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Clinical features and genotyping of patients with primary carnitine deficiency identified by newborn screening

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Abstract

Background: The objective of the study was to investigate clinical and gene mutation characteristics of primary carnitine deficiency (PCD) patients identified by newborn screening using tandem mass spectrometry (MS/MS).
Methods: Tandem mass spectrometry (MS/MS) was applied to screen inherited metabolic disease and seven patients with PCD were diagnosed among 62,568 samples. The *SLC22A5* gene was detected by using diagnosis panel of genetic and metabolic diseases based on Ion Torrent Semiconductor Sequencing Technology.
Results: The initial free carnitine (C0) concentrations of the patients were 6.43 ± 1.36 μmol/L, and the recall screening concentrations were 5.59 ± 0.89 μmol/L. The patients were treated with oral carnitine, so the levels after treatment were 20.24 ± 3.88 μmol/L. All patients had two pathogenic mutation alleles.
Conclusions: The combined application of MS/MS and a next generation sequencing panel could be used for the accurate diagnosis of PCD. The results of genetic diagnosis can guide the assisted reproductive treatment. The prognosis of PCD patients is good after early treatment.

Keywords: free carnitine (C0); primary carnitine deficiency (PCD); *SLC22A5*; tandem mass spectrometry (MS/MS).

Introduction

Primary carnitine deficiency (PCD) is due to abnormalities of carnitine transporters on the cell membrane. Carnitine cannot be transported into the cell, leading to the failure of long-chain fatty acids to enter the mitochondrial matrix involved in fatty acid β oxidation and to provide energy for the body. PCD is one of the most common fatty acid oxidation and metabolic diseases, which is a disease of autosomal recessive inheritance and can be treated. With the application of tandem mass spectrometry (MS/MS) in neonatal screening in China, more and more children receive early screening, early diagnosis and treatment, and the prognosis is good.

Here we reported seven patients with PCD who were screened by MS/MS and diagnosed by DNA sequence analysis. Their clinical and genetic characteristics were analyzed.

Materials and methods

Patients and newborn screening

In total, 62,568 newborns were screened by MS/MS at Nanjing Maternity and Child Health Care Hospital Affiliated to Nanjing Medical University between December 2013 and November 2016. The study was approved by the Nanjing Maternal and Child Health Hospital Ethics Committee.

Diagnostic criteria

The concentration of free carnitine (C0) on dry blood spot (DBS) filter paper cards was determined by MS/MS. The reference value of C0 in our laboratory was 10–50 μmol/L.

Patients were diagnosed with PCD based on following criteria:

(1) C0 <10 μmol/L, excluding maternal carnitine deficiency.
(2) Two pathogenic mutations detected in the *SLC22A5* gene of organic carnitine transporter (OCTN), derived from the father and mother, respectively.

MS/MS analysis

For each DBS card, one 3.2-mm diameter disc (equal to 3.2 μL of blood) was punched into U-96-well microplates and 100 μL of an extract solution was added to each well. After incubating for 45 min, 75 μL of the supernatant was transferred into a fresh V-96-well microplate and let to stand for 2 h at room temperature. This final solution was subsequently used for MS/MS analysis (NeoBase™ Non-derivatized

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MSMS Kit, PerkinElmer, USA). MS/MS analysis was performed using ACQUITY UPLC H-Class XEVO TQD (Waters TQD, USA). Approximately 20 μL of a working solution was directly injected for the analysis. All chromatograms were analyzed with Waters MassLynx v4.1 software (Waters TQD, USA).

DNA sequence analysis and panel detection

A genetic diagnosis panel of genetic metabolic disease covers 51 diseases and 98 genes, of which Panel 1 covers 18 amino acid metabolism diseases and 35 genes, Panel 2 covers 17 diseases and 42 genes of organic acid metabolic diseases and glycogen metabolism diseases and Panel 3 covers 16 fatty acid metabolism diseases and 21 genes. Genomic DNA was extracted from the peripheral blood of patients using the OMEGA Genomic DNA Extraction Kit (OMEGA Biotech, USA). All mutations were verified by Sanger sequencing. The disease spectrum included in the panels are summarized in Table 1.

Treatment

All patients were asymptomatic when diagnosed; 100 mg/kg/day of L-carnitine was given orally, 3 times. The patients had an outpatient

Table 1: Disease spectrum and pathogenic genes included in inherited metabolic disorder target enrichment system kits.

Panel 1		Panel 2		Panel 3	
Disease spectrum	**Pathogenic gene**	**Disease spectrum**	**Pathogenic gene**	**Disease spectrum**	**Pathogenic gene**
PKU	*PAH*	GA I	*GCDH*	PCD	*SLC22A5*
BH4D	*PS*	3-HMG	*HMGCS2*	SCADD	*ACADS*
	QDPR	MCD	*HLCS*	MCADD	*ACADM*
	GCH1		*BTD*	VLCADD	*ACADVL*
	SPR	MCCD	*MCCC1*	LCHAD	*HADHA*
	PCBD1				
MSUD	*BCKDHA*		*MCCC2*	TFP	*HADHA*
	BCKDHB	B-KD	*ACAT1*		*HADHB*
	DBT	MMA	*MUT*	CPT I	*CPT1A*
	DLD		*MCEE*	CPT II	*CPT2*
Hcy	*CBS*		*MMAA*	GA II, MADD	*ETFA*
	MTR		*MMAB*		*ETFB*
	MTHFR		*MMACHC*		*ETFDH*
CTLN I	*ASS1*		*MMADHC*	HADH deficiency	*HADH*
CTLN II	*SLC25A13*		*LMBRD1*	HSD	*SC5DL*
Tyrosinemia	*FAH*	PA	*PCCA*		*HSD3B2*
	TAT		*PCCB*	LCADD	*ACADL*
	HPD	Malonic acidemia	*MLYCD*	CACT deficiency	*SLC25A20*
Argininemia	*ARG1*	Pyruvate carboxylase deficiency	*PC*	5-α-RD	*SRD5A2*
Hyperprolinemia	*PRODH*	PDHCD	*PDHX*	ACAD9 deficiency	*ACAD9*
	ALDH4A1	Pyruvate dehydrogenase phosphatase deficiency	*PDP1*	DECRD	*DECR1*
OTCD	*OTC*	PDC	*DHA1*		*NADK2*
CPS I	*CPS1*	PHI II	*GRHPR*		
ASD	*ASL*	SBCADD	*ACADSB*		
Histidinemia	*HAL*	G6PD	*G6PD*		
Hypermethioninemia	*MAT1A*	GSD	*G6PC*		
	AHCY		*SLC37A4*		
	GNMT		*SLC17A3*		
	ADK		*GAA*		
HHH	*SLC25A15*		*AGL*		
NKH	*GLDC*		*GBE1*		
	AMT		*PYGM*		
	GCSH	MPS	*IDUA*		
			IDS		
Hyperornithinemia	*OAT*		*GNS*		
			HGSNAT		
			NAGLU		
			SGSH		
			GALNS		
AGU	*AGA*		*GLB1*		
			ARSB		
Tyrosinekinase deficiency	*TH*		*GUSB*		
			HYAL1		

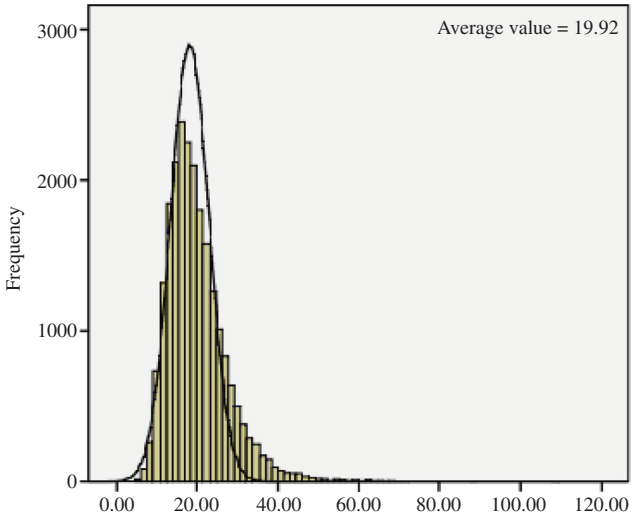

Figure 1: Frequency distribution histogram of C0.

clinical follow-up once every 2 weeks in the initial treatment, and then once every 3 months after serum levels of C0 were normal and stable; C0 levels were regularly reviewed during treatment (C0 at about 20 μmol/L, a variety of other acyl carnitines in the normal range) [1]. During follow-up, carnitine concentration, echocardiogram, electrocardiogram, liver function and creatine kinase were examined regularly. The ages of patients at final follow-up were 0.2–2 years old.

Results

The levels of C0 in DBSs were detected with MS/MS The frequency of C0 was similar to normal distribution (Figure 1) (0.2–99.8% CI: 6.96–56.36, 0.5–99.5% confidence interval [CI]: 7.79–48.65).

Seven cases were diagnosed with PCD by DNA sequence analysis at Nanjing Maternity and Child Health Care Hospital Affiliated to Nanjing Medical University between December 2013 and November 2016. The seven cases included three males and four females, they were full-term children and had no clinical symptoms. The incidence of PCD was 1:8938. Clinical; the genetic characteristics of six cases of children with PCD are shown in Table 2.

Discussion

Karpmi first reported PCD patients in 1975 [2]. PCD prevalence ranges from 1:40,000 to 1:120,000 in different countries or regions: 1:20,000–1:70,000 in the United States [3], 1:40,000 in Japan [4] and 1:1,20,000 in Australia [5], and the incidence of heterozygotes for PCD in the population is 0.5%–1% [6]. Interestingly, the disease is very common in the Faroe Islands, where the prevalence is 1:300 [7]. No data are available about the nationwide prevalence of PCD in China; the incidence of PCD is about 1:45,000 in Shanghai [8, 9] and about 1:22,384 in Zhejiang Province [10]. In this study, 62,568 newborns were screened and seven cases were diagnosed with PCD by MS/MS during December 2013–September 2016 in Nanjing. The incidence of PCD was 1:8938 in Nanjing, higher than that in Shanghai, Hangzhou and other places, considering the geographical differences and shorter statistical years.

To be clear, according to the requirements from Chinese MS/MS-relevant technical documentations, the samples were collected from the baby 72 h after birth. However, some scholars think that the maternal influence could be weaker due to delayed blood sampling time to 4–9 days after parturition, so the delay in the sample collection process could help detect more newborns with positive results [11]. According to the case reports in China and communication with peers, in China the maximum of C0 value in PCD patients is under 11 in 72 h after birth, and most of them are under 10, few are between 10 and 11. So the samples were collected from the baby 72 h after birth and the cutoff value of C0 was determined as 10–50 umol/L.

Table 2: Clinical and genetic characteristics of the seven cases of children with PCD.

Case	Gender	Birth weight, kg	Gestational weeks	C0 level, μmol/L		*SLC22A5* gene mutation	
				Primary screening	After treatment	Mutation site 1	Mutation site 2
1	F	4.15	40	8.46	17.30	c.1400C>G,p.Ser467Cys	c.1400C>G,p.Ser467Cys
2	M	3.85	38+6	5.07	19.12	c.760C>T,p.Arg254X	c.1400C>G,p.Ser467Cys
3	F	3.07	38+6	7.46	26.00	c.1400C>G,p.Ser467Cys	c.1400C>G,p.Ser467Cys
4	F	3.82	41+4	4.94	22.00	c.428C>T, p.Pro143Leu	c.428C>T, p.Pro143Leu
5	F	2.92	36+4	6.22	15.12	c.51C>G, p.Phe17Leu	c.428C>T, p.Pro143Leu
6	M	3.35	39	6.42	21.92	c.51C>G, p.Phe17Leu	c.428C>T, p.Pro143Leu
7	M	3.20	39+6	3.72	16.50	c.1400C>G,p.Ser467Cys	c.1400C>G,p.Ser467Cys

The pathogenic gene of PCD is *SLC22A5*, located on chromosome 5q31.1, which contains 10 exons and 3 introns. More than 110 types of mutations of *SLC22A5* have been detected, mostly missense mutations, and hot spots mutations are different in different regions. Most studies believe that c.760C>T(p.R254X) and c.1400C>G(p.S467C) are the most frequently reported mutations in Chinese patients [10]. During January 2009–December 2014 in the Children' s Hospital, Zhejiang University School of Medicine, 50 neonates were diagnosed with PCD, and the most common mutation of those patients were c.1400C>G(34.3%), c.760C>T(19.4%) and c.51C>G(11.9%) [10]. In this study, four pathogenic mutations were detected in seven child patients, with the frequency of c.1400C>G(p.S467C) being as high as 50% (7/14), confirming previous conclusions.

The diagnosis of PCD mainly depends on blood C0 levels [3, 12], clinical manifestations and gene mutation analysis. In this study, the C0 levels with the primary screening of seven patients were lower than the laboratory cut-off value (<10 μmol/L); the mean was only 6.42 μmol/L, confirming the effectiveness of MS/MS neonatal screening for PCD diagnosis. It was because C0 can be transferred to the fetus through the placenta that the C0 level of plasma in the short time after birth often reflects the C0 level of maternal plasma, and it can lead to false-positive or false-positive diagnosis. Therefore, in our hospital, we detect the mother's C0 levels in the recall of neonatal screening for suspicious positive C0. Also, suspicious positive patients with primary screening values at the cut-off point are followed up several times to avoid a misdiagnosis. In addition, there are some patients who were secondary to carnitine deficiency, such as preterm children, severe malnutrition, dialysis and organic acids or fatty acid metabolic diseases. It is very difficult to diagnose PCD only by the MS/MS screening of C0 and clinical features and genetic diagnosis, thus, is necessary [13]. In this study, the genetic diagnosis of seven cases of PCD patients are based on the second-generation sequencing technology panel method. By comparison with previous simple second-generation sequencing technology, this method could greatly shorten the diagnosis time, avoid misdiagnosis, and encourage early treatment of PCD.

PCD occurs at any age, with primary symptoms such as dilated cardiomyopathy, hepatomegaly, myasthenia, etc. [14]. PCD can cause death if untreated, so early diagnosis and timely treatment is key to improve outcomes. PCD treatment is mainly by oral or intravenous infusion of L-carnitine; intravenous infusion of L-carnitine is mainly applied to critically ill children who cannot eat. In this study, seven children with PCD were identified after neonatal screening, and they had no clinical symptoms. All of them received oral L-carnitine treatment and blood C0 and other acylcarnitine concentrations at the last follow-up were normal; in addition, their growth and development were normal. However, in the clinical we found that most patients with PCD had no obvious clinical symptoms and only needed oral L-carnitine, so that the treatment compliance of patients is poor who do not often continue treatment. Repeated interruption of treatment can induce fat metabolism disorder and even sudden death or other serious consequences [3, 15]. Therefore, the patient's family should be informed about the detailed information of PCD and the patient's condition. Informed consent should be signed to monitor the treatment.

In summary, the use of MS/MS for neonatal screening combined with a second-generation sequencing panel can diagnose PCD. The incidence of PCD is higher in Chinese than in Europeans and Americans. Child patients with PCD have good prognosis after early standard L-carnitine treatment.

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