Biochemical And Genetic Characteristics of Patients With Primary Carnitine Deficiency Identified Through Newborn Screening

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Abstract

Background: Primary carnitine deficiency (PCD) is an autosomal recessive disorder of the carnitine transportation that leads to impaired fatty acid oxidation. Large-scale studies on newborn screening (NBS) for PCD are limited. This study aimed to investigate the biochemical and genetic characteristics of patients with PCD detected by NBS.

Results: A total of 548,247 newborns were screened for PCD between January 2014 and June 2021, 1714 newborns had low free carnitine (C0) levels were called back and forty-nine patients were diagnosed with PCD. The latest incidence rate in Quanzhou, China was estimated to be 1 in 11,189 newborns. NBS results showed that all patients had varying degrees of decreased C0 levels, while seven patients exhibited normal C0 levels during recall review. All patients harbored biallelic pathogenic variants in the *SLC22A5* gene. Nineteen distinct *SLC22A5* variants were detected in the 49 patients, most of the detected variants were clustered in exons 1, 4, and 7. The top eight variants together had an allele frequency of 86.73%. The most common variant was c.760C>T (p.R254*) with an allele frequency of 31.63%, followed by c.51C>G (p.F17L) (17.35%) and c.1400C>G (p.S467C) (16.33%). The C0 level of patients with N/N genotype was significantly lower than that of M/M group. The C0 level of patients with genotypes of R254*/R254* and R254*/F17L were far lower than patients with genotype of R254*/S467C.

Conclusions: This study presented more than 500,000 NBS data with the latest incidence of 1:11,189 in Quanzhou area. The *SLC22A5* variant spectrum in the selected southern Chinese population was updated. Patients with null variants were associated with low C0 levels. It is necessary to combine genetic testing to improve screening efficiency due to PCD patients may have normal C0 levels during NBS and recall review.

Background

Primary carnitine deficiency (PCD, OMIM #212140) is an autosomal recessive disorder of the carnitine transportation that leads to impaired fatty acid oxidation. The causative gene, *SLC22A5* (MIM# 603377), was mapped to chromosome 5q31 and encodes organic cation transporter type 2 (OCTN2) [1, 2]. Patients with this defect experience continuous renal losses of carnitine, defective ketogenesis, and low serum carnitine levels resulting in muscle weakness and pain, as well as cardiomyopathy and arrhythmias [3]. The clinical presentation of PCD shows obvious variability ranging from no clinical symptoms to acute metabolic decompensation early in life, progressive hypertrophic cardiomyopathy later in life, or even sudden death from cardiac arrhythmia [4–7]. Lifelong high dose carnitine supplementation is recommended for patients with PCD regardless of the severity of presentation, and the long-term prognosis is favourable with timely treatment [8].

Newborn screening (NBS) is a useful preventive health measure for early diagnosis, it has been proven to be economically efficient. Given that PCD is a well-treatable metabolic disease and adverse symptoms can be prevented, PCD has been included into many NBS panels worldwide. NBS for PCD is done using

tandem mass spectrometry (MS/MS), by measuring free carnitine (C0) levels below the cut-off value in dried blood spot samples. The diagnosis of PCD after NBS is mainly confirmed by mutational analysis of the *SLC22A5* gene [9, 10].

Although several reports of PCD NBS in the Chinese population have been reported, the screened number and confirmed positive patients were relatively limited [11–13]. The expanded NBS program was implemented in Quanzhou, China since January 2014. So far, more than 500,000 newborns were screened for PCD. In this study, we reported our experience of NBS for PCD over eight years in southern China. We sought to determine the latest incidence, biochemical, and genetic features of PCD identified through NBS. Additionally, we attempted to investigate the relationship between genotype and biochemical phenotype of PCD patients.

Methods

Study population

From January 2014 to June 2021, a total of 548,247 newborns (309,684 males and 238,563 females) who were born in Quanzhou, Fujian province of China were screened using MS/MS in the NBS center at Quanzhou Maternity and Children's Hospital. All the screen-positive newborns and patients genetically diagnosed with PCD (compound heterozygous or homozygous for *SLC22A5* pathogenic variants) were recruited for this study. This study was approved by the Ethical Committee of Quanzhou Maternity and Children's Hospital. Written informed consent was obtained from the parents of all patients.

Nbs And Diagnostic Evaluation

The specimen collection, delivery, and laboratory testing for NBS are as previously described [24]. Newborns with low C0 levels ($C0 < 8.5 \mu\text{mol/L}$, cut-off value: $8\text{--}50 \mu\text{mol/L}$) were called back. Newborns who tested positive on the second screen were referred for further genetic analysis. Between January 2017 and December 2018, a second-tier newborn genetic screening targeting 17 *SLC22A5* variants was added to our NBS program [19]. Targeted next-generation sequencing (NGS) was performed as previously described [27]. Definitive diagnosis was made based on abnormal C0 levels and the presence of biallelic pathogenic variants in *SLC22A5*.

Statistical Analyses

All statistical analyses were done using SPSS 26.0 (SPSS Inc., Chicago, IL, USA). Data were expressed as median $\pm$ standard deviation when normally distributed, as median (interquartile range) when non-normally distributed. One-way ANOVA test and non-parametric test were used for statistic comparisons respectively. Differences between groups were considered statistically significant when $P < 0.05$ (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.)

Results

NBS for PCD

Overall, during the study period 548,247 newborns were screened for PCD. NBS results showed that 1714 newborns had low C0 levels, yielding a positivity rate of 0.31% (1714/548,247). Forty-nine patients were diagnosed with PCD, the positive predictive value (PPV) was approximately 2.86% (49/1714). Consequently, the incidence rate in Quanzhou was estimated to be 1 in 11,189 newborns.

Biochemical Features

The initial NBS results showed that all patients had varying degrees of decreased C0 levels. The mean C0 level at NBS in this cohort was 4.16 ± 1.78 $\mu\text{mol/L}$ (range 1.63–8.25, cut-off value: 8.5–50 $\mu\text{mol/L}$). In comparison, seven patients (14.3%) (no. 15, 16, 26, 27, 30, 37, and 42) exhibited normal C0 levels during recall review. The mean C0 level was increased to 4.69 ± 3.01 $\mu\text{mol/L}$ on the second screen (range 1.05–14.27, cut-off value: 8.5–50 $\mu\text{mol/L}$) (Table 1).

Table 1
Biochemical and genetic characteristics of 49 patients with PCD

Patient no.	Gender	C0	C0-F1	Genotype		References
1	Male	4.61	6.29	c.51C>G (p.F17L)	c.1195C>T(p.R399W)	This study
2	Female	3.28	3.17	c.51C>G (p.F17L)	c.51C>G (p.F17L)	This study
3	Female	2.37	1.05	c.338G>A (p.C113Y)	c.760C>T (p.R254*)	This study
4	Male	7.65	5.04	c.51C>G (p.F17L)	c.1400C>G (p.S467C)	This study
5	Male	3.75	3.19	c.760C>T (p.R254*)	c.1400C>G (p.S467C)	This study
6	Male	2.72	2.86	c.844C>T (p.R282*)	c.1400C>G (p.S467C)	This study
7	Female	2.54	2.29	c.695C>T (p.T232M)	c.760C>T (p.R254*)	This study
8	Male	5.29	6.58	c.51C>G (p.F17L)	c.1195C>T(p.R399W)	This study
9	Female	5.02	5.53	c.428C>T (p.P143L)	c.428C>T (p.P143L)	This study
10	Female	1.63	1.67	c.760C>T (p.R254*)	c.760C>T (p.R254*)	This study
11	Male	2.31	2.76	c.51C>G (p.F17L)	c.1161T>G(p.Y387*)	This study
12	Female	3.49	3.52	c.51C>G (p.F17L)	c.760C>T (p.R254*)	Lin et al. 2020 ^[10]
13	Male	1.96	1.73	c.51C>G (p.F17L)	c.760C>T (p.R254*)	Lin et al. 2021 ^[19]
14	Female	2.40	1.44	c.760C>T (p.R254*)	c.760C>T (p.R254*)	Lin et al. 2021 ^[19]
15	Male	5.78	10.67	c.760C>T (p.R254*)	c.797C>T (p.P266L)	Lin et al. 2021 ^[19]
16	Male	5.95	8.64	c.695C>T (p.T232M)	c.1160A>G (p.Y387C)	Lin et al. 2021 ^[19]
17	Female	7.27	6.66	c.760C>T (p.R254*)	c.797C>T (p.P266L)	Lin et al. 2021 ^[19]
18	Female	5.58	5.59	c.760C>T (p.R254*)	c.1400C>G (p.S467C)	Lin et al. 2021 ^[19]
19	Female	5.34	6.02	c.797C>T (p.P266L)	c.394-1G>A	Lin et al. 2021 ^[19]

C0: free carnitine detected at newborn screening, C0-F1: C0 retested at recall stage, cutoff value: 8.5-50 µmol/L.

Patient no.	Gender	C0	C0-F1	Genotype		References
20	Female	1.78	1.90	c.695C>T (p.T232M)	c.1139C>T (p.A380V)	Lin et al. 2021 ^[19]
21	Male	4.34	4.45	c.51C>G (p.F17L)	c.51C>G (p.F17L)	Lin et al. 2021 ^[19]
22	Female	4.75	4.16	c.760C>T (p.R254*)	c.845G>A (p.R282Q)	Lin et al. 2021 ^[19]
23	Female	3.45	5.24	c.760C>T (p.R254*)	c.1400C>G (p.S467C)	Lin et al. 2021 ^[19]
24	Female	6.82	5.02	c.760C>T (p.R254*)	c.1400C>G (p.S467C)	Lin et al. 2021 ^[19]
25	Male	2.19	2.12	c.822G>A (p.W274*)	c.782_799del ((p.V261_P266del)	Lin et al. 2021 ^[19]
26	Male	2.73	9.84	c.51C>G (p.F17L)	c.1144_1162del (p.V382Cfs*45)	Lin et al. 2021 ^[19]
27	Male	3.00	10.81	c.51C>G (p.F17L)	c.1400C>G (p.S467C)	Lin et al. 2021 ^[19]
28	Male	6.46	5.10	c.695C>T (p.T232M)	c.1400C>G (p.S467C)	Lin et al. 2021 ^[19]
29	Male	3.02	1.77	c.760C>T (p.R254*)	c.760C>T (p.R254*)	Lin et al. 2021 ^[19]
30	Female	6.77	10.05	c.1400C>G (p.S467C)	c.1400C>G (p.S467C)	Lin et al. 2021 ^[19]
31	Female	2.36	1.75	c.760C>T (p.R254*)	c.760C>T (p.R254*)	Lin et al. 2021 ^[19]
32	Female	3.12	2.88	c.760C>T (p.R254*)	c.51C>G (p.F17L)	Lin et al. 2021 ^[19]
33	Male	3.64	3.80	c.695C>T (p.T232M)	c.1139C>T (p.A380V)	Lin et al. 2021 ^[19]
34	Female	3.56	4.31	c.760C>T (p.R254*)	c.1139C>T (p.A380V)	Lin et al. 2021 ^[19]
35	Female	6.27	3.43	c.695C>T (p.T232M)	c.1139C>T (p.A380V)	Lin et al. 2021 ^[19]

C0: free carnitine detected at newborn screening, C0-F1: C0 retested at recall stage, cutoff value: 8.5-50 µmol/L.

Patient no.	Gender	C0	C0-F1	Genotype		References
36	Female	2.70	3.46	c.760C>T (p.R254*)	c.51C>G (p.F17L)	Lin et al. 2021 ^[19]
37	Male	7.35	14.27	c.338G>A (p.C113Y)	c.338G>A (p.C113Y)	Lin et al. 2021 ^[19]
38	Male	8.25	2.51	c.51C>G (p.F17L)	c.338G>A (p.C113Y)	Lin et al. 2019 ^[24]
39	Male	2.45	1.14	c.760C>T (p.R254*)	c.760C>T (p.R254*)	Lin et al. 2019 ^[24]
40	Male	2.8	1.74	c.760C>T (p.R254*)	c.1161T>G(p.Y387*)	Lin et al. 2019 ^[24]
41	Female	6.83	4.59	c.760C>T (p.R254*)	c.1400C>G(p.S467C)	Lin et al. 2019 ^[24]
42	Female	6.22	11.14	c.760C>T (p.R254*)	c.1400C>G(p.S467C)	Lin et al. 2019 ^[24]
43	Female	6.16	4.02	c.695C>T(p.T232M)	c.1400C>G(p.S467C)	Lin et al. 2019 ^[24]
44	Male	6.77	4.5	c.760C>T (p.R254*)	c.1400C>G(p.S467C)	Lin et al. 2019 ^[24]
45	Female	4.91	6.66	c.250T>A(p.Y84N)	c.1400C>G(p.S467C)	Lin et al. 2019 ^[24]
46	Male	3.24	4.05	c.51C>G (p.F17L)	c.1196G>A(p.R399Q)	Lin et al. 2019 ^[24]
47	Male	4.96	5	c.51C>G (p.F17L)	c.1195C>T(p.R399W)	Lin et al. 2019 ^[24]
48	Female	3.15	4.09	c.760C>T (p.R254*)	c.1400C>G(p.S467C)	Lin et al. 2019 ^[24]
49	Female	4.12	1.29	c.760C>T (p.R254*)	c.760C>T (p.R254*)	Lin et al. 2019 ^[24]
C0: free carnitine detected at newborn screening, C0-F1: C0 retested at recall stage, cutoff value: 8.5-50 µmol/L.						

SLC22A5 variants and allele distributions

Forty-nine patients with biallelic pathogenic variants in the *SLC22A5* gene were eventually diagnosed with PCD. A total of 19 distinct *SLC22A5* variants were detected in the 49 patients. Among which 63.2%

(12/19) were missense, 21.1% (4/19) were nonsense, 10.5% (2/19) were frameshift variants, and 5.3% (1/19) affected splicing. All variants have been previously reported, most of the detected variants were clustered in exons 1, 4, and 7. The most common variant was c.760C>T (p.R254*) with an allele frequency of 31.63%, followed by c.51C>G (p.F17L) (17.35%) and c.1400C>G (p.S467C) (16.33%). The other relatively common variants were c.695C>T (p.T232M), c.338G>A (p.C113Y), c.1139C>T (p.A380V), c.797C>T (p.P266L), and c.1195C>T (p.R399W). These eight variants together accounted for 86.73% (85/98) of all mutated alleles (Table 2).

Table 2
SLC22A5 variants and allele distributions in patients with PCD

No.	Location	Variants	Variant type	Mutant allele (No.)	Allele frequency (%)
1	Exon 4	c.760C>T (p.R254*)	Nonsense	31	31.63
2	Exon 8	c.1400C>G (p.S467C)	Missense	16	16.33
3	Exon 1	c.51C>G (p.F17L)	Missense	17	17.35
4	Exon 4	c.695C>T (p.T232M)	Missense	7	7.14
5	Exon 1	c.338G>A (p.C113Y)	Missense	4	4.08
6	Exon 7	c.1139C>T (p.A380V)	Missense	4	4.08
7	Exon 4	c.797C>T (p.P266L)	Missense	3	3.06
8	Exon 7	c.1195C>T (p.R399W)	Missense	3	3.06
9	Exon 2	c.428C>T (p.P143L)	Missense	2	2.04
10	Exon 7	c.1161T>G (p.Y387*)	Nonsense	2	2.04
11	Exon 1	c.250T>A (p.Y84N)	Missense	1	1.02
12	Intron 1	c.394-1G>A	Splice	1	1.02
13	Exon 4	c.782_799del (p.V261_P266del)	Frameshift	1	1.02
14	Exon 7	c.1144_1162del (p.V382Cfs*45)	Frameshift	1	1.02
15	Exon 4	c.822G>A (p.W274*)	Nonsense	1	1.02
16	Exon 5	c.844C>T (p.R282*)	Nonsense	1	1.02
17	Exon 5	c.845G>A (p.R282Q)	Missense	1	1.02
18	Exon 7	c.1160A>G (p.Y387C)	Missense	1	1.02
19	Exon 7	c.1196G>A (p.R399Q)	Missense	1	1.02
Total				98	100.00

Relationship Between Genotype And Biochemical Phenotype

The PCD patients were divided into three groups: (i) N/N (null/null, n=8), (ii) N/M (null/missense, n=22), and (iii) M/M (missense/missense, n=19). As shown in Figure 1a, the median of N/N, N/M, and M/M groups was 2.43, 3.53, and 5.02, respectively. The C0 level of patients with N/N genotype was significantly lower than that of M/M group ($P = 0.001$). Patients with N/N genotype also had low C0 level compared to the N/M group, while no significant difference was observed ($P = 0.055$). The C0 level was no significant difference between N/M and M/M groups ($P = 0.304$).

Besides, patients with c.760C>T (p.R254*) and two other common variants were grouped. There were six patients with genotype of R254*/R254*, four patients with genotype of R254*/F17L, and eight patients with genotype of R254*/S467C. The mean C0 level in patients with genotypes of R254*/R254*, R254*/F17L, and R254*/S467C was 2.66 ± 0.84 , 2.82 ± 0.66 , and 5.32 ± 1.61 $\mu\text{mol/L}$, respectively. The C0 level of patients with genotypes of R254*/R254* and R254*/F17L were far lower than patients with genotype of R254*/S467C ($P = 0.001$ and $P = 0.005$, respectively) (Figure 1b).

Discussion

PCD is the most common fatty acid metabolic disorder in China. NBS for PCD has been successfully implemented in most parts of China, patients usually had better prognosis following early L-carnitine therapy. The incidence of PCD varies greatly throughout China, ranging from 1:8,938 to 1:48,717 [13–15]. The largest scale NBS study in China showed that the incidence of PCD was 1:30,182 in Zhejiang province [8]. This study presented more than 500,000 NBS data, indicating that the latest incidence in Quanzhou area was 1:11,189, which is similar to the incidence recently reported in Guangzhou (1:13,345) and Ningbo (1:16,595) areas [15, 16]. It is noteworthy that the actual incidence of PCD should be higher since current MS/MS-based NBS with poor sensitivity [17, 18].

NBS results showed that all patients with low C0 levels, while some patients may have normal C0 levels during recall review. These patients would have been excluded from conventional NBS, but could be identified by incorporating second-tier genetic screening [19]. However, it is noteworthy that PCD patients are also easily missed during NBS because the C0 levels at birth could be affected by the maternal concentration and therefore cannot reflect the true levels of the newborns themselves. Luo et al. recently conducted a pilot study in which genetic screening was performed on only 1,127 neonates using targeted NGS, and one PCD case of false-negative (C0=11.6 $\mu\text{mol/L}$) was identified [20]. Therefore, NBS for PCD NBS is facing challenges, and it is necessary to combine genetic testing to improve screening efficiency.

This study have updated the *SLC22A5* variant spectrum of a southern Chinese population. The top eight variants together had an allele frequency of 86.73%, which provided an important evidence for the rapid screening of targeted *SLC22A5* variants in the Chinese population. Many studies have shown that c.51C>G (p.F17L), c.760C>T (p.R254*), and c.1400C>G (p.S467C) are the three most common variants in

the Chinese population, but which variant has the highest frequency varies among diverse regions. For instance, c.1400C>G (p.S467C) was the most frequent variant in Jining, Suzhou, Guangzhou, Xuzhou, and Ningbo areas [12, 15, 16, 21, 22]; c.51C>G (p.F17L) was the most common variant in Liuzhou area [23]. By contrast, our data revealed that c.760C>T (p.R254*) was the most common variant in this cohort of patients, and its allele frequency was almost equal to the sum of the other two variants. c.760C>T (p.R254*) is a loss-of-function variant that can cause severe clinical symptoms, it was common in southern China but was rarely detected in northern China [14, 22, 24–26], indicating this variant presented different geographic distribution. Whereas c.1400C>G (p.S467C) with residual function may result in a milder phenotype, it was common in both southern and northern China [14, 22, 24, 26], suggesting this variant was common in the general Chinese population.

Regarding the relationship between genotype and biochemical phenotype, significant difference was observed in C0 levels between patients with N/N and M/M genotypes, and most patients with N/N genotype had low C0 levels compared to the N/M group, indicating patients with null variants were associated with low C0 levels. Notably, patients with N/M genotype may also have very low C0 levels if the missense variant with significantly impaired transport activity. As demonstrated in Figure 1b, no significant difference was observed in C0 levels between patients with genotypes of R254*/R254* and R254*/F17L. In contrast, significant difference was observed in C0 levels between patients with genotypes of R254*/R254* and R254*/S467C because c.1400C>G (p.S467C) retained residual carnitine transport activity.

In summary, this study presented more than 500,000 NBS data with the latest incidence of 1:11,189 in Quanzhou area. The *SLC22A5* variant spectrum in the selected southern Chinese population was updated, the top eight variants together had an allele frequency of 86.73%, and c.760C>T (p.R254*) was the most common variant. Patients with null variants were associated with low C0 levels. It is necessary to combine genetic testing to improve screening efficiency due to PCD patients may have normal C0 levels during NBS and recall review.

Abbreviations

PCD: primary carnitine deficiency; OCTN2: organic cation transporter type 2; NBS: newborn screening; MS/MS: tandem mass spectrometry; C0: free carnitine; NGS: next-generation sequencing; N/N: null/null; N/M: null/missense; M/M: missense/missense.

Declarations

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.

Availability of data and materials

The datasets used and/or analysed during the current study can be obtained from the corresponding author upon a reasonable request.

Conflict of Interest

The authors declare that they have no conflicts of interest.

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Author contributions

YM Lin performed the data analysis, drafted and revised the manuscript; BB Lin performed the statistical analyses; YR Chen, ZZ Zheng, and QL Fu followed the patients and collected the clinical data; WF Zhang assisted with data collection, reviewed and revised the manuscript; WH Lin supervised the research study. All authors read and approved the final manuscript.

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Figures

Figure 1

Comparison of the CO concentrations ($\mu\text{mol/L}$) in PCD patients with different genotypes. N/N: null/null; N/M: null/missense; M/M: missense/missense. Statistically significant differences are indicated by asterisks (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$) above a bracket connecting two groups.