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ORIGINAL ARTICLE

An Integrated Solution for Application of Next-Generation Sequencing in Newborn Screening

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SUMMARY

Background: **Next-generation sequencing (NGS) has greatly improved the diagnostic process for hereditary diseases, and incorporation of NGS into newborn screening (NBS) programs for more actionable diseases has been widely discussed. The aim of this study was to evaluate an integrated solution for application of NGS in newborn screening.**
Methods: **An NGS panel targeting 155 genes related to inborn errors of metabolism, hearing loss, severe combined immunodeficiency, congenital hypothyroidism, and other actionable genetic diseases, was designed. An all-in-one library preparation strategy was developed to combine multiplex PCR target enrichment and sample barcoding. A clinical genetic analysis system was assembled to facilitate bioinformatics analysis and reporting. The integrated solution was validated using 160 samples with known variants.**
Results: **The end-to-end time from DNA isolation to sequencing was approximately 34 hours, and bioinformatics analysis pipeline took 4 hours for 160 samples in parallel. This allowed reporting of results on day 3. All known variants were confirmed by the NGS workflow, and two large insertion/deletions were additionally detected in two cases with previously clinically but not genetically confirmed diseases.**
Conclusions: **The integrated solution for application of NGS in NBS provided reasonable turnaround time to meet the NBS timeframe and could be implemented at scale.**
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Supplementary Data

Table S1. The list of 155 genes and related disorders covered by the NGS panel.

Category	Gene/Locus	Gene/Locus MIM number	Phenotype	Phenotype MIM number	Inheritance
IEMs covered by MS/MS	*PAH*	612349	Phenylketonuria	261600	AR
	PTS	612719	Hyperphenylalaninemia, BH4-deficient, A	261640	AR
	GCH1	600225	Hyperphenylalaninemia, BH4-deficient, B	233910	AR
	QDPR	612676	Hyperphenylalaninemia, BH4-deficient, C	261630	AR
	PCBD1	126090	Hyperphenylalaninemia, BH4-deficient, D	264070	AR
	DNAJC12	606060	Hyperphenylalaninemia, mild, non-BH4-deficient	617384	AR
	FAH	613871	Tyrosinemia, type I	276700	AR
	TAT	613018	Tyrosinemia, type II	276600	AR
	HPD	609695	Tyrosinemia, type III	276710	AR
	BCKDHA	608348	Maple syrup urine disease, type Ia	248600	AR
	BCKDHB	248611	Maple syrup urine disease, type Ib	248600	AR
	DBT	248610	Maple syrup urine disease, type II	248600	AR
	DLD	238331	Dihydrolipoamide dehydrogenase deficiency/Maple syrup urine disease, type III	246900	AR
	ASS1	603470	Citrullinemia, type I	215700	AR
	SLC25A13	603859	Citrullinemia, type II, neonatal-onset	605814	AR
	ARG1	608313	Argininemia	207800	AR
	GLDC	238300	Glycine encephalopathy 1/Nonketotic hyperglycinemia	605899	AR
	ASL	608310	Argininosuccinic aciduria	207900	AR
	OTC	300461	Ornithine transcarbamylase deficiency	311250	XL
	NAGS	608300	N-acetylglutamate synthase deficiency	237310	AR
	OAT	613349	Gyrate atrophy of choroid and retina with or without ornithinemia	258870	AR
	SLC25A15	603861	Hyperornithinemia-hyperammonemia-homocitrullinemia syndrome	238970	AR
	CBS	613381	Homocystinuria due to cystathionine beta-synthase deficiency	236200	AR
	MTHFR	607093	Homocystinuria due to methylenetetrahydrofolate reductase deficiency	236250	AR
	MTR	156570	Homocystinuria-megaloblastic anemia, cblG complementation type	250940	AR

Table S1. The list of 155 genes and related disorders covered by the NGS panel (continued).

Category	Gene/Locus	Gene/Locus MIM number	Phenotype	Phenotype MIM number	Inheritance
IEMs covered by MS/MS	*MTRR*	602568	Homocystinuria-megaloblastic anemia, cbl E type	236270	AR
	MAT1A	610550	Methionine adenosyltransferase (MAT) I/III deficiency	250850	AD, AR
	GNMT	606628	Glycine N-methyltransferase deficiency	606664	AR
	AHCY	180960	Hypermethioninemia with deficiency of S-adenosylhomocysteine hydrolase	613752	AR
	PRODH	606810	Hyperprolinemia, type I	239500	AR
	ALDH4A1	606811	Hyperprolinemia, type II	239510	AR
	CPS1	608307	Carbamoylphosphate synthetase I deficiency	237300	AR
	SPR	182125	Dystonia, dopa-responsive, due to sepiapterin reductase deficiency	612716	AD, AR
	MMACHC	609831	Methylmalonic aciduria and homocystinuria, cblC type	277400	AR
	MMADHC	611935	Methylmalonic aciduria and homocystinuria, cblD type	277410	AR
	LMBRD1	612625	Methylmalonic aciduria and homocystinuria, cblF type	277380	AR
	HCFC1	300019	Methylmalonic aciduria and homocysteinemia, cblX type	309541	XLR
	ABCD4	603214	Methylmalonic aciduria and homocystinuria, cblJ type	614857	AR
	MMUT	609058	Methylmalonic aciduria, mut type	251000	AR
	MMAA	607481	Methylmalonic aciduria, vitamin B12-responsive, cblA type	251100	AR
	MMAB	607568	Methylmalonic aciduria, vitamin B12-responsive, cblB type	251110	AR
	SUCLA2	603921	Mitochondrial DNA depletion syndrome 5 (encephalomyopathic with or without methylmalonic aciduria)	612073	AR
	SUCLG1	611224	Mitochondrial DNA depletion syndrome 9 (encephalomyopathic type with methylmalonic aciduria)	245400	AR
	MCEE	608419	Methylmalonyl-CoA epimerase deficiency	251120	AR
	ACSF3	614245	Combined malonic and methylmalonic aciduria	614265	AR
	CD320	606475	Methylmalonic aciduria, transient, due to transcobalamin receptor defect	613646	AR

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Category	Gene/Locus	Gene/Locus MIM number	Phenotype	Phenotype MIM number	Inheritance
IEMs covered by MS/MS	*ALDH6A1*	603178	Methylmalonate semialdehyde dehydrogenase deficiency	614105	AR
	PCCA	232000	Propionicacidemia	606054	AR
	PCCB	232050	Propionicacidemia	606054	AR
	IVD	607036	Isovaleric acidemia	243500	AR
	GCDH	608801	Glutaricaciduria, type I	231670	AR
	MCCC1	609010	3-Methylcrotonyl-CoA carboxylase 1 deficiency	210200	AR
	MCCC2	609014	3-Methylcrotonyl-CoA carboxylase 2 deficiency	210210	AR
	AUH	600529	3-methylglutaconic aciduria, type I	250950	AR
	TAFAZZIN	300394	Barth syndrome	302060	AR
	HMGCL	613898	3-hydroxy-3-methylglutaryl-CoA lyase deficiency	246450	AR
	HLCS	609018	Holocarboxylase synthetase deficiency	253270	AR
	BTD	609019	Biotinidase deficiency	253260	AR
	ACAT1	607809	Alpha-methylacetoacetic aciduria	203750	AR
	ACADSB	600301	2-Methylbutyryl-CoA dehydrogenase deficiency	610006	AR
	ACAD8	604773	Isobutyryl-CoA dehydrogenase deficiency	611283	AR
	L2HGDH	609584	L-2-hydroxyglutaric aciduria	236792	AR
	ETHE1	608451	Ethylmalonic encephalopathy	602473	AR
	MLYCD	606761	Malonyl-CoA decarboxylase deficiency	248360	AR
	SLC22A5	603377	Carnitine deficiency, systemic primary	212140	AR
	CPT1A	600528	Carnitine palmitoyltransferase I deficiency	255120	AR
	CPT2	600650	Carnitine palmitoyltransferase II deficiency	600649/608836	AR
	SLC25A20	613698	Carnitine-acylcarnitine translocase deficiency	212138	AR
	ACADS	606885	Acyl-CoA dehydrogenase, short-chain, deficiency of	201470	AR
	ACADM	607008	Acyl-CoA dehydrogenase, medium chain, deficiency of	201450	AR
	ACADVL	609575	Acyl-CoA dehydrogenase, very long-chain, deficiency of	201475	AR
	HADHA	600890	Long-chain 3-hydroxyl-CoA dehydrogenase deficiency	609016	AR
	HADHA	600890	Mitochondrial trifunctional protein deficiency 1	609015	AR
	HADHB	143450	Mitochondrial trifunctional protein deficiency 2	620300	AR
	ETFA	608053	Glutaric acidemia IIA	231680	AR
	ETFB	130410	Glutaric acidemia IIB	231680	AR

Table S1. The list of 155 genes and related disorders covered by the NGS panel (continued).

Category	Gene/Locus	Gene/Locus MIM number	Phenotype	Phenotype MIM number	Inheritance
IEMs covered by MS/MS	*ETFDH*	231675	Glutaric acidemia IIC	231680	AR
	HADH	601609	3-hydroxyacyl-CoA dehydrogenase deficiency	231530	AR
	NADK2	615787	2,4-dienoyl-CoA reductase deficiency	616034	AR
Lysosomal storage disorders	*IDUA*	252800	Mucopolysaccharidosis Ih	607014	AR
	IDUA	252800	Mucopolysaccharidosis Ih/s	607015	AR
	IDUA	252800	Mucopolysaccharidosis Is	607016	AR
	IDS	300823	Mucopolysaccharidosis II	309900	XLR
	SGSH	605270	Mucopolysaccharidosis type IIIA (Sanfilippo A)	252900	AR
	NAGLU	609701	Mucopolysaccharidosis type IIIB (Sanfilippo B)	252920	AR
	GALNS	612222	Mucopolysaccharidosis IVA	253000	AR
	GLB1	611458	GM1-gangliosidosis, type I	230500	AR
	GLB1	611458	GM1-gangliosidosis, type II	230600	AR
	GLB1	611458	GM1-gangliosidosis, type III	230650	AR
	GLB1	611458	Mucopolysaccharidosis type IVB (Morquio)	253010	AR
	GUSB	611499	Mucopolysaccharidosis VII	253220	AR
	ARSB	611542	Mucopolysaccharidosis type VI (Maroteaux-Lamy)	253200	AR
	SMPD1	607608	Niemann-Pick disease, type A	257200	AR
	SMPD1	607608	Niemann-Pick disease, type B	607616	AR
	NPC1	607623	Niemann-Pick disease, type C1	257220	AR
	NPC2	601015	Niemann-pick disease, type C2	607625	AR
	GBA	606463	Gaucher disease, type I	230800	AR
	GLA	300644	Fabry disease	301500	XL
	HEXA	606869	Tay-Sachs disease	272800	AR
	GALC	606890	Krabbe disease	245200	AR
	PSAP	176801	Combined SAP deficiency	611721	AR
	PSAP	176801	Gaucher disease, atypical	610539	AR
	PSAP	176801	Krabbe disease, atypical	611722	AR
	PSAP	176801	Metachromatic leukodystrophy due to SAP-b deficiency	249900	AR
	ARSA	607574	Metachromatic leukodystrophy	250100	AR
	GAA	606800	Glycogen storage disease II	232300	AR
	GNPTAB	607840	Mucolipidosis II alpha/beta	252500	AR
	GNPTAB	607840	Mucolipidosis III alpha/beta	252600	AR
Carbohydrate metabolic disorders	*G6PC*	613742	Glycogen storage disease Ia	232200	AR
	SLC37A4	602671	Glycogen storage disease Ib	232220	AR
	SLC37A4	602671	Glycogen storage disease Ic	232240	AR
	AGL	610860	Glycogen storage disease III	232400	AR
	PYGL	613741	Glycogen storage disease VI	232700	AR

Table S1. The list of 155 genes and related disorders covered by the NGS panel (continued).

Category	Gene/Locus	Gene/Locus MIM number	Phenotype	Phenotype MIM number	Inheritance
Carbohydrate metabolic disorders	*PHKA2*	300798	Glycogen storage disease, type IXa	306000	XLR
	GALT	606999	Galactosemia I	230400	AR
	GALK1	604313	Galactosemia II	230200	AR
	GALE	606953	Galactosemia III	230350	AR
	ALDOB	612724	Fructose intolerance, hereditary	229600	AR
	PC	608786	Pyruvate carboxylase deficiency	266150	AR
Treatable epilepsy	*ALDH7A1*	107323	Epilepsy, pyridoxine-dependent	266100	AR
	PNPO	603287	Pyridoxamine 5-prime-phosphate oxidase deficiency	610090	AR
	PHGDH	606879	Neu-Laxova syndrome 1	256520	AR
	PHGDH	606879	Phosphoglycerate dehydrogenase deficiency	601815	AR
	PSAT1	610936	Neu-Laxova syndrome 2	616038	AR
	PSAT1	610936	Phosphoserine aminotransferase deficiency	610992	AR
	SLC2A1	138140	GLUT1 deficiency syndrome 1, infantile onset, severe	606777	AD, AR
	SLC2A1	138140	GLUT1 deficiency syndrome 2, childhood onset	612126	AD
	PSPH	172480	Phosphoserine phosphatase deficiency	614023	AR
	SLC6A8	300036	Cerebral creatine deficiency syndrome 1	300352	XLR
	GAMT	601240	Cerebral creatine deficiency syndrome 2	612736	AR
	GATM	602360	Cerebral creatine deficiency syndrome 3	612718	AR
Hearing loss	*GJB2*	121011	Deafness, autosomal recessive 1A	220290	AR
	GJB3	603324	Deafness, autosomal dominant 2B	612644	AD
	MT-RNR1	561000	Aminoglycoside-induced deafness	-	-
	SLC26A4	605646	Deafness, autosomal recessive 4, with enlarged vestibular aqueduct	600791	AR
	SLC26A4	605646	Pendred syndrome	274600	AR
	OTOF	603681	Deafness, autosomal recessive 9	601071	AR
Severe combined immunodeficiency	*ADA*	608958	Severe combined immunodeficiency due to ADA deficiency	102700	AR
	IL2RG	308380	Severe combined immunodeficiency, X-linked	300400	XLR
	WAS	300392	Neutropenia, severe congenital, X-linked	300299	XLR
	WAS	300392	Thrombocytopenia, X-linked	313900	XLR
	WAS	300392	Wiskott-Aldrich syndrome	301000	XLR
	BTK	300300	Agammaglobulinemia, X-linked	300755	XLR

Table S1. The list of 155 genes and related disorders covered by the NGS panel (continued).

Category	Gene/Locus	Gene/Locus MIM number	Phenotype	Phenotype MIM number	Inheritance
Congenital hypothyroidism	*TSHR*	603372	Hypothyroidism, congenital, nongoitrous 1	275200	AR
	TPO	606765	Thyroid dyshormonogenesis 2A	274500	AR
	TG	188450	Thyroid dyshormonogenesis 3	274700	AR
	DUOX2	606759	Thyroid dyshormonogenesis 6	607200	AR
Hyperbilirubinemia	*G6PD*	305900	G6PD deficiency	300908	XL
	SLC10A1	182396	Hypercholanemia, familial 2	619256	AR
	UGT1A1	191740	Crigler-Najjar syndrome, type I	218800	AR
	UGT1A1	191740	Crigler-Najjar syndrome, type II	606785	AR
	JAG1	601920	Alagille syndrome 1	118450	AD
	ABCC2	601107	Dubin-Johnson syndrome	237500	AR
	ATP8B1	602397	Cholestasis, progressive familial intrahepatic 1	211600	AR
	ABCB11	603201	Cholestasis, progressive familial intrahepatic 2	601847	AR
	ABCB4	171060	Cholestasis, progressive familial intrahepatic 3	602347	AR
	TJP2	607709	Cholestasis, progressive familial intrahepatic 4	615878	AR
Other diseases	*TH*	191290	Segawa syndrome, recessive	605407	AR
	DDC	107930	Aromatic L-amino acid decarboxylase deficiency	608643	AR
	CYP11B1	610613	Adrenal hyperplasia, congenital, due to 11-beta-hydroxylase deficiency	202010	AR
	HSD17B10	300256	HSD10 mitochondrial disease	300438	XLD
	ABCD1	300371	Adrenoleukodystrophy	300100	XLR
	ATP7B	606882	Wilson disease	277900	AR
	ATP7A	300011	Menkes disease	309400	XLR
	LDLR	606945	Hypercholesterolemia, familial, 1	143890	AD, AR
	APOB	107730	Hypercholesterolemia, familial, 2	144010	AD
	PCSK9	607786	Hypercholesterolemia, familial, 3	603776	AD
	LDLRAP1	605747	Hypercholesterolemia, familial, 4	603813	AR
	ABCG8	210250	Sitosterolemia 1	210250	AR
	ABCG5	605459	Sitosterolemia 2	618666	AR
	F9	300746	Hemophilia B	306900	XLR
	CFTR	602421	Cystic fibrosis	219700	AR
	FGFR3	134934	Achondroplasia	100800	AD
	HBB	141900	Thalassemia, beta	613985	AR

Table S2. The NGS results of the 48 patients with clinically or molecularly confirmed previous IEMs.

Patient	Diagnosis	Gene	Nucleotide change	Amino acid change	Zygosity	Mutant allele depth/amplicon depth
P01	G6PD deficiency	*G6PD*	c.185A>G	p.His62Arg	Hemi	75/77
P02	Hyperphenylalaninemia; G6PD deficiency	*PTS*	c.259C>T	p.Pro87Ser	Het	70/134
		PTS	c.286G>A	p.Asp96Asn	Het	64/134
		G6PD	c.1478G>A	p.Arg493His	Hom	232/233
P03	Phenylketonuria	*PAH*	c.728G>A	p.Arg243Gln	Hom	176/177
P04	Epilepsy	*ALDH7A1*	c.1061A>G	p.Tyr354Cys	Hom	613/638
P05	G6PD deficiency	*G6PD*	c.1466G>T	p.Arg489Leu	Hemi	89/89
P06	Carnitine deficiency	*SLC22A5*	c.51C>G	p.Phe17Leu	Het	214/433
		SLC22A5	c.1400C>G	p.Ser467Cys	Het	145/286
		TH	c.1428-1G>A	-	Het	114/256
P07	Glutaricaciduria	*GCDH*	c.892G>A	p.Ala298Thr	Hom	332/332
P08	Propionicacidemia	*PCCB*	c.1087T>C	p.Ser363Pro	Het	79/156
		PCCB	c.1301C>T	p.Ala434Val	Het	233/524
P09	Citrin deficiency	*SLC25A13*	c.852_855del	p.Met285ProfsTer2	Het	114/230
		SLC25A13	IVS16ins3kb *	-	Het	36/25/40
P10	Phenylketonuria	*PAH*	c.441+1G>A	-	Het	113/226
		PAH	c.208_210del	p.Ser70del	Het	242/510
P11	Phenylketonuria; G6PD deficiency	*PAH*	c.940C>A	p.Pro314Thr	Het	41/101
		PAH	c.781C>T	p.Arg261Ter	Het	80/146
		G6PD	c.961G>A	p.Val321Met	Hemi	125/133
P12	Phenylketonuria	*PAH*	c.1256A>G	p.Gln419Arg	Het	84/148
		PAH	c.1084C>A	p.Pro362Thr	Het	124/270
P13	Phenylketonuria	*PAH*	c.1223G>A	p.Arg408Gln	Hom	164/167
P14	Carnitine deficiency	*SLC22A5*	c.760C>T	p.Arg254Ter	Hom	127/127
P15	Phenylketonuria	*PAH*	c.913-7A>G	-	Het	55/108
		PAH	c.770G>T	p.Gly257Val	Het	82/147
P16	2-methylbutyrylglycinuria	*ACADSB*	c.275C>G	p.Ser92Ter	Het	110/229
		ACADSB	c.923G>A	p.Cys308Tyr	Het	59/124
P17	CPT II deficiency	*CPT2*	c.989dup	p.Ile332HisfsTer2	Het	60/143
		CPT2	c.1393G>A	p.Ala465Thr	Het	23/59
P18	Hyperphenylalaninemia	*PTS*	c.84-291A>G	-	Het	52/109
		PTS	c.259C>T	p.Pro87Ser	Het	60/123
P19	G6PD deficiency	*G6PD*	c.793C>T	p.Leu265Phe	Het	69/137
P20	Wilson disease	*ATP7B*	c.3443T>C	p.Ile1148Thr	Het	49/106
		ATP7B	c.2333G>T	p.Arg778Leu	Het	60/123
		GALK1	c.944+2T>G	-	Het	56/128
P21	Citrin deficiency	*SLC25A13*	c.852_855del	p.Met285ProfsTer2	Het	136/276
		SLC25A13	c.103A>G	p.Met35Val	Het	127/246
P22	Phenylketonuria	*PAH*	c.498C>G	p.Tyr166Ter	Het	116/237
		PAH	c.-4173_-407 del3767 *	-	Het	89/41/97
P23	Citrin deficiency	*SLC25A13*	c.852_855del	p.Met285ProfsTer2	Het	80/156
		SLC25A13	c.1638_1660dup	p.Ala554GlyfsTer17	Het	185/398

Table S2. The NGS results of the 48 patients with clinically or molecularly confirmed previous IEMs (continued).

Patient	Diagnosis	Gene	Nucleotide change	Amino acid change	Zygosity	Mutant allele depth/amplicon depth
P24	Methylmalonic aciduria	*MMUT*	c.729_730insTT	p.Asp244LeufsTer39	Het	115/238
		MMUT	c.424A>G	p.Thr142Ala	Het	60/130
P25	Methylmalonic aciduria	*MMACHC*	c.394C>T	p.Arg132Ter	Het	113/250
		MMACHC	c.609G>A	p.Trp203Ter	Het	236/494
		SLC25A20	c.199-10T>G	-	Het	56/150
P26	Phenylketonuria	*PAH*	c.1315+4A>G	-	Het	123/244
		PAH	c.498C>G	p.Tyr166Ter	Het	219/442
P27	Isovaleric acidemia	*IVD*	c.1208A>G	p.Tyr403Cys	Het	132/295
		IVD	c.548C>T	p.Ala183Val	Het	80/164
P28	Carnitine deficiency	*SLC22A5*	c.51C>G	p.Phe17Leu	Hom	210/215
		ACADS	c.1031A>G	p.Glu344Gly	Het	275/500
P29	Phenylketonuria	*PAH*	c.498C>G	p.Tyr166Ter	Hom	231/233
		ACADSB	c.1165A>G	p.Met389Val	Het	135/276
P30	Phenylketonuria	*PAH*	c.721C>T	p.Arg241Cys	Het	127/242
		PAH	c.331C>T	p.Arg111Ter	Het	84/148
P31	Hyperphenylalaninemia	*PTS*	c.155A>G	p.Asn52Ser	Hom	107/108
P32	G6PD deficiency	*G6PD*	c.1478G>A	p.Arg493His	Hemi	131/131
		SLC22A5	c.51C>G	p.Phe17Leu	Het	147/279
P33	Hyperphenylalaninemia	*PTS*	c.259C>T	p.Pro87Ser	Hom	101/103
P34	Carnitine deficiency	*SLC22A5*	c.760C>T	p.Arg254Ter	Hom	252/254
		CPT2	c.338C>T	p.Ser113Leu	Het	48/100
P35	Methylmalonic aciduria	*MMUT*	c.1280G>A	p.Gly427Asp	Het	176/321
		MMUT	c.1853T>C	p.Leu618Pro	Het	64/127
P36	Phenylketonuria; G6PD deficiency	*PAH*	c.721C>T	p.Arg241Cys	Hom	174/178
		G6PD	c.1094C>A	p.Ala365Asp	Hemi	187/190
P37	G6PD deficiency	*G6PD*	c.1478G>A	p.Arg493His	Hemi	177/182
		GBA	c.762-1G>C	-	Het	190/441
P38	Phenylketonuria	*PAH*	c.1197A>T	p.Val399=	Het	327/626
		PAH	c.516G>T	p.Gln172His	Het	225/464
		ATP7B	c.2975C>T	p.Pro992Leu	Het	276/597
P39	Methylmalonic aciduria	*MMAB*	c.519+1G>A	-	Hom	121/123
P40	G6PD deficiency	*G6PD*	c.1466G>T	p.Arg489Leu	Hemi	162/162
		G6PC	c.248G>A	p.Arg83His	Het	27/45
P41	Phenylketonuria	*PAH*	c.721C>T	p.Arg241Cys	Het	89/189
		PAH	c.611A>G	p.Tyr204Cys	Het	275/512
P42	Phenylketonuria	*PAH*	c.728G>A	p.Arg243Gln	Het	102/193
		PAH	c.158G>A	p.Arg53His	Het	84/152
P43	Phenylketonuria	*PAH*	c.781C>T	p.Arg261Ter	Het	153/344
		PAH	c.721C>T	p.Arg241Cys	Het	93/175
P44	Phenylketonuria	*PAH*	c.1238G>C	p.Arg413Pro	Het	146/261
		PAH	c.478C>T	p.Gln160Ter	Het	105/205
P45	Phenylketonuria	*PAH*	c.478C>T	p.Gln160Ter	Hom	116/116
P46	Phenylketonuria	*PAH*	c.464G>A	p.Arg155His	Het	74/129
		PAH	c.1194A>G	p.Lys398=	Het	254/558
P47	Phenylketonuria	*PAH*	c.442-1G>A	-	Het	112/232
P48	Short chain acyl-CoA dehydrogenase deficiency	*ACADS*	c.1031A>G	p.Glu344Gly	Het	236/501
		ACADS	c.79A>C	p.Thr27Pro	Het	58/119

Hom - homozygous, Het - heterozygous. * - Variants only discovered by tNGS.

Table S3. The NGS results of 112 carriers with known variants.

Carrier	Gene	Nucleotide change	Amino acid change	Mutant allele depth/amplicon depth
C01	*PAH*	c.464G>A	p.Arg155His	154/288
	ACADVL	c.1226C>T	p.Thr409Met	172/372
C02	*ASL*	c.281G>A	p.Arg94His	150/298
C03	*CPS1*	c.2407C>T	p.Arg803Cys	195/352
C04	*GJB2*	c.508_511dup	p.Ala171GlufsTer40	86/152
C05	*ATP7B*	c.2304dup	p.Met769HisfsTer26	81/169
C06	*SLC22A5*	c.1400C>G	p.Ser467Cys	130/270
C07	*MCCC1*	c.1518del	p.Glu506AspfsTer17	169/241
C08	*ACADSB*	c.746del	p.Pro249LeufsTer15	197/412
	MCCC1	c.1331G>A	p.Arg444His	228/452
C09	*SLC22A5*	c.428C>T	p.Pro143Leu	114/237
C10	*ATP7B*	c.2333G>T	p.Arg778Leu	51/108
C11	*PAH*	c.722del	p.Arg241ProfsTer100	127/252
C12	*MAT1A*	c.1070C>T	p.Pro357Leu	28/67
C13	*ATP7B*	c.2333G>T	p.Arg778Leu	66/106
C14	*GNPTAB*	c.2715+1G>A	-	57/111
C15	*SLC25A13*	c.852_855del	p.Met285ProfsTer2	133/258
C16	*SLC22A5*	c.1400C>G	p.Ser467Cys	135/281
C17	*MCCC1*	c.639+2T>A	-	111/216
C18	*ACADS*	c.1031A>G	p.Glu344Gly	374/760
C19	*DDC*	c.1297dup	p.Ile433AsnfsTer60	44/84
C20	*PTS*	c.155A>G	p.Asn52Ser	73/143
	PAH	c.331C>T	p.Arg111Ter	138/281
C21	*MMAA*	c.410del	p.Asn137IlefsTer6	164/304
C22	*ATP7B*	c.2804C>T	p.Thr935Met	218/429
C23	*PAH*	c.527G>A	p.Arg176Gln	212/434
C24	*PCBD1*	c.166C>T	p.Gln56Ter	51/99
C25	*SLC22A5*	c.760C>T	p.Arg254Ter	163/232
	ACADVL	c.1349G>A	p.Arg450His	126/253
C26	*HPD*	c.475G>T	p.Glu159Ter	57/134
C27	*ATP7B*	c.2304dup	p.Met769HisfsTer26	75/141
C28	*ETFDH*	c.1255_1258del	p.Thr419ValfsTer9	137/282
C29	*ATP7B*	c.1146_1147del	p.Gln383ThrfsTer21	117/228
C30	*PSAP*	c.640_641del	p.Thr214GlnfsTer17	124/279
C31	*PAH*	c.722_735delGCCTCCGACCTGTGins CCTCCGACCTGT	p.Arg241ProfsTer5	98/203
C32	*MCCC1*	c.1518del	p.Glu506AspfsTer17	129/256
	CBS	c.892C>T	p.Gln298Ter	41/70
C33	*GLB1*	c.245+1G>A	-	129/267
C34	*NPC1*	c.1655-1G>T	-	38/93
C35	*ACADSB*	c.275C>G	p.Ser92Ter	80/184
C36	*ASS1*	c.1087C>T	p.Arg363Trp	77/167
C37	*SMPD1*	c.1133G>A	p.Arg378His	187/319

Table S3. The NGS results of 112 carriers with known variants (continued).

Carrier	Gene	Nucleotide change	Amino acid change	Mutant allele depth/amplicon depth
C38	*PAH*	c.1162G>A	p.Val388Met	183/282
	MMUT	c.1663G>A	p.Ala555Thr	285/454
C39	*TH*	c.457C>T	p.Arg153Ter	39/85
C40	*MMACHC*	c.609G>A	p.Trp203Ter	292/624
C41	*ASS1*	c.892G>A	p.Glu298Lys	36/86
C42	*ETFDH*	c.250G>A	p.Ala84Thr	73/162
C43	*ASS1*	c.910C>T	p.Arg304Trp	80/172
C44	*HLCS*	c.1111del	p.Gly372AlafsTer6	84/188
C45	*GCDH*	c.532G>A	p.Gly178Arg	94/266
C46	*MCCC1*	c.980C>G	p.Ser327Ter	166/310
C47	*SMPD1*	c.847G>A	p.Ala283Thr	184/283
C48	*ASS1*	c.257G>A	p.Arg86His	86/212
C49	*ATP7B*	c.314C>A	p.Ser105Ter	214/454
C50	*PAH*	c.842+2T>A	-	75/145
C51	*GALC*	c.136G>T	p.Asp46Tyr	103/198
C52	*ARG1*	c.124G>T	p.Glu42Ter	58/127
	ACAD8	c.841+1G>A	-	134/253
C53	*MCCC1*	c.1268-1G>A	-	34/81
C54	*MMUT*	c.970G>A	p.Ala324Thr	238/461
	SLC26A4	c.919-2A>G	-	58/134
C55	*GALC*	c.1592G>A	p.Arg531His	75/146
	GAA	c.2237G>A	p.Trp746Ter	36/80
C56	*GJB2*	c.109G>A	p.Val37Ile	73/155
C57	*BCKDHA*	c.14T>C	p.Ile5Thr	22/57
C58	*ASS1*	c.1168G>A	p.Gly390Arg	115/203
C59	*ALDH7A1*	c.1547A>G	p.Tyr516Cys	98/181
C60	*CPT1A*	c.1424G>A	p.Trp475Ter	68/147
C61	*GUSB*	c.397-2A>G	-	238/596
C62	*SLC25A13*	c.1505C>T	p.Pro502Leu	44/96
C63	*ARSB*	c.1577C>A	p.Thr526Asn	107/243
C64	*GBA*	c.1082C>T	p.Ala361Val	45/101
C65	*MMUT*	c.1159A>C	p.Thr387Pro	65/107
C66	*ETFDH*	c.770A>G	p.Tyr257Cys	147/274
	ATP7B	c.3443T>C	p.Ile1148Thr	49/97
	PAH	c.202A>T	p.Arg68Ter	95/185
C67	*GLDC*	c.2815G>A	p.Asp939Asn	36/81
C68	*GALE*	c.643-2A>C	-	46/101
	GJB2	c.109G>A	p.Val37Ile	68/137
C69	*PYGL*	c.2467C>T	p.Gln823Ter	195/365
C70	*SLC26A4*	c.919-2A>G	-	75/156
C71	*SUCLG1*	c.140G>A	p.Arg47Gln	79/150
C72	*GAA*	c.1411_1414del	p.Glu471ProfsTer5	18/50
	GAA	c.[752C>T;761C>T]	p.[Ser251Leu;Ser254Leu]	89/171

Table S3. The NGS results of 112 carriers with known variants (continued).

Carrier	Gene	Nucleotide change	Amino acid change	Mutant allele depth/amplicon depth
C73	*GAMT*	c.472C>T	p.Arg158Cys	41/92
C74	*MAT1A*	c.770G>T	p.Gly257Val	128/204
C75	*PCCB*	c.842G>A	p.Ser281Asn	31/64
C76	*BCKDHA*	c.947G>A	p.Arg316Gln	246/464
C77	*PC*	c.2854A>G	p.Ile952Val	126/208
C78	*SLC37A4*	c.446G>A	p.Gly149Glu	146/336
	ACAT1	c.1094A>G	p.Lys365Arg	63/120
C79	*NPC1*	c.2291C>T	p.Ala764Val	77/148
C80	*SPR*	c.369C>G	p.Tyr123Ter	26/53
C81	*GCH1*	c.162G>T	p.Lys54Asn	432/688
	ACADSB	c.275C>G	p.Ser92Ter	308/630
C82	*MTR*	c.3135C>A	p.His1045Gln	219/460
C83	*ACADVL*	c.1405C>T	p.Arg469Trp	112/239
C84	*PAH*	c.721C>T	p.Arg241Cys	77/137
	SLC25A20	c.47C>T	p.Ala16Val	332/639
C85	*ACADM*	c.50G>A	p.Arg17His	367/789
C86	*GALK1*	c.944+2T>G	-	47/97
C87	*MMUT*	c.1084-10A>G	-	86/154
C88	*CBS*	c.954+2T>C	-	127/265
C89	*ACADVL*	c.105_109dup	p.Arg37LeufsTer26	187/380
C90	*IDUA*	c.911del	p.Val304GlyfsTer13	89/158
C91	*ACADM*	c.449_452del	p.Thr150ArgfsTer4	65/172
C92	*GALNS*	c.1417C>T	p.Gln473Ter	210/478
C93	*IDUA*	c.300-3C>G	-	75/157
	UGT1A1	c.1091C>T	p.Pro364Leu	250/513
C94	*GJB2*	c.299_300del	p.His100ArgfsTer14	61/116
C95	*QDPR*	c.68G>A	p.Gly23Asp	41/123
C96	*GLA*	c.428C>T	p.Ala143Val	167/347
C97	*ATP7B*	c.3766_3767dup	p.Gln1256HisfsTer75	31/63
C98	*GALK1*	c.410del	p.Gly137ValfsTer27	56/127
C99	*ACADVL*	c.829_831del	p.Glu277del	42/87
C100	*GNPTAB*	c.2590dup	p.Glu864GlyfsTer4	70/137
C101	*HLCS*	c.1544G>A	p.Ser515Asn	24/51
C102	*ALDOB*	c.226G>A	p.Gly76Ser	234/509
C103	*G6PC*	c.262del	p.Val88PhefsTer14	12/25
	PNPO	c.322C>T	p.Arg108Cys	335/678
C104	*MMACHC*	c.567dup	p.Ile190TyrfsTer13	59/107
C105	*GAA*	c.-32-13T>G	-	50/101
	UGT1A1	c.1456T>G	p.Tyr486Asp	53/107
C106	*NAGLU*	c.1354G>A	p.Glu452Lys	76/177
C107	*HLCS*	c.782del	p.Gly261ValfsTer20	86/173
C108	*BTD*	c.278A>G	p.Tyr93Cys	140/312

Table S3. The NGS results of 112 carriers with known variants (continued).

Carrier	Gene	Nucleotide change	Amino acid change	Mutant allele depth/amplicon depth
C109	*ACADM*	c.449_452del	p.Thr150ArgfsTer4	76/155
	DLD	c.140T>C	p.Ile47Thr	66/112
C110	*HADHB*	c.209C>G	p.Ser70Ter	171/346
	SLC25A13	c.615+5G>A	-	42/82
C111	*ABCD1*	c.1979G>A	p.Arg660Gln	33/72
	SLC25A13	c.1177+1G>A	-	278/513
C112	*ETFDH*	c.405+1G>A	-	146/274
	CD320	c.262_264del	p.Glu88del	77/159