SHORT REPORT

Heterogeneity of PNPT1 neuroimaging: mitochondriopathy, interferonopathy or both?

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ABSTRACT

Background Biallelic variants in *PNPT1* cause a mitochondrial disease of variable severity. PNPT1 (polynucleotide phosphorylase) is a mitochondrial protein involved in RNA processing where it has a dual role in the import of small RNAs into mitochondria and in preventing the formation and release of mitochondrial double-stranded RNA into the cytoplasm. This, in turn, prevents the activation of type I interferon response. Detailed neuroimaging findings in PNPT1-related disease are lacking with only a few patients reported with basal ganglia lesions (Leigh syndrome) or non-specific signs.

Objective and methods To document neuroimaging data in six patients with PNPT1 highlighting novel findings.

Results Two patients exhibited striatal lesions compatible with Leigh syndrome; one patient exhibited leukoencephalopathy and one patient had a normal brain MRI. Interestingly, two unrelated patients exhibited cystic leukoencephalopathy resembling RNASET2-deficient patients, patients with Aicardi-Goutières syndrome (AGS) or congenital CMV infection.

Conclusion We suggest that similar to RNASET2, PNPT1 be searched for in the setting of cystic leukoencephalopathy. These findings are in line with activation of type I interferon response observed in AGS, PNPT1 and RNASET2 deficiencies, suggesting a common pathophysiological pathway and linking mitochondrial diseases, interferonopathies and immune dysregulations.

INTRODUCTION

Mitochondrial diseases are the most common group of inborn errors of metabolism, with wide phenotypical variability.[1] Primary mitochondrial diseases, which arise from variants in the nuclear or mitochondrial genome and affect multiple mitochondrial functions, have an estimated prevalence of 1:2000–1:5000 and can manifest at any age. Genes encoding mitochondrial proteins reside in both nuclear DNA and in mitochondrial DNA (mtDNA). Variants in more than 300 nuclear genes, as well as mtDNA large deletions and point mutations, are known to cause human diseases.[2] Among the nuclear genes involved in mitochondrial diseases, *PNPT1* was recently identified.[3]

PNPT1 encodes for polynucleotide phosphorylase (PNPase), a conserved homotrimeric exoribonuclease localised in both the mitochondrial matrix and the intermembrane space. It is involved in mtRNA processing and degradation. PNPase has been initially suggested to play a role in RNA import into mitochondria.[3–5] Recent data provide evidence that disrupted PNPase RNA processing lead to the accumulation of double-stranded mtRNAs in the mitochondria that leak into the cytoplasm where they trigger antiviral signalling pathway and subsequent type I interferon response.[6] Patients with biallelic *PNPT1* pathogenic variants exhibit wide clinical heterogeneity ranging from non-syndromic hearing loss[4 7] to multisystemic Leigh disease and other clinical and biochemical features observed in mitochondrial disorders.[8 9] Regardless, neuroimaging findings are scarcely described with only a few reports of basal ganglia lesions (Leigh syndrome)[3 10 11] or non-specific signs[9] such as delayed myelination.[5] Our aim was to exhaustively investigate neuroimaging in a series of six patients with PNPT1 focusing on novel findings.

MATERIALS AND METHODS

PNPT1 variants were identified by whole exome sequencing for patients 1–4 and 6 in three different centres on leucocyte DNA. Based on the suspected autosomal recessive pattern of inheritance, we prioritised genes carrying biallelic variants. The pathogenic variants were selected after filtering against known SNPs reported in dbSNP, 1000 genomes, exome variant server, Intergenic variants and in-house polymorphisms. For patient 5, *PNPT1* variants were identified by targeted next-generation sequencing on leucocyte DNA. We already reported elsewhere data from patients 4–6.[6]

RESULTS

Clinical findings, laboratory parameters and molecular data are summarised in table 1. All of the six patients exhibited clinical and biochemical findings compatible with a mitochondrial encephalopathy: early-onset neurological non-progressive deterioration with lactate accumulation in body fluids (blood and cerebrospinal fluid) with elevated lactate to pyruvate ratio (when determined) for all but one patient (patient 3). Patient 1 exhibited a mild and chronic encephalopathy and reported as alive at age 26 years. Sensorineural deafness was present

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Table 1 Key clinical features from six patients with variants in *PNPT1*

Patients and PNPT1 pathogenic variants	Neonatal period	Age and clinical signs at onset	Age and clinical signs at diagnosis	Laboratory parameters	Clinical course	Brain imaging
Patient 1 c.1525G>A/c.1592C>G p.Val509Ile/p.Thr531Arg	Non-consanguineous parents Male Gestation: 38 weeks Weight: 1984 g Length: 43 cm HC: NA Urgent caesarean section 30-day NICU course for respiratory support	Months 0–12: Hypotonia Developmental delay Strabismus Sensorineural deafness Microcephaly Failure to thrive Cardiac: bicuspid aortic valve, abnormal mitral valve (double-orifice mitral valve) with mild mitral stenosis Meets criteria for NYHA class III	Year 24: Hypoglycaemic Diabetes mellitus Sensorineural deafness Non-progressive-severe encephalopathy Pancytopenia Hepatic fibrosis	Year 18: Insulin growth factor 1: 87 ng/mL (158–497) Year 19: Lactic acid 5 mmol/L (0.50–2.0) Year 25: AST 62 U/L (9–39) ALT 35 U/L (10–52) GGT: 364 U/L (5–64) Alkaline phosphatase 837 U/L (33–120) Total bilirubin: 2.1 mg/dL (0–1.2); direct bilirubin: 0.7 mg/dL (0–0.3); albumin 2.6 g/dL (3.4–5) ETC fibroblasts (spectrophotometry): Mild decrease of CIII (58%/mean control values) and IV activities (60% mean control values). Defective CII activity (50%/mean control values)	Years 5: Scoliosis prior (21% -->65% but slowed in later adolescence (restrictive lung disease as a sequel) Later adolescence: Pancytopenia Hepatic fibrosis Delayed puberty: Primary hypogonadism/growth hormone deficiency Years 18: Non-progressive-severe encephalopathy Years 26: Osteopenia w/closed displaced oblique fracture of calcaneus, humerus with minimal trauma Highest motor milestone: patient is ambulatory with a wide base shuffling gait	25 y five mo MRI: Normal: thick corpus callosum CT: normal
Patient 2 c.1579_1580insGAT/ p.Tyr527* homozygous	Consanguineous parents Male Gestation: 36 weeks Weight: 3020 g Length: 49 cm OFC: 36 cm APGAR: 10/10	Month 4: Frequent crying Mild hypotonia peripheral hypertonia Feeding difficulties Malnutrion Gastro-oesophageal reflux No head control No eyes fixation and following Increased deep tendon reflexes Microcephaly	Years 3: Feeding difficulties Truncal hypotonia Peripheral hypertonia Strabismus Microcephaly Increased deep tendon reflexes Normal auditory evoked potentials	Months 4: Lactic acid 5.2 mmol/L (0.50–2) Lactate/pyruvate: 65.3 (<20) Plasma Alanine: 990 µmol/L (144-400) CSF alanine: 75µmol/L (13-48) CSF Lactic acid 7 mmol/L (1.1–2.8) CSF Lactate/pyruvate ratio: 38 (10-15) Month 7: Lactic acid 2.3 mmol/L (0.50–2.0) Lactate/pyruvate: 55(<20) Month 10: Lactic acid 2.3 mmol/L (0.50–2.0) Lactate/pyruvate: 55 (<20) Months 17: Lactic acid 1.76 mmol/L (0.50–2.0) Month 48: Lactic acid 1.76 mmol/L (0.50–2,0)	Year 4: Hypertrichosis Microcephaly Good eye contact No words Mild spasticity Increased deep tendon reflexes	MRI: 24 months White matter Temporal lobes: bilateral cysts CT: 6 mo: normal findings, no calcifications
Patient 3 c.1399C>T/p.Pro467Ser homozygous	Consanguineous parents Female Gestation: 34 weeks Weight: 1830 g Length: 46 cm OFC: 29.5 cm APGAR: 9/10 Caesarean section for pathological CTG. Short-time hypocalcemia, anaemia. 4 weeks: hospital discharge	First months of life: Restlessness Frequent crying Sleep disturbance feeding problem	Months 5: Failure to thrive Acquired microcephaly Severe psychomotor impairment Trunk hypotonia, Spastic tetraparesis Dystonia No head control No fixation with eyes No smile	Normal metabolic work-up in blood, urine and CSF	Month 10: Seizures Normal sensory nerve conduction velocities Normal visual evoked potentials Normal auditory evoked potentials Year 7: Hypertrichosis Microcephaly Severe psychomotor retardation Absence of contact Striking hyperexcitability Myoclonic seizures and sporadically short secondary generalised seizures: EEG: multifocal epileptiform discharges Spastic tetraparesis Dystonia Rare spontaneous movements Scoliosis Abnormal visual evoked potentials Normal ophthalmological investigation including funduscopy Normal hearing Year 16: alive	seven y four mo MRI: white matter Temporal lobes: bilateral cysts CT: no calficifations
Patient 4 c.1495G>C/c.1519G>T p.Gly499Arg/p.Ala507Ser	Non consanguineous parents Female Gestation: 41 weeks Weight: 2780 g Length: 50 cm OFC: 32 cm APGAR: 10/10	Days 0–5: Right hip congenital luxation Systolic murmur Interventricular communication Gastro oesophageal reflux Sleepiness Feeding difficulties Microcephaly Abnormal eye movements Poor contact	Years 3: Generalised hypotonia Peripheral neuropathy Dystonia Sensorineural deafness Optic atrophy	Months 3: Lactic acid NA CSF Lactic acid 2.4 mmol/L (1–1.90) CSF Lactate/pyruvate ratio 20 (10-15) Months 8: Lactic acid NA CSF Lactic acid 1.55 mmol/L (1–1.90) CSF Lactate/Pyruvate ratio NA Years 3: ETC fibroblasts (spectrophotometry) mildly decreased CV activity: CV/citrate synthase ratio: 0.17 (normal values: 0.28±0.03)	Months 3 Generalised hypotonia Abnormal eye movements Poor contact Sensorineural deafness Optic atrophy Month 17: Generalised hypotonia Peripheral neuropathy Dystonia Sensorineural deafness Optic atrophy Eye contact improvement Movements improvement Year 6: alive	26 months MRI: white matter CT: no calcifications

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Table 1 Continued

Patients and PNPT1 pathogenic variants	Neonatal period	Age and clinical signs at onset	Age and clinical signs at diagnosis	Laboratory parameters	Clinical course	Brain imaging
Patient 5 c.208T>C/c.2137G>T p.Ser70Pro/p.Asp713Tyr	Non-consanguineous parents Male IUGR Gestation: 39 weeks Weight: 2840 g Length: 48.5 cm OFC: 35 cm Apgar: 9/10	Month 3: Axial hypotonia Peripheral hypertonia Nystagmus Poor contact	Month 13: Axial hypotonia Pyramidal signs Dystonia Nystagmus Sensorineural deafness Laryngomalacia	Month 5: Lactic acid 2.5 mmol/L (0.50–2.0) CSF lactic acid 2.5 mmol/L (1–1.90) CSF lactate/pyruvate ratio 15 (10–5) CSF neopterin 101 nmol/L (8-43), homovanillic acid, 3-o-methyldopa, 5-methyltetrahydrofolate: normal ETC fibroblasts (spectrophotometry) mildly decreased CIV activity: CIV/citrate synthase ratio: 0.76 (normal values 1–1.5)	Month 5: Axial hypotonia and limb dystonia Pyramidal signs Nystagmus Normal eye funduscopy Normal electroretinogram Visual evoked potentials conduction delay Month 14: Failure to thrive Axial hypotonia and limb dystonia Nystagmus Microcephaly (HC −2 SD) Year 4: Weight stagnation Axial hypotonia, limb dystonia Nystagmus Progressive encephalopathy	MRI 14 months Leigh
Patient 6 c.407G>A p.Arg136His homozygous	Consanguineous parents Male IUGR Gestation: 39 weeks Weight: 2850 g Length: 47 cm OFC: 34 cm Apgar: 10/10	Days 0–7: Congenital cataract Feeding difficulties Gastro-oesophageal reflux Inconsolable crying Abnormal movements Abnormal auditory evoked potentials	Year 4: Feeding difficulties Truncal hypotonia Peripheral hypertonia Dystonia Congenital cataract Pigmentary retinopathy Nystagmus Deafness Pyramidal syndrome Progressive encephalopathy	Month 4: Lactic acid 7.05 mmol/L (0.50–2.0) Month 7: CSF Lactic acid 2.4 mmol/L (1–1.90) CSF Lactate/pyruvate ratio 17 (10–15) ETC fibroblasts (spectrophotometry) decreased CIV activity: CIV/citrate synthase ratio: 0.55 (normal values 1.28±0.10) ETC muscle (spectrophotometry): multiple RC deficiency except CII: CI/citrate synthase (CS) ratio: 0.12 (normal values 0.18±0.03) CII/CS: 0.51 (0.31±0.04) CIII/CS: 1.56 (2.83±0.34) CIV/CS: 0.95 (1.64±0.22) CV/CS: 0.48 (0.76±0.15) Month 17: Lactic acid 2.1–3.7 mmol/L (0.50–2.0)	Month 3: Feeding difficulties Congenital cataract, nystagmus No contact Abnormal movements Sensorineural deafness Microcephaly (HC −2.5 SD) Month 6: Feeding difficulties Truncal hypotonia Congenital cataract, nystagmus No contact Abnormal movements Sensorineural deafness Month 17: Feeding difficulties Truncal hypotonia Peripheral hypertonia and dystonia Pigmentary retinopathy Nystagmus 4 years 9 months: death: fever, major lactic acidosis	MRI seven mo Leigh

CSF, cerebrospinal fluid; CTG, cardiotocography; ETC, Electron transport chain; HC, head circumference; IUGR, intrauterine growth retardation; NA, not available; NICU, neonatal intensive care unit; NYHA, New York Heart Association.

in four individuals (patients 1, 4, 5 and 6). When investigated, mitochondrial respiratory chain activities were decreased (most often defects in several complexes) in fibroblasts and/or skeletal muscle (table 1). Seven of the *PNPT1* variants were already reported (patients 1, 3, 4, 5 and 6). We describe three novel variants, namely, p.Val509Ile (patient 1), p.Tyr527* (patient 2) and p.Pro467Ser (patient 3). All affect highly conserved residues. The p.Val509Ile and the p.Pro467Ser missense variants are predicted as likely pathogenic by in silico software (online supplemental table S1). The p.Tyr527* variant is predicted as truncating.

For all patients, Sanger sequencing of parental DNA confirmed mutation segregation.

Neuroimaging findings are shown in figure 1. Patient 1 exhibited a near-normal brain MRI apart from a thick corpus callosum. The rest of the patients exhibited either white matter abnormalities (patients 2–4) or basal ganglia involvement (Leigh syndrome, patients 5 and 6). Patients 2 and 3 exhibited similar findings with bilateral leukoencephalopathy with cysts in temporal lobes, while patient 4 exhibited diffuse bilateral white matter abnormalities without cysts. Patients 5 and 6 exhibited bilateral striatal abnormalities suggestive of Leigh syndrome. Patient 5 exhibited a thin corpus callosum.

DISCUSSION

Neuroimaging of PNPT1 deficiency was rarely reported, and our data emphasise high heterogeneity. In a recent report of 17 patients with PNPT1,[9] basal ganglia (putamen or pallidum) hyperintensities, cerebral atrophy, non-specific white matter changes, delayed myelination and thin corpus callosum have been reported. In our cohort, brain MRI abnormalities could be classified into three categories: normal MRI (patient 1), white matter abnormalities (patients 2–4) and Leigh syndrome (patients 5 and 6). Leigh syndrome was previously reported in two other independent patients with PNPT1.[3 11] Regarding white matter involvement, patient 4 exhibited white matter abnormalities compatible with a leukoencephalopathy without cysts and absence of calcifications. In keeping with this, patients 2 and 3 exhibited a cystic leukoencephalopathy with white matter hyperintensity, along with bilateral and symmetric cysts in the temporal lobes. Such a neuroimaging pattern is observed in cystic leukoencephalopathy without megalencephaly due to *RNASET2* mutations[12] and in congenital cytomegalovirus (CMV) infection. Aicardi-Goutières syndrome (AGS) is another recessive disease that can be mistaken for congenital CMV infection. AGS results from dysfunction of proteins involved in nucleic acid metabolism, where self-derived nucleic acid species induce a type I interferon mediated innate immune response.[13] Accordingly, it was recently shown that PNPT1 was preventing the formation and release of mitochondrial double-stranded RNA into the cytosol, with subsequent prevention of the activation of type I interferon response.[6] Interferon-stimulated genes (ISGs) were shown to be overexpressed in blood of patients with PNPT1,[9] including for patient 5.[6] It was not possible to evaluate ISGs in the rest of

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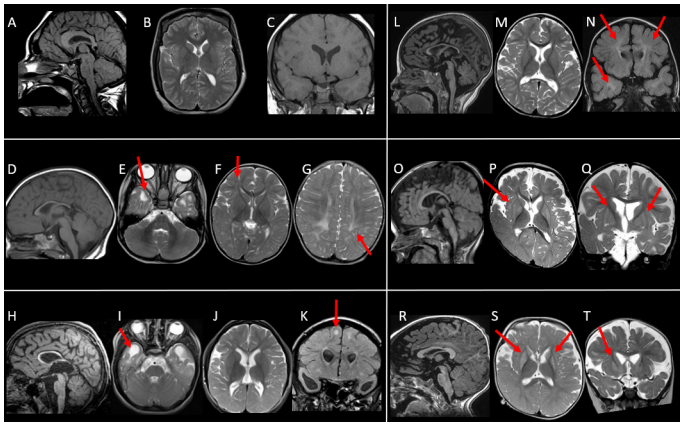


Figure 1 Brain MRI diversity in patients with PNPT1. Patient 1, 25 years old: sagittal T1 (A), axial T2-weighted image (B) and coronal T1 (C) showing thick corpus callosum, no anomalies in white matter or basal ganglia. Patient 2, 2 years old: sagittal T1 (D), axial T2-weighted images at different levels (E–G) showing thin corpus callosum, with cystic leukoencephalopathy in bilateral temporal poles (E, arrow) with periventricular (F, arrow) and centrum semiovale anomalies (G, arrow). Patient 3, 7.5 years old: sagittal T1 (H), axial T2-weighted images at different levels (I,J) and coronal FLAIR weighted image (K) showing thin corpus callosum (H) and mild pons volume loss with cystic leukoencephalopathy in bilateral temporal poles (I, arrow), along with mild subcortical white matter anomalies (K, arrow). Patient 4, 26 months old: sagittal T1 (L), axial T2-weighted image (M) and coronal FLAIR-weighted image (N) showing thin corpus callosum and mild pons volume loss and diffuse white matter anomalies (arrows). Patient 5, 14 months old: sagittal T1 (O), axial T2-weighted image (P) and coronal T2-weighted image (Q) showing thin corpus callosum and bilateral hyperintensities in the basal ganglia (globus pallidus and putamen, arrows in the putamen) suggestive of Leigh syndrome. Note the mild bilateral frontotemporal atrophy and white matter volume loss (P,Q) consistent with microcephaly. Patient 6, 2 years old: sagittal T1 (R), axial T2-weighted image (S) and coronal T2-weighted image (T) showing bilateral hyperintensities in the striatum (arrows in the putamen) suggestive of Leigh syndrome. Note the mild bilateral frontotemporal atrophy and white matter volume loss (S,T) consistent with microcephaly.

our patients. ISGs overexpression was also observed for most of patients with AGS and in some patients with RNASET2.[14] Similarly, increased CSF neopterin was described in patients with AGS,[15] RNASET2[14 16] and PNPT1 (n=1, patient 5[6]).

While these similarities are ascribable to a shared pathophysiological pathway, that is, type I interferon immune response stimulation for PNPT1, RNASET2, AGS and CMV, it is noteworthy that these entities may also share similar neuroimaging pattern. Our data suggest a common pathomechanism at the origin of this cystic leukoencephalopathy. Regardless, in AGS, cystic leukoencephalopathy is not frequently observed as opposed to diffuse white matter abnormalities.[17] Interestingly, it was recently shown that mammalian mitochondrial RNA degradation occurs in the mitochondrial intermembrane space and is operated by RNASET2.[18] Whether and how PNPT1, which has a dual localisation in the intermembrane space and the mitochondrial matrix, and RNASET2 interact is unknown. Similarly, the reason for cystic leukoencephalopathy being present electively in patients 2 and 3 is unclear. Of note, these two individuals carry two different *PNPT1* variants, one truncating (patient 2) and one missense (patient 3) with different predicted effects (online supplemental table S1). The p.Pro467Ser (patient 3) is novel, but a variant affecting the same amino acid (p.Pro467His) has been reported[9] in a patient who did not exhibit cystic leukoencephalopathy.

Further research is mandatory to understand why temporal regions seem electively affected by the presence of cysts in the setting of interferonopathies.

CONCLUSION

Consistent with recent in vitro data showing interferon type 1 pathway activation in the setting of PNPT1 loss of function,[6] our data confirm that PNPT1 deficiency is a novel differential diagnosis for RNASET2-deficient leukoencephalopathy, congenital CMV infection and AGS. Of note, RMND1 is another mitochondrial congenital CMV/AGS mimicker with similar neuroimaging cystic leukoencephalopathy findings in RMD1-deficient individuals.[19] No data on interferon activation are available in RMD1 deficiency. RMND1 encodes a mitochondrial membrane protein involved in mtDNA translation. Similarly, preliminary data recently reported increased expression of ISGs in mtDNA deletion patients.[20] This emphasises the potential relationships between mitochondrial dysfunctions and interferon signalling and highlights wide neuroimaging heterogeneity. It paves the way for future research and potential treatment ramifications in mitochondrial disorders with ISGs activation, such as steroids and/or autoinflammatory disease drugs. We suggest analysing ISGs in patients exhibiting clinical and laboratory findings suggestive of mitochondrial disorders along with cystic leukoencephalopathy.

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REFERENCES

1 Gorman GS, Chinnery PF, DiMauro S, Hirano M, Koga Y, McFarland R, Suomalainen A, Thorburn DR, Zeviani M, Turnbull DM. Mitochondrial diseases. *Nat Rev Dis Primers* 2016;2.
2 Stenton SL, Prokisch H. Genetics of mitochondrial diseases: identifying mutations to help diagnosis. *EBioMedicine* 2020;56.
3 Vedrenne V, Gowher A, De Lonlay P, Nitschke P, Serre V, Boddaert N, Altuzarra C, Mager-Heckel A-M, Chretien F, Entelis N, Munnich A, Tarassov I, Rötig A. Mutation in PNPT1, which encodes a polyribonucleotide nucleotidyltransferase, impairs RNA import into mitochondria and causes respiratory-chain deficiency. *Am J Hum Genet* 2012;91:912–8.
4 von Ameln S, Wang G, Boulouiz R, Rutherford MA, Smith GM, Li Y, Pogoda H-M, Nürnberg G, Stiller B, Volk AE, Borck G, Hong JS, Goodyear RJ, Abidi O, Nürnberg P, Hofmann K, Richardson GP, Hammerschmidt M, Moser T, Wollnik B, Koehler CM, Teitell MA, Barakat A, Kubisch C. A mutation in PNPT1, encoding mitochondrial-RNA-import protein PNPase, causes hereditary hearing loss. *Am J Hum Genet* 2012;91:919–27.
5 Sato R, Arai-Ichinoi N, Kikuchi A, Matsuhashi T, Numata-Uematsu Y, Uematsu M, Fujii Y, Murayama K, Ohtake A, Abe T, Kure S. Novel biallelic mutations in the *PNPT1* gene encoding a mitochondrial-RNA-import protein PNPase cause delayed myelination. *Clin Genet* 2018;93:242–7.
6 Dhir A, Dhir S, Borowski LS, Jimenez L, Teitell M, Rötig A, Crow YJ, Rice GI, Duffy D, Tamby C, Nojima T, Munnich A, Schiff M, de Almeida CR, Rehwinkel J, Dziembowski A, Szczesny RJ, Proudfoot NJ. Mitochondrial double-stranded RNA triggers antiviral signalling in humans. *Nature* 2018;560:238–42.
7 Eaton A, Bernier FP, Goedhart C, Caluseriu O, Lamont RE, Boycott KM, Parboosingh JS, Innes AM, Care4Rare Canada Consortium. Is *PNPT1*-related hearing loss ever non-syndromic? whole exome sequencing of adult siblings expands the natural history of *PNPT1*-related disorders. *Am J Med Genet A* 2018;176:2487–93.
8 Alodaib A, Sobreira N, Gold WA, Riley LG, Van Bergen NJ, Wilson MJ, Bennetts B, Thorburn DR, Boehm C, Christodoulou J. Whole-Exome sequencing identifies novel variants in PNPT1 causing oxidative phosphorylation defects and severe multisystem disease. *Eur J Hum Genet* 2017;25:79–84.
9 Rius R, Van Bergen NJ, Compton AG, Riley LG, Kava MP, Balasubramaniam S, Amor DJ, Fanjul-Fernandez M, Cowley MJ, Fahey MC, Koenig MK, Enns GM, Sadedin S, Wilson MJ, Tan TY, Thorburn DR, Christodoulou J. Clinical spectrum and functional consequences associated with Bi-allelic pathogenic *PNPT1* variants. *J Clin Med* 2019;8. doi:10.3390/jcm8112020. [Epub ahead of print: 19 Nov 2019].
10 Lee JS, Yoo T, Lee M, Lee Y, Jeon E, Kim SY, Lim BC, Kim KJ, Choi M, Chae J-H. Genetic heterogeneity in Leigh syndrome: highlighting treatable and novel genetic causes. *Clin Genet* 2020;97:586–94.
11 Matilainen S, Carroll CJ, Richter U, Euro L, Pohjanpelto M, Paetau A, Isohanni P, Suomalainen A. Defective mitochondrial RNA processing due to PNPT1 variants causes Leigh syndrome. *Hum Mol Genet* 2017;26:3352–61.
12 Henneke M, Diekmann S, Ohlenbusch A, Kaiser J, Engelbrecht V, Kohlschütter A, Krätzner R, Madruga-Garrido M, Mayer M, Opitz L, Rodriguez D, Rüschendorf F, Schumacher J, Thiele H, Thoms S, Steinfeld R, Nürnberg P, Gärtner J. RNASET2-deficient cystic leukoencephalopathy resembles congenital cytomegalovirus brain infection. *Nat Genet* 2009;41:773–5.
13 Crow YJ, Manel N. Aicardi-Goutières syndrome and the type I interferonopathies. *Nat Rev Immunol* 2015;15:429–40.
14 Tonduti D, Orcesi S, Jenkinson EM, Dorboz I, Renaldo F, Panteghini C, Rice GI, Henneke M, Livingston JH, Elmaleh M, Burglen L, Willemsen MAAP, Chiapparini L, Garavaglia B, Rodriguez D, Boespflug-Tanguy O, Moroni I, Crow YJ, Clinical CYJ. Clinical, radiological and possible pathological overlap of cystic leukoencephalopathy without megalencephaly and Aicardi-Goutières syndrome. *Eur J Paediatr Neurol* 2016;20:604–10.
15 Blau N, Bonafé L, Krägeloh-Mann I, Thöny B, Kierat L, Häusler M, Ramaekers V. Cerebrospinal fluid pterins and folates in Aicardi-Goutières syndrome: a new phenotype. *Neurology* 2003;61:642–7.
16 Kuster A, Arnoux J-B, Barth M, Lamireau D, Houcinat N, Goizet C, Doray B, Gobin S, Schiff M, Cano A, Amsallem D, Barnerias C, Chaumette B, Plaze M, Slama A, Ioos C, Desguerre I, Lebre A-S, de Lonlay P, Christa L. Individual contributors who contributed to this work. diagnostic approach to neurotransmitter monoamine disorders: experience from clinical, biochemical, and genetic profiles. *J Inherit Metab Dis* 2018;41:129–39.
17 La Piana R, Uggetti C, Roncarolo F, Vanderver A, Olivieri I, Tonduti D, Helman G, Balottin U, Fazzi E, Crow YJ, Livingston J, Orcesi S. Neuroradiologic patterns and novel imaging findings in Aicardi-Goutières syndrome. *Neurology* 2016;86:28–35.
18 Liu P, Huang J, Zheng Q, Xie L, Lu X, Jin J, Wang G. Mammalian mitochondrial RNAs are degraded in the mitochondrial intermembrane space by RNASET2. *Protein Cell* 2017;8:735–49.
19 Ulrick N, Goldstein A, Simons C, Taft RJ, Helman G, Pizzino A, Bloom M, Vogt J, Pysden K, Diodato D, Martinelli D, Monavari A, Buhas D, van Karnebeek CDM, Dorboz I, Boespflug-Tanguy O, Rodriguez D, Tétreault M, Majewski J, Bernard G, Ng YS, McFarland R, Vanderver A, Care4Rare Canada Consortium. RMND1-Related leukoencephalopathy with temporal lobe cysts and hearing Loss-Another Mendelian Mimicker of congenital cytomegalovirus infection. *Pediatr Neurol* 2017;66:59–62.
20 Martinelli D, Caiello I, Moneta GM, Di Nottia M, Piemonte F, Carrozzo R, Insalaco A, De Benedetti F, Bertini E, Dionisi-Vici C. Interferon signature: a new biomarker to follow disease progression in Pearson and Kearns-Sayre syndrome (Abstract). *J Inherit Metab Dis* 2019;42.

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