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Clinical and molecular characteristics of carnitine-acylcarnitine translocase deficiency: Experience with six patients in Guangdong China

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ABSTRACT

Carnitine-acylcarnitine translocase deficiency (CACTD) is a rare autosomal recessive disorder of mitochondrial fatty acid oxidation that occurs due to mutations in the *SLC25A20* gene. Severe CACTD results in neonatal or infantile sudden death. Herein, we reported six patients with CACTD diagnosed based on biochemical and molecular findings from 5 unrelated families in Guangdong from 2016 to 2017. Among them, five patients presented with hypotonia, nonketotic hypoglycemia, and arrhythmia 2 days after birth, while the other patient presented with respiratory distress, hypotonia, and arrhythmia. Five of the patients died in the neonatal period. Blood acylcarnitine concentrations determination from dried blood spots (DBS) were measured by tandem mass spectrometry (MS/MS). The *SLC25A20* and *CPT2* gene sequences were analyzed by direct Sanger sequencing. *SLC25A20* gene analysis revealed a c.199-10T > G (IVS2-10T > G) homozygous variants in four unrelated patients and a novel mutation c.199-10T > G/c.719-8_c.719-1dupCCCACAG compound heterozygous variants in twins. This report describes the clinical characteristics, biochemical findings and molecular analysis of *SLC25A20* gene of patients with CACTD in Guangdong. And our results show that the c.199-10T > G is likely the most common variant of CACTD in Guangdong population as it accounts for 83% (10/12) of the observed mutant alleles. Individuals with the c.199-10T > G genotype had a severe CACTD phenotype.

1. Introduction

Carnitine-acylcarnitine translocase deficiency (CACTD) (OMIM # 212138) is a rare and life-threatening autosomal recessive disorder of mitochondrial fatty acid oxidation resulting from mutations of the *SLC25A20* gene. The classic phenotype of CACTD is characterized by neonatal hypoketotic hypoglycemia, hyperammonemia, cardiomyopathy, hepatopathy, and myopathy [1]. The estimated incidence of CACTD is 1/60,000 in Hongkong [2] and 1/76,894 in Hunan [3], China. The majority of reported CACTD cases have resulted in unexplained sudden death during the neonatal period [4,5].

The *SLC25A20* gene contains 9 exons and locates on 3p21.31 [6]. To date, at least 42 different pathogenic or possible pathogenic variations have been identified that cause CACTD (HGMD Professional 2018.4), including 20 missense or nonsense mutations, 10 small deletions, 2 small insertions, 1small indel, 4 gross deletions, and 5 splicing mutations. In Asia, the c.199-10T > G splicing mutation is the most frequently reported [3,6–9]. Patients with CACTD with a genotype of c.199-10T > G mutation usually presents with a severe clinical phenotype [3].

In this study, we describe the clinical, biochemical, and molecular characteristics of six Chinese patients with CACTD and summarized the diagnostic process of CACTD.

2. Materials and methods

2.1. Patients

Six patients from five unrelated families with non-consanguineous marriages were enrolled. The diagnosis of CACTD was based on blood acylcarnitine analysis and *SLC25A20* gene sequencing. Three patients, including the twins, were identified from 29,046 newborn screens for inborn errors of metabolism by tandem-mass–spectrometry (MS/MS) during 2017 in Guangzhou Newborn Screening Center at Guangzhou Women and Children's Medical Center (Guangzhou, Guangdong). Two patients were identified by retrospectively analyzing the stored DBS

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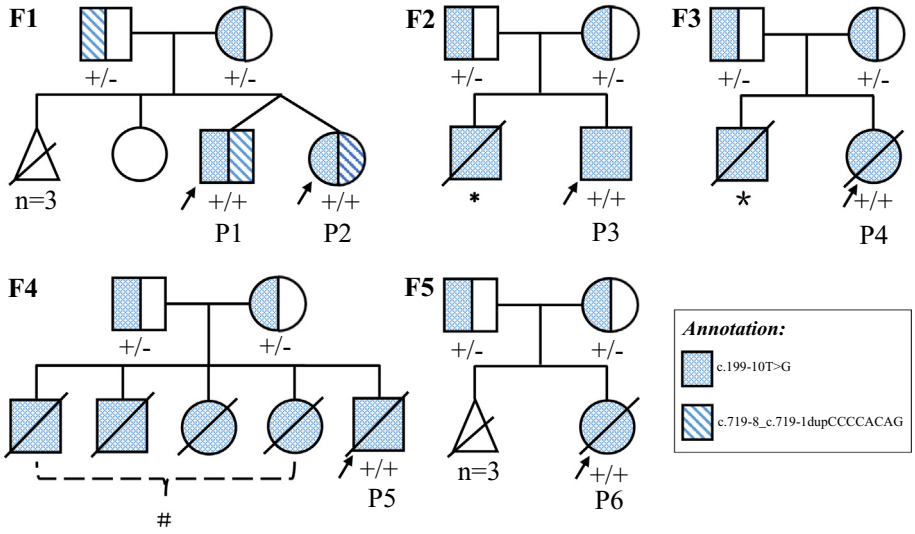

*:Died with similar symptoms at 50 days of age
*:Died with unknown cause in three days after birth
#:Two older brothers and two sisters died suddenly of unknown causes between two and thirty days after birth

Fig. 1. The pedigrees with carnitine-acylcarnitine translocase deficiency (CACTD). *: Died with similar symptoms at 50 days of age. *:Died with unknown cause in three days after birth. #:Two older brothers and two sisters died of unknown causes between two and thirty days after birth.

Table 1
The patients' clinical characteristics.

		Family 1		Family 2	Family 3	Family 4	Family 5
Tissues/Systems	Clinical symptoms[#]	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Heart	Cardiomyopathy	✓	N	✓	✓	N	✓
	Arrhythmias	✓	✓	✓	✓	✓	✓
	Respiratory insufficiency	✓	✓	✓	N	N	✓
Muscle/soft tissue	Hepatic dysfunction	✓	✓	✓	N	N	N
	Skeletal muscle damage	N	N	N	N	N	N
	Rhabdomyolysis	N	N	N	N	N	N
Central Nervous System	Hypotonia	✓	N	✓	✓	✓	✓
	Lethargy	N	✓	N	✓	✓	N
	Irritability	N	N	N	N	N	N
	Seizures	N	✓	N	N	N	N
	Coma	✓	✓	N	N	✓	N
	Hypoketotic hypoglycemia (under fasting conditions)	✓	✓	✓	N	N	✓
Laboratory Abnormalities	Hyperammonemia	✓	✓	N	Un	Un	Un
	Increased serum creatine kinase	✓	✓	✓	Un	N	Un
	Abnormal liver enzymes	✓	✓	✓	Un	N	Un
	Dicarboxylic aciduria	✓	✓	✓	Un	Un	Un
	Low free carnitine	✓	✓	✓	N	N	N
	Increased long-chain acylcarnitines	✓	✓	✓	✓	✓	✓
	Onset in the neonatal period	✓	✓	✓	✓	✓	✓
	Early death may occur	✓	✓	✓	✓	✓	✓
	Later onset with a milder phenotype	N	N	N	N	N	N

#:Refer to the description of clinical symptoms of carnitine-acylcarnitine translocase deficiency in OMIM database.
N: no, indicate no symptoms have been observed.
Un: unknown, it is not clear if the symptoms existed.

during the neonatal and genetic diagnosis from 18 early death neonates (≥32 weeks of gestational age and LBW > 1.5 kg). This group of infants died within 28 days after birth and were part of a cohort of 240,000 neonates born in Guangzhou during 2016(Guangzhou, Guangdong). The sixth patient was identified from a Newborn Screening Center of Shunde (Shunde, Guangdong).

2.2. Newborn screening using tandem mass spectrometry (MS/MS)

Blood amino acids and acylcarnitines from dried blood spots collected from the heels of newborns between 48 h and 7 days after birth were analyzed with a derivatization method using an API3200 triple quadrupole tandem mass spectrometer (AB Sciex, Canada) with an ion spray device. One 3-mm (1/8-in.) diameter spot was punched and dissolved in 100 mL of methanol stock solution containing stable-isotope enriched internal standards (Cambridge Company, USA). After 20 min, the samples were centrifuged. The eluate was evaporated to dryness, reconstituted in 60 mL of 3 N HCl/butanol, placed in sealed micro titer plates, and incubated at 65 °C for 25 min. The resulting mixtures were dried, and the residue was reconstituted in 100 mL

Table 2
Hepatic function and acylcarnitine concentrations in dried blood spots of 6 patients with CACT deficiency.

Patient	Sex	Age of oneset	Age of screened	CK (U/L)	CK-MB (U/L)	LDH (U/L)	HBDH (U/L)	AST (U/L)	ALT (U/L)	C0 (μmol/L)	C2 (μmol/L)	C12 (μmol/L)	C14 (μmol/L)	C16 (μmol/L)	C18 (μmol/L)	C18:1 (μmol/L)	(C16 + C18)/C0	(C16 + C18:1)/C2
P1	M	1d	4d	671	65	513	406	64	24	9.82	12.21	0.22	0.74	7.39	0.86	2.31	0.83	0.79
P2	F	1d	3d	511	133	1855	1444	126	19	8.51	8.69	0.8	0.95	8.82	1.12	3.65	2.13	1.43
P3	M	3d	3d	Un	Un	Un	Un	Un	Un	15.05	5.07	0.6	0.11	15.23	2.14	3.43	1.16	3.68
P4	F	2d	2d	Un	Un	Un	Un	Un	Un	17.85	7.8	1.51	1.93	13.46	1.68	3.44	0.85	2.17
P5	M	2d	2d	907	217	684	604	70	16	7.59	9.77	0.35	0.8	11.39	1.6	3.58	1.72	1.53
P6	F	2d	2d	Un	Un	Un	Un	Un	Un	16.44	21.14	0.92	1.23	15.1	3.59	4.04	1.14	0.91
Normal value				45–390	0–37	159–322	206–309	5–60	9–50	10–50	8–45	0.04–0.4	0.09–0.55	0.82–6.68	0.25–2	0.5–3	0.05–0.41	0.1–0.54
Trend				↑↑	↑↑	↑↑	↑↑	↑	N	N or ↓	N or ↓	N or ↑	N or ↑	↑↑	N or ↑	N or ↑	↑↑	↑↑

M:male, F:female, Un: unknown.

solvent of acetonitrile/water/formic acid (80:20:0.02 by volume). We screened for CACTD through detection of elevated levels of blood C16 and C18 species along with elevated (C16 + C18)/C0 ratio with decreased free carnitine (C0) and acetyl (C2) carnitine.

2.3. Sequencing of SLC25A20 and CPT2

Genomic DNA was extracted from peripheral blood or dried blood spot specimens using the Lab-Aid Nucleic Acid (DNA) Isolation Kit (Xiamen Zee san Biotech Inc., Xiamen, China) according to the manufacturer's protocol. Primers (Table S1) were designed by primer primer 5 software (Biosoft International, Palo Alto, USA). The PCR products were sequenced directly by an ABI 3730 sequencer (Applied Biosystems, Foster City, CA, USA) and the acquired sequences were aligned with *SLC25A20* (NM_000387) or *CPT2* (NM_000098) reference sequences by DNAMAN software (Lynnon Biosoft, Inc., Quebec, Canada), respectively. For identified variants, the PCR and Sanger sequencing were repeated to verify reliability. Pathogenicity of the novel splice site variant was analyzed by MutationTaster (http://www.mutationtaster.org/), NetGene2 Server (http://www.cbs.dtu.dk/services/NetGene2/), SpliceView (http://bioinfo.itb.cnr.it/~webgene/wwwspliceview.html) and Alternative Splice Site Predictor (http://wangcomputing.com/assp/index.html).

3. Results

3.1. Clinical features and acylcarnitine analysis

The pedigree charts of six patients (P1–P6) from five families (F1–F5) were showed in Fig. 1 and the features and acylcarnitine concentrations were showed in Table 1 and Table 2.

Three cases (P1–P3) with CACTD were identified from 29,046 newborns from expanded newborn screening by MS/MS in Guangzhou during 2017. P1 and P2 were male and female twins. All three patients were born after uncomplicated pregnancy and delivery. The patients presented with severe non-ketotic hypoglycemia, hypotonia and poor feeding two days after birth. Cardiac arrest caused by malignant ventricular arrhythmias developed rapidly and responded to cardiac resuscitation and intravenous glucose infusion. Plasma ammonia, lactic acid, serum lactate dehydrogenase (LDH), liver enzymes, uric acid, creatine kinase (CK), CK-MB, and hydroxy butyrate dehydrogenase (HBDH) were all elevated. Acylcarnitine analysis in DBS by MS/MS showed decreased free carnitine C0 and increased C14-acylcanitine, C16-acylcanitine, along with an increased (C16 + C18)/C0 ratio (Table 2). The profile was consistent with either carnitine palmitoyltransferase II (CPTII) or CACTD. Urine organic acids analysis showed increased medium-chain dicarboxylic acids. The first patient died at three days of age due to cardiogenic shock with malignant ventricular arrhythmias and pneumorrhagia. The second patient presented with hypotonia and poor feeding with glucose and amino acids infusion at four days of age. Her condition deteriorated rapidly, including poor responsition, hyperammonia (638 μmol/L (normal range 10–47 μmol/L)), hypoglycemia and arrhythmias after administration of total parenteral nutrition with medium-long chain fatty acids, and she died of at 8 days of age. P3 was treated with glucose, carnitine, argine and fed a MCT enriched formula. His clinical symptoms and abnormal biochemical results were improved and discharged at one month after birth. However, he died suddenly overnight at 2 months after birth with mild respiratory tract infection. Moreover, we were informed during a family history inquiry that he had an elder brother with similar symptoms who died of unknown causes at 50 days.

Two more cases (P4–P5) of CACT deficiency were identified from18 early death neonates in Guangzhou. P4 is a female neonate born after normal pregnancy and delivery with intrauterine growth retardation (GA 38w + 4, BW 2330 g). She manifested decreased responsiveness and lethargy with normal blood glucose in first day after birth. Then she

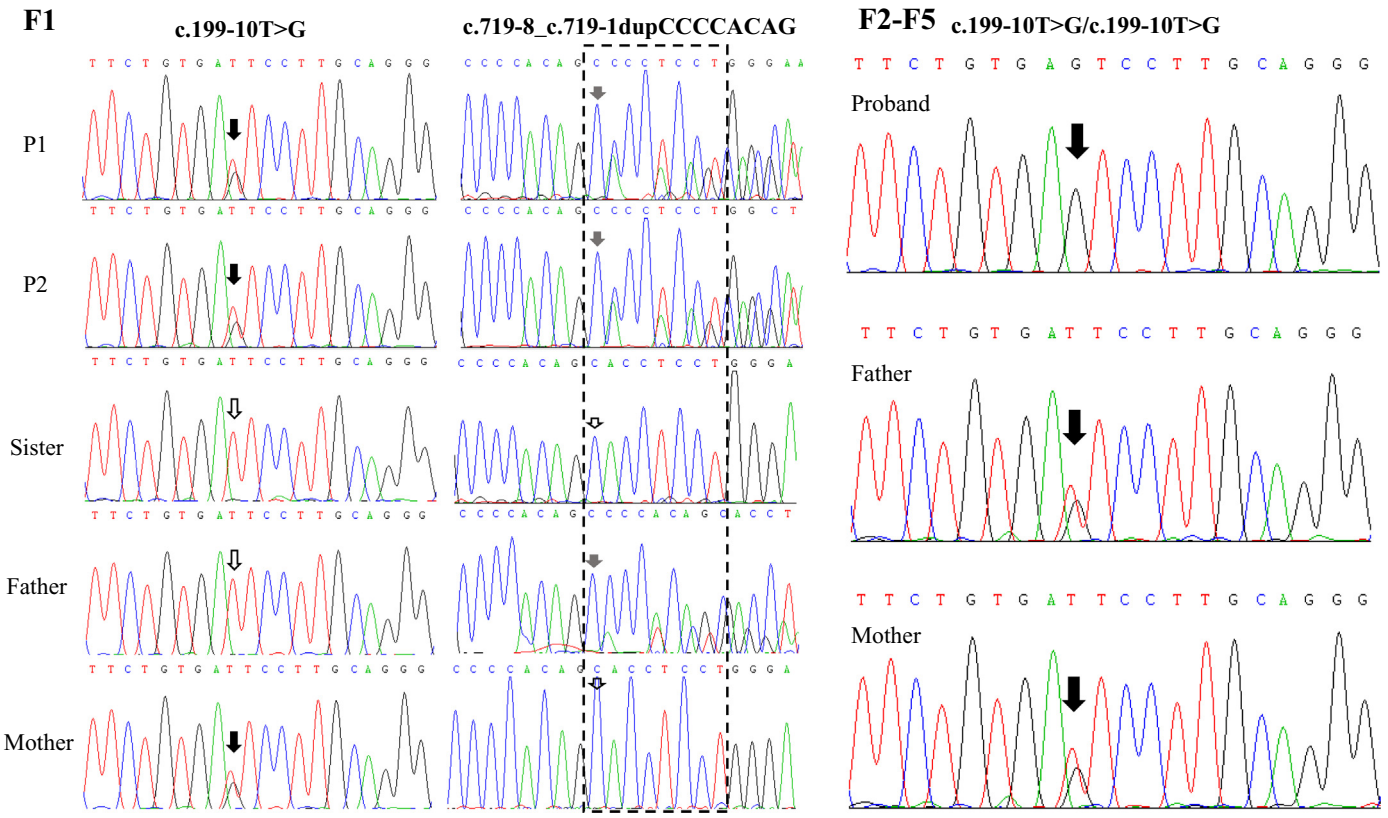

Fig. 2. DNA sequence of *SLC25A20* gene. P1and P2 (F1) were compound heterozygous for a maternally inherited c.199-10 T > G pathogenic variation and a paternally-inherited novel c.719-8_c.719-1dupCCCCACAG variation. Sister was unaffected. The other patients were homozygous mutations of c.199-10 T > G in F2-F5.

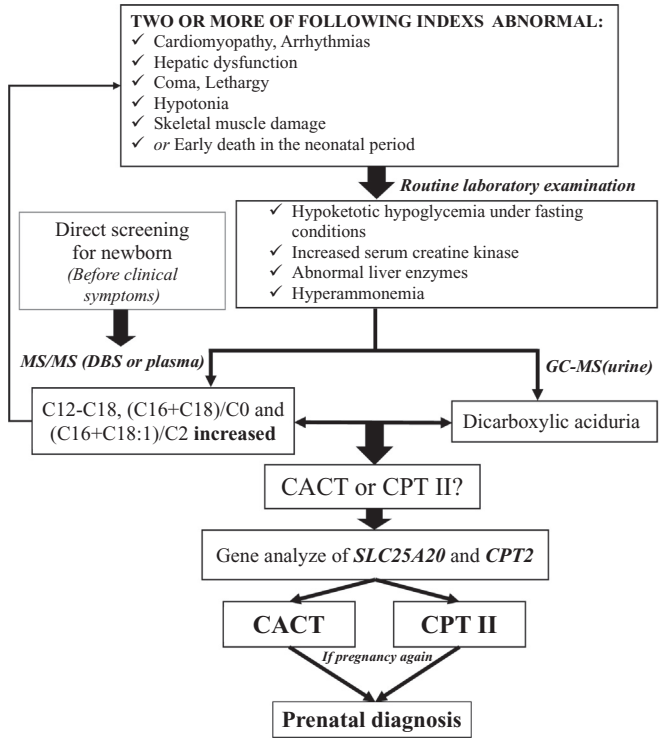

Fig. 3. The diagnostic process of CACTD. CACTD:carnitine-acylcarnitine translocase deficiency, CPT II:CPT II deficiency, myopathic, stress-induced.

developed cyanosis, ventricular tachycardia cardiac arrest culminating in death just three days after birth. Her elder brother died of unknown causes three days after birth in 2012. P5, a male neonate born after an uncomplicated pregnancy and delivery (GA 38w + 6, BW 3040 g) was transferred to the Neonatal Intensive Care Unit in the absence of symptoms because he had a concerning family history for an inborn error of metabolism. Two older brothers and two sisters died of unknown causes between two and thirty days after birth. Unfortunately, he developed respiratory distress, hypotonia, and arrhythmia after receiving intravenous intralipid nutrition.

P6 was identified by the Newborn Screening Center of Shunde in Guangdong with a birth weight of 2500 g. On the second day after birth, an episode of decreased responsiveness and cyanosis with hypoglycemia was noted. She developed ventricular tachycardia and cardiac arrest and died despite supportive treatment.

3.2. Molecular results of SLC25A20 and CPT2 gene

Mutation analysis of the SLC25A20 gene (NM_000387) revealed c.199-10T > G homozygous mutations in 4 unrelated patients (P3–6) in 4 families(F2-F5) and c.199-10T > G/c.719-8_c.719-1dupCCCCACAG compound heterozygous variants in twins (P1, P2)of one family(F1), as shown in Fig. 2. All mutations were inherited. In this study, the known pathogenic variation c.199-10T > G accounted for 83% of the mutant alleles, while a paternally-inherited novel c.719-8_c.719-1dupCCCCACAG variation that affects the splicing and probably affects protein features predicted by MutationTaster, NetGene2 Server, SpliceView and Alternative Splice Site Predictor. Sister was unaffected. Meanwhile, no pathogenic mutation was identified in the *CPT2* gene in these six cases.

4. Discussion

Our results further confirmed that CACTD is present in the Chinese population [8].We identified five cases of CACTD in approximately 50,0000 newborns born in Guangzhou between 2016 and 2017, and estimate an incidence of at least 1:100,000. The first case of CACTD was described in 1992 and only 55 cases were reported worldwide in the two decades that followed [3]. The first three cases of CACTD in the Chinese population were reported in Hong Kong , China [8]. Two more cases were identified from 153,789 screened newborns during three years were reported in 2017 in Hunan province, China [3].

The six cases in this study presented with symptoms in the early neonatal period including hypotonia and cardiomyopathy, although not all had hypoglycemia. Five patients died in the early neonatal period and the other patient died suddenly two months after birth. Furthermore, the family history of P5 included four children who died in the neonatal period of unknown causes, without newborn screening. Even though our results show that some patients presented on second day after birth and then died despite newborn screening, which illustrates newborn screening results is not good enough to save some CACT patients, but early diagnosis and early treatment after newborn screening is still important for assisted reproduction and prenatal diagnosis. And it would also reduce medical disputes in the tense doctor-patient relationship.

Our results reinforced previous genotype/phenotype correlations in CACTD. Previous studies from the Netherlands associated the c.241G > A(G81R) mutation with severe phenotype and the c.955insC mutation (C-terminal extension of 21 amino acids (CACT(121aa))) with a milder disease course [10]. The c.199-10T > G homozygotes were reported to be associated with early death in Asian patients [6,7], especially in China [3,8]. In the two neonatal cases from Hunan province, one c.199-10T > G homozygote died three days after birth due to sudden cardiac arrest. Another compound heterozygous individual with a maternally-inherited c.199-10T > G and a paternally inherited novel c.1A > G mutation died of congestive heart failure six days after birth [3,8]. Moreover, three neonate cases with the same c.199-10T > G homozygous mutations were found in Hong Kong. They were all symptomatic within the first 48 h of age. Two patients experienced cardiac arrest, one survived resuscitation but suddenly developed cardio-respiratory insufficiency and died within a week. In this study, the reported c.199-10T > G was the most frequent mutation. It accounted for 83% of the mutant alleles and was present in all six patients. All six patients had a severe course of CACTD and died in the neonatal period. Hence, our data further reinforce that the c.199-10T > G variant is associated with a severe phenotype of CACTD [11].

Our data also suggest that the (C16 + C18:1)/C2 ratio may be a useful marker that can improve the sensitivity and specificity of CACTD screening by MS/MS. Previous studies of CACTD reported increased C12–C18 (particularly C16 and C18:1 species) concentrations in blood, along with decreased acetyl (C2) carnitine and free carnitine (C0) [9,12]. However, the acylcarnitine profiles in our six cases (Table 2.) sometimes had normal levels of C12, C14, C18 and C18:1, but always had an elevated concentration of C16 along with increased values of the calculated ratios (C16 + C18)/C0 and (C16 + C18:1)/C2.

The sequencing analysis of *SLC25A20* gene, including intron-exon boundaries, revealed that P1 and P2 were both compound heterozygous with 2 mutations: a novel c.719-8_c.719-1dupCCCACAG mutation and a reported c.199-10T > G mutation. Sequence analysis of the parent's DNA extracted from blood indicated that the c.199-10T > G was derived from the maternal allele while the c.719-8_c.719-1dupCCCCACAG from the paternal allele (Fig. 2). However, sequence analysis of their sister's DNA showed no variation.

The diagnostic process of CACTD was summarized based on clinical analysis and literature review (Fig. 3). Although CACTD causes early neonatal death, its clear molecular diagnosis is also important to provide effective reproductive guidance to couples intending future pregnancies.

In conclusion, the c.199-10T > G was the most prevalent mutation of *SLC25A20* and the c.199-10T > G homozygote genotype was associated with a severe phenotype of CACTD. Apart from the common mutation c.199-10T > G, we also have identified a novel c.719-8_c.719-1dupCCCCACAG variant. Additionally, the calculated ratios (C16 + C18)/C0 and (C16 + C18:1)/C2 applied to newborn screening can assist the identification of CACTD-affected newborns.

Conflicts of interest

The authors confirm that they have no competing interests.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cca.2019.05.018.

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