Supplemental Online Content

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This supplemental material has been provided by the authors to give readers additional information about their work.

eAppendix. The statistical methods used for calculating sensitivity, specificity, and PPV

We calculated the sensitivity, specificity, predictive positive value (PPV) and false negative rate (FNR) of AAs/OAs/FAODs and G6PD deficiency by gNBS and bNBS, respectively. That for congenital hypothyroidism was not calculated as only 4 common disease-causing genes were included while dozens of genes were reported to be associated with the disease.

To calculate these parameters, we defined true positive, true negative, false positive, false negative in the study. Newborns lost follow-up, or were with suspicious results by the date of follow-up were excluded. Take AAs/OAs/FAODs by gNBS as an example, cases with conclusive results of both gNBS and MS/MS were divided into four categories:

- True positive: cases with gNBS positive results of AAs/OAs/FAODs (such as phenylketonuria), and was diagnosed with the same AAs/OAs/FAODs (such as phenylketonuria).
- True negative：cases with gNBS results of negative or carrier of AAs/OAs/FAODs (such as phenylketonuria), and was excluded the risk for the same AAs/OAs/FAODs (such as phenylketonuria).
- False positive: cases with gNBS positive results of AAs/OAs/FAODs (such as phenylketonuria)，but was excluded the risk for the same AAs/OAs/FAODs (such as phenylketonuria).
- False negative: cases with gNBS results of negative or carrier of AAs/OAs/FAODs (such as phenylketonuria)，but was diagnosed with the same AAs/OAs/FAODs (such as phenylketonuria).

1. Sensitivity

Formula for sensitivity of AAs/OAs/FAODs:

$$\text{Sensitivity}_{gNBS} = \frac{\text{Patients with AAs/OAs/FAODs and with gNBS positive results in related genes}}{\text{All patients with AAs/OAs/FAODs}}$$

$$\text{Sensitivity}_{bNBS} = \frac{\text{Patients with AAs/OAs/FAODs and with bNBS (MS/MS) positive results}}{\text{All patients with AAs/OAs/FAODs}}$$

Formula for sensitivity of G6PD deficiency:

$$\text{Sensitivity}_{gNBS} = \frac{\text{Patients with G6PD decifiency and with gNBS positive results in } G6PD \text{ gene}}{\text{All patients with G6PD decifiency}}$$

$$\text{Sensitivity}_{bNBS} = \frac{\text{Patients with G6PD decifiency and with bNBS (G6PD screening) positive results}}{\text{All patients with G6PD decifiency}}$$

2. Specificity

Formula for specificity of AAs/OAs/FAODs:

$$\text{Specificity}_{gNBS} = \frac{\text{Newborns without AAs/OAs/FAODs and with gNBS results of negative or carrier in related genes}}{\text{All newborns without AAs/OAs/FAODs}}$$

$$\text{Specificity}_{bNBS} = \frac{\text{Newborns without AAs/OAs/FAODs and with bNBS (MS/MS) negative results}}{\text{All newborns without AAs/OAs/FAODs}}$$

Formula for specificity of G6PD deficiency:

$$\text{Specificity}_{gNBS} = \frac{\text{Newborns without G6PD decifiency and with gNBS negative results in } G6PD \text{ gene}}{\text{All newborns without G6PD decifiency}}$$

$$\text{Specificity}_{bNBS} = \frac{\text{Newborns without G6PD decifiency and with bNBS (G6PD screening) negative results}}{\text{All newborns without G6PD decifiency}}$$

3. PPV

Formula for PPV of AAs/OAs/FAODs:

$$\text{PPV}_{gNBS} = \frac{\text{Patients with AAs/OAs/FAODs and with gNBS positive results in related genes}}{\text{All newborns with gNBS positive results in related genes}}$$

$$\text{PPV}_{bNBS} = \frac{\text{Patients with AAs/OAs/FAODs and with bNBS (MS/MS) positive results}}{\text{All newborns with bNBS (MS/MS) positive results}}$$

Formula for PPV of G6PD deficiency:

$$\text{PPV}_{gNBS} = \frac{\text{Patients with G6PD decifiency and with gNBS positive results in } G6PD \text{ gene}}{\text{All newborns with gNBS positive results in } G6PD \text{ gene}}$$

$$\text{PPV}_{bNBS} = \frac{\text{Patients with G6PD decifiency and with bNBS (G6PD screening) positive results}}{\text{All newborns with bNBS (G6PD decifiency) positive results}}$$

4. FNR

Formula for FNR of AAs/OAs/FAODs:

$$\text{FNR}_{gNBS} = \frac{\text{Patients with AAs/OAs/FAODs and with gNBS results of negative or carrier in related genes}}{\text{All patients with AAs/OAs/FAODs}}$$

$$\mathrm{FNR}_{bNBS} = \frac{\text{Patients with AAs/OAs/FAODs and with bNBS (MS/MS) negative results}}{\text{All patients with AAs/OAs/FAODs}}$$

Formula for FNR of G6PD deficiency:

$$\mathrm{FNR}_{gNBS} = \frac{\text{Patients with G6PD decifiency and with gNBS negative results in } G6PD \text{ gene}}{\text{All patients with G6PD deficiency}}$$

$$\mathrm{FNR}_{bNBS} = \frac{\text{Patients with G6PD decifiency and with bNBS (G6PD screening) negative results}}{\text{All patients with G6PD deficiency}}$$

eTable 1. Diseases and genes in genomic newborn screening.

No	Disease	Screening panel	OMIM	Gene	Transcript	Inheritance mode	Evidence for selection of diseases and genes*	Group
1	Phenylketonuria	tNBS-MS/MS and gNBS	261600	*PAH*	NM_000277.1	AR	BabySeq (A); NC NEXUS（class I）	Disease of Amino Acid Metabolism
2	Tetrahydrobiopterin deficiency	tNBS-MS/MS and gNBS	261640;261630	*PTS;QDPR*	NM_000317.2;NM_000320.2	AR	BabySeq (A); NC NEXUS（class I）	
3	Carbamoylphosphate Synthetase I Deficiency	tNBS-MS/MS and gNBS	237300	*CPS1*	NM_001875.4	AR	BabySeq (A); NC NEXUS（class I）	
4	Maple Syrup Urine Disease	tNBS-MS/MS and gNBS	248600	*BCKDHA;BCKDHB;DBT*	NM_000709.3;NM_183050.2;NM_001918.3	AR	BabySeq (A); NC NEXUS（class I）	
5	Glycine encephalopathy	tNBS-MS/MS and gNBS	605899	*AMT, GLDC*	NM_000481.3, NM_000170.2	AR	BabySeq (A)	
6	Hypermethioninemia	tNBS-MS/MS and gNBS	250850	*MAT1A*	NM_000429.2	AD,AR	BabySeq (C); NC NEXUS（class II）	
7	Hyperornithinemia-Hyperammonemia-Homocitrullinuria Syndrome	tNBS-MS/MS and gNBS	238970	*SLC25A15*	NM_014252.3	AR	BabySeq (A); NC NEXUS（class I）	
8	Citrullinemia type I	tNBS-MS/MS and gNBS	215700	*ASS1*	NM_000050.4	AR	BabySeq (A); NC NEXUS（class I）	
9	Homocystinuria	tNBS-MS/MS and gNBS	236200;236250	*CBS;MTHFR*	NM_000071.2;NM_005957.4	AR	CBS：BabySeq (A); NC NEXUS（class I） MTHFR：BabySeq (B); NC NEXUS（class II）	
10	Argininosuccinic aciduria	tNBS-MS/MS and gNBS	207900	*ASL*	NM_000048.3	AR	BabySeq (A); NC NEXUS（class I）	
11	Argininemia	tNBS-MS/MS	207800	*ARG1*	NM_000045.3	AR	BabySeq (A); NC	

		and gNBS					NEXUS（class I）	
12	Tyrosinemia	tNBS-MS/MS and gNBS	276700;276600;276710	*FAH;TAT;HPD*	NM_000137.2;NM_000353.2;NM_002150.2	AR	FAH; TAT：BabySeq (A); NC NEXUS（class I） HPD：BabySeq (C); NC NEXUS（class II）	
13	Ornithine Transcarbamylase Deficiency	tNBS-MS/MS and gNBS	311250	*OTC*	NM_000531.5	XL	BabySeq (A); NC NEXUS（class I）	
14	Citrin deficiency	tNBS-MS/MS and gNBS	605814	*SLC25A13*	NM_014251.2	AR	BabySeq (A); NC NEXUS（class I）	
15	Hyperprolinemia type I	tNBS-MS/MS and gNBS	239500	*PRODH*	NM_016335.4	AR	BabySeq (C)	
16	2-Methylbutyryl Glycinuria	tNBS-MS/MS and gNBS	610006	*ACADSB*	NM_001609.3	AR	BabySeq (C); NC NEXUS（class II）	Disease of Organic Acid Metabolism
17	3-Methylcrotonyl-CoA carboxylase deficiency	tNBS-MS/MS and gNBS	210200;210210	*MCCC1;MCCC2*	NM_020166.3;NM_022132.4	AR	BabySeq (B); NC NEXUS（class II）	
18	3-Methylglutaconic Aciduria type 1	tNBS-MS/MS and gNBS	250950	*AUH*	NM_001698.2	AR	BabySeq (A)	
19	3-hydroxy-3-methylglutaryl-CoA lyase deficiency	tNBS-MS/MS and gNBS	246450	*HMGCL*	NM_000191.2	AR	BabySeq (A); NC NEXUS（class I）	
20	β-Ketothiolase Deficiency	tNBS-MS/MS and gNBS	203750	*ACAT1*	NM_000019.3	AR	BabySeq (A); NC NEXUS（class I）	
21	Propionicacidemia	tNBS-MS/MS and gNBS	606054	*PCCA, PCCB*	NM_000282.3, NM_000532.4	AR	BabySeq (A); NC NEXUS（class I）	
22	Methylmalonic Acidemia	tNBS-MS/MS and gNBS	251100;251110;277400;251000	*MMAA;MMAB;MMACHC;MMUT*	NM_172250.2;NM_052845.3;NM_015506.2;NM_000255.3	AR	MMAA; MMAB; MMACHC：BabySeq (A); NC NEXUS（class I）	

							MMUT：BabySeq (A)	
23	Multiple carboxylase deficiency	tNBS-MS/MS and gNBS	253270;253260	*HLCS;BTD*	NM_000411.6;NM_000060.2	AR	BabySeq (A); NC NEXUS（class I）	
24	Glutaric Acidemia I	tNBS-MS/MS and gNBS	231670	*GCDH*	NM_000159.2	AR	BabySeq (A); NC NEXUS（class I）	
25	Isobutyryl-CoA dehydrogenase deficiency	tNBS-MS/MS and gNBS	611283	*ACAD8*	NM_014384.2	AR	BabySeq (A)	
26	Isovaleric Acidemia	tNBS-MS/MS and gNBS	243500	*IVD*	NM_002225.3	AR	BabySeq (A); NC NEXUS（class I）	
27	Succinic Semialdehyde Dehydrogenase Deficiency	gNBS only	271980	*ALDH5A1*	NM_001080.3	AR	BabySeq (A)	
28	2,4-Dienoyl-CoA Reductase Deficiency	tNBS-MS/MS and gNBS	616034	*NADK2*	NM_001085411.1	AR	NC NEXUS（class II）	Disease of Fatty Acid Metabolism
29	Short Chain Acyl-CoA Dehydrogenase Deficiency	tNBS-MS/MS and gNBS	201470	*ACADS*	NM_000017.2	AR	BabySeq (C); NC NEXUS（class II）	
30	Multiple Acyl-CoA Dehydrogenase Deficiency	tNBS-MS/MS and gNBS	231680	*ETFDH*	NM_004453.2	AR	BabySeq (A); NC NEXUS（class I）	
31	Acyl-CoA Dehydrogenase Deficiency,Very Long-Chain	tNBS-MS/MS and gNBS	201475	*ACADVL*	NM_000018.3	AR	BabySeq (A); NC NEXUS（class I）	
32	Carnitine-Acylcarnitine	tNBS-MS/MS	212138	*SLC25A20*	NM_000387.5	AR	BabySeq (A); NC	

	Translocase Deficiency	and gNBS					NEXUS（class I）	
33	Carnitine Palmitoyltransferase I Deficiency	tNBS-MS/MS and gNBS	255120	*CPT1A*	NM_001876.3	AR	BabySeq (A); NC NEXUS（class I）	
34	Carnitine palmitoyltransferase II deficiency	tNBS-MS/MS and gNBS	600649/608836	*CPT2*	NM_000098.2	AR	BabySeq (A); NC NEXUS（class I）	
35	Trifunctional Protein Deficiency	tNBS-MS/MS and gNBS	609015	*HADHA, HADHB*	NM_000182.4, NM_000183.2	AR	HADHA：NC NEXUS（class I） HADHB：BabySeq (A); NC NEXUS（class I）	
36	Primary Carnitine Deficiency	tNBS-MS/MS and gNBS	212140	*SLC22A5*	NM_003060.3	AR	BabySeq (A); NC NEXUS（class I）	
37	Long-Chain 3-Hydroxyacyl-Coa Dehydrogenase Deficiency	tNBS-MS/MS and gNBS	609016	*HADHA*	NM_000182.4	AR	BabySeq (A); NC NEXUS（class I）	
38	Medium-Chain Acyl-Coenzyme A Dehydrogenase Deficiency	tNBS-MS/MS and gNBS	201450	*ACADM*	NM_000016.4	AR	BabySeq (A); NC NEXUS（class I）	
39	Malonyl-Coa Decarboxylase Deficiency	tNBS-MS/MS and gNBS	248360	*MLYCD*	NM_012213.2	AR	BabySeq (A); NC NEXUS（class II）	
40	Glucose-6-Phosphate Dehydrogenase Deficiency	tNBS-fluorometric assay and gNBS	300908	*G6PD*	NM_001042351.1	XL	BabySeq (A); NC NEXUS（class I）	G6PD Deficiency
41	Thyroid	tNBS-thyroid-st	274900	*DUOXA2*	NM_207581.3	AR	BabySeq (C); NC	Endocrine Disease

	dyshormonogenesis 5	imulating hormone (TSH) test and gNBS					NEXUS（class I）	
42	Thyroid dyshormonogenesis 6	tNBS-thyroid-stimulating hormone (TSH) test and gNBS	607200	*DUOX2*	NM_014080.4	AR	BabySeq (A); NC NEXUS（class I）	
43	Combined pituitary hormone deficiency 2	tNBS-thyroid-stimulating hormone (TSH) test and gNBS	262600	*PROP1*	NM_006261.4	AR	BabySeq (A); NC NEXUS（class I）	
44	Hypothyroidism Congenital Nongoitrous 1	tNBS-thyroid-stimulating hormone (TSH) test and gNBS	275200	*TSHR*	NM_000369.2	AR	BabySeq (A); NC NEXUS（class I）	
45	Congenital Adrenal Hyperplasia due to 11-beta-Hydroxylase-Deficiency	gNBS only	202010	*CYP11B1*	NM_000497.3	AR	BabySeq (A); NC NEXUS（class I）	
46	17,20-lyase deficiency, isolated	gNBS only	202110	*CYP17A1*	NM_000102.3	AR	reviewed and approved by experts	
47	Adrenal hypoplasia, congenital	gNBS only	300200	*NR0B1*	NM_000475.4	XLR	BabySeq (A)	
48	Cystic fibrosis	gNBS only	219700	*CFTR*	NM_000492.3	AR	BabySeq (A); NC NEXUS（class I）	
49	Krabbe Disease	gNBS only	245200	*GALC*	NM_000153.3	AR	BabySeq (A); NC NEXUS（class II）	Lysosmal Storage Disease
50	Fabry Disease	gNBS only	301500	*GLA*	NM_000169.2	XL	BabySeq (A); NC	

							NEXUS（class I）	
51	Niemann-Pick Disease, type A/B	gNBS only	607616	*SMPD1*	NM_000543.4	AR	BabySeq (A); NC NEXUS（class II）	
52	Niemann-Pick Disease, type D	gNBS only	257220	*NPC1*	NM_000271.4	AR	BabySeq (A); NC NEXUS（class I）	
53	Niemann-Pick Disease type C2	gNBS only	607625	*NPC2*	NM_006432.3	AR	BabySeq (A); NC NEXUS（class I）	
54	Mucopolysaccharidosis type V	gNBS only	607014	*IDUA*	NM_000203.3	AR	BabySeq (A); NC NEXUS（class I）	
55	Mucopolysaccharidosis II	gNBS only	309900	*IDS*	NM_000202.5	XLR	BabySeq (A); NC NEXUS（class I）	
56	Mucopolysaccharidosis type IIIA	gNBS only	252900	*SGSH*	NM_000199.3	AR	BabySeq (A); NC NEXUS（class II）	
57	Mucopolysaccharidosis type IIIB	gNBS only	252920	*NAGLU*	NM_000263.3	AR	BabySeq (A); NC NEXUS（class II）	
58	Mucopolysaccharidosis type IVA	gNBS only	253000	*GALNS*	NM_000512.4	AR	BabySeq (A); NC NEXUS（class I）	
59	GM1-gangliosidosis,type I	gNBS only	253010	*GLB1*	NM_000404.2	AR	BabySeq (A); NC NEXUS（class II）	
60	Mucopolysaccharidosis type VI	gNBS only	253200	*ARSB*	NM_000046.3	AR	BabySeq (A); NC NEXUS（class I）	
61	Mucopolysaccharidosis type VII	gNBS only	253220	*GUSB*	NM_000181.3	AR	BabySeq (A); NC NEXUS（class II）	
62	Galactokinase Deficiency	gNBS only	230200	*GALK1*	NM_000154.1	AR	BabySeq (A); NC NEXUS（class I）	Disease of Glucolipide Metabolism
63	Galactosemia	gNBS only	230400	*GALT*	NM_000155.3	AR	BabySeq (A); NC NEXUS（class I）	
64	Epimerase Deficiency	gNBS only	230350	*GALE*	NM_000403.3	AR	NC NEXUS（class II）	

	Galactosemia							
65	Familial Hyperinsulinemic Hypoglycemia 5	gNBS only	609968	*INSR*	NM_000208.2	AD	BabySeq (A)	
66	Glycogen Storage Disease Type Ia	gNBS only	232200	*G6PC*	NM_000151.3	AR	BabySeq (A); NC NEXUS（class I）	
67	Glycogen Storage Disease type Ib/Ic	gNBS only	232220/232240	*SLC37A4*	NM_001164277.1	AR	BabySeq (A); NC NEXUS（class I）	
68	Glycogen storage disease II	gNBS only	232300	*GAA*	NM_000152.3	AR	BabySeq (A); NC NEXUS（class I）	
69	Glycogen Storage Disease type III	gNBS only	232400	*AGL*	NM_000642.2	AR	BabySeq (A); NC NEXUS（class I）	
70	Glycogen Storage Disease type IV	gNBS only	232500	*GBE1*	NM_000158.3	AR	BabySeq (A); NC NEXUS（class II）	
71	Glycogen Storage Disease type V	gNBS only	232600	*PYGM*	NM_005609.2	AR	NC NEXUS（class I）	
72	Glycogen Storage Disease type VI	gNBS only	232700	*PYGL*	NM_002863.4	AR	BabySeq (A); NC NEXUS（class I）	
73	Glycogen storage disease type IXa	gNBS only	306000	*PHKA2*	NM_000292.2	XLR	BabySeq (A); NC NEXUS（class I）	
74	Glycogen storage disease type IXb	gNBS only	261750	*PHKB*	NM_000293.2	AR	BabySeq (A)	
75	Glycogen storage disease type IXc	gNBS only	613027	*PHKG2*	NM_000294.2	AR	BabySeq (A); NC NEXUS（class I）	
76	Glycogen storage disease type IXd	gNBS only	300559	*PHKA1*	NM_002637.3	XLR	BabySeq (C)	
77	Glycogen Storage Disease type XIV	gNBS only	614921	*PGM1*	NM_002633.2	AR	reviewed and approved by experts	

78	Diabetes, permanent neonatal 2	gNBS only	618856	*KCNJ11*	NM_000525.3	AD	BabySeq (A); NC NEXUS（class I）	
79	Diabetes, permanent neonatal 3	gNBS only	618857	*ABCC8*	NM_000352.3	AD,AR	BabySeq (A); NC NEXUS（class I）	
80	Sitosterolemia, type 1	gNBS only	210250	*ABCG8*	NM_022437.2	AR	reviewed and approved by experts	Disease of Other Metabolism
81	Sitosterolemia, type 2	gNBS only	618666	*ABCG5*	NM_022436.2	AR	BabySeq (A); NC NEXUS（class I）	
82	Hypercholesterolemia, familial,1	gNBS only	143890	*LDLR*	NM_000527.4	AD,AR	BabySeq (A); NC NEXUS（class I）	
83	Cerebrotendinous xanthomatosis	gNBS only	213700	*CYP27A1*	NM_000784.3	AR	BabySeq (A)	
84	X-linked Distal Spinal Muscular Atrophy 3	gNBS only	309400	*ATP7A*	NM_000052.5	XLR	BabySeq (A); NC NEXUS（class I）	
85	X-Linked Hypophosphatemia	gNBS only	307800	*PHEX*	NM_000444.4	XLD	reviewed and approved by experts	
86	Wilson Disease	gNBS only	277900	*ATP7B*	NM_000053.3	AR	BabySeq (A); NC NEXUS（class I）	
87	Cholestasis, progressive familial intrahepatic 1	gNBS only	211600	*ATP8B1*	NM_005603.4	AR	BabySeq (A)	
88	Cholestasis, progressive familial intrahepatic 2	gNBS only	601847	*ABCB11*	NM_003742.2	AR	BabySeq (A)	
89	Cholestasis, progressive familial intrahepatic 3	gNBS only	602347	*ABCB4*	NM_000443.3	AR	BabySeq (A)	
90	Congenital Bile Acid Synthesis Defect 1	gNBS only	607765	*HSD3B7*	NM_025193.3	AR	BabySeq (A)	
91	Hypophosphatasia	gNBS only	146300/241500/2415	*ALPL*	NM_000478.4	AD,AR	BabySeq (A)	

			10					
92	Primary Coenzyme Q10 deficiency 7	gNBS only	616276	*COQ4*	NM_016035.3	AR	reviewed and approved by experts	
93	Pyridoxine-Dependent Epilepsy	gNBS only	266100	*ALDH7A1*	NM_001182.4	AR	NC NEXUS（class I）	
94	Tyrosine Hydroxylase Deficiency	gNBS only	605407	*TH*	NM_199292.2	AR	BabySeq (A); NC NEXUS（class I）	
95	X-Linked Severe Congenital Neutropenia	gNBS only	301000	*WAS*	NM_000377.2	XLR	BabySeq (A)	Immune Disease
96	X-Linked Lymphoproliferative syndrome 1	gNBS only	308240	*SH2D1A*	NM_002351.4	XLR	BabySeq (A)	
97	X-Linked Lymphoproliferative syndrome 2	gNBS only	300635	*XIAP*	NM_001167.3	XLR	reviewed and approved by experts	
98	Chronic granulomatous disease, X-linked	gNBS only	306400	*CYBB*	NM_000397.3	XLR	BabySeq (A)	
99	X-Linked Agammaglobulinemia 1	gNBS only	300755	*BTK*	NM_000061.2	XLR	BabySeq (A)	
100	X-Linked Combined Immunodeficiency	gNBS only	300400	*IL2RG*	NM_000206.2	XLR	BabySeq (A); NC NEXUS（class I）	
101	Severe Combined Immunodeficiency, Autosomal Recessive, T Cell-Negative, B Cell-Negative, Nk Cell-Positive	gNBS only	601457	*RAG1*	NM_000448.2	AR	BabySeq (A); NC NEXUS（class I）	
102	Familial Mediterranean	gNBS only	249100/13	*MEFV*	NM_000243.2	AD,AR	BabySeq (A)	

	Fever		4610					
103	Immunodeficiency with Hyper-IgM, type 1	gNBS only	308230	*CD40LG*	NM_000074.2	XLR	BabySeq (A); NC NEXUS（class I）	
104	Neutropenia, severe congenital 1, autosomal dominant	gNBS only	202700	*ELANE*	NM_001972.2	AD	BabySeq (A)	
105	Kallmann Syndrome 1	gNBS only	308700	*KAL1*	NM_000216.2	XLR	reviewed and approved by experts	Disease of Sex Development
106	Kallmann Syndrome 2	gNBS only	147950	*FGFR1*	NM_023110.2	AD	BabySeq (A)	
107	Kallmann Syndrome 3	gNBS only	244200	*PROKR2*	NM_144773.2	AD	BabySeq (A)	
108	Hypogonadotropic hypogonadism 5 with or without anosmia	gNBS only	612370	*CHD7*	NM_017780.3	AD	BabySeq (A)	
109	Duchenne/Becker Muscular Dystrophy	gNBS only	310200	*DMD*	NM_004006.2	XLR	BabySeq (A)	Neuromuscular Disease
110	Spinal Muscular Atrophy	gNBS only	253550/253300/253400/271150	*SMN1*	NM_000344.3	AR	BabySeq (A)	
111	Early Infantile Epileptic Encephalopathy 6	gNBS only	607208	*SCN1A*	NM_001165963.1	AD	BabySeq (A)	
112	Early Infantile Epileptic Encephalopathy 9	gNBS only	300088	*PCDH19*	NM_001184880.1	XL	reviewed and approved by experts	
113	GLUT1 deficiency syndrome 1, infantile onset, severe	gNBS only	606777	*SLC2A1*	NM_006516.2	AD,AR	BabySeq (A); NC NEXUS（class I）	
114	α-Thalassemia	gNBS only	604131	*HBA1, HBA2*	NM_000558.3, NM_000517.4	AR	BabySeq (A)	Hemoglobinopathies
115	β-Thalassemia	gNBS only	613985	*HBB*	NM_000518.4	AR	BabySeq (A); NC	

							NEXUS（class I）	
116	Diamond-Blackfan Anemia 10	gNBS only	613309	*RPS26*	NM_001029.3	AD	BabySeq (A); NC NEXUS（class I）	Hematological Disease
117	Diamond-Blackfan Anemia 1	gNBS only	105650	*RPS19*	NM_001022.3	AD	BabySeq (A); NC NEXUS（class I）	
118	Diamond-Blackfan Anemia 7	gNBS only	612562	*RPL11*	NM_000975.3	AD	BabySeq (A); NC NEXUS（class I）	
119	Fanconi anemia, complementation group A	gNBS only	227650	*FANCA*	NM_000135.2	AR	BabySeq (A); NC NEXUS（class I）	
120	Familial Hemophagocytic Lymphohistiocytosis 2	gNBS only	603553	*PRF1*	NM_001083116.1	AR	BabySeq (A)	
121	Familial Hemophagocytic Lymphohistiocytosis 3	gNBS only	608898	*UNC13D*	NM_199242.2	AR	BabySeq (A); NC NEXUS（class I）	
122	Gitelman syndrome	gNBS only	263800	*SLC12A3*	NM_000339.2	AR	BabySeq (A)	Urologic Diseases
123	Alport syndrome 1, X-linked	gNBS only	301050	*COL4A5*	NM_000495.4	XLD	BabySeq (A); NC NEXUS（class I）	
124	Alport syndrome 3, autosomal dominant	gNBS only	104200	*COL4A3*	NM_000091.4	AD	BabySeq (A); NC NEXUS（class I）	
125	Alport syndrome 2, autosomal recessive	gNBS only	203780	*COL4A4, COL4A3*	NM_000092.4; NM_000091.4	AR	BabySeq (A); NC NEXUS（class I）	
126	Retinoblastoma	gNBS only	180200	*RB1*	NM_000321.2	AD	BabySeq (A); NC NEXUS（class I）	Ophthalmologic Disease
127	Tuberous Sclerosis 1	gNBS only	191100	*TSC1*	NM_000368.4	AD	BabySeq (A); NC NEXUS（class I）	Multisystem Disorder
128	Tuberous sclerosis 2	gNBS only	613254	*TSC2*	NM_000548.3	AD	BabySeq (A); NC	

							NEXUS（class I）	

* Among the gene-disease pairs, 136 pairs were screened in the North Carolina Newborn Exome Sequencing for Universal Screening (NC NEXUS) project (PMID30851990) and/or Babyseq project (PMID28079900). The remaining 8 gene-disease pairs were manually reviewed and approved by experts.

eTable 2. G6PD deficient patients undetected by the initial screen but confirmed by confirmatory tests

ID	Sex	Gestation week	Weight (g)	Inheritance	Gene	Variant	Initial G6PD screen results (U/gHb)	Confirmatory test results	Clinical management
GP-G004	M	39+1	3480	XLD	*G6PD*	c.[1388G>A];[0]	5.9	G6PD/6PGD=0.9 (normal >1)	Diet plans
GP-G005	M	38	2620	XLD	*G6PD*	c.[1388G>A];[0]	2.7	Specific data was unknown. The patient was followed by phone interview	Not available
GP-G006	M	38+6	2780	XLD	*G6PD*	c.[392G>T];[0]	3.3	Specific data was unknown. The patient was followed by phone interview	Not available
GP-G007	M	39+2	3080	XLD	*G6PD*	c.[392G>T];[0]	3.4	Specific data was unknown. The patient was followed by phone interview	Not available
GP-G008	M	39	3500	XLD	*G6PD*	c.[392G>T];[0]	3.4	Specific data was unknown. The patient was followed by phone interview	Not available
GP-G009	M	39	3640	XLD	*G6PD*	c.[392G>T];[0]	3.0	G6PD/6PGD=0.35 (normal 1.1-2.5)	Diet plans
GP-G010	M	39+4	3030	XLD	*G6PD*	c.[392G>T];[0]	2.7	G6PD/6PGD=0.28 (normal 1.1-2.5)	Diet plans
GP-G011	M	37	3850	XLD	*G6PD*	c.[392G>T];[0]	2.8	Specific data was unknown. The patient was followed by phone interview	Not available
GP-G012	M	39	3600	XLD	*G6PD*	c.[1024C>T];[0]	3.0	G6PD/6PGD=0.2 (normal 1.1-2.5)	Diet plans
GP-G013	M	37+4	2360	XLD	*G6PD*	c.[1024C>T];[0]	2.8	G6PD/6PGD=0.33 (normal 1.1-2.5)	Diet plans

Abbreviation: M, male. XLD, X-link dominant. The initial screen was fluorometric assay for G6PD enzyme activity.

SI conversion factor: To convert U/gHb to nkat/gHb, multiply by 0.0167.

eTable 3. TSH related patients undetected by the initial screen but confirmed by confirmatory tests

ID	Sex	Gestation week	Weight (g)	Inheritance	Gene	Variant	Initial TSH screen results (mIU/L)	Confirmatory test results	Clinical diagnosis	Clinical management
GP-C 10	F	38+2	3250	AR	*DUOX2*	c.[1883delA];[2654G>T]	TSH=5.12	TSH=6.25 mIU/L; FT4=1.12 ng/dL; FT3=392.21 pg/dL	Isolated HT	Active surveillance
GP-C 14	M	39+1	4290	AR	*DUOX2*	c.2048G>T(;)596delC	TSH=3.42	TSH=5.02 mIU/L; FT4=1.1 ng/dL; FT3=399.35 pg/dL	Isolated HT	Active surveillance
GP-C 28	F	40+0	2740	AR	*DUOX2*	c.[2654G>T];[978_979delGGinsTT]	TSH=2.98	TSH=7.78 mIU/L; FT4=1.26 ng/dL; FT3=424.68 pg/dL	Isolated HT	Active surveillance
GP-C 31	F	39+1	3190	AR	*DUOX2*	c.3285_3286delTT(;)2654G>T	TSH=2.21	TSH=31.20 mIU/L; FT4<0.30 ng/dL; FT3=475.97 pg/dL	Congenital hypothyroidism	Treatment with Euthyrox
GP-C 33	F	37+5	3900	AR	*DUOX2*	c.[3329G>A];[1588A>T]	TSH=6.32	TSH=9.88 mIU/L; FT4=0.83 ng/dL; FT3=343.51 pg/dL	Isolated HT	Active surveillance
GP-C 40	F	38+6	2850	AR	*DUOX2*	c.[3693+1G>T];[1588A>T]	TSH=3.68	TSH=15.63 mIU/L; FT4=0.66 ng/dL; FT3=387.79 pg/dL	Isolated HT	Active surveillance
GP-C 43	M	37+5	3050	AR	*DUOX2*	c.[3693+1G>T];[477delC]	TSH=5.35	TSH=8.83 mIU/L; FT4=1.14 ng/dL; FT3=381.82 pg/dL	Isolated HT	Active surveillance
GP-C 44	M	38+0	3160	AR	*DUOX2*	c.[4000C>T];[2635G>A]	TSH=5.12	TSH=92.78 mIU/L; T4=32.90 μg/dL	Congenital hypothyroidism	Treatment with Euthyrox

Abbreviation: M, male. F, female. AR, autosomal recessive. HT, hyperthyrotropinemia. TSH, thyroid-stimulating hormone. FT4, free throxine. FT3, free

triiodothyronine. T4, total thyroxine.
SI conversion factor: To convert FT3 to pmol/L, multiply by 0.0154; to convert FT4 to pmol/L, multiply by 12.871; to convert T4 to nmol/L, multiply by 12.871.

eTable 4. AAs/OAs/FAODs patients undetected by MS/MS but confirmed by confirmatory tests

ID	Sex	Gestation week	Weight (g)	Inheritance	Gene	Variant	Initial MS/MS screen results	Confirmatory test results	Clinical diagnosis	Clinical management
GP-M06	F	38+4	2790	AR	*MMACHC*	c.[482G>A];[565C>T]	C3=2.16 μmol/L C3/C2=0.11	MS/MS: C3=0.66 μmol/L C3/C2=0.05 GC/MS: MMA=73.8 mmol/mol creatinine. MCA=1.38 mmol/mol creatinine. Homocysteine test: Homocysteine=38.5 μmol/L	Methylmalonic Aciduria and Homocystinuria CblC type (OMIM # 277400)	Vitamin B12 injections
GP-M25	M	37+0	2580	AR	*SLC25A13*	c.[1638_1660dup];[852_855del]	Cit=20.13 μmol/L Met=20.63 μmol/L Tyr=117.15 μmol/L	MS/MS: Cit=449.85 μmol/L. Met=261.41 μmol/L. Tyr=256.64 μmol/L Blood test: Bilirubin, direct (conjugated)=40.9 μmol/L. Bilirubin, total=137.4 μmol/L. Bile acids (total)=357.7 μmol/L. Albumin =2.92 g/dL. Alanine transaminase (ALT)=32 U/L. Aspartate transaminase (AST)=85 U/L.	Citrin deficiency (OMIM # 605814)	Treated by lactose-free baby formula, fat-soluble vitamins (A and D), and ursodeoxycholic acid

Abbreviation: M, male. F, female. AR, autosomal recessive. MS/MS, tandem mass spectrometry. GC/MS, gas chromatography-mass spectrometer. AAs/OAs/FAODs, amino acid disorders/organic acid disorders/fatty acid oxidation disorders.

SI conversion factor: To convert Albumin to g/L, multiply by 10; to convert ALT to μkat/L, multiply by 0.0167; to convert AST to μkat/L, multiply by 0.0167.

eTable 5. Indication-based analysis of six AAs/OAs/FAODs patients undetected by the gene panel

ID	Disease	Initial gNBS screen	Indication-based analysis	Note[d]
GC-01[a]	Short Chain Acyl-CoA Dehydrogenase Deficiency	*ACADS*:c.[322G>A];[=]	*ACADS*:c.[322G>A];[779G>T]	c.322G>A was classified as LP. c.779G>T was upgraded from VUS (PM2; PP3) to LP (PM2; PP3; PM3; PP4).
GC-02[a]	Maple Syrup Urine Disease, type II	*DBT*:c.[75_76delAT];[=]	*DBT*:c.[75_76delAT];[1359_1360delAG]	c.75_76delAT was classified as LP. c.1359_1360delAG was upgraded from VUS (PVS1_Moderate; PM2) to LP (PVS1_Moderate; PM2; PM3; PP4).
GC-03[b]	Primary Carnitine Deficiency	*SLC22A5*:c.[1400C>G];[=]	*SLC22A5*:c.[1400C>G];[621G>T]	c.1400C>G was classified as P. c.621G>T was classified as VUS.
GN-01[b]	Isobutyryl-CoA dehydrogenase deficiency	No variant reported	*ACAD8*:c.[473A>G];[1165C>T]	Both variants were classified as VUS.
GN-02[b]	Carnitine palmitoyltransferase II deficiency	No variant reported	*CPT2*:c.[125C>T];[1613delA]	Both variants were classified as VUS.
GN-03[c]	Multiple Acyl-CoA Dehydrogenase Deficiency	No variant reported	Negative	Variants were identified by exome sequencing: NM_000126.4(*ETFA*):c.[659delC];[365G>A]. Both variants were classified as LP. *ETFA* was not in the gene panel.

Abbreviation: AAs/OAs/FAODs, amino acid disorders/organic acid disorders/fatty acid oxidation disorders. P, pathogenic. LP, likely pathogenic. VUS, variant of uncertain significance.

[a] Two infants had genetic results that were indicative and diagnostic as two variants were upgraded from uncertain significance to likely pathogenic because of the associated phenotypic data.

[b] Three infants had genetic results that were indicative but not diagnostic (e.g., one pathogenic/likely pathogenic with one uncertain significance variant, or two uncertain significance variants in related genes).

[c] The infant was affected by multiple Acyl-CoA Dehydrogenase deficiency identified by MS/MS screening. Indication-based analysis did not identify candidate variants. Exome sequencing was further performed on the sample and identified two likely pathogenic variants *in trans* configuration in ETFA (not in the gene panel).

[d] Variants were interpreted based on guidelines recommended by the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (Richards et al. 2015)

eTable 6. Characteristics of 39 patients affected by disorders screened solely by gNBS

#	ID	Sex	Disease	Type	Gene	Variant	Inheritance	Origin	Age of diagnosis	Confirmatory test results	Clinical management
1	GP-E02	M	Wilson Disease	Other metabolic disorder	*ATP7B*	c.[2828G>A];[2755C>G]	AR	Inherited	2 m	Ceruloplasmin=0.13 g/L	Referred to specialists for further treatment
2	GP-E03	F	Wilson Disease	Other metabolic disorder	*ATP7B*	c.[2975C>T];[2333G>T]	AR	Inherited	6 m	Ceruloplasmin=0.05 g/L Copper<0.1 μmol/L	Referred to specialists for further treatment
3	GP-E05	F	Cerebrotendinous xanthomatosis	Other metabolic disorder	*CYP27A1*	c.[1415G>C];[1415G>C]	AR	Inherited	2 m	High cholestanol levels; Blood lipids=4.9 mmol/L	Treatment with gooseoxycholic acid. The liver function test showed slightly abnormal, the bile acid profiles were recovered. The infants had normal growth and development by the date of follow-up.
4	GP-E06	M	Glycogen Storage Disease Type Ia	Glycolipid metabolic disorder	*G6PC*	c.[113A>T];[648G>T]	AR	Inherited	2 m	Alanine transaminase (ALT)=54 U/L Aspartate transaminase (AST)=86 U/L Glucose=4.55 mmol/L Lactic acid=5.9 mmol/L Blood lipids=2.33 mmol/L Liver ultrasound: Hepatomegaly (HP:0002240), abnormal hepatic echogenicity (HP:0031142)	Diet plan to control blood sugar
5	GP-E07	F	Krabbe Disease	Lysosomal storage disorder	*GALC*	c.[1901T>C];[1592G>A]	AR	Inherited	2 m	GALC=1.6 nmol/17h/mg protein	Referred to specialists for further treatment
6	GP-E09	F	Krabbe Disease	Lysosomal storage disorder	*GALC*	c.[1901T>C];[1901T>C]	AR	Inherited	5 m	GALC=0.17 μmol/L/h protein	Referred to specialists for further treatment
7	GP-E11	M	Fabry Disease	Lysosomal storage disorder	*GLA*	c.[335G>A];[0]	XLR	Inherited	1 m	GLA=1.05 nmol/h/mg protein	Active surveillance
8	GP-E12	M	Fabry Disease	Lysosomal storage disorder	*GLA*	c.[640-801G>A];[0]	XLR	Inherited	8 m	GLA=12.8 nmol/h/mg protein	Active surveillance
9	GP-E13	M	Fabry Disease	Lysosomal storage disorder	*GLA*	c.[717A>G];[0]	XLR	Unknown	4 m	GLA=0.47 uM/h	Active surveillance

10	GP-E14	F	Niemann-Pick Disease, type A/B	Lysosomal storage disorder	*SMPD1*	c.[518delT];[995C>G]	AR	Inherited	2 m	ASM=0.62 μmol/L/h	Active surveillance
11	GP-E15	F	Niemann-Pick Disease, type A/B	Lysosomal storage disorder	*SMPD1*	c.[995C>G];[995C>G]	AR	Inherited	1 m	ASM=0.63 μmol/L/h	Active surveillance
12	GP-E16	M	Niemann-Pick Disease, type A/B	Lysosomal storage disorder	*SMPD1*	c.[995C>G];[995C>G]	AR	Inherited	4 m	ASM=0.62 μmol/L/h	Active surveillance
13	GP-E17	M	Niemann-Pick Disease, type A/B	Lysosomal storage disorder	*SMPD1*	c.[995C>G];[995C>G]	AR	Inherited	3 m	ASM=0.36 μmol/L/h	Active surveillance
14	GP-E19	F	Niemann-Pick Disease, type A/B	Lysosomal storage disorder	*SMPD1*	c.[995C>G];[995C>G]	AR	Inherited	3 m	ASM=0.80 μmol/L/h	Active surveillance
15	GP-E20	F	X-Linked Hypophosphatemia	Other metabolic disorder	*PHEX*	c.[1285_1288delGAAG];[1285_1288=]	XL	Inherited	4 m	Phosphorus level=0.88 mmol/L Blood calcium=2.44 mmol/L AKP/ALP=613 U/L Imaging: Osteoporosis (HP:0000939), Abnormality of the metaphysis (HP:0000944), Abnormal diaphysis morphology (HP:0000940).	Treatment with calcitriol and phosphates. The therapeutic effect was unknown as she was failed to follow-up further.
16	GP-E24	F	Retinoblastoma	Eye disorder	*RB1*	c.[763C>T];[=]	AD	*de novo*	2 m	Vision examination: Abnormality of ocular smooth pursuit (HP:0000617), Exotropia (HP:0000577); Visual field examination: Abnormal pupillary light reflex (HP:0007695); Ocular fundus examination: Retinal neoplasm (HP:0012777)	Treatment with systemic chemotherapy and laser therapy. Mass in bilateral fundus was smaller, and B-scan ultrasound showed the calcification.
17	GP-E25	M	Duchenne/Becker Muscular Dystrophy	Neuromuscular disorder	*DMD*	c.[(93+1_94-1)_(960+1_961-1)del];[0] (Exon 3-9 deletion)	XL	Inherited	1 m	CKMM=233 U/L CKMB=7.78 ng/mL	Active surveillance
18	GP-E26	M	Duchenne/Becker Muscular Dystrophy	Neuromuscular disorder	*DMD*	c.[(6912+1_6913-1)_(7309+1_7310-1)del];[0] (Exon 45-50 deletion)	XL	Inherited	1 m	CKMM=11119 U/L CKMB=264 U/L	Active surveillance
19	GP-E27	M	Duchenne/Becker Muscular Dystrophy	Neuromuscular disorder	*DMD*	c.[(6912+1_6913-1)_(7309+1_7310-1)del];[0] (Exon 48-50 deletion)	XL	*de novo*	2 m	CKMM=2598 U/L CKMB=164 U/L	Active surveillance

20	GP-E28	M	Duchenne/Becker Muscular Dystrophy	Neuromuscular disorder	*DMD*	c.[(6912+1_6913-1)_(8027+1_8028-1)del];[0] (Exon 48-54 deletion)	XL	*de novo*	6 m	CKMM=9192 U/L CKMB=97.29 ng/mL	Active surveillance
21	GP-E30	M	Duchenne/Becker Muscular Dystrophy	Neuromuscular disorder	*DMD*	c.[(7098+1_7099-1)_(8027+1_8028-1)];[0] (Exon 49-54 deletion)	XL	Unknown	1 m	CKMM=31455 U/L CKMB=786.6 U/L	Active surveillance
22	GP-E33	M	Duchenne/Becker Muscular Dystrophy	Neuromuscular disorder	*DMD*	c.[(7660+1_7661-1)_(8027+1_8028-1)del)];[0] (Exon 52-54 deletion)	XL	*de novo*	1 m	CKMM=4832 U/L CKMB=176 U/L	Active surveillance
23	GP-E34	F	Early Infantile Epileptic Encephalopathy 6	Neuromuscular disorder	*SCN1A*	c.[302G>A];[=]	AD	*de novo*	4 m	Electroencephalographic: abnormal discharge Magnetic Resonance Imaging: normal	Unknown due to lost to follow-up after clinical diagnosis
24	GP-E36	F	α-Thalassemia	Blood disorder	*HBA1/HBA2*	g.[223300_227103del];[199800_233300del] (-3.7; --THAI)	*AR*	Unknown	15 m	Hb=79 g/L; MCV=49.2 fL; MCH=14.9 pg RBC=5.31 10^12/L	Active surveillance
25	GP-E37	M	α-Thalassemia	Blood disorder	*HBA1/HBA2*	c.427T>C and g.215400_234700del (--SEA) *in trans*	AR	Inherited	6 m	Hb=85 g/L; MCV=64.1 fL; MCH=19.2 pg RBC=4.43 10^12/L	Active surveillance
26	GP-E38	M	α-Thalassemia	Blood disorder	*HBA1/HBA2*	c.369C>G and g.215400_234700del (--SEA)	*AR*	Unknown	10 m	Hb=102 g/L; MCV=59.4 fL; MCH=18.2 pg RBC=5.59 10^12/L HbA2=2.1% ; HbF=4.3%	Active surveillance
27	GP-E39	M	α-Thalassemia	Blood disorder	*HBA1/HBA2*	g.[223300_227103del];[215400_234700del] (-3.7; --SEA)	AR	Inherited	9 m	Hb=95 g/L; MCV=50.8 fL; MCH=14.7 pg RBC=6.46 10^12/L HbA=95.6%; HbA2=1.4%; HbF=3.0%	Active surveillance
28	GP-E40	F	α-Thalassemia	Blood disorder	*HBA1/HBA2*	g.[223300_227103del];[215400_234700del] (-3.7; --SEA)	AR	Inherited	7 m	Hb=70 g/L; MCV=50.9 fl; MCH=14.6 pg RBC=4.79 10^12/L HbA=95.8%; HbA2=1.5%; HbF=2.7%	Active surveillance
29	GP-E41	M	α-Thalassemia	Blood disorder	*HBA1/HBA2*	g.[223300_227103del];[215400_234700del] (-3.7; --SEA)	AR	Unknown	6 m	Hb=93 g/L; MCV=44.9 fL; MCH=14.6 pg	Active surveillance

30	GP-E42	M	α-Thalassemia	Blood disorder	*HBA1/HBA2*	g.[223300_227103del];[215400_234700del] (-3.7; --SEA)	AR	Inherited	11 m	Hb=88 g/L; MCV=48.3 fL; MCH=14.7 pg RBC=5.98 $10^{\wedge 12}$/L HbF=4.3%; HbA=94.2%; HbA2=1.5%	Active surveillance
31	GP-E43	M	α-Thalassemia	Blood disorder	*HBA1/HBA2*	g.[223300_227103del];[215400_234700del] (-3.7; --SEA)	AR	Unknown	8 m	Hb=90 g/L; MCV=49.7 fL; MCH=15.1 pg	Active surveillance
32	GP-E44	F	α-Thalassemia	Blood disorder	*HBA1/HBA2*	g.[223300_227103del];[215400_234700del] (-3.7; --SEA)	AR	Unknown	6 m	Hb=90 g/L; MCV=49.7 fL; MCH=15.3 pg	Active surveillance
33	GP-E46	F	α-Thalassemia	Blood disorder	*HBA1/HBA2*	g.[219817_(223755_224074)del];[215400_234700del] (-4.2; --SEA)	AR	Inherited	5 m	Hb=82 g/L; MCV=51.8 fL; MCH=15.4 pg RBC=5.33 $10^{\wedge 12}$/L HbF=3.9%; HbA=93.3%; HbA2=1.1%; HbH=1.7%	Active surveillance
34	GP-E47	M	α-Thalassemia	Blood disorder	*HBA2*	c.369C>G and g.215400_234700del (--SEA)	AR	Unknown	6 m	Hb=103 g/L; MCV=57.8 fL; MCH=22.5 pg	Active surveillance
35	GP-E48	M	α-Thalassemia	Blood disorder	*HBA2*	c.369C>G and g.215400_234700del (--SEA) *in trans*	AR	Inherited	6 m	Hb=114 g/L; MCV=56.7 fL; MCH=17.9 pg	Active surveillance
36	GP-E49	F	β-Thalassemia	Blood disorder	*HBB*	c.[126_129delCTTT];[-100G>A]	AR	Inherited	10 m	Hb=102 g/L; MCV=54 fL ; MCH=17.6 pg	Active surveillance
37	GP-E50	F	β-Thalassemia	Blood disorder	*HBB*	c.[316-197C>T];[316-197C>T]	AR	Unknown	6 m	Imaging: hepatosplenomegaly (HP:0001433). Blood transfusion.	Treatment with blood transfusion at nine months old.
38	GP-E51	F	β-Thalassemia	Blood disorder	*HBB*	c.[316-197C>T];[316-197C>T]	AR	Unknown	6 m	Imaging: hepatosplenomegaly (HP:0001433). Blood transfusion.	Treatment with blood transfusion at nine months old.
39	GP-E54	F	Tuberous Sclerosis 1	Multisystem disorder	*TSC1*	c.[2503-1G>C];[=]	AD	Unknown	2 m	Ultrasound: Cardiac rhabdomyoma (HP:0009729)	Unknown due to lost to follow-up after clinical diagnosis

F, female. M, male. AR, autosomal recessive. AD, autosomal dominant. XLR, X-linked recessive. m, month.

SI conversion factor: To convert ALT to μkat/L, multiply by 0.0167; to convert AST to μkat/L, multiply by 0.0167; to convert CKMM or CKMB to μkat/L, multiply by 0.0167.

eTable 7. Characteristics of 36 unaffected cases

#	ID	Sex	Disease	Gene	Inheritance	Variant	Screening panel	Initial tNBS screen	Confirmatory Test
1	GP-E01	F	Hypophosphatasia	*ALPL*	AR	c.[529G>A];[979T>C]	Screened by gNBS	Not applicable	Serum phosphorus=2.25 mmol/L Blood calcium=2.46 mmol/L AKP/ALP=213 U/L
2	GP-E08	M	Krabbe disease	*GALC*	AR	c.1901T>C(;)658C>T	Screened by gNBS	Not applicable	GALC=30.56 nmol/17h/mg protein
3	GP-E22	M	Glycogen storage disease type IXd	*PHKA1*	XLR	c.[2606+1G>A];[=]	Screened by gNBS	Not applicable	Alanine transaminase (ALT)=28 U/L Aspartate transaminase (AST)=33 U/L Glucose=4.55 mmol/L Total cholesterol (TG)=4.94 mmol/L
4	GP-E23	M	Glycogen storage disease type IXa	*PHKA2*	XLR	c.[165G>A];[=]	Screened by gNBS	Not applicable	Alanine transaminase (ALT)=17 U/L Aspartate transaminase (AST)=13 U/L Glucose=5.75 mmol/L Total cholesterol (TG)=3.58 mmol/L
5	GP-E35	F	Spinal Muscular Atrophy	*SMN1*	AR	c.[(833+1_834-1)del)];[(833+1_834-1)del)]	Screened by gNBS	Not applicable	With a normal physical examination. Genetic testing revealed 3 copies of SMN2 gene.
6	GP-E52	F	Gitelman syndrome	*SLC12A3*	AR	c.[1456G>A];[c.2548+253C>T]	Screened by gNBS	Not applicable	serum potassium=4.05mmol/L Blood calcium=2.71mmol/L
7	GP-G001	M	Glucose-6-Phosphate Dehydrogenase Deficiency	*G6PD*	XLD	c.[1388G>A];[=]	Screened by tNBS-fluorometric assay	6.1 U/gHb	G6PD/6PGD=1.17
8	GP-G002	M	Glucose-6-Phosphate Dehydrogenase Deficiency	*G6PD*	XLD	c.[1376G>T];[=]	Screened by tNBS-fluorometric assay	3.7 U/gHb	G6PD activity=3.71 U/gHb
9	GP-M04	M	2-Methylbutyryl Glycinuria	*ACADSB*	AR	c.275C>G(;)746delC	Screened by tNBS-MS/MS	C5=0.33 uM C5/C2=0.03	MS/MS: C5=0.21 uM C5/C2=0.01
10	GP-M11	M	Ornithine Transcarbamylase Deficiency	*OTC*	XL	c.[830G>A];[0]	Screened by tNBS-MS/MS	Cit=9.58 umol/L	MS/MS: Cit=9.75 umol/L

11	GP-M12	M	Phenylketonuria	*PAH*	AR	c.[1068C>A];[510T>A]	Screened by tNBS-MS/MS	Phe=134.42 umol/L Phe/Tyr=1.43	MS/MS: Phe=153.43 umol/L Phe/Tyr=1.05 Urine pterins analysis: N=0.88 mmol/molCr; B=0.7 mmol/molCr; B% (B/(N+B))=44.3; DHPR activity: DHPR%=65.6%.
12	GP-M16	M	Phenylketonuria	*PAH*	AR	c.[516G>T];[516G>T]	Screened by tNBS-MS/MS	Phe=117.21 umol/L Phe/Tyr=1.217	MS/MS: Phe=58.93 umol/L; Phe/Tyr=1.41
13	GP-M17	F	Phenylketonuria	*PAH*	AR	c.[728G>A];[532G>A]	Screened by tNBS-MS/MS	PHE:129.02umol/L PHE/TYR=2.07	MS/MS: Phe=153.29 umol/L; Phe/Tyr=1.05
14	GP-M18	M	Phenylketonuria	*PAH*	AR	c.[755G>A];[516G>T]	Screened by tNBS-MS/MS	Phe=79.19 umol/L Phe/Tyr=1.41	MS/MS: Phe=62.72 umol/L; Phe/Tyr=1.11
15	GP-M19	M	Hyperprolinemia type I	*PRODH*	AR	c.1322T>C(;)273+1G>C	Screened by tNBS-MS/MS	Pro=376.00 umol/L	MS/MS: Pro=324.00 umol/L
16	GP-C07	F	Thyroid dyshormonogenesis 6	*DUOX2*	AR	c.[1588A>T];[1588A>T]	Screened by tNBS-TSH test	TSH=7.64 mIU/L	TSH=5.95 mIU/L; FT4=1.21 ng/dL; FT3=328.57 pg/dL
17	GP-C08	M	Thyroid dyshormonogenesis 6	*DUOX2*	AR	c.[1708C>T];[2635G>A]	Screened by tNBS-TSH test	TSH=8.32 mIU/L	TSH=3.80 mIU/L; FT4=1.70 ng/dL; FT3=411.69 pg/dL
18	GP-C11	F	Thyroid dyshormonogenesis 6	*DUOX2*	AR	c.[1883delA];[3616G>A]	Screened by tNBS-TSH test	TSH=2.58 mIU/L	TSH=4.12 mIU/L; FT4=0.94 ng/dL; FT3=373.96 pg/dL
19	GP-C12	M	Thyroid dyshormonogenesis 6	*DUOX2*	AR	c.2048G>T(;)1588A>T	Screened by tNBS-TSH test	TSH=2.10 mIU/L	TSH=1.68 mIU/L
20	GP-C13	F	Thyroid dyshormonogenesis 6	*DUOX2*	AR	c.2048G>T(;)1588A>T	Screened by tNBS-TSH test	TSH=5.12 mIU/L	TSH=3.34 mIU/L
21	GP-C17	F	Thyroid dyshormonogenesis 6	*DUOX2*	AR	c.2635G>A(;)2048G>T	Screened by tNBS-TSH test	TSH=1.97 mIU/L	TSH=1.01 mIU/L; FT4=1.03 ng/dL; FT3=322.08 pg/dL
22	GP-C18	M	Thyroid dyshormonogenesis 6	*DUOX2*	AR	c.[2635G>A];[2048G>T]	Screened by tNBS-TSH test	TSH=1.85 mIU/L	TSH=2.60 mIU/L; FT4=1.19 ng/dL; FT3=502.60 pg/dL
23	GP-C20	F	Thyroid dyshormonogenesis 6	*DUOX2*	AR	c.2654G>T(;)1588A>T	Screened by tNBS-TSH test	TSH=5.24 mIU/L	TSH=3.72 mIU/L; FT4=0.9 ng/dL; FT3=306.49 pg/dL
24	GP-C21	M	Thyroid dyshormonog	*DUOX2*	AR	c.[2654G>T];[1588A>T]	Screened by tNBS-TSH	TSH=2.38 mIU/L	TSH=4.11 mIU/L; FT4=1.21 ng/dL; FT3=459.09 pg/dL

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25	GP-C22	M	Thyroid dyshormonogenesis 6	*DUOX2*	AR	c.2654G>T(;)1708C>T	Screened by tNBS-TSH test	TSH=2.58 mIU/L	TSH=3.65 mIU/L; FT4=1.56 ng/dL; FT3=410.39 pg/dL
26	GP-C23	F	Thyroid dyshormonogenesis 6	*DUOX2*	AR	c.2654G>T(;)1708C>T	Screened by tNBS-TSH test	TSH=3.11 mIU/L	TSH=3.16 mIU/L; FT4=1.12 ng/dL; FT3=387.01 pg/dL
27	GP-C24	F	Thyroid dyshormonogenesis 6	*DUOX2*	AR	c.[2654G>T];[2048G>T]	Screened by tNBS-TSH test	TSH=2.63 mIU/L	TSH=1.44 mIU/L; FT4=1.07 ng/dL;
28	GP-C25	M	Thyroid dyshormonogenesis 6	*DUOX2*	AR	c.2654G>T(;)2403_2406dupCCTG	Screened by tNBS-TSH test	TSH=8.01 mIU/L	TSH=1.38 mIU/L; FT4=1.04 ng/dL; FT3=427.27 pg/dL
29	GP-C27	F	Thyroid dyshormonogenesis 6	*DUOX2*	AR	c.[2654G>T];[605_621delAGCTGGCGTCGGGGCCC]	Screened by tNBS-TSH test	TSH=2.31 mIU/L	TSH=2.13 mIU/L; FT4=1.07 ng/dL; FT3=414.29 pg/dL
30	GP-C30	M	Thyroid dyshormonogenesis 6	*DUOX2*	AR	c.[2654G>T];[2654G>T]	Screened by tNBS-TSH test	TSH=7.44 mIU/L	TSH=2.19 mIU/L; FT4=1.36 ng/dL; FT3=466.88 pg/dL
31	GP-C36	M	Thyroid dyshormonogenesis 6	*DUOX2*	AR	c.[3540T>A];[2654G>T]	Screened by tNBS-TSH test	TSH=11.31 mIU/L	TSH=4.61 mIU/L; FT4=1.15 ng/dL; FT3=399.35 pg/dL
32	GP-C37	F	Thyroid dyshormonogenesis 6	*DUOX2*	AR	c.3616G>A(;)2654G>T	Screened by tNBS-TSH test	TSH=8.66 mIU/L	TSH=5.06 mIU/L; FT4=0.86 ng/dL; FT3=392.86 pg/dL
33	GP-C38	M	Thyroid dyshormonogenesis 6	*DUOX2*	AR	c.[3632G>A];[2048G>T]	Screened by tNBS-TSH test	TSH=0.71 mIU/L	TSH=4.76 mIU/L;FT4=1.54 ng/dL; FT3=444.81 pg/dL
34	GP-C39	F	Thyroid dyshormonogenesis 6	*DUOX2*	AR	c.[3632G>A];[602_603insG]	Screened by tNBS-TSH test	TSH=3.42 mIU/L	TSH=2.43 mIU/L;FT4=1.43 ng/dL; FT3=354.55 pg/dL
35	GP-C41	F	Thyroid dyshormonogenesis 6	*DUOX2*	AR	c.[3693+1G>T];[2654G>T]	Screened by tNBS-TSH test	TSH=9.93 mIU/L	TSH=1.99 mIU/L
36	GP-C42	F	Thyroid dyshormonogenesis 6	*DUOX2*	AR	c.[3693+1G>T];[3632G>A]	Screened by tNBS-TSH test	TSH=3.95 mIU/L	TSH=2.11 mIU/L; FT4=1.38 ng/dL; FT3=501.30 pg/dL

SI conversion factor: To convert ALT to μkat/L, multiply by 0.0167; to convert AST to μkat/L, multiply by 0.0167; to convert CKMM or CKMB to μkat/L, multiply by 0.0167; to convert FT3 to pmol/L, multiply by 0.0154; to convert FT4 to pmol/L, multiply by 12.871; to convert T4 to nmol/L, multiply by 12.871.