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Newborn screening and genetic diagnosis of 3-methylcrotonyl-CoA carboxylase deficiency in Quanzhou,China

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ABSTRACT

Background and aims: 3-Methylcrotonyl-CoA carboxylase deficiency (3-MCCD) is an autosomal recessive leucine catabolism condition caused by 3-methylcrotonyl-CoA carboxylase (3-MCC) deficiency due to *MCCC1/MCCC2* variants. We investigated its incidence and features in Quanzhou, China.
Materials and methods: We screened 643,606 newborns (January 2014 to December 2022) for elevated 3-hydroxyisovalerylcarnitine (C5OH) levels using tandem mass spectrometry (MS/MS). Molecular analyses identified *MCCC1/MCCC2* variants in suspected 3-MCCD cases.
Results: Seventeen neonates, two maternal patients, and one paternal patient had 3-MCCD. Its incidence in the Quanzhou study population was 1/37,859 newborns. All patients and neonates with 3-MCCD exhibited increased C5OH concentrations. Most patients [76.5%(13/17)] had increased urinary 3-methylcrotonylglycine (3-MCG) and 3-hydroxyisovaleric acid (3-HIVA) levels. Eight neonates and all adults with 3-MCCD had secondary carnitine deficiency. We identified seventeen variants, including 6 novel ones.*MCCC1*and *MCCC2* variants were found in 47.1% and 52.9% of patients,with c.1331G > A (31.3%) and c.351_353delTGG (50.0%) being the most prevalent, respectively. Clinical symptoms were observed in 11.8% of patients.
Conclusion: We identified six new *MCCC1/MCCC2* variants, enhancing our understanding of the 3-MCCD molecular profile. Secondary carnitine deficiency occurred in eight neonates and all adult patients. Although clinical symptoms were observed in 11.8% of patients, whether they were related to 3-MCCD remain unclear. Therefore, further studies are required to decide whether 3-MCCD and C5OH indicators should continue to be used.

1. Introduction

3-Methylcrotonyl-CoA carboxylase deficiency (3-MCCD, OMIM#210210) is a metabolic condition related to leucine catabolism caused by a deficiency in 3-methylcrotonyl-CoA carboxylase (3-MCC) due to variants in the *MCCC1* and *MCCC2* genes [1]. It is an autosomal recessive, hereditary disorder characterized by increased urinary excretion of 3-methylcrotonylglycine (3-MCG), 3-hydroxyisovaleric acid (3-HIVA), and 3-hydroxyisovalerylcarnitine (C5OH) and low plasma levels of carnitine [2–4]. *MCCC1* is located on chromosome 3q25–27,contains 19 exons spanning 2580 bp, and encodes 725 amino acid polypeptides. *MCCC2* is located on chromosome 5q12-q13.1, contains 17 exons spanning 2304 bp, and encodes 563 amino acid polypeptides [2]. The clinical manifestations of 3-MCCD can vary widely, ranging from asymptomatic individuals to those experiencing acute metabolic crises, including hyperammonemia, hypoglycemia, metabolic acidosis, and neurological abnormalities [5,6]. Most patients, especially those diagnosed via newborn screening (NBS), are asymptomatic [7–9].

Patients with3-MCCD often have increased C5OH concentrations, which can be detected via expanded NBS using tandemmass spectrometry (MS/MS). However, C5OH elevation is not specific to 3-MCCD; it is also observed in patients with holocarboxylase synthetase deficiency (HLCSD), 3-methylglutaconic acidemia, 3-hydroxy-3-methylglutaryl-

Abbreviations: 3-HIVA, 3-hydroxyisovaleric acid; 3-MCC, 3-methylcrotonyl-CoA carboxylase; 3-MCCD, 3-Methylcrotonyl-CoA carboxylase deficiency; 3-MCG, 3-methylcrotonylglycine; C0, free carnitine; C5OH, 3-hydroxyisovalerylcarnitine; HLCSD, holocarboxylase synthetase deficiency; MS/MS, tandem mass spectrometry; NBS, newborn screening..

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Table 1
Biochemical phenotypes, genotypes, andclinical features of patients with 3-MCCD.

Case	Gender	Age	Biochemical phenotype						Genotype			Clinical phenotype
			Primary C5OH	Primary C0	Recall C5OH	Recall C0	Urine 3-MCG	Urine 3-HIVA	AffectedGene	Variant 1	Variant 2	
1	F	5y 4 m	1.67	16.89	1.32	27.88	**2.14**	**102**	*MCCC1*	c.639 + 5G > T /	c.227_228delTG p. V76Gfs*4	N
2	M	5y 5 m	6.85	9.67	10.5	**3.26**	**35.79**	**470.88**	*MCCC1*	c.1630delA■p. R544Dfs*2	c.1331G > A p.R444H	Hyperammone-mia,lactacemia
3	M	6y 4 m	5.32	11.81	14.16	**6.92**	**16.07**	**736.43**	*MCCC2*	c.351_353delTGG p.G118del	c.592C > T p.Q198*	N
4[H]	M	5y 7 m	8.1	17.05	13.49	**3.57**	**39.4**	**944.9**	*MCCC2*	c.1103delG p.G368Vfs*70	c.1103delG p.G368Vfs*70	N
5[T]	M	3y 9 m	2.46	11.61	4.15	10.81	**67.37**	**71.87**	*MCCC1*	c.1331G > A p.R444H	c.617C > T■ p.A206V	N
6[T]	M	3y 9 m	2.4	13.33	4.16	10.65	**96.38**	**107.63**	*MCCC1*	c.1331G > A p.R444H	c.617C > T■ p.A206V	N
7	M	3y	1.33	20.96	5.91	27.82	0.00	1.18	*MCCC2*	c.351_353delTGG p.G118del	c.914 A > G p.E305G	N
8	F	2y 6 m	1.36	13.03	0.91	31.25	0.00	0.35	*MCCC1*	c.1894C > T p.P632S	c.655_657delins GAAAT■ p.R219Efs*11	N
9	M	2y 2 m	8.19	**5.65**	11.69	**2.57**	**202.68**	**113.53**	*MCCC2*	c.1103delG p. G368Vfs*70	c.351_353delTGG p. G118del	N
10	M	2y 2 m	8.53	**7.7**	13.27	**1.8**	**6.76**	**47.28**	*MCCC2*	c.440delG■p. P147Qfs*2	c.351_353delTGG p.G118del	N
11	F	2y 6 m	1.64	15.87	4.37	39.24	0.34	2.31	*MCCC2*	c.709G > A■ p.G237S	c.351_353delTGGp. G118del	N
12	M	1y 9 m	1.66	24.94	1.24	35.2	0.03	0.52	*MCCC1*	c.1450G > A p.V484M	c.1331G > A p.R444H	N
13	M	1y 9 m	2.13	21.24	2.21	30.37	**3.43**	**21.5**	*MCCC1*	c.639 + 2 T > A p.S164Rfs*3	c.196C > T p.R66C	N
14	F	1y 8 m	6.1	**7.63**	11.96	**3.1**	**306.94**	**148.31**	*MCCC2*	c.592C > T p.Q198*	c.351_353delTGGp. G118del	N
15	F	11 m	10.54	10.88	32.26	**2.67**	**155.06**	**97.05**	*MCCC2*	c.1367C > T p. A456V	c.351_353delTGGp. G118del	Globaldevelop-mental delay at two years old
16[H]	F	11 m	12.54	15.88	12.18	**3.84**	**46.07**	**58.1**	*MCCC2*	c.351_353delTGG p.G118del	c.351_353delTGGp. G118del	N
17	F	3y 7 m	1.76	24.48	2.97	26.9	**1.61**	**24.7**	*MCCC1*	c.639 + 5G > T /	c.1331G > A p.R444H	N
18★[H]	F	32y	16.19	**3.95**	27.76	23.95	**25.74**	**116.47**	*MCCC1*	c.1331G > A p. R444H	c.1331G > A p.R444H	N
19★	F	28y	20.39	**3.6**	Na	Na	Na	Na	*MCCC2*	c.1367C > T p. A456V	c.1538 T > C■ p.F513S	N
20※[H]	M	38y	29.77	**3.64**	36.2	**3.99**	Na	Na	*MCCC1*	c.980C > G p.S327*	c.980C > G p.S327*	N

N, normal; Na, not available; urine 3-MCG, urine 3-methylcrotonylglycine; urine 3-HIVA, urine 3-hydroxyisovaleric acid; y, year; m, month.
C5OH: 3-hydroxyisovalerylcarnitine; C0: free carnitine.
Reference range: C5OH (0.07–0.5 μmol/L), C0 (8.5–50 μmol/L),urine 3-MCG (0.0–1.05 mmol/Mol creatinine), urine 3-HIVA (0.0–6.1 mmol/Mol creatinine) Blood ammonia (10-47 μmol/L), Blood lactic acid (12-16 mg/dL).
■: Novel variants.
H: homozygous variant.
T:Cases5 and 6are identical twins.
★:Mother with 3-MCCD.
※:Father with 3-MCCD.

CoA lyase deficiency, 2-methyl-3-hydroxybutyric acidemia, and beta-ketothiolase deficiency [10–12]. Therefore, differential diagnosis and definitive genetic testing are necessary. Maternal 3-MCCD may also be detected by screening neonates for abnormal C5OH levels [13].

The first case of 3-MCCD ever reported was registered in 1970 [14]. NBS for 3-MCCD has been conducted in many regions of China; however, its incidence varies significantly from region to region. Quanzhou is a prefecture-level city located on the south-east coast of Fujian province, China. By the end of 2023, the permanent population of Quanzhou was about 8.8 million, of which 97.8% are Han and 2.2% are ethnic minorities (http://tjj.quanzhou.gov.cn/). This study aimed to understand the incidence, biochemical, molecular,and clinical features of 3-MCCD in Quanzhou, China. The results of the NBS for 3-MCCD from January 2014 to December 2022 are described.

2. Materials and methods

2.1. Study population

Between January 2014 and December 2022, 643,606 newborns were screened using MS/MS at the Neonatal Disease Screening Center of Quanzhou Maternity and Children's Hospital. This study was approved by the Ethics Committee of the Quanzhou Maternity and Children's Hospital. All parents of the screened newborns provided their informed consent.

2.2. NBS, biochemical, and genetic analysis

Blood samples were collected from newborns72 h after birth. After

Table 2
The frequencies of *MCCC1*gene mutations of the17 neonatal patients with 3-MCCD.

No.	Gene	Nucleotide variants	Amino acid change	Frequencies (%)	Mutationtype	References
1	MCCC1	c.1331G > A	p.R444H	31.3	Missense	Cheng et al. (2023) [20]
2	MCCC1	c.639 + 5G > T	/	12.5	Splicing	Cheng et al. (2023) [20]
3	MCCC1	c.617C > T■	p.A206V	12.5	Missense	This study
4	MCCC1	c.227_228delTG	p.V76Gfs*4	6.3	Frameshift	Grunert et al. (2012) [4]
5	MCCC1	c.1630delA■	p.R544Dfs*2	6.3	Frameshift	This study
6	MCCC1	c.1894C > T	p.P632S	6.3	Missense	Shepard et al. (2015) [32]
7	MCCC1	c.655_657delins GAAAT■	p.R219Efs*11	6.3	Frameshift	This study
8	MCCC1	c.1450G > A	p.V484M	6.3	Missense	Hong et al. (2017) [22]
9	MCCC1	c.639 + 2 T > A	p.S164Rfs*3	6.3	Frameshift	Grunert et al. (2012) [4]
10	MCCC1	c.196C > T	p.R66C	6.3	Missense	Cheng et al. (2023) [20]

MCCC1(NM_020166.5).

delivery to the laboratory, samples were pre-processed according to the operating instruction of NeoBase™ non-derivatized MS/MS kit (PekinElmer, Waltham, MA, USA) [15]. A. MS/MS Screening System (ACQUITY TQD, Waters, Milford, MA, USA) was used to detect the C5OH levels. The C5OH cut-off values were 0.07–0.5 μmol/L. When newborns had two successive increased C5OH level results,the blood C5OH level of their mothers was tested to exclude maternal 3-MCCD. Biochemical tests, including complete blood count, blood gas evaluation, and plasma ammonia concentration, were monitored routinely, and urinary organic acid analysis was performed for differential diagnosis as previously reported [16]. Molecular analysis was conducted when the C5OH levels of either the blood samples of the mother or newborns collected during follow-up were positive.

3. Results

3.1. Newborn screening

Overall, 2487 neonates had elevated C5OH levels during NBS, yielding a positive ratio of 0.39% (2487/643,606). Among the neonates with elevated C5OH, 2460 were successfully recalled for retesting (98.9%) and 17 patients were diagnosed with 3-MCCD, with a positive predictive value (PPV) of 0.69%. Thus, the incidence of 3-MCCD in our population was 1:37,859.One paternal and two maternal patients with 3-MCCD were identified. In addition, one patient was diagnosed with HLCSD.

3.2. Biochemical description

In the 17 neonatal patients with 3-MCCD, the C5OH concentrations fluctuated from 1.33 to 12.54 μmol/L in the initial test and 0.91 to 32.26 μmol/L in the second test. The initial mean C5OH concentration of patients was 4.86 ± 3.68 μmol/L and the follow-up screening mean concentration was 8.63 ± 7.81 μmol/L. Thirteen cases had higher C5OH levels in the follow-up screening than in the initial test, whereas four cases exhibited a reduction in the C5OH levels. The initial free carnitine (C0) concentrations fluctuated from 5.65 to 24.94 μmol/L, and three newborns had low C0 concentrations (5.65–7.7 μmol/L). At the follow-up screening, the C0 concentration fluctuated from 1.8 to 39.24 μmol/L, with eight neonatal cases having low C0 levels (1.8–6.92 μmol/L), three of which had lower C0 levels than in the first test, decreasing from 5.65, 7.7, and 7.63 μmol/L to 2.57, 1.8, and 3.1 μmol/L, respectively. Low C0 levels were most often accompanied by low C2, C3, and C4, followed by low C16 and C18. The C5OH levels of the adult patients fluctuated from 16.19 to 29.77 μmol/L; moreover, all patients had low C0 levels (3.6–3.95 μmol/L). The patient with HLCSD had a high C5OH level (1.37 μmol/L) and low C0 level (6.5 μmol/L).

Urine organic acids were detected in all neonatal patients. The urinary3-MCG and 3-HIVA levels increased in 76.5% (13/17) of the patients from 1.61 to 306.94 and 21.5 to 944.9 mmol/mol creatinine, respectively; the remaining 23.5%(4/17) of patients had normal 3-MCG and 3-HIVA levels. Of the 13 patients whose C5OH levels were further elevated at the follow-up screening, 11 had typical elevations in the urinary 3-HIVA and 3-HCG levels (seven of which had low C0 concentrations). Urine organic acids were detected in one adult patient with increased levels of urinary 3-MCG and 3-HIVA (Table 1).

3.3. Genetic results

In the 17 neonatal patients with 3-MCCD, 17 distinct genetic variants were identified. Eight patients (47.1%) had *MCCC1* variants, all of which were compound heterozygous. Ten distinct *MCCC1* variants were identified, seven of which have previously been reported; three variants (c.617C > T, c.1630delA, and c.655_657delinsGAAAT) were identified for the first time. The most common variant was c.1331G > A(31.3%), followed by c.639 + 5G > T (12.5%) and c.617C > T (12.5%) (Table 2).

Table 3
The frequencies of *MCCC2* gene mutations of the17 neonatal patients with 3-MCCD.

No.	Gene	Nucleotide variants	Amino acid change	Frequencies (%)	Mutationty pe	References
1	MCCC2	c.351_353delTGG	p.G118del	50	Frameshift	Grunert et al. (2012) [4]
2	MCCC2	c.1103delG	p.G368Vfs*70	16.7	Frameshift	Wang et al. (2019) [19]
3	MCCC2	c.592C > T	p.Q198*	11.1	Nonsense	Grunert et al. (2012) [4]
4	MCCC2	c.1367C > T	p.A456V	5.6	Missense	Grunert et al. (2012) [4]
5	MCCC2	c.914 A > G	p.E305G	5.6	Missense	Cheng et al. (2023) [20]
6	MCCC2	c.440delG■	p.P147Qfs*2	5.6	Frameshift	This study
7	MCCC2	c.709G > A■	p.G237S	5.6	Missense	This study

MCCC2(NM_022132.5).

Nine patients (52.9%) had *MCCC2* variants; two had homozygous *MCCC2* variants (c.1103delG and c.351_353delTGG) and seven had compound heterozygous *MCCC2* variants. Seven distinct *MCCC2* variants were identified,five of which have previously been reported; two variants were identified for the first time (c.440delG and c.709G > A). The most frequent variant was c.351_353delTGG (50.0%), followed by c.1103delG (16.7%) and c.592C > T (11.1%) (Table 3). Two of the variants found in adult patients were homozygous (c.1331G > A and c.980C > G) and one was compound heterozygous. Four variants, including a novel c.1538 T > C, were identified (Table 1). One patient with HLCSD carried compound heterozygous variants of c.1741G > A (p.G581S) and c.2159delT (p.L720Pfs*31) in the *HLCS* gene.

3.4. Treatment and follow-up

All eight neonates with low C0 levels were recommended to take an oral L-carnitinesupplement of 50–100 mg/kg, 2–3 times daily. Ultimately, only four patients were treated with carnitine supplements. Of these patients, C0 levels were normal and C5OH levels were elevated further following treatment. Urinary organic acid was not reviewed during follow-up. One patient developed hyperammonemia and lactacemia at 16 days, 43 days, and 8 months after birth, with blood ammonia and lactic acid fluctuating in the 89–156 μmol/L and 25–30 mg/dL ranges, respectively. The other three patients remained asymptomatic. The hyperammonemia patient was treated with L-carnitine and arginine [17,18], and arginine was discontinued after 8 months of age when the blood ammonia level was normal. At the last follow-up before publishing, the patient had no recurrence of hyperammonemia. The mental development and growth of this patient were normal. The other three patients exhibited no symptoms. Of the four patients left untreated, one patient developed global developmental delay at 2 years old and the other three patients remained asymptomatic.

4. Discussion

3-MCCD is the most common organic acid metabolic disorder in many countries [5]. The country with the highest incidence (1:2400) of 3-MCCD worldwide is the Faroe Islands [19]. The incidence ratio of 3-MCCD was estimated to be 1:36,000 in North Carolina [20], 1:41,676 in California [8], 1:43,000 in Israel [21], 1:79,179 in South Korea [22], and 1:84,700 in Germany [7]. No national statistics regarding the prevalence of 3-MCCD in China exist, and the incidence of 3-MCCD varies greatly from region to region in China, ranging from 1:33,412 to 1:83,068 [23–27]. During the 9-year study period, 17 neonates and three adults with 3-MCCD were identified via NBS. The incidence of 3-MCCD in Quanzhou, Fujian Province, was 1:37,859, which is close to that in Jiangsu Province [24].

In this study, most neonatal patients showed higher C5OH concentrations in the follow-up screening, and eight neonatal cases had low C0 levels. Secondary carnitine deficiency was relatively common in patients with 3-MCCD [6,28]. It has been reported that approximately 60% (24/40) of patients with 3-MCCD have low C0 levels, which is consistent with the 15 cases with C0 levels below 5 μmol/L in this study [5]. The decline in C0 levels was consistent with the persistently high C5OH levels [29]. Secondary carnitine deficiency occurs when the C5OH concentration is greater than 5 μmol/L [25]. We found that 53.8% (7/13) of the neonatal patients with higher C5OH levels had low C0 levels in the follow-up screening, and six cases had C0 levels below 4 μmol/L. Patients with low C0 levels had C5OH concentrations greater than 10 μmol/L. All adult patients had C5OH concentrations greater than 16 μmol/L, while C0 levels were below 4 μmol/L. The patients with higher C5OH levels were more likely to have significantly low C0 levels.

In this study, 76.5% of the neonatal patients had increased urinary 3-MCG and 3-HIVA levels. Moreover, 85% of the patients with abnormal urinary organic acids had higher C5OH concentrations in the follow-up screening. This suggests that some patients with increased urinary 3-MCG and 3-HIVA levels may have a delayed excretion of these compounds; therefore, regularly testing these levels is crucial during follow-up. Patients with abnormal urinary organic acid levels usually have a severe secondary carnitine deficiency [30]. All the patients with low C0 levels in the present study had increased urinary 3-MCG and 3-HIVA levels. The presence of typical urinary organic acids indicates severe carnitine consumption and significant blood biochemical abnormalities.

To date, 103 and 113 *MCCC1* and *MCCC2* variants, respectively, have been reported in the Human Gene Mutation Database(HMGD) [31]. In the Faroe Islands, most patients have a homozygous *MCCC1* variant, c.1526delG [19]. In Germany, *MCCC1* variants appear to be dominant,with c.1155 A > C being the most common [25]. In China, *MCCC1* variants also appear to be dominant [24,26]; however, the most frequent *MCCC1* variant varies across regions in China. The c.639 + 2 T > A variant is the most prevalent in Jiangsu Province, Zhejiang Province, and Suzhou City [24–26]. Here, eight newborns (47.1%) with 3-MCCD had *MCCC1* variants,with c.1331G > A (31.3%) being the most prevalent; this variant was also reported in Zhejiang Province (7.1%) [25]. In contrast, *MCCC2* variants are more dominant in Korea and Portugal, with most patients having the c.838G > T [32] and c.1015G > A [33] *MCCC2* variants, respectively. In China, the c.1144_1147delinsTTTT variant is the most prevalent in Zhejiang [25]. Here, nine newborns (52.9%) with 3-MCCD had *MCCC2* variants, with c.351_353delTGG (50.0%) being the most prevalent, with few reports in other parts of China. Our findings suggest that *MCCC1/MCCC2* variants vary according to ethnicity and region. Further studies with more patients are needed to identify variant hotspots. Moreover, we identified three novel *MCCC1* variants and three novel *MCCC*2 variants, enriching the known 3-MCCD mutant spectrum.

Although the genotypic and phenotypic relationship of 3-MCCD has not been determined, our data indicate that biochemical metabolite concentrations are significantly elevated in patients with null variants. All neonatal patients with homozygous frameshift variants or compound heterozygous frameshift and nonsense variants in the *MCCC2* gene had C5OH levels greater than 5 μmol/L. Two patients (No. 2 and 15) with compound heterozygous frameshift and missense variants also had C5OH concentrations higher than 5 μmol/L. We suspect that the enzyme activity of these two missense variants (c.1331G > Ain *MCCC1* and c.1367C > T in *MCCC2*) may be seriously impaired, but further functional studies are needed.

3-MCCD is a low clinical penetrance condition, with less than 10% of the affected individuals experiencing mild symptoms, and less than 1–2% experiencing severe symptoms [7]. A previous study showed that 31% of patients had clinical symptoms; acidosis and hypoglycemia were the most common, and mental retardation, including speech retardation, was the most common chronic symptom observed [5]. With expanded NBS, a growing number of asymptomatic children and adults have been reported [25,29,34,35]; a few adult patients with 3-MCCD have reported severe muscle pain, physical disability, and muscle weakness [21,36]. The causes of global developmental delay are complex [37], which is similar for hyperammonemia [17,38]. Due to parental refusal, we did not perform further examinations for Case 15, such as brain MRI and CMA examinations. Therefore, the patient's clinical symptoms may be caused by other diseases, and it is hard to determine whether the clinical symptoms are related to 3-MCCD.The 3-MCCD symptoms vary greatly, and environmental factors (e.g., infection and catabolic stress) may trigger specific symptoms [39]. Neither biochemical phenotypes nor genotypes could predict which affected individuals would develop symptoms [5,40].

Performing NBS for 3-MCCD is controversial [9,32]. 3-MCCD was excluded from NBS in Israel due to its benign nature [21]. After conducting NBS of 677,852 newborns in Germany, a low benefit-to-harm ratio was achieved; therefore, 3-MCCD was excluded from NBS in this country [7]. Excluding 3-MCCD has been suggested because it may not represent a disease, but a biochemical phenotype [40,41]. However, maintaining the NBS screening for 3-MCCD could provide insights into

its natural progression [42]. Notably, carnitine deficiency is common in patients with 3-MCCD and is associated with cardiomyopathy and death in children [11]. Patients with 3-MCCD have varying degrees of underlying cardiomyopathy [43]. In a previous study, a mother experiencing muscle weakness saw improvements in her symptoms after carnitine supplementation [21]. The data from this study showed that NBS for 3-MCCD produced many false positives (99.31%). Although clinical symptoms were observed in 11.8% of patients, whether they were related to 3-MCCD remain unclear. However, one case of HLCSD with severe clinical symptoms was detected. Therefore, further studies are required to decide whether to retain 3-MCCD and C5OH indicators in NBS.

Since C5OH levels of newborns are affected by maternal factors, some patients with maternal 3-MCCD have been detected following identification of elevated C5OH levels in their children. However, the mother's compliance with the examination was poor, so the actual incidence of maternal 3-MCCD may be higher. In comparison, paternal 3-MCCD cases are rarely reported;the patient in this study was accidentally detected due to the mother's C5OH level being normal and the father requesting further testing. In this case, it should be clear that the elevated C5OH level of the newborn is caused by carrying a variant in the *MCCC1* gene, rather than the influence of the father. This could also explain the high false positive rate of NBS for 3-MCCD, as many false positive cases are carriers of the *MCCC1* and *MCCC2* genes.

5. Conclusion

In summary, 17 neonatal patients with 3-MCCD in Quanzhou, China, were confirmed via NBS, with an incidencerate of 1:37,859. We identified six previously unreported variants that broadened our understanding of the molecular profile of 3-MCCD. Secondary carnitine deficiency occurred in eight neonates and in all adult patients. Although clinical symptoms were observed in 11.8% of patients, it was unclear if they were related to MCCD. Therefore, further studies are needed to determine if 3-MCCD and C5OH indicators should be retained in NBS.

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Author statement

I the undersigned declare that this manuscript is original, has not been published before and is not currently being considered for publication elsewhere. I confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. I further confirm that the order of authors listed in the manuscript has been approved by all involved. I understand that the Corresponding Author is the sole contact for the Editorial process. He/she is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs.

CRediT authorship contribution statement

Weihua Lin: Writing – review & editing, Writing – original draft, Funding acquisition, Formal analysis. **Kunyi Wang:** Validation, Software, Methodology. **Yanru Chen:** Validation, Methodology. **Zhenzhu Zheng:** Validation, Methodology. **Yiming Lin:** Supervision, Project administration.

Declaration of competing interest

The authors declare that they have no competing interests.

Data availability

Data will be made available on request.

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