Homozygous Missense Variation in *PNPLA8* Causes Prenatal-Onset Severe Neurodegeneration

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Established Facts

- *PNPLA8* is associated with mitochondrial myopathy and poor weight gain with lactic acidosis.
- Previously reported patients had truncating variations that lie outside the conserved patatin domain of *PNPLA8*.

Novel Insights

- The authors report a patient with prenatal-onset severe and progressive neurodegeneration with variation in *PNPLA8*.
- The patient presented in this report harbors a novel missense variant in the patatin domain of *PNPLA8*.

Keywords

Congenital microcephaly · Neurodegenerative disorder · Patatin domain · Whole-exome sequencing · *PNPLA8*

Abstract

The patatin-like protein family plays an important role in various biological functions including lipid homeostasis, cellular growth, and signaling. Conserved across species, the patatin domain is shared by all 9 members of the *PNPLA* family without redundancy in the coding sequences. The defective function of *PNPLA2*, *PNPLA6*, and *PNPLA9* are known to cause mitochondrial-related neurodegeneration. Recently, *PNPLA8* has been associated with mitochondrial myopathy and poor weight gain with lactic acidosis in 3 unrelated families. Using

whole-exome sequencing, we identified a homozygous novel missense variation c.1874A>G in the patatin domain of *PNPLA8*. The patient had prenatal-onset severe and progressive neurodegeneration with mortality in infancy.

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Introduction

Mitochondriopathies are a group of clinically heterogeneous genetic disorders caused either by sporadic or inherited variants in genes positioned on nuclear or mitochondrial DNA. The majority of proteins involved in mitochondrial function are encoded by nuclear genes. Decreased mitochondrial respiration is seen in cases with defective

Fig. 1. **a** Pedigree of the family (black arrow indicates the proband). **b** Proband at 5 months of age with microcephaly, receding forehead, thin upper lip, low-set ears and neck hyperextension. **c** Sequence chromatogram of the proband showing homozygous c.1874A>G in *PNPLA8* (NM_015723) and carrier parents. Ultrasonography done at gestational age of 20 weeks showed **d** normal

cerebral development and **e** cerebellar hypoplasia. Postnatal CT scan of the proband at 5 months of age showed atrophy of cerebral hemispheres (**f**) and cerebellum (**g-i**). Hypoplasia of pons is also noted (white arrow in **i**). **j** Schematic representation of *PNPLA8* with the previously reported variants (black) and in our patient (red).

phospholipases A2, which is essential for normal mitochondrial function. *PNPLA8* is one of the predominant calcium-independent phospholipases in mammalian mitochondria. Variations in this gene have been associated with mitochondrial myopathy and poor weight gain with lactic acidosis (OMIM 251950) [Saunders et al., 2015; Shukla et al., 2018]. Here, we report a fourth patient with a sequence variation in *PNPLA8* with prenatal-onset microcephaly, intractable seizures, and spasticity. Our patient harbored a homozygous missense variation in contrast to previous patients who had nonsense sequence variations.

Case Presentation

A nonconsanguineous married woman presented at 20 weeks 1 day of pregnancy with a history of the death of 3 offspring during infancy (shown in Fig. 1a). All of them had intractable seizures with onset during the neonatal period. They did not achieve any developmental milestones. During this pregnancy, ultrasonography at 20 weeks showed normal cerebral development (Fig. 1d) and cerebellar hypoplasia (Fig. 1e). At 21 week 3 days, ultrasonographic pictures of the fetal brain showed cerebellar atrophy (transverse cerebellar diameter of -3.2 Z score) and head circumference (16.7 cm) less than -2 Z score. The microcephaly and cerebellar hypoplasia continued to progress during fetal life. At the

gestational age of 24 weeks, the fetus' head circumference was 19.14 cm (−3 Z score) and abdominal circumference was 18.65 cm (−0.7 Z score). A female baby was delivered at term having a birth weight of 2.5 kg (−1.7 Z score). Record of head circumference at birth was not available. She was in a neonatal intensive care unit soon after birth with intractable seizures and generalized hypertonía. She was brought to us at 5 months of age for an evaluation. She had severe global developmental delay and had not achieved any milestones. She had a receding forehead, thin upper lip, low-set ears, and neck hyperextension (Fig. 1b). Her length was 55 cm (−4 Z score), weight was 3 kg (−5 Z score), and her head circumference was 32 cm (−8 Z score). She had generalized spasticity and opisthotonic posturing. CT of the brain showed a shriveled brain (Fig. 1f) and atrophy of the cerebellar hemispheres (Fig. 1g–i). Comparison with images of prenatal ultrasonography suggested a progressive loss of brain matter and architecture. The child died at 6 months of age. Phenotype and neuroimaging were suggestive of pontocerebellar hypoplasia, especially type 4 and 5 (caused by variations in *TSEN54*), which are known to have onset during prenatal life [Patel et al., 2006; Namavar et al., 2011].

Materials and Methods

Genomic DNA was extracted from peripheral blood lymphocytes using QIAamp DNA Extraction Mini Kit. Chromosomal microarray was done using Affymetrix CytoScan 750K array (Affymetrix, Inc. Santa Clara, CA, USA). Whole-exome sequencing (WES) of the proband was performed on Illumina NextSeq500 platform after library preparation and capture using Illumina Nextera Rapid Capture Exome Kit V1.2 (Illumina, Inc., San Diego, CA, USA). Bioinformatics analysis was done using an in-house pipeline based on BWA-mem, Picard tools, GATK, and Annovar. The coverage of genes known to be causative of pontocerebellar hypoplasia phenotype was also examined. Sanger sequencing of the variant identified was performed on ABI 3100Dx Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

Results

Chromosomal Microarray

No pathogenic/likely pathogenic copy number loss/gain was identified in our patient. Taking 3 Mb as laboratory cut-off value, a total region of 72 Mb with loss of heterozygosity was identified. However, this region did not have any genes relevant to the phenotype observed in our patient.

Whole-Exome Sequencing

Prioritizing for biallelic variants based on family history, a homozygous missense variation c.1874A>G (p. His625Arg) in *PNPLA8* (NM_015723) was identified in the proband. The variant has not been reported in mutation databases like ClinVar and HGMD. This variation

has a minor allele frequency of 0.00003184 (1 heterozygous carrier of the European non-Finnish population) in gnomAD. It is not present in 1000 genomes and in our in-house exomes of 727 individuals. This variation was predicted as deleterious by various in silico prediction tools (MutationTaster, Provean, SIFT, and PolyPhen2). Sanger sequencing of exon 7 was done to validate the results of WES in the proband and showed both parents to be heterozygous for the variant (Fig. 1c). No other variation relevant to the patient's phenotype was detected in the WES data.

Discussion

We report a patient with a novel missense sequence variation in *PNPLA8* characterized by severe and progressive neurodegeneration with documented prenatal onset. The patient had documented microcephaly and cerebellar hypoplasia on second trimester ultrasonography. Postnatal CT scan showed shriveled cerebellum and cerebrum. Recently, Shukla et al. [2018] reported 2 unrelated individuals with biallelic variations in *PNPLA8*. Microcephaly was reported prenatally in one patient with neonatal-onset multifocal clonic seizures but it was more severe in our patient (Table 1). The other patient with compound heterozygous variation achieved all milestones until 13 months after which she developed seizures, neuroregression and progressive weakness, and involuntary movement. She shared clinical similarity with the first patient reported by Saunders et al. [2015], who developed progressive mitochondrial myopathy with dystonia at 1 year of age.

PNPLA8 is a calcium-independent phospholipases A2 (iPLA2s) belonging to a patatin-like protein family and shares homology with patatin, a lipid hydrolase. Having mitochondrial and peroxisomal localization signals in the N-terminal and C-terminal, respectively, it is the predominant phospholipase active in the mammalian mitochondria and peroxisome and is thought to have a role in maintaining lipid homeostasis in these organelles [Hara et al., 2019]. Presently, 9 *PNPLA* genes (*PNPLA1*–*PNPLA9*) are known in humans [Kienesberger et al., 2009]. While most members of *PNPLA* family (*PNPLA2*, *PNPLA3*, *PNPLA5*, and *PNPLA7*) have their role in lipid storage and metabolism [Fischer et al., 2007; Reilich et al., 2011; Mikhailova et al., 2019; Pingitore and Romeo, 2019; Wang et al., 2020], *PNPLA1* is associated with congenital ichthyosis (OMIM #612121). Labonne et al. [2020] recently proposed *PNPLA4* as likely genes for X-linked in-

Table 1. Clinical and molecular findings in patients with pathogenic variation in *PNPLA8*

	Saunders et al. [2015]	Shukla et al. [2018]		Our patient
Patients	1	2	3	4
Origin	NA	India	India/Fiji	India
Consanguinity	NA	+	–	–
Age/Gender	7 years/F	6 days/F	2 years/F	5 months/F
Amino acid change	p.Asn212Hisfs*29 p.Leu759Alafs*4	p.Glu173*	p.Leu759Alafs*4 p.Ser140*	p.His625Arg
Prenatal	Uneventful	Microcephaly reported in third trimester	NA	Cerebellar atrophy and microcephaly reported in second trimester
Family history of similarly affected member	+	–	NA	+
Birth weight, g	3,500 (+1 SD)	2,410 (–2 SD)	NA	2,500 (–1.7 SD)
Disease onset	1 year	Prenatal	13 months	Prenatal
Microcephaly	NA	–4 SD	–4.7 SD	–8 SD
Proximal muscle weakness	+	–	+	NA
Developmental delay	+	NA	+	+
Seizures	+	+	+	+
Tone	Spastic	Spastic	Spastic	Spastic
Dystonia	+	NA	+	NA
Speech	Dysarthria	NA	Nonverbal	Nonverbal
Brain imaging	NA	Hypoplastic cerebellum, prominent cisterna magna, simplified gyral pattern, hypoplasia of corpus callosum	Prominent ventricles, delayed myelination, hypoplastic cerebellum, hypoplasia of corpus callosum	Diffused severe cerebral atrophy, hypoplasia of cerebellum and pons, fourth ventricle communicating with cisterna magna

NA, not available; SD, standard deviation; + present, – absent.

tellectual disability based on comparative genomic mapping study. Variations in *PNPLA6* are reported to cause Oliver-McFarlane syndrome (OMIM# 275400), Laurence-Moon syndrome (OMIM# 245800), and Boucher-Neuhauser syndrome (OMIM# 215470). These disorders have variable overlapping features of intellectual impairment, hypogonadism, and retinal degeneration. Additionally, *PNPLA6* variations are also causative of spastic paraplegia type 39 (OMIM# 612020). Variations in *PNPLA9* consequently result in neurodegeneration with brain iron accumulation 2B (OMIM# 610217), infantile neuroaxonal dystrophy 1 (OMIM# 256600), and Parkinson disease type 14 (OMIM# 612953).

Our patient harbored a missense variant in the patatin domain of *PNPLA8*. All 3 previously reported patients had truncating variations that lie outside this domain (Fig. 1j). The patatin domain is shared by all 9 members of the *PNPLA* family without redundancy in the coding sequences and is conserved across species. Thus, it may be suggested that missense variation in the patatin domain can result in severe phenotypes and early infantile lethality comparable to truncating variations outside it (Table 1). A similar phenotype was recapitulated in mouse models with *PNPLA8* null variants [Mancuso et al., 2009]. The cellular consequence of missense variation in the patatin domain, however, remains to be elucidated by functional validation.

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Statement of Ethics

The study was approved by the institutional ethics committee. Informed written consent was obtained from the parents for genetic testing and publication of clinical details and photographs.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Suzena Masih drafted the manuscript and performed the Sanger sequencing of the variant identified in WES. Whole-exome data analysis and variant identification was done by Amita Moirangthem. Clinical evaluation of the presented case and overall supervision of the study was done by Shubha R Phadke. All authors discussed the results and contributed to the final manuscript.

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