BRIEF REPORT

Gene mutation and pedigree analysis of tetrahydrobiopterin deficiency in a Uyghur family of China

Long Li¹ | Yulan Qin² Yajie Su¹ | Haili Jiang¹ | Nuerya Rejiafu¹ |
Mingzhu Li¹ | Ayijiamali Muhetaer¹ | Yongqiao Liu¹ | Yan Ren¹

¹Neonatal Department, People's Hospital of Xinjiang Uygur Autonomous Region, Urumqi, Xinjiang, China

²Shihezi University, Shihezi, Xinjiang, China

Correspondence

Long Li, Neonatal Department, People's Hospital of Xinjiang Uygur Autonomous Region, Urumqi, Xinjiang, China.
Email: lilong65@126.com

Funding information

This work was supported by the National Natural Science Foundation of China (grant NO. 81741102)

Background: Tetrahydrobiopterin (BH₄) deficiency is an autosomal recessive disorder, which is caused by an enzyme deficiency involved in its synthetic or metabolic pathways. Clinical symptoms may include microcephaly, hypoevolutism, severe ataxia, and seizures. The purposes of this study are to analyze the genotype-phenotype and the pedigree of the first case of BH₄ deficiency in the Uyghur of China.

Methods: (a) This patient received tandem mass spectrometry, urinary neopterin and biopterin analysis, and determination of dihydropteridine reductase (DHPR) activity in dried blood spots. (b) Blood DNA samples of this patient and her three family members were collected for gene sequencing and mutation analysis.

Results: (a) The basic urinary neopterin and biopterin were 1.07 mmol/mol Cr and 3.12 mmol/mol Cr, respectively, and biopterin percentage was 74.42%. The DHPR activity of this patient was 31.11% of normal control. (b) Sanger sequencing of PAH gene in this patient was negative but positive of her sister, which carries 2 heterozygous mutation c.781C>T and c.1238G>C. Next-generation sequencing on the patient identified a homozygous mutation in the quinoid dihydropteridine reductase (QDPR) gene at c.508G>A, which was confirmed by Sanger sequencing.

Conclusion: (a) The patient was the first case of clinical diagnosis of BH₄ deficiency in the Uighur. And there are two types of hyperphenylalaninemia (HPA) in the same family. (b) The mild HPA patient with severe nervous system damage should pay more attention to the BH₄ deficiency. (c) Using next-generation sequencing technology can increase the mutation detection rate when the hereditary diseases are highly suspected in clinic.

KEYWORDS

dihydropteridine reductase, gene mutation, hyperphenylalaninemia, tetrahydrobiopterin deficiency, Uyghur

1 | INTRODUCTION

Hyperphenylalaninemia (HPA) is an autosomal recessively metabolic disorder which is characterized by abnormally high concentrations of blood phenylalanine (Phe) and production of phenylketones. HPA may be caused by the deficiency of the hepatic phenylalanine hydroxylase (PAH) or its obligatory cofactor, tetrahydrobiopterin (BH₄).¹

BH₄ is the cofactor for the enzyme PAH, and for tryptophan and tyrosine hydroxylases. The deficiency of BH₄ compromises the hydroxylation of these three aromatic amino acids and the synthesis of the corresponding neurotransmitter precursors, and then reduces the production of neurotransmitters such as 5-HT and dopamine. Patients with BH₄ deficiency usually show signs of progressive neurological impairment when treated with a Phe-restricted diet al.

one.² So far, five different enzymes dihydropteridine reductase (DHPR), GTP cyclohydrolase I (GTPCH), 6-pyruvoyltetrahydropterin synthase (PTPS), sepiapterin reductase (SR), and pterin-4 α -carbinolamine dehydratase (PCD) have been identified in the synthesis or recycling of BH₄.³

Since the newborn screening was carried out in Xinjiang China, more than 200 cases of Uighur patients were diagnosed with PAH deficiency. Recently, we did Sanger sequencing of PAH gene for these patients, but only one did not detect the mutation of PAH. This child was diagnosed BH₄ deficiency with a QDPR mutation by next-generation sequencing. More interesting, we tracked the family mutation spectrum and found that the 7-year-old sister was PAH-deficient HPA. Therefore, we report on the genotype-phenotype of the child and the pedigree as follows.

2 | CASE REPORT

2.1 | Materials and methods

2.1.1 | Patient and clinical data

The criteria of genetic testing were approved by the ethics committees of People's Hospital of Xinjiang Uyghur Autonomous Region (2017010). The study obtained the written informed consent from the patient's parents, and was conducted in accordance with the ethical principles of the Declaration of Helsinki. Pretest counseling was performed by physicians.

We present a girl who is 13-month-old. She was born by cesarean section at 36⁺² weeks. Her birthweight was 1600 g. Amniotic fluid, umbilical cord, and placenta were normal. Apgar scores were 8 at 1 and 5 minutes. She was less active with grunting, tachypnea, and hypotonia after birth. Because of premature birth and tachypnea, she was admitted into the Neonatal Department of People's Hospital of Hotan Prefecture in Xinjiang. Blood glucose was only 0.1 mmol/L (2.2-7.0 mmol/L) after admission. Admission diagnoses were aspiration of amniotic fluid syndrome, premature infant, and low birthweight infant. She recovered and was discharged from hospital. Neonatal screening was carried out until 3 months after birth, and the value of blood Phe concentration was 6.2 mg/dL. The parents did not pay attention to the disease, the child was only re-collected dried blood spots (DBSs) at 13 months, and the value of Phe concentration was 13.41 mg/dL. Physical examination showed she had white skin and yellow hair without rat urine smell. The girl had attention deficit and she was floppy. Her blood glucose, blood cell count, and electrolytes were all normal. Head Computed Tomography (CT) showed white matter dysplasia and have a deep brain sulcus. Her parents refused other auxiliary examinations. She was the fourth child of nonconsanguineous parents of Uyghur ethnicity. She has 2 older brothers and 1 older sister. The two brothers did not have the symptoms which were associated with phenylketonuria and were able to study and live normally.

Her older sister also had mental retardation and was not able to walk until 7 years old. We collected the blood sample for Phe detection from her sister, and the value of Phe in the DBSs was 18 mg/dL.

2.1.2 | Biochemistry test for differential diagnosis

The dried blood spots (DBSs) and urine sample of the patient were sent to institute of Xin Hua Hospital of Shanghai for tandem mass spectrometry, urinary pterin analysis, and determination of DHPR activity. For economic reasons, the older sister only performed tandem mass spectrometry.

2.1.3 | Genetic test and mutation analysis

All specimens were sequenced from 13 exons of PAH gene. If the result of sequencing was negative or only a single heterozygous mutation was found, the next-generation sequencing was performed. Then, we will validate it by Sanger sequencing if the results are positive.

DNA isolation

We collected the peripheral venous blood about 2 mL of the patient, the older sister and their parents. DNA isolation was performed using the QIAamp DNA Blood Mini Kit (250) (QIAGEN, Vienna, Austria).

Polymerase chain reaction condition and Sanger sequencing

PCR primers were designed to amplify all 13 exons and the surrounding introns of the PAH gene, all 7 exons and the surrounding introns of the QDPR gene.

PCR was carried out on the Gene Amp PCR system 9700 (Applied Biosystems, CA). In brief, reaction mixtures were made up in a volume of 20 μ L containing 0.1 μ g of genomic DNA, 10 mmol/L of each primer, 0.25 mmol/L of each dNTP in 1 $\times$ reaction buffer and 1 U Taq DNA polymerase. (Takara Bio, Inc., Shiga, Japan).

The PCR cycles of PAH gene were carried out as follows: 30 seconds initial denaturation at 94°C followed by 12 cycles of amplification, each including 10 seconds denaturation at 94°C, 10 seconds annealing at 58-64°C (Each cycle down 0.5°C), and 45 seconds extension at 72°C. This was followed by 25 cycles of amplification, each including 10 seconds denaturation at 94°C, 10 seconds annealing at 57°C, and 45 seconds extension at 72°C.

The PCR conditions of QDPR gene included an initial denaturation step for 5 minutes at 95°C, 1 minute at 95°C, 1 minute at 64°C(except for exon1 at 62°C.) and 1 minute in 72°C and a final extension for 10 minutes at 72°C for 28-30 cycles.

Amplification cycles were followed by a 4 minutes final extension at 72°C and then cooled to 10°C. The PCR products were sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) with an ABI 3730 Genetic Analyzer. The sequence data were analyzed by the Mutation Surveyor Software (Softgenetics) using PAH RefSeq NM_000277.1 and QDPR RefSeq NM_000320.2.

Next-generation sequencing

Exome capture was accomplished using the TruSeq™ Exome Enrichment Kit (Illumina) and sequencing was performed using the Illumina HiSeq 2500 platform following the manufacturer's protocol. It covers a minimum of 98% of the genome with 20× coverage and is compared to a human reference sequence.

Data analysis

Variants were annotated using Variant Effect Predictor (VEP-Ensembl 73). Functional exonic and splicing variants were considered in the analysis. This contains loss of function (stop, frameshift, and splice) or missense (nonsynonymous and nonframeshift) mutations, these mutations are predicted to have high and moderate impact, respectively. The three major databases for known pathogenic and likely pathogenic mutations were used including ClinVar, OMIM, and HGMD Professional. Numerous missense functional predictors, noncoding regulatory region annotations, and others were included. To be able to filter out variants of high frequency, the allelic frequencies were annotated from the public datasets from the 1000 Genomes, the Exome Sequencing Project

(EVS6500) and Exome aggregation Consortium (ExAC), Kyoto, and Dutch allelic frequency databases. All aligned reads, variants, and annotations were stored according to genomic position in the WuXi NextCODE Genomically Ordered Relational Database (GORdb) to facilitate more rapid access by the Clinical Sequence Analyzer (CSA) user interface, Sequence Miner, and the WuXi NextCODE Genome Browser.

2.2 | Result

2.2.1 | Biochemistry test

The tandem mass spectrometry results of the patient showed phenylalanine (Phe) 533.48 μmol/L (normally 20-120 μmol/L) and phenylalanine/tyrosine ratio 11.8 (normally 0.5-2.00), suggesting hyperphenylalaninemia.

The tandem mass spectrometry results of the older sister showed phenylalanine (Phe) 738.66 μM (normally 20-120 μmol/L) and phenylalanine/tyrosine ratio 12.8 (normally 0.5-2.00), suggesting hyperphenylalaninemia.

FIGURE 1 The sister and parents' PAH gene Sanger sequencing results.(As can be seen from the figure, the mutation gene of the sister was derived from the parental PAH mutant genes c.781C>T (p.R261RX) and c.1238G>C (p.R413RP), respectively)

FIGURE 2 Next-generation sequencing and QDPR Sanger sequencing results in the patient with DHPR deficiency. (A): Next-generation sequencing showed homozygous missense mutation c.508G>A in QDPR of the patient. B: Sanger sequencing traces confirmed the change c.508G>A, QDPR)

The basic urinary neopterin of the patient was 1.07 mmol/mol Cr (normally 0.29-2.61 mmol/mol Cr), biopterin was 3.12 mmol/mol Cr (normally 0.35-2.67 mmol/mol Cr).

The DHPR activity of this patient was 0.93 nmol/min./ (5 mmdisc) (normally 1.02-3.35 nmol/min./ (5 mmdisc)), which were 31.11% of normal control.

2.2.2 | Gene mutation analysis

Sanger sequencing of PAH gene in this patient was negative, but her sister was detected 2 heterozygous mutation c.781C>T and c.1238G>C on the two allele of PAH gene, which can lead to PAH deficiency. Further analysis of parental samples confirmed that they were heterozygous carriers of the two mutations, respectively (Figure 1). The next-generation sequencing identified a homozygous mutation c.508G>A in the two allele of quinoid dihydropteridine reductase (QDPR) gene (Figure 2), which can lead to BH₄ deficiency (DHPR deficiency). Sanger sequencing of QDPR gene in this patient validates the homozygous mutation of c.508G>A in QDPR (Figure 2).

Pedigree mutation sites and mutation analysis of the family are shown in Figure 3 and Table 1.

3 | DISCUSSION

Hyperphenylalaninemia (HPA) is caused by phenylalanine hydroxylase deficiency (PAHD) (98%) or BH₄ deficiency (2%).⁴ Internationally, PTPS deficiency is a major subset of cause (accounts

FIGURE 3 Segregation analysis of QDPR gene mutations and pedigree of the family examined in the present

TABLE 1 Gene mutations identified in this family

Family member	Gene	Base change	Amino acid	Mutation type	Exon	Zygosity	Inheritance	Source of variation
Patient	QDPR	c.508G>A	p.G170S	Missense	5	hom	AR	NS
Sister	PAH	c.781C>T	p.R261X	Nonsense	7	het	AR	Paternal
Sister	PAH	c.1238G>C	p.R413P	Missense	12	het	AR	Maternal

AR, autosomal recessive inheritance; Het: heterozygote; Hom, homozygous; NS, not search.

for 65%), followed by DHPR deficiency (accounts for 25%).⁵ GTPCH and PCD deficiencies are relatively uncommon, each accounting for approximately 4%.⁶ In the mainland of China, PTPS deficiency accounts for 96% and DHPR deficiency only accounts for 2.4%, so the incidence of DHPR in China was very low.^{7,8} In 1975, the first case of DHPR deficiency was reported by Butler.⁹ DHPR deficiency is a rare autosomal recessively inherited metabolic disorder which is caused by mutations in the *QDPR* gene. The human *QDPR* gene (OMIM 612676) on chromosome 4p15.3 consists of seven exons and encodes for a protein of 244 amino acids.¹⁰ To date, At least 35 different mutations are described in BIOMDB and HGMD.

Infants with BH₄ deficiency usually appear normal at birth, and untreated early infancy showed only an increase in blood Phe concentration. The impairment of nervous system function becomes apparent over time. Signs and symptoms of this condition include intellectual disability, microcephaly, mental and growth retardation, hypotonia, and epilepsy. Differential diagnosis between PAHD and BH₄ deficiencies is based on the analysis of pterin pattern in urine; DHPR activity in DBSs; analysis of biogenic amines, pterins and folates in cerebrospinal fluid (CSF); and BH₄ loading test. Molecular genetic analysis of causative genes is an ultimate diagnostic method. The patient should be treated with low-phenylalanine diet, BH₄ supplementation, folic acid, and correction of neurotransmitter homeostasis in the central nervous system (dopamine, serotonin). Early treatment can greatly improve the prognosis.¹¹

In this case of DHPR deficiency, white skin, hypotonia, serious nervous system dysfunction and other clinical manifestations are similar to previous reports of clinical manifestations of BH₄ deficiency. In the differential diagnosis, the blood Phe concentration was slightly elevated; urinary pterin analysis showed no significant abnormalities; DHPR activity was 31.11% of the normal enzyme activity. Lu et al previously reported DHPR activity decreased significantly—the lowest enzyme activity decreased to 3.13% of normal activity, up to normal activity of 13.67%.¹² Therefore, the DHPR enzyme activity of our case was not significantly reduced. Considering the enzyme activity test results are affected by many factors, the patient was diagnosed as DHPR deficiency until received the genetic testing. So the definite diagnosis depends on genetic testing.

The next-generation sequencing detected a homozygous mutation in the *QDPR* gene c.508G>A (p.G170S) in this child. The mutation was previously reported as a pathogenic mutation. In vitro experiments presumed that it could produce abnormal enzyme structure, increasing by four times the Km for NADH compared to the wild-type enzyme, and reduced DHPR mRNA expression.^{12,13}

Tracking the pedigree mutation spectrum, Sanger sequencing of the sister detected two complex *PAH* heterozygous mutations c.781C>T and c.1238G>C. Both the two variants have been reported as pathogenic variants in the *PAH* Consortium database and associated with a classic phenylketonuria (PKU) phenotype.¹⁴ The mutation c.1238G>C is the most common mutation in *PAH* deficiency in the Asian population and the Uygur of China.¹⁵ Sanger sequencing of the parents found that the two mutation sites of the sister can be detected, which in line with single-gene recessive inheritance.

Due to cognitive limitations of the parents and family economic reasons, the patient failed to timely diagnosis after birth, and parents refused further treatment for the patient, resulting in serious nervous system damage. At present, most patients routinely accept newborn screening, so that diagnosis and early treatment of HPA could be made within 1 month after birth. It is difficult to distinguish the patient's phenotype in clinical. Genetic testing of all HPA patients is essential for a clear diagnosis. Gene test of children with better control of blood Phe concentration and serious nervous system symptoms is strongly recommended for finding other types of HPA such as BH₄ deficiency.

In conclusion, this is the first reported case of Uighur BH₄ deficiency, which provides a reference for Uighur BH₄ gene mutation research in the future. For the differential diagnosis, genetic testing should be performed. To avoid any delay in diagnosis and treatment, it is important to combine Sanger sequencing and next-generation sequencing technology for confirming hereditary diseases. At present, more relevant disease research is still needed to provide a more scientific treatment programs for clinical practice.

ACKNOWLEDGMENTS

The authors thank the family for their participation.

ETHICAL APPROVAL

This study was approved by the Ethics Committee of People's Hospital of Xinjiang Uygur Autonomous Region (2017010).

AUTHOR CONTRIBUTIONS

Li L gave clinic information guidance. Qing YL and Su YJ wrote and revised the manuscript. Jiang HL, Liu YQ, and Ren Y collected the case data. Nuerya, Ayjamali, and Li MZ provided clinical genetic diagnosis. All authors read and approved the final manuscript.

ORCID

Yulan Qin <http://orcid.org/0000-0003-0112-1050>

REFERENCES

1. Blau N, Hennermann JB, Langenbeck U, Lichter-Konecki U. Diagnosis, classification, and genetics of phenylketonuria and tetrahydrobiopterin (BH4) deficiencies. *Mol Genet Metab*. 2011;104(suppl):S2-S9.
2. Werner ER, Blau N, Thöny B. Tetrahydrobiopterin: biochemistry and pathophysiology. *Biochem J*. 2011;438(3):397-414.
3. Foroozani H, Abiri M, Salehpour S, et al. Molecular characterization of QDPR gene in Iranian families with BH4 deficiency: reporting novel and recurrent mutations. *JIMD Rep*. 2015;21:123-130.
4. Yilmaz E, Cali F, Romano V, et al. Molecular basis of mild hyperphenylalaninaemia in Turkey. *J Inherit Metab Dis* 2000;23(5):523-527.
5. Blau N, Yue W, Perez B. [database on the Internet]. UK: Connecting rare disease researchers worldwide. C2006 - 2018 [cited 2018 Apr 5]. Available from: <http://www.biopku.org/home/biopku.asp>.
6. Blau N, Barnes I, Dhondt JL. International database of tetrahydrobiopterin deficiencies. *J Inherit Metab Dis*. 1996;19(1):8-14.
7. Gu X, Wang Z, Ye J, Han L, Qiu W. Newborn screening in China: phenylketonuria, congenital hypothyroidism and expanded screening. *Ann Acad Med Singapore*. 2008;37(12 Suppl):107-110.
8. Ye J, Yang YL, Yu WM, et al. Demographics, diagnosis and treatment of 256 patients with tetrahydrobiopterin deficiency in mainland China: results of a retrospective, multicentre study. *J Inherit Metab Dis*. 2013;36:893-901.
9. Kaufman S, Holtzman NA, Milstien S, Butler LJ, Krumholz A. Phenylketonuria due to a deficiency of dihydropteridine reductase. *N Engl J Med*. 1975;293(16):785-790.
10. Dianzani I, De SL, Smooker PM, et al. Dihydropteridine reductase deficiency: physical structure of the QDPR gene, identification of two new mutations and genotype-phenotype correlations. *Hum Mutat*. 1998;12(4):267-273.
11. Ponzone A, Spada M, Ferraris S, Dianzani I, de Sanctis L. Dihydropteridine reductase deficiency in man: from biology to treatment. *Med Res Rev*. 2004;24(2):127-150.
12. Lu DY, Ye J, Han LS, et al. QDPR, gene mutation and clinical follow-up in Chinese patients with dihydropteridine reductase deficiency. *World J Pediatr*. 2014;10(3):219-226.
13. Liu TT, Chiang SH, Wu SJ, Hsiao KJ. Tetrahydrobiopterin-deficient hyperphenylalaninemia in the Chinese. *Clin Chim Acta*. 2001;313(1-2):157-169.
14. Jeannessonthivisol E, Feillet F, Chéry C, et al. Genotype-phenotype associations in French patients with phenylketonuria and importance of genotype for full assessment of tetrahydrobiopterin responsiveness. *Orphanet J Rare Dis*. 2015;10:158-179.
15. Xuan-Lin FU, Wang R, Jiang HE, Yang X, Zhou HY, Wu-Zhong YU. Application of next-generation sequencing in detection of phenylketonuria gene mutation. *Chin J Birth Health Heredity*. 2017;5:11-13.

How to cite this article: Li L, Qin Y, Su Y, et al. Gene mutation and pedigree analysis of tetrahydrobiopterin deficiency in a Uyghur family of China. *J Clin Lab Anal*. 2019;33:e22665. <https://doi.org/10.1002/jcla.22665>