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First Case Report of EX3del4765 Mutation in *PAH* Gene in Asian Population

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Abstract

Introduction: Phenylketonuria (PKU) is an autosomal recessive inborn error of phenylalanine metabolism, which is caused by mutation in phenylalanine hydroxylase (*PAH*) gene. Most of the *PAH* mutations are missense mutations (67%), which are followed by small or large deletions (13%).

Case Presentation: We reported a patient with classic PKU and his parents harboring a large deletion in exon 3 (EX3del4765) of *PAH* gene. This is the first case report of EX3del4765 in Asian patients with PKU.

Conclusions: This finding may help improve early detection, differential diagnosis, genetic counseling, and even treatment of patients with PKU.

Keywords: Gene Deletion, Phenylalanine Hydroxylase, Phenylketonuria

1. Background

Phenylketonuria (PKU) is caused due to various genetic alterations of phenylalanine hydroxylase (PAH) enzyme, which is involved in conversion of L-phenylalanine (L-Phe) to L-tyrosine (L-Tyr) in phenylalanine metabolic pathway. The classic PAH deficiency is considered, when the serum concentration of unchanged phenylalanine (Phe) crosses the level of 1200-μmol/L (1). The Phe concentration in the range of 600 to 1200-μmol/L is diagnosed as mild PKU, and values less than 600-μmol/L are classified as hyperphenylalaninemia (HPA).When the treatment program of a patient with PKU did not start at the early weeks of neonatal period developmental delay, mental retardation and microcephaly are caused by accumulation of toxic byproducts of Phe within its metabolic pathway.

PAH gene is located on chromosome 12q23.2, spanning 171 kbp and includes 13 exons, which encode a polypeptide of 452 amino acids (2). More than 520 various pathogenic mutations have been reported in all 13 exons of *PAH* gene which could be found in the *PAH* Mutation Analysis Consortium Database. Missense mutations constitute 67% of the total *PAH* mutations, which could result in different clinical manifestations based on their effects on structure and function of PAH enzyme. Small or large exon deletions are the second frequent genetic alterations of *PAH* gene, which comprises 13% of the total mutations listed in *PAH* mutation database (http:// www.PAHdb.mcgill.ca). Although complete deletion of exon 3 of *PAH* gene has been previously reported in European patients, to our knowledge, it has not been identified among Asian population, especially in Iranian patients with PKU yet. This report aimed to describe the same but the first Iranian patient with classic PKU who was homozygous for deletion of *PAH* exon 3.

2. Case Presentation

A 15-day-old male was referred to Genomic Research Center in summer 2014 in Tehran due to high level of blood Phe detected in the routine national framework of PKU neonatal screening (Table 1). He was born to consanguineous parents from East Azerbaijan province of Iran. His parents were first cousins with no family history of PKU (Figure 1). Due to persistent high level of phenylalanine in blood (1404-μmol/L), the patient was diagnosed as classic PKU.

Molecular genetic analysis was performed to confirm PKU diagnosis in the child. After obtaining approval from the Medical Ethics Committee of Shahid Beheshti University of Medical Sciences, and informed consent from patient family, peripheral blood samples from the patient and his parents were collected. Genomic DNA was extracted by salting out method. Extracted genomic DNA was amplified by polymerase chain reaction (PCR) technique by ABI GeneAmp 9700 device, using *PAH* gene primers amplifying exons 1 - 13 and exon-intron boundaries. Then, PCR products were sequenced using the ABI Prism 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) (Primer sequences and PCR programs are available upon request). During PCR of *PAH* exons in the patient, exon 3 was not amplified. Further analysis was conducted by designing two primers within the flanking introns of exon 3 (introns 2 and 3). In normal individuals, the segment size between two primers is 5885 bps (which does not amplify in regular PCR). In our patient, a 1120 bps segment was amplified (Figure 2) that demonstrated a large deletion of 4765 bps (g.21560-26324del4765) containing exon 3, and results were confirmed by Sanger sequencing (Figure 3) too. As a result, heterozygous status of parents for the same deletion was revealed by genotyping analysis.

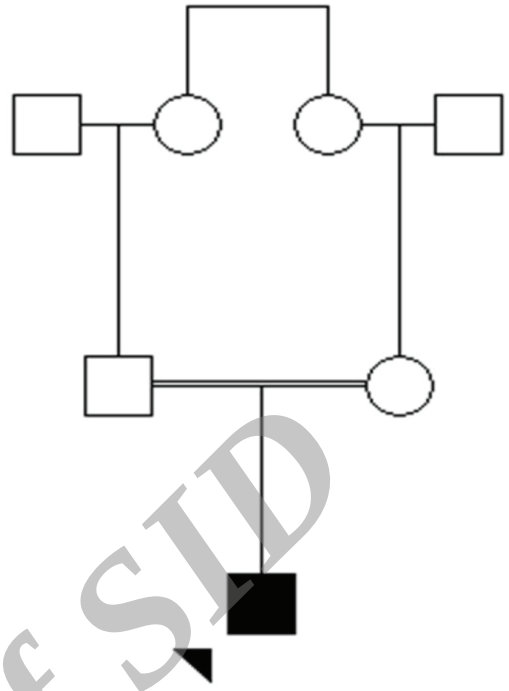


Figure 1. Family Pedigree of the Patient

Table 1. Plasma Amino Acid High Performance Liquid Chromatography Results of the Patient at 15th Day of Life. The Value of Phenylalanine Was About 16 Fold Greater Than Normal

Amino Acid	Value, µmol/L	Normal Range
Alanine	189	195 - 560
Valine	104	123 - 310
Isoleucine	23	21 - 110
Serine	121	80 - 230
Aspartic Acid	2	2 - 25
Glutamic Acid	76	26 - 240
Glutamine	442	345 - 685
Lysine	53	80 - 240
Tyrosine	37	10 - 145
Arginine	29	10 - 80
Glycine	285	135 - 350
Leucine	66	60 - 190
Threonine	87	60 - 205
Asparagine	32	20 - 80
Methionine	23	12 - 40
Phenylalanine	1404	32 - 85
Ornithine	47	28 - 110
Histidine	63	54 - 120
Citrulline	21	10 - 45

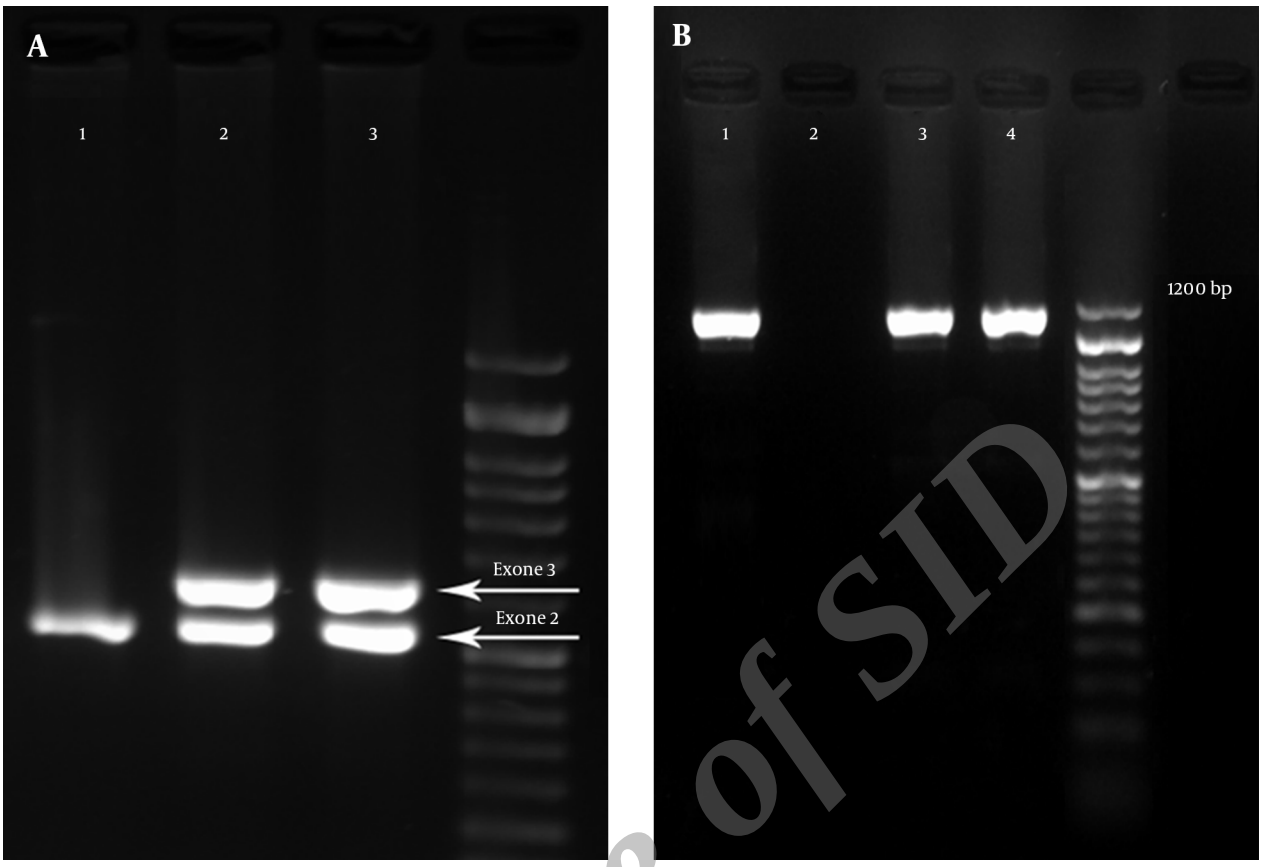
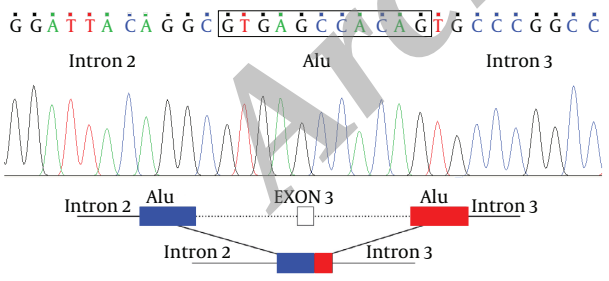


Figure 2. A, gel electrophoresis of PCR products of exon 2 and 3; 1, absence of exon 3 in patient, 2 and 3, presence of exon 3 in the father and mother. B, gel electrophoresis of PCR products using primers for introns 2 and 3; 1, 1120 base pair band due to deletion in patient; 2, absence of the band in normal control; 3 and 4, presence of the band in heterozygous father and mother.

Figure 3. Sequencing Result of 1120 bps Product in Patient Show Deletion of Large Segment Containing Exon 3



The Deletion Occurred Within Alu Repeat Segment.

3. Discussion

PKU is an autosomal recessive metabolic disorder caused by various molecular alterations in either *PAH* gene or the gene encoding its cofactor, tetrahydrobiopterin (BH4). Multiple pathogenic and nonpathogenic variations have been reported in all 13 exons of *PAH* gene. Deletions constitute 13% of all kinds of mutations identified in *PAH* gene, however large exon deletions comprise less than 7% of all the deletions. We reported a new case of 4765 bps deletion (EX3del4765) encompassing the entire exon 3 of *PAH* gene, which has been never described in Asian and Iranian patients. This deletion initially was identified in patients with PKU from Czech Republic by performing MLPA (Multiplex Ligation-Dependent Probe Amplification) and long range PCR. Also, it has been reported in patients with PKU of other European populations such as Italian, Polish, and Slovene, which could be an example of founder effect (3-5).

EX3del4765 is produced as a result of intra-chromosomal, unequal homologous recombination between Alu-Alu repeats, which is the major cause of large genomic rearrangements. Alu repeats are interspersed repetitive elements comprising 10% of the total human genome, and are found in untranslated regions, introns, and even interagenic regions of genome (6).

Exon 3 of *PAH* gene is involved in encoding the regulatory domain of PAH enzyme (7), which lies at the N-terminal of the protein. This domain also determines the specificity of PAH enzyme to Phe. Regulatory domain spans within the 1/3 of the total length of the N-terminal of PAH protein and contains an auto-regulatory sequence (ARS). ARS plays a fundamental role in the prevention of

substrate from binding to enzyme active site (8). ARS also modulates the entrance of substrate, BH4, and catecholamine inhibitors to control the Phe activation. Regulatory domain facilitates access of both substrate and cofactor through interaction with active site of catalytic domain, therefore either partial or complete deletion of it, could interfere with its proper function (9).Considering the direct effects of various pathogenic mutations on the PAH enzyme activity and the serum level of Phe, accurate identification of the *PAH* gene mutations could have a determining role in treatment schedule of PKU patients. Despite the lack of enough investigations, it is demonstrated that some of the *PAH* gene mutations are more responsive to BH4 therapy (10, 11). Moreover, determination of specific *PAH* gene mutation in patients can lead to early initiation of Phe-free regimen, which can prevent future severe neurological complications. Ascertaining the precise type of underlying molecular defect in *PAH* gene, particularly those occurring in regulatory domain, not only confirms the diagnosis, but also could help health care providers to afford the best treatment program. In addition, identification of the mutation type could help family genetic counseling, especially in the prenatal diagnosis (PND) of future pregnancies and this is one of the strong points of our study.

Finally, discovering the deletion of exon 3 in *PAH* gene in Iranian population could assist us in better molecular diagnosis of patients with PKU and their family members.

Footnotes

Authors' Contribution: Study concept, design and supervision: Mohammad Miryounesi; performing PCR: Ziba Soltani; contribution in primer design: Feyzollah Hashemi-Gorji; drafting of the manuscript: Fatemeh Karami; critical revision of the manuscript: Mahdieh Talebzadeh; and administrative, technical, and material support: Vahidreza Yassaee.

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