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Supplementary Table S1. Phenotypic characteristics and mitochondrial and nuclear DNA variant profiles of the mitochondrial involvement group

Case ID	Sex	Onset age	Patient phenotype	Gene / locus	Genetic variant	Amino acid substitution	Zygosity / Heteroplasmy	ACMG class	ClinVar associated condition	ACMG evidence	Genetic test
PM 01	M	At birth	Impaired exercise capacity, facies myopathica, hypotonia, global developmental delay, tonic-clonic seizures, pyramidal tract signs, optic nerve atrophy, strabismus, renal dysfunction, peripheral neuropathy, RVH, cognitive impairment, dysmorphism, elevated plasma alanine, episodic hypoglycemia, MRI: Leigh-like lesions.	*MT-TL1*	m.3243A>G	–	Heteroplasmic (blood – 62.6%)	P	MELAS, LS, MIDD	PS4, PM2, PP5	qPCR-HRM
PM 02	M	15 y	Visual impairment, dyschromatopsia, exercise-induced fatigue, recurrent migraine, optic nerve atrophy, positive family history, neuroimaging evidence of optic neuritis.	*MT-ND1*	m.3460G>A	p.Ala52Thr	Heteroplasmic (blood – 96%)	P	LHON	PS4, PM2, PP3, PM1, PP5	qPCR-HRM
PM 03	F	3 m	Developmental delay, hypotonia, loss of acquired milestones, infantile spasms, tonic-clonic seizures, optic nerve atrophy, feeding difficulties, urinary organic acid abnormalities, elevated plasma alanine, MRI: Leigh-like lesions.	*MT-ATP6*	m.8993T>G	p.Leu156Arg	Heteroplasmic (blood – 83.2%)	P	NARP, LS	PM3, PS2, PM2, PM5, PP3, PP5	qPCR-HRM

Abbreviations: ACMG – American College of Medical Genetics and Genomics; ACP – Acylcarnitine profile; AD – Autosomal dominant; AR – Autosomal recessive; HCM – Hypertrophic cardiomyopathy; F – female; LHON – Leber's Hereditary Optic Neuropathy; LP – Likely-pathogenic; LS – Leigh syndrome; LVH – Left ventricular hypertrophy; MELAS – Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-like Episodes; m – months; M – male; MIDD – Maternally Inherited Diabetes and Deafness; MM – Mitochondrial myopathy; MRI – Magnetic Resonance Imaging; mtDNA – mitochondrial DNA; NARP – Neuropathy, Ataxia, and Retinitis Pigmentosa; nDNA – Nuclear DNA; P – Pathogenic; qPCR-HRM – quantitative Polymerase Chain Reaction - High-Resolution Melting; RAH – Right atrial hypertrophy; RVH – Right ventricular hypertrophy; seq. – sequencing; SNHL – Sensorineural hearing loss; VUS – Variant of Uncertain Significance; WES – Whole Exome Sequencing; y – years.

ACMG Evidence Criteria: PVS1 – pathogenic very strong (null variant in a gene where loss-of-function is a known disease mechanism); PS1 – pathogenic strong (same amino acid change as a known pathogenic variant); PS2 – pathogenic strong (confirmed de novo occurrence); PS4 – pathogenic strong (significant increase in variant prevalence in cases vs. controls); PM1 – pathogenic moderate (located in a mutational hotspot or critical functional domain); PM2 – pathogenic moderate (extremely low frequency in gnomAD population databases); PM3 – pathogenic moderate (detected in trans with a pathogenic variant); PM5 – pathogenic moderate (different amino acid change at the same residue as a known pathogenic variant); PP1 – pathogenic supporting (cosegregation with disease in multiple affected family members); PP2 – pathogenic supporting (missense variant in a gene with a low rate of benign missense variation); PP3 – pathogenic supporting (computational tools unanimously support a deleterious effect); PP5 – pathogenic supporting (reported as pathogenic by a reputable source without available raw evidence); BS1 – benign strong (allele frequency is higher than expected for the disorder); BP4 – benign supporting (computational evidence suggests no functional impact); 1A – contains a genomic region with established dosage sensitivity; 2A-2E – various degrees of overlap with dosage-sensitive regions, specific gene components, or established clinical syndromes; 2F-2G – assessment based on genomic content and intra-genic involvement within a dosage-sensitive region; 2H – overlap with known benign genomic regions; 3C – quantitative assessment of protein-coding genes within the interval; 4L-4O – inheritance and segregation analysis, including confirmed or unconfirmed de novo status and transmission from affected or unaffected progenitors.

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Supplementary Table S1. Continued

Case ID	Sex	Onset age	Patient phenotype	Gene / locus	Genetic variant	Amino acid substitution	Zygosity / Heteroplasmy	ACMG class	ClinVar associated condition	ACMG evidence	Genetic test
PM 04	F	2 y	Ophthalmoplegia, strabismus, developmental delay, impaired exercise capacity, rhabdomyolysis, loss of acquired milestones, recurrent migraine, tonic-clonic seizures, stroke-like episodes, brainstem involvement, cognitive impairment, ataxia, positive family history, elevated plasma alanine, MRI: stroke-like lesions.	*MT-TL1*	m.3243A>G	–	Heteroplasmic (blood – 70.3%)	P	MELAS, LS, MIDD	PS4, PM2, PP5	qPCR-HRM
				OPA1	c.323C>T	p.Ala108Val	Heterozygous, AD	VUS	Autosomal dominant optic atrophy	PM2	WES
PM 05	M	14 y	Central scotoma, bilateral sequential vision loss, nystagmus, optic nerve atrophy, exercise-induced fatigue, recurrent migraine, positive family history, neuroimaging evidence of optic neuritis.	*MT-ND4*	m.11778G>A	p.Arg340His	Homoplasmic	P	LHON	PM2, PM5, PP3, PP5	qPCR-HRM
PM 06	M	6 m	Impaired exercise capacity, global developmental delay, hypotonia, cerebellar ataxia, peripheral neuropathy, loss of acquired milestones, brainstem involvement, tonic-clonic seizures, nystagmus, hematological abnormalities, cognitive impairment, elevated plasma alanine, MRI: Leigh-like lesions.	*MT-ATP6*	m.8993T>G	p.Leu156Arg	Heteroplasmic (blood – 91.8%)	P	NARP, LS	PM3, PS2, PM2, PM5, PP3, PP5	qPCR-HRM
PM 07	F	20 y	Painless central vision loss, nystagmus, optic nerve atrophy, fatigability, recurrent migraine, positive family history.	*MT-ND1*	m.3460G>A	p.Ala52Thr	Heteroplasmic (blood – 92%)	P	LHON	PS4, PM2, PP3, PM1, PP5	qPCR-HRM

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Supplementary Table S1. Continued

Case ID	Sex	Onset age	Patient phenotype	Gene / locus	Genetic variant	Amino acid substitution	Zygosity / Heteroplasmy	ACMG class	ClinVar associated condition	ACMG evidence	Genetic test
PM 08	M	7 m	Generalized hypotonia, failure to thrive, impaired exercise capacity, tonic-clonic seizures, brainstem involvement, positive family history, elevated plasma alanine.	*MT-TL1*	m.3243A>G	–	Heteroplasmic (blood – 56.1%)	P	MELAS, LS, MIDD	PS4, PM2, PP5	qPCR-HRM
PM 09	F	3 y	Neurodevelopmental delay, ophthalmoplegia, recurrent migraine, spasticity, dysphagia, loss of acquired milestones, tonic-clonic seizures, optic nerve atrophy, nystagmus, strabismus, feeding difficulties, MRI: diffuse brain volume loss.	*MT-TN*	m.5667G>A	–	Homoplasmic	P	External ophthalmoplegia	PM2	Targeted mtDNA Sanger seq.
PM 10	F	At birth	Global developmental delay, impaired exercise capacity, hypotonia, loss of acquired milestones, brainstem involvement, hyperkinesia, spastic cerebral palsy, myoclonus, infantile spasms, congenital partial eyelid ptosis, positive family history, cognitive impairment, elevated plasma alanine, ethylmalonic aciduria, altered ACP, lactate peak detected by magnetic resonance spectroscopy, MRI: leukomalacia, and focal gliotic changes.	*ETHE1*	c.487C>G	p.Arg163Gly	Homozygous, AR	P	Ethylmalonic encephalopathy	PM1, PP2, PM2, PM5, PP3	Targeted nDNA Sanger seq.

Abbreviations: ACMG – American College of Medical Genetics and Genomics; ACP – Acylcarnitine profile; AD – Autosomal dominant; AR – Autosomal recessive; HCM – Hypertrophic cardiomyopathy; F – female; LHON – Leber's Hereditary Optic Neuropathy; LP – Likely-pathogenic; LS – Leigh syndrome; LVH – Left ventricular hypertrophy; MELAS – Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-like Episodes; m – months; M – male; MIDD – Maternally Inherited Diabetes and Deafness; MM – Mitochondrial myopathy; MRI – Magnetic Resonance Imaging; mtDNA – mitochondrial DNA; NARP – Neuropathy, Ataxia, and Retinitis Pigmentosa; nDNA – Nuclear DNA; P – Pathogenic; qPCR-HRM – quantitative Polymerase Chain Reaction - High-Resolution Melting; RAH – Right atrial hypertrophy; RVH – Right ventricular hypertrophy; seq. – sequencing; SNHL – Sensorineural hearing loss; VUS – Variant of Uncertain Significance; WES – Whole Exome Sequencing; y – years.

ACMG Evidence Criteria: PVS1 – pathogenic very strong (null variant in a gene where loss-of-function is a known disease mechanism); PS1 – pathogenic strong (same amino acid change as a known pathogenic variant); PS2 – pathogenic strong (confirmed de novo occurrence); PS4 – pathogenic strong (significant increase in variant prevalence in cases vs. controls); PM1 – pathogenic moderate (located in a mutational hotspot or critical functional domain); PM2 – pathogenic moderate (extremely low frequency in gnomAD population databases); PM3 – pathogenic moderate (detected in trans with a pathogenic variant); PM5 – pathogenic moderate (different amino acid change at the same residue as a known pathogenic variant); PP1 – pathogenic supporting (cosegregation with disease in multiple affected family members); PP2 – pathogenic supporting (missense variant in a gene with a low rate of benign missense variation); PP3 – pathogenic supporting (computational tools unanimously support a deleterious effect); PP5 – pathogenic supporting (reported as pathogenic by a reputable source without available raw evidence); BS1 – benign strong (allele frequency is higher than expected for the disorder); BP4 – benign supporting (computational evidence suggests no functional impact); 1A – contains a genomic region with established dosage sensitivity; 2A-2E – various degrees of overlap with dosage-sensitive regions, specific gene components, or established clinical syndromes; 2F-2G – assessment based on genomic content and intra-genic involvement within a dosage-sensitive region; 2H – overlap with known benign genomic regions; 3C – quantitative assessment of protein-coding genes within the interval; 4L-4O – inheritance and segregation analysis, including confirmed or unconfirmed de novo status and transmission from affected or unaffected progenitors.

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Supplementary Table S1. Continued

Case ID	Sex	Onset age	Patient phenotype	Gene / locus	Genetic variant	Amino acid substitution	Zygosity / Heteroplasmy	ACMG class	ClinVar associated condition	ACMG evidence	Genetic test
PM 11	F	11 m	Exercise-induced fatigue, hypotonia, SNHL, ataxia, growth delay, papilloretinal coloboma, brainstem involvement, elevated plasma alanine, altered ACP, MRI: leukoencephalopathy and encephalomalacia.	*MT-RNR1*	m.1494C>T	–	Homoplasmic	LP	SNHL	PM2, PP5	Targeted mtDNA Sanger seq.
PM 12	F	At birth	Motor developmental delay, hypotonia, impaired exercise capacity, feeding difficulties, recurrent vomiting, gastroesophageal reflux, metabolic acidosis, altered ACP, MRI: microcephaly and prominent subarachnoid spaces.	*TWNK*	c.1000C>T	p. Arg334Ter	Heterozygous, AD	P	Progressive external ophthalmoplegia with mitochondrial DNA deletions	PS4, PVS1, PM2, PP5	WES
PM 13	F	1 m	Developmental delay, hypotonia, impaired exercise capacity, pyramidal tract signs, startle-type seizures, myoclonus, SNHL, cognitive impairment, dysmorphism, MRI: basal ganglia signal abnormalities and hemispheric asymmetry.	*MT-ND4*	m.12018C>G	p.Thr420Ser	Homoplasmic	VUS	LS, Epilepsy, Mitochondrial cardiomyopathy, Hypotonia, Deafness	PM2	Targeted mtDNA Sanger seq.
				MT-TH	m.12201T>C	–	Heteroplasmic	LP	SNHL	PM2, PP5	

Abbreviations: ACMG – American College of Medical Genetics and Genomics; ACP – Acylcarnitine profile; AD – Autosomal dominant; AR – Autosomal recessive; HCM – Hypertrophic cardiomyopathy; F – female; LHON – Leber's Hereditary Optic Neuropathy; LP – Likely-pathogenic; LS – Leigh syndrome; LVH – Left ventricular hypertrophy; MELAS – Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-like Episodes; m – months; M – male; MIDD – Maternally Inherited Diabetes and Deafness; MM – Mitochondrial myopathy; MRI – Magnetic Resonance Imaging; mtDNA – mitochondrial DNA; NARP – Neuropathy, Ataxia, and Retinitis Pigmentosa; nDNA – Nuclear DNA; P – Pathogenic; qPCR-HRM – quantitative Polymerase Chain Reaction - High-Resolution Melting; RAH – Right atrial hypertrophy; RVH – Right ventricular hypertrophy; seq. – sequencing; SNHL – Sensorineural hearing loss; VUS – Variant of Uncertain Significance; WES – Whole Exome Sequencing; y – years.

ACMG Evidence Criteria: PVS1 – pathogenic very strong (null variant in a gene where loss-of-function is a known disease mechanism); PS1 – pathogenic strong (same amino acid change as a known pathogenic variant); PS2 – pathogenic strong (confirmed de novo occurrence); PS4 – pathogenic strong (significant increase in variant prevalence in cases vs. controls); PM1 – pathogenic moderate (located in a mutational hotspot or critical functional domain); PM2 – pathogenic moderate (extremely low frequency in gnomAD population databases); PM3 – pathogenic moderate (detected in trans with a pathogenic variant); PM5 – pathogenic moderate (different amino acid change at the same residue as a known pathogenic variant); PP1 – pathogenic supporting (cosegregation with disease in multiple affected family members); PP2 – pathogenic supporting (missense variant in a gene with a low rate of benign missense variation); PP3 – pathogenic supporting (computational tools unanimously support a deleterious effect); PP5 – pathogenic supporting (reported as pathogenic by a reputable source without available raw evidence); BS1 – benign strong (allele frequency is higher than expected for the disorder); BP4 – benign supporting (computational evidence suggests no functional impact); 1A – contains a genomic region with established dosage sensitivity; 2A-2E – various degrees of overlap with dosage-sensitive regions, specific gene components, or established clinical syndromes; 2F-2G – assessment based on genomic content and intra-genic involvement within a dosage-sensitive region; 2H – overlap with known benign genomic regions; 3C – quantitative assessment of protein-coding genes within the interval; 4L-4O – inheritance and segregation analysis, including confirmed or unconfirmed de novo status and transmission from affected or unaffected progenitors.

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Supplementary Table S1. Continued

Case ID	Sex	Onset age	Patient phenotype	Gene / locus	Genetic variant	Amino acid substitution	Zygosity / Heteroplasmy	ACMG class	ClinVar associated condition	ACMG evidence	Genetic test
PM 14	M	2 m	Global delay in cognitive and motor milestones, exercise-induced fatigue, hypotonia, ophthalmoplegia, strabismus, myoclonus, ataxia, RVH, heart failure, congenital heart defects, reactive pancreatitis, hematological abnormalities, dysmorphism, episodic hypoglycemia, hyperlactatemia.	*POLG*	c.752C>T	p. Thr251Ile	Compound heterozygous, AR	P	Progressive external ophthalmoplegia	PS4, PM2, PP2, PP5	WES
					c.1760C>T	p. Pro587Leu				PS4, PM1, PP2, PM2, PP3, PP5	
				1p36	–	–	–	P	Chromosome 1p36 deletion syndrome	1A, 2A-2E, 2F-2G, 2H, 3C, 4L-4O	
PM 15	M	At birth	Marked hypotonia, developmental delay, polymorphic seizures, feeding difficulties, optic nerve atrophy, extrapyramidal signs, cardiac conduction defects, cognitive impairment, microcephaly, metabolic acidosis, MRI: lissencephaly, global atrophy, and basal ganglia signal changes.	*MT-TS2*	m.12250C>T	–	Homoplasmic	VUS	MELAS	PM2, BP4	Targeted mtDNA Sanger seq.
				MT-TL2	m.12320A>G	–	Heteroplasmic	P	MM	PM2	
PM 16	F	1 m	Hypotonia, exercise intolerance, rhabdomyolysis, global developmental delay, recurrent migraine, brainstem involvement, tonic-clonic seizures, cardiac conduction defects, feeding difficulties, recurrent vomiting, dysmorphism, elevated plasma alanine, MRI: white matter signal abnormalities.	*MT-ND3*	m.10197G>A	p.Ala47Thr	Homoplasmic	LP	LS	PS2, PM2, PP3, PP5	Targeted mtDNA Sanger seq.

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Supplementary Table S1. Continued

Case ID	Sex	Onset age	Patient phenotype	Gene / locus	Genetic variant	Amino acid substitution	Zygosity / Heteroplasmy	ACMG class	ClinVar associated condition	ACMG evidence	Genetic test
PM 17	M	1 m	Impaired exercise capacity, marked hypotonia, ophthalmoplegia, global developmental delay, brainstem involvement, febrile seizures, nystagmus, flaccid cerebral palsy, peripheral neuropathy, cerebellar ataxia, cognitive impairment, dysmorphism, elevated plasma alanine, MRI: global brain volume loss and encephalomalacic changes.	*MT-ATP6*	m.8873G>C	p.Gly116Ala	Homoplasmic	VUS	LS	PM2	Targeted mtDNA Sanger seq.
				MT-CYB	m.14792C>T	p.His16Tyr	Homoplasmic	VUS	LS	PM2	
				MT-CYB	m.15117T>C	p.Met124Thr	Heteroplasmic	VUS	LS	PM2	
PM 18	M	7 y	Severe hypotonia, global developmental delay, impaired exercise capacity, ataxia, rhabdomyolysis, loss of acquired milestones, peripheral neuropathy, brainstem involvement, feeding difficulties, cognitive impairment, metabolic acidosis, urinary organic acid abnormalities, MRI: basal ganglia signal abnormalities.	*MT-ND1*	m.4142G>A	p.Cys39Gly	Homoplasmic	LP	LS	PM2	Targeted mtDNA Sanger seq.
PM 19	F	4 m	Facies myopathica, hypotonia, developmental delay, polymorphic seizures, infantile spasms, HCM, recurrent vomiting, intestinal obstruction, optic nerve atrophy, strabismus, feeding difficulties, cognitive impairment, MRI: basal ganglia signal abnormalities.	*MT-ATP8*	m.8528T>C	p.Trp55Arg	Heteroplasmic	LP	HCM	PM3, PP1, PM2, PVS1, PP5	Targeted mtDNA Sanger seq.
				MT-ATP6	m.9194A>G	p.His223Arg	Homoplasmic	VUS	LS	PM2	

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Case ID	Sex	Onset age	Patient phenotype	Gene / locus	Genetic variant	Amino acid substitution	Zygosity / Heteroplasmy	ACMG class	ClinVar associated condition	ACMG evidence	Genetic test
PM 20	F	2 m	Neurodevelopmental delay, impaired exercise capacity, hypotonia, brainstem involvement, tonic-clonic seizures, ophthalmoplegia, strabismus, nystagmus, severe neonatal liver failure, cognitive impairment, episodic hypoglycemia, hyperlactatemia.	*POLG*	c.1283T>C	p.Leu428Pro	Homozygous, AR	LP	Progressive sclerosing poliodystrophy	PM2, PP3, PP2, PP5	Targeted nDNA Sanger seq.
PM 21	M	1.5 y	Impaired exercise capacity, hypotonia, global developmental delay, loss of acquired milestones, SNHL, brainstem involvement, RVH, recurrent vomiting, diarrhea, ataxia.	*MT-TL1*	m.3264T>C	–	Homoplasmic	VUS	MIDD	PM2, PP5	Targeted mtDNA Sanger seq.
PM 22	M	At birth	Hypertonia, impaired psychomotor development, exercise intolerance, pyramidal tract signs, tonic-clonic seizures, optic nerve atrophy, nystagmus, LVH, coarctation of the aorta, peripheral neuropathy, cognitive impairment, dysmorphism, elevated plasma alanine, MRI: hemispheric asymmetry, diffuse cerebral atrophy, leukomalacia, and encephalomalacic changes.	*MT-ATP6*	m.8612T>C	p.Leu29Pro	Homoplasmic	VUS	LS	PM2, PP5	Targeted mtDNA Sanger seq.
				MT-ND3	m.10237T>C	p.Ile60Thr	Homoplasmic	VUS	LHON	PP3, BS1, PP5	
				MT-ND6	m.14466T>C	p.Thr70Ala	Homoplasmic	VUS	LS	PM2, PM5, PM1	

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Supplementary Table S1. Continued

Case ID	Sex	Onset age	Patient phenotype	Gene / locus	Genetic variant	Amino acid substitution	Zygosity / Heteroplasmy	ACMG class	ClinVar associated condition	ACMG evidence	Genetic test
PM 23	F	At birth	Hypotonia, tonic-clonic seizures, recurrent vomiting, feeding difficulties, failure to thrive, impaired exercise capacity, cardiac conduction defects, dysmorphism, elevated plasma alanine, MRI: cerebral atrophy and severely delayed myelination of the cerebral white matter.	*MT-CO2*	m.8241T>G	p.Phe219Cys	Homoplasmic	VUS	MIDD	PM2, PP3	Targeted mtDNA Sanger seq.
				MT-ATP8	m.8514C>T	p.Pro50Leu	Homoplasmic	VUS	LS	PM2	
				MT-ND4	m.10932C>T	p.Ser58Phe	Homoplasmic	VUS	LS	PM2, BP4	
PM 24	M	At birth	Global developmental delay, impaired exercise capacity, pyramidal tract signs, infantile spasms, myoclonus, optic nerve atrophy, strabismus, nystagmus, recurrent vomiting, dysmorphism, metabolic acidosis, MRI: bilateral mesial temporal sclerosis, global brain volume loss, basal ganglia involvement, and bilateral thalamic hyperdensity.	*MT-CO2*	m.8202T>C	p.Phe206Ser	Homoplasmic	VUS	LS	PM2, PP3	Targeted mtDNA Sanger seq.
				MT-CO2	m.8252C>T	p.Pro223Ser	Heteroplasmic	VUS	LS	PM2	
				MT-CO3	m.9247G>A	p.Ser14Asn	Homoplasmic	VUS	LS	PM2	
PM 25	F	4 m	Severe hypotonia, facies myopathica, dystonia, brainstem involvement, tonic-clonic seizures, reactive pancreatitis, failure to thrive, peripheral neuropathy, cerebellar ataxia, cognitive impairment, urinary organic acid abnormalities, elevated blood ammonia levels, MRI: diffuse brain volume loss.	*MT-CO2*	m.7698T>C	p.Val38Ala	Homoplasmic	VUS	LS	PM2	Targeted mtDNA Sanger seq.
				MT-ATP6	m.8615T>C	p.Leu30Ser	Homoplasmic	VUS	LS	PM2	
				MT-ND5	m.12545C>T	p.Thr70Met	Homoplasmic	VUS	LS	BS1	

Abbreviations: ACMG – American College of Medical Genetics and Genomics; ACP – Acylcarnitine profile; AD – Autosomal dominant; AR – Autosomal recessive; HCM – Hypertrophic cardiomyopathy; F – female; LHON – Leber's Hereditary Optic Neuropathy; LP – Likely-pathogenic; LS – Leigh syndrome; LVH – Left ventricular hypertrophy; MELAS – Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-like Episodes; m – months; M – male; MIDD – Maternally Inherited Diabetes and Deafness; MM – Mitochondrial myopathy; MRI – Magnetic Resonance Imaging; mtDNA – mitochondrial DNA; NARP – Neuropathy, Ataxia, and Retinitis Pigmentosa; nDNA – Nuclear DNA; P – Pathogenic; qPCR-HRM – quantitative Polymerase Chain Reaction - High-Resolution Melting; RAH – Right atrial hypertrophy; RVH – Right ventricular hypertrophy; seq. – sequencing; SNHL – Sensorineural hearing loss; VUS – Variant of Uncertain Significance; WES – Whole Exome Sequencing; y – years.

ACMG Evidence Criteria: PVS1 – pathogenic very strong (null variant in a gene where loss-of-function is a known disease mechanism); PS1 – pathogenic strong (same amino acid change as a known pathogenic variant); PS2 – pathogenic strong (confirmed de novo occurrence); PS4 – pathogenic strong (significant increase in variant prevalence in cases vs. controls); PM1 – pathogenic moderate (located in a mutational hotspot or critical functional domain); PM2 – pathogenic moderate (extremely low frequency in gnomAD population databases); PM3 – pathogenic moderate (detected in trans with a pathogenic variant); PM5 – pathogenic moderate (different amino acid change at the same residue as a known pathogenic variant); PP1 – pathogenic supporting (cosegregation with disease in multiple affected family members); PP2 – pathogenic supporting (missense variant in a gene with a low rate of benign missense variation); PP3 – pathogenic supporting (computational tools unanimously support a deleterious effect); PP5 – pathogenic supporting (reported as pathogenic by a reputable source without available raw evidence); BS1 – benign strong (allele frequency is higher than expected for the disorder); BP4 – benign supporting (computational evidence suggests no functional impact); 1A – contains a genomic region with established dosage sensitivity; 2A-2E – various degrees of overlap with dosage-sensitive regions, specific gene components, or established clinical syndromes; 2F-2G – assessment based on genomic content and intra-genic involvement within a dosage-sensitive region; 2H – overlap with known benign genomic regions; 3C – quantitative assessment of protein-coding genes within the interval; 4L-4O – inheritance and segregation analysis, including confirmed or unconfirmed de novo status and transmission from affected or unaffected progenitors.

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Supplementary Table S1. Continued

Case ID	Sex	Onset age	Patient phenotype	Gene / locus	Genetic variant	Amino acid substitution	Zygosity / Heteroplasmy	ACMG class	ClinVar associated condition	ACMG evidence	Genetic test
PM 26	F	3 m	Global developmental delay, hypotonia, tonic-clonic seizures, pyramidal tract signs, strabismus, dilated cardiomyopathy, RAH, feeding difficulties, urinary organic acid abnormalities, metabolic acidosis, altered ACP, MRI: diffuse brain volume loss.	*MT-ATP6*	m.9125C>T	p.Thr200Ile	Homoplasmic	VUS	LS	PM2	Targeted mtDNA Sanger seq.
				MT-ND6	m.14423G>A	p.Ser84Leu	Heteroplasmic	VUS	LS	PM2, BP4	
				MT-CYB	m.15132T>C	p.Met129Thr	Homoplasmic	VUS	Mitochondrial cardiomyopathy	PM2	
PM 27	F	6 m	Facies myopathica, impaired exercise capacity, global developmental delay, pyramidal tract signs, dystonia, tonic-clonic seizures, dysarthria, flaccid cerebral palsy, cortical blindness, optic nerve atrophy, nystagmus, cognitive impairment, dysmorphism, positive family history, peripheral neuropathy, MRI: diffuse brain volume loss.	*MT-ATP8*	m.8558C>T	p.Pro65Ser	Homoplasmic	VUS	Mitochondrial cardiomyopathy	PM2, PM1, PP5	Targeted mtDNA Sanger seq.
				MT-ATP6	m.8785C>T	p.Leu87Phe	Homoplasmic	VUS	LS	PM2	
				MT-ATP6	m.8873G>C	p.Gly116Ala	Homoplasmic	VUS	LS	PM2	
PM 28	M	At birth	Marked hypotonia, severe fetal growth restriction, feeding difficulties, gastric hemorrhage, hematological abnormalities, acute respiratory distress with moderate sternal retractions, metabolic acidosis.	*DGUOK*	c.437G>A	p.Ser146Asn	Homozygous, AR	VUS	Mitochondrial DNA depletion syndrome	PM2, PP3, PP2	WES

Abbreviations: ACMG – American College of Medical Genetics and Genomics; ACP – Acylcarnitine profile; AD – Autosomal dominant; AR – Autosomal recessive; HCM – Hypertrophic cardiomyopathy; F – female; LHON – Leber's Hereditary Optic Neuropathy; LP – Likely-pathogenic; LS – Leigh syndrome; LVH – Left ventricular hypertrophy; MELAS – Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-like Episodes; m – months; M – male; MIDD – Maternally Inherited Diabetes and Deafness; MM – Mitochondrial myopathy; MRI – Magnetic Resonance Imaging; mtDNA – mitochondrial DNA; NARP – Neuropathy, Ataxia, and Retinitis Pigmentosa; nDNA – Nuclear DNA; P – Pathogenic; qPCR-HRM – quantitative Polymerase Chain Reaction - High-Resolution Melting; RAH – Right atrial hypertrophy; RVH – Right ventricular hypertrophy; seq. – sequencing; SNHL – Sensorineural hearing loss; VUS – Variant of Uncertain Significance; WES – Whole Exome Sequencing; y – years.

ACMG Evidence Criteria: PVS1 – pathogenic very strong (null variant in a gene where loss-of-function is a known disease mechanism); PS1 – pathogenic strong (same amino acid change as a known pathogenic variant); PS2 – pathogenic strong (confirmed de novo occurrence); PS4 – pathogenic strong (significant increase in variant prevalence in cases vs. controls); PM1 – pathogenic moderate (located in a mutational hotspot or critical functional domain); PM2 – pathogenic moderate (extremely low frequency in gnomAD population databases); PM3 – pathogenic moderate (detected in trans with a pathogenic variant); PM5 – pathogenic moderate (different amino acid change at the same residue as a known pathogenic variant); PP1 – pathogenic supporting (cosegregation with disease in multiple affected family members); PP2 – pathogenic supporting (missense variant in a gene with a low rate of benign missense variation); PP3 – pathogenic supporting (computational tools unanimously support a deleterious effect); PP5 – pathogenic supporting (reported as pathogenic by a reputable source without available raw evidence); BS1 – benign strong (allele frequency is higher than expected for the disorder); BP4 – benign supporting (computational evidence suggests no functional impact); 1A – contains a genomic region with established dosage sensitivity; 2A-2E – various degrees of overlap with dosage-sensitive regions, specific gene components, or established clinical syndromes; 2F-2G – assessment based on genomic content and intra-genic involvement within a dosage-sensitive region; 2H – overlap with known benign genomic regions; 3C – quantitative assessment of protein-coding genes within the interval; 4L-4O – inheritance and segregation analysis, including confirmed or unconfirmed de novo status and transmission from affected or unaffected progenitors.

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Supplementary Table S1. Continued

Case ID	Sex	Onset age	Patient phenotype	Gene / locus	Genetic variant	Amino acid substitution	Zygosity / Heteroplasmy	ACMG class	ClinVar associated condition	ACMG evidence	Genetic test
PM 29	F	3 m	Impaired exercise capacity, hypotonia, brainstem involvement, tonic-clonic seizures, feeding difficulties, failure to thrive, peripheral neuropathy, cognitive impairment, elevated plasma ammonia and alanine, increased transaminases, MRI: diffuse brain volume loss.	*MT-CO2*	m.7746A>G	p.Asn54Ser	Homoplasmic	VUS	LS	PM2	Targeted mtDNA Sanger seq.
				MT-ATP6	m.9125C>T	p.Thr200Ile	Homoplasmic	VUS	LS	PM2	
				MT-ND5	m.12479T>C	p.Leu48Pro	Homoplasmic	VUS	LS	PM2	
PM 30	F	2 y	Ophthalmoplegia, hypotonia, developmental delay, brainstem involvement, nystagmus, dysmorphism, peripheral neuropathy, positive family history, metabolic acidosis.	*MT-ND1*	m.4180A>G	p.Asn292Asp	Homoplasmic	VUS	LS	PM2	Targeted mtDNA Sanger seq.
				MT-ND4L	m.10663T>C	p.Val65Ala	Heteroplasmic	LP	LHON	PS2, PM2, PP3, PP5	
PM 31	M	9 m	Hypotonia, developmental delay, impaired exercise capacity, tonic-clonic seizures, positive family history, cerebellar ataxia, stereotypies, intellectual disability, dysmorphism, MRI: diffuse brain volume loss.	*MT-ND2*	m.4953A>G	p.Ile162Val	Homoplasmic	VUS	LS	PM2	Targeted mtDNA Sanger seq.
				MT-ND4	m.10931 T>C	p.Ser58Pro	Homoplasmic	VUS	LS	BS1	
				MT-TE	m.14687 A>G	–	Homoplasmic	VUS	Intellectual disability, MM	BP4	
PM 32	M	10 m	Exercise-induced fatigue, hypotonia, loss of acquired milestones, extrapyramidal signs, endocrine and growth abnormalities, subclinical hypothyroidism, stereotypies, cognitive impairment, positive family history, elevated blood ammonia levels, MRI: diffuse brain volume loss.	*MT-TK*	m.8328G>A	–	Homoplasmic	VUS	MM, exercise intolerance	PM2, PP3	Targeted mtDNA Sanger seq.
				MT-ATP8	m.8373A>T	p.Gln3Leu	Heteroplasmic	VUS	LS	PM2	
				MT-ATP8	m.8418T>C	p.Leu18Pro	Homoplasmic	VUS	Optic neuropathy	PM2	

Abbreviations: ACMG – American College of Medical Genetics and Genomics; ACP – Acylcarnitine profile; AD – Autosomal dominant; AR – Autosomal recessive; HCM – Hypertrophic cardiomyopathy; F – female; LHON – Leber's Hereditary Optic Neuropathy; LP – Likely-pathogenic; LS – Leigh syndrome; LVH – Left ventricular hypertrophy; MELAS – Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-like Episodes; m – months; M – male; MIDD – Maternally Inherited Diabetes and Deafness; MM – Mitochondrial myopathy; MRI – Magnetic Resonance Imaging; mtDNA – mitochondrial DNA; NARP – Neuropathy, Ataxia, and Retinitis Pigmentosa; nDNA – Nuclear DNA; P – Pathogenic; qPCR-HRM – quantitative Polymerase Chain Reaction - High-Resolution Melting; RAH – Right atrial hypertrophy; RVH – Right ventricular hypertrophy; seq. – sequencing; SNHL – Sensorineural hearing loss; VUS – Variant of Uncertain Significance; WES – Whole Exome Sequencing; y – years.

ACMG Evidence Criteria: PVS1 – pathogenic very strong (null variant in a gene where loss-of-function is a known disease mechanism); PS1 – pathogenic strong (same amino acid change as a known pathogenic variant); PS2 – pathogenic strong (confirmed de novo occurrence); PS4 – pathogenic strong (significant increase in variant prevalence in cases vs. controls); PM1 – pathogenic moderate (located in a mutational hotspot or critical functional domain); PM2 – pathogenic moderate (extremely low frequency in gnomAD population databases); PM3 – pathogenic moderate (detected in trans with a pathogenic variant); PM5 – pathogenic moderate (different amino acid change at the same residue as a known pathogenic variant); PP1 – pathogenic supporting (cosegregation with disease in multiple affected family members); PP2 – pathogenic supporting (missense variant in a gene with a low rate of benign missense variation); PP3 – pathogenic supporting (computational tools unanimously support a deleterious effect); PP5 – pathogenic supporting (reported as pathogenic by a reputable source without available raw evidence); BS1 – benign strong (allele frequency is higher than expected for the disorder); BP4 – benign supporting (computational evidence suggests no functional impact); 1A – contains a genomic region with established dosage sensitivity; 2A-2E – various degrees of overlap with dosage-sensitive regions, specific gene components, or established clinical syndromes; 2F-2G – assessment based on genomic content and intra-genic involvement within a dosage-sensitive region; 2H – overlap with known benign genomic regions; 3C – quantitative assessment of protein-coding genes within the interval; 4L-4O – inheritance and segregation analysis, including confirmed or unconfirmed de novo status and transmission from affected or unaffected progenitors.

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Supplementary Table S1. Continued

Case ID	Sex	Onset age	Patient phenotype	Gene / locus	Genetic variant	Amino acid substitution	Zygosity / Heteroplasmy	ACMG class	ClinVar associated condition	ACMG evidence	Genetic test
PM 33	M	1 m	Neurodevelopmental delay, RVH, hypotonia, optic nerve atrophy, tonic-clonic seizures, pyramidal tract signs, cortical blindness, MRI: hemispheric asymmetry and global cerebral volume loss.	*MT-ND1*	m.4184T>C	p.Phe293Ser	Homoplasmic	LP	LHON	PM2	Targeted mtDNA Sanger seq.
				MT-ND4	m.11360A>G	p.Met201Val	Homoplasmic	VUS	LS, seizures, LHON	PM2	
PM 34	M	3 m	Hypotonia, developmental delay, polymorphic seizures, optic nerve atrophy, cortical blindness, nystagmus, mild cognitive impairment, positive family history.	*MT-ND2*	m.5038T>C	p.Met190Thr	Homoplasmic	VUS	LS	PM2	Targeted mtDNA Sanger seq.
				MT-ND6	m.14482C>G	p. Met64Ile	Homoplasmic	LP	LHON	PS4, PS1, PM5, PM2, PM1, PP3, PP5	
PM 35	F	6 m	Exercise-induced fatigue, hypotonia, developmental delay, stroke-like episodes, dyskinetic cerebral palsy, polymorphic seizures, optic nerve atrophy, strabismus, cardiac conduction defects, elevated plasma alanine, MRI: stroke-like lesions and basal ganglia involvement.	*MT-ND1*	m.4171C>A	p.Leu289Met	Homoplasmic	VUS	LHON, LS	PM2	Targeted mtDNA Sanger seq.
				MT-ND1	m.4193T>C	p.Leu296Pro	Homoplasmic	VUS	LS	PM2	
				MT-CO2	m.7698T>C	p.Val38Ala	Homoplasmic	VUS	LS	PM2	

Abbreviations: ACMG – American College of Medical Genetics and Genomics; ACP – Acylcarnitine profile; AD – Autosomal dominant; AR – Autosomal recessive; HCM – Hypertrophic cardiomyopathy; F – female; LHON – Leber's Hereditary Optic Neuropathy; LP – Likely-pathogenic; LS – Leigh syndrome; LVH – Left ventricular hypertrophy; MELAS – Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-like Episodes; m – months; M – male; MIDD – Maternally Inherited Diabetes and Deafness; MM – Mitochondrial myopathy; MRI – Magnetic Resonance Imaging; mtDNA – mitochondrial DNA; NARP – Neuropathy, Ataxia, and Retinitis Pigmentosa; nDNA – Nuclear DNA; P – Pathogenic; qPCR-HRM – quantitative Polymerase Chain Reaction - High-Resolution Melting; RAH – Right atrial hypertrophy; RVH – Right ventricular hypertrophy; seq. – sequencing; SNHL – Sensorineural hearing loss; VUS – Variant of Uncertain Significance; WES – Whole Exome Sequencing; y – years.

ACMG Evidence Criteria: PVS1 – pathogenic very strong (null variant in a gene where loss-of-function is a known disease mechanism); PS1 – pathogenic strong (same amino acid change as a known pathogenic variant); PS2 – pathogenic strong (confirmed de novo occurrence); PS4 – pathogenic strong (significant increase in variant prevalence in cases vs. controls); PM1 – pathogenic moderate (located in a mutational hotspot or critical functional domain); PM2 – pathogenic moderate (extremely low frequency in gnomAD population databases); PM3 – pathogenic moderate (detected in trans with a pathogenic variant); PM5 – pathogenic moderate (different amino acid change at the same residue as a known pathogenic variant); PP1 – pathogenic supporting (cosegregation with disease in multiple affected family members); PP2 – pathogenic supporting (missense variant in a gene with a low rate of benign missense variation); PP3 – pathogenic supporting (computational tools unanimously support a deleterious effect); PP5 – pathogenic supporting (reported as pathogenic by a reputable source without available raw evidence); BS1 – benign strong (allele frequency is higher than expected for the disorder); BP4 – benign supporting (computational evidence suggests no functional impact); 1A – contains a genomic region with established dosage sensitivity; 2A-2E – various degrees of overlap with dosage-sensitive regions, specific gene components, or established clinical syndromes; 2F-2G – assessment based on genomic content and intra-genic involvement within a dosage-sensitive region; 2H – overlap with known benign genomic regions; 3C – quantitative assessment of protein-coding genes within the interval; 4L-4O – inheritance and segregation analysis, including confirmed or unconfirmed de novo status and transmission from affected or unaffected progenitors.

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Supplementary Table S1. Continued

Case ID	Sex	Onset age	Patient phenotype	Gene / locus	Genetic variant	Amino acid substitution	Zygosity / Heteroplasmy	ACMG class	ClinVar associated condition	ACMG evidence	Genetic test
PM 36	F	At birth	Facies myopathica, marked hypotonia, developmental delay, tonic-clonic seizures, myoclonus, cerebrovascular hemorrhage, optic nerve atrophy, feeding difficulties, dysmorphism, RVH, ethylmalonic aciduria, metabolic acidosis, altered ACP, MRI: leukomalacia and diffuse cerebral volume loss.	*MT-ATP8*	m.8536A>T	p.Lys57Asn	Homoplasmic	LP	Sporadic myoclonus epilepsy	PM2	Targeted mtDNA Sanger seq.
PM 37	M	1 m	Impaired exercise capacity, hypotonia, failure to thrive, dilated cardiomyopathy, RVH, hematological abnormalities, feeding difficulties, altered ACP.	*MT-CYB*	m.14849T>C	p.Ser35Pro	Homoplasmic	VUS	Mitochondrial cardiomyopathy, LS, Septo-optic dysplasia	PM2	Targeted mtDNA Sanger seq.

Abbreviations: ACMG – American College of Medical Genetics and Genomics; ACP – Acylcarnitine profile; AD – Autosomal dominant; AR – Autosomal recessive; HCM – Hypertrophic cardiomyopathy; F – female; LHON – Leber's Hereditary Optic Neuropathy; LP – Likely-pathogenic; LS – Leigh syndrome; LVH – Left ventricular hypertrophy; MELAS – Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-like Episodes; m – months; M – male; MIDD – Maternally Inherited Diabetes and Deafness; MM – Mitochondrial myopathy; MRI – Magnetic Resonance Imaging; mtDNA – mitochondrial DNA; NARP – Neuropathy, Ataxia, and Retinitis Pigmentosa; nDNA – Nuclear DNA; P – Pathogenic; qPCR-HRM – quantitative Polymerase Chain Reaction - High-Resolution Melting; RAH – Right atrial hypertrophy; RVH – Right ventricular hypertrophy; seq. – sequencing; SNHL – Sensorineural hearing loss; VUS – Variant of Uncertain Significance; WES – Whole Exome Sequencing; y – years.

ACMG Evidence Criteria: PVS1 – pathogenic very strong (null variant in a gene where loss-of-function is a known disease mechanism); PS1 – pathogenic strong (same amino acid change as a known pathogenic variant); PS2 – pathogenic strong (confirmed de novo occurrence); PS4 – pathogenic strong (significant increase in variant prevalence in cases vs. controls); PM1 – pathogenic moderate (located in a mutational hotspot or critical functional domain); PM2 – pathogenic moderate (extremely low frequency in gnomAD population databases); PM3 – pathogenic moderate (detected in trans with a pathogenic variant); PM5 – pathogenic moderate (different amino acid change at the same residue as a known pathogenic variant); PP1 – pathogenic supporting (cosegregation with disease in multiple affected family members); PP2 – pathogenic supporting (missense variant in a gene with a low rate of benign missense variation); PP3 – pathogenic supporting (computational tools unanimously support a deleterious effect); PP5 – pathogenic supporting (reported as pathogenic by a reputable source without available raw evidence); BS1 – benign strong (allele frequency is higher than expected for the disorder); BP4 – benign supporting (computational evidence suggests no functional impact); 1A – contains a genomic region with established dosage sensitivity; 2A-2E – various degrees of overlap with dosage-sensitive regions, specific gene components, or established clinical syndromes; 2F-2G – assessment based on genomic content and intra-genic involvement within a dosage-sensitive region; 2H – overlap with known benign genomic regions; 3C – quantitative assessment of protein-coding genes within the interval; 4L-4O – inheritance and segregation analysis, including confirmed or unconfirmed de novo status and transmission from affected or unaffected progenitors.

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Supplementary Table S2. Phenotypic characteristics and genetic variant profiles of the alternative genetic disorders group

Case ID	Sex	Onset age	Patient phenotype	Gene / locus	Genetic variant	Amino acid substitution	Zygosity / Heteroplasmy	ACMG class	ClinVar associated condition	ACMG evidence	Genetic test
PNM 01	F	3 m	Delayed developmental milestones, impaired exercise capacity, febrile seizures, tonic-clonic seizures, cognitive impairment including attention and memory difficulties; altered ACP, MRI: leukomalacic alterations and cerebral volume loss.	*SCN1A*	c.2589+3 A>T	–	Heterozygous, AD	P	Developmental Epileptic Encephalopathy 6A	PS3, PS2, PM2, PP3, PP5	WES
PNM 02	M	At birth	Exercise-induced fatigue, hypotonia, growth delay, hematological abnormalities, hepatic steatosis, repetitive episodes of vomiting, dysmorphism, episodic hypoglycemia, hyperlactatemia.	*ALDOB*	c.113-1 _115del GGTA	–	Homozygous, AR	P	Hereditary Fructosuria	PM3, PVS1, PM2, PP5	Targeted Sanger seq.
PNM 03	M	1 m	Global developmental delay, spasticity, hyperkinesia, tonic-clonic seizures, ataxic cerebral palsy, strabismus, hematological abnormalities, dysmorphism, cognitive impairment, microcephaly, positive family history, MRI: pontocerebellar hypoplasia.	*TSEN54*	c.919G>T	p.Ala307Ser	Homozygous, AR	P	Pontocerebellar hypoplasia, type 2A	PM3, PP1, PM2, PP5	WES

Abbreviations: ACMG – American College of Medical Genetics and Genomics; aCGH – array Comparative Genomic Hybridization; ACP – Acylcarnitine profile; AD – Autosomal dominant; AR – Autosomal recessive; F – female; LP – Likely-pathogenic; m – months; M – male; MLPA – Multiplex Ligation-dependent Probe Amplification; MRI – Magnetic Resonance Imaging; NGS – Next-Generation Sequencing; P – Pathogenic; RVH – Right ventricular hypertrophy; seq. – sequencing; SNHL – Sensorineural hearing loss; VUS – Variant of Uncertain Significance; WES – Whole Exome Sequencing; WGS – Whole Genome Sequencing; y – years.

ACMG Evidence Criteria: PVS1 – pathogenic very strong (null variant in a gene where loss-of-function is a known disease mechanism); PS1 – pathogenic strong (same amino acid change as a known pathogenic variant); PS2 – pathogenic strong (confirmed de novo occurrence); PS3 – pathogenic strong (well-established functional studies supportive of a damaging effect on the gene product); PS4 – pathogenic strong (significant increase in variant prevalence in cases vs. controls); PM1 – pathogenic moderate (located in a mutational hotspot or critical functional domain); PM2 – pathogenic moderate (extremely low frequency in gnomAD population databases); PM3 – pathogenic moderate (detected in trans with a pathogenic variant); PM5 – pathogenic moderate (different amino acid change at the same residue as a known pathogenic variant); PM6 – pathogenic moderate (assumed de novo occurrence without confirmation of paternity and maternity); PP1 – pathogenic supporting (cosegregation with disease in multiple affected family members); PP2 – pathogenic supporting (missense variant in a gene with a low rate of benign missense variation); PP4 – pathogenic supporting (patient's phenotype or family history is highly specific for a disease with a single genetic etiology); PP5 – pathogenic supporting (reported as pathogenic by a reputable source without available raw evidence); BS1 – benign strong (allele frequency is higher than expected for the disorder); BP4 – benign supporting (computational evidence suggests no functional impact); BP6 – benign supporting (reputable source reports variant as benign); BP7 – benign supporting (synonymous variant for which computational algorithms predict no effect on splicing); 1A – contains a genomic region with established dosage sensitivity; 2A-2E – various degrees of overlap with dosage-sensitive regions, specific gene components, or established clinical syndromes; 2F-2G – assessment based on genomic content and intra-genic involvement within a dosage-sensitive region; 2H – overlap with known benign genomic regions; 3C – quantitative assessment of protein-coding genes within the interval; 4L-4O – inheritance and segregation analysis, including confirmed or unconfirmed de novo status and transmission from affected or unaffected progenitors.

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Supplementary Table S2. Continued

Case ID	Sex	Onset age	Patient phenotype	Gene / locus	Genetic variant	Amino acid substitution	Zygosity / Heteroplasmy	ACMG class	ClinVar associated condition	ACMG evidence	Genetic test
PNM 03`	F	1 m	Global developmental delay, spasticity, hyperkinesia, febrile seizures, tonic-clonic seizures, flaccid cerebral palsy, strabismus, gastroesophageal reflux, cognitive impairment, microcephaly, dysmorphism, positive family history; metabolic acidosis, MRI: pontocerebellar hypoplasia.	*TSEN54*	c.919G>T	p.Ala307Ser	Homozygous, AR	P	Pontocerebellar hypoplasia, type 2A	PM3, PP1, PM2, PP5	WES
PNM 04	M	1 m	Generalized hypotonia, severe psychomotor delay, impaired exercise capacity, ataxia, hyperkinesia, polymorphic seizures, cortical blindness, optic nerve atrophy, cognitive impairment, dysmorphism, elevated blood ammonia levels.	*FOXG1*	c.693C>A	p.His231Gln	Heterozygous, AD	LP	Rett syndrome, congenital variant	PM1, PP2, PM2, PM5, PP3	WES
PNM 05	F	1 m	Hypotonia, global developmental delay, tonic-clonic seizures, myoclonus, recurrent migraine, strabismus, auditory impairment, peripheral neuropathy, ataxia, cognitive impairment, dysmorphism, metabolic acidosis.	*PPP2R5D*	c.592G>A	p.Glu198Lys	Heterozygous, AD	P	Hogue-Janssens Syndrome	PS3, PS2, PM1, PP2, PM2, PP5	WES
PNM 06	M	At birth	Stroke-like episodes, hypotonia, developmental delay, tonic-clonic seizures, infantile spasms, hyperactivity, hematological abnormalities, hyperlactatemia, MRI: stroke-like lesions.	*SCN8A*	c.4447G>A	p.Glu1483Lys	Heterozygous, AD	P	Seizures, Benign Familial Infantile, 5	PS4, PP1, PS3, PM1, PP2, PM2, PP3, PP5	WGS

Abbreviations: ACMG – American College of Medical Genetics and Genomics; aCGH – array Comparative Genomic Hybridization; ACP – Acylcarnitine profile; AD – Autosomal dominant; AR – Autosomal recessive; F – female; LP – Likely-pathogenic; m – months; M – male; MLPA – Multiplex Ligation-dependent Probe Amplification; MRI – Magnetic Resonance Imaging; NGS – Next-Generation Sequencing; P – Pathogenic; RVH – Right ventricular hypertrophy; seq. – sequencing; SNHL – Sensorineural hearing loss; VUS – Variant of Uncertain Significance; WES – Whole Exome Sequencing; WGS – Whole Genome Sequencing; y – years.

ACMG Evidence Criteria: PVS1 – pathogenic very strong (null variant in a gene where loss-of-function is a known disease mechanism); PS1 – pathogenic strong (same amino acid change as a known pathogenic variant); PS2 – pathogenic strong (confirmed de novo occurrence); PS3 – pathogenic strong (well-established functional studies supportive of a damaging effect on the gene product); PS4 – pathogenic strong (significant increase in variant prevalence in cases vs. controls); PM1 – pathogenic moderate (located in a mutational hotspot or critical functional domain); PM2 – pathogenic moderate (extremely low frequency in gnomAD population databases); PM3 – pathogenic moderate (detected in trans with a pathogenic variant); PM5 – pathogenic moderate (different amino acid change at the same residue as a known pathogenic variant); PM6 – pathogenic moderate (assumed de novo occurrence without confirmation of paternity and maternity); PP1 – pathogenic supporting (cosegregation with disease in multiple affected family members); PP2 – pathogenic supporting (missense variant in a gene with a low rate of benign missense variation); PP4 – pathogenic supporting (patient's phenotype or family history is highly specific for a disease with a single genetic etiology); PP5 – pathogenic supporting (reported as pathogenic by a reputable source without available raw evidence); BS1 – benign strong (allele frequency is higher than expected for the disorder); BP4 – benign supporting (computational evidence suggests no functional impact); BP6 – benign supporting (reputable source reports variant as benign); BP7 – benign supporting (synonymous variant for which computational algorithms predict no effect on splicing); 1A – contains a genomic region with established dosage sensitivity; 2A-2E – various degrees of overlap with dosage-sensitive regions, specific gene components, or established clinical syndromes; 2F-2G – assessment based on genomic content and intra-genic involvement within a dosage-sensitive region; 2H – overlap with known benign genomic regions; 3C – quantitative assessment of protein-coding genes within the interval; 4L-4O – inheritance and segregation analysis, including confirmed or unconfirmed de novo status and transmission from affected or unaffected progenitors.

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Supplementary Table S2. Continued

Case ID	Sex	Onset age	Patient phenotype	Gene / locus	Genetic variant	Amino acid substitution	Zygosity / Heteroplasmy	ACMG class	ClinVar associated condition	ACMG evidence	Genetic test
PNM 07	M	4 m	Neurodevelopmental delay, hypotonia, coarctation of the aorta, clinical hypothyroidism, repetitive episodes of vomiting, feeding difficulties, dysmorphism, altered ACP.	*7q11.23*	–	–	–	P	Williams-Beuren syndrome	1A, 2A-2E, 2F-2G, 2H, 3A, 4L-4O	MLPA
PNM 08	M	4 y	Hypostature, subclinical hypothyroidism, sagittal sinus thrombosis, nephrotic syndrome, glomerulonephritis, ascites, cardiac conduction defects, impaired exercise capacity, dysmorphism.	*SMARCAL1*	c.1933C>T	p.Arg645Cys	Homozygous, AR	P	Schimke Immuno-Osseous Dysplasia	PM3, PS3, PM2, PM5, PM1, PP3, PP5	NGS panel
PNM 09	F	At birth	Bulbar syndrome, severe psychomotor delay, hypotonia, polymorphic seizures, myoclonus, nystagmus, feeding difficulties, hematological abnormalities, cognitive impairment, elevated plasma alanine, altered ACP.	*SCN2A*	c.2638G>A	p.Ala880Thr	Heterozygous, AD	P	Developmental and Epileptic Encephalopathy, 11	PS4, PM1, PP2, PM2, PM5, PP3, PP5	WES
PNM 10	M	1 m	Exercise-induced fatigue, developmental delay, brainstem involvement, tonic-clonic seizures, optic nerve atrophy, positive family history.	*ALDH7A1*	c.1482-1G>T	–	Homozygous, AR	P	Pyridoxine-Dependent Epilepsy	PM3, PVS1, PM2, PP5	NGS panel

Abbreviations: ACMG – American College of Medical Genetics and Genomics; aCGH – array Comparative Genomic Hybridization; ACP – Acylcarnitine profile; AD – Autosomal dominant; AR – Autosomal recessive; F – female; LP – Likely-pathogenic; m – months; M – male; MLPA – Multiplex Ligation-dependent Probe Amplification; MRI – Magnetic Resonance Imaging; NGS – Next-Generation Sequencing; P – Pathogenic; RVH – Right ventricular hypertrophy; seq. – sequencing; SNHL – Sensorineural hearing loss; VUS – Variant of Uncertain Significance; WES – Whole Exome Sequencing; WGS – Whole Genome Sequencing; y – years.

ACMG Evidence Criteria: PVS1 – pathogenic very strong (null variant in a gene where loss-of-function is a known disease mechanism); PS1 – pathogenic strong (same amino acid change as a known pathogenic variant); PS2 – pathogenic strong (confirmed de novo occurrence); PS3 – pathogenic strong (well-established functional studies supportive of a damaging effect on the gene product); PS4 – pathogenic strong (significant increase in variant prevalence in cases vs. controls); PM1 – pathogenic moderate (located in a mutational hotspot or critical functional domain); PM2 – pathogenic moderate (extremely low frequency in gnomAD population databases); PM3 – pathogenic moderate (detected in trans with a pathogenic variant); PM5 – pathogenic moderate (different amino acid change at the same residue as a known pathogenic variant); PM6 – pathogenic moderate (assumed de novo occurrence without confirmation of paternity and maternity); PP1 – pathogenic supporting (cosegregation with disease in multiple affected family members); PP2 – pathogenic supporting (missense variant in a gene with a low rate of benign missense variation); PP4 – pathogenic supporting (patient's phenotype or family history is highly specific for a disease with a single genetic etiology); PP5 – pathogenic supporting (reported as pathogenic by a reputable source without available raw evidence); BS1 – benign strong (allele frequency is higher than expected for the disorder); BP4 – benign supporting (computational evidence suggests no functional impact); BP6 – benign supporting (reputable source reports variant as benign); BP7 – benign supporting (synonymous variant for which computational algorithms predict no effect on splicing); 1A – contains a genomic region with established dosage sensitivity; 2A-2E – various degrees of overlap with dosage-sensitive regions, specific gene components, or established clinical syndromes; 2F-2G – assessment based on genomic content and intra-genic involvement within a dosage-sensitive region; 2H – overlap with known benign genomic regions; 3C – quantitative assessment of protein-coding genes within the interval; 4L-4O – inheritance and segregation analysis, including confirmed or unconfirmed de novo status and transmission from affected or unaffected progenitors.

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Case ID	Sex	Onset age	Patient phenotype	Gene / locus	Genetic variant	Amino acid substitution	Zygosity / Heteroplasmy	ACMG class	ClinVar associated condition	ACMG evidence	Genetic test
PNM 11	M	4 m	Marked hypotonia, global developmental delay, impaired exercise capacity, febrile seizures, flaccid cerebral palsy, repetitive episodes of vomiting, feeding difficulties, RVH, nephrotic syndrome, peripheral neuropathy, positive family history, cognitive impairment, hyperlactatemia, urinary organic acid abnormalities, elevated ketone bodies, altered ACP, elevated blood ammonia levels, lactate peak detected by magnetic resonance spectroscopy, MRI: diffuse volume loss and encephalomalacia.	*PCCA*	c.937C>T	p.Arg313Ter	Homozygous, AR	P	Propionic Acidemia	PM3, PS3, PVS1, PM2, PP5	Targeted Sanger seq.
PNM 12	F	4 m	Developmental delay, hypotonia, flaccid cerebral palsy, peripheral neuropathy, auditory impairment, severe congenital heart defects, bilateral multicystic kidneys, diffuse ecchymoses, hepatosplenomegaly, feeding difficulties, positive family history, dysmorphism, altered ACP.	*GLB1*	c.176G>A	p.Arg59His	Homozygous, AR	P	GM1 Gangliosidosis	PM3, PP1, PS3, PM1, PP2, PM2, PM5, PP3, PP5	NGS panel

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ACMG Evidence Criteria: PVS1 – pathogenic very strong (null variant in a gene where loss-of-function is a known disease mechanism); PS1 – pathogenic strong (same amino acid change as a known pathogenic variant); PS2 – pathogenic strong (confirmed de novo occurrence); PS3 – pathogenic strong (well-established functional studies supportive of a damaging effect on the gene product); PS4 – pathogenic strong (significant increase in variant prevalence in cases vs. controls); PM1 – pathogenic moderate (located in a mutational hotspot or critical functional domain); PM2 – pathogenic moderate (extremely low frequency in gnomAD population databases); PM3 – pathogenic moderate (detected in trans with a pathogenic variant); PM5 – pathogenic moderate (different amino acid change at the same residue as a known pathogenic variant); PM6 – pathogenic moderate (assumed de novo occurrence without confirmation of paternity and maternity); PP1 – pathogenic supporting (cosegregation with disease in multiple affected family members); PP2 – pathogenic supporting (missense variant in a gene with a low rate of benign missense variation); PP4 – pathogenic supporting (patient's phenotype or family history is highly specific for a disease with a single genetic etiology); PP5 – pathogenic supporting (reported as pathogenic by a reputable source without available raw evidence); BS1 – benign strong (allele frequency is higher than expected for the disorder); BP4 – benign supporting (computational evidence suggests no functional impact); BP6 – benign supporting (reputable source reports variant as benign); BP7 – benign supporting (synonymous variant for which computational algorithms predict no effect on splicing); 1A – contains a genomic region with established dosage sensitivity; 2A-2E – various degrees of overlap with dosage-sensitive regions, specific gene components, or established clinical syndromes; 2F-2G – assessment based on genomic content and intra-genic involvement within a dosage-sensitive region; 2H – overlap with known benign genomic regions; 3C – quantitative assessment of protein-coding genes within the interval; 4L-4O – inheritance and segregation analysis, including confirmed or unconfirmed de novo status and transmission from affected or unaffected progenitors.

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Supplementary Table S2. Continued

Case ID	Sex	Onset age	Patient phenotype	Gene / locus	Genetic variant	Amino acid substitution	Zygosity / Heteroplasmy	ACMG class	ClinVar associated condition	ACMG evidence	Genetic test
PNM 13	M	2 m	Failure to thrive, hypotonia, polymorphic seizures, myoclonus, infantile spasms, nystagmus, repetitive episodes of vomiting, feeding difficulties, subclinical hypothyroidism, RVH, nephrotic syndrome, bilateral multicystic kidneys, hematological abnormalities, dysmorphism, hyperlactatemia, episodic hypoglycemia, urinary organic acid abnormalities, elevated blood ammonia levels, altered ACP.	*PEX1*	c.2T>C	p.Met1Thr	Compound heterozygous, AR	P	Zellweger Leukodystrophy, Peroxisome Biogenesis Disorder 1A	PM3, PS1, PVS1, PM2, PP5	WGS
					c.1810G>A	p.Gly604Arg		VUS	Zellweger Spectrum Disorders	PM2, PP3	
PNM 14	M	3 m	Exercise-induced fatigue, developmental delay, brainstem involvement, drug-resistant seizures, myoclonus, stereotypies, growth delay, chronic pancreatitis, dysmorphism, MRI: white matter signal alterations.	*GABRA1*	c.-442dupG	–	Heterozygous, AD	VUS	Juvenile myoclonic epilepsy	BS1, BP4	WGS
PNM 15	F	10 m	Hypotonia, motor dysfunction, optic nerve atrophy, hyperopia, strabismus, speech delay, cognitive impairment, dysmorphism.	*RALA*	c.68G>C	p.Gly23Ala	Heterozygous, AD	LP	Hiatt-Neu-Cooper Neurodevelopmental Syndrome	PS4, PM2, PP3, PP5	WES
PNM 16	F	1.5 y	Neurodevelopmental delay, hypotonia, febrile seizures, polymorphic seizures, precocious puberty, mild cognitive impairment, positive family history, dysmorphism.	*NSD1*	c.5622+1 G>A	–	Heterozygous, AD	P	Sotos syndrome	PVS1, PS2, PM2, PP5	WGS

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Supplementary Table S2. Continued

Case ID	Sex	Onset age	Patient phenotype	Gene / locus	Genetic variant	Amino acid substitution	Zygosity / Heteroplasmy	ACMG class	ClinVar associated condition	ACMG evidence	Genetic test
PNM 17	F	4 m	Cerebellar ataxia, hypotonia, developmental delay, optic nerve atrophy, hydronephrosis, RVH, peripheral neuropathy, cognitive impairment, dysmorphism, hyperlactatemia.	*SOX11*	c.685G>T	p.Asp229Tyr	Heterozygous, AD	VUS	Coffin-Siris syndrome	PM2	WES
PNM 18	F	3 m	Exercise-induced fatigue, hypotonia, developmental delay, spasticity, tonic-clonic seizures, loss of acquired milestones, flaccid cerebral palsy, hyperkinesia, joint contractures, ataxia, gastroesophageal reflux, dysmorphism, hyperlactatemia, MRI: leukodystrophic lesions.	*GBA1*	c.1448T>C	p.Leu483Pro	Homozygous, AR	P	Gaucher disease	PM3, PS3, PM1, PP2, PM2, PM5, PP3, PP5	WES
PNM 19	M	2 y	Speech and cognitive delay, dysarthria, tonic-clonic seizures, myoclonus, repetitive episodes of vomiting, abdominal pain, stereotypies, hyperactivity, autism spectrum disorder, sleep disturbances, urinary organic acid abnormalities, MRI: white matter signal alterations.	*CACNA1A*	c.4570G>A	p.Glu1524Lys	Heterozygous, AD	LP	Developmental and epileptic encephalopathy	PM2, PM1, PP2, PP3	WGS

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Case ID	Sex	Onset age	Patient phenotype	Gene / locus	Genetic variant	Amino acid substitution	Zygosity / Heteroplasmy	ACMG class	ClinVar associated condition	ACMG evidence	Genetic test
PNM 20	F	1 m	Impaired exercise capacity, motor developmental delay, cardiac conduction abnormalities, congenital partial palpebral ptosis, growth delay, peripheral neuropathy, cognitive impairment, dysmorphism, elevated ketone bodies, episodic hypoglycemia.	*SCN10A*	c.4777A>T	p.Ile1593Phe	Heterozygous, AD	VUS	Brugada syndrome	PM2, PP3	WGS
PNM 21	M	At birth	Significant global developmental impairment, impaired exercise capacity, hypotonia, ataxia, hyperkinesia, startle-type seizures, cognitive impairment, congenital heart defects, positive family history, dysmorphism.	*4p16.3*	–	–	–	P	Wolf-Hirschhorn syndrome	1A, 2A-2E, 2H, 3C, 4L-4O	MLPA
PNM 21`	F	At birth	Facies myopathica, impaired exercise capacity, ataxia, brainstem involvement, startle-type seizures, severe developmental delay and cognitive impairment, hypotonia, strabismus, hematological abnormalities, recurrent vomiting, dysmorphism, positive family history, hyperlactatemia, MRI: diffuse brain volume loss.	*4p16.3*	–	–	–	P	Wolf-Hirschhorn syndrome	1A, 2A-2E, 2H, 3C, 4L-4O	MLPA

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ACMG Evidence Criteria: PVS1 – pathogenic very strong (null variant in a gene where loss-of-function is a known disease mechanism); PS1 – pathogenic strong (same amino acid change as a known pathogenic variant); PS2 – pathogenic strong (confirmed de novo occurrence); PS3 – pathogenic strong (well-established functional studies supportive of a damaging effect on the gene product); PS4 – pathogenic strong (significant increase in variant prevalence in cases vs. controls); PM1 – pathogenic moderate (located in a mutational hotspot or critical functional domain); PM2 – pathogenic moderate (extremely low frequency in gnomAD population databases); PM3 – pathogenic moderate (detected in trans with a pathogenic variant); PM5 – pathogenic moderate (different amino acid change at the same residue as a known pathogenic variant); PM6 – pathogenic moderate (assumed de novo occurrence without confirmation of paternity and maternity); PP1 – pathogenic supporting (cosegregation with disease in multiple affected family members); PP2 – pathogenic supporting (missense variant in a gene with a low rate of benign missense variation); PP4 – pathogenic supporting (patient's phenotype or family history is highly specific for a disease with a single genetic etiology); PP5 – pathogenic supporting (reported as pathogenic by a reputable source without available raw evidence); BS1 – benign strong (allele frequency is higher than expected for the disorder); BP4 – benign supporting (computational evidence suggests no functional impact); BP6 – benign supporting (reputable source reports variant as benign); BP7 – benign supporting (synonymous variant for which computational algorithms predict no effect on splicing); 1A – contains a genomic region with established dosage sensitivity; 2A-2E – various degrees of overlap with dosage-sensitive regions, specific gene components, or established clinical syndromes; 2F-2G – assessment based on genomic content and intra-genic involvement within a dosage-sensitive region; 2H – overlap with known benign genomic regions; 3C – quantitative assessment of protein-coding genes within the interval; 4L-4O – inheritance and segregation analysis, including confirmed or unconfirmed de novo status and transmission from affected or unaffected progenitors.

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Case ID	Sex	Onset age	Patient phenotype	Gene / locus	Genetic variant	Amino acid substitution	Zygosity / Heteroplasmy	ACMG class	ClinVar associated condition	ACMG evidence	Genetic test
PNM 22	M	4 m	Exercise-induced fatigue, hypotonia, delayed psychomotor development, spastic cerebral palsy, brainstem involvement, tonic-clonic seizures, hyperkinesia, cerebellar ataxia, chronic pancreatitis, positive family history, hyperlactatemia, urinary organic acid abnormalities, altered ACP.	*TCAP*	c.88C>G	p.Ser31Cys	Homozygous, AR	VUS	Limb-Girdle Muscular Dystrophy Type 2G	PM2	NGS panel
PNM 23	M	6 m	Severe delay in psychomotor development, ataxia, pyramidal tract signs, RVH, tonic-clonic seizures, optic neuropathy, cardiac conduction defects, peripheral neuropathy, metabolic acidosis.	*SCN2A*	c.3967A>G	p.Met1323Val	Heterozygous, AD	P	Developmental and epileptic encephalopathy, 11	PS4, PM1, PP2, PM2, PP3, PP5	WES
PNM 24	M	6 m	Severe developmental delay, extrapyramidal signs, dysarthria, tonic-clonic seizures, ataxia, impaired exercise capacity, severe neonatal hepatic failure, subclinical hypothyroidism, cognitive impairment, dysmorphism, altered ACP, elevated plasma alanine.	*15q11–q13 Imprinting defect*	–	–	–	P	Angelman syndrome	1A, 2A-2E, 2H, 3A, 4L-4O	MLPA

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ACMG Evidence Criteria: PVS1 – pathogenic very strong (null variant in a gene where loss-of-function is a known disease mechanism); PS1 – pathogenic strong (same amino acid change as a known pathogenic variant); PS2 – pathogenic strong (confirmed de novo occurrence); PS3 – pathogenic strong (well-established functional studies supportive of a damaging effect on the gene product); PS4 – pathogenic strong (significant increase in variant prevalence in cases vs. controls); PM1 – pathogenic moderate (located in a mutational hotspot or critical functional domain); PM2 – pathogenic moderate (extremely low frequency in gnomAD population databases); PM3 – pathogenic moderate (detected in trans with a pathogenic variant); PM5 – pathogenic moderate (different amino acid change at the same residue as a known pathogenic variant); PM6 – pathogenic moderate (assumed de novo occurrence without confirmation of paternity and maternity); PP1 – pathogenic supporting (cosegregation with disease in multiple affected family members); PP2 – pathogenic supporting (missense variant in a gene with a low rate of benign missense variation); PP4 – pathogenic supporting (patient's phenotype or family history is highly specific for a disease with a single genetic etiology); PP5 – pathogenic supporting (reported as pathogenic by a reputable source without available raw evidence); BS1 – benign strong (allele frequency is higher than expected for the disorder); BP4 – benign supporting (computational evidence suggests no functional impact); BP6 – benign supporting (reputable source reports variant as benign); BP7 – benign supporting (synonymous variant for which computational algorithms predict no effect on splicing); 1A – contains a genomic region with established dosage sensitivity; 2A-2E – various degrees of overlap with dosage-sensitive regions, specific gene components, or established clinical syndromes; 2F-2G – assessment based on genomic content and intra-genic involvement within a dosage-sensitive region; 2H – overlap with known benign genomic regions; 3C – quantitative assessment of protein-coding genes within the interval; 4L-4O – inheritance and segregation analysis, including confirmed or unconfirmed de novo status and transmission from affected or unaffected progenitors.

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Case ID	Sex	Onset age	Patient phenotype	Gene / locus	Genetic variant	Amino acid substitution	Zygosity / Heteroplasmy	ACMG class	ClinVar associated condition	ACMG evidence	Genetic test
PNM 25	F	3 m	Failure to thrive, impaired exercise capacity, hypotonia, tonic-clonic seizures, stereotypies, repetitive episodes of vomiting, peripheral neuropathy, dysmorphism, cognitive impairment, episodic hypoglycemia.	*9p13.1p12*	–	–	–	P	Microdeletion of 9p13.1–p12	1A, 2F-2G, 3A	aCGH
PNM 26	M	At birth	Spastic cerebral palsy, developmental delay, impaired exercise capacity, tonic-clonic seizures, strabismus, optic nerve atrophy, cognitive impairment, hydrocephalus, altered ACP.	*RP1L1*	c.591C>A	p.Tyr197Ter	Heterozygous, AD	P	Occult macular dystrophy	PVS1, PM2	WES
PNM 27	M	8 m	Delay in physical and mental development, impaired exercise capacity, pyramidal tract signs, tonic-clonic seizures, strabismus, optic nerve atrophy, repetitive episodes of vomiting, feeding difficulties, hyperlactatemia, elevated plasma alanine, episodic hypoglycemia.	*GALT*	c.563A>G	p.Gln188Arg	Homozygous, AR	P	Galactosemia	PM3, PM1, PP2, PM2, PP3, PP5	Targeted Sanger seq.
PNM 28	F	6 m	Impaired exercise capacity, severe psychomotor delay, hypotonia, cerebellar ataxia, drug-resistant seizures, myoclonus, hyperkinesia, stereotypies, bruxism, strabismus, repetitive episodes of vomiting, cognitive impairment, elevated blood ammonia levels, MRI: white matter signal alterations.	*MECP2*	c.806del	p.Ala269ValfsTer32	Heterozygous, X-linked dominant	P	Rett syndrome	PVS1, PM2	WGS

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ACMG Evidence Criteria: PVS1 – pathogenic very strong (null variant in a gene where loss-of-function is a known disease mechanism); PS1 – pathogenic strong (same amino acid change as a known pathogenic variant); PS2 – pathogenic strong (confirmed de novo occurrence); PS3 – pathogenic strong (well-established functional studies supportive of a damaging effect on the gene product); PS4 – pathogenic strong (significant increase in variant prevalence in cases vs. controls); PM1 – pathogenic moderate (located in a mutational hotspot or critical functional domain); PM2 – pathogenic moderate (extremely low frequency in gnomAD population databases); PM3 – pathogenic moderate (detected in trans with a pathogenic variant); PM5 – pathogenic moderate (different amino acid change at the same residue as a known pathogenic variant); PM6 – pathogenic moderate (assumed de novo occurrence without confirmation of paternity and maternity); PP1 – pathogenic supporting (cosegregation with disease in multiple affected family members); PP2 – pathogenic supporting (missense variant in a gene with a low rate of benign missense variation); PP4 – pathogenic supporting (patient's phenotype or family history is highly specific for a disease with a single genetic etiology); PP5 – pathogenic supporting (reported as pathogenic by a reputable source without available raw evidence); BS1 – benign strong (allele frequency is higher than expected for the disorder); BP4 – benign supporting (computational evidence suggests no functional impact); BP6 – benign supporting (reputable source reports variant as benign); BP7 – benign supporting (synonymous variant for which computational algorithms predict no effect on splicing); 1A – contains a genomic region with established dosage sensitivity; 2A-2E – various degrees of overlap with dosage-sensitive regions, specific gene components, or established clinical syndromes; 2F-2G – assessment based on genomic content and intra-genic involvement within a dosage-sensitive region; 2H – overlap with known benign genomic regions; 3C – quantitative assessment of protein-coding genes within the interval; 4L-4O – inheritance and segregation analysis, including confirmed or unconfirmed de novo status and transmission from affected or unaffected progenitors.

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PNM 29	M	6 m	Severe developmental delay, brainstem involvement, febrile seizures, polymorphic seizures, RVH, liver dysfunction, cognitive impairment, dysmorphism, MRI: hippocampal volume loss.	*PSAT1*	c.777A>T	p.Lys259Asn	Homozygous, AR	VUS	Phosphoserine aminotransferase deficiency	PM2	NGS panel
PNM 30	M	2 m	Spastic cerebral palsy, psychomotor impairment, tonic-clonic seizures, impaired exercise capacity, cognitive impairment, feeding difficulties, dysmorphic features, MRI: altered cortical folding pattern.	*MACF1*	c.12767 G>A	p.Arg4256Gln	Heterozygous, AD	VUS	Lissencephaly 9 with complex brainstem malformation	PM2, PP2	WES
PNM 31	M	9 m	Hypotonia, developmental delay, drug-resistant seizures, myoclonus, infantile spasms, peripheral neuropathy, cognitive impairment, MRI: diffuse brain volume loss.	*PAH*	c.1222C>T	p.Arg408Trp	Homozygous, AR	P	Phenylketonuria	PS3, PM3, PP4, PM1, PP2, PM2, PM5, PP3, PP5	Targeted Sanger seq.
PNM 32	M	4 y	Impaired exercise capacity, hypotonia, developmental delay, tonic-clonic seizures, strabismus, optic nerve atrophy, feeding difficulties, cognitive impairment, hyperlactatemia, urinary organic acid abnormalities, elevated blood ammonia levels, MRI: leukodystrophic lesions.	*GK*	c.1A>G	–	Hemizygous, X-linked recessive	VUS	Inborn glycerol kinase deficiency	PM2, PVS1	WES
				NARS2	c.392A>G	p.Lys131Arg	Homozygous, AR	VUS	Combined oxidative phosphorylation defect type 24	PM2	

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Supplementary Table S2. Continued

Case ID	Sex	Onset age	Patient phenotype	Gene / locus	Genetic variant	Amino acid substitution	Zygosity / Heteroplasmy	ACMG class	ClinVar associated condition	ACMG evidence	Genetic test
PNM 33	F	1 m	Neurodevelopmental and growth delay, impaired exercise capacity, hypotonia, absence seizures, myoclonus, hematological abnormalities, feeding difficulties, positive family history, elevated ketone bodies, hyperlactatemia.	*ANO5*	c.-162A>G	–	Homozygous, AR	VUS	Limb-Girdle Muscular Dystrophy	PM2, BP7	NGS panel
PNM 34	M	3 y	Impaired exercise capacity, hypotonia, developmental delay, loss of acquired milestones, brainstem involvement, strabismus, peripheral neuropathy, hyperlactatemia.	*DARS2*	c.492+2 T>C	–	Homozygous, AR	P	Leuko-encephalopathy with brain stem and spinal cord involvement-high lactate syndrome	PM3, PVS1, PM2, PP5	WGS
PNM 35	M	1 y	Neurodevelopmental delay, hypotonia, growth retardation, subclinical hypothyroidism, ADHD, autism spectrum disorder, elevated plasma alanine, elevated ketone bodies, MRI: encephalomalacia.	*TRIO*	c.6852del insACCA	p.Ser2284del insArgPro	Heterozygous, AD	VUS	Intellectual developmental disorder, autosomal dominant 63, with macrocephaly	PVS1, PM2	WGS
PNM 36	M	1 y	Exercise-induced fatigue, hypotonia, pyramidal tract signs, developmental delay, loss of acquired milestones, delayed psychomotor development, primary hypothyroidism, cognitive impairment, MRI: white matter signal alterations.	*NPC1*	c.2039dup	p.Leu680 PhefsTer9	Homozygous, AR	P	Niemann-Pick Disease	PVS1, PM2, PP5	WGS

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SUPPLEMENTARY TABLES
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Supplementary Table S2. Continued

Case ID	Sex	Onset age	Patient phenotype	Gene / locus	Genetic variant	Amino acid substitution	Zygosity / Heteroplasmy	ACMG class	ClinVar associated condition	ACMG evidence	Genetic test
PNM 37	M	1 y	Hypotonia, developmental delay, febrile seizures, repetitive episodes of vomiting, hepatomegaly, cognitive impairment, stereotypies, elevated blood ammonia levels, hyperlactatemia.	*G6PD*	c.710T>C	p.Phe237Ser	Hemizygous, X-linked recessive	LP	Anemia, nonspherocytic hemolytic, due to G6PD deficiency	PM1, PP2, PM2, PP3	WGS
				CFTR	c.1392G>T	p.Lys464Asn	Heterozygous, AD	P	Hereditary Chronic Pancreatitis	PS4, PM1, PP2, PM5, PP3, PP5	
PNM 38	F	10 m	Dystonia, hypotonia, motor developmental delay, absence seizures, impaired exercise capacity, cognitive impairment, autism spectrum disorder, positive family history.	*GRIK2*	c.965T>C	p.Leu322Pro	Heterozygous, AD	VUS	Neuro-developmental disorder with impaired language and ataxia and with or without seizures	PM2, PP3	WGS
PNM 39	F	8 m	Generalized hypotonia, severe psychomotor delay, hyporeflexia, polymorphic seizures, nystagmus, cognitive impairment, optic nerve atrophy, microcephaly, elevated blood ammonia levels.	*SCN8A*	c.1779G>A	p.Glu593Asp	Heterozygous, AD	VUS	Developmental and Epileptic Encephalopathy, 13	PM2	WGS
PNM 40	M	4 m	Generalized hypotonia, delayed motor and somatic development, hypospadias, sleep disturbances, stereotypies, dysmorphism.	*TCF4*	c.1739G>A	p.Arg580Gln	Heterozygous, AD	P	Pitt-Hopkins Syndrome	PP4, PS3, PS4, PM6, PM1, PP2, PM2, PM5, PP3, PP5	WGS

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Case ID	Sex	Onset age	Patient phenotype	Gene / locus	Genetic variant	Amino acid substitution	Zygosity / Heteroplasmy	ACMG class	ClinVar associated condition	ACMG evidence	Genetic test
PNM 41	M	15 y	Exercise-induced fatigue, brainstem involvement, growth retardation, hepatosplenomegaly, hepatic cirrhosis, gastroesophageal reflux, reactive pancreatitis, positive family history, metabolic acidosis, MRI: basal ganglia signal abnormalities.	*ATP7B*	c.3207C>A	p.His1069Gln	Compound heterozygous, AR	P	Wilson Disease	PM3, PP1, PS3, PM1, PP2, PM2, PM5, PP3, PP5	WES
					c.2183A>G	p.Asn728Ser				PM3, PM1, PP2, PM2, PM5, PP3, PP5	
PNM 42	F	1 m	Developmental delay, hypotonia, polymorphic seizures, infantile spasms, SNHL, cognitive impairment, hyperlactatemia, MRI: vermian hypoplasia.	*CPA6*	c.619C>G	p.Gln207Glu	Compound heterozygous, AR	VUS	Familial Temporal Lobe Epilepsy 5)	PM2, BP6	WGS
					c.799G>A	p.Gly267Arg				PM2, PP3, BP6	

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