

Clinical and Gene Analysis of Fatty Acid Oxidation Disorders Found in Neonatal Tandem Mass Spectrometry Screening

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Objective: To investigate the clinical and gene mutation characteristics of fatty acid oxidative metabolic diseases found in neonatal screening.

Methods: A retrospective analysis was performed on 29,948 neonatal blood tandem mass spectrometry screening samples from January 2018 to December 2021 in our neonatal screening centre. For screening positive, recall review is still suspected of fatty acid oxidation metabolic disorders in children as soon as possible to improve the genetic metabolic disease-related gene detection package to confirm the diagnosis. All diagnosed children were followed up to the deadline.

Results: Among 29,948 neonates screened by tandem mass spectrometry, 14 cases of primary carnitine deficiency, six cases of short-chain acyl coenzyme A dehydrogenase deficiency, two cases of carnitine palmitoyltransferase-I deficiency and one case of multiple acyl coenzyme A dehydrogenase deficiency were recalled. Except for two cases of multiple acyl coenzyme A dehydrogenase deficiency that exhibited [manifestations], the other 21 cases were diagnosed pre-symptomatically. Eight mutations of *SLC22A5* gene were detected, including c.51C>G, c.403G>A, c.506G>A, c.1400C>G, c.1085C>T, c.706C>T, c.1540G>C and c.338G>A. Compound heterozygous mutation of *CPT1A* gene c.2201T>C, c.1318G>A, c.2246G>A, c.2125G>A and *ETFA* gene c.365G>A and c.699_701delGTT were detected, and new mutation sites were found.

Conclusion: Neonatal tandem mass spectrometry screening is an effective method for identifying fatty acid oxidative metabolic diseases, but it should be combined with urine gas chromatography-mass spectrometry and gene sequencing technology. Our findings enrich the gene mutation profile of fatty acid oxidative metabolic disease and provide evidence for genetic counselling and prenatal diagnosis in families.

Keywords: fatty acid oxidative metabolic disorder, primary carnitine deficiency, multiple acyl coenzyme A dehydrogenase deficiency, carnitine palmitoyltransferase-I deficiency, short-chain acyl-coenzyme A dehydrogenase deficiency

Introduction

Fatty acid oxidation disorders (FAODs) are a general term for a group of diseases related to the oxidative metabolism of fatty acids in mitochondria;¹ dysfunction of any enzymes involved in the entry of fatty acids into mitochondria or in the β -oxidation of fatty acids results in impaired fatty acid oxidation, impaired energy supply and accumulation of intermediate metabolites, thus affecting organs with high energy requirements such as the liver, myocardium, skeletal muscle, brain² and even causing sudden death. Patients with FAOD showed a highly heterogeneous clinical spectrum. The most common clinical manifestations include hypoglycaemia, liver dysfunction, cardiomyopathy, rhabdomyolysis and skeletal muscle disease, as well as some subtypes of peripheral neuropathy and retinopathy. Despite efforts to detect FAOD through neonatal screening and manage patients early, symptomatic episodes can be sudden and severe and even lead to death. Therefore, rapid and accurate identification of key signs and symptoms in patients with FAOD is essential for managing metabolic decompensation and preventing severe comorbidities.³ Kang et al from Korea identified 14 FAODs (mean age 54.8 ± 4.8 days) in newborn screenings over 14 years. Three patients with VLCADD or LCHAD/

MTP deficiency developed recurrent rhabdomyolysis or cardiomyopathy, one patient died of cardiomyopathy and the other ten patients had normal neuron development and no symptoms during follow-up after therapeutic intervention.⁴ A study by Maguolo et al in Italy on newborn screening between 2014 and 2019 found an overall prevalence of FAODs at 1/4316, which also confirmed the importance of tailored follow-up and treatment.⁵ Therefore, we screened the newborns in our hospital to study the clinical and gene mutation characteristics of neonatal fatty acid oxidation metabolic diseases in China. Tandem mass spectrometry (TMS) has been widely used for the rapid diagnosis of inborn errors of metabolism due to its sensitivity, specificity and ability to analyse dozens of metabolites simultaneously.⁶ Previous studies have shown that the clinical diagnosis of FAOD is diverse but not specific for clinical symptoms, while pre-symptomatic diagnosis and treatment can be achieved with the help of newborn screening TMS and genetic testing methods⁶ to avoid acute metabolic crises. Therefore, strengthening the understanding of fatty acid oxidative metabolic disease and achieving early diagnosis can improve the prognosis of children as much as possible.

The aim of this study was to investigate the clinical characteristics and genetic mutation of fatty acid oxidative metabolic disorders detected by newborn screening and to provide a basis for family genetic counselling and prenatal diagnosis. A total of 23 cases of fatty acid oxidative metabolic disorders detected by TMS from January 2018 to December 2021 in our hospital were included and analysed in this study.

Data and Methods

Study Design

Between January 2018 and December 2021, blood samples from a total of 29,948 newborns were selected for primary screening of blood acylcarnitine profiles and analysed by TMS; those with abnormal free carnitine (C0) or acylcarnitine profiles were recalled for follow-up. This study was approved by the Ethics Committee of Maternal and Child Health Hospital of Hubei Province.

Medical Examination After Recall

Family members of newborns with positive primary screening were interviewed for medical history, family history, maternal history, dietary history, drug history, etc. Physical examination for newborns was performed, and C0 and acylcarnitine concentrations were rechecked. Those who were still positive on recheck were also checked for the following indicators: urinary organic acid analysis, urinary ketone bodies, blood gas analysis, blood glucose and biochemistry, liver, gallbladder, spleen and kidney ultrasound, cardiac ultrasound, brain MRI scan and mutation analysis of relevant pathogenic genes.

Blood Sample Analysis by TMS and Urine Organic Acids Analysis by Gas Chromatography-Mass Spectrometry Detection

Blood was collected from the bottom of the foot or vein, dried on a special filter paper sample card, processed according to the operating instructions of the kit and analysed with TMS to detect the concentrations of various amino acids, C0 and acylcarnitine. The blood acylcarnitine profile was analysed at the same time for mothers whose children had low C0. Fresh urine was processed and tested for urine organic acids. Sample processing and computer analysis for chromatography-mass spectrometry (GC-MS) are as described above.⁷ Data analyses were conducted using AMDIS software (Version 2.71) linked to NIST Mass Spectral Search Program for the NIST/EPA/NIH Mass Spectral Library (Version 2.0F, built Oct. 8, 2008). The criteria for suspecting FAOD are based on the levels of C0 and various acyl carnitine in blood tandem mass spectrometry, compared with the reference values of quality control standards, as a basis for screening or diagnosis.

Gene Mutation Analysis

For the children who were still positive in the rediagnosis, informed consent was obtained from their guardians, and medical ethics approval was obtained from the hospital. Mutation analysis of FAOD-related pathogenic genes was performed using the Genetic Metabolic Etiology Diagnostic Panel (covering 175 genes), after searching literature

databases such as China Knowledge Network, Vipul, Wanfang, PubMed and professional versions of databases, such as HGMP, dbSNP, PloyPhen2, SIFT, MutationTaster and other software, to obtain the suspected pathogenic mutations. Sanger sequencing was used for intra-lineage validation to determine the genetic origin of the pathogenic mutations.

Results

General Information

Twenty-three cases were recalled due to abnormal blood acylcarnitine spectrum. Except for two cases with multiple acyl coenzyme A dehydrogenase deficiency that exhibited [manifestations], the other 21 cases were diagnosed pre-symptomatically.

There were 14 cases of primary carnitine deficiency (PCD), nine females and five males, all born at full term with birth weights of 3.2 to 3.8 kg and aged 29 days. At the time of recall (four months), seven cases had carnitine jaundice, seven cases had liver dysfunction and three cases had anaemia. None of them had hepatomegaly, convulsions, coma or muscle weakness, etc. There were six cases of SCADD. They were all female, born at full term and had no liver dysfunction, no muscle hypotonia and no symptoms of muscle weakness. There was one case of MADD, recalled at three months, who was a female child. She was born at full term with G2P1 and a birth weight of 2.7 kg. The crying was obvious in the first month after birth. Facial swelling and an anaemic appearance appeared in the third month, with poor mental health and drowsiness. Her liver was 4 cm below the right rib. She had muscle strength hypotonia and died at the age of four months after her parents gave up treatment for three days. There were two cases of CPTID: one male born at full-term normal delivery with G1P1 and one female born at full-term normal delivery. Their birth weights were 3.5 ~ 3.8 kg. One case was hospitalised twice in the neonatal period for “neonatal hyperbilirubinemia” and “neonatal hyperbilirubinemia with neonatal pneumonia”. At the time of recall (44 days), the newborn had jaundice, no hepatomegaly, no abnormal muscle strength, no convulsions, coma and Reye’s syndrome; the other newborn had no hepatomegaly, no liver dysfunction and no muscle strength abnormalities at the time of recall (40 days).

Biochemical Analysis Results

Among the 14 cases with PCD, seven had elevated ALT and AST, six had elevated total bilirubin (TBIL) and indirect bilirubin (IBIL) and ten had low actual bicarbonate (AB); The creatine kinase (CK), creatine kinase isoenzyme (CKMB), lactate dehydrogenase (LDH) and alpha-hydroxybutyrate dehydrogenase (HBDH) were normal or mildly elevated; the total cholesterol (TC), triglycerides (TG), blood gas pH and lactate (Lac) were normal, and one case had moderate anaemia. Among the six cases with SCADD, the AB of all remained at a low level. One case had elevated ALT and AST, and one case had elevated TBIL and IBIL. In one case with MADD, liver enzymes and muscle enzymes were significantly elevated, and triglycerides were especially elevated, accompanied by high levels of lactic acid and metabolic acidosis with decreased hemoglobin, and the result of liver ultrasound indicated that this case had hepatomegaly and fatty liver. In the two newborns with CPTID, the normal liver enzymes, mild jaundice, and normal muscle enzymes, lipids, and blood gas analysis results were observed. None of the 23 FAOD cases had hypoglycemia or hyperammonia, and there were no abnormalities in cardiac ultrasound, urological ultrasound, or brain MRI plain scan. These results are shown in [Table 1](#).

Blood Sample Analysis Results Using Tandem Mass Spectrometry (TCM) and Urine Organic Acids Analysis by Gas Chromatography–Mass Spectrometry Detection

In 14 cases of PCD, free carnitine ranged from 2.56 to 8.76 $\mu\text{mol/L}$ and maternal acyl carnitine was normal; in six cases of SCADD, blood analysis by TCM indicated significantly elevated butyryl carnitine (C4); in one case of MADD, TCM results showed elevated butyryl carnitine (C4), 18-ene acyl carnitine (C18) and multiple long-chain acyl carnitines. In one case of CPTID, the acyl carnitine profile results revealed that C0 was elevated, while hexadecenoyl carnitine (C16) and octadecenoyl carnitine (C18) were reduced, and the ratio C0/(C16+C18) was significantly increased. All 23 cases of FAOD shared normal urinary organic acid results. These findings are presented in [Table 2](#).

Table 1 Biochemical Results of 23 Cases with FAOD

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8	Case 9	Case 10	Case 11	Case 12	Case 13	Case 14	Case 15	Case 16	Case 17	Case 18	Case 19	Case 20	Case 21	Case 22	Case 23	Reference Range	
Disease type	PCD	PCD	PCD	PCD	PCD	PCD	PCD	PCD	PCD	PCD	PCD	PCD	PCD	PCD	SCADD	SCADD	SCADD	SCADD	SCADD	SCADD	MADD	CPT1D	CPT1D		
ALT (U/L)	26	85.6	30	31.8	43	80	85.6	29	25	47	70.6	41.9	32.6	33.5	32	33	34	35	36	67	130.7	38.7	23		0–40
AST (U/L)	39	70.1	44	55.8	50	76.7	70.1	37	31	35	68.4	29.4	40.9	31.7	40	34	35	36	37	58	255.3	55	33		0–40
TBIL (umol/L)	45.5	6	17.8	130.8	7.3	9.3	12.4	7.1	9.4	34.7	40.8	41.8	22.1	13.5	6.7	14.2	7.2	43.7	5.3	8.8	11	52.2	3.8		0–24
IBIL (umol/L)	36.9	3.7	11	122.4	6.4	8.1	10.9	5.3	7.9	29.9	38.1	39.1	19.2	11.1	6.6	13.5	6.8	35.3	3.8	7.6	5	44.2	2.6		1.7–17.3
CK (U/L)	162	218	134	106	186	82	218	106	240	168	109	110	212	300	167	295	149	170	174	193	1601	82	167		50–310
CKMB (U/L)	65.2	39.5	43	49.7	28.9	30.7	39.5	26.1	21	29	40.7	51.1	32.7	23.8	40.2	49.8	22.7	23.6	44.2	35.2	670.1	30.5	40.2		0–25
LDH (U/L)	408	350	350	251	405	308	350	267	187	281	299	217	301	198	171	252	303	277	175	146	1403	303	271		120–300
HBDH (U/L)	352	281	272	197	273	216	281	194	203	159	106	196	211	129	208	109	237	201	112	213	1307	226	208		72–182
TC (mmol/L)	3.75	5.03	4.26	3.1	3.1	3.5	5.03	2.10	3.04	3.36	2.91	1.93	1.61	2.98	1.89	1.83	3.43	0.99	1.52	2.11	4.29	3.1	1.89		<5.2
TG (mmol/L)	1.07	2.24	1.19	0.74	0.78	1.93	2.24	0.49	1.73	2.15	1.66	0.73	1.50	0.81	0.33	1.07	0.61	1.36	2.02	0.61	12.71	0.39	0.33		0–2.26
PH	7.355	7.358	7.4	7.42	7.37	7.42	7.4	7.38	7.31	7.33	7.43	7.41	7.39	7.45	7.41	7.38	7.32	7.4	7.43	7.42	7.32	7.42	7.41		7.35–7.45
Lac (mmol/L)	1.4	1.7	1.5	1	1.27	0.8	1.2	0.97	0.7	1.8	1.1	0.9	3.0	1.8	2	0.9	0.3	1.91	0.73	0.77	5	1.9	2		0–1.7
AB (mmol/L)	22	21.8	22.6	22.3	–3	–1.8	–3.47	–2.9	–2.4	–2.65	1.9	–1.5	3.0	0.9	–2.3	–2.13	–1.3	–0.3	0.7	1.7	15.1	20.2	–3.3		22–27
HGB (g/L)	120	122	89	140	109	116	114	110	117	111	98	120	100	106	104	118	112	101	92	103	81	111	98		110–160

Abbreviations: FAOD, Fatty acid oxidation disorders; ALT, glutamic pyruvic transaminase; AST, glutamic-oxalacetic transaminase; TBIL, total bilirubin; IBIL, indirect bilirubin; CK, creatine kinase; CKMB, creatine phosphokinase isomerase; ldh, lactic dehydrogenase; hbdh, hydroxybutyric dehydrogenase; TC, total cholesterol; TG, triglyceride; PH, Hydrogen ion concentration index; Lac, lactic acid; AB, antibody; HGB, hemoglobin.

Table 2 Blood Free Carnitine, Acyl Carnitine Spectrum and Mutant Gene Types in 23 Cases

Case	At Initial Screening (umol/L)	Reference Range (umol/L)	Mutant Gene	Allele 1			Allele 2		
				Nucleotide Alteration	Amino Acid Change	Whether to Report	Nucleotide Alteration	Amino acid Change	Nucleotide Alteration
1	C0= 2.56	9.5–60	SLA22A5	c.51C>G	p.F17L	Yes	c.51C>G	p.F17L	Yes
2	C0=5.04	9.5–60	SLA22A5	c.403G>A	p.V135M	No	c.506G>A	p.R169Q	Yes
3	C0=6.01	9.5–60	SLA22A5	c.51C>G	p.F17L	Yes	c.1400C>G	p.S467C	Yes
4	C0=4.25	9.5–60	SLA22A5	c.1400C>G	p.S467C	Yes	c.1085C>T	p.S362L	Yes
5	C0= 2.63	9.5–60	SLC22A5	c.51C>G	p.F17L	Yes	c.51C>G	p.F17L	Yes
6	C0= 3.58	9.5–60	SLC22A5	c.51C>G	p.F17L	Yes	c.51C>G	p.F17L	Yes
7	C0= 5.04	9.5–60	SLC22A5	c.403G>A	p.V135M	No	c.506G>A	p.R169Q	Yes
8	C0= 5.6	9.5–60	SLC22A5	c.51C>G	p.F17L	Yes	c.1400C>G	p.S467C	Yes
9	C0= 5.46	9.5–60	SLC22A5	c.1400C>G	p.S467C	Yes	c.760C>T	p.R254Ter	Yes
10	C0= 7.3	9.5–60	SLC22A5	c.51C>G	p.F17L	Yes	c.1400C>G	p.S467C	Yes
11	C0= 4.37	9.5–60	SLC22A5	c.51C>G	p.F17L	Yes	c.760C>T	p.R254Ter	Yes
12	C0= 7.32	9.5–60	SLC22A5	c.760C>T	p.R254Ter	Yes	c.1400C>G	p.S467C	Yes
13	C0= 8.76	9.5–60	SLC22A5	c.1400C>G	p.S467C	Yes	c.1540G>C	p.G514R	Yes
14	C0= 3.81	9.5–60	SLC22A5	c.1400C>G	p.S467C	Yes	c.338G>A	p.C113Y	Yes
15	C4= 3.15	0.06–0.5	ACADS	c.1031A>G	p.E344G	Yes	c.1031A>G	p.E344G	Yes
16	C4= 0.71	0.06–0.5	ACADS	c.981–983del	p.T328del	Yes	c.1031A>G	p.E344G	Yes
17	C4= 0.79	0.06–0.5	ACADS	c.795+1G>A	/	No	c.250G>A	p.V84M	Yes
18	C4= 1.85	0.06–0.5	ACADS	c.164C>T	p.P55L	Yes	c.1195C>T	p.R399W	Yes
19	C4= 0.56	0.06–0.5	MMACHC	c.609G>A	W203Ter	No	/	/	No
20	C4= 1.62	0.06–0.5	ACADS	c.989G>A	p.R330H	Yes	c.989G>A	p.R330H	Yes
21	C4=0.67	0.06–0.5	ETFA	c.365G>A	p.R122K	Yes	c.699_701delGTT	p.234_234del	No
	C5=1.07	0.04–0.7							
	C6=0.42	0.02–0.15							
	C8=0.5	0.02–0.25							
	C10=0.56	0.01–0.3							
	C12=0.77	0.03–0.4							
	C14=1.00	0.06–0.45							
	C14:1=0.76	0.02–0.35							
	C16:1=0.91	0.03–0.5							
	C18:2=0.94	0.06–0.8							
22	C0=94.71	9.5–60	CPT1A	c.2201T>C	p.F734S	Yes	c.1318G>A	p.A440T	No
	C16=0.03	0.32–6.5							
	C18=0.02	0.13–1.7							
	C0/(C16+C18) = 1894	1.9–42							
23	C0=208.79	9.5–60	CPT1A	c.2246G>A	p.R749H	Yes	c.2125G>A	p.G709R	Yes

Abbreviations: C0, free carnitine; C4, butyryl carnitine; C5, isovaleryl carnitine; C6, capryl carnitine; C8, octyl carnitine; C10, decanoyl carnitine; C12, lauroyl carnitine; C14, myristoyl carnitine; (C14:1), myristoyl carnitine; C16, palmitoyl carnitine; (C16:1), palmitoyl carnitine; C18, Octadecyl carnitine; (C18:2), octadecadienyl carnitine.

Gene Mutation Analysis and Results

A total of eight missense mutations were found in 14 PCD cases by sequencing the SLC22A5 gene: c.51C>G, c.403G>A, c.506G>A, c.1400C>G, c.1085C>T, c.706C>T, c.1540G>C and c.338G>A; among them, c.403G>A and p.V135M were novel mutations not included in the Human Gene Mutation Database (HGMD) or dbSNP databases and were predicted to be pathogenic by SIFT software. c.51C>G pure mutations occurred in the first, fifth and sixth cases. Among the six SCADD cases, acyl-CoA dehydrogenase gene mutations were found in cases 15–18 and 20, while methylmalonic acidemia with homocystinuria gene mutations were identified in case 19. The ETFA gene in one MADD case revealed two heterozygous mutations—c.365G>A and c.699_701delGTT. The former mutation was recorded in the HGMD Professional database and predicted to be pathogenic by PolyPhen-2 and SIFT software, while the latter deletion mutation has not been reported; Mutation Taster prediction results suggest it is a pathogenic locus. In two carnitine palmitoyltransferase I deficiency (CPTID) cases, two heterozygous mutations (c.2201T>C and c.1318G>A) were detected in the CPT1A gene of one case: c.1318G>A was a newly discovered mutation, and both were predicted to be potentially pathogenic by PolyPhen2 and SIFT software; another case also had heterozygous mutations of c.2246G>A and c.2125G>A in the CPT1A gene. These findings are shown in Table 2.

Treatment and Follow-Up

Fourteen cases of PCD were treated orally with levocaine 100 mg/kg/d. After treatment, C0 was maintained at normal levels without hypoglycaemia, and liver function, cardiac enzymes and lipids were all in the normal range at the time of follow-up. The treatment of six children with SCADD mainly consisted of dietary management, avoiding prolonged fasting and hypoglycaemia and regular monitoring of blood carnitine levels. After treatment, C0 was maintained at normal levels without hypoglycaemia, and liver function, cardiac enzymes and lipids were all in the normal range at the time of follow-up. One case of MADD was given vitamin B2 (100 mg, three times per day), coenzyme Q10 (10 mg, three times per day) and levocarnitine (1000 mg/d, intravenously). The parents discontinued the treatment after three days, and the child passed away one month after being discharged from the hospital. Cases with CPTID were fed a low-fat, medium-chain triglyceride-rich formula, and they grew and developed normally, with free carnitine maintained at normal levels and elevated C16 and C18 compared to before. All these patients are under continuous follow-up now.

Discussion

FAOD Prevalence

FAOD is an autosomal recessive inherited disease with over 15 different clinical descriptions affecting FAO, mainly including MADD, PCD, CPTID, carnitine acylcarnitine translocase deficiency, VLCADD, medium/short chain hydroxyacyl-CoA dehydrogenase deficiency, short-chain acyl-coenzyme A dehydrogenase deficiency (SCADD), long-chain 3-hydroxyacyl-CoA dehydrogenase or mitochondrial trifunctional protein and MADD.⁸

FAOD comprises two primary categories: carnitine-dependent fatty acid transport disorders and abnormal fatty acid β -oxidation in mitochondria (Figure 1). In this study, PCD and CPTID belong to the former, while MADD falls within the latter category. In China, some scholars estimated the overall prevalence of FAOD as approximately 1/15382 via newborn screening, with PCD being 1/23862 – the highest prevalence among FAOD.⁹ Conversely, other scholars suggested that MADD has the highest prevalence among FAOD.¹⁰ In our current study, out of 23 cases of FAOD, there were 14 instances of PCD, a relatively low proportion of MADD and one rare case of CPTID. This differs from the composition ratio of FAOD observed in other parts of China. The reasons for these differences could be the varying ages of onset for FAOD diseases (ranging from birth to adulthood), the distinct study subjects examined and the disparities in regional data. Additionally, our sample size was not large enough to draw definitive conclusions about prevalence rates.

PCD Symptoms and Gene Detection

PCD is caused by a defect in the SLC22A5 gene, which results in a loss of function of its encoded high-affinity carnitine transporter, OCTN2. This leads to a decrease in carnitine transfer from the intestine to the blood and from the blood to the cells. The reduced transport eventually results in a lack of tissue cell carnitine and an inability of long-chain fatty

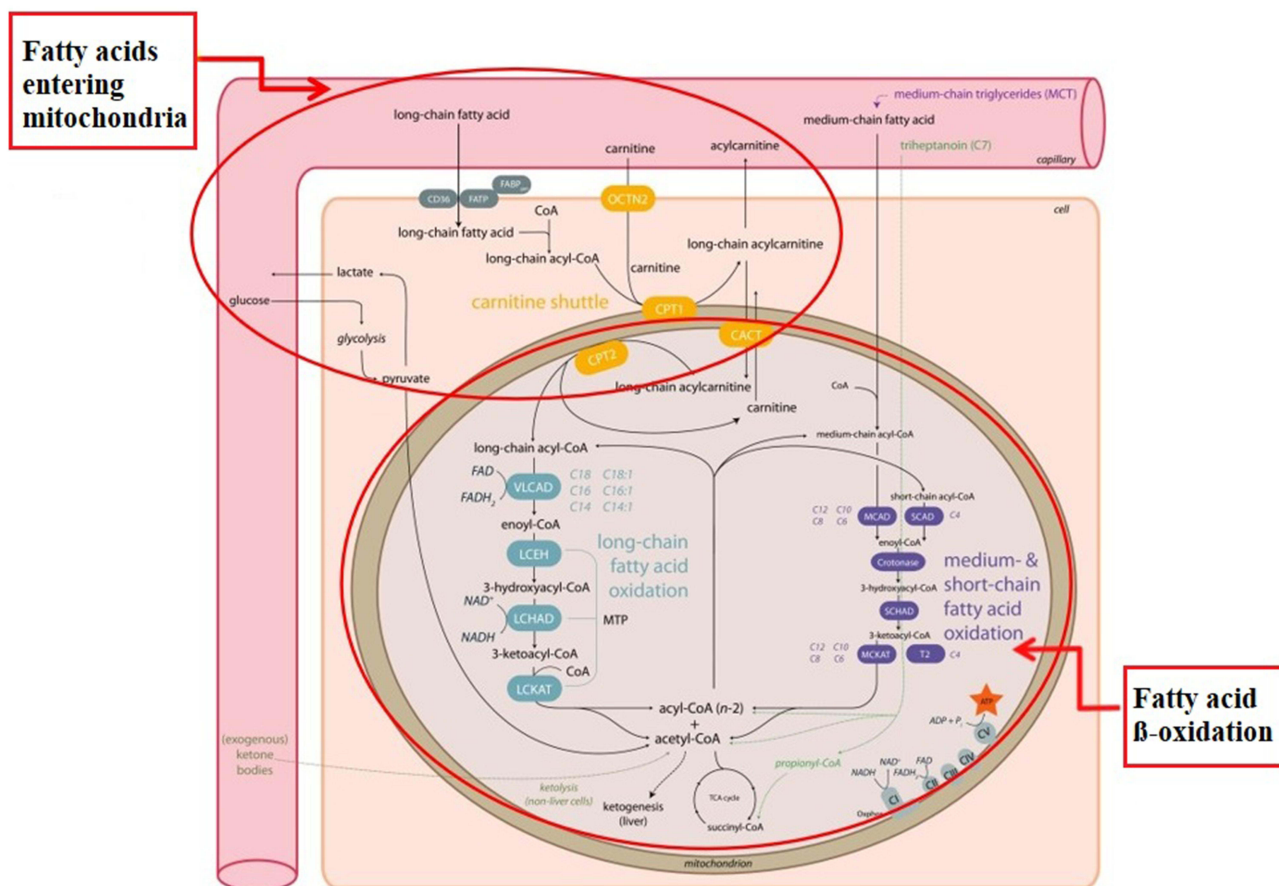


Figure 1 The mechanisms of FA transport to mitochondria and oxidation.

acids to pass through the mitochondrial membrane for fatty acid β -oxidation.¹¹ The age of carnitine deficiency onset ranges from neonatal to adult years, predominantly between one month and seven years, with clinical symptoms varying from asymptomatic to developing cardiomyopathy, hypoglycaemia, hepatopathy, myasthenia and metabolic acidosis. A few cases have reported convulsions, impaired consciousness, Reye's syndrome-like episodes and gastrointestinal symptoms such as abdominal pain, vomiting, gastro-oesophageal reflux and even anaemia. Two instances of infantile PCD with jaundice reported in India had hepatomegaly,^{12,13} whereas a large cohort study in France showed nearly universal hepatomegaly in FAOD (92%).¹⁴ In the current study, there were two cases of jaundice and one case of poor liver function among 14 cases of PCD; nevertheless, none had hepatomegaly or other manifestations such as hypoglycaemia or cardiomyopathy. Liver damage was considered an extension of neonatal hyperbilirubinaemia or infantile hepatitis syndrome, also suggesting non-specific and heterogeneous clinical symptoms of PCD. PCD is a potentially lethal disease regardless of the presence or absence of symptoms and their early onset.¹⁵ Still, it is also a condition with specific therapeutic agents and a better prognosis with timely treatment. The rate of SLC22A5 mutations detected by newborn screening and OCTN2 gene sequence analysis of their mothers is higher than that of subjects with clinically suspected PCD,¹⁶ so the combination of newborn screening and SLC22A5 genes can lead to pre-symptomatic diagnosis and intervention, greatly improving the prognosis. The minimum limit of C0 detection in neonatal screening is 10 $\mu\text{mol/L}$. In clinical practice, it is also vital to exclude factors of maternal origin, medication (cyclomycin, valproate na, pivalic acid-containing antibiotics,¹⁷ etc.), nutritional factors, haemodialysis and reduced free carnitine due to renal tubular dysfunction, prematurity and hereditary organic acidaemia or other abnormalities of mitochondrial fatty acid metabolism. These factors can be identified by combining maternal free carnitine levels and dietary habits, history of drug use, family history, presence of organic acid and other acyl carnitine profiles inherited from blood tandem mass spectra and genetic

tests. Currently, more than 180 mutations have been reported in the SLC22A5 gene, with hotspot mutations differing by race and region. In this study, the c.51C>G and c.1400C>G mutations had the highest frequency, with one new mutation, c.403G>A, also being reported. The relationship between PCD genotype and clinical phenotype remains unclear; however, as newborn screening becomes more widespread and our understanding of PCD deepens, additional newly found mutations and their effects on protein structure will be reported. PCD patients generally have a favourable prognosis if treated pre-symptomatically.¹⁸

CPTID Symptoms and Gene Analysis

CPTID is a further fatty acid translocation disorder that inhibits long-chain acyl coenzyme A from entering mitochondrial β -oxidation due to defective carnitine palmitoyltransferase I (CPTIA), the gene responsible for preventing long-chain acyl coenzyme A from binding to free carnitine. CPTID can be asymptomatic and identified during newborn screening, or it can manifest during acute illnesses such as starvation, fever, or vomiting, with altered consciousness, convulsions, coma, hepatomegaly, or even sudden death due to hepatic encephalopathy. Generally, it does not impair skeletal muscle or the heart.⁹ In this case, the child had mild jaundice and predominantly elevated IBIL, considered a non-CPTID-specific symptom caused by neonatal hyperbilirubinaemia. However, a Korean scholar reported a case of CPTID with jaundice as the first symptom and predominantly elevated direct bilirubin, suggesting that FAOD, including CPTID, should be regarded as one of the aetiologies of cholestatic hepatitis in children.¹⁹ TMS screening result is characterised by elevated blood C0, reduced long-chain acyl carnitine (C16, C18, etc.) and increased C0/(C16+C18). In one instance, the diagnosis of CPTID was delayed in a newborn due to total reliance on C0 and insufficient consideration of C0/(C16+C18) during screening. C0 returned to normal upon retesting.²⁰ As not all children with CPTID exhibit elevated free carnitine levels, reduced C18 combined with an elevated C0/(C16+C18) ratio has high diagnostic value for CPTID in terms of sensitivity and specificity.²¹ Gessner et al²² reported that C0/(C16+C18) ratios above 100 can achieve 100% positive predictive value; however, the possibility of false negatives by TMS detection still requires consideration. Over 20 mutant loci of the CPTIA gene have been discovered, most of which appear as individual cases or in only a few family lines.²³ The compound heterozygous mutation on exon eight and exon one in our child, neither constituting a polymorphic locus, occurs with very low frequency in the population. The variant c.2201T>C was also reported by Cui et al²⁴ in 2017, and no c.1318G>A was identified after searching the database, which PolyPhen2 and SIFT software predicted to be a pathogenic mutation, enriching the CPTIA gene spectrum.

SCADD Symptoms and Gene Detection

SCADD is a metabolic disorder of short-chain fats due to a deficiency of short-chain acyl-coenzyme A dehydrogenase.²⁵ The process of fatty acid β -oxidation occurs in the mitochondria. Fatty acids are very long carbon chains that are gradually shortened into units of two carbons during oxidation. Each shortening process undergoes four steps: from acyl coenzyme A to enoyl coenzyme A, hydroxyl coenzyme A, ketolipid coenzyme A and finally back to acyl coenzyme A. Nevertheless, the metabolism of fatty acids of varying lengths sometimes necessitates different enzymes, which increases the diversity of enzymes involved and consequently makes the diseases caused more complex.²⁶ Short-chain acyl coenzyme A dehydrogenase deficiency arises from an insufficiency in the enzyme responsible for metabolising short-chain fatty acids and makes diagnosis more challenging due to its nonspecific clinical presentation. Suspicion of abnormal metabolism of short-chain fatty acids is usually observed alongside tandem mass spectrometry blood film examination with an increase in the concentration of the item C4-carnitine. In this case report, all six children with SCADD had significantly elevated C4 levels; however, none of them experienced significant adverse symptoms. Following dietary management, the children's C4 levels returned to normal.

SCADD Symptoms and Gene Analysis

MADD is caused by mutations in the ETF and ETFDH genes, which are crucial transporters in the fatty acid β -oxidation electron transfer process, resulting in impaired function of multiple dehydrogenases in the mitochondrial respiratory chain, failure to transfer electrons generated by dehydrogenation, and impaired energy metabolism. It is rare in neonatal screening and the prevalence of this disease in Zhejiang Province is 1:465316,¹⁸ which is categorised into three types

according to the age of onset and clinical features: type I is neonatal onset with congenital developmental abnormalities such as polycystic kidney; type II is neonatal onset without congenital developmental abnormalities; type III, also known as late onset, may manifest in infancy as episodes of hypoglycemia, acidosis, liver damage, encephalopathy, hypertrophic cardiomyopathy or Reye's syndrome, while adult-onset clinical manifestations are relatively mild and mainly present as intermittent muscle weakness involving the skeletal muscles of the trunk and proximal extremities.²⁷ The neonatal seizure type mostly dies in the first few days of life due to hypoketotic hypoglycemia, metabolic acidosis, and encephalopathy. This case of MADD with lethargy, hepatopathy, myopathy and metabolic acidosis in infancy is classified as a type III MADD with infantile onset according to the above-mentioned classification. It is worth noting that triglycerides are significantly elevated and a fatty liver is evident in this child. The pathogenesis of this case is the blockage of the fatty acid β -oxidation process in the liver, which causes lipid accumulation in the hepatocytes,²⁸ and triglyceride catabolism is blocked and significantly elevated in the blood. The absence of monitored hypoglycemia suggests that the metabolic disorder is not in the decompensated phase. The acylcarnitine profile of this case with MADD was characterised by a general elevation of medium- and long-chain acylcarnitines and normal urinary organic acids. Although MADD is often called "glutaric acidemia type II" because of the large amount of glutaric acid and other dicarboxylic acids excreted in the urine, when analysing the blood acylcarnitine profiles and urinary organic acid results, it cannot be easily diagnosed as FAOD because of the elevated dicarboxylic acids in the urine, nor can it be denied to be FAOD because of the absence of elevated dicarboxylic acids in the urine, and dicarboxylic acids can also be increased in other diseases. Even if the blood acylcarnitine profile is normal in newborn screening, the acylcarnitine may not be elevated due to the interictal period and other reasons, and sometimes false negatives may occur. Taiwan reported five cases of false-negative children in newborn screening, three of which were MADD,²⁹ so attention should be paid to the missed diagnosis of MADD in newborn screening. Significantly elevated levels of multiple medium and long chain acyl carnitines may suggest one of several diseases, including carnitine palmitoyltransferase-II deficiency, carnitine acyl carnitine shiftase deficiency, very long chain acyl coenzyme A dehydrogenase deficiency and MADD; it cannot be confirmed which disease it is and requires the use of gene sequences to confirm the diagnosis.³⁰ MADD can result from mutations in any of the three genes, ETFDH, ETFA, and ETFB; yet, it is primarily due to mutations in the ETFDH gene, with fewer reports of mutations in the ETFA and ETFB genes.³¹ ETFA and ETFB mutations mainly cause neonatal-type seizures, while ETFDH mutations lead to delayed MADD.³² However, a study summarising 350 cases of MADD discovered that ETFA gene mutations were present in 5% of patients with late-onset MADD³² and that some patients with MADD showed significant results with riboflavin (vitamin B2) treatment – termed riboflavin-responsive MADD (RR-MADD). Current studies reveal that almost all RR-MADD cases involve ETFDH gene mutations;^{33,34} nonetheless, Cotelli et al³⁵ proposed that MADD cases responding favourably to riboflavin might also be due to ETF genes or even other unknown mutation types. Thus, the association between MADD genotypes and phenotypes requires further investigation through large samples. Zhang et al³⁶ compared the clinical characteristics of children with MADD to those of adult patients and concluded that although both exhibit late-onset MADD, the disease is more severe in children and there is a significant relationship between age of onset and disease prognosis. In the present case, a double heterozygous mutation in the ETFA gene was identified in the child; however, he sadly passed away after halting treatment. Based on the age of onset and genotype, his prognosis was estimated to be poor.

Conclusion

Newborn screening using TMS is an effective method for the early identification of FAOD. Gene sequencing can be used to confirm the diagnosis of FAOD and provide a basis for genetic counselling and prenatal diagnosis of family lines. Our study provided new gene mutation sites and enriched the gene mutation spectrum of fatty acid oxidative metabolic diseases. As the onset ages of diseases in FAOD range from birth to adulthood, different research subjects lead to different conclusions. The sample size of our study is not large enough, and there may be regional differences. More clinical cases will be included in the follow-up study.

Disclosure

The authors report no conflicts of interest in this work.

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