**QDPR gene mutation and clinical follow-up in Chinese patients with dihydropteridine reductase deficiency**

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**Background:** This study aimed to investigate the mutation spectrum of the QDPR gene, to determine the effect of mutations on dihydropteridine reductase (DHPR) structure/function, to discuss the potential genotype-phenotype correlation, and to evaluate the clinical outcome of Chinese patients after treatment.

**Methods:** Nine DHPR-deficient patients were enrolled in this study and seven of them underwent neonatal screening. QDPR gene mutations were analyzed and confirmed by routine methods. The potential pathogenicity of missense variants was analyzed using Clustal X, PolyPhen program and Swiss-PDB Viewer 4.04 OSX software, respectively. The clinical outcomes of the patients were evaluated after long-term treatment.

**Results:** In 10 mutations of the 9 patients, 4 were novel mutations (G20V, V86D, G130S and A175R), 4 were reported by us previously, and 2 known mutations were identified. R221X was a hotspot mutation (27.7%) in our patients. Eight missense mutations probably had damage to protein. Six patients in this series were treated with a good control of phenylalanine level. The height and weight of the patients were normal at the age of 4 months to 7.5 years. Four patients, who underwent a neonatal screening and were treated early, showed a normal mental development. In 2 patients diagnosed late, neurological symptoms were significantly improved.

**Conclusions:** The mutation spectrum of the QDPR gene is different in the Chinese population. Most mutations are related to severe phenotype. The determination of DHPR activity should be performed in patients with hyperphenylalaninemia. DHPR-deficient patients who were treated below the age of 2 months may have a near normal mental development.

**Key words:** dihydropteridine reductase; hyperphenylalaninaemia; tetrahydrobiopterin

**Introduction**

Hyperphenylalaninaemia (HPA) can arise from the deficiency of tetrahydrobiopterin (BH4), which is an obligate cofactor of a series of aromatic amino acid hydroxylases (phenylalanine, tyrosine and tryptophan hydroxylase), alkylglycerol mono-oxygenase (AGMO) and three NO synthase (NOS) isoenzymes.\(^1\) BH4 deficiency is caused by defects in one of enzymes responsible for its synthesis and recycling, of which 6-pyruvoyl-tetrahydropterin synthase (PTPS) deficiency is the most common cause (accounts for 56%) and dihydropteridine reductase (DHPR) deficiency (MIM 261630) is the second one (accounts for 32%) in the world. DHPR catalyzes the conversion of quinonoid dihydrobiopterin (qBH2) to 5,6,7,8-BH4 in a nicotinamide adenine dinucleotide (phosphate) [NAD(P)H]-dependent reaction involving in directing hydride transfer from the reduced nicotinamide to the quinoid dihydrobiopterin.\(^2-4\) Both NADPH and NADH are active and play a role as an electron donor, though NADH is the preferred cofactor.\(^5\) The main metabolic derangements caused by DHPR deficiency are HPA, impaired production of monoamine neurotransmitter derivatives of tyrosine and tryptophan, and lessened 5-methyltetrahydrofolic acid (5MTHF) in cerebrospinal fluid (CSF).\(^6\) Accordingly, the main clinical symptoms are dystonia, progressive microcephaly and growth retardation besides HPA-phenotype.

DHPR deficiency 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.\[7\] The active enzyme might be a homodimer with two bound NADH molecules per dimer.\[8\] In 1975, the first case of DHPR deficiency was reported.\[9\] To date, at least 35 different mutations are described in BIOMDB and HGMD (Fig. 1). However, there are only four Chinese DHPR deficient-patients from Taiwan registered in the BIODEF Database.

In the mainland of China, newborn screening for HPA was carried out in 1981 and differential diagnosis for BH4 deficiency was made by analysis of urine pterins until 2002, when DHPR activity determination was first performed in our laboratory. Since we reported the first case of DHPR deficiency in 2008,\[10\] PTPS deficiency has not been the only cause of BH4 deficiency in the mainland of China. According to a recent study of 256 Chinese patients with BH4 deficiency, PTPS deficiency accounts for 96% and DHPR deficiency only accounts for 2.4% in the mainland of China.\[11\]

So far, we have diagnosed 12 patients with DHPR deficiency. Although we have reported **QDPR** gene mutations of four patients before, we didn't analyze them further. First, this study aims to investigate **QDPR** mutation spectrum of Chinese patients, to analyze the effect of mutations on DHPR structure and function, and to discuss the potential correlation between genotype and phenotype. Second, it is to evaluate the clinical outcome of our patients after treatment.

**Methods**

**Patients**

Nine DHPR-deficient patients (6 males and 3 females), who had been diagnosed from 2007 to August 2013 in our clinic, were enrolled in this study after informed consent was obtained from their parents and the study was approved by the institutional ethical committee. All of the patients were born at full term after an uneventful pregnancy of non-consanguineous couples and about half of them were born in southern China.

Clinical and biochemical data of the 9 patients are summarized in Table 1. Seven patients (P1, P2, P3, P4, P5, P6, P9) were diagnosed as having HPA by neonatal screening and diagnosed with DHPR deficiency at median (Q1, Q3) age of 1.80 (1.67, 2.50) months by urinary pterin analysis using high performance liquid chromatography and DHPR activity determination using the method we described previously.\[12\] Three (P3, P4, P5) of the 9 patients had typical clinical features such as microcephaly, drowsiness, feeding difficulty, psychomotor milestone delay. Two patients (P7, P8) were diagnosed by high-risk screening. Patient 7 presented with yellow hair, microcephaly, dystonia, hyperspasms, motor delay and mental retardation at 5 months of age and was diagnosed with DHPR deficiency at 1.5 years old. Patient 8 presented with microcephaly, dystonia and delayed psychomotor milestone (e.g. poor head control) at 11 months old and was diagnosed with DHPR deficiency at the age of 1.25 years. Head circumferences at diagnosis were normal in four asymptomatic patients, but abnormal (≤P3) in five symptomatic patients of same age and sex.

![Fig. 1. Mutations listed in BIOMDB/HGMD and identified by the present study. Mutations listed in BIOMDB and HGMD (black), four novel mutations (red) and four mutations we reported previously (blue). ATG: initiation codon; TAG: stop codon.](image-url)
In this series, the median (Q1, Q3) blood Phe concentration was 750 (563.5, 1309.5) μmol/L. Two patients (P1, P9) presented elevated biopterin ratio. The medium (Q1, Q3) DHPR activity of all patients was significantly reduced to 0.23 (0.13-0.54) nmol/min per 5 mmdisc, which is equivalent to 6.00 (3.13, 13.67) % significantly reduced to 0.23 (0.13-0.54) nmol/min per 5 mmdisc of all patients was 0.55 (0.31, 1.91) nmol/min per 5 mmdisc.

Detection of gene mutation
Genomic DNA templates were extracted from peripheral blood leukocytes obtained from the patients and their parents as well as 50 healthy individuals using the TIANamp Blood DNA kit (TIANGEN Biotech, Beijing, China). The exonic and flanking intronic regions of the QDPR gene were amplified by polymerase chain reaction (PCR) and primers have been described previously.[10] Amplification products were separated and sequenced directly by an ABI 3700 sequencer (Applied Biosystems, Foster City, California, United States). Analyzed sequences were compared with cDNA and genomic DNA sequences in GenBank (accession number QDPR_NM_000320). Novel gene variants were confirmed after ruling out polymorphism by directly sequencing for their parents and 50 healthy individuals. Mutation nomenclature followed the recommendation of the Human Genome Variation Society (http://www.hgvs.org/mutnomen).[13]

Prediction of the potential pathogenic effect of missense mutations
Multiple sequence alignments were performed by Clustal X to investigate evolutionary conservation of amino acid residues across orthologous genes. PolyPhen program was conducted to predict the impact of missense variants on protein function (http://genetics.bwh.harvard.edu/pph2/). Protein plots were generated by a Swiss-PDB Viewer 4.0 using PDB entry code of 1hdr which was solved by X-ray diffraction at a resolution of 2.50 Å.[13]

Treatment
Our patients were treated with a low Phe formula or BH4 (10-15 mg/kg,d), combined with neurotransmitter precursors L-dopa/cabidopa and 5-hydroxytryptophan (5-HTP). The starting doses of L-dopa and 5-HTP were 1 mg/kg per day, with an increase of 1 mg/kg every 5-7 days as required to achieve target concentrations for the corresponding age[16] and adjusted according to clinical symptoms and blood prolactin concentration which was often elevated in DHPR-deficient patients due to a low concentration of dopamine. Folinic acid (15 mg/d) was supplied as well and anti-folic drugs had to be avoided in such patients.[17]

Follow-up
Blood Phe concentration was monitored every week to once a month. The physical development (height, weight and head circumference) and mental development

Table 1. Characteristics of clinical and biochemical data in DHPR-deficient patients

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Sex</th>
<th>Age at diagnosis</th>
<th>Clinical symptoms</th>
<th>Head circumference</th>
<th>Blood Phe levels (μmol/L)</th>
<th>Urinary pterins (mmol/mol Cr)</th>
<th>Biopterin (μmol/L)</th>
<th>NEOPT (μmol/L)</th>
<th>Relative to normal (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>2.5 mon</td>
<td>No</td>
<td>Yes</td>
<td>1200   750 1680 603 1419 520</td>
<td>11.04 9.22 4.15 8.60 1.91 6.90</td>
<td>1.31 2.78 1.37 3.48 1.16 2.79</td>
<td>54.66 23.29 24.80 28.80 37.70 28.81</td>
<td>3.25 126 600 600 527 1020</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>50 d</td>
<td>Yes</td>
<td>Yes</td>
<td>0 h   750 972 585 - - -</td>
<td>0.53 0.13 0.31 0.23 0.07 0.22</td>
<td>0.53 0.13 0.31 0.23 0.07 0.22</td>
<td>325 282 682 72 - -</td>
<td>284 395 - - -</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>54 d</td>
<td>Yes</td>
<td>No</td>
<td>2 h   450 837 362 - - -</td>
<td>1.16 4.23 0.12 0.55 0.64 0.67</td>
<td>0.53 0.13 0.31 0.23 0.07 0.22</td>
<td>325 282 682 72 - -</td>
<td>284 395 - - -</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>5 mon</td>
<td>No</td>
<td>No</td>
<td>4 h   750 972 585 - - -</td>
<td>0.53 0.13 0.31 0.23 0.07 0.22</td>
<td>0.53 0.13 0.31 0.23 0.07 0.22</td>
<td>325 282 682 72 - -</td>
<td>284 395 - - -</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>71 d</td>
<td>Yes</td>
<td>Yes</td>
<td>6 h   750 972 585 - - -</td>
<td>0.53 0.13 0.31 0.23 0.07 0.22</td>
<td>0.53 0.13 0.31 0.23 0.07 0.22</td>
<td>325 282 682 72 - -</td>
<td>284 395 - - -</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>1.5 y</td>
<td>Yes</td>
<td>No</td>
<td>8 h   450 837 362 - - -</td>
<td>0.53 0.13 0.31 0.23 0.07 0.22</td>
<td>0.53 0.13 0.31 0.23 0.07 0.22</td>
<td>325 282 682 72 - -</td>
<td>284 395 - - -</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>1.25 y</td>
<td>Yes</td>
<td>Yes</td>
<td>24 h 750 972 585 - - -</td>
<td>0.53 0.13 0.31 0.23 0.07 0.22</td>
<td>0.53 0.13 0.31 0.23 0.07 0.22</td>
<td>325 282 682 72 - -</td>
<td>284 395 - - -</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>1 mon</td>
<td>Yes</td>
<td>No</td>
<td>- - -</td>
<td>- - -</td>
<td>- - -</td>
<td>- - -</td>
<td>- - -</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>1 mon</td>
<td>Yes</td>
<td>Yes</td>
<td>- - -</td>
<td>- - -</td>
<td>- - -</td>
<td>- - -</td>
<td>- - -</td>
</tr>
</tbody>
</table>

*: The percentile of head circumference compared to healthy children for the same age and sex in China; †: normal reference: (0.29-7.49) nmol/min per 5 mmdisc; ‡: normal reference: neopterin: (0.29-7.49) nmol/min per 5 mmdisc; -: not detected; DHPR: dihydropteridine reductase; BH4: tetrahydrobiopterin; Cr: creatinine; M: male; F: female.
were followed up for all treated patients every 3-6 months during the treatment. Physical development was assessed using the infant Gesell developmental quotient (DQ)\(^{[18]}\) (normal score > 85) in children under 3 years old. Magnetic resonance imaging (MRI) was performed for some patients. Liver and kidney function test, blood and urine routine examinations were conducted routinely to monitor the adverse effect of drugs.

**Results**

**Gene mutation detection**

Ten mutations including 9 missense mutations and 1 deletion mutation were identified in the 9 patients with a detection rate of 100%. Four mutations [c.388G>A (p.G130S), c.59G>T (p.G20V), c.257T>A (p.V86D), c.523GC>AG (p.A175R)] were novel. Four mutations [c.515C>T (p.P172L), c.311G>A (p.C104Y), c.630-3delC (IVS6-3delC), c.451G>C (p.G151R)] were reported by our team previously.\(^{[10,11]}\) Two mutations [c.661C>T (p.R221X), c.508G>A (p.G170S)] were reported by others (Fig. 1). Six patients (66.67%) were homozygous. In this study, the nonsense R221X mutation was a hotspot mutation with a frequency of 27.8% (5/18). The polymorphisms of novel variants were excluded.

**Prediction of the potential pathogenic effect of missense mutations**

The conservativeness of amino acid residues and the effect of the mutations on protein function are shown in Table 2. Seven amino acid residues were highly conserved, and two were relatively conserved. Eight missense mutations were predicted "probably" or "possibly" to damage protein function, while C104Y was predicted as a benign mutation.

Human DHPR crystallographic structure seen with a Swiss-PDB Viewer 4.04 showed that the overall structure of human DHPR (Fig. 2) is a α/β protein with a central twisted β-sheet flanked on each side by a layer of α-helices and that the β-sheet has seven parallel strands and a single antiparallel strand at one edge leading to the C-terminus of the protein.\(^{[8]}\) DHPR structure altered by eight missense mutations was analyzed (Fig. 3). The results were as follows: 1) The wide type residue G20 is located in the NAD(P)H binding site. Four hydrogen bonds are formed from G20, two of which are formed with S24 and another two formed with G17 and G23, respectively. However, the mutated V20 residue (G20V) engenders clashes with NAD(P)H, thus easily affecting the interactions with the nucleotide (Fig. 3A). 2) The wide type residue V86 forms one hydrogen bond with Y16, while the mutated D86 residue (V86D), an acidic amino acid carrying negative charge, produces a new H-bond with A87 (Fig. 3B). 3) The wide-type residue C104, located in the αE helix, is buried in the core of the protein. Four hydrogen bonds are formed between C104 and L100 and G108, whereas the mutated Y104 (C104Y) loses one H-bond with F101 and forms clashes with L100 (Fig. 3C). 4) The wild-type residue G130 is located in β-strand between αE

![Fig. 2. 3D-structure of dihydropteridine reductase. The homodimer is presented in yellow (α subunit) and blue (β subunit), respectively. Seven missense mutations we reported (purplish red spheres), two known mutations (brown spheres) and NAD(P)H (red) are shown.](image-url)

**Table 2. Predicted effect of 10 different mutations of QDPR gene detected in 9 Chinese DHPR-deficient patients**

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Exon</th>
<th>Mutation at nucleotide level</th>
<th>Change at protein level</th>
<th>Predicted protein domain</th>
<th>Conservation</th>
<th>PolyPhen prediction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>c.311G&gt;A</td>
<td>C104Y</td>
<td>αE involved in dimerization</td>
<td>Relatively conserved</td>
<td>Benign</td>
</tr>
<tr>
<td>2'</td>
<td>7</td>
<td>c.630-3delC</td>
<td>IVS6-3delC</td>
<td>Next to the acceptor of splicing site in intron 6</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>2'</td>
<td>4</td>
<td>c.388G&gt;A</td>
<td>G130S</td>
<td>β-strand between αE and αF</td>
<td>Highly conserved</td>
<td>Probably damaging</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>c.59G&gt;T</td>
<td>G20V</td>
<td>Nucleotide binding site</td>
<td>Highly conserved</td>
<td>Probably damaging</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>c.451G&gt;C</td>
<td>G151R</td>
<td>Tyr-(Xaa)3-Lys motif</td>
<td>Highly conserved</td>
<td>Probably damaging</td>
</tr>
<tr>
<td>5, 7, 9</td>
<td>7</td>
<td>c.661C&gt;T</td>
<td>R221X</td>
<td>Probable truncated protein</td>
<td>Highly conserved</td>
<td>Probably damaging</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>c.257T&gt;A</td>
<td>V86D</td>
<td>In an analogous fragment of DHFR</td>
<td>Highly conserved</td>
<td>Probably damaging</td>
</tr>
<tr>
<td>7'</td>
<td>5</td>
<td>c.515C&gt;T</td>
<td>P172L</td>
<td>αF-βF interface</td>
<td>Highly conserved</td>
<td>Probably/possibly damaging</td>
</tr>
<tr>
<td>8'</td>
<td>5</td>
<td>c.508G&gt;A</td>
<td>G170S</td>
<td>αF-βF interface</td>
<td>Highly conserved</td>
<td>Probably damaging</td>
</tr>
<tr>
<td>8'</td>
<td>5</td>
<td>c.523GC&gt;AG</td>
<td>A175R</td>
<td>αF-βF interface</td>
<td>Relatively conserved</td>
<td>Possibly damaging</td>
</tr>
</tbody>
</table>

*: patients with compound-heterozygous mutations. NA: not analyzed; DHFR: dihydrofolate reductase.
and αF. Two hydrogen bonds are formed between G130 with K127 and A176, whereas the mutated S130 residue (G130S) forms two new hydrogen bonds with D81 and V80 respectively (Fig. 3 D). 5) The wild-type residue G151 is located very close to the active site (G150). G151-α subunit forms two hydrogen bonds with M147-α subunit and G155-α subunit, whereas the mutated R151 (G151R), an alkaline amino acid carrying positive charge, forms a new hydrogen bond with L160-β subunit and produces clashes with T144-α subunit and S163-β subunit. This mutation may affect the function of the protein by changing the local structure surrounding the active site (Fig. 3 E). 6) The highly conserved wild-type residue G170 is located on the αF-βF interface and only glycine is flexible enough to make the unusual torsion angle. The mutated S170-α subunit (G170S) forms two new H-bonds with A95-β subunit and K96-β subunit, which will force the local backbone into an incorrect conformation (Fig. 3F). 7) The wild-type residue at codon 172 is a rigid proline inducing a special backbone conformation, which may be required at this position. P172 forms one H-bond with A175, but the P172L substitution produces a clash with L126 (Fig. 3G). 8) The wild-type residue A175, which is located on the αF-βF interface as G170 and P172, forms one H-bond with P172. The mutant R175 (A175R) introduces positive charge at this position and is less hydrophobic, inducing a new H-bond with M171 (Fig. 3H).

**Treatment and follow-up**

Six of the 9 patients (P1, P2, P6, P7, P8, and P9) received formal therapy after diagnosis at median (Q1, Q3) age of 2.15 (1.5, 16) months. Two of them (P2, P7) were treated with BH4 (10-15 mg/kg per day), but patient 7 switched to a low Phe diet after BH4 therapy for one year due to economic problem. The other four patients received a phenylalanine-restricted diet. All of them also received neurotransmitter precursors L-dopa, 5-hydroxytryptophan (5-HTP) substitution and folinic acid (15 mg/day). The maximum dosages of L-dopa and 5-HTP were 14 mg/kg per day and 8 mg/kg per day, respectively. Three patients (P3, P4, P5) gave up treatment because of heavy economy burdens or poor

![Fig. 3. 3D structures of dihydropteridine reductase bearing wild-type (purplish red) and eight missense mutations (green). A: Gly20Val (G 20V); B: Val86Asp (V86D); C: Cys104Tyr (C104Y); D: Gly130Ser (G130S); E: Gly151Arg (G151R); F: Gly170Ser (G170S); G: Pro172Leu (P172L); H: Ala175Arg (A175R). NAD(P)H (red); hydrogen bond (green dotted line); clash (pink dotted line); missing hydrogen bond (grey dotted line).](image-url)
compliance for treatment.

The median (Q1, Q3) age was 3 (1.27, 6) years old in 6 treated patients at the last visit. Blood Phe concentration had been controlled within target concentration during the treatment. Two patients (P7, P8) diagnosed late showed marked improvement of neurological symptoms. In patient 7, the frequency of hyperspasms decreased gradually and disappeared after 6 months' treatment. The great incensement of muscle strength and considerable progress in the physical and mental development were also observed. His DQ score was 95 at the age of 5.5 years, but language development was delayed obviously. At age of 7.5 years old, he was subjected to education in kindergarten. Patient 8, a female, presented poor head control and hypotonia in the lower limbs before treatment at the age of 1.25 years. After 6-month treatment, these symptoms were improved greatly and she could walk alone at the age of 1.75 years. Four patients (P1, P2, P6, P9) who underwent neonatal screening and received formal treatment at the age of 2.5 months were asymptomatic with normal mental development. DQ score was 106 at the age of 7 months in patient 1 and 102 at 2.67 years in patient 2. The physical development of all patients including height and weight were within normal ranges (P25-P90) compared with healthy children of same age and sex. Brain MRI/CT scans showed normal results in four patients (P5, P6, P7, P8).

Some patients suffered from diarrhea due to the side effect of 5-HTP at the beginning of the treatment. By the end of the follow-up, we did not find any adverse effects of these agents on liver and kidney function. The results of blood and urine examinations were also normal.

Discussion

Hyperphenylalaninemia is caused by phenylalanine hydroxylase (PAH) deficiency (98%) or by BH4 defects (2%). In the mainland of China, the overall incidence of HPA is 1:11 763 and BH4 deficiency accounts for 12.9% of HPA cases in Shanghai. The incidence of BH4 deficiency is higher in southern than in northern Chinese. DHPR-deficient patient had a higher level of urinary bipterin, but only two patients were consistent with some of those, particularly newborns of breast feeding, had a normal profile of urinary pterin. This finding suggested that DHPR deficiency could not be diagnosed by urinary pterin alone. Since the incidence of DHPR deficiency is very low in China, more patients will be diagnosed when DHPR activity determination is used in differential diagnosis of HPA.

To present, at least 35 mutations spreading over the QDPR gene have been described. Common mutations are associated with different ethnic background. R221X and G23D are found to be common in patients of Mediterranean origin. G170S mutation was identified in two Chinese siblings of a consanguineous marriage and also in one of our patients. In the present study, the 9 mutations were not found in other ethnic populations, suggesting Chinese patients have different spectrum of QDPR gene mutation. We analyzed the conservativeness of amino acid replacement and predicted potential pathogenic effect on DHPR structure/function. However, the reference structure was solved at a resolution of 2.5 Å, which is limited to predict the structural effects of mutations. These changes couldn't be found before the appearance of crystal structures of specific mutations.

The combination therapy of low phenylalanine diet/BH4, neurotransmitter precursors and folic acid is very effective for DHPR-deficient patients. In our patients, those who were treated early had normal physical development and better intelligence scores. However, in those who were diagnosed late, mental retardation was still noted although their clinical symptoms were remarkably improved. Supplementary folic acid is essential to the treatment of the patients. However, BH4 substitution in DHPR-deficient patients is not recommended because such patients should be treated with a large dose of BH4 (8-20 mg/kg) to control their Phe levels, which result in accumulation of BH2. BH2 can induce inhibitory effects on aromatic amino acid hydroxylases and neuronal NOS. The side effects of BH4 include headache, throat pain, diarrhea, abdominal pain, etc. Therefore, HPA has to be managed by diet in DHPR-deficient patients.

The levels of 5-hydroxyindoleacetic acid and homovanillic acid in CSF are indicative of severe or mild forms of BH4 deficiency, especially in patients without neurological symptoms. Severe patients usually present dystonia and mental retardation, whereas mild patients have no neurologic symptoms. However, the measurement of neurotransmitters metabolic products in CSF was not performed in the mainland of China, and most patients underwent neonatal screening and were treated immediately after differential diagnosis within two months after birth. Hence the clinical symptoms of these patients were rarely seen. In addition, the difference in DHPR activities of our patients was not related to their genotype because patients 5 and 9 were homozygous for R221X mutation, but their activities were minimal and maximal in the 9 patients, respectively. Thus it is difficult to distinguish the phenotypes (severe vs. mild) of the asymptomatic patients. But it is clear if the patient presents typical neurological symptoms before treatment and the
corresponding genotype is not mild.

In our series, three patients (P2, P7, P8) had heterozygous mutations: 1) Patient 2 is a compound heterozygote of G130S/IVS6-3delC. It is difficult to predict the phenotype related to these mutations because good clinical outcome is based on early diagnosis and treatment at 50 days after birth. Some mutations within introns may induce aberrant splicing and create or activate novel splice sites, resulting in inappropriate inclusion of intronic sequences.\[30,31\] We attempt to do mRNA analysis to find whether IVS6-3delC can influence the splicing sites, but the parents refused. 2) Patients 7 and 8 who were diagnosed late and presented with severe neurological symptoms carried compound heterozygote of P172L/R221X and G170S/A175R, respectively. The non-missense mutation (R221X) is related to a severe form.\[32\] The G170S mutation can increase the Km for NADH by four times compared to the wild type enzyme.\[26\] As a whole, the four mutations are related to severe phenotype.

Another six patients had homozygous mutations: 1) Patient 1 carried a homozygous mutation (C104Y), which is located the inαE encoded by codons 100-124 involving in dimerization.\[15,33\] PolyPhen analysis showed C104Y was benign. Patient 1 was asymptomatic with normal head circumference (P75) at diagnosis (2.5 months), whereas severe patients without treatment presented clinical signs at 2 months after birth. At last visit, the patient was accepted into a kindergarten with normal physical and mental development. Hence C104Y mutation may be related to a mild phenotype. 2) Patient 3 was a homozygous of G20V. Glycine 20 is characterized by a NADH binding fold, named the β-α-β-fold or "Rossmann fold",\[19\] which contains three highly conserved glycines in the order of Gly(18)-X-Gly(20)-X-X-Gly(23) in DHPR. G20 at this position is significant for NAD(P)H to be bound without intervention from an amino acid side chain. Patient 3 had classic manifestations with severe microcephaly at the age of 2 months. We consider G20V mutation may have a dramatic steric effect on NADH binding and result in severe phenotype. 3) Patient 4 was homozygous for G151R. The residue glycine 151 is located in a Tyr(150)-(Xaa)3-Lys(154)-containing motif, which is important to the proton transfer that may initiate or complete substrate reduction.\[4\] This patient presented delayed psychomotor milestones and obviously delayed language development. We estimate that G151R mutation may affect the catalytic reductive mechanism and result in severe phenotype. 4) Patient 6 was homozygous for V86D. This patient underwent prenatal screening and received formal therapy within 2 months. She had no symptoms at the last follow-up. There is no direct evidence to predict the severity of induced disease, resulting from this mutation. 5) Two patients (P5, P9) were homozygous for R221X, as mentioned above.

In conclusion, Chinese patients have QDPR gene mutations different from other ethnics. R221X is also a mutation in Chinese patients and should be confirmed by further investigation. Six mutations including G20V, G151R, G170S, P172L, A175R, and R221X were related to severe phenotype, and one mutation (C104Y) was related to mild phenotype. Further research into QDPR gene expression is required. The results of this study suggest that DHPR-deficient patients, who have undergone neonatal screening and been treated within 2 months, have a near normal mental development.

**Funding:** This study was supported by grants from the Major Program of Shanghai Committee on Science and Technology (11dz1950300) and the National Key Technology R&D Program (2012BAI09B04).

**Ethical approval:** This study was approved by the Ethics Committee of Xinhua Hospital (XHEC-D-2012-042).

**Competing interest:** All authors declare no conflict of interest.

**Contributors:** Lu DY and Ye J conceived and designed this study. Lu DY did data analysis and wrote the draft. Ye J revised the manuscript and acted as the guarantor of the study. Han LS, Qiu WJ, Zhang HW, and Gu XF reviewed critically the article. Zhou JD, Bao PZ, and Zhang YF did laboratory work (PCR, sequencing, dihydropteridine reductase activity determination). All authors approved the final version of the article for publication.

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Received October 12, 2013
Accepted after revision January 10, 2014