

Molecular diagnosis of Phenylketonuria in 157 Families and Prenatal Diagnosis of Phenylketonuria

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Abstract

Background Phenylketonuria (PKU) is a genetic metabolic disease with a relatively higher incidence, but only a few studies about the prenatal diagnosis of PKU have been reported so far in China. The aim of this study was to characterize the spectrum of mutations in PAH gene in PKU probands and the prenatal diagnosis of PKU in north China.

Methods A total of 157 families in which PKU patients had been diagnosed were included in the study. The 13 exons and their flanking sequences of PAH gene were amplified by PCR and sequenced in the probands. If none or only one mutant allele was found in the probands, the sample was subjected to MLPA for large deletions/duplication detection in PAH gene. Prenatal diagnosis was performed for pregnant women in these families.

Results Pathogenic mutation in PAH was found in 2 alleles in 148 probands and in one allele in 7 probands, and the mutation was not detected in 2 probands. There were 289 point mutants, 10 frame-shift mutations and 4 large deletions with a total of 80 kinds of mutations. The most prevalent mutations were R243Q (17.2%), EX6-96A>G (8.6%) and V399V (8.0%). We also found a novel mutation of 163_164insATAT. Prenatal diagnosis of 95 families found 21 healthy fetus (20.4%), 52 carriers (50.5%) and 30 patients (29.1%), and the accuracy of prenatal diagnosis was confirmed after birth of the fetuses.

Conclusion We present here a spectrum of mutations in PAH gene in PKU patients in north China. Prenatal diagnosis for PKU is useful for PKU families to prevent birth of another PKU case.

Background

PKU is an autosomal recessive genetic disease caused by mutations in *PAH* gene coding phenylalanine hydroxylase, a key enzyme in the metabolism of phenylalanine. Early dietary therapy improves most of the neuropsychological disorders, but is hard to be maintained for a long period of time [1]. To date more than 700 mutations in *PAH* gene, including [missense](#), splicing, nonsense, insertion and deletion mutations, have been identified. The distribution of the mutations are heterogeneous in ethnic groups. [Genetic](#) testing and prenatal [diagnosis](#) can prevent PAH families from transmitting PKU to their progeny. However, only a few reports about the prenatal diagnosis of PKU from north of China are found in the literature. Here we summarized the results of mutation detection in 157 probands and prenatal diagnosis of 103 fetuses from 95 PKU families.

Methods

Participants

A total of 157 probands with their parents were examined for the mutations in *PAH* gene during the period from May 2012 to December 2018 at Peking University First Hospital. The age of the probands ranged from one month to 17 years, with the male to female ratio of 1:0.92. Most of them lived in north of China.

All of the probands had high levels of plasma phenylalanine concentration (>2mg/dL), and the diagnosis of tetrahydrobiopterin (BH4) deficiency had been excluded by a BH4-loading test.

PCR-direct sequencing

Genomic DNA was isolated from peripheral blood of the probands and their parents by a QuickGene DNA whole blood kit. The 13 exons and their flank sequences were amplified by PCR that containing 50 ng DNA, 200 µmol dNTPs, 1× reaction buffer, 10 pmol of each primer, and 2.5 units of Taq DNA polymerase in a total volume of 50 µl. PCR products were purified and then sequenced in an ABI 3730xl DNA Analyzer. Sequencing results were compared with FAH cDNA (NM_000277) and its genomic sequences (GRCh38/hg38). Detected mutations were further searched in the 4 databases (<http://www.pahdb.mcgill.ca>, www.biopku.org/pah/, <http://www.biopku.org/biopku/>, <https://www.ncbi.nlm.nih.gov/clinvar/>, <http://www.hgmd.cf.ac.uk/ac/>). Novel mutations not found in these databases were evaluated by online softwares of SIFT, PROVEAN and POLYPHEN2 to predict pathogenic effects of the mutant proteins. Mutations were compared between the proband and his/her parents to comprehend the inheritance of the mutant allele(s) from parents to their progeny.

MLPA analysis

For probands without mutation or only one mutation found, their DNA samples were subjected to MLPA (MLPA P055 kit, MRC-Holland, Amsterdam, Netherlands) for the detection of large insertions/deletions or duplications in *PAH* gene. The MLPA products were separated in ABI 3130XL Genetic Analyzer and analyzed by Coffalyser.Net

Prenatal diagnosis

Ninety-five parents of the 157 families identified pathogenic mutations in *FAH* gene asked for [prenatal diagnosis](#) when the mothers became pregnant again, in which 8 families asked for prenatal diagnosis twice because of two times of pregnancies. Informed consent was obtained from the parents. DNA samples were extracted from chorionic villi, amniotic fluid or cord blood using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) and subjected to the same PCR-direct sequencing and/or MLPA procedures as described before. In addition, PCR amplification of the 5 STR markers nearby *PAH* and separation of the PCR products by ABI 3130XL Genetic Analyzer were also included to exclude false results due to maternal blood contamination.

Results

Spectrum of mutations in *PAH* gene

Among the 157 probands, pathogenic mutation in *PAH* was found in 2 alleles in 148 probands and in one allele in 7 probands, and the mutation was not detected in 2 probands. There are totally 303 mutations

including 289 point mutants, 10 frame shift mutations and 4 large deletions. Table 1 lists the spectrum of the 303 mutations we detected, in which the mutation of 163_164insATAT is a novel mutation not stored in the 4 databases. The most prevalent mutations are R243Q, EX6-96A>G and V399V accounting for 17.2%, 8.6% and 8.0% of the mutant alleles, respectively.

Table 1. Classification of the *PAH* mutations in the 148 probands

Point mutant			Frame shift mutation		Large deletion/duplication	
Missense	Nonsense	Splicing	Insert	Delete	Duplication	Deletion
53	7	12	2	4	0	2
Total		72	6		2	

The highest prevalence of exon and its flanking sequences where mutations locate in *PAH* gene is exon 7, followed by exon 11, exon 12, exon 6, exon 3, exon 5 and exon 2 (Figure 1).

Figure 1. The exons and their flanking sequences where mutations locate

MLPA analysis

MLPA performed in 13 probands and found 2 large deletions of exon 1 and its upstream region in 2 probands and exon 4/exon 5 in other 2 probands.

Prenatal diagnosis

Prenatal diagnosis of PKU was performed in 103 fetuses in 95 PKU families; 30 fetuses (29.1%) were identified as PKU (carrying 2 mutations), 52 (50.5%) as PKU carriers (carrying one mutation), and 21 (20.4%) as normal fetuses (no mutation found).

3 carriers' spouses accepted prenatal counseling and *PAH* sequencing. One was found a pathogenic mutant (c.1068C>A, p.Y356X), which is shown in Figure 2. Advice of PKU prenatal diagnosis was given to them.

Figure 2. pedigree of one PKU family

Discussion

PAH gene locates in human chromosome 12q23.2, consisting of 13 exons that encode a polypeptide of 452 amino acid residues. Abnormal *PAH* blocks the transformation of phenylalanine to tyrosine, causing

the accumulation of phenylalanine, which is then metabolized into phenylpyruvate. Phenylpyruvate and its metabolites are toxic, eventually resulting in severe mental retardation and neurobehavioral abnormalities in children. Neonatal screening for PKU are useful for the early treatment of PKU. During the period from 1999 to 2009 in Beijing, a total of 1,166,218 newborns were screened for PKU, and a total of 145 newborns were confirmed to have PKU with the incidence of 1/8,064 [2]

In this cohort of PKU probands, the 3 prevalent mutations of R243Q, EX6–96A>G and V399V accounted for 33.8% of the mutations, similar to the reports from other regions in China[3–5] and from Korea[6]. In contrast in Japan, the most prevalent mutation is R413P[7]. The mutations of R243Q and EX6–96A>G have been reported in Chinese PKU cases; the R243Q mutation derived an abnormal PAH that only has <10% of normal PAH activity in a eukaryotic cell expression system[8]. The novel mutation of 163_164insATAT we found causes frame shift and premature termination of the polypeptide chain and is definitely a pathogenic mutation.

Three pathogenic mutations in a proband were detected in two of our probands. One carried c.59A>C and c.60G>C from mother and c.721C>T from father, and the other carried c.194T>C and c.510T>A from mother and c.739G>C from father. The c.59A>C and c.60G>C mutations in one allele have been reported previously[9,10]. The genotype of 3 pathogenic mutations in a patient is not uncommon, for an example, a total 35 PKU patients with this genotype were detected from 796 PKU patients by next-generation sequencing[3].

Thirteen probands not carrying two mutations in two alleles were subjected to MLPA, and two large deletions, exon 1 and its upstream region in 2 probands and exon 4/exon 5 in other 2 probands were identified in these probands. Chen et al. reported that 3 large deletion alleles (exon 1 and its upstream region, exon 4/exon 5, and exon 5) were disclosed in 17 PKU families without having two pathogenic mutations[11]. Yan et al. identified 24 (51.1%) large deletion/duplication alleles by MLPA in 22 of the 43 PKU patients with none or only one mutant allele, in which Ex1del3758 was detected in 10 cases and Ex4_5del in 4 cases; the authors considered that Ex1del3758 large deletion is a hotspot in Chinese PKU patients, similar to our findings[12].

Figure 3. Ratio of the mutants in this cohort of PKU patients

No hotspot mutation exists in PKU patients in north China, as the results we present here (Figure 3). In our study, the highest prevalence of exon and its flanking sequences where mutations locate was exon 7, followed by exon 11, exon 12, exon 6, exon 3, exon 5 and exon 2. Zhang et al. examined mutations in *PAH* gene of exons 3, 5, 6, 7, 10, 11 and 12 in 40 PKU families and demonstrated that exon 7 concentrated the most mutations, followed by exons 6, 11 and 3, also similar to our results[13].

The next-generation sequencing technology has become a powerful tool for the diagnosis of genetic diseases. Regular PCR-Sanger sequencing and MLPA could detect 96.5% (303/314) pathogenic alleles in *PAH* gene in this cohort of PKU patients, suggesting that the classic methods are still useful in the genetic diagnosis of PKU with regard to cost and efficiency.

In families that both the husband and wife carry a mutation in *PAH* gene, the possibility of giving birth to a PKU baby is 25%. Prenatal diagnosis is the exclusive way for PKU families to prevent birth of another PKU case. Technologically, genotyping of several STR markers must be included to prevent misdiagnosis due to maternal blood contamination in fetal samples[14]. The 6 highly polymorphic STR markers we used for linkage analysis locate around *PAH* gene, two upstream of, 3 downstream of, and one in intron 3 of *PAH* gene. In case of maternal blood contamination in fetal samples, DNA extracted from cultured amniotic fluid cells or chorionic villi cells is the only way to get accurate results.

Conclusions

Here we present a spectrum of mutations in *PAH* gene in PKU patients in north China. No hotspot mutation was found. The mutations were frequently detected in exon 7. Prenatal diagnosis is an essential way to prevent the birth of PKU cases, but related information is rare in China. We show the results of prenatal diagnosis for PKU in north China.

Declarations

Abbreviations

BH4: Tetrahydrobiopterin; MLPA: Multiplex ligation-dependent probe amplification; PAH: Phenylalanine hydroxylase; PKU: Phenylketonuria; STR: Short Tandem Repeats

Ethics approval and consent to participate

This study was approved by the Research Ethics Committee of Peking University First Hospital. Signed informed consent was obtained from the probands and their family members.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed in the current study are available from the corresponding author on request.

Competing interests

The authors declare that they have no competing interests.

Funding

Not applicable.

Authors' contributions

YM designed the methodology and supervised the study. HP participated in design and coordination. YX executed PCR and [sequencing](#), collected and analyzed data, and drafted the manuscript. QG confirmed the PKU case through clinical examinations. HW executed STR genotyping. SW executed MLPA experiment. PP and XZ extracted DNA from samples. All authors have read and approved the manuscript.

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References

- [1] [Blau N, van Spronsen FJ, Levy HL](#). Phenylketonuria. *Lancet* 376 (2010):1417–1427.
- [2] [Yang HH, Zhang YM, Qin JL, Qiu L, Ding H](#). Study on neonatal screening for phenylketonuria during the last decades in Beijing. *CJCHC* Mar. 2010, Vol 18, No.3.
- [3] [Li N, Jia HT, Liu Z, Tao J, Chen S, Li XH, Deng Y, Jin X, Song JP, Zhang LT, Liang Y, Wang W & Zhu J](#). Molecular characterisation of phenylketonuria in a Chinese mainland population using next-generation sequencing. *Scientific Reports*. 2015 Oct 27;5:15769.
- [4] [Song F, Qu YJ, Yang YL, Jin YW, Zhang YM, Wang H, Yu WZ](#). The mutant spectrum of phenylalanine hydroxylase gene in Northern Chinese. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi*. 2007 Jun;24(3):241–6.

- [5] He J, Wang HZ, Xu FL, Yang X, Wang R, Zou HY, Yu WZ. Mutation analysis of the PAH gene in children with phenylketonuria from the Qinghai area of China. *Zhongguo Dang Dai Er Ke Za Zhi*. 2015 Nov;17(11):1221–7.
- [6] Lee DH, Koo SK, Lee KS, Yeon YJ, Oh HJ, et al. The molecular basis of phenylketonuria in Koreans. *J Hum Genet* 2004;49:617–21.
- [7] Okano Y, Kudo S, Nishi Y, Sakaguchi T, Aso K. Molecular characterization of phenylketonuria and tetrahydrobiopterin-responsive phenylalanine hydroxylase deficiency in Japan. *J. Hum. Genet.* 2011; 56: 306–12.
- [8] Wang T, Okano Y, Eisensmith RC, Lo WH, Huang SZ, Zeng YT, Yuan LF, Liu SR, Woo SL. Missense mutations prevalent in Orientals with phenylketonuria: molecular characterization and clinical implications. *Genomics*. 1991 Jun;10(2):449–56.
- [9] Wang L, Wang X, He B, Cai N, Li W, Lou C, Xin S, Wu Q, Yu W, Qiang R. Mutation analysis of the phenylalanine hydroxylase gene and prenatal diagnosis of phenylketonuria in Shaanxi, China. *J Pediatr Endocrinol Metab*. 2017 Nov 27;30(12):1305–1310.
- [10] Yan Y, Zhang C, Jin X, Zhang Q, Zheng L, Feng X, Hao S, Gao H, Ma X. Mutation spectrum of PAH gene in phenylketonuria patients in Northwest China: identification of twenty novel variants. *Metab Brain Dis*. 2019 Feb 12.
- [11] Chen C, Zhao ZH, Ren YL, Kong XD. Characteristics of PAH gene variants among 113 phenylketonuria patients from Henan Province. *Chin J Med Genet*, 2018 December Vol.35. No.6:791–5.
- [12] Yan YS, Yao FX, Hao SJ, Zhang C, Chen X, Feng X, Yang T, Huang SZ. [Analysis of large deletion of phenylalanine hydroxylase gene in Chinese patients with phenylketonuria](#). *Zhonghua Yi Xue Za Zhi*. 2016 Apr 12;96(14):1097–102.
- [13] Zhang YM, Qin JL, Qiu L, Li SH, Lu GX, Song F, Jin YW, Wang H, Zhang T. The PKU neonatal screening, diagnosis, treatment and genic mutational analysis in Beijing area. *Chinese Journal of Child Health Care*. 2003, 11(6):366–367, 388.
- [14] Liu N, Kong XD, Zhao DH, Wu QH, Li XL, Guo HF, Cui LX, Jiang M, Shi HR. Prenatal diagnosis of Chinese families with phenylketonuria. *Genet Mol Res*. 2015 Nov 19;14(4):14615–28.

Figures

Figure 1. The exons and their flanking sequences where mutations locate

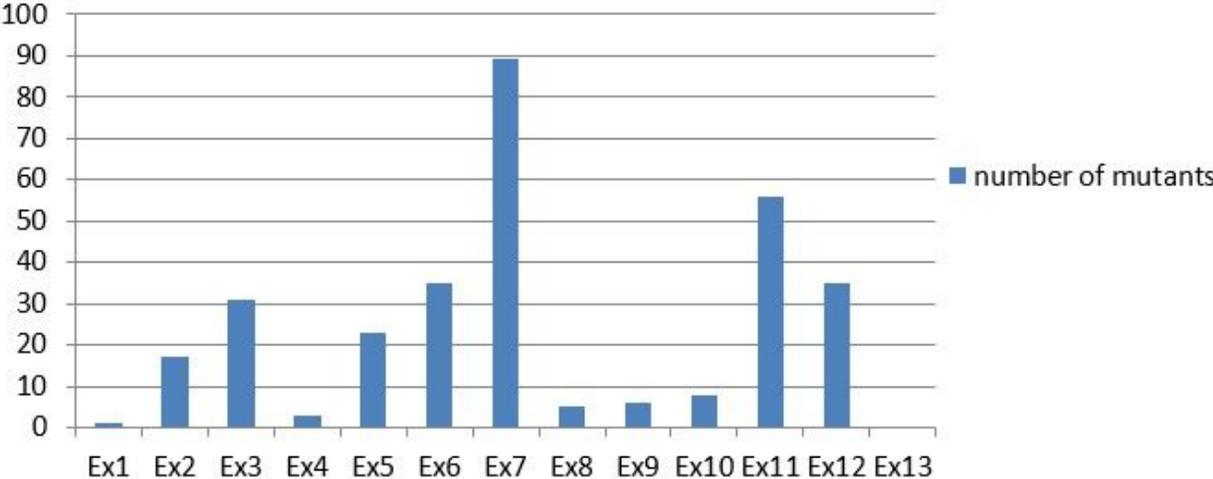


Figure 1

The exons and their flanking sequences where mutations locate

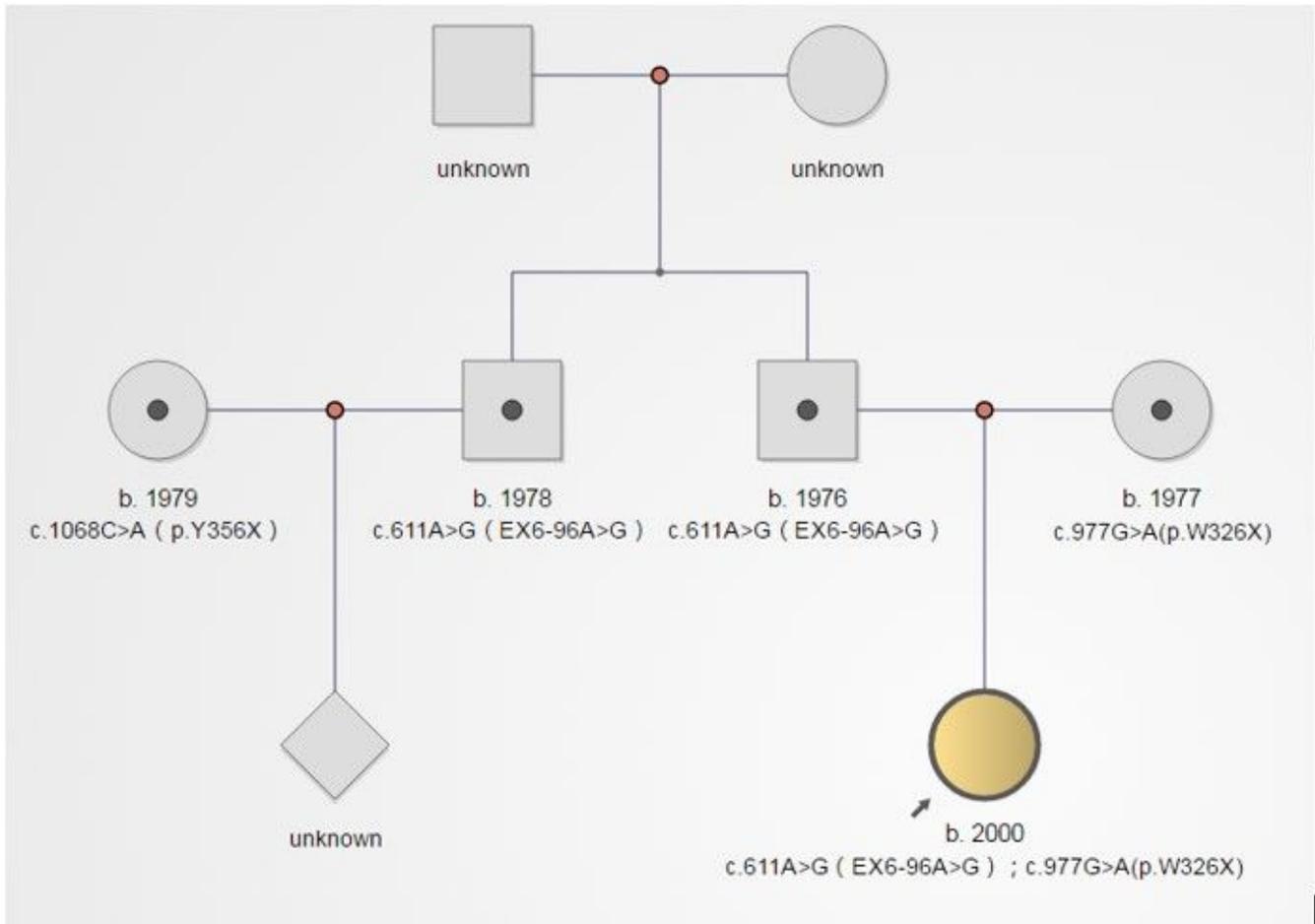


Figure 2. pedigree of one PKU family

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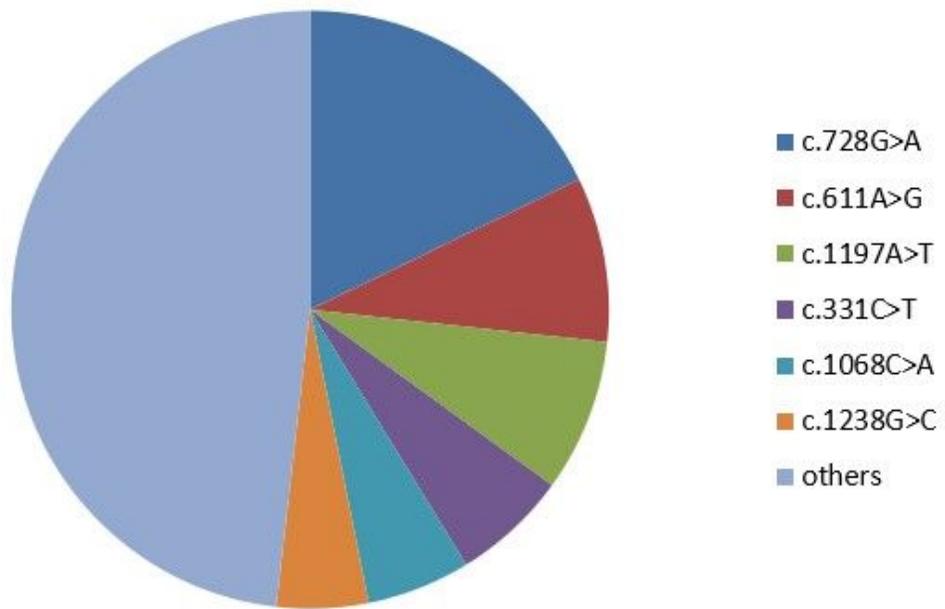


Figure 3

Ratio of the mutants in this cohort of PKU patients