REVIEW ARTICLE

Autosomal Recessive Spastic Ataxia of Charlevoix-Saguenay (ARSACS): a once obscure neurodegenerative disease with increasing significance for neurological research

Xinlu (Crystal) Li[1]*

[1] Department of Biochemistry, McGill University, Montreal, QC

*Email Correspondence:
xinlu.li@mail.mcgill.ca

Keywords:
Autosomal Recessive Spastic Ataxia of Charlevoix-Saguenay (ARSACS): A complex early-onset neurodegenerative disease found in high prevalence in the population of the Charlevoix-Saguenay region in Quebec due to founder effect.
Founder Effect: Significant reduction in genetic variability and increase in homozygosity level in a population descending from a small founder population geographically isolated from its ancestral population.
Chaperones: Important protein-folding quality control machineries that promote and maintain the proper folding of cellular proteins and target misfolded proteins for degradation.
Mitochondrial Fission/Fusion Dynamics: The delicate balance between the fission and fusion of mitochondria that is essential for its quality control, function and distribution in the cell.

Abstract

Background: Autosomal Recessive Spastic Ataxia of Charlevoix-Saguenay (ARSACS) is a rare cerebellar ataxia occurring in the Charlevoix-Saguenay population in Quebec with high incidence as a result of founder effects. Following the discovery of the gene responsible for the disease, many other patient groups have been identified worldwide and the characterization of the gene product, sacsin, has unveiled similarities between the pathogenic mechanism of ARSACS and those of other major neurodegenerative disease.

Summary: The core symptoms of ARSACS consist of a triad of early-onset cerebellar ataxia, peripheral neuropathy and spasticity, which is accounted by degeneration of Purkinje neurons. The gene responsible for the disease is located on chromosome 13q11 and encodes for the chaperone sacsin. Drp-1, a GTPase crucial for regulating mitochondrial fission/fusion dynamics, has been identified as a potential substrate of sacsin, suggesting a link between the pathogenic mechanisms of ARSACS and prevalent neurodegenerative diseases such as Alzheimer's, Parkinson's and Huntington's diseases.

Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is a complex hereditary neurodegenerative disorder characterized by a triad of early childhood-onset cerebellar ataxia (uncoordinated movements due to defects in the cerebellum (1)), peripheral neuropathy (peripheral nerve damages resulting in a variety of sensorimotor symptoms (2)) and pyramidal tract signs (traits such as spasticity, abnormal reflexes and loss of ability to perform fine motor movements due to defects in the neurons relaying signals from the cerebral cortex or midbrain to the spinal cord (3)) (4). The disease was initially named after its high incidence in the French-Canadian population of Charlevoix-Saguenay region in northeastern Quebec as a result of founder effect (5) but was later also discovered in many other regions and ethnic groups worldwide (6). *SACS*, the gene whose mutations are responsible for the ARSACS' symptoms, was identified by Engert *et al.* in 2000(7). The gene product, sacsin, constitutes one of the largest known human proteins and bioinformatics and biochemical characterizations of peptide domains in sacsin suggest that it performs its cellular function by integrating the ubiquitin-proteasome system and the chaperone pathways (6). Furthermore, a recent study of a transgenic sacsin-knockout mouse model associated the loss of sacsin's function with the disruption in neuronal mitochondrial fission/fusion dynamics (8). This finding in turn significantly elevated the research significance of ARSACS, since the disruption in neuronal mitochondrial dynamics underlies many prevalent neurodegenerative disorders such as Alzheimer's, Parkinson's and Huntington's diseases (9). Therefore, the purpose of this review is to delineate major clinical, genetic and biochemical findings on ARSACS to highlight the increasing research significance of this once obscure disease.

Clinical Features of ARSACS

The clinical features of ARSACS were first reported in 1978 by Bouchard *et al.* in a group of French-Canadian patients in Quebec whose families mostly originated from the Charlevoix-Saguenay region, where around forty families had migrated from Quebec city in the 1600's (5). The features were "remarkably homogenous" and were inherited in an autosomal recessive fashion, distinguishing the disease from most other classes of ataxia disorders except for Friedreich's Ataxia (also autosomal recessive) (5). Subsequent electrophysiological studies (i.e. electromyography and nerve conduction studies) (10), as well as brain biopsy studies using light microscopy (11), computed cerebral tomography (12) and magnetic resonance imaging (13), distinguished ARSACS from FA by pinpointing the anatomical origin of ARSACS to degeneration of the Purkinje neurons in the cerebellum, the region responsible for motor coordination and balance.

Clinical manifestation in all ARSACS patients reported by Bouchard *et al.* begins with gait unsteadiness observed in toddlers (12 to 18 months old) learning to walk (5). Spasticity (abnormal tensing of muscles) subsequently develops in the peripheries of the body and progressively increases in severity over time, accompanied by peripheral neuropathy (neuronal degeneration) and amyotrophy (muscle wasting) (5). The progression of the symptoms is much slower than in other forms of ataxia and severity of the symptoms can remain stable for a long period of time and suddenly worsen over the course of a few years (normally during early adulthood) (5). The individual is usually wheelchair-bound by the age of thirty or forty with life expectancy reduced to about fifty years of age (5).

The ARSACS Gene

A major turning point in ARSACS research occurred when *SACS*, the gene responsible for the disease, was identified by Engert *et al.* in 2000 via a series of haplotype and linkage analyses and physical gene mapping among French-Canadian ARSACS patients (7). The gene was located on the long arm of chromosome 13 (13q11) was found to contain a 12,794-base pair (bp) exon, the largest that had been identified in vertebrates (7). Furthermore, sequence analysis showed extensive conservation between mouse and human *SACS* (14). Population screening revealed a single base-pair deletion at position 6,594 (6594delT) in most ARSACS patients and a nonsense mutation at position 5254 (5254 C-T) in a small proportion of ARSACS patients (7). Both mutations were predicted to lead to the production of truncated protein products with their functions completely abolished (7). These findings, along with the migration history of the Chalevoix-Saguenay (CS) region, attributed the prevalence of the disease amongst the CS population to the increased homozygosity level in the population due to founder effect (7). In addition, though the large size of the *SACS* exon makes sequencing an overly expensive and time-consuming means for diagnosis, several fragment-based sequencing techniques were developed that could more efficiently identify patients harboring specific mutations in *SACS* (15, 16).

"ARSACS Goes Global"

The identification of *SACS* and advances in genetic linkage study tools resulted in the diagnosis of an increasing number of ARSACS patients in many other regions of the world. The trend started as early as in 2000, when several Tunisian patients previously diagnosed with another class of cerebellar ataxia were found to harbor mutations at loci linked to *SACS* (17). Since then, many other ARSACS patient groups have been identified in Italy (18-20), Japan (21-27), Britain (28), France (29-31), Spain (32-34), Netherlands (35) and Belgium (16, 36, 37). All identified ARSACS patients shared the core symptoms of ARSACS (early onset spasticity and ataxia increasing in severity over time accompanied by dysarthria/slurred speech, nystagmus/vision impairment and amyotrophy/muscle wasting) despite a few minor clinical variations, such as later onset and the lack of retinal striation or mental retardation. In fact, the trend of globalization is not unique to ARSACS. *Gomez et a*l. pointed out in an editorial that the "spreading" of ARSACS embodies a prevailing trend in the realm of neurological diseases: Several recessive neurological disorders originally considered to be restricted to a particular ethnic group or geographical location were later found to be present in many other populations around the world and Gomez *et al.* predicted that more neurological diseases were to follow suit (38).

With the identification of ARSACS patients worldwide, an increasing number of mutations in *SACS* were also revealed. To date, over 70 *SACS* mutations have been discovered in 13 countries (6). It is interesting to note that founder effect seems to play a role only in the Quebec patient population (6). Most of the currently identified *SACS* mutations are frameshift or nonsense point mutations residing within the exon (13, 16, 18, 19, 21-24, 36, 39-44) identified by *Engert et al.* However, the discovery of several point mutations and macrodeletions upstream of the exon in ARSACS patients (13, 16, 20, 25, 36, 40, 45) led to the upstream extension of the putative *SACS* gene to contain eight additional exons (6). In 2009, reverse transcriptase-polymerase chain reaction (RT-PCR) analysis of human brain mRNA, in conjunction with northern blot analysis and in-situ hybridization on a number of human tissues, confirmed that the *SACS* encodes a nine-exon transcript that is expressed in a variety of tissues including fibroblasts, skeletal muscle and the brain, particularly in the Purkinje cells of the cerebellum (46).

The Sacsin Protein

The gene product of SACS in human is a relatively large protein (~4500 amino acids) named *sacsin* (8). Based on its amino acid sequence, the secondary structure of sacsin has been predicted to consist of

two leucine zippers (a leucine-rich motif known to mediate protein dimerization (47)), three coiled coils and one hydrophilic domain in the carboxyl-terminal half of the peptide (7). To date, bioinformatics analyses have identified five distinct peptide domains in sacsin and their respective functions have been characterized to varying extents (See Fig. 1 for the location of the domains and selected ARSACS-causing mutations in sacsin) (48).

The N-terminal end of sacsin contains an ubiquitin-like domain (UbL) that was first reported by Parfitt *et al.* in 2009. Co-immunoprecipitation experiments have demonstrated the ability of N-terminal region of sacsin (up to and including the UbL domain) to directly interact with the 19S cap of the 26S proteasome, the primary protein-degradation machinery in eukaryotic cells (46). Nevertheless, this does not exclude the possibility that the observed interaction between the N-terminal region of sacsin and the proteasome could be due polyubiquitination-mediated targeting of sacsin to the proteasome instead of direct interaction between the sacsin-UbL domain and the proteaosome (46).

Towards the C-terminal direction, the next discernable structural entity consists of three SRRs (sacsin repeat regions), each of which is a supra-domain (complex of multiple independent peptide domains exerting synergistic functions) consisting of an N-terminal domain homologous to the Hsp90 (a chaperone)-ATPase domain and a hydrophobic C-terminal domain of unknown function (49). The sacsin-SRRs were first described by Engert *et al.* upon their identification of the SACS gene in 2000 and the ATPase activity of the N-terminal domain of the first SRR was verified by Anderson *et al.* to be similar to that of yeast Hsp90 (with turnover rates of 2.5/min and 1/min, respectively) (7, 49). In addition, ARSACS-causing missense mutations D168Y and R2703C in the first and third SRRs were both shown to abolish the

ATPase activity of the entire protein, thereby confirming the non-redundancy in the functions of the three SRRs in sacsin (15, 18). Bis-1-anilinoaphthalene 8-sulfonate (a molecule that becomes highly fluorescent when interacting with exposed hydrophobic surface of proteins) fluorescence measurement experiment revealed substantially increased surface hydrophobicity of the mutant SRR compared with the wild-type, indicating disruption in the normal folding of the supra-domain. However, since the study also demonstrated that the mutant protein remained soluble in the cell, the mutant SRR is most likely still able to fold to the extent of avoiding aggregation but with structural alterations significant enough to abrogate its ATPase activity (49).

The domain C-terminal to the third SRR was identified as XPCB domain by Kamionka *et al.* in 2004 (50). The sacsin XPCB-domain was found to exhibit 35% sequence identity with the hHR23 XPCB domain (50). hHR23 had been known to bind to and stabilize XPC, initiator of the global genome repair pathway. The hHR23/XPC complex is implicated in group C xeroderma pigmentosum (XP-C), a rare nucleotide

excision repair deficiency disorder characterized by markedly increased photosensitivity and propensity for developing UV-induced skin cancer (50).

Interestingly, the hHR23 protein also contains an UbL domain, which has been shown to interact with the 19S regulatory subunit of proteasome *in vivo* (51). The 19S subunit of the proteasome has been found to exhibit independent chaperone-like activity, which was suggested to help maintain the proper conformation of the highly hydrophobic XPC molecule on XPCB (49). Since sacsin also contains an UbL domain, the sacsin-XPCB domain most likely function in a similar fashion as hHR23 and delivers the binding partner of sacsin to the proteasome or chaperones. However, the binding partner as well as the cellular activity of the sacsin-XPCB is yet to be identified.

The penultimate domain at the C-terminus of sacsin is the J-domain, first reported by Parfitt *et al.* in 2009 (46). J-domains are integral components of the DnaJ/Hsp40-class chaperones and mediate the interaction between Hsp40 and Hsp70 to enhance Hsp70's ATPase and substrate-binding activity (52). The Hsp40-Hsp70 complex constitutes the primary cellular machinery in assisting protein folding and quality control (52). Sequence alignment between sacsin- and Hsp40/DnaK-J-domains revealed a 60% similarity over 30 residues and the ability of the sacsin J-domain to recruit and stimulate Hsp70 and stimulate its chaperone activity was verified by in vivo complementation assays in bacteria (46). To date, the only identified ARSACS-causing mutation in the sacsin J-domain is the compound heterozygous mutation K1715X/R4331Q, with the arginine occupying a position known to be critical for the function of J-domains in most proteins (15). Note that since the R4331Q mutation alone did affect the ability of the sacsin J-domain to rescue the function of mutant bacterial DnaJ (Hsp40 class chaperone) with nonfunctional J-domain, both mutations most likely need to be present to exert a perceptible effect on the function of sacsin J-domain (46). The fact that K1715X
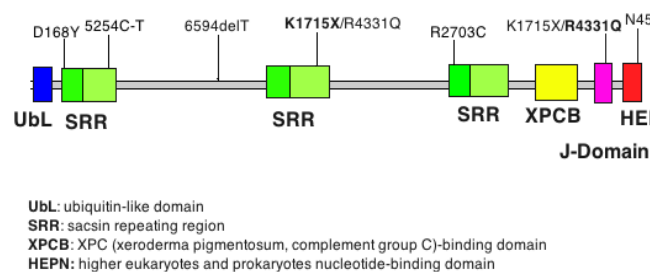
Fig. 1
A schematic diagram of sacsin showing the known structural domains and selected ARSACS-causing mutations. Note that the numbers in 6594delT and 5254C-T represent the position of the mutated nucleotide whereas the numbers in the rest of the mutations presented in the diagram indicate the position of the mutated amino acid.

resides within the second SRR suggests that the function of the sacsin J-domain may require its interaction with SRR, whose ATPase activity may contribute to J-domain's recruitment and stimulation of Hsp70.

Finally, the 110-residue region at the very C-terminus of sacsin constitutes the higher eukaryotes and prokaryotes nucleotide-binding (HEPN) domain, first reported by Crynberg *et al.* in 2003 (48). As its name suggests, the HEPN domain is known to bind nucleotides and a number of HEPN domain-containing proteins, such as the kanamycin nucleotidyl transferase family proteins, which are implicated in bacterial antibiotic resistance (48). The ability of the HEPN domain of human sacsin to bind to various nucleotides (i.e. ATP, ADP, GTP, ADP and etc.) was verified by Kozlov *et al.* in 2011 via nuclear magnetic resonance (NMR) spectroscopy and isothermal titration calorimetry (ITC) (53). Crystal structure of the human sacsin-HEPN domain revealed that it exists as a dimer, which interacts with nucleotides electrostatically via a symmetric binding pocket formed at the dimer interface (53). The ARSACS-causing mutation N4549D at the dimer interface was shown to hinder the proper folding and the dimerization of the domain, which is likely responsible for its loss of function (53). Interestingly, the sacsin-HEPN domain did not exhibit any GTPase or ATPase activity (53) and many suggested that the sacsin HEPN domain most likely functions as an "energy stockroom" to supply nucleotides (particularly GTP and ATP) for sacsin's chaperone-like activity (48, 53). However, there is a lack of direct evidence demonstrating the utilization of the HEPN-recruited nucleotides by sacsin or its binding partners and the "energy stockroom" notion therefore remains largely theoretical.

Fitting Together Pieces of the Puzzle

Experimental evidence accumulated to date strongly suggests that the sacsin protein functions as a Hsp40 family chaperone in neurons (54). The RegA region (N-terminal region of sacsin including UbL domain, the first SRR domain and the linker region preceding the second SRR domain) in human sacsin has been demonstrated to be capable of maintaining denatured firefly luciferase (FLuc, model protein-folding client in chaperone studies) in a soluble state and moderately facilitate its refolding independently from ATP, an activity characteristic of Hsp40 family chaperones (54). In addition, Reg A has been shown to be able to enhance the yield of the bacterial Hsp70 chaperone system in the absence of DnaJ (49). This observation, in addition to the presence of a J-domain in the sacsin protein, signifies that the structure of sacsin is well-suited for delivering misfolded or denatured proteins to the Hsp70 system (54). In addition, siRNA studies demonstrated that sacsin is able to reduce the number and sizes of the insoluble nuclear ataxin-1 inclusion bodies (hallmark of many cerebellar ataxia disorders) in neurons and further corroborated sacsin's potential role as a neuronal chaperone (46).

Recently, Girard *et al.* reported a transgenic sacsin-knockout (KO)

mouse model that helped to elucidate several important aspects of the underlying pathogenic mechanism of ARSACS (8). The mouse model showed normal brain morphology and phenotypes (compared to control mice) at birth but displayed progressively decreasing number of Purkinje neurons from 120 days after birth, consistent with autopsy reports of significantly reduced number of Purkinje neurons in ARSACS patients (8). This observation confirmed age-dependent Purkinje neuron degeneration as one of the main pathological features of ARSACS and excluded developmental causes from the etiology of the disease. In addition, immunofluorescence experiments revealed sacsin's intracellular localization on the cytoplasmic side of mitochondria and small interference RNA (siRNA)-mediated knockdown of sacsin demonstrated remarkable changes in the morphology, function and transportation of mitochondria in sacsin-deficient neurons (8). Fluorescence recovery after photobleaching (FRAP) confirmed that mitochondria in sacsin-knockdown cells are more elongated and interconnected, indicating defective mitochondrial fission; fluorescence measurement with tetramethylrhodamine methyl ester (TMRM) showed a significantly weaker membrane potentials of these mitochondria, signifying diminished oxidative phosphorylation activity and ATP production (53). Immunofluorescence with mitochondrial marker in sacsin-knockdown neurons showed that the mitochondria in these cells fail to be delivered effectively along the dendrites but cluster within the soma and the dendritic region proximal to the soma of the neurons (8). Furthermore, the decrease in the number of dendrites as well as the thickened and disorganized morphology of the remaining dendrites in these neurons suggest that the defect in mitochondrial transport had led to degeneration of these neurons (8).

Interestingly, dynamin-related protein 1 (Drp1), a GTPase crucial for mitochondrial fission, has been found to co-immunoprecipitate and partially colocalize with sacsin in neurons (8). In light of sacsin's potential role as a molecular chaperone, Girard *et al.* suggested that sacsin most likely serves to maintain the properly-folded, functional conformation of Drp-1 either directly or indirectly by recruiting the Hsp70 family chaperones (8). Absence of sacsin from cells would therefore increase the number of misfolded, dysfunctional Drp-1 and subsequent disruption of the fission/fusion dynamics of mitochondria. The ensuing impairments in mitochondrial function and transport would in turn result in degeneration of neurons (8). The effect would be particularly detrimental to Purkinje neurons, in which the maintenance of extensively branched dendrites heavily depends the energy produced by mitochondria as well as their ability to be transported effectively along the dendrites (35). The end-result would be impairments in the cerebellum's ability to regulate and relay signals to the peripheries of the body, leading to a variety of sensory-motor symptoms such as those observed in ARSACS patients.

Disruption in the mitochondrial fission/fusion dynamics is a common theme among many neurodegenerative diseases (9). In addition, Drp1 is suggested to be implicated in many of these diseases as

well (9). Mitochondria in the neurons of Alzheimer's disease patients exhibit a spherical and fragmented morphology, which is accompanied by significant changes in the expression levels of mitochondrial fission and fusion proteins (55). This is thought to be a result of amyloid β fibril accumulation in the neurons and is hypothesized to be a Drp-1 mediated process (55). Similarly, aberrant mitochondrial fragmentation is also observed in the neurons of Parkinson's disease (PD) patients and leucine-rich repeat kinase 2 (LRRK2), the protein implicated in the most common form of PD, is hypothesized to regulate mitochondrial fission by interacting with Drp1 (56). Furthermore, a recent study attributed excessive mitochondrial fragmentation observed in neurons of Huntington's disease patients to the overstimulation of Drp-1 by huntingtin aggregates (57). Therefore, there seem to be a strong link between the pathogenic mechanisms of ARSACS and these neurodegenerative diseases, adding another level of significance to the study of ARSACS (58). In fact, it would be interesting to examine whether knocking-out or downregulating sacsin expression in neurons presented with these neurodegenerative diseases would be able to partially restore the normal mitochondrial fission/fusion dynamics.

However, disruptions in mitochondrial morphology is a common response to possible defects in a variety of mitochondrial proteins as well as the accumulation of oxidative stress (9). It is possible that sacsin affects the "health" of the mitochondria indirectly through another binding partner (or even another mechanism). Therefore, it is necessary to confirm the direct interaction between sacsin and Drp1 and its downstream cellular effects, as well as to clarify the functions of sacsin's domains and their mechanism of interaction, in order to confirm the mitochondrial model of ARSACS pathogenesis and its relation to other neurological diseases.

Conclusions

Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS), whose primary symptoms consist of a triad of early-onset cerebellar ataxia, peripheral neuropathy and pyramidal tract signs (16), was first reported to occur in high prevalence in the Charlevoix-Saguenay population in Quebec as an evolutionary product of founder effect (5). Additional patient populations have since been identified in over thirteen countries worldwide (38). *SACS*, the gene implicated in the disease, has been mapped to the long arm of chromosome 13 (13q11) and has been found to encode the 437kDa protein sacsin, one of the largest human proteins ever identified (7). To date, over 70 ARSACS-causing missense mutations, non-sense mutations and macrodeletions have been found in patient populations identified so far and the majority of these mutations reside within the large exon 9 (6). Bioinformatic studies have identified five distinct domains within the sacsin protein, namely (from the N- to C-terminus) the UbL domain, SRR domains, XPCB domain, J-domain and HEPN domain. Furthermore, the available structural and functional information on

sacsin's domains, as well as sacsin's intracellular localization pattern, suggest that sacsin most likely functions as a special class of Hsp40 chaperone that helps to regulate the dynamics of mitochondrial fission/fusion in the Purkinje neurons of cerebellum by interacting with Drp1 (8). Since disruptions in mitochondrial fission/fusion dynamics via Drp-1 mediated processeses is considered to be the underlying cause of many neurodegenerative diseases such as Alzheimer's (55), Parkinson's (56) and Huntington's diseases (57), the study of the once obscure disease ARSACS holds great importance in understanding the pathogenic mechanisms of neurodegenerative diseases and their relations to mitochondrial functions in neurons.

Acknowledgements

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