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Expanded newborn screening for inherited metabolic disorders and genetic characteristics in a southern Chinese population

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ABSTRACT

To evaluate the incidence, disease spectrum, and genetic characteristics of inherited metabolic disorders (IMDs) of newborns in Quanzhou area, China. We analyze the expanded newborn screening results of IMDs detected by tandem mass spectrometry (MS/MS) during 5 years. Suspected positive patients were diagnosed through next-generation sequencing and validated by Sanger sequencing. In addition, multiplex ligation-dependent probe amplification technology has also been applied to assist in diagnosis of diseases with deletion or duplication mutations. A total of 364,545 newborns were screened, 130 IMDs were identified yielding an incidence of 1:2804. In addition, 9 cases of maternal disorders were also identified by our MS/MS newborn screening program. There were 42 newborns with amino acid disorders (1:8680), 39 with organic acid disorders (1:9347), and 49 with fatty acid oxidation disorders (1:7440). Unlike other studies, our study indicated that fatty acid oxidation disorder has the highest proportion (37.7%), particularly primary carnitine deficiency (PCD) with incidence up to 1:10,126 was the most common disorder in the region. The recurrent mutations of relatively common diseases like PCD, phenylalanine hydroxylase deficiency, short-chain acyl-CoA dehydrogenase deficiency, citrin deficiency, glutaric acidemia type I, isobutyryl-CoA dehydrogenase deficiency, and multiple acyl-CoA dehydrogenase deficiency in this region were also clearly elucidated. Therefore, our data indicated that IMDs are never uncommon in Quanzhou, the disease spectrum and genetic backgrounds were clearly elucidated, contributing to the treatment and prenatal genetic counseling of these disorders in this region.

1. Introduction

Inherited metabolic disorders (IMDs) are a group of genetic disorders caused by defective enzymes, cofactors, or transporters in metabolic pathways. Many IMDs have serious consequences to the affected neonate, including lethargy, irreversible mental retardation, physical handicaps, coma, and even death. Albeit individually rare, IMDs are collectively numerous. If early accurate diagnosis was made, many of these severe complications can be prevented or significantly reduced in a considerable number of diseases. Newborn screening (NBS) is a valuable preventive health measure for early diagnosis, which has been shown to be diagnostically effective and economically efficient. In particular, the introduction of tandem mass spectrometry (MS/MS) technology enabled the simultaneous determination and quantification of several amino acids and acylcarnitines in a single test [1]. Consequently, MS/MS has been applied wildly for the screening of IMDs that include disorders of amino acids, organic acidurias, and fatty acid oxidiation disorders [2]. In China, MS/MS based NBS was launched in

Abbreviations: IMD, Inherited metabolic disorder; NBS, Newborn screening; MS/MS, Tandem mass spectrometry; DBS, Dried blood spot; NGS, Next-generation sequencing; PCR, Polymerase chain reaction; MLPA, Multiplex ligation-dependent probe amplification; NKH, Non-ketotic hyperglycinemia; PA, Propionic academia; PPV, Positive predictive value; PAHD, Phenylalanine hydroxylase deficiency; CD, Citrin deficiency; UCD, Urea cycle disorder; BH4D, tetrahydrobiopterin deficiency; MAT I/III, Methionine adenosyltransferase I/III; OTCD, Ornithine carbamoyltransferase deficiency; SBCADD, Short/branched chain acyl-CoA dehydrogenase deficiency; GA-I, Glutaric acidemia type I; IBDHD, Isobutyryl-CoA dehydrogenase deficiency; 3-MCCD, 3-methylcrotonyl CoA carboxylase dedicency; IVA, Isovaleric acidemia; MMA, Methylmalonic academia; FAODs, Fatty acid oxidation disorders; PCD, Primary carnitine deficiency; MADD, Multiple acyl-CoA dehydrogenase deficiency; SCADD, Short-chain acyl-CoA dehydrogenase deficiency; VLCADD, Very-long-chain acyl-CoA dehydrogenase deficiency; CPT-I, Carnitine palmitoyl transferase-I deficiency; PTPS, 6-Pyruvoyl-tetrahydropterin synthase; LSM, Lipid storage myopathies; C2, Aceylcarnitine; C3, Propionylcarnitine; C5, Isovalerylcarnitine/2-methylbutyrylcarnitine; C5DC, Glutarylcarnitine; C5OH, 3-Hydroxyisovalerylcarnitine/3-hydroxy-2-methylbutyrylcarnitine; C0, Free carnitine; C8, Octanoylacrnitine; C10, Decanoylcarnitine; C12, Dodecanoylcarnitine; C14, Myristoylcarnitine

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2004 and the average incidence of IMDs was 1:3795 in a pilot study [3]. However, subsequent studies have shown that the incidence and disease spectrum of IMDs varied greatly in different regions of China, especially between the south and north. The expanded NBS program incorporating MS/MS for newborns was begun in Quanzhou since January 2014. Herein, we report our five-year experience with MS/MS expanded NBS, which include the incidence, disease spectrum, and genetic characteristics of IMDs.

2. Materials and methods

2.1. Newborn screening

In total, 364,545 infants (209,136 males and 155,409 females) born in Quanzhou, China, between January 2014 to November 2018 were enrolled for expanded NBS by MS/MS. Blood samples were collected by heel stick and spotted on Whatman 903 filter paper. Blood collection for NBS is recommended between 3 and 7 days of life. Dried blood spot (DBS) samples were delivered by cold-chain transportation to the NBS center of Quanzhou Maternity and Children's Hospital within 5 days. DBS samples were pre-processed following the instruction of NeoBase™ non-derivatized MS/MS kit (PekinElmer, USA), then an ACQUITY TQD mass spectrometer (Waters, Milford, MA, USA) was used to analyze amino acids and aclycarnitines in DBS samples. The cutoff values were initially set by reference to the worldwide collaborative project and other screening centers [4,5], and were adjusted over time as the number of samples increased. Newborns with clear aberrant initial screening results were immediately referred to confirmatory tests, including biochemical and genetic analysis. Newborns with mild initial screening results were recalled for repeated test, if the second test was still positive, the patient was referred to confirmatory tests. This study was approved by the Ethical Committee of Quanzhou Maternity and Children's Hospital. Written informed consents were obtained from all the infants' patients.

2.2. Genetic analysis

Genetic analysis was performed by Genuine Diagnostics Company (Hangzhou, Zhejiang, China). Briefly, DBS or peripheral whole blood of suspected positive patients were referred to laboratory, and genomic DNA was extracted using Qiagen Blood DNA mini kits (Qiagen, Hilden, Germany) according to the manufacturer's protocol. DNA samples of the probands were quantified using a Qubit® dsDNA HS Assay Kit (Invitrogen, Carlsbad, CA, USA) and then were taken for next-generation sequencing (NGS). The target sequencing panel of 306 known genes involved in inherited metabolic disorders were applied. Genomic DNA was sheared to an approximate mean fragment length of 200-base pair (bp) using the Covaris LE220 (Covaris, Woburn, MA). Sheared DNA was used for library preparation of targeted regions by multiplex polymerase chain reaction (PCR). The library concentration and amplicon size were determined using an Agilent High Sensitivity DNA Kit (Agilent, Santa Clara, CA, USA). The prepared sample libraries were sequenced by Illumina NextSeq 500 platform (Illumina Inc., San Diego, CA, USA) in paired end mode, generating 150-bp paired end reads and the data were analyzed by NextSeq 500 Reporter. All variants identified by NGS were further validated by Sanger sequencing of the patients and available their parents. In addition, multiplex ligation-dependent probe amplification (MLPA) technology has also been applied to assist in diagnosis of diseases where NGS did not find pathogenic mutations but suspected to have deletion or duplication mutations, such as non-ketotic hyperglycinemia (NKH) and propionic academia (PA).

2.3. Diagnosis

Definitive diagnosis was made by metabolic disease specialists based on the patients' biochemical performance, genetic mutations, and clinical symptoms. Only patients diagnosed by genetic analysis were included in this study.

Table 1
Positive cases detected by MS/MS newborn screening.

Disorders	Positive cases	Frequency
Amino acid disorders	**42**	**1:8680**
Phenylalanine hydroxylase deficiency (PAHD)	14	1:26,039
Citrin deficiency (CD)	10	1:36,455
Tetrahydrobiopterin deficiency (BH4D)	4	1:91,136
Non-ketotic hyperglycinemia (NKH)	3	1:121,515
Methionine adenosyltransferase I/III (MAT I/III) deficiency	3	1:121,515
Hyperprolinemia	1	1:364,545
Urea cycle disorders	**7**	**1:52,078**
Citrullinemia type I (CTLN1)	2	1:182,273
Argininosuccinate layse deficiency (ASLD)	2	1:182,273
Ornithine transcarbamylase deficiency (OTCD)	2	1:182,273
Carbamoylphosphate synthetase deficiency (CPSD)	1	1:364,545
Organic acid disorders	**39**	**1:9347**
Short/branched chain acyl-CoA dehydrogenase deficiency (SBCADD)	12	1:30,379
Glutaric acidemia type I (GA-I)	7	1:52,078
Isobutyryl-CoA dehydrogenase deficiency (IBDHD)	6	1:60,758
3-methylcrotonyl CoA carboxylase dedicency (3-MCCD)	5	1:72,909
Isovaleric acidemia (IVA)	4	1:91,136
Methylmalonic academia (MMA)	3	1:121,515
Propionic academia (PA)	2	1:182,273
Fatty acid oxidation disorders	**49**	**1:7440**
Primary carnitine deficiency (PCD)	36	1:10,126
Multiple acyl-CoA dehydrogenase deficiency (MADD)	5	1:72,909
Short-chain acyl-CoA dehydrogenase deficiency (SCADD)	4	1:91,136
Very-long-chain acyl-CoA dehydrogenase deficiency (VLCAD)	3	1:121,515
Carnitine palmitoyl transferase-I deficiency (CPT I)	1	1:364,545
Total numbers	130	1:2804
Total numbers including materal disorders	139	1:2623

3. Results

3.1. Newborn screening

Among the 364,545 screened newborns, 4897 newborns were suspected positive in the first screening, yielding a positive ratio of 1.34%. Among which 4809 were successfully recalled for retesting (98.2%), and 130 patients were finally confirmed with IMDs, the positive predictive value (PPV) was 2.7%. Twenty-two types of IMDs were diagnosed in 130 confirmed cases, the overall IMDs detection incidence was 1:2804. Of these, 42 (32.3%) newborns with amino acid disorders, 39 (30%) with organic acid disorders, and 49 (37.7%) with fatty acid oxidation disorders. The incidences of amino acid disorders, organic acid disorders, and fatty acid oxidation disorders were 1:8680, 1:9347, and 1:7440, respectively. In addition, 9 cases of maternal disorders were also identified by our MS/MS NBS program. The overall incidence reached 1:2623 when all these maternal disorders were included in statistical analysis (Tables 1 and 2).

3.2. Amino acid disorders

Totally ten types of amino acid disorders were detected, phenylalanine hydroxylase deficiency (PAHD) was the most common disorder (14/42, 33.3%), followed by citrin deficiency (CD, 10/42, 23.8%), urea cycle disorders (UCDs, 7/42, 16.7%), tetrahydrobiopterin deficiency (BH4D, 4/42, 9.5%), NKH (3/42, 7.1%), and methionine adenosyltransferase I/III (MAT I/III) deficiency (3/42, 7.1%). Hyperprolinemia were relatively rare. The concentrations of phenylalanine and the

Table 2
Biochemical and genetic characteristics of nine patients with maternal disorders.

No.	Abnormal parameter and concentration (μmol/L)[a]	Disorders	Affected gene	Genotype	
				Allele 1	Allele 2
1	C5DC: 1.07, C0: 4.0	Glutaric acidemia type I	*GCDH*	c.1244-2A > C	c.1244-2A > C
2	C5DC: 0.76, C0: 2.46	Glutaric acidemia type I	*GCDH*	c.1063C > T (p.R355C)	c.769C > T (p.R257W)
3	C50H: 20.39, C50H/C0: 5.66, C5OH/C8: 2039, C0: 3.6	3-methylcrotonyl CoA carboxylase dedicency	*MCCC2*	c.1367C > T (p.A456V)	**c.1538 T > C (p.F513S)**[b]
4	C50H: 16.19, C50H/C0: 4.1, C5OH/C8: 1619, C0: 3.95	3-methylcrotonyl CoA carboxylase dedicency	*MCCC1*	c.1331G > A (p.R444H)	c.1331G > A (p.R444H)
5	C0: 2.21	Primary carnitine deficiency	*SLC22A5*	c.760C > T (p.R254X)	c.1400C > G (p.S467C)
6	C0: 4.39	Primary carnitine deficiency	*SLC22A5*	c.517delC (p.L173Cfs)	c.797C > T (p.P266L)
7	C0: 2.48	Primary carnitine deficiency	*SLC22A5*	c.760C > T (p.R254X)	c.1400C > G (p.S467C)
8	C0: 1.32	Primary carnitine deficiency	*SLC22A5*	c.51C > G (p.F17 L)	c.1144_1162del (p.V382Cfs*45)
9	C0: 1.69	Primary carnitine deficiency	*SLC22A5*	c.760C > T (p.R254X)	c.51C > G (p.F17 L)

[a] Reference range: C5DC: 0.03–0.3 μmol/L; C5OH: 0.07–0.5 μmol/L, C50H/C0: 0–0.02, C50H/C8: 1.22–18; C0: 9–50 μmol/L.
[b] Novel mutations are in bold character.

Table 3
Levels of abnormal parameters for different disorders of amino acid metabolism.

Amino acidemias (n = 42)	n (%)	Abnormal parameter	Concentration mean (range) (μmol/L)	Reference range (μmol/L)
Phenylalanine hydroxylase deficiency (PAHD)	14 (33.3)	Phe	155.21 (106.40–646.74)	24–105
		Phe/Tyr	2.49 (0.80–7.92)	0.16–1.25
Citrin deficiency (CD)	10 (23.8)	Cit	110.39 (17.85–217.89)	5.5–30
		Cit/Arg	8.42 (1.64–18.35)	0.35–15
Tetrahydrobiopterin deficiency (BH4D)	4 (9.5)	Phe	375.78 (375.78–761.23)	24–105
		Phe/Tyr	8.51 (6.19–11.86)	0.16–1.25
Non-ketotic hyperglycinemia (NKH)	3 (7.1)	Gly	1303.05 (971.01–1711.17)	165–900
Methionine adenosyltransferase I/III deficiency	3 (7.1)	Met	75.45 (47.88–90.59)	6–40
		Met/Phe	1.79 (1.22–2.33)	0.12–0.73
Citrullinemia type I (CTLN1)	2 (4.9)	Cit	67.78 (45.51–90.05)	5.5–30
		Cit/Arg	25.42 (4.65–46.18)	0.35–15
Argininosuccinate layse deficiency (ASLD)	2 (4.8)	Cit	219.53 (171.18–266.87)	5.5–30
		Cit/Arg	24.85 (19.43–30.26)	0.35–15
Ornithine transcarbamylase deficiency (OTCD)	2 (4.8)	Cit	2.61 (2.55–2.66)	5.5–30
		Orn/Cit	42.49 (32.07–52.91)	2.54–19
Carbamoylphosphate synthetase deficiency (CPSD)	1 (2.4)	Cit	4.13	5.5–30
		Orn/Cit	22.69	2.54–19
Hyperprolinemia	1 (2.4)	Pro	991.04	80–530

phenylalanine/tyrosine ratios in patients with BH4D appear to be significantly higher than those in PAHD. All but one of the patients with CD and UCDs had citrulline concentrations outside the reference range. One patient with CD had false negative screening result was missed by NBS, NBS results showed that the patient had citrulline concentration of 17.85 μmol/L was within the reference range (reference range: 5.5–30 μmol/L), but was identified later due to the patient manifested cholestatic jaundice at 2 months old (Table 3).

Fifteen different mutations in *PAH* gene were detected in 14 patients with PAHD, the most common mutation was c.158G > A (p.R53H) (25%), followed by c.721C > T (p.R242C) (14.3%). The most frequent *PTS* gene mutation in patients with BH4D was c.155A > G (p.N52S), which accounted for 50% of mutant alleles. Six different mutations in *SLC25A13* gene were detected in 10 patients with CD, the most common mutation was c.852_855del (p.M285Pfs*2) (45%), followed by c.615 + 5G > A (p.A206Vfs*7) (20%), c.1638_1660dup (p.A554Gfs*17) (15%), and IVS16ins3kb (p.A584Vfs*2) (10%). For UCDs, both patients with ornithine carbamoyltransferase deficiency (OTCD) were male and carried hemizygous mutations, the rest were homozygous or compound heterozygotes. For MAT I/III deficiency, two patients were heterozygous for the dominant c.791G > A (p.R264H) mutation. For NKH, three patients are siblings and all harbored heterozygous mutation c.2680A > G with deletion of exon 3 (Supplementary file 1: Table S1).

3.3. Organic acid disorders

There were seven types of organic acid disorders, among which the most common disorder was short/branched chain acyl-CoA dehydrogenase deficiency (SBCADD, 12/39, 30.8%), followed by glutaric acidemia type I (GA-I, 7/39, 17.9%), isobutyryl-CoA dehydrogenase deficiency (IBDHD, 6/39, 15.4%), 3-methylcrotonyl CoA carboxylase dedicency (3-MCCD, 5/39, 12.8%), isovaleric academia (IVA, 4/39, 10.3%), methylmalonic academia (MMA, 3/39, 7.7%), and PA (2/39, 5.1%). In addition, 4 cases of maternal disorders (two with GA-I and two with 3-MCCD) were identified in this group. The initial NBS results showed that the concentrations of C5 in patients with SBCADD were slightly elevated, while the ratios of C5/C2 and C5/C3 were within normal range. The concentrations of C5DC in neonatal patients with GA-I seem to higher than those in maternal GA-I cases. In contrast, the concentrations of C5OH and the ratios of C5OH/C0 and C5OH/C8 in neonatal patients with 3-MCCD were significantly lower those in maternal 3-MCCD cases. It is noteworthy that the free carnitine levels of all maternal cases were significantly reduced (Tables 2 and 4).

Eight different mutations in the *ACADSB* gene were identified in 12 patients with SBCADD. The most common mutation was c.1165A > G (33.3%), followed by c.275C > G (20.8%). In patients with GA-I, the predominant mutation in *GCDH* gene was c.1244-2A > C, with an allele frequency of 57.14%. The most common mutation in *ACAD8* gene in patients with IBDHD was c.286G > A (7/14, 50%), which has been reported in our previous article. Three patients with 3-MCCD have compound heterozygous mutations in *MCCC1* and two with mutations

Table 4
Levels of abnormal parameters for different organic acid disorders.

Organic acidemias (n = 39)	n (%)	Abnormal parameter	Concentration mean (range) (μmol/L)	Reference range (μmol/L)
Short/branched chain acyl-CoA dehydrogenase deficiency (SBCADD)	12 (30.8)	C5	0.47 (0.36–0.69)	0.03–0.35
		C5/C2	0.03 (0.02–0.04)	0–0.04
		C5/C3	0.26 (0.17–0.39)	0.02–0.42
Glutaric acidemia type I (GA-I)	7 (17.9)	C5DC	2.37 (1.45–3.79)	0.03–0.3
Isobutyryl-CoA dehydrogenase deficiency (IBDHD)	6 (15.4)	C4	1.25 (0.83–1.94)	0.08–0.45
		C4/C2	0.13 (0.03–0.19)	0–0.03
		C4/C3	0.7 (0.53–1.63)	0.04–0.39
3-methylcrotonyl CoA carboxylase dedicency (3-MCCD)	5 (12.8)	C50H	4.58 (0.95–6.85)	0.07–0.5
		C50H/C0	0.35 (0.03–0.71)	0–0.02
		C50H/C8	134.25 (31.67–342.5)	1.22–18
Isovaleric acidemia (IVA)	4 (10.3)	C5	2.1 (0.4–7.17)	0.03–0.35
		C5/C2	0.21 (0.02–0.7)	0–0.04
		C5/C3	6.95 (0.17–26.56)	0.02–0.42
Methylmalonic academia (MMA)	3 (7.7)	C3	10.2 (7.05–11.95)	0.3–4.5
		C3/C0	0.86 (0.71–1.13)	0.02–0.2
		C3/C2	0.73 (0.61–0.85)	0.01–0.2
Propionic academia (PA)	2 (5.1)	C3	9.54 (7.33–11.74)	0.3–4.5
		C3/C0	0.91 (0.44–1.38)	0.02–0.2
		C3/C2	0.64 (0.5–0.77)	0.01–0.2

in *MCCC2*. The remaining patients were either compound heterozygous or homozygous mutations, no high frequent mutation was found (Supplementary file 2: Table S2).

3.4. Fatty acid oxidation disorders (FAODs)

Only five types of FAODs were detected among the 49 cases. Primary carnitine deficiency (PCD) was the most common disease in this group, which accounted for 73.47%, followed by multiple acyl-CoA dehydrogenase deficiency (MADD, 5/49, 10.20%), short-chain acyl-CoA dehydrogenase deficiency (SCADD, 4/49, 8.16%), and very-long-chain acyl-CoA dehydrogenase deficiency (VLCADD, 3/49, 6.12%). The carnitine palmitoyl transferase-I deficiency (CPT-I) was comparatively rare. In addition, 5 patients with maternal PCD were also picked up. The levels of free carnitine in all patients with PCD were significantly reduced, especially in maternal patients. All but one patients with MADD showed significantly elevated concentrations of C8, C10, C12, and C14. The patient showed only elevated levels of C5 in the initial NBS suggestive of SBCADD or IVA, ultimately, was diagnosed as MADD by genetic testing (Tables 2 and 5).

Totally twenty-one mutations were identified in *SLC22A5* gene in neonatal patients with PCD. The most common mutation was c.760C > T (p.R254X) (21/72, 29.17%), followed by c.1400C > G (p.S467C) (13/72, 18.06%) and c.51C > G (p.F17 L) (9/72, 12.5%). Likewise, the most frequent mutations in maternal patients were c.760C > T (p.R254X) (3/10, 30%), c.1400C > G (p.S467C) (2/10, 20%), and c.51C > G (p.F17 L) (2/10, 20%). In patients with MADD, six different *ETFDH* gene mutations were identified and the most common mutation was c.250G > A (p.A84T) (5/10, 50%). The rest of patients in this group all harbored compound heterozygous mutations and there were no high frequency mutations (Supplementary file 3: Table S3).

4. Discussion

The incidence of IMDs detected by MS/MS varies greatly among different countries. In the United States, the estimated incidence ranges between 1:3367 in California and 1:4300 in North Carolina [6,7]. The overall incidence in Australia is 1:2855 [8]. The incidence of IMDs in many European countries is similar, such as 1:2105 in Italy, 1:2396 in Portugal, 1:2920 in West Germany, and 1:2960 in Spain [9–12]. For Asian countries, it occurs in 1:2800 newborns in South Korea, 1:2916 in Malaysia, 1:3159 in Singapore, and 1:9330 in Japan [13–16]. In China, the incidence varies greatly in various regions, such as 1:1178 in Jinan, 1:5626 in Zhejiang, and 1:6219 in Taiwan [5,17,18]. Additionally, a previous pilot study showed that the average incidence of IMDs detected by MS/MS is 1:3795 in Chinese population [3]. The overall incidence of IMDs detected by MS/MS in Quanzhou is 1:2826, which is

Table 5
Levels of abnormal parameters for different disorders of fatty acid oxidation disorders.

Fatty acid oxidation disorders (n = 48)	n (%)	Abnormal parameter	Concentration mean (range) (μmol/L)	Reference range (μmol/L)
Primary carnitine deficiency (PCD)	36 (73.47)	C0	4.91 (2.19–7.94)	9–50
Multiple acyl-CoA dehydrogenase deficiency (MADD)	5 (10.20)	C4	0.39 (0.2–0.58)	0.08–0.45
		C6	0.17 (0.06–0.4)	0.01–0.09
		C8	0.54 (0.07–0.99)	0.01–0.15
		C10	1.04 (0.13–1.8)	0.02–0.2
		C12	1.29 (0.08–3.05)	0.01–0.24
		C14	0.98 (0.08–1.98)	0.02–0.37
Short-chain acyl-CoA dehydrogenase deficiency (SCADD)	4 (8.16)	C4	1.10 (0.93–1.32)	0.08–0.45
		C4/C2	0.12 (0.07–0.2)	0–0.03
		C4/C3	0.83 (0.65–1.19)	0.04–0.39
Very-long-chain acyl-CoA dehydrogenase deficiency (VLCAD)	3 (6.12)	C14	1.44 (0.98–2.3)	0.02–0.37
		C14:1	2.74 (1.43–4.02)	0.02–0.26
		C14:1/C2	0.31 (0.07–0.44)	0–0.01
		C14:1/C16	0.72 (0.24–1.22)	0.01–0.09
Carnitine palmitoyl transferase-I deficiency (CPT I)	1 (2.04)	C0	113.46	9–50
		C16	2.48	0.15–6.0
		C18	0.81	0.1–2
		C0/(C16 + C18)	34.49	2.4–35

comparable to other studies in Malaysia, South Korea, Australia, Germany, and Spain. However, we must keep in mind that the different NBS panel could substantially affect the figures. Notably, the PPV of this study is much lower than other NBS programs (German: 11.3% PPV, Australia: 12.6% PPV, and Singapore 18% PPV), which may be due largely to the lack of second-tier testing, limited experience and other underlying reasons.

PAHD and CD were the two most common amino acid disorders. The incidence of PAHD ranges from 1:2600 in Turkey to 1:143,000 in Japan [19]. PAHD has an incidence of 1:11,614 in the Chinese population and it is confirmed that PAHD has a higher incidence in the north [3]. Our data showed that PAHD with an incidence of 1:26,039 is close to 1: 20,445 in Zhejiang province [20], but is much lower than that in Jining city of north China (1:4391) [17], which is in accordance with previous reports. In the mainland of China, 6-Pyruvoyl-tetrahydropterin synthase (PTPS) deficiency is a major subset of BH4D (account for 96%) [21], and all of our BH4D patients were PTPS deficiency. On the contrary, CD is more common in south China than that in northern, our current incidence of CD is 1:36,455, which is much lower than the calculated incidence based on carrier rate of 1:48 in south China [22]. Therefore, the true incidence of CD may be underestimated since the natural history of this condition where citrulline levels may be not elevated and is easily missed by NBS, as was observed in one patient in this cohort. The overall incidence of UCD varies between 1:50,000 and 1:8000 live births, the prevalence of 1:52,078 by our NBS program is similar to the recent cross-border study from Germany, Austria and Switzerland, which with an estimated cumulative incidence of 1:51,946 for UCDs [23]. The worldwide incidence of MAT I/III deficiency remains unknown, the estimated incidence of 1:121,515 in this study is close to 1:107,850 in Hokkaido, Japan [24]; but was much lower than 1:22,874 in Spain and 1:26,000 in Portugal [25,26]. Genetically, the mutation c.158G > A (p.R53H) was classified as associated with mild PAHD [20], consistent with this, most patients with PAHD (12/14, 85.7%) in this cohort were mild hyperphenylalaninemia (MHP, Phe less than 360 μmol/L) and 58.3% patients harbored this mutation. The most common *PTS* gene mutation in our PTPS deficiency patients was c.155A > G (p.N52S), which account for 50% is in line with previous report that c.155A > G (p.N52S) occurs predominantly in south China [21]. The c.852_855del (p.M285Pfs*2), c.615 + 5G > A (p.A206Vfs*7), c.1638_1660dup (p.A554Gfs*17), and IVS16ins3kb (p.A584Vfs*2) have been clearly elucidated as hot-spot mutations in the Chinese CD patients [22]. Similarly, these four mutations together account for 90% of *SLC25A13* gene mutations in this study. The c.791G > A (p.R264H) was the most frequently autosomal dominant mutation [25], and two of the three patients were observed to harbor this mutation.

Of particular note, the most common organic acid disorder was SBCADD (1:30,379), which previously reported has particularly high frequency (1:132) in the Hmong ethnic population [27]. This is obviously different from other domestic studies, for instance, MMA was the most common organic aciduria in Zhejiang and Shandong province, especially the incidence of the latter was as high as 1:3920 [28]. By contrast, only three MMA cases were detected in our study, the estimated incidence of MMA was 1 in 121,515 infants in Quanzhou. The incidence of GA-I is about 1:100,000 worldwide, GA-I as the second most common organic aciduria in this group is similar to in the Australian population [8]. Besides, GA-I was the most common organic aciduria in Spanish, with an incidence of 1:35,027 [9]. The incidence of IBDHD remains unclear, our group previously reported this disease for the first time in China, and our current incidence was 1:60,758 [29]. 3-MCCD was the most common organic aciduria such as in North Carolina and Australia [6,8], whereas it was ranked fourth in our study with an incidence of 1:72,909. More than 3 million NBS data from Germany showed that the incidence of IVA is 1:65,000 [30]. In this study, we found 4 cases of IVA with an incidence of 1:91,136. In addition, four cases of maternal disorders were identified due to the abnormal NBS results observed in neonates, all of these mothers had secondary carnitine deficiency. Genetically, the *ACADSB* gene mutation c.1165A > G was reported previously as a founder mutation in the Hmong ethnic patients with SBCADD, likewise, the most common mutation in our group was c.1165A > G (33.3%). Moreover, we found another high frequency mutation c.275C > G in Chinese population. The c.1244-2A > C in *GCDH* was a recurrent mutation in Chinese population [31], similarly, c.1244-2A > C account for 57.14% of mutant alleles. Previously, patients with IBDHD had already been reported by our group, the predominant mutation in *ACAD8* was c.286G > A (50%), which might be a hot-spot mutation in Chinese population [29].

The combined incidence of FAODs is 1:9300 from reports out of Australia, Germany, and the USA, but it is rarely identified in Asian countries, which even raises the question of whether to exclude FAODs from screening panel in Asia [32]. Furthermore, spectrum analysis of common IMDs in Chinese patients by Han et al. showing that the FAODs were less common in China, which only accounted for 13% of the detected cases [33]. However, the highest proportion of IMDs in this study is FAOD, which is significantly different from previous observations. PCD represented the majority of FAODs with incidence up to 1:10,126. PCD was not only the most common FAOD, but also it was the most frequent disorder in this study, with an incidence even higher than PAHD. In addition to the newborns with PCD, an additional 5 mothers was found to be affected with PCD. MADD as the second most common FAOD, the incidence was approximately 1:72,909 in our area. MADD was the most common lipid storage myopathies (LSM) in China [34], so we speculate that the disease should account for a higher proportion of FAODs, but actually it was less found in many NBS programs. The possible explanation is that the acylcarnitine profiles used for screening MADD may be mild and atypical, even cannot detectable during acute episodes. Thus MADD might be missed by NBS, as observed in one of our patients, who showed only slight elevation of C5 level but no increase in other medium and long chain acylcarnitines. Besides, other FAODs such as SCADD and VLCADD have also been found in a few cases, with the incidence of 1:91,136 and 1:121,515, respectively. However, MCADD as the most prevalent FAODs in Australia, Europe, and the United States [7,8,10,11,35], by contrast was not detected in our study, indicating that this disease is relatively rare in Chinese population. Genetically, the c.760C > T (p.R254X) in *SLC22A5* gene was a founder mutation in Chinese population, in line with this, c.760C > T (p.R254X) was the most frequent mutation both in neonatal and maternal patients with PCD. Interestingly, previous reports revealed that c.760C > T (p.R254X) was lower in asymptomatic mother and newborns [36]. Our data showed that the c.760C > T (p.R254X), c.1400C > G (p.S467C), and c.51C > G (p.F17 L) were three most common mutations in patients with PCD. The c.250G > A (p.A84T) in *ETFDH* gene was reported to with high frequency in China, Thailand, and Singapore, especially the frequency was up to 81.1% in Fujian province [34]. In this study, the frequency of this mutation was as high as 50%, which also confirmed previous reports.

In summary, we presented expanded NBS results with MS/MS in a southern Chinese population. The prevalence, disease spectrum, and genetic characteristics of confirmed IMDs were clarified. Our experience shows a different story that FAODs has the highest proportion, particularly PCD was the most common disorder in the region. The recurrent mutations of relatively common diseases like PCD, PAHD, SBCADD, CD, GA-I, IBDHD, and MADD in this region were also clearly elucidated. NBS may miss part of the IMDs, the performance of our NBS program needs to be improved, and the combination of MS/MS with gene screening is necessary in future.

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Competing interests

The authors declare that they have no competing interests.

References

[1] D.H. Chace, T.A. Kalas, E.W. Naylor, Use of tandem mass spectrometry for multianalyte screening of dried blood specimens from newborns, Clin. Chem. 49 (2003) 1797–1817.

[2] B. Wilcken, V. Wiley, J. Hammond, K. Carpenter, Screening newborns for inborn errors of metabolism by tandem mass spectrometry, N. Engl. J. Med. 348 (2003) 2304–2312.

[3] X.T. Shi, J. Cai, Y.Y. Wang, W.J. Tu, W.P. Wang, L.M. Gong, et al., Newborn screening for inborn errors of metabolism in mainland China: 30 years of experience, JIMD Rep. 6 (2012) 79–83.

[4] D. McHugh, C.A. Cameron, J.E. Abdenur, M. Abdulrahman, O. Adair, S.A. Al Nuaimi, et al., Clinical validation of cutoff target ranges in newborn screening of metabolic disorders by tandem mass spectrometry: a worldwide collaborative project, Genet. Med. 13 (2011) 230–254.

[5] D.M. Niu, Y.H. Chien, C.C. Chiang, H.C. Ho, W.L. Hwu, S.M. Kao, et al., Nationwide survey of extended newborn screening by tandem mass spectrometry in Taiwan, J. Inherit. Metab. Dis. 33 (2010) S295–S305.

[6] D.M. Frazier, D.S. Millington, S.E. McCandless, D.D. Koeberl, S.D. Weavil, S.H. Chaing, et al., The tandem mass spectrometry newborn screening experience in North Carolina: 1997-2005, J. Inherit. Metab. Dis. 29 (2006) 76–85.

[7] L. Feuchtbaum, J. Carter, S. Dowray, R.J. Currier, F. Lorey, Birth prevalence of disorders detectable through newborn screening by race/ethnicity, Genet. Med. 14 (2012) 937–945.

[8] D.C. Kasper, R. Ratschmann, T.F. Metz, T.P. Mechtler, D. Moslinger, V. Konstantopoulou, et al., The national Austrian newborn screening program - eight years experience with mass spectrometry. Past, present, and future goals, Wien. Klin. Wochenschr. 122 (2010) 607–613.

[9] M.L. Couce, D.E. Castineiras, M.D. Boveda, A. Bana, J.A. Cocho, A.J. Iglesias, et al., Evaluation and long-term follow-up of infants with inborn errors of metabolism identified in an expanded screening programme, Mol. Genet. Metab. 104 (2011) 470–475.

[10] L. Vilarinho, H. Rocha, C. Sousa, A. Marcao, H. Fonseca, M. Bogas, et al., Four years of expanded newborn screening in Portugal with tandem mass spectrometry, J. Inherit. Metab. Dis. 33 (Suppl. 3) (2010) S133–S138.

[11] M. Lindner, G. Gramer, G. Haege, J. Fang-Hoffmann, K.O. Schwab, U. Tacke, et al., Efficacy and outcome of expanded newborn screening for metabolic diseases—report of 10 years from South-West Germany, Orphanet J. Rare Dis. 6 (2011) 44.

[12] G. la Marca, S. Malvagia, B. Casetta, E. Pasquini, M.A. Donati, E. Zammarchi, Progress in expanded newborn screening for metabolic conditions by LC-MS/MS in Tuscany: update on methods to reduce false tests, J. Inherit. Metab. Dis. 31 (Suppl. 2) (2008) S395–S404.

[13] Z.M. Yunus, S.A. Rahman, Y.S. Choy, W.T. Keng, L.H. Ngu, Pilot study of newborn screening of inborn error of metabolism using tandem mass spectrometry in Malaysia: outcome and challenges, J. Pediatr. Endocrinol. Metab. 29 (2016) 1031–1039.

[14] J.S. Lim, E.S. Tan, C.M. John, S. Poh, S.J. Yeo, J.S. Ang, et al., Inborn error of metabolism (IEM) screening in Singapore by electrospray ionization-tandem mass spectrometry (ESI/MS/MS): an 8 year journey from pilot to current program, Mol. Genet. Metab. 113 (2014) 53–61.

[15] H.R. Yoon, K.R. Lee, S. Kang, D.H. Lee, H.W. Yoo, W.K. Min, et al., Screening of newborns and high-risk group of children for inborn metabolic disorders using tandem mass spectrometry in South Korea: a three-year report, Clin. Chim. Acta 354 (2005) 167–180.

[16] S. Yamaguchi, Newborn screening in Japan: restructuring for the new era, Ann. Acad. Med. Singap. 37 (2008) 13–15.

[17] K. Guo, X. Zhou, X. Chen, Y. Wu, C. Liu, Q. Kong, Expanded newborn screening for inborn errors of metabolism and genetic characteristics in a Chinese population, Front. Genet. 9 (2018) 122.

[18] X.W. Huang, J.B. Yang, F. Tong, R.L. Yang, H.Q. Mao, X.L. Zhou, et al., Screening for neonatal inborn errors of metabolism by electrospray ionization-tandem mass spectrometry and follow-up, Chinese J. Pediatrics 49 (2011) 765–770.

[19] J.J. Mitchell, Y.J. Trakadis, C.R. Scriver, Phenylalanine hydroxylase deficiency, Genet. Med. 13 (2011) 697–707.

[20] T. Chen, W. Xu, D. Wu, J. Han, L. Zhu, F. Tong, et al., Mutational and phenotypic spectrum of phenylalanine hydroxylase deficiency in Zhejiang Province, China, Sci. Rep. 8 (2018) 17137.

[21] J. Ye, Y. Yang, W. Yu, H. Zou, J. Jiang, R. Yang, et al., Demographics, diagnosis and treatment of 256 patients with tetrahydrobiopterin deficiency in mainland China: results of a retrospective, multicentre study, J. Inherit. Metab. Dis. 36 (2013) 893–901.

[22] W.X. Lin, H.S. Zeng, Z.H. Zhang, M. Mao, Q.Q. Zheng, S.T. Zhao, et al., Molecular diagnosis of pediatric patients with citrin deficiency in China: SLC25A13 mutation spectrum and the geographic distribution, Sci. Rep. 6 (2016) 29732.

[23] S. Nettesheim, S. Kolker, D. Karall, J. Haberle, R. Posset, G.F. Hoffmann, et al., Incidence, disease onset and short-term outcome in urea cycle disorders -cross-border surveillance in Germany, Austria and Switzerland, Orphanet J. Rare Dis. 12 (2017) 111.

[24] M. Nagao, T. Tanaka, M. Furujo, Spectrum of mutations associated with methionine adenosyltransferase I/III deficiency among individuals identified during newborn screening in Japan, Mol. Genet. Metab. 110 (2013) 460–464.

[25] M.L. Couce, M.D. Boveda, C. Garcia-Jimemez, E. Balmaseda, I. Vives, D.E. Castineiras, et al., Clinical and metabolic findings in patients with methionine adenosyltransferase I/III deficiency detected by newborn screening, Mol. Genet. Metab. 110 (2013) 218–221.

[26] E. Martins, A. Marcao, A. Bandeira, H. Fonseca, C. Nogueira, L. Vilarinho, Methionine adenosyltransferase I/III deficiency in Portugal: high frequency of a dominantly inherited form in a small area of Douro high lands, JIMD Rep. 6 (2012) 107–112.

[27] S.C. Van Calcar, M.W. Baker, P. Williams, S.A. Jones, B. Xiong, M.C. Thao, et al., Prevalence and mutation analysis of short/branched chain acyl-CoA dehydrogenase deficiency (SBCADD) detected on newborn screening in Wisconsin, Mol. Genet. Metab. 110 (2013) 111–115.

[28] B. Han, Z. Cao, L. Tian, H. Zou, L. Yang, W. Zhu, et al., Clinical presentation, gene analysis and outcomes in young patients with early-treated combined methylmalonic acidemia and homocysteinemia (cblC type) in Shandong province, China, Brain and Development 38 (2016) 491–497.

[29] Y. Lin, W. Peng, M. Jiang, C. Lin, W. Lin, Z. Zheng, et al., Clinical, biochemical and genetic analysis of Chinese patients with isobutyryl-CoA dehydrogenase deficiency, Clin. Chim. Acta 487 (2018) 133–138.

[30] R. Ensenauer, R. Fingerhut, E.M. Maier, R. Polanetz, B. Olgemoller, W. Roschinger, et al., Newborn screening for isovaleric acidemia using tandem mass spectrometry: data from 1.6 million newborns, Clin. Chem. 57 (2011) 623–626.

[31] C.S. Lee, Y.H. Chien, S.F. Peng, P.W. Cheng, L.M. Chang, A.C. Huang, et al., Promising outcomes in glutaric aciduria type I patients detected by newborn screening, Metab. Brain Dis. 28 (2013) 61–67.

[32] M. Lindner, G.F. Hoffmann, D. Matern, Newborn screening for disorders of fatty-acid oxidation: experience and recommendations from an expert meeting, J. Inherit. Metab. Dis. 33 (2010) 521–526.

[33] L. Han, F. Han, J. Ye, W. Qiu, H. Zhang, X. Gao, et al., Spectrum analysis of common inherited metabolic diseases in Chinese patients screened and diagnosed by tandem mass spectrometry, J. Clin. Lab. Anal. 29 (2015) 162–168.

[34] J. Xi, B. Wen, J. Lin, W. Zhu, S. Luo, C. Zhao, et al., Clinical features and ETFDH mutation spectrum in a cohort of 90 Chinese patients with late-onset multiple acyl-CoA dehydrogenase deficiency, J. Inherit. Metab. Dis. 37 (2014) 399–404.

[35] J. Oerton, J.M. Khalid, G. Besley, R.N. Dalton, M. Downing, A. Green, et al., Newborn screening for medium chain acyl-CoA dehydrogenase deficiency in England: prevalence, predictive value and test validity based on 1.5 million screened babies, J. Med. Screen. 18 (2011) 173–181.

[36] L. Han, F. Wang, Y. Wang, J. Ye, W. Qiu, H. Zhang, et al., Analysis of genetic mutations in Chinese patients with systemic primary carnitine deficiency, Eu. J. Med. Genet. 57 (2014) 571–575.