Human Genetics (2019) 138:1409–1417
https://doi.org/10.1007/s00439-019-02077-7

ORIGINAL INVESTIGATION

Compound heterozygous mutations in SNAP29 is associated with Pelizaeus-Merzbacher-like disorder (PMLD)

Lorida Llaci[1,2] · Keri Ramsey[1,2] · Newell Belnap[1,2] · Ana M. Claasen[1,2] · Chris D. Balak[1,2] · Szabolcs Szelinger[1,2] · Wayne M. Jepsen[1,2] · Ashley L. Siniard[1,2] · Ryan Richholt[1,2] · Tyler Izat[1] · Marcus Naymik[1,2] · Matt De Both[1,2] · Ignazio S. Piras[1,2] · David W. Craig[1,2,3] · Matthew J. Huentelman[1,2] · Vinodh Narayanan[1,2] · Isabelle Schrauwen[1,2,4] · Sampathkumar Rangasamy[1,2]

Received: 28 July 2019 / Accepted: 22 October 2019 / Published online: 20 November 2019

Abstract

Pelizaeus-Merzbacher-like disease (PMLD) is an autosomal recessive hypomyelinating leukodystrophy, which is clinically and radiologically similar to X-linked Pelizaeus-Merzbacher disease (PMD). PMLD is characterized by early-onset nystagmus, delayed development (motor delay, speech delay and dysarthria), dystonia, hypotonia typically evolving into spasticity, ataxia, seizures, optic atrophy, and diffuse leukodystrophy on magnetic resonance imaging (MRI). We identified a 12-year-old Caucasian/Hispanic male with the classical clinical characteristics of PMLD with lack of myelination of the subcortical white matter, and absence of the splenium of corpus callosum. Exome sequencing in the trio revealed novel compound heterozygous pathogenic mutations in *SNAP29* (p.Leu119AlafsX15, c.354DupG and p.0?, c.2T > C). Quantitative analysis of the patient's blood cells through RNA sequencing identified a significant decrease in *SNAP29* mRNA expression, while western blot analysis on fibroblast cells revealed a lack of protein expression compared to parental and control cells. Mutations in *SNAP29* have previously been associated with cerebral dysgenesis, neuropathy, ichthyosis, and keratoderma (CEDNIK) syndrome. Typical skin features described in CEDNIK syndrome, such as generalized ichthyosis and keratoderma, were absent in our patient. Moreover, the early onset nystagmus and leukodystrophy were consistent with a PMLD diagnosis. These findings suggest that loss of SNAP29 function, which was previously associated with CEDNIK syndrome, is also associated with PMLD. Overall, our study expands the genetic spectrum of PMLD.

Introduction

Pelizaeus-Merzbacher disease (PMD; MIM #312080) is a rare, X-linked recessive hypomyelinating leukodystrophy with prevalence among males of 1:200,000–1:500,000. PMD is a prototypic hypomyelinating leukodystrophy (HLD) with classic clinical features including congenital nystagmus, developmental delay, ataxia, progressive spasticity, and diffuse abnormality of the white matter on brain magnetic resonance imaging (MRI). PMD is caused by mutations in *PLP1*, encoding for myelin proteolipid protein, a major protein component of central nervous system myelin (Madry et al. 2010). The clinically similar disease, Pelizaeus-Merzbacher-like disease (PMLD; MIM #608804), shares several features of PMD; however, patients with PMLD typically possess better cognitive and motor function. In addition, PMLD is usually inherited in an autosomal recessive manner in both males and females (Biancheri et al. 2013) and exhibits

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00439-019-02077-7) contains supplementary material, which is available to authorized users.

✉ Isabelle Schrauwen
isabelle.schrauwen@gmail.com

✉ Sampathkumar Rangasamy
srangasamy@tgen.org

[1] Center for Rare Childhood Disorders, Translational Genomics Research Institute, Phoenix, AZ, USA

[2] Neurogenomics Division, Translational Genomics Research Institute, Phoenix, AZ, USA

[3] Present Address: Department of Translational Genomics, University of Southern California, Los Angeles, CA, USA

[4] Present Address: Center for Statistical Genetics, Department of Neurology, Gertrude H. Sergievsky Center, Columbia University Medical Center, New York, NY, USA

genetic heterogeneity, given that the disease is caused by mutations in one of several genes (Supplementary Table 1).

We identified novel compound heterozygous mutations in *SNAP29* (p.Leu119AlafsX15, c.354DupG and p.0?, c.2T > C) in a patient with the clinical features of PMLD. *SNAP29* is a member of the *SNAP25* gene family, encoding a soluble *N*-ethylmaleimide-sensitive attachment receptor (SNARE) protein that is important in prophase kinetochore formation during mitosis (Morelli et al. 2016). Loss-of-function mutations in *SNAP29* have been reported in patients with cerebral dysgenesis, neuropathy, ichthyosis, and palmoplantar keratoderma (CEDNIK) syndrome (MIM # 609528). CEDNIK disease is characterized by microcephaly, severe neurologic impairment, psychomotor retardation, failure to thrive, facial dysmorphism, palmoplantar keratoderma, late-onset ichthyosis, roving eye movement, and various degrees of cerebral dysgenesis on brain MRI. Epidermal knockout mouse models of *SNAP29* show reduced body size, an ichthyosiform appearance, and die shortly after birth, confirming the importance of *SNAP29* (Schiller et al. 2016). In addition, loss of SNAP29 has been shown to affect cell migration and endocytosis in CEDNIK patients (Rapaport et al. 2010).

In this report, we describe a male child with a PMLD phenotype consisting of congenital nystagmus, hypotonia, developmental delay, mild optic atrophy, and lack of myelination of the subcortical white matter on an MRI scan with improvement over time. Whole exome sequencing (WES) led to the identification of compound heterozygous mutations in *SNAP29,* while no variants were detected in genes known to cause PMLD, such as *AIMP1* and *MAG.* Additionally, no variants were detected in *HSPD1*, a gene previously associated with leukodystrophy (Yamamoto et al. 2018). Clinical testing did not reveal any variants in *PLP1* or *GJC2*, previously associated with PMD and PMLD, respectively. Our finding expands the spectrum of disorders associated with *SNAP29* and suggests that *SNAP29* mutations should also be considered in cases of suspected PMLD.

Case report

A 12-year-old Caucasian/Hispanic male was diagnosed with hypotonic cerebral palsy, whose findings include congenital nystagmus, muscle hypotonia, delayed development, lack of myelination in the subcortical white matter, and absence of the splenium of the corpus callosum. He was born by vaginal delivery, full-term and product of a second pregnancy to a 21-year-old woman who had one healthy child (G2 P1-2). The pregnancy was uneventful, and the child weighed 7 lb 2 oz. He showed mild neonatal jaundice, which did not require phototherapy, and was discharged home 24 h later as a healthy neonate. His mother noted oscillatory horizontal eye movements shortly after birth, and he took a long time to feed. He was very quiet, and hardly ever cried. Because of poor visual fixation and a head lag, he was referred to neurology and ophthalmology at 3 months of age. Initial examination revealed that the patient's head circumference was at the 50th percentile. He did not fix and follow, had intermittent exotropia, mild optic atrophy, and continuous horizontal pendular nystagmus. He was hypotonic in his trunk and had a significant head lag. His strength seemed normal and his tendon reflexes were brisk; both the crossed adductor reflex and Babinski's sign were present. MRI of the brain and orbits at 4 months of age was normal, without features of septo-optic dysplasia (Fig. 1a, b). Genetic testing (Nemours Children's Hospital, Orlando, FL, USA) showed neither duplication nor sequence alteration of the proteolipid protein 1 (*PLP1*) gene.

The patient had global developmental delay, but made slow and steady progress. He started to roll over at 14 months, but was not able to crawl or maintain sitting. By 15 months, he could finger feed and hold a cup, but could not push up in a prone position. Examination continued to show pendular horizontal nystagmus, severe hypotonia and head lag on traction, preserved tendon reflexes, and Babinski's sign. No dysmorphic features or abnormalities of his skin were noted at that time. Follow-up MRI of the brain at 21 months revealed absent splenium of the corpus callosum and normal myelination in the anterior corpus callosum, posterior limb of the internal capsule, and the cerebellum (Fig. 1c, d). There was diffusely abnormal myelination in the cerebral white matter of all lobes (centrum semiovale and subcortical white matter) suggestive of a leukodystrophy or severe delay in myelination. The optic nerves were small and the septum pellucidum was intact. A CT scan of the head at 2 years showed no calcifications within the brain, but diffuse low density of the white matter.

At 3 years of age, he could not get to a sitting position. He was hypotonic in the trunk, and would flop forward if unsupported. At 4 years, he could maintain sitting posture, was able to scoot on his back, could stand briefly with support and feed himself. At 5.5 years, he communicated primarily by signing, and knew 30–40 signs. He moved by rolling over or scooting on his back, and could propel himself in his wheelchair. At 6.5 years, he could manipulate his wheelchair, and could stand and take a few steps with support. He was learning to use a walker and a gait trainer. He learned to say a few words. The MRI scans were repeated when he was 5 (Fig. 1e, f) and 10 years old (Fig. 1g, h). Both these studies showed severely delayed or arrested myelination of the cerebral white matter with some myelination in the genu of the corpus callosum and the brainstem. The splenium of the corpus callosum was absent, unchanged from the scan performed at 21 months. Chromosomal microarray analysis (Oligo Array v. 6.5; Baylor College of Medicine, Houston, TX, USA) was normal. Screening for congenital disorders of

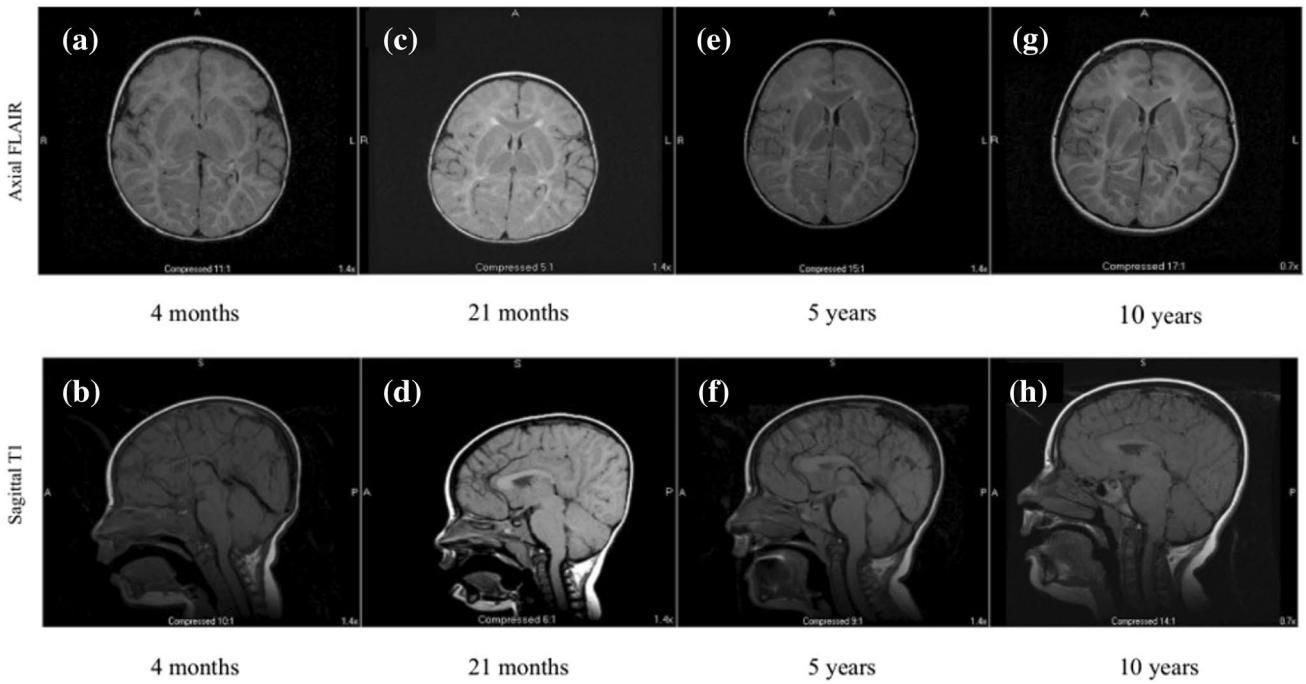

Fig. 1 Evolution of Brain MRI findings with age—4 months, 21 months, 5 years and 10 years. **a**–**d** Axial FLAIR images showing lack of myelination in the centrum semiovale and subcortical white matter. **e**–**h** Sagittal T1-weighted images showing stable truncation of the posterior corpus callosum (absent splenium)

glycosylation (carbohydrate-deficient transferrin assay) was normal. Urine organic acids showed features of mild ketosis (elevated acetoacetate), plasma lactate was mildly elevated at 2.4 mmol/L (normal up to 2.2 mmol/L) and pyruvate was 0.19 mmol/L (normal being 0.3–0.7 mmol/L). Genetic testing for mutations in gap junction protein gamma 2 (*GJC2)* (Nemours Children's Hospital, New Orleans, FL) was found to be negative. At 14 years of age, the patient was diagnosed by a dermatologist with acne in the face, chest and back, and some verruca papules (warts). He was considered to have areas of dry skin, and no signs of ichthyosis or keratoderma (Supplementary Fig. 1).

The clinical features characterized by the presence of early onset of nystagmus and absence of myelination initially led to the differential diagnosis of PMD. However, absence of a genetic diagnosis for PMD established Pelizaeus-Merzbacher-like disease (PMLD) as the primary diagnosis.

Results

Whole exome sequencing

Exome sequencing in the nuclear family led to the identification of compound heterozygous mutations in *SNAP29* in the patient: (a) c.2T > C (p.0?) in exon 1 (chr22:21213400:T > C), which shifts the in-frame translation initiation codon 65 codons downstream, and (b) c.354dupG (p.Leu119AlafsX15) in exon 2 (chr22:21224735:T > TG) resulting in a frame-shift mutation (NM_004782.3). These mutations were confirmed by Sanger sequencing in the patient by a CLIA-certified laboratory (GeneDx, Gaithersburg, USA). The c.2T > C was reported by GeneDx in the homozygous state in a patient with CEDNIK features and was classified as pathogenic. The second variant (c.354dupG) was previously reported in a patient with CEDNIK syndrome (Farwell et al. 2015). Additionally, the variant was reported by Illumina Clinical Services Laboratory as a variant of unknown significance in a homozygous state. The same variant was also reported in ClinVar as a pathogenic by GeneDx in the homozygous state, but the phenotypic information is not available. The c.2T > C variant showed a combined annotation-dependent depletion (CADD) score (Kircher et al. 2014) of 18.9, while the frame-shift mutation in the middle of the protein (p.Leu119AlafsX15) is expected to create a loss-of-function allele. No other likely damaging candidate variants consistent with our patient's phenotype were found in WES analysis (Supplementary Table 2). In particular, we did not identify any pathogenic variants in GJC2, *HSPD1*, *AIMP1, MAG, TUBB4A, FAM126A,* or *RARS,* genes causing HLD including PMD or PMLD (Feinstein et al. 2010; Lossos et al. 2015). Trio analysis indicates that the c.2T > C (p.0?) mutation was inherited from the father, while the

c.354dupG (p.Leu119AlafsX15) mutation was inherited from the mother.

RNA and protein expression

SNAP29 expression was significantly lower in the patient's blood cells, compared to five unaffected and unrelated young controls ($p = 0.003$; fold change: $-0.6\times$; Fig. 2a, b). No difference in exon usage was seen between the patient and the controls. Western blotting analysis of the primary dermal fibroblast cells from the patient showed an absence of full-length SNAP29 protein expression (Fig. 2c). However, the use of a c-terminal antibody does not exclude the expression of a short-truncated protein (p.Leu119AlafsX15) due to the frame-shift c.354dupG mutation. RNA sequencing analysis of the patient cells showed that the only transcribed allele of the *SNAP29* gene included the c.2T > C allele, and that the maternally inherited c.354dupG frame-shift mRNA is not expressed and may have undergone nonsense-mediated decay (NMD), as would have been predicted in this case by the 50-bp rule. We also found upregulation of two gene-encoding proteins that directly interact with SNAP29 ($p = 0.014$ and $p = 0.030$ for *EHD1* and *COX6B1,* respectively; Fig. 2a) in our patient's blood sample. Genome-wide analysis also showed several genes that were significantly differentially expressed between the patient and the controls, shown in Supplementary Fig. 2.

Methods

DNA sequencing

Exome libraries were prepared with the TruSeq DNA sample preparation and Exome Enrichment kit (62 Mb; Illumina, San Diego, CA, USA), following the manufacturer's protocol. Sequencing was performed by 101 bp paired-end sequencing on a HiSeq 2000 instrument (Illumina).

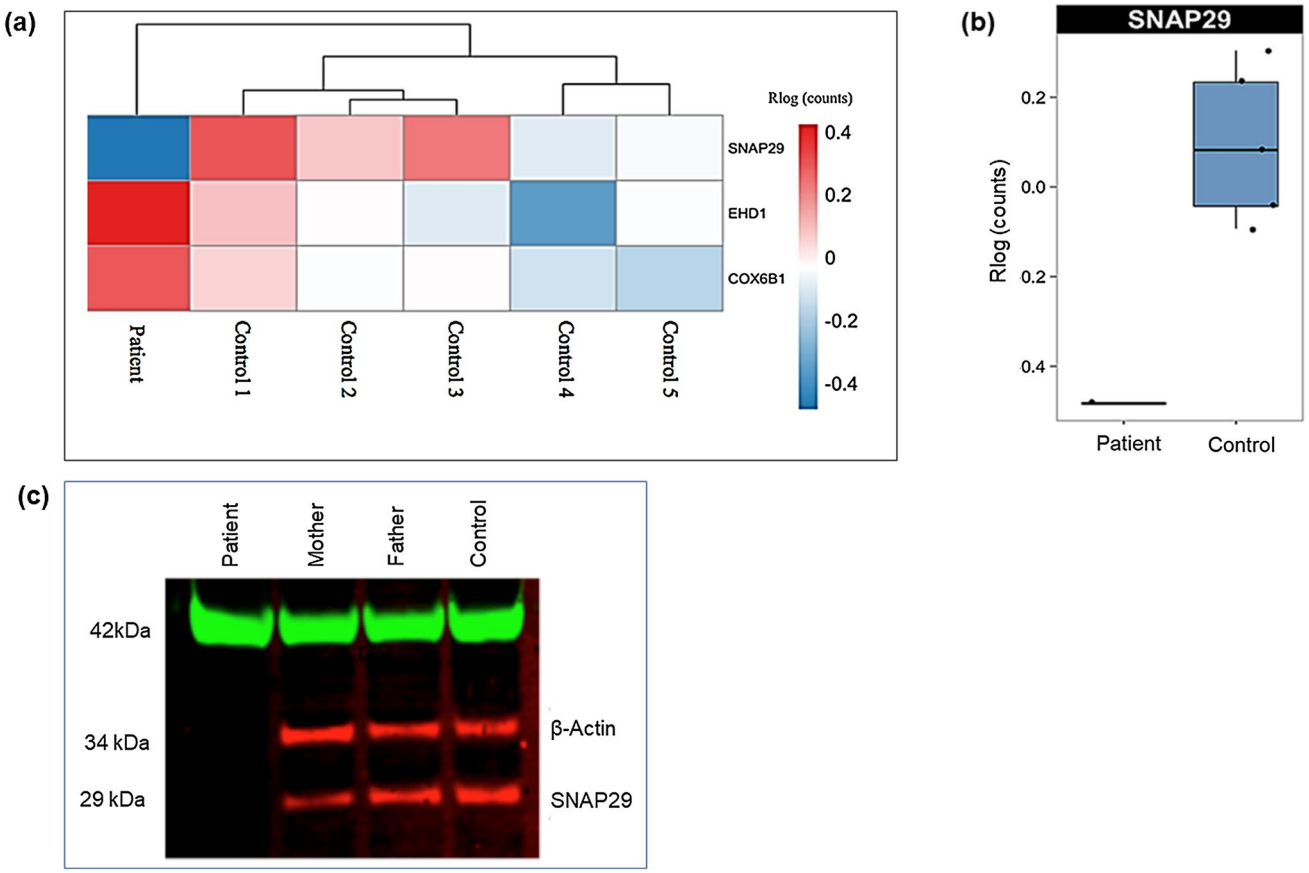

Fig. 2 **a** Heatmap of the mRNA expression for *SNAP29* and the mRNAs encoding the SNAP29-interacting proteins *EHD1* and *COX6B1* in the patient and five age-matched controls. SNAP29 expression is significantly lower in the patient ($p = 0.003$ for *EHD1* and *COX6B1*). Expression of both interactors is upregulated in the patient compared to the controls ($p = 0.014$ and $p = 0.030$). Expression values are plotted as Rlog(counts). **b** Boxplot of *SNAP29* mRNA expression in the patient and five age-matched controls. Expression values are plotted as Rlog(counts). **c** Western blot analysis of primary skin-derived fibroblast cell lysates from the patient, mother, father, and control were probed with mouse anti-B-actin (control, marked in green) and rabbit anti-SNAP29 (marked in red)

Filtered reads were aligned to the human genome (Hg19/GRCh37) using the Burrows-Wheeler transform (BWA-MEM v0.7.8) (Li and Durbin 2009). PCR duplicates were marked using Picard (v1.128) and base quality recalibration, and indel realignment was performed using the Genome Analysis Toolkit (GATK v3.3) (McKenna et al. 2010). Variants were called jointly with HaplotypeCaller, recalibrated with GATK, and annotated with dbNSFP (v3.1) and snpEff (v3.2a) for protein-coding events. Prediction scores were loaded from dbNSFP (v3.1) and used for filtering (Cingolani et al. 2012). An annotated variant list was generated from the trios and variants were filtered for known genetic models including de novo, recessive, compound heterozygous, and X-linked. We utilized several methods to identify candidate variants from the exome data, such as population frequency by referencing the Exome Aggregation Consortium (ExAC) database and Genome Aggregation Database (gnomAD) (Lek et al. 2016), Combined Annotation-Dependent Depletion (CADD) (Kircher et al. 2014), Genomic Evolutionary Rate Profiling (GERP) (Cooper et al. 2010), MutationTaster (Schwarz et al. 2010), and Polyphen 2 (Adzhubei et al. 2010). Sanger sequencing was performed on the patient and his unaffected brother by the CLIA-certified laboratory GeneDx (Gaithersburg, MD, USA), and on the parents by the authors. In short, the target areas of the gene were PCR amplified and capillary sequencing was performed. A bi-directional sequence was assembled, aligned to reference gene sequences based on human genome build GRCh37.70/UCSC hg19 and analyzed for known familial sequence variant(s) (Applied Biosystems, Foster City, CA, USA).

Patient characteristics and sample collection

All subjects were consented into a research protocol at TGen (20120789). The study protocol and consent procedure were approved by the Western Institutional Review Board. Blood samples were obtained from the patient, a 12-year-old boy and his parents. Blood samples were also collected from five healthy, unrelated, age-matched controls. Fibroblast cell lines from the study participants were established from 3 mm skin biopsy punches and cultured for 2 weeks in primary fibroblast media containing the following: Minimal Essential Media (Invitrogen, Carlsbad, CA, USA), 20% FBS (American Type Culture Collection, Manassas, VA, USA), Penicillin/Streptomycin and Amphotericin (Sigma-Aldrich, St. Louis, MO, USA), and Plasmocin (InvivoGen, San Diego, CA, USA) (Villegas and McPhaul 2005). Written informed consent for publication of clinical details and clinical images was obtained from the legally authorized representative and the participant's family.

RNA sequencing

RNA was extracted from whole blood using the PAXgene Blood RNA Isolation Kit (Qiagen, Hilden, Germany), and prepared with the TruSeq RNA Library Preparation Kit v2 (non-stranded; Illumina). Barcoded libraries were pooled (8-plex) and run on two lanes of a flow cell on an Illumina HiSeq 2000 instrument. Sequencing was performed using 2 × 83 bp reads. The dataset was aligned to the current reference genome GRCh37 using RNAstar (v2.4.0) (Widmann et al. 2012), and quality control was performed using Picard RnaSeqMetrics (v1.128). Gene and exon read counts were generated using HTSeq (Anders et al. 2014). Normalization, differential expression analysis, and correction for multiple testing were performed with DESeq 2 (Love et al. 2014). We investigated SNAP29 expression and its direct interactors. SNAP29 interaction partners were downloaded from BioGRID (v3.4). Resulting counts were transformed and plotted as regularized logarithms (rlog). Plots were made in R v3.1.2 (www.R-project.org). Heatmaps (Fig. 2a and Supplementary Fig. 2) were made using Euclidean distances and the complete linkage clustering method using the heatmap R-package.

Western blotting

Fibroblast cells were lysed using 1 × Cell Lysis Buffer (Cell Signaling Technology, Danvers, MA, USA), including a Halt Protease and Phosphatase Inhibitor Cocktail (Sigma-Aldrich, St. Louis, MO, USA). A BCA Protein Assay (Thermo Fisher Scientific, Waltham, MA, USA) was used to measure the protein concentration, and 50 µg of each lysate was used for western blotting. The samples were denatured at 70 °C for 10 min in Bolt Sample Reducing Reagent (Life Technologies, Carlsbad, CA, USA) and loaded onto a Bolt 4–12% Bis–Tris gel, together with a SeeBlue Pre-Stained Standard (Life Technologies, Carlsbad, CA, USA), and electrophoresed at 200 V for 32 min. The samples were transferred onto a PVDF membrane for 6 min at 12 V, using an iBlot 2 Dry Blotting System (Thermo Fisher Scientific, Waltham, MA, USA). The membrane was blocked in 5% goat serum for 1 h at room temperature, and incubated overnight with the following primary antibodies in filtered TBST: C-terminal rabbit anti-SNAP29 (1/500) (ab138500; Abcam, Cambridge, UK) or mouse anti-B-actin (1/1000) (8H10D10; Santa Cruz Biotechnology, Dallas, TX, USA) as a loading control. Next, the membrane was incubated in the following secondary antibodies in filtered TBST for 1 h at room temperature: anti-rabbit IgG (5366P; 1/5000; Cell Signaling Technology, MA, USA), anti-mouse IgG (5257P; 1/5000; Cell Signaling Technology, MA, USA) and followed by four washes (5 min each) with filtered TBST and two washes (5 min each) with filtered PBS. The membrane was

visualized using an Odyssey CLx Imaging system (LI-COR Biosciences, Nebraska, USA).

Discussion

In the present study, we report compound heterozygous mutations (p.Leu119AlafsX15, c.354DupG and p.0? c.2T > C) in *SNAP29* (22q11.21) associated with Pelizaeus-Merzbacher-like disease (PMLD). The c.354dupG (p.Leu119AlafsX15) *SNAP29* mutation causes a frameshift and results in a premature stop codon. This variant is predicted to cause loss of normal protein function through protein truncation or nonsense-mediated mRNA decay. The other variant, c.2T > C (p.0?), either shifts the in-frame translation initiation codon 65 codons downstream, thereby completely disrupting the protein product, or prevents protein translation. Both RNA sequencing of peripheral blood samples (Fig. 2a, b) and western blotting (Fig. 2c) of protein extracted from patient-derived fibroblasts confirmed loss of SNAP29 expression, when comparing the affected individual to his parents and other controls.

Mutations in *SNAP29* have previously been related to CEDNIK syndrome, identified by ichthyosis of late onset, palmoplantar keratoderma, microcephaly, facial dysmorphism or roving eye movement (Sprecher et al. 2005). All seven patients originally described by Sprecher et al. were from two unrelated consanguineous Arab families and presented with microcephaly, facial dysmorphism, psychomotor retardation, palmoplantar keratoderma, and ichthyosis. The probands in the family shared the same homozygous 1 bp deletion (c.220delG) resulting in a frameshift and a premature termination of translation. Another homozygous 1 bp deletion (c.223delG) in *SNAP29* was reported in two sisters from a consanguineous Jordanian family (Ben-Salem et al. 2015) and both were diagnosed with CEDNIK syndrome. Loss-of-function mutations by insertions in *SNAP29* (c.486insA) have also been reported (Fuchs-Telem et al. 2011) in two siblings from a consanguineous Pakistani family who showed a similar phenotype to CEDNIK patients described by Sprecher et al. A homozygous nonsense mutation in this *SNAP29* (c.85C > T) was reported in a Jordanian American patient with global developmental delay, dysgenesis of the corpus callosum, dysmorphic features, polymicrogyria, optic nerve dysplasia, and gaze apraxia (Hsu et al. 2017).

As such, there is a consistency of Arab families to present with *SNAP29* mutations causing CEDNIK syndrome. In contrast, our patient is a Caucasian/Hispanic male who presented with congenital nystagmus, hypotonia, delayed development, mild optic atrophy, and lack of myelination of the subcortical white matter on an MRI scan, which improved over time. This phenotype is consistent with the hypomyelinating leukodystrophy Pelizaeus-Merzbacher disease (PMD). However, the child showed no mutations in the PMD-related gene (*PLP1*). The phenotype of PMLD is broad, however, our patient presented with a suggestive phenotype of PMLD such as nystagmus, motor developmental delay, and central hypotonia during infancy. Signs of upper motor neuron dysfunction (including brisk tendon reflexes, and Babinski sign) and gait abnormalities were present. Brain MRI revealed severely delayed myelination of the cerebral white matter with some myelination in the genu of the corpus callosum and the brainstem. The presence of the hypomyelinating pattern is consistent with a PMD diagnosis. However, the presence of nystagmus, hypotonia, and hypomyelination along with the absence of mutation in gPLP1 gene known to cause PMD strongly suggest that the identified *SNAP29* compound heterozygous mutation is a novel genetic cause of PMLD. Yet, some of the clinical symptoms such as hypotonia, delayed motor milestones, feeding difficulties, and abnormality of the corpus callosum observed in CEDNIK syndrome overlapped with our patient described here (Table 1).

SNAP29 is an essential protein that is expressed in neurons, oligodendrocytes, Schwann, and Purkinje cells. Schardt et al. reported SNAP29 expression in mice before birth, with levels increasing into adulthood, indicating the importance of SNAP29 throughout development and adulthood. SNAP29 interacts with Rab3A GTPase, and they both regulate neurotransmitter release at the presynaptic terminal, a very crucial role in exocytosis during glial cell myelination (Schardt et al. 2009). As a part of the SNAP25 family of proteins, SNAP29 functions as a SNARE complex disassembly regulator and helps in the recycling of the components of this complex after fusion inside the cell (Su et al. 2001). As such, a mutation in *SNAP29* would severely disrupt myelin formation, resulting in the neurodevelopmental problems seen in our patient.

Diggle et al. (2017) described three boys previously diagnosed with a homozygous *TUBA8* mutations (Abdollahi et al. 2009) that could not be explained by the lack of phenotype in the TUBA8 knockout mouse model. Interestingly, WES analysis confirmed a homozygous frameshift *SNAP29* mutation (c.487dupA) to be the most likely cause of the phenotype for these patients, which include optic nerve hypoplasia and polymicrogyria (PMG), consistent with CEDNIK syndrome. However, the absence of dysmorphic features, palmoplantar keratoderma or peripheral neuropathy in these patients could be an indicator of the different manifestation of the disease. In addition, SNAP29 has been described as a major modifier of variable expressivity in 22q11.2 DS (McDonald-McGinn et al. 2013, 2015). Phenotypic variability was documented in a subset of patients with 22q11.2 DS who had hemizygous SNAP29 mutations on the non-deleted chromosome. As such, the presence of a

Table 1 List of phenotypes for CEDNIK, PMLD, and the patient presented here

#	Clinical Characteristics	CEDNIK	Patient in this study	PMLD
1	Facial Dysmorphism	+	-	Mild/-
2	Roving eye movement	+	-	-
3	Hypotonia	+	+	+
4	Delayed motor milestones	+	+	+
5	Severe neurological impairment	+	-	+/-
6	Microcephaly	+/-	-	+/-
7	Diffuse leukodystrophy on MRI	-	+	+
8	Abnormality of corpus callosum	+	+	-
9	Various degrees of cerebral dysgenesis	+	-	-
10	Ichthyosis of late onset	+	-	-
11	Palmoplantar Keratoderma	+	-	-
12	Nystagmus	-	+	+
13	Progressive spasticity	-	-	+
14	Dystonia	-	-	+
15	Ataxia	-	-	+
16	Scoliosis	-	-	+
17	Speech delay	+	+	+
18	Seizures	-	-	+
19	Hearing loss	+	-	+
20	Optic atrophy	+	+	+
21	Feeding difficulties	+	+	+
22	Progressive spasticity	-	-	+
23	Babinski sing	-	+	-

compound heterozygous mutation in *SNAP29* in our patient is possible to cause a different phenotype from what has been observed so far.

An expanding number of Mendelian disorders have shown that mutations in the same gene can exhibit a wide range of phenotype and a broader clinical diagnosis (Zhu et al. 2014). Genetic modifiers, epigenetic factors, and mutation location have been shown to cause dissimilar or overlapping phenotypes in Mendelian disorders. Our patient with compound heterozygous *SNAP29* mutations adds to the numerous examples of the same gene causing different diseases.

Concluding remarks

We found compound heterozygous loss-of-function mutations in *SNAP29* in a patient with PMLD. This finding expands the spectrum of *SNAP29*-associated disorders.

Acknowledgements The authors thank the family for participating in this study and all the previous members of the C4RCD research group not included in the author list.

Funding This work was supported by the TGen Foundation funding to Center for Rare Childhood Disorders (C4RCD).

Data deposition and access The exome sequencing datasets have been added to the Database of Genotypes and Phenotypes (dbGaP; http://www.ncbi.nlm.nih.gov/gap) under project phs000816. Both *SNAP29* variants have been reported to ClinVar (htpp://www.ncbi.nlm.nih.gov/clinvar/) under variation IDs 279932 and 279894 (NM_004782.3). The raw sequence data of the father, mother, and child were submitted to the Sequence Read Archive (http://www.ncbi.nlm.nih.gov/sra) with the following Biosample ID numbers: SAMN05687153 (father), SAMN05687449 (mother), SAMN05687373 (child) (DNA), and SAMN05687278 (child) (RNA).

Compliance with ethical standards

Conflict of interest The authors have no competing interests to disclose.

Informed consent Written informed consent for publication of clinical details and clinical images was obtained from the legally authorized representative and the patient's family. The study protocol and consent procedure was approved by the Western Institutional Review Board (WIRB; study number 20120789).

References

Abdollahi MR, Morrison E, Sirey T, Molnar Z, Hayward BE, Carr IM, Sheridan E (2009) Mutation of the variant alpha-tubulin TUBA8 results in polymicrogyria with optic nerve hypoplasia. Am J Hum Genet 85(5):737–744. https://doi.org/10.1016/j.ajhg.2009.10.007

Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, Sunyaev SR (2010) A method and server for predicting damaging missense mutations. Nat Methods. https://doi.org/10.1038/nmeth0410-248

Anders S, Pyl PT, Huber W (2014) HTSeq—a python framework to work with high-throughput sequencing data. Bioinformatics (Oxford, England) 31(2):166–169. https://doi.org/10.1093/bioinformatics/btu638

Ben-Salem S, Nara S, Al-Shamsi AM, Valle D, Ali BR, Al-Gazali L (2015) A new Arab family with CEDNIK syndrome suggests a possible founder effect for the c.223delG mutation. J Dermatol 42(8):821–822

Biancheri R, Rosano C, Denegri L, Lamantea E, Pinto F, Lanza F, Filocamo M (2013) Expanded spectrum of Pelizaeus-Merzbacher-like disease: literature revision and description of a novel GJC2 mutation in an unusually severe form. Eur J Hum Genet 21(1):34–39

Cingolani P, Platts A, Wang LL, Coon M, Nguyen T, Wang L, Ruden DM (2012) A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of Drosophila melanogaster strain w1118; iso-2; iso-3. Fly 6(2):80–92. https://doi.org/10.4161/fly.19695

Cooper GM, Goode DL, Ng SB, Sidow A, Bamshad MJ, Shendure J, Nickerson DA (2010) Single-nucleotide evolutionary constraint scores highlight disease-causing mutations. Nat Methods. https://doi.org/10.1038/nmeth0410-250

Diggle CP, Martinez-Garay I, Molnar Z, Brinkworth MH, White E, Fowler E, Sheridan E (2017) A tubulin alpha 8 mouse knockout model indicates a likely role in spermatogenesis but not in brain development. PLoS One 12(4):e0174264. https://doi.org/10.1371/journal.pone.0174264

Farwell KD, Shahmirzadi L, El-Khechen D, Powis Z, Chao EC, Tippin Davis B, Tang S (2015) Enhanced utility of family-centered diagnostic exome sequencing with inheritance model-based analysis: results from 500 unselected families with undiagnosed genetic conditions. Genet Med 17(7):578–586. https://doi.org/10.1038/gim.2014.154

Feinstein M, Markus B, Noyman I, Shalev H, Flusser H, Shelef I, Birk OS (2010) Pelizaeus-merzbacher-like disease caused by AIMP1/p43 homozygous mutation. Am J Hum Genet 87(6):820–828. https://doi.org/10.1016/j.ajhg.2010.10.016

Fuchs-Telem D, Stewart H, Rapaport D, Nousbeck J, Gat A, Gini M, Sprecher E (2011) CEDNIK syndrome results from loss-of-function mutations in SNAP29. Br J Dermatol 164(3):610–616. https://doi.org/10.1111/j.1365-2133.2010.10133.x

Hsu T, Coughlin CC, Monaghan KG, Fiala E, McKinstry RC, Paciorkowski AR, Shinawi M (2017) Phenotypic and molecular characterization of an additional patient and review of the literature. Child Neurol Open. https://doi.org/10.1177/2329048X17733214

Kircher M, Witten DM, Jain P, O'roak BJ, Cooper GM, Shendure J (2014) A general framework for estimating the relative pathogenicity of human genetic variants. Nat Genet 46(3):310–315. https://doi.org/10.1038/ng.2892

Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, Exome Aggregation Consortium (2016) Analysis of protein-coding genetic variation in 60,706 humans. Nature 536(7616):285–291. https://doi.org/10.1038/nature19057

Li H, Durbin R (2009) Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25(14):1754–1760. https://doi.org/10.1093/bioinformatics/btp324

Lossos A, Elazar N, Lerer I, Schueler-Furman O, Fellig Y, Glick B, Peles E (2015) Myelin-associated glycoprotein gene mutation causes Pelizaeus-Merzbacher disease-like disorder. Brain 138(9):2521–2536. https://doi.org/10.1093/brain/awv204

Love MI, Huber W, Anders S (2014) Moderated estimation of fold change and dispersion for RNA-Seq data with DESeq2. bioRxiv. Cold Spring Harbor Labs J. https://doi.org/10.1101/002832

Madry J, Hoffman-Zacharska D, Królicki L, Jakuciński M, Friedman A (2010) PLP1 gene duplication as a cause of the classic form of Pelizaeus-Merzbacher disease—case report. Neurol Neurochir Pol 44(5):511–515. https://doi.org/10.1016/S0028-3843(14)60142-0

McDonald-McGinn D, Fahiminiya S, Revil T, Nowakowska B, Suhl J, Bailey A, Jerome-Majewska L (2013) Hemizygous mutations in SNAP29 unmask autosomal recessive conditions and contribute to atypical findings in patients with 22q11.2DS. J Med Genet 50:80–90. https://doi.org/10.1136/jmedgenet-2012-101320

McDonald-McGinn DM, Sullivan KE, Marino B, Philip N, Swillen A, Vorstman JAS, Bassett AS (2015) 22q11.2 deletion syndrome. Nat Rev Dis Primers. https://doi.org/10.1038/nrdp.2015.71

McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, DePristo MA (2010) The genome analysis toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res 20(9):1297–1303. https://doi.org/10.1101/gr.107524.110

Morelli E, Mastrodonato V, Beznoussenko GV, Mironov AA, Vaccari T (2016) An essential step of kinetochore formation controlled by the SNARE protein Snap29. Embo J 35(20):1–40. https://doi.org/10.15252/embj.201693991

Rapaport D, Lugassy Y, Sprecher E, Horowitz M (2010) Loss of snap29 impairs endocytic recycling and cell motility. PLoS One. https://doi.org/10.1371/journal.pone.0009759

Schardt A, Brinkmann BG, Mitkovski M, Sereda MW, Werner HB, Nave K (2009) The SNARE protein SNAP-29 interacts with the GTPase Rab3A: implications for membrane trafficking in Myelinating Glia. J Neurosci Res 87:3465–3479. https://doi.org/10.1002/jnr.22005

Schiller SA, Seebode C, Wieser GL, Goebbels S, Möbius W, Horowitz M, Emmert S (2016) Establishment of two mouse models for CEDNIK syndrome reveals the pivotal role of SNAP29 in epidermal differentiation. J Investig Dermatol 136(3):672–679. https://doi.org/10.1016/j.jid.2015.12.020

Schwarz JM, Rödelsperger C, Schuelke M, Seelow D (2010) MutationTester evaluates disease-causing potential of sequence alterations. Nat Methods. https://doi.org/10.1038/nmeth0810-575

Sprecher E, Ishida-Yamamoto A, Mizrahi-Koren M, Rapaport D, Goldsher D, Indelman M, Mandel H (2005) A mutation in SNAP29, coding for a SNARE protein involved in intracellular trafficking, causes a novel neurocutaneous syndrome characterized by cerebral dysgenesis, neuropathy, ichthyosis, and palmoplantar keratoderma. Am J Hum Genet 77(2):242–251. https://doi.org/10.1086/432556

Su Q, Mochida S, Tian JH, Mehta R, Sheng ZH (2001) SNAP-29: general SNARE protein that inhibits SNARE disassembly and is implicated in synaptic transmission. Proc Natl Acad Sci USA 98(24):14038–14043. https://doi.org/10.1073/pnas.251532398

Villegas J, McPhaul M (2005) Establishment and culture of human skin fibroblasts. In: Frederick MA (ed) Current protocols in

molecular biology, chapter 28, unit 283. Wiley, Hoboken. https://doi.org/10.1002/0471142727.mb2803s71

Widmann J, Stombaugh J, McDonald D, Chocholousova J, Gardner P, Iyer MK, Knight R (2012) RNASTAR: an RNA STructural alignment repository that provides insight into the evolution of natural and artificial RNAs. RNA (New York, NY) 18(7):1319–1327. https://doi.org/10.1261/rna.032052.111

Yamamoto T, Yamamoto-Shimojima K, Ueda Y, Imai K, Takahashi Y, Imagawa E, Miyake N, Matsumoto N (2018) Independent occurrence of de novo *HSPD1* and *HIP1* variants in brothers with different neurological disorders—leukodystrophy and autism. Human Genome Var 5:18. https://doi.org/10.1038/s41439-018-0020-z

Zhu X, Need AC, Petrovski S, Goldstein DB (2014) One gene, many neuropsychiatric disorders: lessons from Mendelian diseases. Nat Neurosci. https://doi.org/10.1038/nn.3713