Supplemental Data Table S5. Mutation classification, diagnosis, and turnaround time in 37 control samples

ID	Genes	NT alteration	AA alteration	Zygosity	NewbornSeq	Conventional classification	ACMG classification	Causality	TAT using Sanger sequencing (days)	Diagnosis
PC1	*ACADS*	c.1031A>G	p.E344G	Hom	Detected	EP	LP	C	52	SCAD deficiency
	PCCA	c.1598G>T	p.W533L	Het	Detected	KP	LP	I		
	CYP21A2	c.293-13C>G	NA	Het	Detected	KP	LP	I		
	PDHA1	c.751A>C	p.M251L	Het	Detected	KP	LP	I		
PC2	*ASS1*	c.892G>A	p.E298K	ComHet	Detected	KP	LP	C	31	CTLN1
		c.689G>C	p.G230A		Detected	KP	LP	C		
PC3	*PAH*	c.442-1G>A	NA	Hom	Detected	KP	P	C	19	PKU
	PCCA	c.1598G>T	p.W533L	Het	Detected	KP	LP	I		
	PCCB	c.1217G>T	p.G406V	Het	Detected	EP	VUS	I		
	PDHA1	c.751A>C	p.M251L	Hom	Detected	KP	LP	I		
PC4	*PAH*	c.1256A>G	p.Q419R	ComHet	Detected	KP	LP	C	14	PKU
		c.1268T>C	p.V423A		Detected	EP	VUS	C		
	CBS	c.1552T>C	p.Y518H	Het	Detected	EP	VUS	I		
	ASS1	c.970G>A	p.G324S	Het	Detected	KP	LP	I		
PC5	*PAH*	c.833C>T	p.T278I	ComHet	Detected	KP	LP	C	24	PKU
		c.491T>C	p.I164T		Detected	KP	LP	C		
	CPS1	c.1507G>T	p.V503L	Het	Detected	EP	VUS	I		
	PDHA1	c.751A>C	p.M251L	Het	Detected	KP	LP	I		
PC6	*GLDC*	c.2258A>C	p.H753P	ComHet	Detected	KP	LP	C	11	NKH
		c.2285G>T	p.G762V		Detected	EP	VUS	C		
PC7	*PAH*	c.728G>A	p.R243Q	ComHet	Detected	EP	VUS	C	11	PKU
		c.975C>G	p.Y325*		Detected	EP	VUS	C		
	DUOXA2	c.738C>T	p.Y246Y	Het	Detected	KP	LP	I		
PC8	*MCCC2*	c.313G>C	p.G105R	ComHet	Detected	EP	VUS	C	136	3-MCC deficiency
		c.1252A>T	p.I418F		Detected	EP	VUS	C		
	SLC25A15	c.292T>A	p.L98M	Het	Detected	EP	VUS	I		
	TPO	c.1475G>A	p.R492Q	Het	Detected	KP	LP	I		
PC9	*GALE*	c.207C>A	p.D69E	ComHet	Detected	KP	LP	C	15	Galactosemia
		c.505C>T	p.R169W		Detected	KP	LP	C		
	SLC7A7	c.422A>G	p.N141S	Het	Detected	EP	VUS	I		
	PDHA1	c.751A>C	p.M251L	Het	Detected	KP	LP	I		
PC10	*MCCC2*	c.838G>T	p.D280Y	Hom	Detected	KP	LP	C	21	3-MCC deficiency
	PCCA	c.1676G>T	p.W533L	Het	Detected	KP	LP	I		
	DUOXA2	c.738C>G	p.Y246Y	Het	Detected	KP	LP	I		
PC11	*PAH*	c.728G>A	p.R243Q	ComHet	Detected	EP	VUS	C	29	PKU
		c.1238G>C	p.R413P		Detected	KP	LP	C		
	PDHA1	c.751A>C	p.M251L	Het	Detected	KP	LP	I		

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Supplemental Data Table S5. Continued

ID	Genes	NT alteration	AA alteration	Zygosity	NewbornSeq	Conventional classification	ACMG classification	Causality	TAT using Sanger sequencing (days)	Diagnosis
PC12	*PAH*	c.442-1G>A	NA	ComHet	Detected	KP	P	C	19	PKU
		c.1197A>T	p.V399V		Detected	KP	LP	C		
	ACADM	c.697T>A	p.X233K	Het	Detected	EP	LP	I		
	DBT	c.916T>C	p.S306P	Het	Detected	EP	VUS	I		
	IVD	c.751A>C	p.S251R	Het	Detected	EP	VUS	I		
PC13	*MUT*	c.1481T>A	p.L494X	ComHet	Detected	KP	P	C	6	MMA
		c.322C>T	p.R108C		Detected	KP	LP	C		
	IVD	c.1054T>G	p.F352V	Het	Detected	KP	LP	I		
	CPS1	c.2759G>A	p.R920Q	Het	Detected	EP	VUS	I		
PC14	*PAH*	c.721C>T	p.R241C	ComHet	Detected	KP	LP	C	14	PKU
		c.1162G>A	p.V388M		Detected	EP	VUS	C		
	DUOXA2	c.738C>G	p.Y246Y	Het	Detected	KP	LP	I		
PC15	*ASS1*	c.421-2A>G	NA	ComHet	Detected	KP	P	C	16	CTLN1
		c.970G>A	p.G324S		Detected	KP	LP	C		
PC16	*ASS1*	c.421-2A>G	NA	Hom	Detected	KP	P	C	15	CTLN1
	PAH	c.158G>A	p.R53H	Het	Detected	KP	LP	I		
PC17	*MUT*	c.1105C>T	p.R369C	ComHet	Detected	KP	LP	C	13	MMA
		c.1481T>A	p.L494X		Detected	KP	P	C		
	GALK1	c.593C>T	p.A198V	Het	Detected	KP	LP	I		
PC18	*PAH*	c.158G>A	p.R53H	ComHet	Detected	KP	LP	C	3	PKU
		c.1238G>C	p.R413P		Detected	KP	LP	C		
	SLC7A7	c.498T>G	p.I166M	Hom	Detected	EP	VUS	I		
PC19	*PAH*	c.611A>G	p.Y204C	Hom	Detected	KP	LP	C	19	PKU
PC20	*PAH*	c.721C>T	p.R241C	ComHet	Detected	KP	LP	C	17	PKU
		c.775G>A	p.A259T		Detected	KP	LP	C		
	GALK1	c.593C>T	p.A198V	Het	Detected	KP	LP	I		
PC21	*PAH*	c.442-1G>A	NA	ComHet	Detected	KP	P	C	27	PKU
		c.1066-3C>T	NA		Detected	KP	LP	C		
	DUOX2	c.1462G>A	p.G488R	Het		KP	P	I		
PC22	*PAH*	c.721C>T	p.R241C	ComHet	Detected	KP	LP	C	12	PKU
		c.728G>A	p.R243Q		Detected	EP	VUS	C		
	PCCA	c.1598G>T	p.W533L	Het	Detected	KP	LP	I		
	PDHA1	c.751A>C	p.M251L	Het	Detected	KP	LP	I		
PC23	*PAH*	c.320A>G	p.H107R	ComHet	Detected	KP	LP	C	26	PKU
		c.1068C>A	p.Y356*		Detected	EP	LP	C		
	GALK1	c.593C>T	p.A198V	Het	Detected	KP	LP	C		
	CPT2	c.1559C>T	p.P520L	Het	Detected	KP	VUS	I		

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Supplemental Data Table S5. Continued

ID	Genes	NT alteration	AA alteration	Zygosity	NewbornSeq	Conventional classification	ACMG classification	Causality	TAT using Sanger sequencing (days)	Diagnosis
PC24	*PAH*	c.728G>A	p.R243Q	ComHet	Detected	KP	VUS	C	21	PKU
		c.1197A>T	p.V399V		Detected	KP	LP	C		
	PDHA1	c.751A>C	p.M251L	Het	Detected	KP	LP	I		
	MLYCD	c.1247T>A	p.L416Q	Het	Detected	EP	VUS	I		
PC25	*PAH*	c.442-1G>A	NA	ComHet	Detected	KP	P	C	22	PKU
		c.611A>G	p.Y204C		Detected	KP	LP	C		
	AHCY	c.758C>A	p.A253D	Het	Detected	EP	VUS	I		
	HLCS	c.710T>G	p.L237R	Het	Detected	EP	VUS	I		
PC26	*ASS1*	c.910C>T	p.R304W	ComHet	Detected	KP	LP	C	21	CTLN1
		c.970G>A	p.G324S		Detected	KP	LP	C		
	DUOX2	c.T4541C	p.V1514A	Het	Detected	EP	VUS	I		
	GCSH	c.C203G	p.T68R	Het	Detected	EP	VUS	I		
	BCKDHA	c.C735A	p.D245E	Het	Detected	EP	VUS	I		
	HLCS	c.T710G	p.L237R	Het	Detected	EP	VUS	I		
PC27	*GALT*	c.286-299del	NA	ComHet	Detected	EP	LP	C	NA	GAL
		c.378-1G>C	NA		Detected	EP	LP	C	NA	
NC1	*ACADSB*	c.848A>G	p.Y283C	Het	Detected	EP	LP	I	NA	NA
NC2	*DUOXA2*	c.413dupA	p.Y138X	Het	Detected	KP	LP	I	NA	NA
NC3	*GALK1*	c.593C>T	p.A198V	Het	Detected	KP	VUS	I	NA	NA
NC4	*CPS1*	c.1147C>A	p.P383T	Het	Detected	EP	VUS	I	NA	NA
NC5	*ETFA*	:c.478C>T	p.R160X	Het	Detected	EP	P	I	NA	NA
NC6	*MTR*	c.3339delC	p.R1113fs	Het	Detected	EP	LP	I	NA	NA
	PCCA	c.1598G>T	p.W533L	Het	Detected	EP	VUS	I		
	PRODH	c.998T>C	p.L333P	Het	Detected	KP	VUS	I		
NC7	*HAL*	c.203C>T	p.P68L	Het	Detected	EP	LP	I	NA	NA
NC8	*ND*	ND	ND	ND	ND	ND	ND	ND	ND	ND
NC9	*ALDH4A1*	c.1387dupG	p.A463fs	Het	Detected	EP	LP	I	NA	NA
	PCCB	c.1304A>G	p.Y435C	Het	Detected	KP	LP	I		
NC10	*ND*	ND	ND	ND	ND	ND	ND	ND	ND	ND

Reference sequences of *ACADS*, *PCCA*, *CYP21A2*, *PDHA1*, *ASS1*, *PAH*, *PCCB*, *CBS*, *CPS1*, *GLDC*, *DUOXA2*, *MCCC2*, *SLC25A15*, *TPO*, *GALE*, *SLC7A7*, *ACADM*, *DBT*, *IVD*, *MUT*, *GALK1*, *DUOX2*, *CPT2*, *MLYCD*, *AHCY*, *HLCS*, *GCSH*, *BCKDHA*, and *GALT* were NM_000017. NM_001127692, NM_000500.7, NM_001173456, NM_054012, NM_000277, NM_001127692, NM_000071, NM_001122634, NM_000170, NM_207581, NM_022132, NM_014252, NM_175722, NM_001008216, NM_001126105, NM_001286044, NM_001918, NM_001159508, NM_000255, NM_000154, NM_014080, NM_000098, NM_012213, NM_000687, NM_000411, NM_004483, NM_000709, and NM_000155, respectively.

Abbreviations: KP, known pathogenic based on the Human Genome Mutation Database (DM) or ClinVar (pathogenic) databases; EP, expected pathogenic; LP, likely pathogenic; VUS, variant of unknown significance; NA, not applicable; NT, nucleotide; AA, amino acid; Het, heterozygous; ComHet, compound heterozygous; Hom, homozygous; P, Pathogenic; TAT, turnaround time; ND, Not detected; I, incidental mutations; C, causal mutations.