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Insights into *SACS* pathological attributes in autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS)☆

Khaled A. Aly, Mohamed Taha Moutaoufik, Mara Zilocchi, Sadhna Phanse and Mohan Babu

Abstract

Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is a rare early-onset neurodegenerative disease caused by mutations in the *SACS* gene, encoding Sacsin. Initial functional annotation of Sacsin was based on sequence homology, with subsequent experiments revealing the Sacsin requirement for regulating mitochondrial dynamics, along with its domains involved in promoting neurofilament assembly or resolving their bundling accumulations. ARSACS phenotypes associated with *SACS* loss-of-function are discussed, and how advancements in ARSACS disease models and quantitative omics approaches can improve our understanding of ARSACS pathological attributes. Lastly in the perspectives section, we address gene correction strategies for monogenic disorders such as ARSACS, along with their common delivery methods, representing a hopeful area for ARSACS therapeutics development.

Addresses

Department of Biochemistry, University of Regina, Regina, Saskatchewan, Canada

Corresponding author: Babu, Mohan (mohan.babu@uregina.ca)

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Keywords

ARSACS, Chaperone-like activity, Disease models, Intermediate filaments, Mitochondrial dynamics, Quantitative omics, Sacsin, *SACS* mutations.

Introduction

Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is an early-onset neurodegenerative disease characterized by loss of Purkinje cells in the cerebellum, peripheral neuropathy, hypermyelination, and thickening of retinal fibers [1]. Mutations in the *SACS* gene, comprising 10 exons, cause ARSACS [1]. The first *SACS* exon is a non-coding component, while the remaining exons are coding regions located within the long arm of chromosome 13. The *SACS* gene encodes Sacsin, a large multidomain protein (~520 kDa) in humans, posing difficulties in Sacsin biochemical characterization. ARSACS was first described in the Charlevoix and Saguenay regions of Quebec (Canada) from which the disease name has been derived. Owing to founder effect, 96% of ARSACS patients in these Canadian regions share one (c.8844del) common mutation [2]. To date, over 400 *SACS* mutations have been discovered, which are thought to be pathogenic as per the data retrieved from CLinVar (archive of human genomic variants) database.

Annotating Sacsin function from sequence homology analysis

Sacsin function has been initially predicted from its sequence and putative domain compositions. Sequence analysis shows that Sacsin comprises an internal repeat region, termed *SACS* repeated region (SRR), that occurs thrice throughout the protein length (Figure 1a). The SRR region shares homology with the heat shock protein 90 (HSP90), suggesting that SRR mediates chaperone-like activity [3]. While classical HSP90 hydrolyzes ATP with its activity inhibited by compounds such as geldanamycin and radicicol [4], these inhibitors fail to halt ATP hydrolysis in the case of Sacsin, indicating that despite their predicted homology, SRR and HSP90 respond differently to ATP hydrolysis inhibitors [3]. Subsequent reports undertook computational and sequence alignment approaches to expand SRRs into larger domains. Three large internal repeats were thus named Sacsin-internal repeats (SIRPTs 1–3), since they share repeated subdomains [5]. The remaining three smaller domains include ubiquitin-like (Ubl), J-domain, and higher eukaryotic–prokaryotic nucleotide binding (HEPN) domain (Figure 1a).

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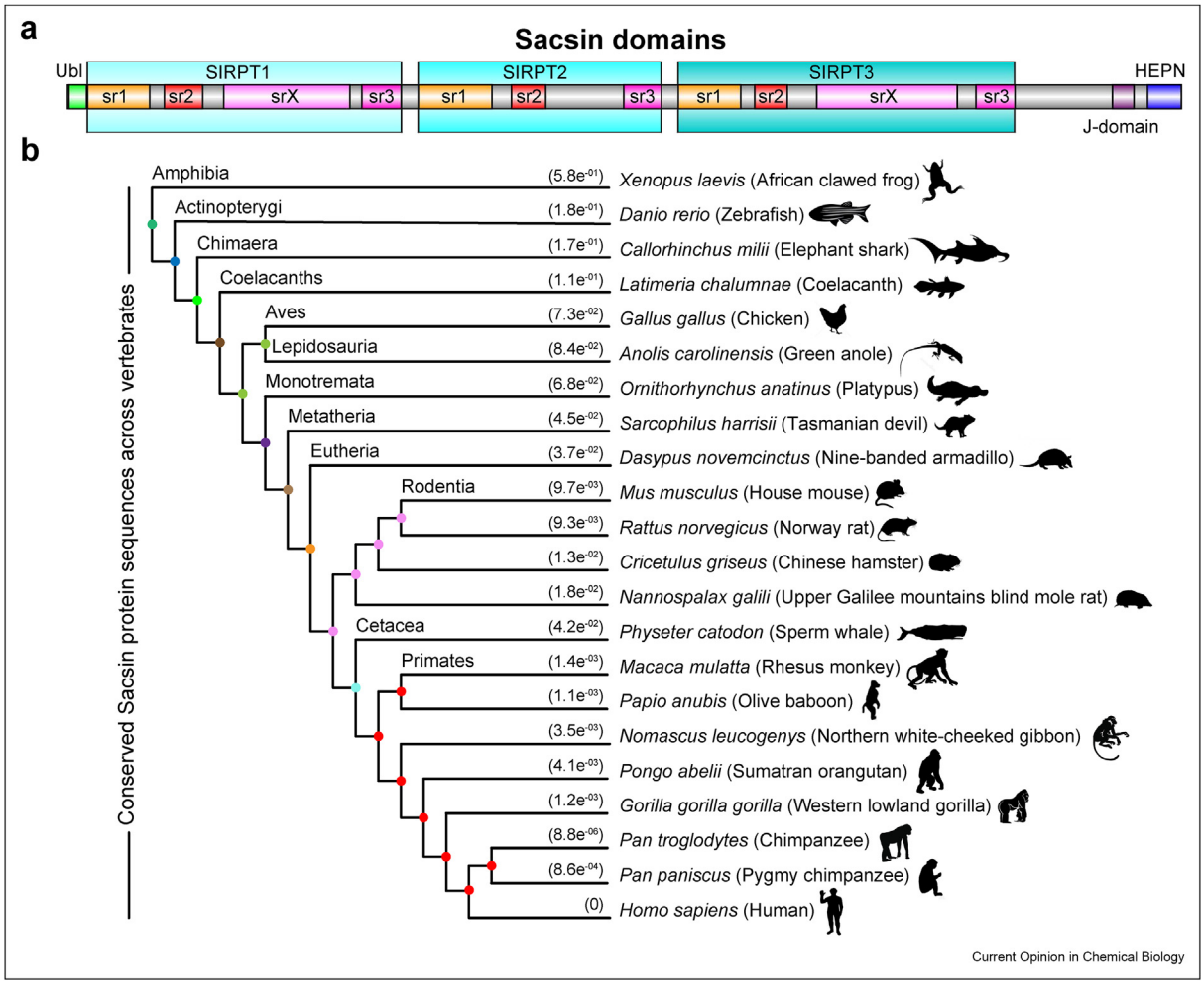
Figure 1



Sacsin protein conservation. (**a**) Domain composition of Sacsin. (**b**) Sacsin conservation patterns across vertebrates. Phylogenetic reconstruction, implying evolutionary distances between organisms spanning classes or orders under the subphylum Vertebrata for Sacsin. Values in parenthesis represent phylogenetic distances.

Evidence suggests that *SACS* evolved from duplication and fusion events of simpler homologs of the SIRPT superdomain coding region [3]. It thus becomes conceivable that Sacsin functions in an integrated multidomain fashion. Notably, Sacsin with its 3 distinct SIRPT domains is exclusively found in vertebrates within the animal kingdom [5] (Figure 1b). While having ancestral lineages to single domain SIRPT-like proteins, it appears that over the course of evolution the 3 SIRPT domains gave rise to Sacsin upon extensive fusion and duplication events.

Experimental support to Sacsin function

In addition to computationally annotating Sacsin function [2,3], earlier experimental reports describe Sacsin as a chaperone due to its tendency to inhibit ataxin-1 inclusions [6]. This was based on the discovery of a truncated J-domain that acts as HSP40, which can complement for the loss of endogenous HSP40 in the Gram-negative bacterium, *Escherichia coli*. Despite this evidence, inclusion bodies or protein aggregates of ataxin-1 have not been described in neurons of ARSACS patients in post-mortem studies [7,8], nor can subcellular assemblies be detected in *SACS* siRNA knockdown [9] or CRISPR-mediated knockout cells [10]. Besides, *SACS* loss-of-function leads to neurofilament network abnormalities in the brains of *Sacs*$^{-/-}$ mice, ARSACS patient fibroblasts, and also in engineered *Sacs*$^{-/-}$ cell lines [11–14]. Confocal imaging of ARSACS patients' dermal fibroblasts show abnormal vimentin cytoskeleton that appears as bundles of perinuclear accumulations, and the same phenotype was observed with different *SACS* mutations, which validates earlier reports showing unusual neurofilament accumulations in *Sacs*$^{-/-}$ primary

neurons [14]. Similar to *SACS* knockout mice that display early-onset ataxia with neurofilament bundling in many neurons, mice carrying the R272C missense homozygote mutation, reported in ARSACS, also display the same *SACS* knockout bundling phenotype [12], confirming a link between neurofilament accumulations and *SACS* loss-of-function.

Ectopically expressing full-length wild-type *SACS* to complement its loss-of-function results in resolving the bundling phenotype in motor neurons, revealing an essential role for Sacsin in regulating neurofilament dynamics. In addition, the neurofilament bundling patterns caused by *SACS* mutations can be potentially reversed [13]. In support of this, the role of each Sacsin domain in *de novo* assembly of neurofilaments was examined in $SW13^{vim-}$ model cells, since they lack endogenous intermediate filaments. Individual myc-tagged Sacsin domains co-produced with both, the neurofilament light and heavy polypeptide proteins, revealed an important role for both the HEPN and SIRPT domains in promoting neurofilament assembly [13].

To examine possible reversal of the neurofilament bundling phenotype, various Sacsin domains were independently produced in the motor neurons of 6-week-old $Sacs^{-/-}$ spinal cord dorsal root ganglion cultures, followed by confocal examination, which revealed the Sacsin J-domain as being the most efficient in resolving pathological neurofilament accumulations, with the Ubl domain being less efficient, and in accordance with the chaperone hypothesis of Sacsin, ectopic expression of HSPA1A compensates for *SACS* loss-of-function and mimics the J-domain in resolving the neurofilament accumulations 3 days post microinjection. These data confirm a pivotal role for Sacsin in regulating the neurofilament assembly process, with *SACS* mutations responsible for their pathological accumulations associated with ARSACS [13].

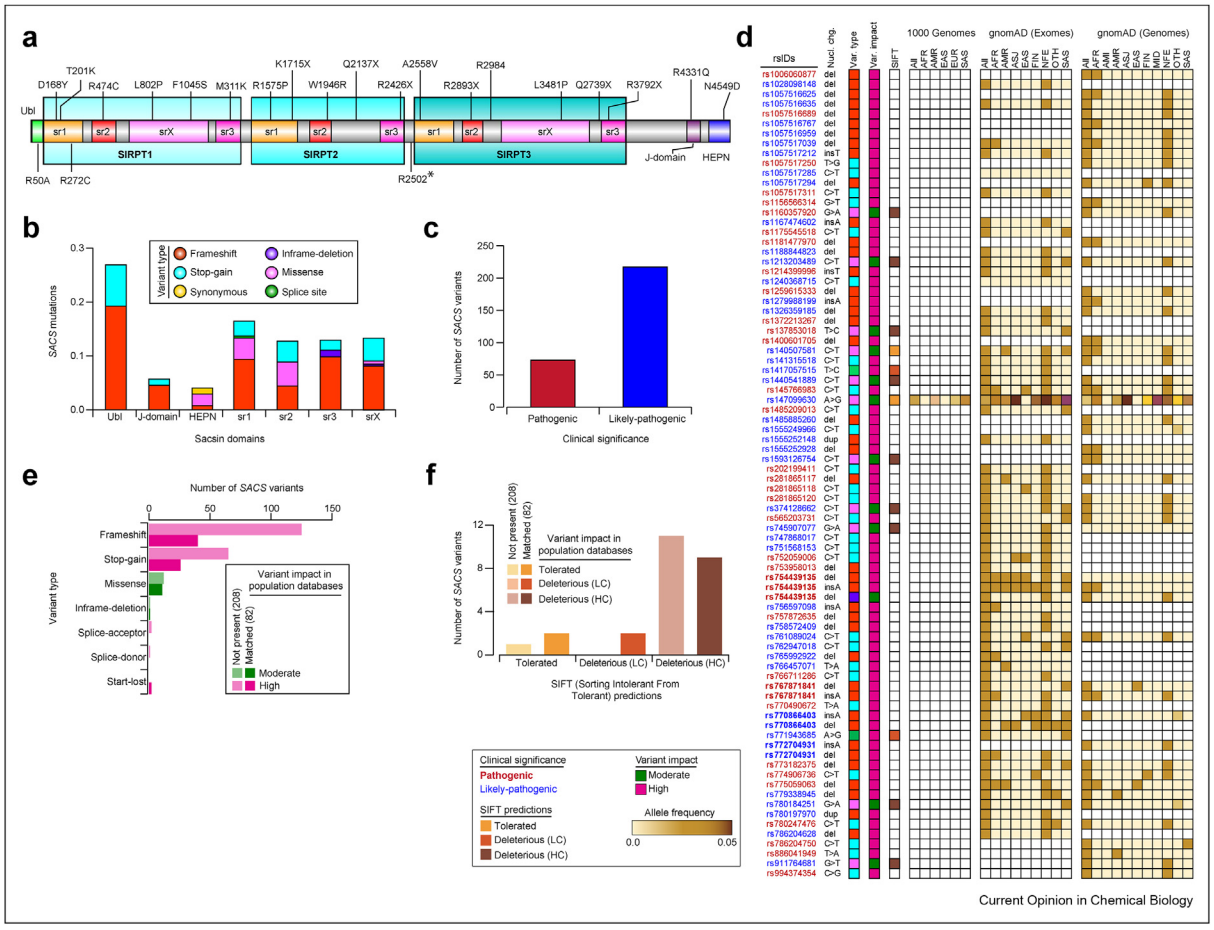
On another front, Sacsin localizes to the mitochondria, as validated by experiments performed on hippocampal neurons, Cos-7 and HeLa cells, primary neurons, ARSACS patient fibroblasts, *SACS* knockout mice, and organotypic mice brain slice cultures [15]. Sacsin mitochondrial localization plays a seminal role in regulating mitochondrial dynamics. In the healthy neurons, the N-terminus of Sacsin interacts with the Dynamin related protein-1 (Drp1) GTPase, another mitochondrial-associated protein that critically regulates mitochondrial fission [16,17]. Either Sacsin or Drp-1 disruption increases mitochondrial network interconnections due to impaired fission, with pathological consequences impeded the mitochondrial transport. Accordingly, Sacsin knockdown shows clustered mitochondria that accumulate in the soma and dendrites, along with altered dendritic morphology, which are reported in ARSACS [15].

In addition to Sacsin's role in regulating mitochondrial dynamics and neurofilament assembly, there has been an ongoing interest in understanding how various *SACS* mutations are linked to ARSACS disease symptoms or severity, especially because the 290 pathological *SACS* variants we extracted from the single nucleotide polymorphism database (dbSNP) are spread throughout the entire gene length (Figure 2a), with most mutations mapped to Ubl domain in the form of frameshifts, while HEPN domain encompasses the least number of mutations reported in ARSACS to date, the majority of which are missense mutations (Figure 2b). Among the 290 *SACS* variants, nearly three-fourth (75%, 217 of 290) are likely-pathogenic, while one-fourth (25%, 73 of 290) are pathogenic (Figure 2c) in ClinVar database.

Systematic analysis of genetic variants shared among populations can provide deep insights into population history [18]. We therefore explored how often the pathogenicity of the compiled *SACS* variants is common or restricted among populations across the world. Following ACMG variant classification guidelines, we mapped the allele frequency (AF) for 290 *SACS* variants against the most extensively used public population genetic repositories (i.e., 1000 Genomes Project, and Genome Aggregation Database (gnomAD) containing full exome and genome sequencing data; Figure 2d). While we were unable to map *SACS* variants to superpopulations in the 1000 Genomes Project either due to lack of allele frequency or their clinical significance being uncertain or likely benign, rsIDs were matched to a non-redundant set of 82 variants with relatively low ($\leq$ 0.05%) frequency in gnomAD populations. Strikingly, we found over one-tenth (16%, 45 of 290) of the *SACS* variants with low-frequency alleles (AF = 0 to $6.8e^{-3}$) are overrepresented ($P = 2.0e^{-19}$, hypergeometric test) in the non-Finnish European (NFE) individuals than other population groups (Figure 2d), indicating a possible genetic drift (i.e., loss of alleles from a population by chance). Nevertheless, more than three-fourth (84%, 245 of 290) of *SACS* variants that were noted to be pathogenic or likely-pathogenic were absent in the population databases. Collectively, our results are consistent with the notion that variants either missing from gnomAD or present at a low allele frequency are evidence for pathogenicity [19] in ARSACS compared to high allele frequency that can be a resultant of low penetrance in monogenic disease [20].

Next, we probed how often 82 *SACS* variants and the rest (208) that were not present in gnomAD lead to a consequence as in missense, frameshift, stop-gain, or other mutation outcomes. Our meta-analysis indicated that a vast majority (83%, 66 of 290) of the frame-shift and stop-gain variants have high (i.e., disruptive) impact on Sacsin protein production, whereas less than one-fifth (14%, 11 of 290) of the missense variant exhibit moderate (i.e., non-disruptive) impact on Sacsin

Figure 2



SACS mutations in various encoded domains. **(a)** Representative Sacsin amino acid substitutions associated with ARSACS. **(b)** Variant types, containing 290 pathological *SACS* mutations compiled from dbSNP database. (**c**) *SACS* variants displaying likely-pathogenic or pathogenic in ClinVar database. (**d**) Heatmap indicates allele frequencies for a non-redundant set of 82 variants along with their rsIDs and changes in nucleotide bases (Nucl. chg.) in population repositories. The variant (Var) type corresponds to the color-scheme shown in Figure 2b. AFR, African; AMR, American; EAS, East Asian; EUR, European; SAS, Southeast Asian; ASJ, Ashkenazi Jewish; FIN, Finnish; NFE, Non-Finnish European, OTH, Other; AMI, Amish; MID, Middle Eastern. (**e**) Variants that were matched (82) and the rest (208) not present in the population databases grouped into high (i.e., disruptive) and moderate (i.e., non-disruptive) impact. (**f**) SIFT predicted deleterious (LC, low confidence; HC, high confidence) and tolerated variants. The variant impact and SIFT predictions correspond to the color-scheme shown in Figures 2e and f.

(Figure 2e). Since disease-causing amino acid substitutions affect protein function [21], we applied SIFT (Sorting Intolerant From Tolerant) algorithm [21] to 290 *SACS* variants containing different mutations. SIFT predicted one-tenth (10%, 22 of 290) of the variants to be "deleterious" (SIFT score 0.0 to 0.05) with missense alleles being the major players, while 1% (3 of 290) of *SACS* variants were "tolerated" (SIFT score >0.05; Figure 2f).

Besides gaining knowledge on the pathogenicity of aforesaid *SACS* variants, steadfast SACS sequencing will be a robust validation tool of suspected ARSACS patients, which can highlight the family pedigree information. For example, routine brain magnetic resonance imaging performed on an outpatient revealed superior cerebellar vermis atrophy with corpus callosum abnormalities [22]. This patient is a child to consanguineous parents who expressed early childhood, ataxic gait, frequent falls, dysarthria, and muscle cramps. Classical genetic testing for Friedreich ataxia failed to detect pathological repeats in cognate *FXN* gene. But whole-exome sequencing unveiled a new homozygous SACS deletion (NM_014363.5:c.429_430 delTT: p.Trp144ValfsTer39) [22]. Subsequent segregation analysis through Sanger sequencing revealed the

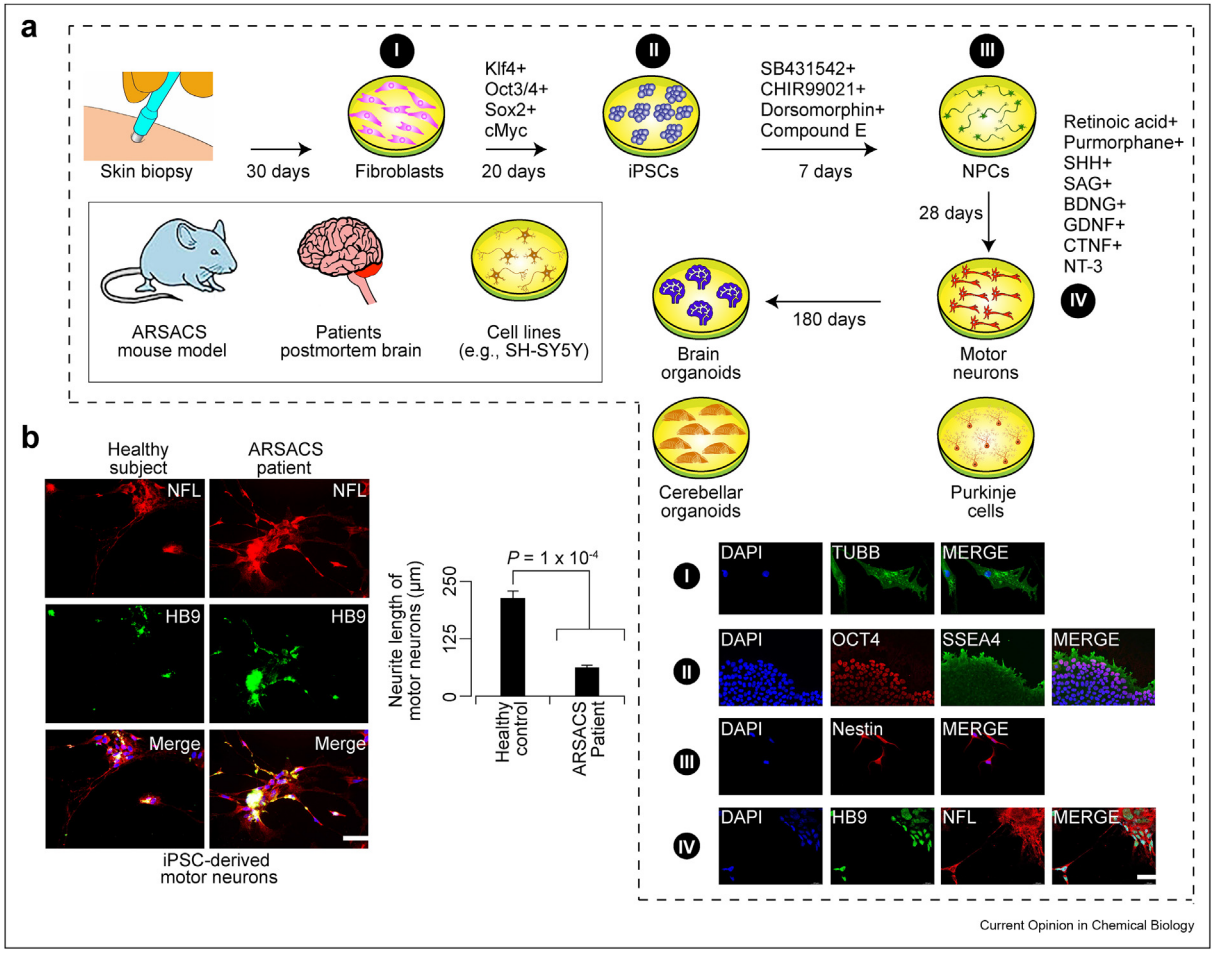
patient's sibling is a homozygous carrier for the same mutation, and both parents were found to be heterozygous. This example underlines how clinical sequencing is a requisite that aids in devising ARSACS management plans, while considering carrier status of close family members.

Notably, a recent report analyzed large sets of skin fibroblast samples obtained from ARSACS patient cohorts, and found that regardless of the mutation site in the *SACS* gene, Sacsin protein levels are either undetectable or reduced in all patients despite varied mRNA transcript levels or an uninterrupted translation mechanism, highlighting rapid posttranslational ubiquitination and degradation, protein instability, or aggregation of altered Sacsin as the outcomes in ARSACS regardless of the mutation site [23]. This further complicates the full characterization of how domain-specific alterations in Sacsin play a role in the ARSACS pathophysiology.

Advancements in disease models and their significance in ARSACS research

Although cell lines and animal models are still used to unravel the role of altered Sacsin in ARSACS, recent developments of close-to-natural and reliable cellular models offer novel strategies to elucidate the pathophysiological mechanisms of this neurodegenerative disorder (Figure 3a). Indeed, despite the generation of *SACS* knockout or the introduction of *SACS* mutations

Figure 3



ARSACS disease models. **(a)** In addition to *SACS* knockout mice and cell lines or the post-mortem brains, patient dermal fibroblast (I) reprogramming into induced pluripotent stem cells (iPSCs, II) and subsequent differentiation into neural progenitor cells (NPCs, III) and maturation into motor neurons or Purkinje cells (IV) are shown along with micrographs stained for cell state-specific markers (scale bar, 20 µm: I–III; 60 µm: IV) and for nuclei with DAPI (blue), offers great advantage to generate various organoids. **(b)** Micrographs of immunostained cells positive for neurofilament (NFL) and HB9 motor neuron markers in healthy and ARSACS patient (*SACS*: c.4568G > A (p.W1523*); c. 9305T > A (p.L3102*)) iPSC-derived motor neurons are shown along with neurite length measurements. Significance ($P = 1 \times 10^{-4}$) by Student's two-sided *t*-test.

in neuronal-like cell lines, such as SH-SY5Y cells, to consistently study Sacsin [10,24], these model cell lines cannot recapitulate the complex neurodegeneration mechanisms affecting Purkinje cells and motor neurons in ARSACS. Thus, the use of animal models (Figure 3a) has improved our understanding of ARSACS by allowing researchers to study brain areas and neuronal types directly impacted by this disorder. In particular, Sacsin pathophysiology was successfully studied in *SACS* knockout transgenic mouse models, which displayed axonal swellings and degeneration, as well as loss of Purkinje cells and intermediate filament accumulations as the primary disease phenotypes [14,15]. However, animal models still do not replicate the disease phenotype [12,14,25] due to differences in the anatomy, metabolism, and behavior between mice and humans, thus imposing restrictions on ARSACS research.

The advent of a more patient-oriented view drove the introduction of human-derived models that were able to recapitulate the genetic background of patients. In particular, blood and brain autoptic samples have always been widely used for the identification of disease biomarkers [26] and the investigation of pathological mechanisms [27,28], respectively. Conversely, human skin biopsies offer an easily accessible source of proliferating fibroblasts, which mirror the risk factors of patients and share similarities with the biochemical alterations found in the neurons affected by the disease [29,30]. For example, a study conducted on cell lines, primary neuron cultures and knockout mice revealed that targeted disruption of Sacsin caused alterations in mitochondrial morphology and function, with mitochondrial damages also present in primary skin fibroblasts obtained from ARSACS patients [15]. However, given the different functions carried out by these cells in the human body compared to neurons [31–33] or the post-mortem effects on brain molecular pathways [34], the use of these human samples come with limitations. The advent of induced pluripotent stem cell (iPSC) technology led to the subsequent generation of various neuronal cell types and 3D brain organoids with ease (Figure 3a). Specially, iPSC-derived neurons form functional synapses that share similar electrophysiological properties of brain neurons, and resemble the pathological processes underlying the disease [35,36]. In the case of ARSACS, the development of protocols for the differentiation of Purkinje cells and motor neurons is thus needed to understand the pathological pathways driving ARSACS. As a proof of principle, we show the generated iPSC-derived motor neurons from an ARSACS patient (Figure 3a), using an established protocol [37]. These advancements can facilitate the characterization of pathological features associated with ARSACS such as motor neuron length, which was significantly ($P = 1 \times 10^{-4}$) shorter in ARSACS patients (~60 μm) than their healthy counterparts (~213 μm, Figure 3b).

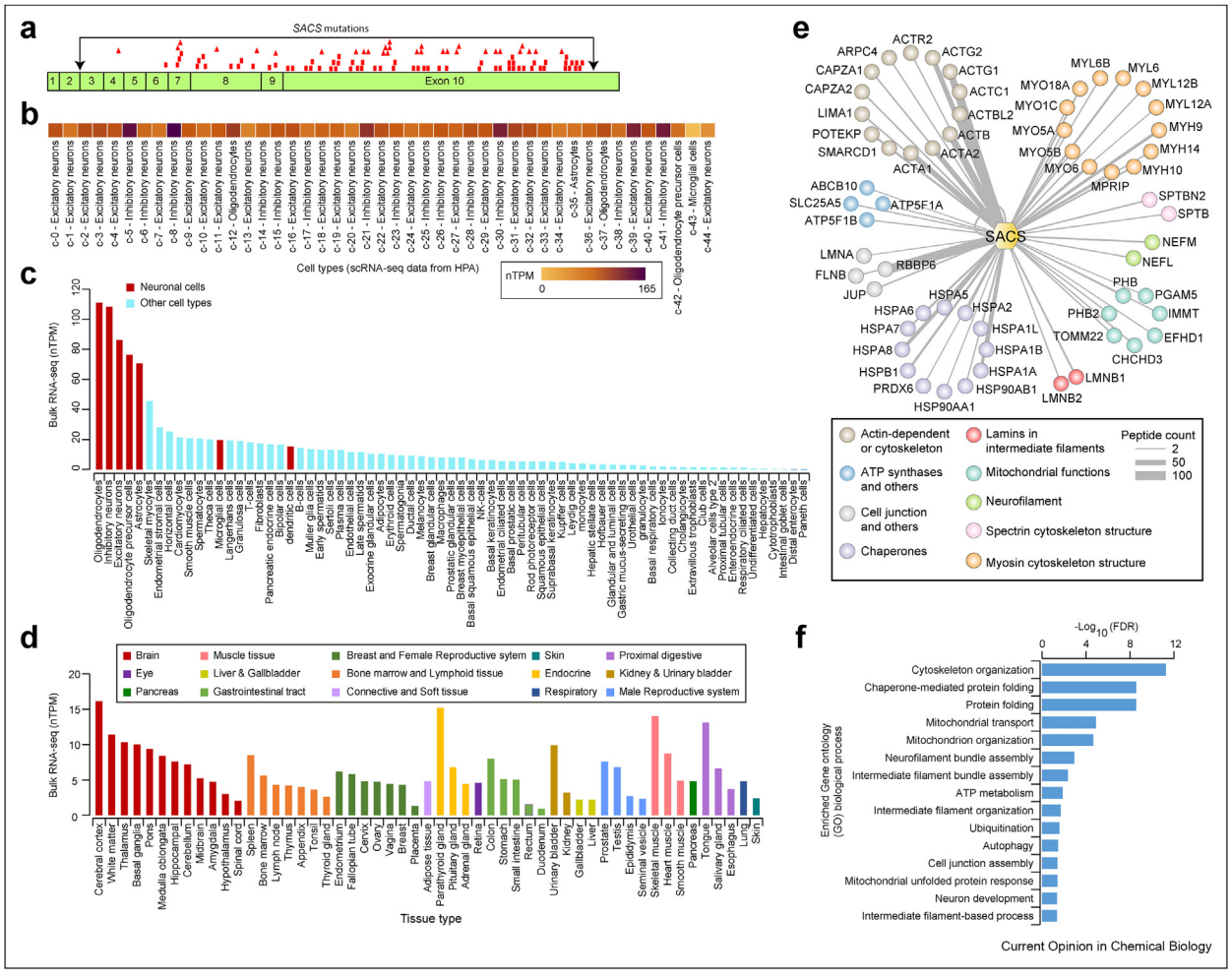
Despite iPSC-derived neurons offering promise for the study of complex human diseases, such as ARSACS, they cannot mirror the higher complexity of brain regions, which are composed not only of neurons, but also of astrocytes, glial cells, and oligodendrocytes [38]. These limitations can be overcome by the generation of 3D brain organoids, which resemble the multidimensional complexity of the human brain [38–40]. Additionally, the 3D tissues offer a unique opportunity to study the pathological mechanisms of ARSACS in various stages of neuronal development and discover the earliest cellular defects in a patient-derived system.

Multilayered omics in understanding the ARSACS molecular mechanisms

Areas such as single-cell transcriptomics [41], proteomics, or quantitative omics, with the advent of mass spectrometry [42], can improve our understanding of the ARSACS pathophysiology. The ongoing effort in identifying *SACS* mutation loci, frequency, and the types of mutations (Figure 4a) [43] can expand the repertoire linking *SACS* mutations to disease phenotypes. In addition, functional transcriptomics offer better understanding of the molecular mechanisms of ARSACS beyond the mere genomics level. For example, RNA-sequencing performed on *SACS* knockout SH-SY5Y cells compared to the wild-type control revealed more than 1,500 upregulated and 1,700 downregulated genes related to RNA processing, mitochondrial organization, protein folding, programmed cell death, autophagy, and others [10].

Although bulk RNA-sequencing (RNA-seq) or single-cell RNA-seq of *SACS* transcript were expressed in excitatory and inhibitory neurons, and in the major glial cells (i.e., astrocytes and oligodendrocytes) of the brain compared to other cell types (Figure 4b–c), *SACS* is noticeably expressed in brain regions and in a multitude of other organs (Figure 4d), highlighting organ-specific roles, which remains largely understudied. Advancement in Sacsin research beyond the immediate scope of brain tissues can therefore reveal additional comorbidities linked to ARSACS. For example, the parathyroid hormone (PTH) is a key metabolic factor required to regulate calcium levels in the bloodstream. PTH is also known to cross the blood-brain barrier, where PTH receptors on brain cells are recognized for subsequent intracellular regulation of calcium levels in different brain tissues [44]. Elevated PTH levels associate with calcium overloading, apoptosis, cerebral hyperintensities, vascular dementia, and Alzheimer's disease manifestations [45]. Thus, Sacsin abundance in parathyroid gland (Figure 4d) may indicate ARSACS-linked PTH pathological attributes when *SACS* is altered, a possibility that warrants further investigation. When more multi-organ *SACS* single-cell transcriptomics data from different patients becomes available, this will continue to refine our understanding

Figure 4



Gene expression and physical associations of *SACS*. (**a**) Graphical representation of *SACS* mutations associated with ARSACS is displayed as different symbols (missense mutations in squares; insertions/duplications/deletions in triangles). (**b–d**) Single-cell RNA (**b**)- and bulk-RNA (**c, d**) sequencing data from the Human Protein Atlas, showing *SACS* transcripts levels (represented as normalized transcript per million, nTPM) in various clusters of excitatory and inhibitory neurons and in the major glial cells (i.e., astrocytes and oligodendrocytes) of the brain (b), neuronal vs. other cell types (c), as well as for different tissue (d) types. (**e**) Sacsin association with proteins from indicated processes by immunoprecipitation coupled with mass spectrometry in SH-SY5Y neuronal-like cells using Sacsin antibody (ABN1019, EMD Millipore). Interactions filtered at 95% confidence in all 3 biologically independent experiments with SEQUEST matches (performed at 20 pp. fragment ion mass tolerance) evaluated using STATQUEST algorithm by assigning confidence scores to putative matches of peptides and proteins at 1% false discovery rate (FDR) for all identifications. (**f**) Sacsin binding partners enriched (adjusted *P*-value by Benjamini-Hochberg FDR correction) for Gene Ontology biological process annotation terms.

of ARSACS pathology in brain tissues and other body organs.

On the proteomics front, ARSACS has been linked to dysregulation patterns in bioprocesses such as neuro-inflammation and synaptogenesis using aptamer-based proteomic platform [24]. As well, purification of Sacsin bait protein in differentiated SH-SY5Y human neuronal-like cells, with Sacsin antibody, using immunoprecipitation (IP) and MS [46] revealed Sacsin association with human proteins involved in cytoskeleton, cell junctions, neurofilaments, mitochondrial functions, and chaperone-assisted protein folding activities, among others (Figure 4e). In fact, the putative Sacsin interaction partners involved in these processes are also significantly (FDR ≈ 5%) enriched according to the Gene Ontology (GO) annotation terms. Similar attempts on ARSACS-derived pathological tissues or iPSC-derived neurons differentiated further into more specific neuron types (e.g., motor neurons or Purkinje cells) from ARSACS vs. healthy subjects can uncover disrupted or altered patterns of macromolecular assemblies, thereby prioritizing candidate interacting Sacsin proteins for therapeutic interventions to tackle ARSACS.

Conclusion and perspectives

Since ARSACS is a monogenic disorder characterized by *SACS* loss-of-function mutations, therapeutic options for loss-of-function diseases are significantly harder to develop when compared with their gain-of-function counterparts, with cancer therapeutics among the exceptions since tumor suppressor genes loss-of-function is exploited to develop synthetic lethality-based cancer drugs to target parallel genes that are activated upon tumor suppressor gene disruptions [47]. To date, pharmacological treatments for ARSACS management are scarce and only alleviate disease symptoms without addressing its underlying molecular mechanisms, with baclofen as an example used to manage spasticity and minimize tendon shortening [48].

In the case of ARSACS, gene specific therapies represent a sound path to correct *SACS* mutations or compensate for its mechanistic loss. Gene specific therapies aim to silence or manipulate altered RNAs using antisense oligonucleotides (ASOs) or small interfering RNAs (siRNAs) that modulate the expression of altered mRNAs. ASO binding to its target mRNA triggers RNase H-mediated degradation or target slice sites of the exon of interest to mediate exon skipping, or alternately using small molecules that mediate exon inclusion. In addition, siRNAs recognition of their mRNA target results in target degradation, with the delivery methods of siRNAs ranging from lipophilic derivatives, siRNA-receptor ligand, siRNA-antibody or aptamer, dynamic polyconjugates, and exosomes, among others. These strategies are already yielding measured success in neurological diseases. For example, several ASOs that are either FDA-approved or in clinical trials to correct motor neuron disorders are available for targeting *SMN1*, *SMN2*, *SOD1*, *C9orf72*, and *XPO1* mutations associated with amyotrophic lateral sclerosis (ALS) [49]. However, recent work suggests that *SACS* mutations lead to undetectable or significantly reduced protein levels in ARSACS regardless of the gene mutation site [23], this renders ASOs approach, albeit necessary in other disorders, dispensable in ARSACS due to *SACS* gene silencing by default. Subsequently, viral or non-viral delivery of an exogeneous healthy copy of *SACS* can ultimately compensate for its loss-of-function. While viral vectors are more efficient in gene delivery than their non-viral counterparts, they remain challenged by non-targeted transfections, transient gene expression, low transgene levels due to potential insertional mutations as well as host immune neutralization [50].

Despite the challenges, ~500 clinical trials based on viral gene therapy delivery are in progress [50]. This is in addition to CRISPR/Cas9 genome editing tools that are facing technical and/or regulatory hurdles, genome editing-based therapeutics are also rapidly advancing, and there already exists genome-editing based therapeutics in the pipelines to treat Huntington's disease as well as familial amyloid polyneuropathy. These ongoing efforts will gradually bring us closer to the era of developing promising therapies to manage ARSACS and other neurological disorders.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

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References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest

1. Ding M, Weng C, Fan S, Cao Q, Lu Z: **Purkinje cell degeneration and motor coordination deficits in a new mouse model of autosomal recessive spastic ataxia of charlevoix-saguenay**. *Front Mol Neurosci* 2017, **10**:121.

2. ** Engert JC, Berube P, Mercier J, Dore C, Lepage P, Ge B, Bouchard JP, Mathieu J, Melancon SB, Schalling M, *et al.*: **ARSACS, a spastic ataxia common in northeastern Quebec, is caused by mutations in a new gene encoding an 11.5-kb ORF**. *Nat Genet* 2000, **24**:120–125.
First report of the gene SACS mutations linked to ARSACS.

3. * Anderson JF, Siller E, Barral JM: **The sacsin repeating region (SRR): a novel Hsp90-related supra-domain associated with neurodegeneration**. *J Mol Biol* 2010, **400**:665–674.
First Sacsin computational study that sheds light on the repeat regions within the protein.

4. Hadden MK, Lubbers DJ, Blagg BS: **Geldanamycin, radicicol, and chimeric inhibitors of the Hsp90 N-terminal ATP binding site**. *Curr Top Med Chem* 2006, **6**:1173–1182.

5. * Romano A, Tessa A, Barca A, Fattori F, de Leva MF, Terracciano A, Storelli C, Santorelli FM, Verri T: **Comparative analysis and functional mapping of SACS mutations reveal novel insights into sacsin repeated architecture**. *Hum Mutat* 2013, **34**:525–537.
In depth computational analysis of Sacsin revealed the presence of repeated supra and subdomains within the protein.

6. * Parfitt DA, Michael GJ, Vermeulen EG, Prodromou NV, Webb TR, Gallo JM, Cheetham ME, Nicoll WS, Blatch GL, Chapple JP: **The ataxia protein sacsin is a functional co-chaperone that protects against polyglutamine-expanded ataxin-1**. *Hum Mol Genet* 2009, **18**:1556–1565.
Functional assignment of Sacsin as a chaperone.

7. Stevens JC, Murphy SM, Davagnanam I, Phadke R, Anderson G, Nethisinghe S, Bremner F, Giunti P, Reilly MM: **The ARSACS phenotype can include supranuclear gaze palsy and skin lipofuscin deposits**. *J Neurol Neurosurg Psychiatry* 2013, **84**:114–116.

8. Martin MH, Bouchard JP, Sylvain M, St-Onge O, Truchon S: **Autosomal recessive spastic ataxia of Charlevoix-Saguenay: a report of MR imaging in 5 patients**. *AJNR Am J Neuroradiol* 2007, **28**:1606–1608.

9. Bradshaw TY, Romano LE, Duncan EJ, Nethisinghe S, Abeti R, Michael GJ, Giunti P, Vermeer S, Chapple JP: **A reduction in Drp1-mediated fission compromises mitochondrial health in**

autosomal recessive spastic ataxia of Charlevoix Saguenay. *Hum Mol Genet* 2016, **25**:3232–3244.

10. Morani F, Doccini S, Sirica R, Paterno M, Pezzini F, Ricca I, Simonati A, Delledonne M, Santorelli FM: **Functional transcriptome analysis in ARSACS KO cell model reveals a role of sacsin in autophagy**. *Sci Rep* 2019, **9**, 11878.

11. Duncan EJ, Lariviere R, Bradshaw TY, Longo F, Sgarioto N, Hayes MJ, Romano LEL, Nethisinghe S, Giunti P, Bruntraeger MB, *et al.*: **Altered organization of the intermediate filament cytoskeleton and relocalization of proteostasis modulators in cells lacking the ataxia protein sacsin**. *Hum Mol Genet* 2017, **26**:3130–3143.

12. Lariviere R, Sgarioto N, Marquez BT, Gaudet R, Choquet K, McKinney RA, Watt AJ, Brais B: **Sacs R272C missense homozygous mice develop an ataxia phenotype**. *Mol Brain* 2019, **12**: 19.

13. * Gentil BJ, Lai GT, Menade M, Lariviere R, Minotti S, Gehring K, Chapple JP, Brais B, Durham HD: **Sacsin, mutated in the ataxia ARSACS, regulates intermediate filament assembly and dynamics**. *Faseb J* 2019, **33**:2982–2994.

Assessing neurofilament bundling patterns caused by SACS mutations in mutiple cell types.

14. Lariviere R, Gaudet R, Gentil BJ, Girard M, Conte TC, Minotti S, Leclerc-Desaulniers K, Gehring K, McKinney RA, Shoubridge EA, *et al.*: **Sacs knockout mice present pathophysiological defects underlying autosomal recessive spastic ataxia of Charlevoix-Saguenay**. *Hum Mol Genet* 2015, **24**:727–739.

15. * Girard M, Lariviere R, Parfitt DA, Deane EC, Gaudet R, Nossova N, Blondeau F, Prenosil G, Vermeulen EG, Duchen MR, *et al.*: **Mitochondrial dysfunction and Purkinje cell loss in autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS)**. *Proc Natl Acad Sci U S A* 2012, **109**:1661–1666.

Describes mitochondrial dysfunction or mislocalization as the basis for ARSACS and assigns a biological role for Sacsin in the regulation of mitochondrial dynamics.

16. Reddy PH, Reddy TP, Manczak M, Calkins MJ, Shirendeb U, Mao P: **Dynamin-related protein 1 and mitochondrial fragmentation in neurodegenerative diseases**. *Brain Res Rev* 2011, **67**:103–118.

17. * Smirnova E, Shurland DL, Ryazantsev SN, van der Bliek AM: **A human dynamin-related protein controls the distribution of mitochondria**. *J Cell Biol* 1998, **143**:351–358.

Important report on the involvement of Sacsin in mitochondrial dynamics, most notablly fission.

18. Genomes Project C, Auton A, Brooks LD, Durbin RM, Garrison EP, Kang HM, Korbel JO, Marchini JL, McCarthy S, McVean GA, *et al.*: **A global reference for human genetic variation**. *Nature* 2015, **526**:68–74.

19. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, *et al.*: **Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American college of medical genetics and genomics and the association for molecular pathology**. *Genet Med* 2015, **17**:405–424.

20. Ghosh R, Harrison SM, Rehm HL, Plon SE, Biesecker LG: **ClinGen sequence variant interpretation working G: updated recommendation for the benign stand-alone ACMG/AMP criterion**. *Hum Mutat* 2018, **39**:1525–1530.

21. Ng PC, Henikoff S, SIFT: **Predicting amino acid changes that affect protein function**. *Nucleic Acids Res* 2003, **31**:3812–3814.

22. Habibzadeh P, Tabatabaei Z, Inaloo S, Nashatizadeh MM, Synofzik M, Ostovan VR, Faghihi MA: **Case report: expanding the genetic and phenotypic spectrum of autosomal recessive spastic ataxia of charlevoix-saguenay**. *Front Genet* 2020, **11**, 585136.

23. ** Longo F, De Ritis D, Miluzio A, Fraticelli D, Baets J, Scarlato M, Santorelli FM, Biffo S, Maltecca F: **Assessment of sacsin turnover in patients with ARSACS: implications for molecular diagnosis and pathogenesis**. *Neurology* 2021, **97**: e2315–e2327.

Analyzing a pool of SACS mutaitons led to the discovery that regardless of SACS mutation site, protein prodcuts are always ubiquitinated and degraded.

24. Morani F, Doccini S, Chiorino G, Fattori F, Galatolo D, Sciarrillo E, Gemignani F, Zuchner S, Bertini ES, Santorelli FM: **Functional network profiles in ARSACS disclosed by aptamer-based proteomic technology**. *Front Neurol* 2020, **11**, 603774.

25. Ady V, Toscano-Marquez B, Nath M, Chang PK, Hui J, Cook A, Charron F, Lariviere R, Brais B, McKinney RA, *et al.*: **Altered synaptic and firing properties of cerebellar Purkinje cells in a mouse model of ARSACS**. *J Physiol* 2018, **596**:4253–4267.

26. Barro C, Zetterberg H: **The blood biomarkers puzzle - a review of protein biomarkers in neurodegenerative diseases**. *J Neurosci Methods* 2021, **361**, 109281.

27. Love S: **Post mortem sampling of the brain and other tissues in neurodegenerative disease**. *Histopathology* 2004, **44**: 309–317.

28. Zilocchi M, Finzi G, Lualdi M, Sessa F, Fasano M, Alberio T: **Mitochondrial alterations in Parkinson's disease human samples and cellular models**. *Neurochem Int* 2018, **118**:61–72.

29. Kalman S, Garbett KA, Janka Z, Mirnics K: **Human dermal fibroblasts in psychiatry research**. *Neuroscience* 2016, **320**: 105–121.

30. Auburger G, Klinkenberg M, Drost J, Marcus K, Morales-Gordo B, Kunz WS, Brandt U, Broccoli V, Reichmann H, Gispert S, *et al.*: **Primary skin fibroblasts as a model of Parkinson's disease**. *Mol Neurobiol* 2012, **46**:20–27.

31. Lundgaard I, Osorio MJ, Kress BT, Sanggaard S, Nedergaard M: **White matter astrocytes in health and disease**. *Neuroscience* 2014, **276**:161–173.

32. Lemons JM, Feng XJ, Bennett BD, Legesse-Miller A, Johnson EL, Raitman I, Pollina EA, Rabitz HA, Rabinowitz JD, Coller HA: **Quiescent fibroblasts exhibit high metabolic activity**. *PLoS Biol* 2010, **8**, e1000514.

33. Glaros T, Larsen M, Li L: **Macrophages and fibroblasts during inflammation, tissue damage and organ injury**. *Front Biosci* 2009, **14**:3988–3993.

34. Lewis DA: **The human brain revisited: opportunities and challenges in postmortem studies of psychiatric disorders**. *Neuropsychopharmacology* 2002, **26**:143–154.

35. Kang S, Chen X, Gong S, Yu P, Yau S, Su Z, Zhou L, Yu J, Pan G, Shi L: **Characteristic analyses of a neural differentiation model from iPSC-derived neuron according to morphology, physiology, and global gene expression pattern**. *Sci Rep* 2017, **7**, 12233.

36. McKinney CE: **Using induced pluripotent stem cells derived neurons to model brain diseases**. *Neural Regen Res* 2017, **12**: 1062–1067.

37. Bianchi F, Malboubi M, Li Y, George JH, Jerusalem A, Szele F, Thompson MS, Ye H: **Rapid and efficient differentiation of functional motor neurons from human iPSC for neural injury modelling**. *Stem Cell Res* 2018, **32**:126–134.

38. Amin ND, Pasca SP: **Building models of brain disorders with three-dimensional organoids**. *Neuron* 2018, **100**:389–405.

39. * Pasca SP: **The rise of three-dimensional human brain cultures**. *Nature* 2018, **553**:437–445.

Important report on 3D disease model development and their contribution to better understanding the pathophyisiology of neurological diseases under close-to-natural setting.

40. Birey F, Andersen J, Makinson CD, Islam S, Wei W, Huber N, Fan HC, Metzler KRC, Panagiotakos G, Thom N, *et al.*: **Assembly of functionally integrated human forebrain spheroids**. *Nature* 2017, **545**:54–59.

41. Moutaoufik MT, Malty R, Amin S, Zhang Q, Phanse S, Gagarinova A, Zilocchi M, Hoell L, Minic Z, Gagarinova M, *et al.*: **Rewiring of the human mitochondrial interactome during neuronal reprogramming reveals regulators of the respirasome and neurogenesis**. *iScience* 2019, **19**: 1114–1132.

42. Aly KA, Moutaoufik MT, Phanse S, Zhang Q, Babu M: **From fuzziness to precision medicine: on the rapidly evolving proteomics with implications in mitochondrial connectivity to rare human disease**. *iScience* 2021, **24**, 102030.

43. Synofzik M, Soehn AS, Gburek-Augustat J, Schicks J, Karle KN, Schule R, Haack TB, Schoning M, Biskup S, Rudnik-Schoneborn S, *et al.*: **Autosomal recessive spastic ataxia of Charlevoix Saguenay (ARSACS): expanding the genetic, clinical and imaging spectrum**. *Orphanet J Rare Dis* 2013, **8**:41.

44. Lourida I, Thompson-Coon J, Dickens CM, Soni M, Kuzma E, Kos K, Llewellyn DJ: **Parathyroid hormone, cognitive function and dementia: a systematic review**. *PLoS One* 2015, **10**, e0127574.

45. Hagstrom E, Kilander L, Nylander R, Larsson EM, Michaelsson K, Melhus H, Ahlstrom H, Johansson L, Lind L, Arnlov J: **Plasma parathyroid hormone is associated with vascular dementia and cerebral hyperintensities in two community-based cohorts**. *J Clin Endocrinol Metab* 2014, **99**:4181–4189.

46. Malty RH, Aoki H, Kumar A, Phanse S, Amin S, Zhang Q, Minic Z, Goebels F, Musso G, Wu Z, *et al.*: **A map of human mitochondrial protein interactions linked to neurodegeneration reveals new mechanisms of redox homeostasis and NF-kappaB signaling**. *Cell Syst* 2017, **5**:1–14.

47. Setton J, Zinda M, Riaz N, Durocher D, Zimmermann M, Koehler M, Reis-Filho JS, Powell SN: **Synthetic lethality in cancer therapeutics: the next generation**. *Cancer Discov* 2021, **11**:1626–1635.

48. Picher-Martel V, Dupre N: **Current and promising therapies in autosomal recessive ataxias**. *CNS Neurol Disord: Drug Targets* 2018, **17**:161–171.

49. Brenner D, Ludolph AC, Weishaupt JH: **Gene specific therapies - the next therapeutic milestone in neurology**. *Neurol Res Pract* 2020, **2**:25.

50. Wang Y, Bruggeman KF, Franks S, Gautam V, Hodgetts SI, Harvey AR, Williams RJ, Nisbet DR: **Is viral vector gene delivery more effective using biomaterials?** *Adv Healthc Mater* 2021, **10**, e2001238.