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RESEARCH REPORT

Molecular Characterization of *QDPR* Gene in Iranian Families with BH4 Deficiency: Reporting Novel and Recurrent Mutations

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Abstract Newborn screening for PKU has been in practice in Iran since 2007. Some hyperphenylalaninemia cases have tetrahydrobiopterin (BH4) biosynthesis deficiency/disorder. Several genes including *QDPR* (encodes DHPR enzyme, the necessary cofactor for PAH activity) have been associated with the BH4. Mutations have been previously described in the *QDPR* gene. The incidence of BH4 deficiency is expected to be higher in Iran due to high rate of consanguineous marriages.

We identified a total of 93 BH4-deficient families. A multiplex set of STR markers linked to 4 genes responsible for the BH4 deficiency (i.e., *GCH1*, *PCBD1*, *PTS,* and *QDPR* genes) was used to quickly determine which gene may be responsible to cause the disease. Mutation analysis of *QDPR* gene revealed some known and novel mutations. Our findings show that no common mutation predominates, and they are scattered in the gene in our population.

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Introduction

Hyperphenylalaninemia is presented either as a result of phenylalanine hydroxylase (PAH, EC 1.14.16.1) deficiency or rarely as a form of tetrahydrobiopterin (BH_4) deficiency. It is estimated that the overall prevalence of BH4 deficiencies is about 1 in 10^6 live births in the western population, but this estimation is higher in some Mediterranean countries (Blau et al. 2001). PAH is a hepatic enzyme that needs the BH4 as a necessary cofactor for proper functioning.

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BH4 is also an essential cofactor for tyrosine and tryptophan hydroxylases, the critical enzyme in monoamine neurotransmitters (Wang et al. 2012). So BH4 deficiency can also leads to defect in neurotransmitters. As a result, BH4-deficient patients show neurological deterioration when they are only treated with phenyl restricted diet (Wang et al. 2006). So the differential diagnosis of the BH4 deficiency from phenylketonuria (PKU, MIM 261600) is very essential and should be initiated as soon as possible to reduce neurological impairment in the affected individuals.

BH4 deficiencies include heterogeneous disorders with several genes involved in the etiology of the disease. BH4 is synthesized from guanosine triphosphate (GTP) by GTP cyclohydrolase 1 (GTPCH; EC 3.5.4.16), sepiapterin reductase (SR; EC 1.1.1.153), and 6-pyruvoyl-tetrahydropterin synthase (PTPS; EC 4.3.2.12) and recycled from pterin-4-α carbinolamin by pterin-4-α-carbinolamin dehydrogenase (PCD; EC 4.2.1.96) and dihydropteridine reductase (DHPR; EC 1.6.99.7) enzymes (Blau et al. 2005). Therefore, five different genes have been identified in the synthesis or recycling of BH4.

DHPR deficiency is one form of BH4 deficiency. This deficiency is presented by psychomotor retardation, myoclonic epilepsy, microcephaly, febrile attacks, hypertonia of the trunk with limb hypertonia, and fatal course because of neurotransmitters. Patients also show defective folate metabolism which recuperate by phe-restricted diet. Diagnosis is confirmed by measuring pterins and neurotransmitter metabolites in urine and cerebrospinal fluid. Measurement of the DHPR enzyme activity in dried blood spot or fibroblast is also possible (Smith and Brenton 1996).

The mode of inheritance of the DHPR deficiency is autosomal recessive. Therefore, a higher number of patients are expected in the countries with high rate of consanguineous marriage. In this study, 93 BH4-deficient families were studied. All participants were subjected to homozygosity mapping by linked STR markers. Twenty-four families were homozygous for STR markers linked to *QDPR* locus. Full gene sequencing was done for all 24 samples.

Materials and Methods

Sample Criteria

The samples were collected from families with hyperphenylalaninemia referred to Kawsar Human Genetic Research Center and Department of Biochemistry, Pasteur Institute of Iran since 2008.

Inclusion criteria were having at least two children (either both or one being affected) born to consanguineous parents or both parents coming from the same small village and also showing autosomal recessive mode of inheritance. All families completed a questionnaire and signed informed consent.

Differential diagnosis between PAH- and BH4-deficient patients was performed according to the published diagnostic criteria (Blau et al. 2011).

Molecular Genetic Studies

Five milliliters of peripheral blood was collected from each patient, parents, and relatives (if needed) in EDTA. DNA was extracted from peripheral blood for all participants using salting-out method (Miller et al. 1988). Homozygosity mapping by the STR markers flanking the *QDPR* gene was conducted to indirectly track the probable mutated gene. Table 1 shows the STR markers used in this study and their locations, for each gene. Primer sequences are available upon request.

We carried out direct sequencing of *QDPR* gene by using specific primers amplifying seven exons and the exon/intron boundaries (Table 2).

PCR amplification was performed in a 25 μL reactions; briefly, 1.7 μL of AMS 10X buffer (Cinnagen, Tehran, Iran), 1U Taq DNA polymerase (Kawsar Biotech Co., KBC, Tehran, Iran; KBC) 0.24 μM of each primer, 0.66 μL of $MgCl_2$ (100 mM), 0.4 μL dNTP (40 mM), 12.3 μL ddH_2O, and 1.66 DMSO μL (Sigma, USA) were mixed. The PCR conditions included an initial denaturation step for 5 min at 95°C, 1 min at 95°C, 1 min at 64°C (except for exon 1 at 62°C), and 1 min in 72°C and a final extension for 10 min at 72°C for 28–30 cycles.

PCR products were directly sequenced using BigDye Terminator kit (Thermo Fisher Scientific, Life Technologies, USA, TS) according to manufacture protocol, and the samples were run on an ABI3130XL Genetic Analyzer at KBC facility. Sequences were compared with human genomic and cDNA of the *QDPR* gene, and variations were checked with NCBI reference sequence (NC_000004.12).

Result

From 93 investigated families, 24 affected probands showed homozygosity to all STR markers linked to the *QDPR* gene. This suggested high probability of disease segregation with the *QDPR* gene. All seven exons and exon/intron boundaries of the gene in the probands were sequenced, and detected mutations were confirmed in the parents. We identified different new and previously reported mutations in the *QDPR* gene (Tables 3 and 4).

Sixteen different types of mutations were found. Ten of them had not been reported previously. Most of the mutations

Table 1 STR markers used this study

STR marker	Location	Repeated seq	Length of PCR product
D4QDPRSD0. 2	Chr4:17464963–17465289	TATC	327
D4QDPRSD2	Chr4:17281517–17281740	CTAT	224
D4SQDPRSD9. 6	Chr4:16519399–16519589	ATCC	191
D4QDPRSD10	Chr4:16483281–16483592	TAGA	312
D4QDPRSU13	Chr4:18845242–18845393	GATA	129–162
D4QDPRSU17	Chr4:19234187–19234517	TATAGA	314–342

Table 2 List of QDPR exon amplification primers and product sizes (bp)

Exon number	Primer sequences	Primer length (bp)	Product size (bp)
1	F: 5′-TTACACTTCACAAATTAATGCTCGT-3′ R: 5′-AAACAGGAATAGACGCGTAGACC-3′	25 23	605
2	F: 5′-CCCTCATTTCTATGTGTGACTCTT-3′ R: 5′-CAAAGGAAGAACATACAGCCAG-3′	24 24	247
3	F: 5′-TCTTCCGTCTAATTCTCAAAGC-3′ R: 5′-GTGTATATCCCGGAATCTTTACA-3′	22 23	363
4	F: 5′-TGTGCTGTTTGTGTTAGACCTTG-3′ R: 5′-ATCTATCTGTTAAGCAGCTTAGAGG-3′	23 25	408
5	F: 5′-GAGGAGGCCAGATGCAGCTA-3′ R: 5′-GTGGAAAGCTACAGTCAGACAAAC-3′	20 24	315
6	F: 5′-GTGCCAGAGGCTCTAGGTTGTC-3′ R: 5′-CGGAATCTCAGAGTAGCTGGACT-3′	22 23	377
7	F: 5′-GTGCCAGAGGCTCTAGGTTGTC-3′ R: 5′-AGTTAACAGAGATCAACGGATGC-3′	22 23	412

Table 3 Types of mutation, amino acids, and number seen for previously reported mutation (NM_000320)

Codon and nucleotide changes	c.DNA change	Amino acid change	No. seen	Genotype	References
Cd18GGC>GAC	c.[233G>A];[233G>A]	p. [(Gly18Asp)]	4	Homozygous	Scrutton et al. (1990)
Cd 23GGT>GAT	c.[248G>A];[248G>A]	p. [(Gly23Asp)]	1	Homozygous	Romstad et al. (2000)
Cd150TAC>TGC	c.[629A>G];[629A>G]	p. [(Tyr150Cys)]	3	Homozygous	(Dianzani et al. 1993)
Cd221CGA>TGA	c.[661C>T];[661C>T]	*p.(R221*)*	2	Homozygous	Smooker and Cotton (1995)
Cd158CAC>TAC	c.[652C>T];[652C>T]	p. [(His158Tyr)]	1	Homozygous	Smooker et al. (1993)
Cd97DelC	c.472delC	–	1	Homozygous	Romstad et al. (2000)

were missense mutations and only two were homozygous deletion of a nucleotide. We also found two nonsense mutations. From 12 missense mutations, two would cause termination of codon. Two different types of mutations were seen for codon 115 (exon 4). One creates a termination codon and the other causes substitution of leucine to serine. p. [(Gly18Asp)] was found to be the most common mutation which was seen in four different families. Types and frequencies of mutations are shown in Tables 3 and 4.

The pathogenicity of identified mutations were checked in related websites such as Polyphen-2 (Adzhubei et al. 2010), SIFT (Kumar et al. 2009), and also HOPE project (Venselaar et al. 2010). Results and suggested effects are shown in Table 4.

Table 4 Novel mutations identified in this study and related information (NM_000320)

Codon and nucleotide changes	Type of mutation	Exon	Amino acid change	cDNA change	No. seen	SIFT suggestion	Polyphen suggestion
Cd115 TCG>TAG	Nonsense	4	*p.(S115*)*	c.[344C>A]; [344C>A]	2	Tolerated	–
Cd17 GGC>TGC	Missense	1	p. [(Gly17Cys)]	c.[49G>T]; [49G>T]	1	Damaging	Probably damaging sensitivity: 0.00; specificity: 1.00
Cd217 del A	Deletion	7	–	c.649 del A	1	–	–
Cd185 CTG>CCG	Missense	6	p. [(Leu185Pro)]	c.[554 T>C]; [554 T>C]	1	Damaging	Probably damaging sensitivity: 0.00; specificity: 1.00
Cd89 GGA>AGA	Missense	3	p. [(Gly89Arg)]	c.[265G>A]; [265G>A]	1	Damaging	Probably damaging sensitivity: 0.00; specificity: 1.00
Cd 115 TCG>TTG	Missense	4	p. [(Ser 115Leu)]	c.[344C>T]; [344C>T]	1	Tolerated	Probably damaging sensitivity: 0.00; specificity: 1.00
Cd225 GGA>AGA	Missense	7	p. [(Gly225Arg)]	c.[673G>A]; [673G>A]	1	Damaging	Probably damaging sensitivity: 0.00; specificity: 1.00
Cd 237 ACG>ATG	Missense	7	p. [(Thr237Met)]	c.[710C>T]; [710C>T]	1	Damaging	Probably damaging sensitivity: 0.00; specificity: 1.00
Cd 114 ACA>ATA	Missense	4	p. [(Thr114Ile)]	c.[341C>T]; [341C>T]	2	Damaging Score: 0.02	Probably damaging score: 0.998; sensitivity: 0.27; specificity: 0.99
Cd 163 AGC>AAC	Missense	5	p. [(Ser163Asn)]	c.[488G>A]; [488G>A]	1	Damaging Score: 0.01	Possibly damaging score: 0.885 sensitivity: 0.82; specificity: 0.94

Discussion

Frequency of PKU in Iran has been estimated to be about one per 5000 live births (Setoodeh et al. 2015). The above study and also data obtained at the molecular level show that approximately 3–5 of the hyperphenylalaninemia cases ended up being BH4. This number is about 3–5 per 100,000 live births. This number is higher than 1 in 10^6 reported in some Mediterranean countries (Blau et al. 2001).

We found that most of the mutations (10 out of 16) were novel, perhaps because of disease rarity in other populations: eight missense, one nonsense, and one single nucleotide deletion mutations. The *p. (S115*)* mutation changes serine to a stop codon in exon 4. This causes a truncated protein that lacks the active site of the enzyme. The active site of the enzyme, which is also a proton acceptor site, is situated on the 150th amino acid. As a result of this mutation, protein synthesis stops before reaching the active site. It can be postulated that the enzyme should have no or very little activity. However, SIFT predicted that this mutation can be tolerated. However, we can argue that the phenotype of the affected child is more aligned with our claim.

DHPR has a NADH binding fold at the N-terminus formed by two β-sheets and an α-helix, named the 13c43-fold or the "Rossmann fold" (Scrutton et al. 1990). Within this fold, there is a motif that contains three highly conserved glycine residues in the order of Gly-X-Gly-X-X-GIy. These glycines are positioned in codons 18, 20, and 23. Two mutations have already been reported in these codons (Romstad et al. 2000), and we found them again in five Iranian families. We found a novel mutation in codon 17: p. [(Gly17Cys)] (Table 4). This mutation substitutes a hydrophilic amino acid cysteine with neutral glycine. This residue is 100% conserved, and its substitution can disturb the multimeric interactions and affect the catalytic activity. Therefore, this change has a high potency for being pathogenic. The result of Polyphen2 and SIFT also confirms this (Kumar et al. 2009; Adzhubei et al. 2010; Venselaar et al. 2010).

Substitution of p. [(Gly23Asp)] (Table 3) has been found to be a frequent mutation in patients of Mediterranean origin (Smooker et al. 1993). We did not see this mutation. However, we observed that p. [(Gly18Asp)] mutation was more frequent in the Iranian population. The mentioned mutation had been reported in a Turkish patient with severe phenotype (Romstad et al. 2000).

Four amino acid substitutions, namely, p. [(Gly17Cys)], p. [(Leu185Pro)], p. [(Gly225Arg)], and p. [(Thr237Met)], are potentially pathogenic since they are not present in the single nucleotide polymorphism database (Exome Variant Server, http://evs.gs.washington.edu/EVS/). This was supported by bioinformatics predictions by Polyphen-2 (Adzhubei et al. 2010) which indicated that all of these are

Table 5 Distribution of different abnormalities in PTPS- and DHPR-deficient patients

	Mean age at diagnosis	CNS abnormality	Convulsions	Abnormal movement	Impaired tonus	No symptoms	Retardation
PTPS	2 month	9	12	4	15	4	16
%	–	30	40	13.3	50	13.3	53
DHPR	2 month	11	20	3	24	6	20
%	–	45.8	83	12.5	100	25	83

"probably damaging" (score 1.00), and SIFT (Kumar et al. 2009) indicated that they may be "damaging" (score 0).

Regarding the p. [(Leu185Pro)] mutation, proline is a very rigid residue that might abolish the required flexibility of the protein at the highly conserved position 185. Since proline residue is smaller than leucine, this will cause a possible loss of external interactions and prevents multimeric contacts. The 225th amino acid has been shown to be 100% conserved residue (Venselaar et al. 2010), and it would damage the protein if it is replaced with an arginine which is a bigger amino acid with different charge. Also, methionine in comparison with threonine at residue 237 shows differences in hydrophobicity. Therefore, it is expected that this substitution affects hydrogen bond formation. This threonine residue is much conserved and also methionine is a bigger residue; therefore, it is expected that it cannot be buried in the core of the protein. In conclusion, based on the HOPE project's (Venselaar et al. 2010) information, these changes are possibly damaging to the protein.

Also, other mutations including p. [(Gly89Arg)], p. [(Ser 115Leu)], p. [(Thr114Ile)], p. [(His158Tyr)], and p. [(Ser163Asn)] are potentially pathogenic because they have not been reported in the single nucleotide polymorphism database (Exome Variant Server). Moreover, bioinformatics prediction by Polyphen-2 and SIFT indicated that these changes are either "probably damaging" or "damaging" with different scores (see Table 4). The p.[(Gly89Arg)] amino acid substitution introduces a bigger and a less hydrophobic residue at this position that can disturb the multimeric interactions. At the 89th amino acid, only glycine is flexible enough to make torsion angles, so mutation into arginine will force the local backbone into an incorrect conformation which may disturb the local structure. At 115th, wild-type residue is a highly conserved glycine that forms a hydrogen bond with the serine on 111th position. Therefore, mutation in this site will cause loss of hydrogen bonds in the core of the protein and as a result disturbs correct folding. Besides, at this position the mutant leucine residue is bigger than serine and probably will not be buried in the core of the protein which is important for the main activity of the protein.

By substitution of threonine with isoleucine at amino acid 114, the multimeric contacts would be disturbed. Since the mutated isoleucine is located in a domain that is important for the main activity of the protein, differences between the wild-type and mutant residue can damage the core structure of this important domain and thereby affect the catalytic activity. The histidine at position 158 is a conserved residue and is buried in the core of the protein. The mutant tyrosine residue is bigger and probably will not fit in the space neatly. Also, because of different hydrophobicity, the mutation p.H158Y will cause loss of hydrogen bonds in the core of the protein and as a result disturbs correct folding.

Finally, mutation p. [(Ser163Asn)] changes serine into asparagine at position 163 which can damage the protein. One reason to support this claim is that the wild type is highly conserved. Serine forms a hydrogen bond with the glutamine on position 159, so because of the size difference, the mutant residue cannot make the same hydrogen bond as the original wild-type residue. The mutant asparagine residue is bigger with less hydrophobicity, so it can disturb interactions with other molecules or other parts of the protein which results in a defective protein.

Regarding genotype/phenotype correlation in our patients, we conclude that no clear correlation was observed between various mutations and also the type of the gene involved. One reason could be due to the age of admission, age at treatment, prior management by other physicians, parental adherence to the physicians' orders, etc. (see Table 5). Molecular analysis of the remaining families is ongoing.

Number of recurrent mutations found was limited in the studied population. This may be due to either small sample size or the heterogeneity of Iranian population. Since BH4 deficiency is a rare disease, therefore, 24 samples may not be few. This finding may also be supported by a suggestion (Blau et al. 2001) that recurrent mutations, in unrelated individuals with BH4 deficiency, are more likely to be due to recurrent mutations at the CpG dinucleotide rather than founder effects. Multi-founder effect hypothesis may also be valid in large and heterogeneous populations like Iran, with several ethnicities and long history of civilization and human migrations (Najmabadi et al. 2003).

Take-Home Message of the Article

QDPR accounts for the majority of BH4 deficiency with a variety of mutations in Iran.

Compliance with Ethics Guidelines

Hannaneh Foroozani, Maryam Abiri, Shadab Salehpour, Hamideh Bagherian, Zohreh Sharifi, Mohammad Reza Alaei, Shohreh Khatami, Azadmeh S, Aria Setoodeh, Leyli Rejali, Farzaneh Rohani, and Sirous Zeinali declare that they have no conflict of interest.

Contribution of Authors in Project

Maryam Abiri: Interpretation of data and drafting of manuscript

Hannaneh Foroozani: Data collection and doing molecular genetic testing in laboratory

Shadab Salehpour: Clinical diagnosis of patients and responding for clinical comments of the reviewers

Hamideh Bagherian: Genetic counselor of medical genetics laboratory of Kawsar Human Genetics Research Center

Zohreh Sharifi: Primer designing

Shohreh Khatami: Performing biochemical tests for patients and analysis of data

Mohammad Reza Alaei, Aria Setoodeh, Farzaneh Rohani: Clinical diagnosis of patients

Leyli Rejali: Sequencing of pcr product

Sara Azadmehr: Doing molecular genetic testing in laboratory

Sirous Zeinali: Supervisor of the project and edit of manuscript

References

Adzhubei IA, Schmidt S, Peshkin L et al (2010) A method and server for predicting damaging missense mutations. Nat Methods 7:248–249

Blau N, Thony B, Cotton RG (2001) Disorders of tetrahydrobiopterin and related biogeninc amines. In: Scriver CR, Beaudet A, Sly WS, Valle D, Childs B, Vogelstein B (eds) The metabolic and molecular bases of inherited disease. McGraw Hill, New York, pp 1275–1776

Blau N, Bonafe L, Blaskovics M (2005) Disorders of phenylalanine and tetrahydrobiopterin metabolism. In: Blau N, Duran M, Blaskovics M, Gibson K (eds) Physician's guide to the laboratory diagnosis of metabolic disease. Chapman & Hall, London, pp 89–106

Blau N, Hennermann JB, Langenbeck U, Lichter-Konecki U (2011) Diagnosis, classification, and genetics of phenylketonuria and tetrahydrobiopterin (BH4) deficiencies. Mol Genet Metab 104: S2–S9

Dianzani I, Howells DW, Ponzone A, Saleeba JA, Smooker PM, Cotton RG (1993) Two new mutations in the dihydropteridine reductase gene in patients with tetrahydrobiopterin deficiency. J Med Genet 30:465–469

Kumar P, Henikoff S, Ng PC (2009) Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. Nat Protoc 4:1073–1081

Miller S, Dykes D, Polesky H (1988) A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16:1215

Najmabadi H, Neishabury M, Sahebjam F et al (2003) The Iranian Human Mutation Gene Bank: a data and sample resource for worldwide collaborative genetics research. Hum Mutat 21:146–150

Romstad A, Kalkanoglu HS, Coskun T et al (2000) Molecular analysis of 16 Turkish families with DHPR deficiency using denaturing gradient gel electrophoresis (DGGE). Hum Genet 107:546–553

Scrutton NS, Berry A, Perham RN (1990) Redesign of the coenzyme specificity of a dehydrogenase by protein engineering. Nature 343:38–43

Setoodeh A, Yarali B, Rabbani A et al (2015) Tetrahydrobiopterin responsiveness in a series of 53 cases of phenylketonuria and hyperphenylalaninemia in Iran. Mol Genet Metab Rep 2:77–79

Smith I, Brenton D (1996) Hyperphenylalaninemias. In: Fernandes J, Saudubray J, van den Berghe G (eds) Inborn metabolic diseases: diagnosis and treatment. Springer-Verlag, Berlin, pp 147–160

Smooker PM, Cotton RG (1995) Molecular basis of dihydropteridine reductase deficiency. Hum Mutat 5:279–284

Smooker PM, Howells DW, Cotton RG (1993) Identification and in vitro expression of mutations causing dihydropteridine reductase deficiency. Biochemistry 32:6443–6449

Venselaar H, te Beek TA, Kuipers RK, Hekkelman ML, Vriend G (2010) Protein structure analysis of mutations causing inheritable diseases. An e-science approach with life scientist friendly interfaces. BMC Bioinformatics 11:548

Wang L, Yu W-M, He C et al (2006) Long-term outcome and neuroradiological findings of31 patients with 6-pyruvoyltetrahydropterin synthase deficiency. J Inherit Metab Dis 29:127–134

Wang W, Gao J, Wang J, Liu C, Meng Y (2012) Cloning, expression and enzymatic properties analysis of dihydrofolate reductase gene from the silkworm, Bombyx mori. Mol Biol Rep 39:10285–10291