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A Neurodegenerative Mitochondrial Disease Phenotype Due to Biallelic Loss-of-function Variants in *PNPLA8* Encoding Calcium-independent Phospholipase A2γ

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Abstract

Animal studies have demonstrated the critical roles of the patatin-like protein family plays in cellular growth, lipid homeostasis and second messenger signaling the nervous system. Of the nine known calcium-independent phospholipase A2γ, only *PNPLA2*, *PNLPA6, PNPLA9* and most recently a single patient with *PNPLA8* are associated with mitochondrial-related neurodegeneration. Using whole exome sequencing, we report two unrelated individuals with variable but similar clinical features of microcephaly, severe global developmental delay, spasticity, lactic acidosis, and progressive cerebellar atrophy with biallelic loss-of-function variants in *PNPLA8*.

Keywords

Patatin-like protein family; *PNPLA8*; Exome sequencing; Mitochondrial disorder

INTRODUCTION

Phospholipases A2 are essential for maintenance of normal mitochondrial function and defects can lead to decreased mitochondrial respiration [Kienesberger et al., 2009]. The Ca

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2+-independent phospholipases A2 (iPLA $_2$s) are part of a diverse family of PLA_2s that hydrolyze membrane phospholipids and do not require calcium for either translocation or activity. They are also designated as group VI $iPLA_2$s. The $iPLA_2$s are included in the patatin-like protein family due to their shared homology with patatin, a lipid hydrolase and are also referred to as patatin-like phospholipase domain containing proteins (PNPLAs). Currently, nine PNPLAs (PNPLA1–9) are known in humans [Kienesberger et al., 2009]. Recently, bi-allelic loss-of-function variants in *PNPLA8* have been reported in a child with progressive muscle weakness, hypotonia, seizures, poor weight gain, and lactic acidosis [Saunders et al., 2015].

We report two additional unrelated individuals with microcephaly, spasticity, lactic acidosis and cerebellum and brainstem volume loss. Our report validates the progressive neurodegenerative phenotype associated with loss-of-function variants in *PNPLA8*.

METHODS

We evaluated two families from two medical centers, with institutional ethics committee approval at both centers. Informed consent was obtained for the use of clinical and research findings for publication.

Patient 1

A female child was evaluated on the sixth postnatal day in the neonatal intensive care unit for congenital microcephaly and seizures. She was the second born child to a consanguineous couple with a two-year old healthy female sibling (Figure 1A). The prenatal period was unremarkable. Parents mentioned that the third trimester ultrasound suggested microcephaly, but documents of head size were unavailable for review. The infant was born full term via normal vaginal delivery. Weight was 2410 gm (−2 SD), length 48 cm (−1 SD) and head circumference 29 cm (−4 SD) at birth. On the second day of life, she had clonic movements in all four limbs and excessive cry. She was treated with phenobarbital and phenytoin for seizure control.

On exam, microcephaly and overriding cranial sutures were noted. She had multifocal clonic seizures and spasticity of all four limbs. Brain MRI showed microcephaly with simplified gyral pattern, hypoplasia of corpus callosum, prominent cisterna magna with hypoplastic cerebellum, brainstem atrophy (Figure 2A–C). Magnetic resonance spectroscopy (MRS) showed an elevated lipid-lactate peak. TORCH testing (immunoblotting for toxoplasmosis, rubella, cytomegalovirus, herpes simplex virus 1 and 2) and bacterial culture for blood was negative. Hematology, serum sodium, potassium, calcium, magnesium, C-reactive protein, renal functions tests were normal. Phenotype data can be accessed at www.phenomecentral.org (ID: P0004651).

Patient 2

This patient presented at two-years of age. She was born to non-consanguineous parents. By history, she had normal development until about 13 months of age. She was able to independently sit, stand, cruise, and babble. However, she then regressed concomitantly with several episodes of epilepsy partialis continua and difficult to control focal seizures. At first

exam at 2 years of age, head circumference was approximately at the 50% percentile. Initial developmental assessment at 21 months of age demonstrated a performance at an 8-month level. Her development has remained severely stagnated at this level over the ensuing years. Seizures are well controlled on two medications. At 2 years, she was non-verbal and required gastrostomy tube for nutrition. Central hypotonia together with muscle weakness contributes to her night requirement for continuous positive airway treatment. She demonstrated acquired microcephaly; head circumference did not significantly increase after the age of 3 years. Currently, at the age of 15 years, her head circumference was 49 cm (−4.7 SD). She developed involuntary choreiform movements, dystonia, proximal muscle weakness and absent muscle stretch reflexes. Evolving severe contractures at the hamstrings, biceps and ankles were observed. Initial brain MRI at 20 months of age revealed prominent ventricles and delayed myelination. Over the ensuing decade there was cortical volume loss in the cerebellum, brainstem, bilateral parietal and occipital lobes and corpus callosum thinning (Figure 2D–2L). MRS imaging revealed elevated lipid-lactate peak was persistently present within the parenchyma and CSF. Muscle biopsy demonstrated enlarged and irregular shaped mitochondria on electron microscopy with surrounding membrane whorls. Electron dense deposits, lipid droplets, pools of glycogen and empty vacuoles, disrupted cristae. Electron transport chain enzymatic activities of complexes I – IV were within normal ranges. EEG demonstrated multifocal epileptiform discharges with background slowing that persistently remained present over multiple studies. Visual evoked potentials at 5 years of age demonstrated severe reduced amplitudes with prolonged latencies. Multifocal electroretinographic testing demonstrated grossly normal retinal function. Echocardiography detected hypertrophic cardiomyopathy with subaortic stenosis. Blood lactate was found to be elevated in several occasions. In addition, at age 14 years, the patient had significantly elevated growth and differentiation factor 15 (1293 pg/ml; normal values <750 pg/ml). Sleep studies demonstrated obstructive sleep apnea with restrictive lung disease. Phenotype data can be accessed at www.phenomecentral.org (ID: P0004652).

Whole exome sequencing (WES) was performed in patient 1 and patient 2parents (trio). Further details of the test performed are given in the supplementary data.

RESULTS

Clinical and molecular findings in cases with pathogenic variation in *PNPLA8* are summarized in Table 1.

In patient, 1 we identified a homozygous nonsense variant in *PNPLA8* [(c.517G>T, p.(Glu173Ter), NM_001256007.1] (Figure 1B). Patient 2 was found to possess compound variants in *PNPLA8*, c.2275_2276delCT; p.(Leu759AlafsTer4) and c.419C>A; p.(Ser140Ter). The variant was confirmed by Sanger sequencing in the proband. Segregation analysis confirmed that the variants were inherited from carrier parents, consistent with an autosomal recessive pattern (Figure 1C).

DISCUSSION

Biallelic loss-of-function variants in *PNPLA8* were found in two unrelated individuals. The phenotypes of the probands were variable but shared many similar features. P1 presented in the newborn period with congenital microcephaly, spasticity and seizures. However, P2 had normal development till one year followed by neuro-regression, multiple episodes of epilepsy partialis continua, focal seizures, progressive weakness and involuntary movements. P2 shared similarity in clinical presentation to the previously reported individual with variants in *PNPLA8*. Of the nine PNPLAs, *PNPLA2*, *PNLPA6* and *PNPLA9* have been implicated in human diseases. *PNPLA2*, is associated with neutral lipid storage disease with myopathy (MIM #610717) [Reilich et al., 2011]. *PNLPA6* defects cause Boucher-Neuhäuser and Gordon Holmes syndromes, and spastic paraplegia 39 (MIM #215470, #612020, #612020) [Deik et al., 2014; Synofzik et al., 2015; Topaloglu et al., 2014; Wortmann et al., 2015]. PNPLA9 (Group VIA phospholipase) is associated with infantile neuronal axonal dystrophy (neurodegeneration with brain iron accumulation 2A; NBIA2A), neurodegeneration with brain iron accumulation 2B (NBIA2B) and Parkinson disease 14 (MIM #603604, #610217, #612953) [Gregory et al., 2008; Shi et al., 2011].

The gene, *PNPLA8* is located at 7q31 and encodes a protein containing 782 amino acids. This enzyme is known to increase prostaglandin E2 (PGE2) production via cyclooxygenase (COX)-1 and -2, which contributes to cell growth and tumorigenesis [Moon et al., 2012]. It has protective effects against oxidant and cytokine-induced cell death. The PNPLA8 (Group VIB phospholipase or iPLA2γ) protein has been localized to the inner mitochondrial membrane and is involved in regulation of oxidative stress. PNPLA8 is specific to sn-1 and sn-2 phospholipases with multiple transcription start sites and multiple methods of proteolytic processing [Liu et al., 2017; Tanaka et al., 2000; Tang et al., 1997]. *Pnpla8* null mice show increased levels of reactive oxygen species resulting in liver mitochondrial swelling, mitochondrial permeability transition pore opening, and cytochrome c release from mitochondria, which trigger the intrinsic apoptotic pathway. Histological findings in P2 of abnormal mitochondria with concentric lamellar membranes and inclusions were in concordance with the observations in the null-mice and the patient reported by Saunders *et al.* [Saunders et al., 2015]. The null mice also show growth retardation, impaired learning, decreased exercise endurance, enhanced insulin sensitivity, a thin body habitus, cold intolerance, and increased mortality. They exhibit a mitochondrial neurodegenerative disorder characterized by degenerating mitochondria, autophagy and cognitive decline [Mancuso et al., 2009; Mancuso et al., 2007]. A similar phenotype of progressive neurodegeneration is evident in the affected individuals with this condition as well.

The variants observed in *PNPLA8*, including the ones in our study are loss-of-function pathogenic variants, which introduce a premature stop codon (Figure 1E). These variants are very likely to result in a truncated protein or undergo nonsense mediated decay as evidenced by near absence of this protein in patient reported by Saunders *et al.* The clinical picture in the three patients with pathogenic variants in *PNPLA8* is similar but variable. This variability in phenotypic expression and MRI findings are not uncommon in mitochondrial disease, but the precise mechanism for these abnormalities remains unclear [Gorman et al.,

2016; Saneto 2017; Saneto et al., 2008; Saneto and Naviaux 2010]. Also, due to limited number of individuals, a genotype-phenotype correlation cannot be deduced at present.

In summary, we report two additional unrelated individuals with neurodegeneration of variable severity harboring loss-of-function variants in *PNPLA8*. Further, the human phenotype appears to have striking similarity with progressive neurodegeneration observed in null mice. The observed phenotype is consistent across the other genes of the patatin-like protein family and suggests that variants of these genes constitute a distinct class of disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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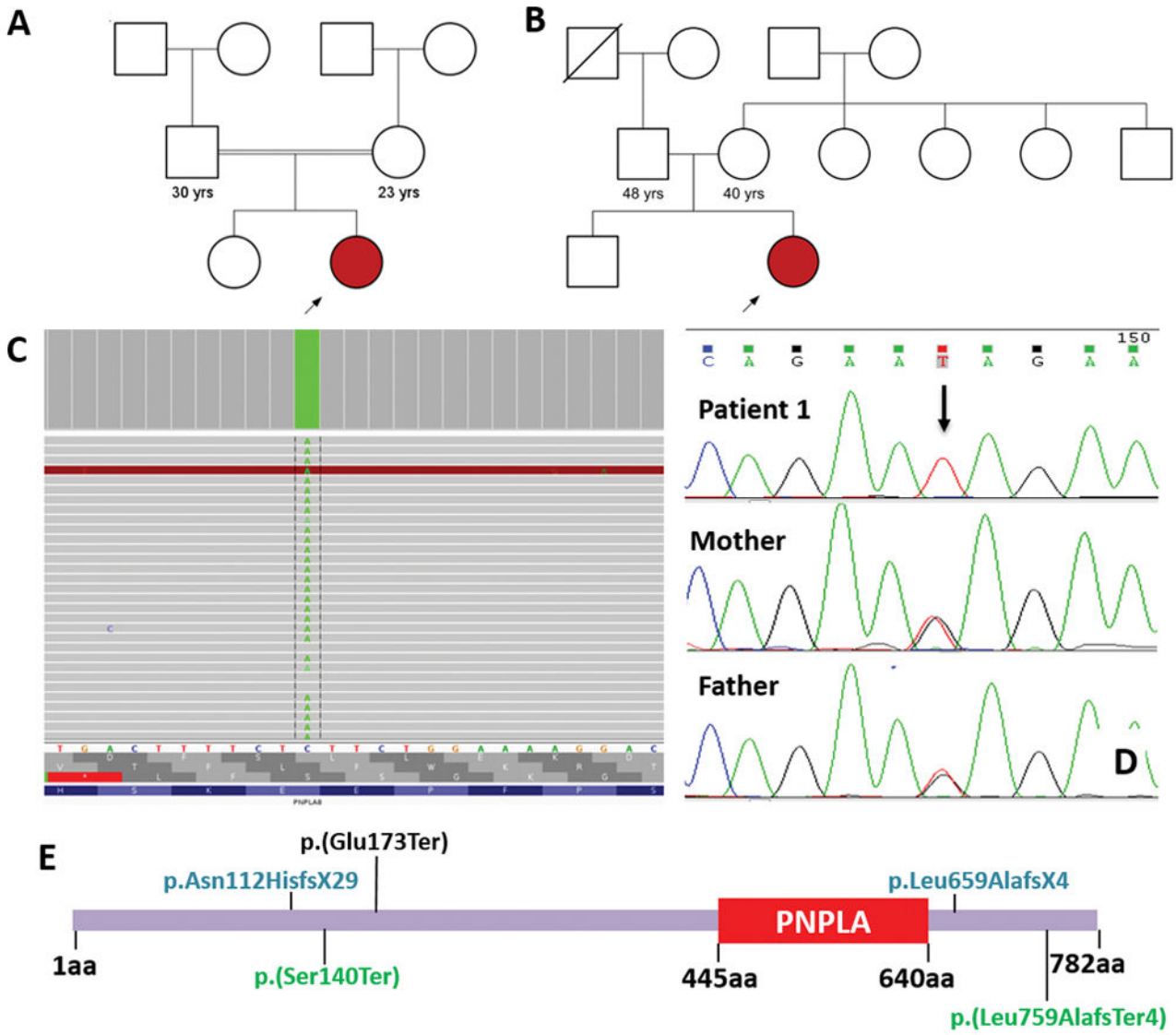

Figure 1.

Family pedigree of patient 1 (A) and patient 2 (B), Integrative Genomics Viewer snapshot of the variant (C), Sanger sequencing of the variant, c.517G>T [p.(Glu173Ter), NM_001256007.1] in homozygous state in patient 1 and heterozygous state in the parents (D). A schematic representation of PNPLA8 with the reported variants (blue), variants in patient 1 (black), patient 2 (green) in our study (E).

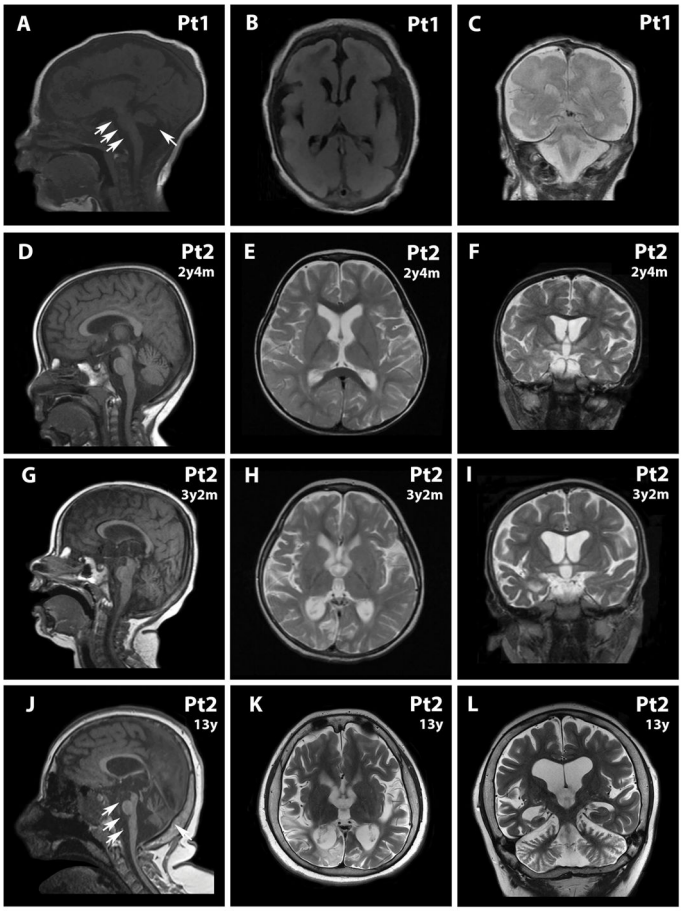

Figure 2.

Brain MR Images of *PNPLA8* mutation-positive patients. A–C, brain MRI of patient 1 at age (day 2) years showing microcephaly, diffuse simplification of the gyral pattern with diffuse atrophy resulting in increased extra-axial space, and a small, uprotated cerebellar vermis with diffuse hypoplasia and atrophy of the cerebellar hemispheres and brain stem (arrows). D–L, brain MRI of patient 2 at age 2 years 4 months (D–F), 3 years and 2 months (G–I), and 13 years (J–L) showing progressive and diffuse atrophy of the cerebrum, cerebellum and brainstem (arrows).

Table 1

Comparison of clinical and molecular findings in cases with pathogenic variation in *PNPLA8 (n=3)*

Phenotype	**Saunders *et al*. 2015**	**Patient 1**	**Patient 2**
Origin	NA	India	India/Fiji
Consanguinity	NA	Yes	No
Gender	Female	Female	Female
Age at last assessment	7 years	10 days	14 years
Age of presentation	2 years	At birth	13 months
Coding DNA change	c.334_337delAATT, c.1975_1976delAG	c.517G>T	c.2275_2276delCT; c.419C>A
Amino acid change	p.Asn112HisfsX29, p.Leu659AlafsX4	p.(Glu173Ter)	p.(Leu759AlafsTer4); p.(Ser140Ter)
Birth weight (gm/SD)	3500 (+1 SD)	2500 (−1 SD)	NA
Birth length (cm/SD)	NA	48 (−1 SD)	NA
Progressive muscle weakness	+	Not applicable	+
Regression of motor milestones	+	Not applicable	+
Seizures	+	+	+
Involuntary movements	Dystonia and dysmetria	Not applicable	Dystonia and choreiform movements
Proximal muscle weakness	+	−	+
Tone	Spastic	Spastic	Spastic
Deep tendon reflexes	NA	Elicitable	Absent
Brain imaging			
Hypoplastic cerebellum	−	+	+
Simplified gyral pattern	−	+	−
Hypoplasia of corpus callosum	−	+	+
Elevated lipid-lactate peak (MRS)	−	+	+
Muscle biopsy	Abnormal	NA	Abnormal
EEG findings	Recurrent left temporal lobe spikes, slow wave discharges exacerbated by sleep	NA	Multifocal generalized slow epileptiform discharges
Echocardiography	NA	NA	Hypertrophic cardiomyopathy
Ophthalmological findings	NA	NA	VEP-absent/ERG mildly abnormal

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Phenotype	Saunders *et al*. 2015	Patient 1	Patient 2
Other investigations *	Normal hematological tests, renal function test, karyotype, microarray	Normal hematological tests, TORCH serology testing, renal function tests	Normal hematological tests, TORCH serology testing, renal function tests, neurotransmitter studies, karyotype
Lactic acidosis	+	NA	+
Blood pyruvate	Elevated	NA	NA
Mitochondrial DNA sequencing	No variation	NA	No variation

Abbreviations: + present, − absent, NA not avaiable, SD standard deviation, USG ultrasonography, EEG electroencephalography, CSF cerebrospinal fluid, TSH thyroid stimulating hormone, MRS magnetic resonance spectroscopy, CSF cerebrospinal fluid, VEP visual evoked potential, ERG electroretinogram,

* Includes hematological tests, TORCH (toxoplasmosis, rubella, cytomegalovirus, herpes simplex virus 1 and 2), renal function tests, neurotransmitter studies on CSF, karyotype, microarray.