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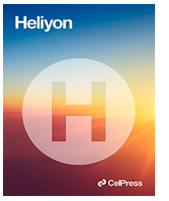

Newborn screening for fatty acid oxidation disorders in a southern Chinese population

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ABSTRACT

Background and aims: Fatty acid oxidation disorders (FAODs) are a group of autosomal recessive metabolic diseases included in many newborn screening (NBS) programs, but the incidence and disease spectrum vary widely between ethnic groups. We aimed to elucidate the incidence, disease spectrum, and genetic features of FAODs in a southern Chinese population.
Materials and methods: The FAODs screening results of 643,606 newborns from 2014 to 2022 were analyzed.
Results: Ninety-two patients were eventually diagnosed with FAODs, of which 61 were PCD, 20 were MADD, 5 were SCADD, 4 were VLCADD, and 2 were CPT-IAD. The overall incidence of FAODs was 1:6996 (95 % CI: 1:5814–1:8772) newborns. All PCD patients had low C0 levels during NBS, while nine patients (14.8 %) had normal C0 levels during the recall review. All but one MADD patients had elevated C8, C10, and C12 levels during NBS, while eight patients (40 %) had normal acylcarnitine levels during the recall review. The most frequent *SLC22A5* variant was c.760C > T (p.R254*) with an allele frequency of 29.51 %, followed by c.51C > G (p.F17L) (17.21 %) and c.1400C > G (p.S467C) (16.39 %). The most frequent *ETFDH* variant was c.250G > A (p.A84T) with an allelic frequency of 47.5 %, followed by c.524G > A (R175H) (12.5 %), c.998A > G (p.Y333C) (12.5 %), and c.1657T > C (p.Y553H) (7.5 %).
Conclusion: The prevalence, disease spectrum, and genetic characteristics of FAODs in a southern Chinese population were clarified. PCD was the most common FAOD, followed by MADD. Hotspot variants were found in *SLC22A5* and *ETFDH* genes, while the remaining FAODs showed great molecular heterogeneity. Incorporating second-tier genetic screening is critical for FAODs.

1. Introduction

Fatty acid oxidation disorders (FAODs) are a group of autosomal recessive metabolic diseases caused by defective enzymes involved in the transport and oxidation of fatty acids in the mitochondria [1]. FAOD subtypes are classified primarily based on the length of the

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fatty acid disrupted during metabolism or the deficiency of specific fatty acid transport protein, including primary carnitine deficiency (PCD), multiple acyl-CoA dehydrogenase deficiency (MADD), carnitine-acylcarnitine translocase deficiency (CACTD), carnitine palmitoyltransferase I/II deficiency (CPT-IAD/CPT-IID), very long chain acyl-CoA dehydrogenase deficiency (VLCADD), long chain hydroxyacyl-CoA dehydrogenase deficiency or tri-functional protein deficiency (LCHADD/TFPD), short chain acyl-CoA dehydrogenase deficiency (SCADD), medium chain acyl-CoA dehydrogenase deficiency (MCADD), and medium/short chain hydroxyacyl-CoA dehydrogenase deficiency (M/SCHADD). These defects have a wide variety of clinical presentations, including liver dysfunction, hypoketotic hypoglycemia, cardiomyopathy, skeletal myopathy, and episodic rhabdomyolysis [2]. Without timely detection, patients with FAODs may have serious and potentially life-threatening consequences. If early detection and timely treatment was made, potentially acute life-threatening events of most FAODs can be prevented.

Newborn screening (NBS) for inherited metabolic disorders, including FAODs, is now routinely performed in many countries. FAODs can be detected by quantitative analysis of acylcarnitine profiles and/or free carnitine (C0) levels using tandem mass spectrometry [3,4]. Confirmation requires further genetic testing or enzymatic analysis. However, the subtypes of FAODs included in NBS programs differ across countries, the incidence and disease spectrum of FAODs vary considerably between ethnic groups [5].

Expanded NBS was performed in Quanzhou, China since January 2014. Over 600,000 newborns have been screened thus far. Moreover, second-tier genetic testing was carried out for PCD and MADD since 2017 [6,7]. Herein, we report our nine years' experience of NBS for FAODs in southern China. The aim of this study was to figure out the incidence, disease spectrum, and genetic features of FAODs identified by NBS.

2. Methods

2.1. Study population

A total of 643,606 newborns were screened using tandem mass spectrometry at the Quanzhou Maternity and Children's Hospital, China between January 2014 and December 31, 2022. Newborns with abnormal NBS results indicative of FAODs and those genetically diagnosed with FAODs were recruited for this study. This study was approved by the Ethics Committee of the Quanzhou Maternity and Children's Hospital (reference number: 2021-IRB-029). Written informed consent was obtained from the parents of all the patients included in the study.

2.2. NBS and genetic diagnosis

The detailed procedure of NBS is as described in our previous article [8]. Newborns with positive screening results indicative of FAODs were referred for repeat screening. Matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) assay was incorporated into our NBS program and used for second-tier screening for PCD and MADD as previously described [6,7]. Newborns with extremely abnormal initial screening, positive repeat screening, and positive genetic screening results were classified as highly suspected patients. All these patients underwent further Sanger sequencing and/or targeted next-generation sequencing analysis [8]. Newborns with homozygous and compound heterozygous pathogenic variants in disease-causing genes relevant to FAODs were defined as positive patients. Newborns with one or no pathogenic variants were excluded from this study.

2.3. Data analysis

Prevalence (P) was calculated by dividing the number of positive patients by the total number of screened newborns (N). The confidence interval (CI) was calculated as:

$$95\%\ CI = P \pm 1.96 \times ((P \times (1-P)/N))^{1/2}$$

Table 1
Incidences of fatty acid oxidation disorders (FAODs) in Quanzhou, China.

Disorders	Positive cases	Incidence
Primary carnitine deficiency (PCD)	61	1:10551 (95 % CI: 1:8403–1:14,085)
Multiple acyl-CoA dehydrogenase deficiency (MADD)	20	1:32180 (95 % CI: 1:22,222–1:58,824)
Short chain acyl-CoA dehydrogenase deficiency (SCADD)	5	1:128721 (95 % CI: 1:66,667–1:1,042,753)
very long chain acyl-CoA dehydrogenase deficiency (VLCADD)	4	1:160902 (95 % CI: 1:83,333–1:8,064,516)
Carnitine palmitoyltransferase I deficiency (CPT-IAD)	2	1:321803
Total	92	1:6996 (95 % CI: 1:5814–1:8772)

The 95 % CI cannot be calculated for CPT-IAD due to its extremely low incidence.

Table 2
Acylcarnitine profiles and genetic features of 61 patients with PCD.

Case	Gender	NBS	RC	Genotype	
1	Female	C0 = 8.41	C0 = 6.81	c.51C > G (p.F17L)	c.1400C > G (p.S467C)
2	Male	C0 = 6.91	C0 = 3.28	c.760C > T (p.R254*)	c.1400C > G (p.S467C)
3	Female	C0 = 2.40	C0 = 2.33	c.760C > T (p.R254*)	c.290T > C (p.L97P)
4	Male	C0 = 5.35	C0 = 5.14	c.760C > T (p.R254*)	c.1400C > G (p.S467C)
5	Male	C0 = 4.93	C0 = 7.57	c.760C > T (p.R254*)	c.797C > T (p.P266L)
6	Male	C0 = 5.31	C0 = 4.42	c.695C > T (p.T232 M)	c.1400C > G (p.S467C)
7	Male	C0 = 5.91	C0 = 6.95	c.51C > G (p.F17L)	c.797C > T (p.P266L)
8	Female	C0 = 2.02	C0 = 1.60	c.760C > T (p.R254*)	c.C1411T (p.R471C)
9	Female	C0 = 7.53	C0 = 3.65	c.839C > T (p.S280F)	c.1400C > G (p.S467C)
10	Female	C0 = 5.42	**C0=14.79**	c.51C > G (p.F17L)	c.252C > T (p.Y84Y)
11	Male	C0 = 6.57	**C0=9.03**	c.797C > T (p.P266L)	c.1139C > T (p.A380V)
12	Male	C0 = 4.61	C0 = 6.29	c.51C > G (p.F17L)	c.1195C > T (p.R399W)
13	Female	C0 = 3.28	C0 = 3.17	c.51C > G (p.F17L)	c.51C > G (p.F17L)
14	Female	C0 = 3.25	C0 = 2.78	c.51C > G (p.F17L)	c.51C > G (p.F17L)
15	Female	C0 = 2.37	C0 = 1.05	c.338G > A (p.C113Y)	c.760C > T (p.R254*)
16	Male	C0 = 7.65	C0 = 5.04	c.51C > G (p.F17L)	c.1400C > G (p.S467C)
17	Male	C0 = 3.75	C0 = 3.19	c.760C > T (p.R254*)	c.1400C > G (p.S467C)
18	Male	C0 = 2.72	C0 = 2.86	c.844C > T (p.R282*)	c.1400C > G (p.S467C)
19	Female	C0 = 2.54	C0 = 2.29	c.695C > T (p.T232 M)	c.760C > T (p.R254*)
20	Male	C0 = 5.29	C0 = 6.58	c.51C > G (p.F17L)	c.1195C > T (p.R399W)
21	Female	C0 = 5.02	C0 = 5.53	c.428C > T (p.P143L)	c.428C > T (p.P143L)
22	Female	C0 = 1.63	C0 = 1.67	c.760C > T (p.R254*)	c.760C > T (p.R254*)
23	Male	C0 = 2.31	C0 = 2.76	c.51C > G (p.F17L)	c.1161T > G (p.Y387*)
24	Female	C0 = 3.49	C0 = 3.52	c.51C > G (p.F17L)	c.760C > T (p.R254*)
25	Male	C0 = 1.96	C0 = 1.73	c.51C > G (p.F17L)	c.760C > T (p.R254*)
26	Female	C0 = 2.40	C0 = 1.44	c.760C > T (p.R254*)	c.760C > T (p.R254*)
27	Male	C0 = 5.78	**C0=10.67**	c.760C > T (p.R254*)	c.797C > T (p.P266L)
28	Male	C0 = 5.95	**C0=8.64**	c.695C > T (p.T232 M)	c.1160A > G (p.Y387C)
29	Female	C0 = 7.27	C0 = 6.66	c.760C > T (p.R254*)	c.797C > T (p.P266L)
30	Female	C0 = 5.58	C0 = 5.59	c.760C > T (p.R254*)	c.1400C > G (p.S467C)
31	Female	C0 = 5.34	C0 = 6.02	c.797C > T (p.P266L)	c.394-1G > A
32	Female	C0 = 1.78	C0 = 1.90	c.695C > T (p.T232 M)	c.1139C > T (p.A380V)
33	Male	C0 = 4.34	C0 = 4.45	c.51C > G (p.F17L)	c.51C > G (p.F17L)
34	Female	C0 = 4.75	C0 = 4.16	c.760C > T (p.R254*)	c.845G > A (p.R282Q)
35	Female	C0 = 3.45	C0 = 5.24	c.760C > T (p.R254*)	c.1400C > G (p.S467C)
36	Female	C0 = 6.82	C0 = 5.02	c.760C > T (p.R254*)	c.1400C > G (p.S467C)
37	Male	C0 = 2.19	C0 = 2.12	c.822G > A (p.W274*)	c.782_799del ((p.V261_P266del)
38	Male	C0 = 2.73	**C0=9.84**	c.51C > G (p.F17L)	c.1144_1162del (p.V382Cfs*45)
39	Male	C0 = 3.00	**C0=10.81**	c.51C > G (p.F17L)	c.1400C > G (p.S467C)
40	Male	C0 = 6.46	C0 = 5.10	c.695C > T (p.T232 M)	c.1400C > G (p.S467C)
41	Male	C0 = 3.02	C0 = 1.77	c.760C > T (p.R254*)	c.760C > T (p.R254*)
42	Female	C0 = 6.77	**C0=10.05**	c.1400C > G (p.S467C)	c.1400C > G (p.S467C)
43	Female	C0 = 2.36	C0 = 1.75	c.760C > T (p.R254*)	c.760C > T (p.R254*)
44	Female	C0 = 3.12	C0 = 2.88	c.760C > T (p.R254*)	c.51C > G (p.F17L)
45	Male	C0 = 3.64	C0 = 3.80	c.695C > T (p.T232 M)	c.1139C > T (p.A380V)
46	Female	C0 = 3.56	C0 = 4.31	c.760C > T (p.R254*)	c.1139C > T (p.A380V)
47	Female	C0 = 6.27	C0 = 3.43	c.695C > T (p.T232 M)	c.1139C > T (p.A380V)
48	Female	C0 = 2.70	C0 = 3.46	c.760C > T (p.R254*)	c.51C > G (p.F17L)
49	Male	C0 = 7.35	**C0=14.27**	c.338G > A (p.C113Y)	c.338G > A (p.C113Y)
50	Male	C0 = 8.25	C0 = 2.51	c.51C > G (p.F17L)	c.338G > A (p.C113Y)
51	Male	C0 = 2.45	C0 = 1.14	c.760C > T (p.R254*)	c.760C > T (p.R254*)
52	Male	C0 = 2.8	C0 = 1.74	c.760C > T (p.R254*)	c.1161T > G (p.Y387*)
53	Female	C0 = 6.83	C0 = 4.59	c.760C > T (p.R254*)	c.1400C > G (p.S467C)
54	Female	C0 = 6.22	**C0=11.14**	c.760C > T (p.R254*)	c.1400C > G (p.S467C)
55	Female	C0 = 6.16	C0 = 4.02	c.695C > T (p.T232 M)	c.1400C > G (p.S467C)
56	Male	C0 = 6.77	C0 = 4.5	c.760C > T (p.R254*)	c.1400C > G (p.S467C)
57	Female	C0 = 4.91	C0 = 6.66	c.250T > A (p.Y84 N)	c.1400C > G (p.S467C)
58	Male	C0 = 3.24	C0 = 4.05	c.51C > G (p.F17L)	c.1196G > A (p.R399Q)
59	Male	C0 = 4.96	C0 = 5	c.51C > G (p.F17L)	c.1195C > T (p.R399W)
60	Female	C0 = 3.15	C0 = 4.09	c.760C > T (p.R254*)	c.1400C > G (p.S467C)
61	Female	C0 = 4.12	C0 = 1.29	c.760C > T (p.R254*)	c.760C > T (p.R254*)

NBS: newborn screening, RC: recall, C0: free carnitine, reference range: 8.5–50 μmol/L.
The C0 levels within the reference range are given in bold.

3. Results

3.1. NBS

Among the 643,606 screened newborns, 2961 (0.46 %) newborns had abnormal NBS results indicative of FAODs. Ninety-two patients were eventually diagnosed with FAODs, comprising five subtypes, of which 61 were PCD, 20 were MADD, 5 were SCADD, 4 were VLCADD, and 2 were CPT-IAD. The overall incidence of FAODs was 1:6996 (95 % CI: 1:5814–1:8772) newborns, PCD was the most common FAOD with an incidence of 1:10,551 (95 % CI: 1:8403–1:14,085) newborns, followed by MADD (Table 1).

3.2. Acylcarnitine profile analysis

All PCD patients had low C0 levels during NBS, while nine patients (14.8 %) (nos. 10, 11, 27, 28, 38, 39, 42, 49, and 54) had normal C0 levels during the recall review. The mean C0 concentrations at NBS and recall review were 4.44 ± 1.78 μmol/L and 4.89 ± 3.14 μmol/L, respectively (Table 2). All but one MADD patients had elevated C8, C10, and C12 levels during NBS, while eight patients (40 %) (nos. 1, 4, 7–10, 14, and 18) had normal acylcarnitine levels during the recall review. The mean C8, C10, and C12 concentrations at NBS were 0.59 ± 0.63 μmol/L, 0.92 ± 0.67 μmol/L, and 1.14 ± 0.95 μmol/L, respectively. However, the mean C8, C10, and C12 concentrations at the recall stage were reduced to 0.19 ± 0.08 μmol/L, 0.32 ± 0.15 μmol/L, and 0.27 ± 0.18 μmol/L, respectively. Of note, patient 2 showed only slightly elevated C5 level at NBS, and the C5 level during the recall review was 0.78 μmol/L (Table 3). All SCADD patients had elevated C4 levels and corresponding ratios at NBS and recall review. All VLCADD patients had markedly elevated

Table 3
Acylcarnitine profiles and genetic features of 20 patients with MADD.

Case	Gender	NBS	RC	Genotype	
1	Female	C6 = 0.06,C8 = 0.16,C10 = 0.31,C12 = 0.38,C14 = 0.42	**C6=0.05,C8=0.06,C10=0.08, C12=0.05,C14=0.15**	c.250G > A (p.A84T)	c.1657T > C (p.Y553H)
2	Male	C5 = 0.44	C5 = 0.78	c.250G > A (p.A84T)	c.770A > G (p.Y257C)
3	Female	C6 = 1.46,C8 = 2.72,C10 = 2.76,C12 = 3.95,C14 = 5.07	C6 = 0.73,C8 = 1.27,C10 = 1.69,C12 = 1.7,C14 = 1.02	c.1691-3C > G	c.929A > G (p.Y310C)
4	Male	C6 = 0.13,C8 = 0.32,C10 = 0.6,C12 = 0.53,C14 = 0.41	**C6=0.06,C8=0.08,C10=0.1, C12=0.07,C14=0.13**	c.998A > G (p.Y333C)	c.998A > G (p.Y333C)
5	Male	C6 = 0.15,C8 = 0.33,C10 = 0.55,C12 = 0.55,C14 = 0.39	C6 = 0.19,C8 = 0.55,C10 = 0.84,C12 = 0.39,C14 = 0.31	c.250G > A (p.A84T)	c.524G > A (R175H)
6	Female	C6 = 0.17,C8 = 0.5,C10 = 0.8,C12 = 0.48,C14 = 0.31	C6 = 0.46,C8 = 1.17,C10 = 1.57,C12 = 0.49,C14 = 0.25	c.250G > A (p.A84T)	c.1845_1846insCAAT (p.G616fs*8)
7	Female	C6 = 0.19,C8 = 0.42,C10 = 0.9,C12 = 1.41,C14 = 0.89	**C6=0.06,C8=0.1,C10=0.15, C12=0.12,C14=0.09**	c.250G > A (p.A84T)	c.250G > A (p.A84T)
8	Male	C6 = 0.21,C8 = 0.38,C10 = 0.72,C12 = 0.9,C14 = 0.52	**C6=0.02,C8=0.02,C10=0.04, C12=0.03,C14=0.05**	c.250G > A (p.A84T)	c.250G > A (p.A84T)
9	Male	C6 = 0.15,C8 = 0.5,C10 = 0.85,C12 = 0.75,C14 = 0.49	**C6=0.06,C8=0.13,C10=0.17, C12=0.14,C14=0.18**	c.250G > A (p.A84T)	c.1845_1846insCAAT (p.G616fs*8)
10	Male	C6 = 0.08,C8 = 0.25,C10 = 0.42,C12 = 0.26,C14 = 0.25	**C6=0.02,C8=0.03,C10=0.03, C12=0.03,C14=0.11**	c.250G > A (p.A84T)	c.1657T > C (p.Y553H)
11	Female	C6 = 0.96,C8 = 1.95,C10 = 2.27,C12 = 2.41,C14 = 2.11	ND	c.524G > A (R175H)	c.524G > A (R175H)
12	Female	C6 = 0.11,C8 = 0.22,C10 = 0.35,C12 = 0.55,C14 = 0.44	C6 = 0.13,C8 = 0.35,C10 = 0.54,C12 = 0.65,C14 = 0.41	c.250G > A (p.A84T)	c.380T > A (p.127H)
13	Male	C6 = 0.24,C8 = 0.46,C10 = 0.89,C12 = 1,C14 = 0.47	C6 = 0.07,C8 = 0.17,C10 = 0.3,C12 = 0.14,C14 = 0.11	c.250G > A (p.A84T)	c.524G > A (R175H)
14	Female	C6 = 0.13,C8 = 0.29,C10 = 0.58,C12 = 0.84,C14 = 0.42	**C6=0.04,C8=0.07,C10=0.09, C12=0.05,C14=0.07**	c.998A > G (p.Y333C)	c.998A > G (p.Y333C)
15	Male	C6 = 0.3,C8 = 0.39,C10 = 0.69,C12 = 1.35,C14 = 1.17	C6 = 0.06,C8 = 0.11,C10 = 0.23,C12 = 0.29,C14 = 0.24	c.250G > A (p.A84T)	c.524G > A (R175H)
16	Female	C6 = 0.1,C8 = 0.25,C10 = 0.42,C12 = 0.95,C14 = 0.71	C6 = 0.09,C8 = 0.25,C10 = 0.39,C12 = 0.39,C14 = 0.28	c.250G > A (p.A84T)	c.250G > A (p.A84T)
17	Female	C6 = 0.15,C8 = 0.27,C10 = 0.53,C12 = 0.77,C14 = 0.56	C6 = 0.11,C8 = 0.27,C10 = 0.53,C12 = 0.34,C14 = 0.21	c.250G > A (p.A84T)	c.250G > A (p.A84T)
18	Male	C6 = 0.33,C8 = 0.62,C10 = 1.36,C12 = 3.05,C14 = 1.98	**C6=0.04,C8=0.11,C10=0.16, C12=0.11,C14=0.09**	c.250G > A (p.A84T)	c.998A > G (p.Y333C)
19	Female	C6 = 0.25,C8 = 0.76,C10 = 1.4,C12 = 1.05,C14 = 0.38	C6 = 0.05,C8 = 0.17,C10 = 0.22,C12 = 0.07,C14 = 0.06	c.380T > A (p.127H)	c.1657T > C (p.Y553H)
20	Female	C6 = 0.4,C8 = 0.99,C10 = 1.8,C12 = 1.5,C14 = 0.76	C6 = 0.1,C8 = 0.21,C10 = 0.4,C12 = 0.35,C14 = 0.27	c.250G > A (p.A84T)	c.1399G > C (p.G467R)

NBS: newborn screening, RC: recall, ND: no data.
Reference range: C5: 0.03–0.35 μmol/L, C6: 0.01–0.09 μmol/L, C8: 0.01–0.15 μmol/L, C10: 0.02–0.2 μmol/L, C12: 0.01–0.24 μmol/L, C14: 0.02–0.37 μmol/L.
The acylcarnitines within the reference range are given in bold.

C14:1 levels at NBS, along with C14:1/C2 and C14:1/C16 ratios. Notably, the C14:1 concentration of patient 8 was in the normal reference range at recall review, and the corresponding ratios were mildly increased. CPT-IAD patients had either elevated C0 levels or C0/(C16+C18) at NBS. Of note was that patient 11 had normal C0 concentration at recall review, but the C0/(C16+C18) ratio was significantly elevated (Table 4).

3.3. Genetic features

Twenty-four distinct *SLC22A5* variants were identified in PCD patients. The most frequent variant was c.760C > T (p.R254*) with an allele frequency of 29.51 %, followed by c.51C > G (p.F17L) (17.21 %) and c.1400C > G (p.S467C) (16.39 %). The other variants with relatively high frequency were c.695C > T (p.T232 M), c.797C > T (p.P266L), c.338G > A (p.C113Y), c.1139C > T (p.A380V), and c.1195C > T (p.R399W) (Table 5). Ten distinct *ETFDH* variants were identified in MADD patients. The most frequent *ETFDH* variant was c.250G > A (p.A84T) with an allelic frequency of 47.5 %, followed by c.524G > A (R175H) (12.5 %), c.998A > G (p.Y333C) (12.5 %), and c.1657T > C (p.Y553H) (7.5 %) (Table 6). Seven *ACADS* variants, eight *ACADVL* variants, and four *CPT1A* variants were identified in SCADD, VLCADD, and CPT-IAD patients, respectively. No high frequent variants were found in these three disorders.

4. Discussion

The incidence and disease spectrum of FAODs vary widely between ethnic groups. A combined incidence of 1:9300 newborns was described from reports of Australia, Germany, and the USA [5]. FAODs are being increasingly detected following expanded NBS, and high incidence of 1:4316 newborns was reported in a recent study from Italy [3]. The incidence of FAODs also varies greatly throughout China, with reported incidences ranging from 1:7466 to 1:15,125 newborns [9,10]. This study described the experience of NBS for FAODs in a southern Chinese population, with five subtypes and 92 patients being identified. The incidence of FAODs in our population was 1:6996 newborns, which is similar to the incidence rate reported in Liuzhou city (1:7466) [10], but higher than that reported in Jining city (1:15125) [11] and Zhejiang province (1:12261) [12].

Many large-scale NBS studies have shown that PCD was the most prevalent FAOD in China [13,14]. Consistent with previous studies, PCD was the most common FAOD in the selected population, with a high incidence of around 1:10,000 newborns. NBS for PCD is useful in early diagnosis and timely treatment which results in favourable outcome. However, the biochemical-based NBS had poor performance, leading to high rate of false positives and occasional false negatives [15,16]. The study presented the acylcarnitine profiles of 61 PCD patients during NBS and recall review. It is noteworthy that a small numbers of patients (14.8 %) had normal C0 levels during recall review. These patients would have been missed if second-tier genetic testing had not been performed. Incorporating second-tier genetic testing into NBS is crucial to improve the screening performance [6]. As one of the most common inherited metabolic disease in China, more than 1000 cases of PCD have been reported, and the mutation spectrum of *SLC22A5* gene has been

Table 4
Acylcarnitine profiles and genetic features of patients with SCADD, VLCADD, and CPT-IAD.

Case	Gender	NBS	RC	Affected gene	Genotype		Disorders
1	Male	C4 = 1.2, C4/C2 = 0.08, C4/C3 = 0.65	C4 = 1.03, C4/C2 = 0.11, C4/C3 = 0.56	*ACADS*	c.293A > G(p.Y98C)	c.1031A > G(p.E344G)	SCADD
2	Female	C4 = 0.96, C4/C2 = 0.07, C4/C3 = 0.74	C4 = 1.05, C4/C2 = 0.07, C4/C3 = 0.66	*ACADS*	c.164C > T(p.P55L)	c.815G > A(p.R272H)	SCADD
3	Female	C4 = 0.93, C4/C2 = 0.12, C4/C3 = 0.72	C4 = 1.04, C4/C2 = 0.12, C4/C3 = 1.11	*ACADS*	c.293A > G(p.Y98C)	c.1195C > T(p.R399W)	SCADD
4	Female	C4 = 1.17, C4/C2 = 0.07, C4/C3 = 0.95	C4 = 1.32, C4/C2 = 0.2, C4/C3 = 1.19	*ACADS*	c.1031A > G (p.E344G)	c.1130C > T (p.P377L)	SCADD
5	Female	C4 = 1.79, C4/C2 = 0.13, C4/C3 = 2.36	C4 = 1.57, C4/C2 = 0.17, C4/C3 = 2.34	*ACADS*	c.682G > A (p.E228K)	c.682G > A (p.E228K)	SCADD
6	Male	C14:1 = 3.32, C14:1/C2 = 0.19, C14:1/C16 = 0.73	C14:1 = 2.62, C14:1/C2 = 0.23, C14:1/C16 = 0.56	*ACADVL*	c.1055T > C(p.M352T)	c.1838G > A (p.R613Q)	VLCADD
7	Female	C14:1 = 4.02, C14:1/C2 = 0.44, C14:1/C16 = 0.7	C14:1 = 2.05, C14:1/C2 = 0.49, C14:1/C16 = 0.96	*ACADVL*	c.1349G > A(p.R450H)	c.1434+2T > C	VLCADD
8	Male	C14:1 = 1.43, C14:1/C2 = 0.07, C14:1/C16 = 0.24	**C14:1=0.19**, C14:1/C2 = 0.04, C14:1/C16 = 0.11	*ACADVL*	c.1376G > A(p.R459Q)	c.1434G > A(p.M478I)	VLCADD
9	Female	C14:1 = 2.77, C14:1/C2 = 0.41, C14:1/C16 = 1.22	C14:1 = 1.88, C14:1/C2 = 0.33, C14:1/C16 = 1.1	*ACADVL*	c.520G > A(p.V174 M)	c.896_898del(p.K299del)	VLCADD
10	Female	C0 = 113.46, C0/(C16+C18) = 34.49	C0 = 102.54, C0/(C16+C18) = 48.83	*CPT1A*	c.734G > A (p.R245Q)	c.1336G > A (p.G446S)	CPT-IAD
11	Male	C0 = 79.65, C0/(C16+C18) = 379.29	**C0=49.16**, C0/(C16+C18) = 204.83	*CPT1A*	c.1131G > C (p.E377D)	c.272T > C (p.L91P)	CPT-IAD

NBS: newborn screening, RC: recall.
Reference range: C4: 0.08–0.5 μmol/L, C4/C2: 0–0.03, C4/C3: 0.04–0.42; C14:1: 0.02–0.26 μmol/L, C14:1/C2: 0–0.01, C14:1/C16: 0.01–0.09; C0: 8.5–50 μmol/L, C0/(C16+C18): 0–40.
The acylcarnitines within the reference range are given in bold.

Table 5
The detected *SLC22A5* variants and their frequencies in patients with PCD.

No.	Location	Variants	Mutant allele (No.)	Allele frequency (%)
1	Exon 4	c.760C > T (p.R254*)	36	29.51
2	Exon 8	c.1400C > G (p.S467C)	21	17.21
3	Exon 1	c.51C > G (p.F17L)	20	16.39
4	Exon 4	c.695C > T (p.T232 M)	9	7.38
5	Exon 4	c.797C > T (p.P266L)	7	5.74
6	Exon 1	c.338G > A (p.C113Y)	4	3.28
7	Exon 7	c.1139C > T (p.A380V)	4	3.28
8	Exon 7	c.1195C > T (p.R399W)	3	2.46
9	Exon 2	c.428C > T (p.P143L)	2	1.64
10	Exon 7	c.1161T > G (p.Y387*)	2	1.64
11	Exon 1	c.250T > A (p.Y84 N)	1	0.82
12	Exon 1	c.252C > T (p.Y84Y)	1	0.82
13	Exon 1	c.290T > C (p.L97P)	1	0.82
14	Intron 1	c.394-1G > A	1	0.82
15	Exon 4	c.782_799del (p.V261_P266del)	1	0.82
16	Exon 7	c.1144_1162del (p.V382Cfs*45)	1	0.82
17	Exon 4	c.822G > A (p.W274*)	1	0.82
18	Exon 5	c.839C > T (p.S280F)	1	0.82
19	Exon 5	c.844C > T (p.R282*)	1	0.82
20	Exon 5	c.845G > A (p.R282Q)	1	0.82
21	Exon 7	c.1139C > T (p.A380V)	1	0.82
22	Exon 7	c.1160A > G (p.Y387C)	1	0.82
23	Exon 7	c.1196G > A (p.R399Q)	1	0.82
24	Exon 8	c.1411C > T (p.R471C)	1	0.82

Table 6
The detected *ETFDH* variants and their frequencies in patients with MADD.

No.	Location	Variants	Mutant allele (No.)	Allele frequency (%)
1	Exon 3	c.250G > A (p.A84T)	19	47.50
2	Exon 5	c.524G > A (R175H)	5	12.50
3	Exon 9	c.998A > G (p.Y333C)	5	12.50
4	Exon 12	c.1657T > C (p.Y553H)	3	7.50
5	Exon 3	c.380T > A (p.127H)	2	5.00
6	Exon 13	c.1845_1846insCAAT (p.G616fs*8)	2	5.00
7	Exon 7	c.770A > G (p.Y257C)	1	2.50
8	Intron 12	c.1691-3C > G	1	2.50
9	Exon 8	c.929A > G (p.Y310C)	1	2.50
10	Exon 11	c.1399G > C (p.G467R)	1	2.50

gradually clarified. This study further updated the mutation spectrum of *SLC22A5*, confirming previous studies that c.760C > T (p.R254*), c.1400C > G (p.S467C), and c.51C > G (p.F17L) are the three most common mutations in the Chinese population [17,18]. Notably, c.760C > T (p.R254*) is common in southern China but it is less frequent in northern China [11]. Several other relatively common *SLC22A5* pathogenic variants were also presented, providing valuable basis for the rapid mutation screening.

Riboflavin responsive MADD (RR-MADD) as the most common cause of lipid storage myopathy in China, more than 700 cases of RR-MADD have been reported so far, but the majority of patients were diagnosed clinically and rarely detected by NBS [19]. This study revealed that MADD was the second most common FAOD, the incidence was 1:32,180 newborns in the study population and up to 1:25,880 newborns during the combined genetic screening period [7]. The high detection rate of MADD was due to the use of second-tier genetic screening. Although MADD patients had the combined elevation of short-, medium- and long-chain acylcarnitines at NBS, acylcarnitine profiles may be normal or had only a few mild elevations at recall review. This may be due to these patients are in the anabolic state. Our data showed that 40 % of MADD patients had normal acylcarnitine profiles during recall review, such that these patients would be missed by conventional NBS. Moreover, MADD patients may present with atypical acylcarnitine profiles at NBS, as demonstrated by the patient with only elevated C5 level. NBS for MADD is therefore challenging, combining metabolic testing with second-tier genetic screening for MADD is necessary. In contrast to sporadic variants in other ethnic groups, recurrent *ETFDH* variants were mainly identified in the mainland Chinese population [20]. c.250G > A (p.A84T) was a founder mutation in southern China, c.770A > G (p.Y257C) and c.1227A > C (p.L409F) were widespread in the Chinese population [21–23]. c.250G > A (p.A84T) with a high frequency of 47.5 %, which coincided with previous viewpoint that c.250G > A was the most common mutation in southern China. Interestingly, c.770A > G (p.Y257C) was detected only once, and c.1227A > C (p.L409F) was not found in this cohort of patients. However, we found that c.524G > A (12.5 %) and c.998A > G (12.5 %) were relatively common, which suggested that the mutational spectrum of *ETFDH* gene vary greatly between northern and southern China.

SCADD is now defined as a biochemical phenotype rather than a metabolic disorder, its inclusion in NBS programs remains controversial. The identification of SCADD would increase substantial parental anxiety, it is recommended that SCADD be removed

from NBS panels or that SCADD be retained but release patients from follow-up [24]. SCADD was the third most common FAOD, which was relatively common in several NBS centers in China [25–27]. The incidence in this study was much lower than that reported in Jiangsu province (1:26,800) [25]. This may due to the uncertain clinical significance of this disorder leading to fewer families underwent further genetic diagnosis. Regarding the mutation spectrum of *ACADS* gene, previous studies have shown that c.1031A > G (p.E344G) was the most common variant in China, followed by c.164C > T (p.P55L) [9]. Inconsistent with previous studies, *ACADS* variants were sporadic in our population, seven distinct variants were detected in the five patients.

VLCADD is the second most frequent FAOD in the USA and Europe, the incidence is estimated to be between 1:30,000 and 1:100,000 live births [28]. This defect has now become the most common FAOD in Japan after the implementation of expanded NBS, with an incidence of 1:93,000 newborns [29]. However, VLCADD was rarely detected in most NBS centers in China, except for the relatively high incidence of 1:66,934 and 1:48,717 newborns reported in Suzhou city and Xi'an city [13,30]. In accordance with previous studies [11,26,27], VLCADD with a relatively low incidence of 1:160,902 newborns in our population. Elevation of C14:1and abnormal acylcarnitine ratios are the primary biomarkers for VLCADD. However, NBS for VLCADD had poor specificity since healthy individual also showed elevated C14:1 levels after fasting as a physiological response to lipolysis, resulting in overlapping C14:1 levels in VLACDD patients, heterozygotes, and healthy individuals [31]. Although all our patients showed elevated C14:1 levels during NBS, one patient with normal C14:1 level during recall review is also easily missed. Genetically, recurrent *ACADVL* variants have only been reported in a few ethnic groups. c.848T > C (p.V283A) is the most common mutation in the United States and Germany [31,32], c.1820G > C (p.C607S) is a common variant unique Japan [29]. Although c.1349G > A (p.R450H) was considered to be a common variant in the Chinese population, few VLACDD patients have been reported in China so far [33]. In this study, eight different variants were detected in four patients, suggesting that the wide allelic heterogeneity characteristic of VLACDD in the Chinese population.

CPT-IAD appears to be extremely rare in the general population. According to the NBS data from Germany, Australia, and the USA, the incidence of CPT-IAD is as low as 1:750,000 to 1:2,000,000 newborns [5]. This defect was rarely detected in the Chinese population, with an overall incidence of 1:546,128 newborns in mainland China [14]. Consistent with previous reports, only two CPT-IAD patients were detected in the selected population with an incidence of 1:321,803 newborns. Genetically, *CPT1A* high frequency variants appear to be unique in a few ethnic groups. c.2129G > A (p.G710E) is a founder mutation in the Alaskan and American Hutterite populations, with a high carrier frequency of 1:16 [34]. c.1436C > T (p.P497L) is highly prevalent among the Inuit, First Nations, and Alaska Native populations [35]. The genetic profiles of CPT-IAD in the Chinese population remains unclear due to the limited number of CPT-IAD patients.

MCADD was the most common FAOD in Australia, Europe, and the United States, with an incidence between 1:4900 and 1:24,900 newborns in North America and Europe [36]. It was relatively rare among Asians, with an incidence of 1:108,000 newborns in Japan [37]. The incidence varies greatly among different regions in China. For example, the incidence in Henan province, Lianyungang city of Jiangsu province, and Shanghai was 1:40,842 [38], 1:61,299 [26], and 1:100,428 newborns [39], respectively, while the incidence in Jining city of Shandong province was 1:171,411 newborns [11]. Remarkably, this disorder was not detected in our NBS program, indicating that MCADD may be very rare in southern China. The mutation spectrum of *ACADM* gene varies between regions and ethnicities. The pathogenic variant, c.985A > G (K329E), occurs frequently in European population [40,41]. c.449_452del (p.T150fs*4) is the most prevalent variant in East Asian (Japanese, Korean, and Chinese) populations [37,39,42]. Recently, c.1085G > A (p.G362E) was found to be more common in northern China [38]. However, only a few MCADD patients have been reported in China so far, the genetic background of MCADD require further investigation.

In summary, the prevalence, disease spectrum, and genetic characteristics of FAODs in a southern Chinese population were clarified. The overall incidence of FAODs in our population was 1:6996 newborns, with PCD being the most common FAOD, followed by MADD. Hotspot variants were found in *SLC22A5* and *ETFDH* genes, while the remaining FAODs showed great molecular heterogeneity. Incorporating second-tier genetic screening is critical for FAODs since some patients with PCD and MADD may have normal markers during recall review, these patients would be missed under conventional NBS protocols.

Ethics approval and consent to participate

This study was approved by the Ethical Committee of Quanzhou Maternity and Children's Hospital and was performed in accordance with the Declaration of Helsinki. Written informed consent was obtained from the parents of all infants for collection of samples and publication of medical data.

Consent for publication

Consent was obtained from the parents of all patients for publication.

Data availability statement

Data included in article/supp. Material/referenced in article.

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Additional information

No additional information is available for this paper.

CRediT authorship contribution statement

Yiming Lin: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Chunmei Lin:** Data curation, Formal analysis, Investigation, Methodology, Resources, Validation. **Bangbang Lin:** Data curation, Formal analysis, Investigation, Software, Validation. **Zhenzhu Zheng:** Investigation, Methodology, Resources, Validation, Visualization. **Weihua Lin:** Investigation, Methodology, Validation. **Yanru Chen:** Investigation, Methodology, Validation. **Dongmei Chen:** Conceptualization, Funding acquisition, Supervision. **Weilin Peng:** Conceptualization, Project administration, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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List of abbreviations

FAODs fatty acid oxidation disorders
PCD primary carnitine deficiency
MADD multiple acyl-CoA dehydrogenase deficiency
CACTD carnitine-acylcarnitine translocase deficiency
CPT-IAD/CPT-IID carnitine palmitoyltransferase I/II deficiency
VLCADD very long chain acyl-CoA dehydrogenase deficiency
LCHADD/TFPD long chain hydroxyacyl-CoA dehydrogenase deficiency or tri-functional protein deficiency
SCADD short chain acyl-CoA dehydrogenase deficiency
MCADD medium chain acyl-CoA dehydrogenase deficiency
M/SCHADD medium/short chain hydroxyacyl-CoA dehydrogenase deficiency
NBS newborn screening
MALDI-TOF MS matrix-assisted laser desorption/ionization-time of flight mass spectrometry
CI confidence interval
C0 free carnitine
C5 isovalerylcarnitine
C6 hexanoylcarnitine
C8 octanoylcarnitine
C10 decanoylcarnitine
C12 dodecanoylcarnitine
C14 tetradecenoylcarnitine
C16 almitoylcarnitine
C18 stearylcarnitine.

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