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Case Reports

Genotype of Mild 6-pyruvoyl-tetrahydropterin Synthase Deficiency: Three Case Reports and a Literature Review

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Abstract ***Objectives:*** To analyse the characteristics of mutation in PTS gene in Chinese patients with mild 6-pyruvoyl-tetrahydropterin synthase deficiency (M-PTSD). ***Methodology:*** Clinical and genetic data of three newborns with M-PTSD were collected from the Newborn Screening Center of Zhejiang Province, China. The characteristics of gene mutation in the selected patients were analysed together with local and international literature related to PTS gene and M-PTSD. ***Results:*** Nine M-PTSD patients have been reported in China, including three with the IVS1-291A>G/ D96N genotype, two with the L93M/ N52S genotype, and one each with the IVS1-291A>G/N52S, IVS1-291A>G/L127F, V56M/R25G, and V56M/ T106M genotypes. Four M-PTSD patients have been reported abroad: one each with the genotypes IVS1-322A>T/IVS1-322A>T, L26F/V124L, N47D/D116G, and R16C/C370_383 (K120→stop). ***Conclusion:*** Results showed ethnic differences in the M-PTSD related mutations. In the Chinese population, IVS1-291A>G, L93M, and V56M are mild-type mutations, and IVS1-291A>G is the most common benign mutation (27.8%). M-PTSD is not rare among individuals from mainland China, and newborns screened with PTSD should undergo mutation detection as soon as possible in order for physicians to select the appropriate therapeutic regimen and avoid over-treatment.

Key words 6-pyruvoyl-tetrahydropterin synthase deficiency; Medication treatment; Mutation; Tetrahydropterin deficiency

Introduction

6-pyruvoyl-tetrahydrobiopterin synthase deficiency (PTSD) is the most common type of tetrahydrobiopterin deficiency around the world.[1-3] It represents a heterogeneous group of progressive neurological disorders caused by autosomal recessively inherited mutations affecting 6-pyruvoyl-tetrahydropterin synthase (PTS), the second enzyme in the biosynthetic pathway for tetrahydrobiopterin (BH4).[1,2] As assessed by cerebrospinal fluid (CSF) measurement of catecholamines and serotonin metabolites or clinical outcomes, it is divided into at least two different phenotypes, the more common severe central form and the rare mild peripheral form.[1-2] Recently more and more PTSD cases were found in China due to more newborn screening and differential diagnosis being performed for hyperphenylalaninaemia (HPA).[4] Many of the PTSD diagnosed late are retarded[2] and it is difficult to distinguish mild (M-PTSD) from severe types, so that these newborns were often treated by combination therapy with BH4, dopamine and serotonin immediately. Meanwhile, the rapid development of sequencing technology and the study for relationship between genotype and phenotype of PTSD make it possible to diagnose M-PTSD by genotype. But reports about it are still scarce.

Between September 1999 and June 2014, there are 5,912,779 newborns screened for HPA at the Newborn

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Screening Center of Zhejiang Province, China. Of these, 35 PTSD were diagnosed. The incidence of PTSD was 1 in 168,937, including three cases of M-PTSD. We report the details of these three cases in the present study.

Case Presentation

Methods and Results

Genetic examination: Genetic examination was conducted after informed consent was obtained from the parents. Peripheral blood specimens of the newborns and their parents were collected. Total DNA was extracted from the blood spots by using QIAamp DNA Investigator Kit (Qiagen GmbH, Hilden, Germany).

Mutation genotyping was performed using polymerase chain reaction (PCR). The Typer 4.0 genotyping software (Sequenom, San Diego, CA, USA) was used to design PCR primers and single-base extension assays of mutation sites. All primers were synthesized by Integrated DNA Technologies (Coralville, IA, USA). PCR was performed using reagents and an iPLEX Gold reagent kit from Sequenom (San Diego, CA, USA).

Mass spectrometry was conducted using the Nanodispenser RS1000 and MassARRAY Analyzer 4 systems with Typer 4.0 software (Sequenom).

Case 1

The patient was a male born at 40 weeks of gestation with a birth weight (BW) of 3.7 kg. Newborn screening on Day 3 after birth showed increased blood phenylalanine (Phe) levels and Phe-to-tyrosine ratio (Phe/Tyr), i.e., 257 μmol/L (range, 23-100 μmol/L) and 2.52 (range, 0.1-1.5) respectively. The BH4 loading test was positive, urinary pterin analysis showed low biopterin / (biopterin+ neopterin) [B%], and dihydropteridine reductase (DHPR) activity showed normal, which suggested the diagnosis of PTSD (Table 1). Combination therapy was immediately implemented. The patient is now 5 years and 6 months, with normal clinical outcomes (Table 1). PTSD was finally diagnosed by PTS gene mutation detection at age 4 years (Figure 1).

Case 2

The patient was a female born at 39 weeks of gestation with a BW of 3.5 kg. Newborn screening on Day 3 showed elevated blood Phe levels (156 μmol/L) and Phe/Tyr ratio (1.8). Urinary pterin analysis and a DHPR activity assay suggested the diagnosis of PTSD. The patient was suggested to have the above-mentioned combination therapy, but rejected by her parents. Diagnosis was made using PTS gene mutation detection after one month (Figure 1). At present, the patient is 21 months old and has normal clinical outcomes (Table 1).

Case 3

The patient was a male born at 37 weeks of gestation with a BW of 3.1 kg. Screening showed blood Phe levels of 248 μmol/L. Results of urinary pterin analysis and a DHPR activity assay suggested PTSD, which was finally diagnosed by gene detection (Figure 1). His parents did not allow any medication but agreed for follow up. Now the patient is 18 months old with normal clinical outcomes (Table 1).

The parents of all three newborns were of Han nationality (Chinese), all in good health and from non-consanguineous family. The patients' births were all natural, with a mean Apgar score of 10.

Literature Review

A keyword search of "PTSD" in the Chinese databases VIP (http://www.cqvip.com) and CNKI (http://www.cnki.net) (before June 2014) yielded 4 studies on M-PTSD patients carrying the PTS gene. Of these, cases 4 and 5 were siblings, a sister and brother, respectively (Table 1). A keyword search of "PTSD" and "PTS" in the international database PubMed (http://www.ncbi.nlm.nih.gov/pubmed) (before June 2014) yielded 6 studies on M-PTSD genes (Table 1).

M-PTSD accounted for 8.6% (3/35) of all PTSD cases in our hospital, and 12.5%,[1] 20%[2] respectively from previous reports. All patients, whether treated nor untreated, were normal clinically during follow-up (Table 1). Nine M-PTSD patients have been reported in China, including 6 from previous reports and 3 from this report (Table 1). While four M-PTSD patients have been reported abroad (Table 1). In China, IVS1-291A>G accounts for 27.8% (5/18) of M-PTSD mutation sites. Splicing and missense mutations are the majority mutation types for M-PTSD (Table 1).

Discussion

The incidence of PTSD is significantly higher in the Chinese (especially the southern Chinese population) than

in Caucasians.[1] In recent years, an increasing number of PTSD patients have been identified because of widespread screening for HPA. In China, PTSD is commonly diagnosed by the BH4 loading test, urinary pterin analysis, and DHPR activity assay. Gene detection has not been used extensively. Severe PTSD affects brain neurotransmitter (NTM) levels and generally has a poor prognosis. Once diagnosed, early PTSD is often treated by combination therapy with BH4, dopamine, and serotonin.[4]

Clinically, PTSD can be divided into the severe type and the mild type. M-PTSD does not significantly changes brain NTM levels but only increases blood Phe level; it causes no other neurological symptoms and therefore generally requires no treatment or single BH4 to maintain normal Phe level.[2] In the present study, the blood Phe levels detected during newborn screening for M-PTSD varied from 247 to 1100 μmol/L. The data suggests that mild and severe types of PTSD cannot be distinguished solely on the basis of blood

Table 1 Summary of information from M-PTSD patients from this and previous studies

Case No.	Year of reporting	Nationality	Age at diagnosis	Serum Phe levels (μmol/L)	Neopterin (mmol/mol creatinine)
1	2014	Chinese	10 days	16-997	12.83
2	2014	Chinese	1 month	29-335	4.07
3	2014	Chinese	1 month	170-279	3.50
4 and 5 [1]	2009	Chinese	After 3 months		
6 [1]	2009	Chinese	After 3 months		
7 [8]	2004	Chinese		25-198	
8 [9]	2009	Moroccan	Neonatal screening	1100	5.04
9 [2]	2001	German	3 months and 1 week	165-541	18.70
10 [10]	1999	Italian	3 weeks	420	
11 [6]	1998	Chinese	13 years	820	
12 [6]	1998	Chinese	7 years	710	
13 [3]	1994	Pakistani	1 month	360	

Case No.	Biopterin (mmol/mol creatinine)	B%	Dihydropteridine reductase activity (% of controls)	PTSD activity (% of controls)	Mutation 1	Effect of mutation 1
1	0.20	1.53	91.6	ND	c.IVS1-291A>G	Splicing error
2	0.15	3.55	179.1	ND	c.IVS1-291A>G	Splicing error
3	0.12	3.31	89.0	ND	c.IVS1-291A>G	Splicing error
4 and 5 [1]				ND	c.155A>G(p.N52S)	Missense
6 [1]				ND	c.IVS1-291A>G	Splicing error
7 [8]				ND	c.IVS1-291A>G	Splicing error
8 [9]	<0.01			1.4(RBC)3.1(fibroblasts)	c.IVS1-322A>T	Splicing error
9 [2]	0.65	3.0		5(RBC)0.45(fibroblasts)	c.78G>A(p.L26F)	Missense
10 [10]		1.7		5.9(RBC)0.16(fibroblasts)	c.139A>G(p.N47D)	Missense
11 [6]					c.166G>A(p.V56M)	Missense
12 [6]					c.166G>A(p.V56M)	Missense
13 [3]					c.55C>T(p.R16C)	Missense

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Table 1 Summary of information from M-PTSD patients from this and previous studies (cont'd)

Case No.	Mutation 2	Effect of mutation 2	NTM in CSF	The BH4 loading test	Age at treatment	Age at drug withdrawal
1	c.155A>G (p.N52S)	Missense	ND	Positive	10 days	9 months
2	c.286G>A (p.D96N)	Missense	ND	ND	ND	ND
3	c.379C>T(p.L127F)	Missense	ND	ND	ND	ND
4 and 5 [1]	c.277C>A(p.L93M)	Missense	ND			
6 [1]	c.286G>A (p.D96N)	Missense	ND			
7 [8]	c.286G>A (p.D96N)	Missense	ND			
8 [9]	c.IVS1-322A>T	Splicing error	Normal	ND	3 months	
9 [2]	c.370G>T(p.V124L)	Missense	Normal	Positive	3 months and 1 week	5 years
10 [10]	c.347A>G(p.D116G)	Missense	Normal	ND	5 weeks	22 months
11 [6]	c.74G>A(p.R25G)	Missense	ND		ND	ND
12 [6]	c.317C>T(p.T106M)	Missense	ND		ND	ND
13 [3]	ΔC370_C383(K120→stop)	Frame shift	Normal	Positive	ND	ND

Case No.	Dose of BH4 (mg/kg)	Dose of dopamine(mg/kg)	Dose of serotonin(mg/kg)	Physical development	Neurological development
1	1	5	5	Normal	Normal
2	ND	ND	ND	Normal	Normal
3	ND	ND	ND	Normal	Normal
4 and 5 [1]				Normal	Normal
6 [1]				Normal	Normal
7 [8]				Normal	Normal
8 [9]	1-2	10	10	Normal	Normal
9 [2]	2.0	ND	ND	Normal	Normal
10 [10]	2.2-5.0	1.8-4.8	1.8-4.0	Normal	Normal
11 [6]				Normal	Mild mental deficiency(IQ 75)
12 [6]				Normal	Mild mental deficiency(IQ 76)
13 [3]	ND	ND	ND	Normal	Normal

B% = Biopterin / (Biopterin+ Neopterin); ND (not done); Blank (no information); [ ] (for reference number)

Positive in the BH4 loading test means Phe levels normalized after taking BH4 4-6 hours;

The Bayley Scales of Infant Development in case 1-3 have done and shows normal;

Frequency of gene in Chinese: c.IVS1-291A>G 27.8%, D96N 16.7%, N52S 16.7%, L93M 11.1%, V56M 11.1%, L127F 5.6%, R25G 5.6%, T106M 5.6%;

Reported relationship between genotype and phenotype: c.IVS1-291A>G mild[1,8], V56M mild[6], c.IVS1-322A>T mild[9], D116G mild[10], R16C mild[3], D96N severe[1,6], N52S severe[1,6], L127F severe[1,7], R25G severe[6].

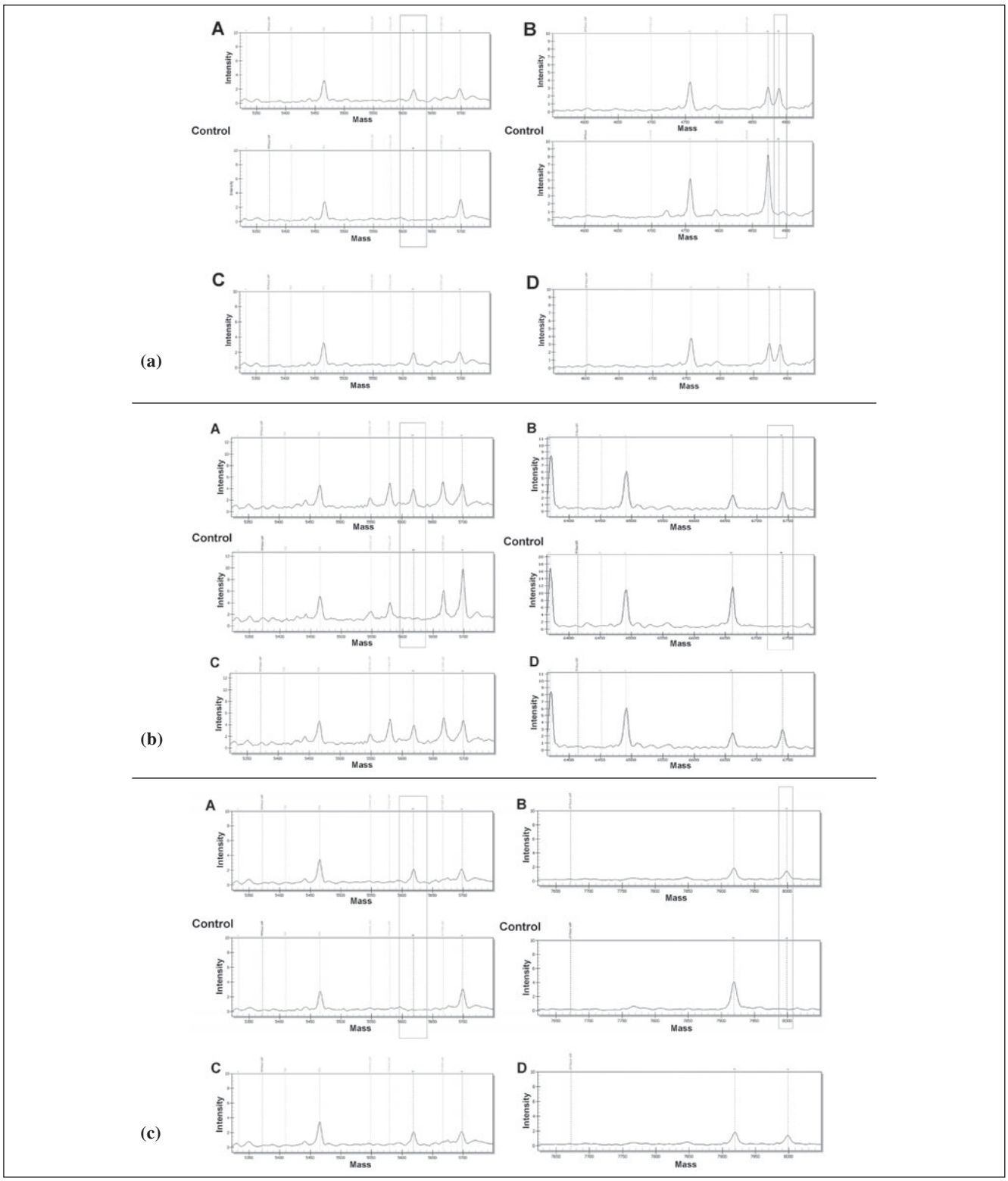

Figure 1 (a) *Mutations of case 1 and his parents.* A: IVS1-291A>G in case 1; B: 155A>G in case 1; C: IVS1-291A>G in father; D: 155A>G in mother. *(*b) *Mutations of case 2 and her parents.* A: IVS1-291A>G in case 2; B: 286A>G in case 2; C: IVS1-291A>G in mother; D: 286A>G in father. (c) *Mutations of case 3 and his parents.* A: IVS1-291A>G in case 3; B: 379C>T in case 3; C: IVS1-291A>G in mother; D: 379C>T in father.

Phe levels. Dudesek et al[2] found that L26F, V124L showed no measurable PTS activity in COS-1 cells but the heterozygote with L26F and V124L showed M-PTSD. It was suggested that the PTS enzyme activity assay was also ineffective in distinguishing between the mild and severe types. Although NTM levels in the CSF are indicative of M-PTSD,[2] CSF examination is invasive and therefore may not find widespread clinical application, and it was rarely accepted by patients' parents in China.

In 1992, Thöny et al first cloned the PTS gene,[5] which is positioned at 11q22.3-23.3 and comprises 6 exons. To date, more than 80 mutations in PTS gene have been reported in the HGMD database, and there are some reports about the correlation between PTSD phenotype and genotype.[1-3] We summarised information on 18 alleles of 9 M-PTSD patients from mainland China and found IVS1-291A>G to be the most common mutation (27.8%). Further, in our cases, when the patient carried the IVS1-291A>G or L93M genotypes, even if the other allele was associated with severe-type PTSD, the symptoms manifested as M-PTSD. Similarly, Liu et al[6] reported a heterozygous V56M mutation that clinically manifested as M-PTSD. Our data showed splicing and missense mutations are the majority mutation types for M-PTSD and the M-PTSD genotype was very different between Chinese and other populations.

Data also showed that M-PTSD accounted for 8.6%-20% of all PTSD cases. IVS1-291A>G was previously found as a hotspot mutation of the PTS gene in Chinese,[1] suggesting that mild-type PTSD gene is not rare in Chinese.

Thus, M-PTSD related mutations show ethnic differences and are not rare in the Chinese population. IVS1-291A>G, L95M, and V56M in PTS gene are mild-type PTSD related mutations in Chinese, and IVS1-291A>G is the most common mutation. We recommend that early genetic testing be performed in newborns identified with PTSD by screening in order to select an appropriate therapeutic regimen and thereby avoid wasting energy and financial resources caused by over-treatment.

Consent

The written informed consent was obtained from the parents of the children who served as subjects of the investigation. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

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Competing Interests

The authors declare that they have no competing interest.

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