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SHORT REPORTS

ARSACS as a Worldwide Disease: Novel *SACS* Mutations Identified in a Consanguineous Family from the Remote Tribal Jammu and Kashmir Region in India

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Abstract

Autosomal recessive spastic ataxia of Charlevoix–Saguenay (ARSACS) is a rare neurodegenerative disorder characterized by the triad of early-onset cerebellar ataxia, peripheral sensorimotor neuropathy, and lower limb spasticity. Here, we present a 28-year-old male patient with symptoms of ARSACS and mild intellectual disability from a consanguineous family of tribal J&K, India. Whole exome sequencing unraveled a novel homozygous frameshift *SACS* mutation (Cys2869ValfsTer15) in the patient. In addition to the well-established ARSACS imaging features, MRI revealed T2 hyperintense rim around the thalami ("bithalamic stripes") demonstrating that this feature might serve as an additional supportive diagnostic imaging marker for ARSACS. Moreover, retinal nerve fiber layer thickening which has recently been proposed as a diagnostic biomarker for ARSACS was present on routine optic coherence tomography (OCT) also in this patient, indicating that it might indeed present a relatively universal diagnostic biomarker for ARSACS. In sum, our findings extend the geographical distribution of ARSACS to even very remote tribal regions in Asia (such as the Rajouri region of J&K, India) and extend the mutational and imaging spectrum of ARSACS. They provide further support that brain imaging and OCT markers might serve as diagnostic biomarkers for ARSACS in patients with novel *SACS* mutations, applicable even in remote regions of the world to identify and confirm ARSACS disease.

Keywords ARSACS · Consanguinity · Whole exome · Tribal India · J&K

Introduction

Autosomal recessive spastic ataxia of Charlevoix–Saguenay (ARSACS) (Online Mendelian Inheritance in Man database [OMIM] #270550), caused by mutations in *SACS*, was first described 40 years ago in the regions of Charlevoix and Saguenay-Lac-St-Jean in Quebec, Canada [1]. It is classically characterized by a triad of slowly progressive early-onset cerebellar ataxia, pyramidal spasticity, and axonal-demyelinating sensorimotor peripheral neuropathy, later often complicated by frequent dysphagia [2, 3].

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Recent studies indicate that ARSACS is not limited to Quebec, but variably present worldwide, including Europe and Asia [4–7]. In fact, with now more than 170 *SACS* mutations having been reported, ARSACS is now appreciated as one of the most frequent causes of spastic ataxia worldwide [8, 9].

With advances in whole exome sequencing (WES), researchers have been successful in identifying genetic causes for disorders in consanguineous families in even remote tribal regions. Here, we report a novel homozygous frameshift mutation in *SACS* (NM_014363:exon10:c.8605delT:p.Cys2869ValfsTer15) identified by WES in a consanguineous family from tribal region of Rajouri, Jammu and Kashmir (J&K), India, with ARSACS. Our findings extend the mutational and geographic spectrum of ARSACS, and add further support for brain and retinal imaging biomarkers that help to identify and confirm ARSACS disease even in remote regions of the world.

Subjects and Methods

Family and Patient Selection

We studied four members of a family including the index patient, his unaffected parents, and his unaffected brother (for pedigree, see Fig. 1). The family was of tribal origin from Rajouri district of J&K, India. This study was carried out under a protocol approved by the ethical committee of Baba Ghulam Shah Badshah University, Rajouri, J&K. Written informed consent was obtained from all participants or their legal guardians.

Clinical Examination

Clinical examination was carried out on the index patient using MRI and optical coherence tomography (OCT). Routine biochemical tests (AFP, cholesterol, TSH, free T4, and albumin) were performed in the index patient to exclude biomarker findings characteristic of ataxia telangiectasia, hypothyroidism, and ataxia with oculomotor apraxia 1 and 2, respectively.

Whole Exome Sequencing

Genomic DNA was isolated from collected blood samples using QIAamp kit (Qiagen, Hilden, Germany). WES was performed on 50 ng of genomic DNA from index patient. Exome libraries were constructed from the genome DNA by shearing to a mean fragment length of 200 bp, and then captured using the IDT xGen Exome capture kit. Exome of the index patient was captured together using one IDT xGen oligo pool, and then sequenced on one lane of Illumina's HiSeq 4000. This resulted in a mean of 47.90 million reads, 56× mean coverage across the exome's target regions, and 98.60% target region coverage at 10 reads or higher (for details, see supplementary data; see Table S1 for exome metrics).

Fig. 1 **a** Pedigree of the index patient, demonstrating consanguineous parents (arrow indicates index patient). **b** Evolutionary conservation of the novel SACS variant. Linear representation of functional domains of SACS with location of p. Cys2869ValfsTer15 mutation. The mutation occurs at a position highly conserved through orthologs in Xenopus. Ubiquitin-like, ubiquitin-like domain; J, J domain; HEPN, higher eukaryotes and prokaryotes nucleotide-binding domain. **c**–**h** MRI of the index subject revealing the characteristic MRI findings of ARSACS. MRI of the index subject reveals atrophy of the superior cerebellar vermis (**c**, FLAIR), short thinning of the rostral corpus callosum (**d**, FLAIR), bilateral hypointense stripes in the paramedian pons (**e**, FLAIR; **f**, T2), a bilateral hyperintense lateral pons (**g**, FLAIR) and a hyperintense rim around both thalami (**h**, T2). **i**–**j** OCT peripapillary circular scan showing increased RNFL thickness across the entire circumference of the optic nerve head (average thickness right eye 183.6 μm, left eye 176.1 μm; normal range 95.7–98.7 μm)

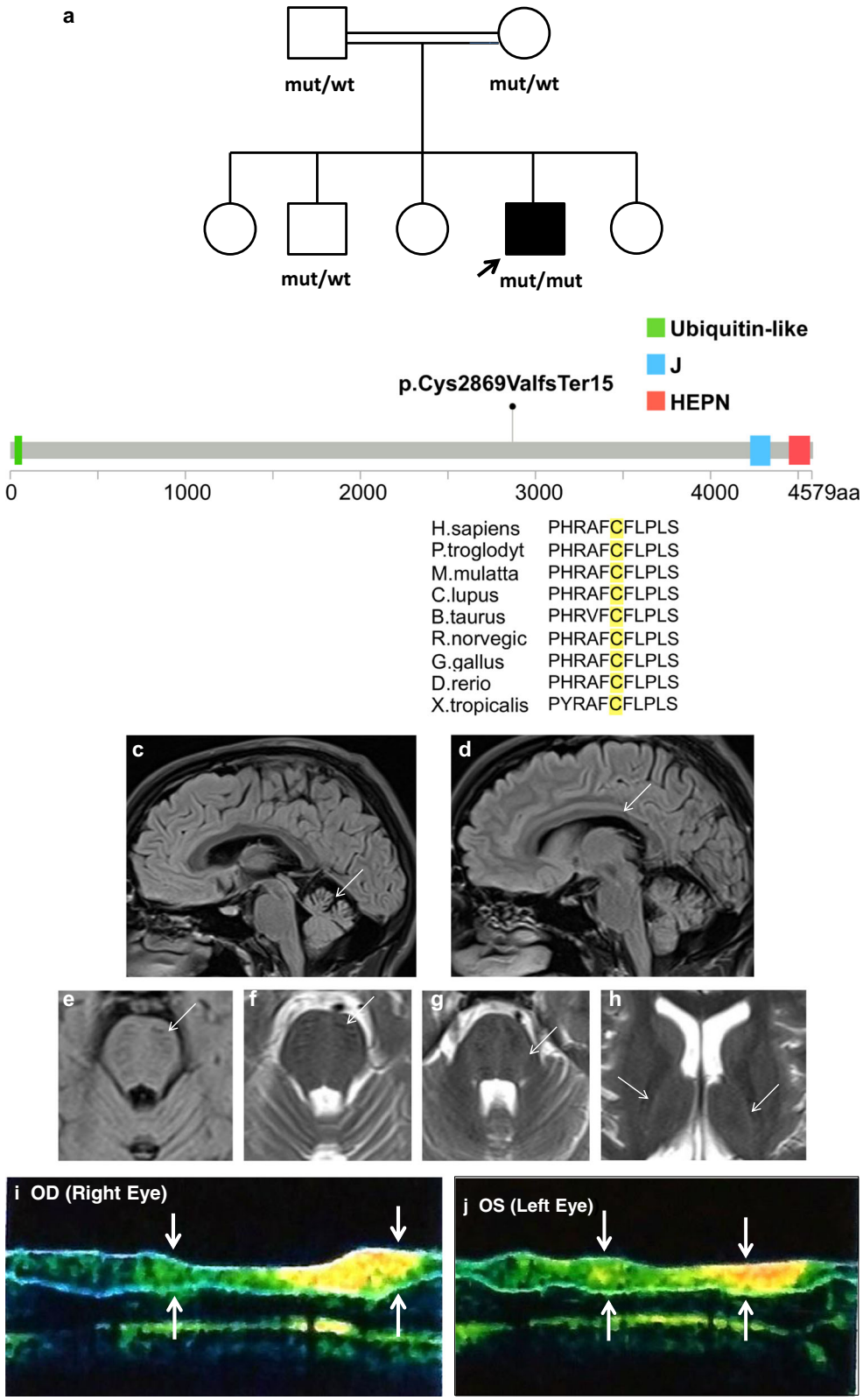
Co-segregation Analysis

The candidate variant was confirmed by co-segregation analysis using Sanger sequencing (for details, see supplementary data; Fig. S1).

Results

Whole Exome Analysis of the Index Patient

We filtered for rare (MAF ≤ 0.01 in 1000Genome, EVS, and ExAC) homozygous variants that exhibited high-quality sequence reads (pass GATK Variant Score Quality Recalibration [VSQR], have a minimum 4 total reads, and have a minimum 80% alternate allele ratio). Further, variants that are not predicted to disrupt canonical splice site and are non-exonic were removed. Only LoF variants (nonsense, canonical splice site, frameshift indels, and start-loss) with phyloP100way_vertebrate score ≥ 1 and missense variants and non-frameshift indels with CADD ≥ 20 and GERP++_RS ≥ 0 were considered potentially damaging to the disease. All variants passed the filtering were visually inspected and Blat was performed against the reference genome (hg19) to make sure reads harboring the mutation map to a single locus in the genome. With the criteria above, a total of seven homozygous variants were identified in IDE2 (Table 1). Additionally, genes were annotated with brain-specific expression value in a form of reads per kilobase transcript



per million reads (RPKM) from the GTEx database (https://gtexportal.org/home/) as well as human brain-specific expression data obtained during the first 4 weeks of development in transcript per kilobase million (TPM) [10]. Genes with RPKM < 1 in brain based on GTEx database and TPM < 1 based on fetal brain expression were

Table 1 Homozygous variants

ID	Gene	cDNA	Protein	Type	pLI	OMIM	phyloP100way_vertebrate	GERP++_RS
IDE2	*SACS*	c.8605delT	p.C2869Vfs*15	frameshift_deletion	0.00	Spastic ataxia, Charlevoix–Saguenay type, AR	.	.
	TENM1	c.A5396G	p.N1799S	Dmis	1.00	.	6.27	5.54
	PLXNB3	c.C4210T	p.L1404F	Dmis	0.10	.	2.62	4.84
	QPCTL	c.C1091T	p.T364M	Dmis	0.00	.	7.63	5.71
	CD96	c.C131T	p.T44I	Dmis	0.00	C syndrome, AD	− 0.05	4.33
	TSPAN1	c.G690A	p.M230I	Dmis	0.00		6.40	5.36
	GPC3	c.G1354A	p.V452M	Dmis	0.99	Simpson-Golabi-Behmel syndrome, type 1, XLR	2.92	5.07

ID	ExAC	gnomAD_WES	gnomAD_WGS	CADD	MetaSVM	Brain_GTEx_FPKM	FetalBrain_TPM
IDE2	.	4.07E−06	.	.	.	2.84	3.34
	0.0004	0.0004	.	24.10	D	2.83	0.17
	0.0006	0.0005	0.0001	27.40	T	11.59	0.77
	0.0046	0.0051	0.0055	29.30	D	3.18	1.66
	0.0004	0.0004	3.23E−05	29.20	T	0.04	0.00
	0.003	0.0026	0.0026	26.40	T	0.92	0.03
	0.006	0.0062	0.0029	33.00	T	0.34	0.15

considered as not expressed in the brain. Genes expressed in the brain (*SACS*, *TENM1*, *PLXNB3*, *QPCTL*) were prioritized (Table 1). Subsequent segregation analysis of the NM_014363: exon10:c.8605delT: p.Cys2869ValfsTer15 *SACS* variant in the family using Sanger sequencing confirmed homozygosity of the variant in the index patient, whereas unaffected parents and sibling were heterozygous for the wild-type allele variant (supplementary data; Fig. S1). None of the variants in the other genes co-segregated with the disease in the family. The frameshift *SACS* variant is extremely rare (1 allele among 250,698 alleles in gnomAD; novel in ExAC) and introduces a premature stop codon 14 amino acids downstream of the mutated locus, predicted to lead to truncated protein.

Clinical Characteristics

The 28-year-old male index patient presented with an early-onset (14 months), slowly progressive, spastic-ataxic disorder, and mild intellectual disability (Wechsler Intelligence Scale). On neurological examination, he showed a spastic-ataxic gait, moderate lower limb spasticity with Babinski sign, broken-up smooth pursuit, and gaze evoked nystagmus. In addition, examination revealed severe peripheral neuropathy in the lower limbs, including distal muscle weakness and atrophy and loss of Achilles reflexes. The patient had started independent walking at 18 months of age. Gait difficulties and frequent falls were noticed at 3 years of age with progressive worsening thereafter. In the teen years, the features of gait and limb ataxia were accompanied by dysarthria, spasticity in the lower limbs and peripheral neuropathy.

Diagnostic Imaging Biomarker Candidates

To investigate whether recently proposed imaging ARSACS biomarkers would be applicable also to an ARSACS patient with a novel *SACS* mutation and from a very different region of the world, MRI and OCT were performed. MRI revealed the characteristic MRI features of ARSACS (Fig. 1) including *hypo*intense pontine stripes, a *hyper*intense lateral pons as very ARSACS specific signs, and—as less specific, but still characteristic feature—atrophy of the superior vermis and thinning of the rostral corpus callosum (Fig. 1). Interestingly, also a T2 *hyper*intense rim around both thalami was observed. Retinal imaging using OCT demonstrated thickening of the retinal nerve fiber layer (RNFL) in both eyes (right eye: 184 μm, left eye: 176 μm; normal range: 96–99 μm) as typically seen in ARSACS patients (see supplementary data; Fig. S2).

Discussion

Autosomal recessive cerebellar ataxias (ARCAs) are heterogeneous diseases, with > 100 genes having been reported [2, 9, 11, 12]. Lack of genetic testing, pleiotropic phenotypes, and the high variability in age of onset often lead to difficulties in diagnosis. While Sanger sequencing for each disease gene is expensive and time-consuming, whole or targeted exome sequencing is often more effective in identifying the underlying molecular diagnosis. Moreover, as also demonstrated here, they help to add to our knowledge of the geographic distribution of those genetic diseases, which have long been thought to be constrained to certain restricted regions only (e.g., ARSACS only to French-Canadian or at least Western populations). Here, we investigated a consanguineous family from the remote Rajouri district of J&K, India, with segregation analysis available for a total of four individuals from the family. WES identified a novel homozygous mutation c.8605delT:p.Cys2869ValfsTer15 in *SACS* thus expanding both the mutational spectrum as well as the geographic distribution of ARSACS disease, demonstrating that it is present even in very remote tribal regions in Asia. Protein containing patient biomaterial for investigating the exact truncation of the sacsin protein was unfortunately not available, but in silico predictions of the frameshift SACS variant clearly predict a premature stop codon 14 amino acids downstream of the mutated locus with clear prediction of a truncated protein.

Few studies have been carried out on Indian patients with ARSACS [7, 13–15]. To our knowledge, this is the first study on ARSACS from the remote Jammu and Kashmir region of India. Our ARSACS case from the J&K region shares the characteristic key MRI findings of ARSACS, namely, the highly specific linear pontine T2-*hypo*intensities with a T2-*hyper*intense lateral pons and as less specific, but still highly characteristic signs like thinning of the rostral corpus callosum and atrophy of the superior cerebellar vermis [6]. Moreover, our case adds evidence that also T2 *hyper*intense rim around the thalami ("bithalamic stripes")—a feature reported before, but not yet widely appreciated as a helpful biomarker for ARSACS—might serve as an additional diagnostic imaging biomarker for ARSACS [16].

Likewise, retinal nerve fiber layer thickening—which has recently been proposed as a presumably reliable diagnostic biomarker for ARSACS—was observed in our ARSACS case [17, 18]. This adds further support that RNFL thickening might indeed present a relatively universal diagnostic biomarker for ARSACS, present even in patients with novel *SACS* mutations and from very different parts of the world where it has not yet been studied so far.

Cognitive decline and intellectual disability have been reported previously in individuals with ARSACS [8]. Approximately 50% of ARSACS patients were reported to be intellectually disabled in a recently conducted study [2]. The mild intellectual disability observed in the index patient of our study further corroborates this notion. However, while verbal

and physical aggressive behavior with progressive cognitive decline has been reported on patients with ARSACS, our index patient did not display any such behavioral pattern [19].

Conclusions

In summary, using WES and variant filtering, we identified a novel mutation in *SACS* causing ARSACS in a patient from a consanguineous family from the remote Rajouri district of J&K, India. Our study thus expands the mutational and geographic spectrum of ARSACS disease, demonstrating that it is present even in very remote tribal parts of the world outside French Canada. Moreover, it highlights diagnostic MRI and OCT imaging biomarkers which might help to identify ARSACS patients or to confirm patients with *SACS* variants of uncertain significance (VUS). They are applicable even in remote regions of the world and to unexplained ataxia patients with novel *SACS* mutations also outside Canada or Europe.

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Compliance with Ethical Standards All the procedures described in the text have been conducted in accordance with ethical principles.

Conflict of Interest The authors declare that they have no conflict of interests.

References

1. Bouchard JP, Barbeau A, Bouchard R, Bouchard RW. Autosomal recessive spastic ataxia of Charlevoix Saguenay. Can J Neurol Sci. 1978;5:61–9.
2. Pilliod J, Moutton S, Lavie J, Maurat E, Hubert C, Bellance N, et al. New practical definitions for the diagnosis of autosomal recessive spastic ataxia of Charlevoix-Saguenay. Ann Neurol. 2015;78:871–86. https://doi.org/10.1002/ana.24509.
3. Vogel AP, Rommel N, Oettinger A, Stoll LH, Kraus EM, Gagnon C, et al. Coordination and timing deficits in speech and swallowing in autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS). J Neurol. 2018;265:2060–70. https://doi.org/10.1007/s00415-018-8950-4.
4. Ogawa T, Takiyama Y, Sakoe K, Mori K, Namekawa M, Shimazaki H, et al. Identification of a SACS gene missense mutation in ARSACS. Neurology. 2004;62:107–9. https://doi.org/10.1212/01.WNL.0000099371.14478.73.
5. Bouhlal Y, Amouri R, El Euch-Fayeche G, Hentati F. Autosomal recessive spastic ataxia of Charlevoix-Saguenay: an overview. Parkinsonism Relat Disord. 2011;17:418–22. https://doi.org/10.1016/j.parkreldis.2011.
6. Synofzik M, Soehn AS, Gburek-Augustat J, Schicks J, Karle KN, Schule R, et al. Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) expanding the genetic, clinical and imaging spectrum. Orphanet J Rare Dis. 2013;8:41. https://doi.org/10.1186/1750-1172-8-41.
7. Agarwal PA, Upasani PA, Ramprasad VL. Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) first report of clinical and imaging features from India, and a novel SACS gene duplication. Mov Disord Clin Pract. 2017;4:775–7. https://doi.org/10.1002/mdc3.12520.
8. Ali Z, Klar J, Jameel M, Khan K, Fatima A, Raininko R, et al. Novel SACS mutations associated with intellectual disability epilepsy and widespread supratentorial abnormalities. BMC Med Genet. 2017;18:144–11. https://doi.org/10.1016/j.jns.2016.10.032.
9. Synofzik M, Nemeth AH. Recessive ataxias. Handb Clin Neurol. 2018;155:73–89.
10. Gerrard DT, Berry AA, Jennings RE, Hanley KP, Bobola N, Hanley NA, et al. An integrative transcriptomic atlas of organogenesis in human embryos. Elife. 2016;24:5. https://doi.org/10.7554/eLife.15657.
11. Fogel BL, Perlman S. Clinical features and molecular genetics of autosomal recessive cerebellar ataxias. Lancet Neurol. 2007;6:245–57. https://doi.org/10.1016/S1474-4422(07)70054-6.
12. Zeng H, Tang JG, Yang YF, Tan ZP, Tan JQ. A novel homozygous SACS mutation identified by whole-exome sequencing in a consanguineous family with autosomal recessive spastic ataxia of Charlevoix-Saguenay. Cytogenet Genome Res. 2017;152:16–21. https://doi.org/10.1159/000477428.
13. Menon MS, Shaji CV, Kabeer AK, Parvathy G. SACS gene-related autosomal recessive spastic ataxia of Charlevoix-Saguenay from South India. Arch Med Health Sci. 2016;1:22–124. https://doi.org/10.4103/2321-4848.183359.
14. Biswas A, Varman M, Yoganathan S, Subhash PK, Mani S. Teaching NeuroImages: autosomal recessive spastic ataxia of Charlevoix-Saguenay: typical MRI findings. Neurology. 2018;90(3):1271–2. https://doi.org/10.1212/WNL.0000000000005252.
15. Pandi S, Deshpande A, Khardenavi S. Case report of autosomal recessive spastic ataxia of Charlevoix-Saguenay. J Mahatma Gandhi Inst Med Sci. 2014;191:62–4. https://doi.org/10.4103/0971-9903.126253.
16. Oguz KK, Haliloglu G, Temucin C, Gocmen R, Has AC, Doerschner K, et al. Assessment of whole-brain white matter by DTI in autosomal recessive spastic ataxia of Charlevoix-Saguenay. AJNR Am J Neuroradiol. 2013;34:1952–7. https://doi.org/10.3174/ajnr.A3488.
17. Parkinson MH, Bartmann AP, Clayton LM, Nethisinghe S, Pfundt R, Chapple JP, et al. Optical coherence tomography in autosomal recessive spastic ataxia of Charlevoix-Saguenay. Brain. 2018;141(4):989–99. https://doi.org/10.1093/brain/awy028.
18. Desserre J, Devos D, Sautiere BG, Debruyne P, Santorelli FM, Vuillaume I, et al. Thickening of peripapillar retinal fibers for the diagnosis of autosomal recessive spastic ataxia of Charlevoix-Saguenay. Cerebellum. 2011;10(4):758–62. https://doi.org/10.1007/s12311-011-0286-x.
19. Mignarri A, Tessa MA, Carluccio A, Rufa E, Storti G, Bonelli C, et al. Cerebellum and neuropsychiatric disorders: insights from ARSACS. Neurol Sci. 2014;35:95–7. https://doi.org/10.1007/s10072-013-1592.