial loss of function may be sufficient to cause ARSACS disease [30]. This finding about partial loss of sacsin function causing ARSACS is also consistent with a recent study by the group of Francesca Maltecca using patients' skin fibroblasts [31].

While much insight has been gained from ARSACS mouse models, human cellular models are currently being developed using human induced pluripotent stem cells (iPSC) technology in efforts to better understand the human pathology at the cellular level [32–34].

Here, we review the most recent data regarding clinical aspects, genetics as well as cellular pathophysiology of ARSACS. We also highlight recent strategies, including human iPSC-derived models, to investigate the pathophysiology of ARSACS and test potential therapeutics.

Clinical Features of ARSACS

ARSACS presents a mixed picture of pyramidal, cerebellar, and neuropathic features. Manifestations start early in childhood, and there is a high level of variability among individuals in terms of clinical presentation, severity, and progression [2] (Table 1). The neuropathy in ARSACS is demyelinating with secondary axonal degeneration [35]. The ARSACS clinical spectrum is broad, and there are patients who do not systematically display all three components of the classical triad. Other symptoms include hearing loss [36–38], palatal tremor, sleep disorders, generalized seizures [4, 39, 40], concentration problems [41], neuropsychiatric disorders [42] and autonomic dysfunction, including bladder urge incontinence, erectile dysfunction, and constipation in patients with long disease duration [2, 4]. Generalized seizures have been reported among family members, regardless of the mutation type [40, 41]. In general, ARSACS patients can suffer spasms, cramps, and mild dystonia. Ataxic gait, with upper and lower limb dysmetria is common and requires symptom management. Dysarthria with explosive speech is also part of the clinical picture of ARSACS, with about half of patients presenting with dysphonia and poor pitch control [43]. Swallowing difficulties were reported in up to 50% of ARSACS patients, making dysphagia an important issue in this population [43]. Some atypical cases of ARSACS do not present ataxia or adult-onset ataxia [7, 44], spasticity or peripheral neuropathy [45, 46], retinal striations [10, 15, 47, 48], or they even display a Charcot-Marie tooth-like phenotype [2, 49].

Patients tend to develop a striated peripapillary retina, which could be caused by an increased peripapillary retinal nerve-fiber layer (RNFL) thickness (average > 119 µm on optical coherence tomography) [50, 51]. This finding was first interpreted as hypermyelinated fibers and suggested being pathognomonic for a diagnosis of ARSACS disease [10, 15, 47, 48]. Rezende Filho et al. recently reported new retinal disturbances consisting of increased macular

Table 1 Clinical features of autosomal-recessive spastic ataxia of Charlevoix–Saguenay

Common features
Pyramidal signs
Spasticity predominantly in lower limbs*
Increased deep tendon reflexes*
Bilateral abnormal plantar response*
Contractures and tendon retractions
Pes cavus*
Bladder urge incontinence*
Cerebellar signs
Gait ataxia*
Appendicular ataxia
Dysarthria*
Sensorimotor axonal neuropathy
Demyelinating with secondary axonal degeneration
Decreased proprioception in lower limbs*
Distal amyotrophy more severe in the lower limbs*
Gradual weakening of ankle reflex with complete loss*
Neuro-ophthalmologic findings
Striated peripapillary retina*
Saccadic alteration of smooth ocular pursuit
Supranuclear vertical gaze deficits
Gaze-evoked nystagmus
Uncommon features
No ataxia or adult-onset ataxia
No spasticity
Lack of neuropathy
No retinal striation
Macular microcysts, inner retina dentate appearance
Club feet
Hearing loss
Palatal tremor
Dysphagia
Sleep disorders signs
Generalized seizures
Intellectual disability
Concentration problems
Mitral valve prolapse*

*Typical clinical features of ARSACS in French Canadians

thickness with loss of foveal depression, dentate appearance of the inner retina, papillomacular fold, and macular microcysts [17]. Other early nonprogressive ocular signs include saccadic alteration of smooth ocular pursuit, supranuclear vertical gaze deficits, and gaze-evoked nystagmus [1, 52, 53].

Both the extent and intensity of symptoms progress from childhood to late adulthood and generally result in premature death. Regarding cognitive manifestations, intellectual disability and cognitive decline have been reported in a few adult patients. The symptoms seem to be variable in ARSACS and often co-occur with additional complications [7, 16, 41, 54]. For instance, in a cohort of 17 patients with ARSACS, Batz et al. reported one case of mental retardation and two cases of mild cognitive impairment [7]. In a case report of one patient, Hara and al. found impairment of recent memory and calculation with mild emotional incontinence [47]. Also, in a cohort of 21 Dutch patients, Vermeer and al. found two patients displaying mild cognitive problems. One of these patients had suffered from meningitis and had experienced epileptic seizures. The other patient had suffered from strokes [16]. Ali and al. reported two families accounting for a total of eight individuals with ARSACS that were also affected by intellectual disability and behavioral abnormalities [41]. Another study conducted on 41 ARSACS patients reported intellectual disability and/or difficulties at school in up to 50% of patients [4] which is in line with recent MRI findings indicating involvement of supratentorial structures [2, 54, 55]. The study mentioned that intellectual disability was mild in nature. However, neuropsychological evaluations were rarely available, making it challenging to provide a comprehensive description of the intellectual challenges faced by these patients. The study emphasizes the need for more detailed neuropsychological assessments to better understand the cognitive impairments associated with ARSACS.

With over 300 people diagnosed with the disease, ARSACS patients in Quebec represent about 1/3 of all cases worldwide [1, 50]. This group is of particular interest because clinical manifestations of ARSACS were first described in this population, in which over 92% of individuals carry the same homozygous c.6594delT mutation as will be discussed in the next section. As with ARSACS patients in general, the first sign of cerebellar ataxia commonly appears in childhood, during the gait learning process. Parents often report a tendency to fall in their affected children [1, 50]. Lower limb spasticity is observable early when there is little sign of cerebellar dysfunction (12–18 months), and this worsens as the disease progresses, causing gait abnormalities such as scissoring [56]. Hyperreflexia with polyclonic reflexes and Babinski signs are present in the first two decades of life, and reflexes eventually disappear in the third decade. Quebec patients generally suffer from early-onset spastic ataxia, RNFL hypertrophy and hand or foot deformities. Most patients eventually develop motor axonal polyneuropathy with distal muscle weakness and peripheral amyotrophy [57, 58]. Also, extremity deformities such as clawing, clubfeet, pes planus, and pes cavus can accompany spasticity and peripheral neuropathy, including axonal-demyelinating sensorimotor neuropathy [1, 50, 59]. Little intra- and extrafamilial phenotypic variability has been observed among affected individuals [1]. A study on the Quebec population reported that ARSACS patients become wheelchair-bound at a mean age of 41 years (range 17–57 years), but some patients may experience severe walking limitations in their early adulthood [57]. The average age of death is 51 years. However, better symptom management, such as wearing foot orthoses, has improved patients' quality of life in the last two decades and might have positively influenced life expectancy. Several patients have survived into their 70 s [57].

The prevalence of epilepsy recently reported in ARSACS patients of 40 years old from Quebec (9.3%) [60] is much higher than that of epilepsy in the Canadian population (0.6%) [61]. This prevalence is slightly lower than the one found by Duquette (15.5%) [59] among pediatric patients, but could be potentially explained by the clinical observation that epilepsy tends to resolve with age in this population. Recent data are somewhat higher than reported by Bouchard (7.2%) [62] in the original description of the disease in an adult population. However, further studies are needed to understand the connection between epilepsy and ARSACS.

Additionally, while the verbal IQ of Quebec patients is considered to be within normal limits [58, 62], a decrease in nonverbal mental abilities (object assembly and digit symbols) has been reported at adult age 55 [1]. When observed, mild learning disabilities become evident in patients at the elementary school level as they can be slower to master handwriting [59]. Outside the neurological field, there are few clinical abnormalities. Cardiovascular evaluation revealed mitral valve prolapse in a majority of examined Quebec patients [62].

Diagnosis

Unequivocal diagnosis of ARSACS must be established by molecular testing. However, when molecular testing is not possible or consented by the patient, a detailed family history, physical examination and characteristic MRI or optic coherence tomography features, can provide some suggestive clinical findings that can lead to a diagnosis of ARSACS. In fact, both brain imaging and optic coherence tomography markers are proposed as helpful biomarkers for ARSACS in patients with novel *SACS* mutations [11, 63, 64]. The highly specific linear pontine T2 hypointensities with a T2-hyperintense lateral pons, and less specific,

thinning of the rostral corpus callosum and atrophy of the superior cerebellar vermis [2] are key characteristic MRI findings of ARSACS. Likewise, RNFL thickening adds further support to ARSACS diagnosis and has been proposed as a relatively universal diagnostic biomarker for ARSACS, present even in patients from very different parts of the world [64].

Genetic Basis of ARSACS

ARSACS is a monogenic, autosomal recessive disease. The gene responsible, *SACS,* has been discovered in 2000 by fine structure linkage disequilibrium mapping and positional cloning [20, 65]. *SACS* is located on chromosome 13q12.12 [21, 65] and consists of 10 exons, including nine coding exons forming an open reading frame of 13,737 base pairs. The last coding exon is very large and spans more than 12.8 kb [20, 66]. *SACS* mRNA has been detected by in situ hybridization in all areas of the human brain, especially in the cerebral cortex, hippocampus and cerebellum, but was also found in skeletal muscle, pancreas and connective tissue [20, 67].

To date, over 200 different mutations have been identified in the *SACS* gene (Human Gene Mutation Database [http://www.hgmd.cf.ac.uk]). These consist of various types of mutations: 37.4% missense, 11.7% nonsense, 16.2% frameshift including both insertions and deletions, and 1.4% duplications (Fig. 1) [68]. Furthermore, an intragenic deletion of exons 3–5, a whole-gene deletion of *SACS* and the contiguous gene *SGCG* in addition to one chromosomal deletion including *SACS* gene were also reported in several patients [37, 38, 69, 70]. Most *SACS* mutations (80%) are located within the large exon 10. All mutations, including those outside exon 10, may lead to different levels of sacsin protein expression, which may explain the differences observed when comparing the phenotypes of different patients. In total, 48.3% of all mutations were found to be highly pathogenic, 24.3% were computationally pathogenic, 6.8% were computationally likely pathogenic and 1.4% were likely benign [68].

Most French Canadian patients harbor the homozygous nucleotide deletion c.6594delT (94%) due to a founder effect. Heterozygosity with the nonsense mutation (c.5254C > T) has also been reported (2%), both leading to protein truncation [20]. After mapping of the ARSACS locus and gene identification, missense and nonsense mutations of the *SACS* gene have been found in Tunisia [10], Turkey [15], Italy [8, 12], Japan [13, 46–48], Spain [9] and Belgium [36]. The phenotype of these patients is largely comparable to that identified in Quebec.

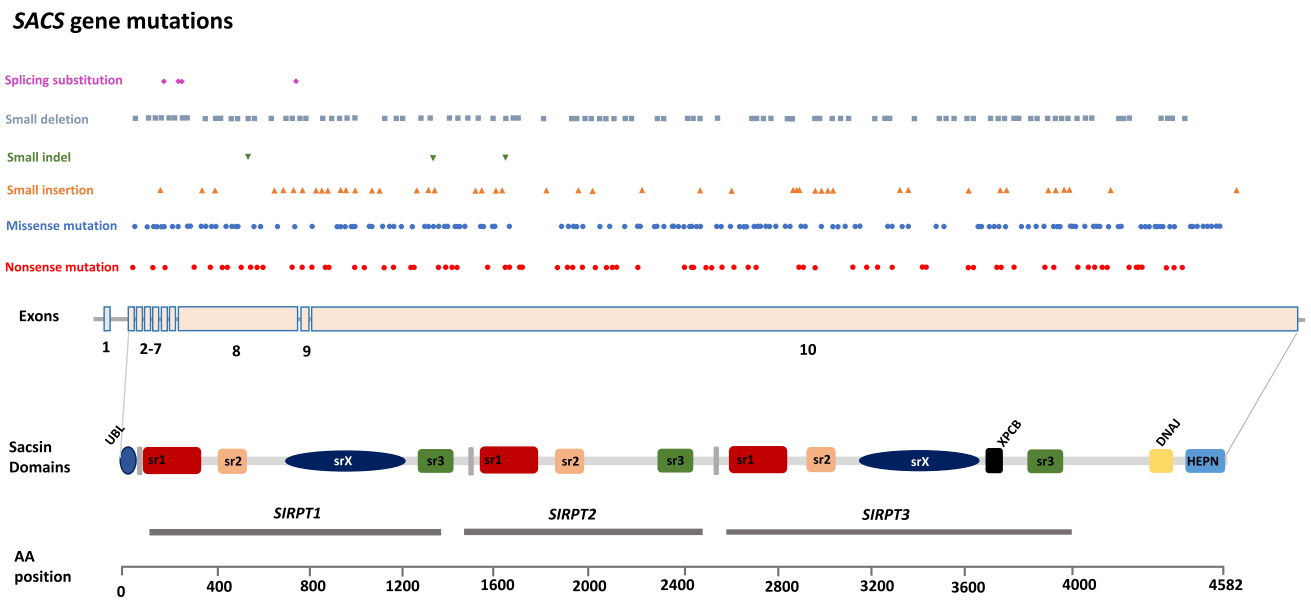

Fig. 1 SACS mutations. Graphical overview of mutations found in SACS gene (Human Gene Mutation database, except gross deletions and gross insertion). Different mutation types are marked with different symbols and colors: missense mutations = blue circles; nonsense mutations = red circles; small deletion = squares; small insertion = triangles with upward orientation; small insertion/deletion (indel) = triangles with downward orientation; splicing substitution = diamonds. Acronyms: SIRPT1 (Sacsin Internal RePeaT 1; amino acids 84–1,374), SIRPT2 (Sacsin Internal RePeaT 2; amino acids 1,444–2,443), SIRPT3 (Sacsin Internal RePeaT 3; amino acids 2,512–3,896), sr1 (Sacsin Repeat 1), ubl (Ubiquitin-like domain), sr2 (Sacsin Repeat 2), srX (Sacsin Repeat X), sr3 (Sacsin Repeat 3), XPCB (XPC-binding domain), DNAJ (DnaJ domain), and HEPN (Higher Eukaryotes and Prokaryotes Nucleotide-binding domain)

Genotype–Phenotype Correlation

The clinical spectrum in ARSACS is broad and ranges from the core phenotype, consisting of an early-onset progressive spastic ataxia with sensorimotor polyneuropathy, to less common clinical presentations and atypical phenotypes. As we will discuss, while some reports suggest potential genotype–phenotype correlations, others argue against it. This complexity is exemplified by the observation that the same genetic mutation can result in widely varying clinical presentations, both between different families (inter-familial) and within the same family (intra-familial). This variability in phenotypic expression, despite identical genetic alterations, challenges the establishment of straightforward genotype–phenotype correlations in ARSACS [3].

The clinical phenotype of ARSACS patients in Quebec tends to be more uniform, and no phenotypic differences between patients homozygous for the c.6594delT mutation and those harboring the compound heterozygous mutations c.6594delT/c.5254C > T have been described [71]. However, the average age of wheelchair dependence ranges from 17 to 58 depending on patients [62], indicating that variation exists even in a genetically similar group. Gagnon et al. (2018) described French Canadian ARSACS patients sharing the same mutation (c.6594delT) and they reported a high level of variability within disease stage with regards to clinical presentation and disease severity at different ages [72]. Interestingly, Hara et al. (2005) described two Japanese siblings with a mutation c.6543delA adjacent to the mutation c.6594delT and resulting both in a premature stop codon at amino acid residue 2202 [47]. Although the mutations are located close to each other, Japanese siblings exhibited distinct clinical features compared to ARSACS patients in the French Canadian population. These features included the absence of RNFL hypertrophy, a later onset of the disease, and the presence of dementia and external ophthalmoplegia. In addition to highlighting the variability of the disease phenotype in genetically similar patients, this observation suggests that RNFL hypertrophy is not caused by the loss of function of sacsin [47].

The clinical picture of patients born outside Quebec is often different. Although the phenotype is still quite constant, clinical variations such as later onset [10, 66], absence of spasticity [46], absence of RNFL hypertrophy [9, 12, 47, 48, 73], mental retardation [8, 47, 48, 73, 74], ophthalmoplegia [47], amenorrhea and hyperlipidemia [48] have been widely reported in non-Quebec patients. A recent systemic review describing the phenotypic characteristics of 162 ARSACS cases and excluding the well-defined French Canadian original cases, found that age of onset is mainly affected by the pathogenicity of the mutation, the presence of volume loss within the superior and middle components of the cerebellar vermis and RNFL hypertrophy [68].

Hearing impairment has been reported in ARSACS patients, generally associated with a macrodeletion in the *SACS* gene [36, 38]. Two deletions (1.54/1.5 Mb) including six genes (*SGCG*, *SACS*, *TNFRSF19*, *MIPEP*, *C1QTNF9* and *SPATA13*) were reported in a Belgian and two Italian ARSACS patients with hearing loss [36, 38]. Individuals with a microdeletion of 13q12.12 that encompasses *SACS* (and a pathogenic variant on the other allele) have a slightly different phenotype consisting of hearing loss and learning difficulties in addition to the typical features of ARSACS [36, 38]. However, another 1.33-Mb deletion encompassing six genes (*SACS*, *SGCG*, *TNFRSF19*, *MIPEP*, *C1QTNF9* and a part of *SPATA13*) was reported in a Chinese ARSACS patient without hearing loss [70]. None of these genes have a known role in hearing impairment [70]. It could be possible that a lack of about 1.5 Mb on chromosome 13q12.1 influences the expression of nearby genes related to either cochlear (that is, those encoding connexin-26 and connexin-30 on chromosome 13q12) or retinal function (connexin-46 on 13q11) [38].

In summary, several examples illustrate the complexity of establishing a clear genotype–phenotype correlation in ARSACS. This include reports of different age of onset within families harboring the same mutation [75], the suggestion that missense variants in the *SACS* gene cause a milder phenotype compared to truncating mutations [41], and the finding of extensive phenotypic overlap when comparing patients with truncating versus missense *SACS* variants [4, 7, 76]. However, the mutation location appears to be unrelated to clinical variations. Indeed, it was noticed that patients bearing mutations leading to the loss of more than 3000 residues of the sacsin protein, showed a uniform clinical phenotype compared to patients presenting with mutations affecting only the C-terminal region of the protein [16, 77]. Since all the mutations occurred upstream of both the DnaJ motif and the HEPN (Higher Eukaryotes and Prokaryotes Nucleotide-binding) domain in the C-terminal region, and presumably lead to the loss of these domains, the ARSACS phenotype was always associated with the loss of the DnaJ motif [78]. A recent study from the group of Francesca Maltecca proposed a new explanation on the complexity of establishing a genotype to phenotype correlation in ARSACS. The authors propose that a lack of genotype–phenotype correlation in ARSACS could be explained by a mechanism of co-translational ubiquitination and degradation of nascent mutant sacsin protein. Such a mechanism would prevent the synthesis of an unfolded full-length protein, leading to a loss of function regardless of the specific mutation type [31]. This finding underscores the complexity of ARSACS and the necessity for further studies to elucidate the underlying mechanisms contributing to its clinical variability. They also propose a model in which, in the presence of frameshift or nonsense variations,

SACS mRNA is degraded and in the presence of missense variations, sacsin fails to fold and undergoes co-translational degradation. More recently, the same group also showed this phenomenon to occur in peripheral blood mononuclear cells isolated from carriers of a SACS missense variant [79].

Sacsin Protein

Sacsin is a large 520-kDa protein, consisting of 4579 amino acids (GenBank accession no. NP_055178.3). Sacsin is highly expressed in the brain, with the highest levels found in the Purkinje cells followed by precerebellar nuclei and corticospinal motor neurons [80]. Sacsin is also present in fibroblasts, skeletal muscle, skin and at low levels in the pancreas [62]. The subcellular localization of sacsin in a cultured neuroblastoma-derived cell line was predominantly cytoplasmic and also in close proximity to mitochondria [28, 80].

Structurally, sacsin is a multidomain protein that comprises a ubiquitin-like (UBL) domain [80], three large sacsin repeat regions called Sacsin Internal RePeats (*SIRPT*1, 2 and 3) comprising over 80% of the protein [81], and a J-domain immediately followed by a higher eukaryotes and prokaryotes nucleotide-binding domain (HEPN) [78, 80–82] (Fig. 1). Experimental data suggest that the full-length sacsin protein exists as a dimer both in solution and in cellular environments, primarily mediated by the C-terminal HEPN domain's dimerization interface [27, 78].

The UBL domain was shown to interact with the proteosomal 20S alpha subunit C8 and to bind to the 26S proteasome, implicating sacsin in the proteosomal degradation pathway [27, 80]. The isolated sacsin UBL domain does not form a stable dimer in solution. However, it is proposed that in the context of a pre-existing dimeric protein, it may adopt the swapped dimer configuration observed in the crystal structure [27].

The three *SIRPT* internal repeats are each divided into sub-repeats namely *sr1*, *sr2*, *sr3* and *srX* [83]. The second repeat does not contain *srX,* making *SIRPT2* smaller than *SIRPT1* and *SIRPT3*. Within each of *SIRPT*, the first half of *sr1* shows sequence homology to the nucleotide-binding domain (NBD) and Hsp90 suggesting an Hsp90-like chaperone function and a quality control function [83, 84].

The J-domain (also known as DnaJ domain) is a Hsp40 homolog chaperone [80]. This domain was demonstrated to functionally substitute for the bacterial Hsp40 chaperone in an in vivo bacterial complementation assay technique. It could assist Hsp70 by enhancing ATPase activity [81, 82, 84, 85].

The HEPN domain immediately follows the J-domain at the C-terminus of sacsin. Its molecular function is unknown, but a comparative bioinformatics analysis predicted that the HEPN domain functions as either an RNase domain or a non-catalytic RNA-binding domain [86]. The.

close physical association of the HEPN and J-domains suggests that the nucleotide-binding activity of the HEPN domain may increase local ATP concentration, which could assist the J-domain in stimulating Hsp70 ATPase activity or stabilize the interaction between DnaJ, ATP, and Hsp70 (84). The HEPN domain could serve to supply nucleotides to other domains of sacsin or to its interacting partners (77). A high-resolution crystal structure of HEPN revealed that this domain forms a stable dimer that mediates sacsin dimerization and has a high-affinity binding site for GTP without GTPase activity (77, 83).

The C-terminal domain of sacsin protein also includes a xeroderma pigmentosum complementation domain (XPCB), which interacts with Ube3A ubiquitin protein ligase and ataxin-3. Mutations in the genes encoding Ube3A and ataxin-3 have been associated with autism spectrum disorders [87] and spinocerebellar ataxia type 3 [88], respectively. However, there is no evidence that mutations in the sacsin XPCB domain itself are directly associated with these disorders.

Sacsin Function

Despite the identification of sacsin's domains, its cellular role remains incompletely understood. The architecture of domains in sacsin suggests that the protein may subserve a chaperone function in protein quality control, mitochondrial dynamic regulation and neurofilaments (NF) assembly regulation roles.

Chaperone Activity

The presence of both a functional UBL and J-domain suggests that sacsin is involved in the neuronal chaperone-proteasome pathway. One role of sacsin in neurons could be to recruit other chaperones to prevent the aggregation of misfolded proteins and send terminally misfolded proteins to the proteasome for degradation [84]. Through its DnaJ domain, sacsin interacts with ligases, such as parkin, which ubiquitinate various cargos. This ubiquitination is linked to both proteasomal protein degradation and mechanisms like autophagy or mitophagy [89]. Recent preliminary data using a sacsin knockout cell line suggested that sacsin loss of function may result in impaired autophagic flux, inefficient degradation system and subsequent impairment of mitochondrial accumulation [29]. In addition, a study of sacsin chaperone activity showed some protective function against toxicity of polyglutamine-expanded ataxin-1, which is mutated in spinocerebellar ataxia type 1. *Sacsin* siRNA knockdown in SH-SY5Y cells resulted in increased toxicity in cells expressing mutant ataxin-1, whereas there was no

toxicity observed in cells expressing ataxin-1 containing a normal amount of glutamine residues [80].

Mitochondrial Dynamic Regulation

Decreased mitochondrial function is a feature of ARSACS. Bradshaw and al. showed that oxidative phosphorylation is impaired with a decreased expression of nuclear genes encoding respiratory chain complex components in both sacsin knockdown cells and ARSACS patient's fibroblasts. Sacsin has been demonstrated to interact with dynamin-related protein 1 (Drp1), and both proteins co-localize on the outer mitochondrial membrane. This interaction suggests that sacsin may regulate mitochondrial dynamics by either recruiting Drp1 and maintaining its functional conformation or directly aiding in mitochondrial fission. Experimental evidence indicates that a deficiency in sacsin activity, due to mutations, shifts mitochondrial dynamics towards fusion. This shift results in the accumulation of enlarged, swollen, and interconnected mitochondria, which are ineffective in providing energy for neurons. Consequently, this mitochondrial dysfunction leads to the degeneration of Purkinje cells [28, 90]. Moreover, patient's fibroblasts lacking sacsin showed an increased reactive oxygen species (ROS) production [90, 91].

Intermediate Filament Assembly Regulation

Disorganization of the NF network showing abnormal bundling of NFs has been described in multiple neuronal populations including Purkinje cells and cortical motor neurons both in *Sacs* knockout mice [92] and in postmortem ARSACS human brains [57, 62, 93]. Peripheral neuropathy is also associated with ARSACS, and similar NF bundles were found in cultured *Sacs*-/- spinal motor neurons and dorsal root ganglia (DRG) sensory neurons [94]. The ectopic expression of sacsin domains was shown to modify NF assembly *Sacs*-/- motor neurons. While expression of the *SIRPT2*, *SIRPT3* and HEPN domains had no or minimal effects, the expression of the J-domain was most effective in resolving NF bundles. Moreover, upregulating expression of heat shock proteins also resolved NF bundles, indicating that this endogenous chaperone system can compensate to some extent for sacsin deficiency [94].

Purkinje Cell Pathology in ARSACS

Sacsin is highly expressed in the cerebellum and particularly in the Purkinje cells, which are the principal neurons of the cerebellar cortex. Post-mortem and brain imaging studies in ARSACS patients have demonstrated a characteristic early atrophy of the anterior cerebellar vermis accompanied by Purkinje cell loss, followed by the degeneration of cerebellar hemispheres and other brain regions [50, 93]. The observed Purkinje cell loss appears to be progressive, with more advanced cases presenting with a greater amount of atrophy [57, 62].

The characteristic region-specific and progressive loss of Purkinje cells has been recapitulated in both ARSACS mouse models; *Sacs*-/- mice and the *Sacs*^{R272C} knock-in model [30, 92]. As in human patients, progressive Purkinje cell loss in these models was observed specifically in cerebellar anterior lobules from three months of age. Interestingly, within the anterior cerebellum, zebrin-negative Purkinje cells were found to be uniquely susceptible to cell death [95]. The characteristic patterning of the cerebellum relates to distinct molecular identities and physiological activity of the Purkinje neurons across the cerebellar cortex [96, 97]. Zebrin, also known as aldolase C, was one of the first molecules identified to be expressed in an evolutionary conserved striped pattern [98]. Zebrin-negative Purkinje cells have been found to have higher intrinsic firing properties [99], which requires large amounts of energy to be sustained [100]. Thus, zebrin-negative Purkinje cells may be uniquely vulnerable to the mitochondrial deficits caused by mutant sacsin as these would negatively impact the amount of energy available in these neurons. However, zebrin is only one of many molecules that are differentially expressed in Purkinje cell subpopulations [101]. Further characterization of the molecular identity of the affected Purkinje cells in ARSACS is likely to provide more clues as to their selective vulnerability in the disease.

In the *Sacs*-/- mouse model, Purkinje cell loss is observed at late stages of the disease and well after the onset of motor symptoms [92]. This suggests that it is not the loss but the dysfunction of Purkinje cells that is the major driver of disease. Indeed, functional and structural changes in Purkinje cells have been identified at early stages of disease in ARSACS mouse models. Synaptic input and firing rate of Purkinje cells as well as their output to the deep cerebellar nuclei were found to be reduced in *Sacs*-/- mice during the early stages of the disease [102]. Firing rate deficits were also observed in the *Sacs*^{R272C} mouse [30]. Notably, firing rate deficits were limited to cerebellar anterior lobules that later display Purkinje cell death, which is consistent with the hypothesis that their specific physiological properties might render these Purkinje cells uniquely vulnerable in ARSACS.

A recent proteomic and transcriptomic analysis of cerebellar tissue in *Sacs*-/- mice identified gene expression changes related to disrupted calcium homeostasis [103]. Although these experiments were done on bulk tissue and not the specifically vulnerable Purkinje cell populations, altered calcium homeostasis might be linked to the altered Purkinje cell firing properties observed in these mice.

In addition to functional changes, several structural deficits are observed in ARSACS Purkinje cells at early stages

of disease including axonal torpedoes that are characteristic of axonal degeneration [92]. Characteristic NF bundling was observed at early stages in Purkinje cell soma and dendrites [30, 92]. Disorganization of the NF network in Purkinje cells has been linked to subsequent deficits in mitochondrial localization and dynamics [92, 103]. However, others have argued that it might be the disrupted mitochondrial dysfunction as a direct result of sacsin deficiency that leads to morphological changes in Purkinje cell dendrites [28]. Recent work by Márquez et al. has demonstrated that improving mitochondrial function through the administration of mitoquinol mesylate (MitoQ) prevented the progression of motor deficits and Purkinje cell loss in the *Sacs*-/- mouse model [104]. However, MitoQ treatment did not rescue the Purkinje cell firing deficits in the *Sacs*-/- animals. This suggests that the observed mitochondrial deficits arise independently and/or downstream of the firing alterations in sacsin-deficient Purkinje cells.

iPSC modeling to study ARSACS

While much insight into the genetic and molecular mechanisms underlying ARSACS has been gained from cell and animal models, this has not yet been progressed into disease-modifying therapies for patients. Translating findings from preclinical models into therapies has been a general challenge in the field of neurodegenerative diseases and as such, there is a desire for alternative, human-centric model systems that might have more predictive value in a clinical setting [105, 106]. The advent of iPSC technology holds great promise for the modeling of central nervous system (CNS) diseases using relevant human cell types and organ-like structures from a stem cell state and without the need for invasive surgical techniques (Fig. 2). First described by Yamanaka in 2006 [107], iPSCs have the potential to be differentiated into any cell type of the body, including neurons and glia. Of particular interest, the use of patient-derived somatic cells for the generation of iPSCs followed by neural differentiation allows the investigation of disease-affected specific CNS cell types in the context of patient-specific genetic backgrounds. Building on lessons from developmental studies, protocols have been developed for the differentiation of iPSCs into neurons from a range of structures in the CNS including the cerebellum [108–110]. Protocols for the differentiation of human iPSCs into cerebellar neurons are based on methods that were first established for the differentiation of mouse and human embryonic stem cells [111–114]. These protocols were subsequently adapted for iPSCs, allowing the culture of both, dissociated cerebellar neurons and three-dimensional cerebellar organoids [115–119]. Human iPSC-derived Purkinje cells can be obtained under either culture conditions and, albeit still relatively immature, show expression of specific markers, characteristic morphological features and electrophysiological hallmarks [116, 118–122].

Recently, researchers have begun to investigate the effects of sacsin mutations in iPSC-derived motor neurons and Purkinje cells from patients with ARSACS (122). Purkinje cells differentiated from ARSACS patient iPSCs displayed some disease features including decreased levels of sacsin and NF aggregation along neurites. Characteristic NF bundling that has been observed in human ARSACS brain and ARSACS mouse models (see above) was not detected in the iPSC-derived models, possibly due to the short time of the differentiated neurons in culture. While there was no significant difference in Drp1 expression in the patient-derived cells compared to controls, potential mitochondrial deficits were not further investigated. This first study demonstrating that Purkinje cells can be generated from ARSACS patient-derived iPSCs and display some of the characteristic ARSACS pathological signatures open exciting new avenues for research into the specific effects of sacsin mutations on human Purkinje cell function. It will be interesting to determine whether the functional deficits that have previously been described in ARSACS fibroblast and mouse models can be recapitulated in iPSC-derived Purkinje cells. Moreover, important insights might be gained in understanding the mechanisms driving human Purkinje cell vulnerability in ARSACS by using more advanced cerebellar organoids combined with the assessment of neuronal activity [116, 122]. Further technological advances in the iPSC field such as the addition of vasculature and the culture of assembloids are poised to offer new ways of studying complex neurological diseases [123]. Ultimately, the identification of patient-specific cellular phenotypes using iPSC-derived models promises to advance the screening for therapeutics and personalized medicine for ARSACS.

Therapeutic Options in ARSACS

The therapeutic approaches for ARSACS are currently limited to supportive care delivered by the multidisciplinary team, which partially improves some cerebellar and non-cerebellar manifestations but fails to halt the progression of the disease. Treatment is particularly challenging in ARSACS, where complex co-morbid damage to extracerebellar neural systems places additional disease burden on the ataxia patients, including pyramidal, visual, cognitive, and peripheral nerve damage [2]. Until now, there are two kinds of therapeutic interventions that have been evaluated in ARSACS patients. These consist of occupational and physical interventions for which the findings need to be confirmed by means of future clinical trials. For example, a recent four-week controlled pilot study on seven ARSACS patients using home-based speech treatment software program showed preliminary proof-of-concept evidence such

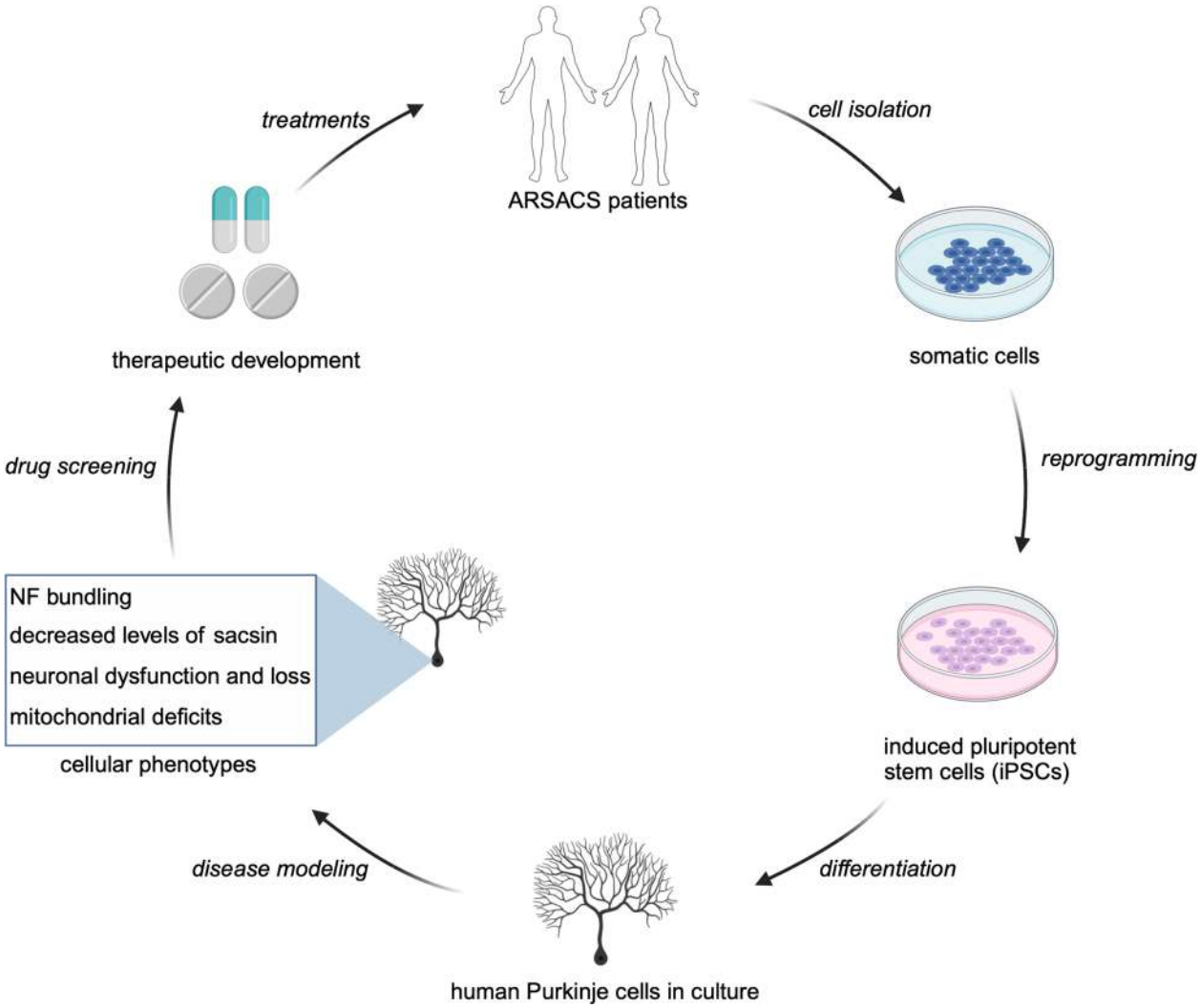

Fig. 2 iPSC-based disease modeling for ARSACS. Somatic cells such as fibroblasts or blood cells are obtained from ARSACS patients and reprogrammed into induced pluripotent stem cells (iPSCs) through the overexpression of pluripotency-inducing transcription factors. Patient and control iPSCs are differentiated into disease-relevant cell types including Purkinje cells following protocols that mimic cerebellar developmental cues in vitro. Cell culture models (both dissociated and organoid cultures) can be used for the investigation of ARSACS cellular phenotypes and as a platform to screen for treatments that will improve patients' quality of life. Figure created with BioRender.com

as improvements in intelligibility (ability to be understood) during connected speech tasks and enhanced naturalness (degree to which individuals sound "different" from healthy peers), suggesting that ataxia-tailored speech treatment might be effective in cerebellar degenerative diseases [43].

In 2018, Audet and coworkers published the first study aimed at assessing the impact of an exercise program on the physical fitness and functional capacity of patients affected by ARSACS [124]. An eight-week specific training program, including physical activities, strength-power and aerobic training were conducted in twelve early-onset ARSACS patients. This preliminary study demonstrated that physical training does not have a deleterious effect on their musculoskeletal and/or cardiorespiratory functions. In addition, it showed improvement of physical fitness and functional capacity of ARSACS patients and provided an encouraging sign that it is possible to help these patients maintain or regain their autonomy. In the early stages of the disease, oral medications like baclofen can help control spasticity [125–127]. Exercise may help avoid tendon shortening and joint contractures, which could help delay major functional disabilities until severe muscle weakness or cerebellar ataxia happens [124]. Urinary urgency and incontinence may be controlled with low doses of amitriptyline or oxybutynin [125].

Conclusion

ARSACS is a complex neurodegenerative disorder caused by mutations in the *SACS* gene. The resulting sascin deficiency has been shown to impair protein quality control, disrupt the mitochondrial dynamic network and NFs, and causes neuronal cell death. These processes affect the morphology and function of Purkinje cells, ultimately causing Purkinje cell loss and cerebellar atrophy. Purkinje cell loss is causing ataxia in ARSACS but other neuron populations can be affected, leading to additional neurological symptoms outside the scope of this review. Overall, the resultant phenotype is characterized by spasticity, ataxia, polyneuropathy, retinal changes and—in some cases—late cognitive decline and hearing loss. A disease-modifying treatment is currently lacking, and symptomatic management trials are being evaluated. Over almost 25 years, many studies have been conducted in order to understand the molecular and cellular phenotype of ARSACS and how this relates to the genotype. These efforts have allowed a comprehensive characterization of the cell biological role of sacsin and the underlying pathology in ARSACS and, together with recently developed human iPSC models, set a promising scenario for future therapeutic trials.

Author Contributions IHS : Writing – original draft MB : Writing – original draft, project administration, MB has contributed equaly with IHS to this work,VMZS :Writing – original draft, LR : review & editing, EBEB : Writing – review & editing, ND : Writing – review & editing

Funding No funding was involved in the preparation of this manuscript.

Data Availability No datasets were generated or analysed during the current study.

Declarations

Ethical Approval Not applicable.

Competing Interests The authors declare no competing interests.

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