Review Article

Phenylketonuria from genetics to clinics: An Iranian prospect

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Abstract
Phenylketonuria (PKU) is the most common autosomal recessive disorder of amino acid metabolism. The disease is caused mainly by mutations in the phenylalanine hydroxylase *(PAH)* gene, encoding phenylalanine hydroxylase (PAH) enzyme. The PAH enzyme deficiency results in the elevation of phenylalanine in the blood, which may cause severe irreversible mental retardation in the affected individuals. More than 500 different disease causing mutations have been identified in the *PAH* gene. Direct and indirect molecular approaches have been developed for carrier detection and prenatal diagnosis of PKU disease. Population distribution of the *PAH* gene mutations and the PKU disease varies in different countries. In view of relatively high prevalence of the disease in Iranian population, investigations toward the elucidation of molecular aspects of the disease were required. In the present article, clinical and molecular basis of the PKU disease, with emphasis on the studies performed in Iranian population, were reviewed.

Keywords**:** phenylalanine hydroxylase; phenylketonuria; mutation; haplotype; polymorphic markers; Iranian population

INTRODUCTION

Phenylketonuria (PKU; OMIM 261600) is the most common genetic disorder of amino acid metabolism which is caused mainly by deficiency of the hepatic enzyme phenylalanine hydroxylase (PAH; EC 1.14.16.1), as a result of the mutations in the gene encoding the enzyme (Madden, 2004; Liu *et al*., 2001; Scriver and Kaufman, 2001). Phenylalanine hydroxylase converts L-phenylalanine (Phe) to tyrosine as the first step in the catabolic metabolism of Phe using molecular oxygen and tetrahydrobiopterin (BH4) as a cofactor (Erlandsen and Stevens, 1999).

Deficiency in PAH enzyme could result in the elevation of phenylalanine and its metabolic derivatives in blood and other fluids in the body, especially urine and cerebrospinal fluid (Madden, 2004). This may cause profound mental retardation, microcephaly and seizures most likely due to the metabolic imbalances in the central nervous system (Scriver and Kaufman, 2001; Erlandsen and Stevens, 1999). Moreover, deficiency in the biosynthesis of BH4 due to the lack of dihydrofolate reductase enzyme activity may lead to a similar condition as the deficiency in PAH activity (Lindnerref *et al*., 2003).

There are different degrees of hyperphenylalaninemia (HPA): i) classical phenylketonuria, which is the most severe form of the disease (serum phenylalanine above >20 mg/dl); ii) non-PKU hyperphenylalaninemia (non-PKU HPA) (serum phenylalanine about 8-12 mg/dl) which is a moderate form of the disease. Patients with moderate PKU usually does not require dietary restriction because the PAH enzyme has a lower than general limits of activity; iii). Variant PKU (serum phenylalanine is about 12-16 mg/dl) which has phenotypic variability due to allelic variation at the

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PAH locus. The individuals with variant PKU need to be under diet with low phenylalanine, but less strict than the classic one (Scriver and Kaufman, 2001).

The PAH Enzyme and its pathologic role: The human PAH enzyme has two forms in the body; tetramer (active) and dimer form (inactive) (Fig. 1). The enzyme contains a C- terminal catalytic domain, an N- terminal regulatory domain and a tetramerization domain (Madden, 2004). The mechanism of regulation of PAH enzyme activity involves the activation by phenylalanine, inhibition by BH4 and additional activation by phosphorylation (Madden, 2004). Phosphorylation at amino acid serine at position 16 of the regulatory domain of the enzyme is mediated by cyclic adenosine monophosphate (cAMP)- dependendent protein kinase. This reduces the concentration of phenylalanine required for the activation of PAH enzyme and modulates the activity of the enzyme (Kappock and Caradonna, 1996).

Phenylalanine (Phe) is considered as a large neutral amino acid (LNAA). LNAAs such as phenylalanine, tyrosine, tryptophan, threonine, isoleucine, leucine, valine, methionine, and histidine, compete with each other for transport across the blood-brain barrier (BBB) via the L-type amino acid carrier. Deficiency of PAH enzyme results in the accumulation of Phe in the plasma, and the elevated plasma Phe impairs the uptake of other LNAAs into brain. The elevated brain Phe concentration and depletion LNAAs seems to be the most important factors for disturbed brain development and dysfunction in PKU (Pietz *et al*., 1999).

The *PAH* Gene: The *PAH* gene is located on the long arm of chromosome 12 in the region q22-q24 (with genomic position 101,756,233 base pair to 101,835,510) (Scriver *et al*., 2003; Scriver, 2007). It contains 13 exons, in which the exons and introns are variable in size from 1 to 23 kb within 90-100 kb of DNA. The shortest exon and intron is exon 9 (57 bp) and intron 10 (556 bp), respectively. The longest one is exon 13 (892 bp) and intron 2 (17,874 bp). At the 5'

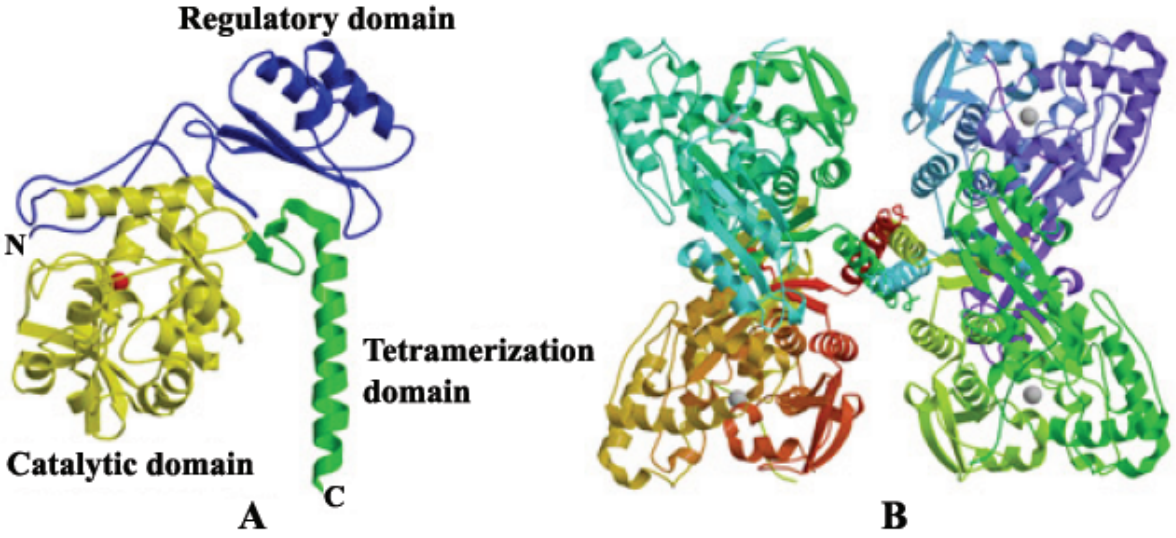

Figure 1. A schematic representation of crystallographic structure of phenylalanine hydroxylase. A: Overall structure of the full-length monomer model based on the crystals structures of various truncations of PheOH, and B: the model of the full-length tetramer of the enzyme (Stevens *et al*., 1997).

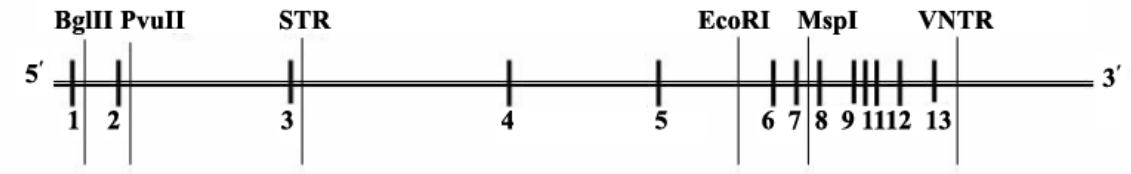

Figure 2. Schematic map of polymorphic markers, *Bgl*II, *Pvu*II(a), *Eco*RI, *Msp*I, STR and VNTR at the *PAH* gene. The numbered boxes correspond to the exons. The Figure was compiled and redrawn from different data.

end, the *PAH* gene contains large introns and compact exons, but the introns at the 3' end are smaller (Fig. 2). Overall, the *PAH* gene has large non-coding region compared to its coding regions (Scriver *et al*., 2003).

The PAH genomic sequence and its flanking regions span 171,266 base pairs. The 5' untranslated region (UTR) covers almost 27 kbp, and contains several control cis-elements which play important role in the regulation of the expression of the gene. The 3' sequence downstream to the poly (A) site in the last exon (exon 13) is about 64.5 kbp (Scriver *et al*., 2003; Konecki *et al*., 1992).

The *PAH* gene regulatory promoter region is characterized by: i) lack of TATA-like sequence at the -3' region; ii) the presence of multiple GC-rich domains with specific features of many housekeeping genes; iii) the utilization of multiple transcription initiation sites; iv) the absence of characterized tissue-specific transcription factor binding sites; v) the presence of two essential but ubiquitous cis-elements in its proximal promoter region (Blake *et al*., 1990).

Three putative protein binding sites have been identified in the proximal region of the human *PAH* gene promoter. These include: i) PAH-A site (-106 to -83 nucleotides) containing a unique palindromic sequence (TGGGCAGGTGACCTGGAGC); ii) PAH-B site (-73 to -50 nucleotides) containing CCCTCCC repeats; and iii) PAH-C site (-10 to +5 nucleotides). Sites A and B seems to be the essential regulatory elements for transcriptional activity of the *PAH* gene (Wang *et al*., 1994).

There are 28 polymorphic markers in the *PAH* gene, consisting of two multiallelic marker with variable number of tandem repeats (VNTR), a short number of tandem repeats (STR), eight biallelic restriction fragment length polymorphism (RFLP) markers and 19 single number polymorphisms (SNPs) (Scriver *et al*., 2003; DiLella *et al*., 1986 a). A diagram of the polymorphic markers at the *PAH* gene is shown in Figure 2. The VNTR marker is located 3 kb downstream of the last exon and contains an AT-rich minisatellite region (70%) responsible for the *Hind*III RFLP. The STR marker with a tetra-nucleotide core repeat sequence $(TCTA)_n$ located about 200bp downstream of exon 3 (Goltsov *et al*., 1993). These polymorphic markers have proven to have a great value in molecular diagnosis of PKU disease, especially in prenatal diagnosis and carrier detection of the disease (Vallian and Barahimi, 2005; Scriver *et al*., 2003).

Population study of the markers revealed at least 16 alleles for the STR and VNTR loci in different populations. Analysis of the VNTR marker indicated the presence of alleles with 3, 6, 7, 8, 9 and 12 repeats with relatively high frequency. It seems that alleles with more than 12 and less than 3 repeat are less frequent and may be limited to few populations (Goltsov *et al*. 1993). In two studies performed in Iranian population in our laboratory and others, almost all the VNTR alleles including the allele with 13 copies were reported. This may suggest that Iran could have one of the most heterogeneous populations in the world (Fazeli and Vallian, 2009 a; Vallian and Barahimi, 2005; Kamkar *et al*., 2003). Interestingly, the frequency spectrum of VNTR alleles in Iranian normal chromosomes was found different from mutant chromosomes (Hosseini-Mazinani *et al*., 2008). These data could be useful and applicable in the determination of the origin of the PAH mutations.

***PAH* Gene Expression:** The human *PAH* gene is expressed in liver (hepatocytes) and kidney (mainly proximal renal tubule epithelial cells). Proximal region of the *PAH* promoter contains necessary and important cis-elements for its tissue-specific expression. During embryonic development, transcription of the *PAH* gene and its expression begins at a very early stage in human. Whereas in rats and mice, it begins as late as 2-3 days before birth (Lichter-Konecki *et al*., 1999; Wang *et al*., 1994).

Homeoprotein hepatocyte nuclear factor 1a (HNF1a), is one of the factors which acts in concert with ubiquitous transcription factors to regulate liver-specific Promoters. The HNF1a binding sites are located -3.5 kb at the 5' flanking region of the human *PAH* gene (Lei and Kaufman, 1998). Defects in HNF1a could result in *PAH* transcriptional deficiency due to the lack of an open chromatin configuration. It seems that this relates to hypermethylation of the *PAH* promoter-enhancer regions. HNF1a has an important role in the onset of the *PAH* gene activation via the maintenance of unmethylated status of promoter-enhancer regions during liver development (Bristeau *et al*., 2001; Viollet *et al*., 2001; Pontoglio *et al*., 2000; Cereghini, 1996).

The *PAH* gene mutations: Mutations in the *PAH* gene result in the deficiency of phenylalanine hydroxylase (PAH) enzyme, a main characteristic of PKU). More than 500 different *PAH* mutations have been identified in the *PAH* gene. About 90% of the mutations are point mutations (Birk Moller *et al*., 2007). Most of the point mutations occur in exon 5 (PAH residue 148) to exon

12 (PAH residue 438) region of the gene (Erlandsen *et al*., 2003). Among the mutations identified, missense mutations comprise the most frequent ones. A full update list of mutations and their frequency in different populations is available at: http://www.mcgill.ca/*PAHdb*.

According to the genotype/phenotype correlation at the *PAH* gene, mutations could be categorized into three groups: i) mutations affecting both PAH kinetics and stability, ii) mutations that alter kinetic properties, but also have no effect on the PAH structure, and iii) mutations displaying normal kinetics, but with reduced stability *in vitro* and *in vivo*. For example, mutation R252W is a missense mutation that involves the catalytic domain of the PAH enzyme (amino acids 143-410). *In vitro* expression analysis showed that this mutation could almost fully affect the activity of the enzyme (<1%) (Erlandsen *et al*., 2003).

Population incidence of PKU in Iranians: During the last few years several studies have been performed on PKU families in Iranian population. Although the exact population incidence of the disease is not clearly elucidated in Iranian population as no national newborn screen has been performed yet. However, reports indicated a relatively high incidence of the disease in the Iranian population from 1:3627 (Koochmeshgi *et al*., 2002) to 1:3700 (27.2:100000) (Golbahar and Honardar, 2002). In another report from a neonatal screening for PKU from 2000 to 2005 in Fars province, PKU was detected in newborn babies with incidence of 1:4698 (Senemar *et al*., 2009). Furthermore, a recent report indicated a much lower incidence of the disease of 1.7-1.5:10000 (Habib *et al*., 2010). Interestingly, the incidence of PKU due to BH4 deficiency was very low to 1:76966 (Karamifar *et al*., 2010).

The incidence of the PKU disease seems to be high among the mentally retarded individuals residing in institutions. The study performed by Vallian and coworkers in Isfahan (Vallian *et al*., 2003), and other studies indicated a high incidence of the disease among mentally retarded individuals from 2.1-5% (Ghiasvand *et al*., 2009). These investigations may suggest the PKU disease as one of the main causes of mental retardation in Iranian population. PKU disease was observed more frequent in children of consanguineous couples than non-consanguineous ones in Iran (Mokhtari and Bagga, 2003; Golbahar and Honardar, 2002; Kabiri, 1995). Therefore, the high incidence of consanguineous marriages could be related to the high population incidence of the disease among Iranians.

Population distribution of the *PAH* gene mutations: The *PAH* gene mutations have been studied in many populations around the world. Interestingly, some pathogenic mutations, such as R408W (Trp> Arg at amino acid 408 of the PAH protein) and IVS10nt-11 (in intron 10 nucleotide 11), occur at fairly quite high frequency in most of the studied populations, suggesting a role of founder effect for these mutations.

In our previous study performed in Iranian population, a number of PAH mutations were identified, which could be considered as the first report of PAH mutation spectrum in Iranian population (Vallian *et al*., 2003). These include R252W (15.38%), Q232Q (13.46%), R261Q (7.69%), delL364 (7.69%), IVS10-11g>a (5.77%), L333F (5.77%), V245V (5.77%) and S67P (3.85%). Mutations Q232Q and V245V, although silent, but since they were the only mutations found in the PKU affected individuals, were discussed as they interfere with tRNA trafficking in the cell (Vallian *et al*., 2003). The position of PAH mutations

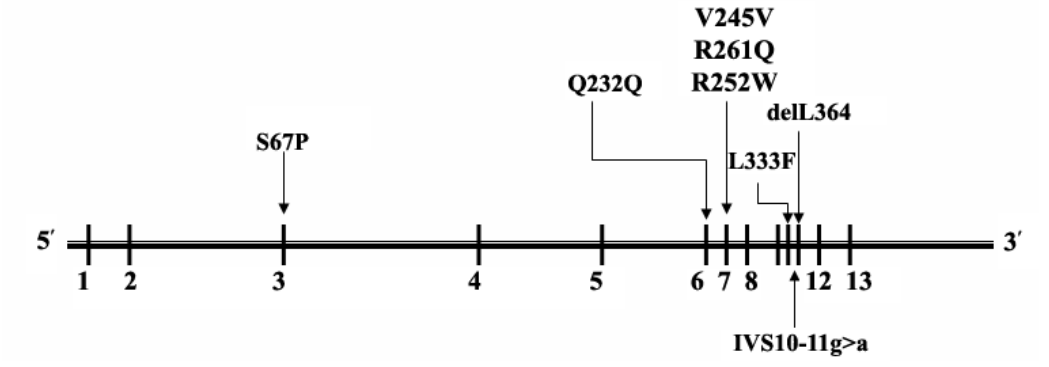

Figure 3. A diagram of a number of PAH mutations found in Iranian population, especially in Isfahan population. The numbered boxes correspond to the exons.

in *PAH* gene region detected in these Iranian population is shown in Figure 3. Among the mutations identified, S67P (missense mutation) showed the lowest frequency (3.55%), and the mutation R252W seemed to be the predominant one with the frequency of 16% (Table 1). Comparison between the frequency of PAH mutations in different populations indicated that the prevalence of R252W is 2-8%, and for Q232Q and V245V (silent mutations) varies from 2-45 % (Scriver *et al*., 2003). The mutation R261Q with the frequency of 7.1% in Iranian population is almost similar to the frequency reported from Turkey, which may be useful in the identification of PKU mutations in the Middle East region (Vallian *et al*., 2003).

In a recent study, 26 different mutations and 18 polymorphisms were reported among Iranian PKU patients. In this study, IVS10-11g>a mutation was reported as the mutation with the highest frequency (24.6%) (Zare-Karizi *et al*., 2010). Although the frequency of this mutation is low in Isfahan (central province of Iran), but this mutation was observed with high frequency in north of Iran. The mutations R252W, delL364, R261Q and L333F as long as two polymorphisms Q232Q and V245V were observed in this study as well as our previous study in Isfahan population (Zare-Karizi *et al*., 2010; Vallian *et al*., 2003). The IVS10-11g>a mutation was also found at high frequency (19.3%) in Iranian Azari Turkish Patients (Bonyadi *et al*., 2010). The PAH mutation spectrum of Azeri Turkish patients seems to be different from PKU patients in Isfahan. In fact, some mutations seem to be general to Iranian population (eg. IVS10-11g>a). However, different mutations may be specific to different regions of Iranian population (e.g. R252W in Isfahan). Therefore, more investigation on different populations and ethnics of Iranians are required to fully elucidate the PKU mutation spectrum in Iranian population.

The frequencies shown correspond to the following references: Iran (Vallian *et al*., 2003), Cuba (Desviat *et al*., 2001), Russia (Sueoka *et al*., 1999), Northern Ireland (Zschocke *et al*., 1995), Chile (Perez *et al*., 1999) and Brazil (Acosta *et al*., 2001).

The Haplotype and linkage disequilibrium (LD) Analysis of *PAH* Gene: PKU disease is highly heterogeneous at the molecular level. Therefore, mutation analysis at the *PAH* gene is a time-consuming and cost-effective procedure. Nowadays, the molecular markers linked to the *PAH* gene have been used in carrier detection and prenatal diagnosis in PKU families (Fazeli and Vallian, 2009 a). The haplotype data is often much more informative than only the genotype data (Zhao *et al*., 2003). Studies in European populations, in which PKU was a common disease, showed that haplotype frequency varied between different regions of the world (Kidd *et al*., 2000). Although the frequency of the PKU disease in Iranian populations is not precisely known yet (see above), it seems that Iran is one of the regions with relatively high incidence of the disease. The study on Iranian population also showed that haplotype frequency varied in Iranian population, and that the haplotype phasing was performed with great difficulty in Iranian families. The genotyping of other family members was necessary in most of the cases (Fazeli and Vallian, 2009 a,b; Vallian *et al*., 2009).

It has been reported that specific PAH haplotypes were associated with some mutations at the *PAH* gene.

Table 1. Comparison of a number of PKU mutations in Iranian patients with other populations of the world.

Mutation	Frequency (%)					
	Iran	Cuba	Russia	Northern Ireland	Chile	Brazil
R252W	15.38	5.3	1.7	1.7	7.8	6.5
Q232Q	13.46	0	0	0	0	0
R261Q	7.69	15.8	6.7	1.2	3.7	12.2
delL364	7.69	0	0	0	0	0
IVS10-11g>a	5.77	7.0	0	0	6.7	17.4
L333F	5.77	0	0	0.8	0	0
V245V	5.77	0	0	0	0	0
S67P	3.85	0	0	0.4	0	0
Other mutations	0	71.9	70	95.6	81.8	63.9
Unknown mutations	34.62	0	21.6	0.4	0	0

In a study performed in Denmark, two haplotypes were found significantly more common among PKU chromosomes than normal chromosomes (DiLella *et al*., 1986 b; DiLella *et al*., 1987; Kidd, 1987; Kidd *et al*., 2000). Up to now, more studies in different populations were performed to identify haplotypes associated with different PAH mutations (Desviat *et al*., 1997; Perez *et al*., 1997; Lucca *et al*., 1998; Perez *et al*., 1999; Zschocke and Hoffmann, 1999; Sueoka *et al*., 1999; Acosta *et al*., 2001; Desviat *et al*., 2001; O' Donnell *et al*., 2002; Stojiljkovic *et al*., 2007).

The studies of LD (linkage disequilibrium) in different populations indicated that LD among normal polymorphic markers differs in various populations of the world (Kidd *et al*., 2000; DeMille *et al*., 2002). These studies indicated less LD in Africans and significant LD in Europeans (Kidd *et al*., 2000). It should be noted that, LD was observed among the polymorphic markers located across both the ends of the *PAH* gene. However, LD was not significant between the polymorphic markers at the opposite ends of the *PAH* gene (Chakraborty *et al*., 1987; Daiger *et al*., 1989).

In our study on *Bgl*II, *Eco*RI and VNTR markers located in *PAH* gene region, no significant LD was observed between these markers in Iranian population (Fazeli and Vallian, 2009 a,b). In this study, the haplotype phase of *Bgl*II-*Eco*RI-VNTR at 20 family trios was determined. This function was investigated by the use of PedPhase program. The haplotype phasing at eight pedigrees was successfully performed. In addition to haplotype phasing, the origin of haplotype phase (maternal or parental) was distinguished in these pedigrees. The genotyping data of remainder families was not enough for haplotype phasing. The estimation of haplotype frequency of *Bgl*II-*Eco*RI-VNTR by using PedPhase and PHASE programs showed that the diversity of *Bgl*II-*Eco*RI-VNTR haplotype was high in the studied population. Therefore, the inferring of *Bgl*II- *Eco*RI-VNTR haplotype phase at most pedigrees in Iranian population required genotyping data obtained from other family members. The haplotype phase of only 32 from 60 individuals was determined by the use of PedPhase program. The comparison of haplotype frequency obtained from the two programs, PHASE and PedPhase, indicated that eight haplotypes had frequency higher than 5% (i. e. informative haplotypes). Since the genotyping data of single marker at PAH gene region was not informative in some families, the haplotype phasing could be used as a suitable method for detection of PKU disease. The high diversity of *Bgl*II-*Eco*RI-VNTR haplotype in Iranian population caused that the haplotype phasing was difficulty done in Iranian family trios. In this case, the genotyping of other family members is necessary in most cases. These data were consistent with high variety of PAH haplotypes observed in Iranian population (Fazeli and Vallian, 2009 a,b).

Evidence of balancing selection in *PAH* gene: It has been reported that PKU carriers in Ireland and West Scotland show protection against the toxic effects of ochratoxin A. PKU carrier women in these areas have low spontaneous abortion rate, as well. These observations and other molecular genetic data suggested an important role for balancing selection in individuals with PKU disease (Woolf *et al*., 1975; Woolf, 1976; Saugstad, 1977; Krawczak and Zschocke, 2003). We have recently investigated whether two polymorphisms, *Bgl*II and *Eco*RI, located in *PAH* gene were subjected to selective pressure in Isfahan population. The results indicated the presence of balancing selection on these polymorphisms in Isfahan population (Fazeli and Vallian, 2010).

Genotype/phenotype correlations: Correlation between mutations in *PAH* gene and clinical phenotypes have been demonstrated. A number of mutations have been related to mild PKU phenotype and mutations such as R297P, A322G, T380M were shown to be associated only with a non-PKU hyperphenylalaninemia. Some mutations led to the complete loss of enzyme activity and others were associated with a residual activity of the PAH enzyme. The combinations of these mutations would result in a full spectrum of phenotypes ranging from classic PKU to mild non-PKU. As an example among 31 mutations found on the residues involving the active site, mutation D143G was associated with a severe PKU phenotype (Erlandsen *et al*., 2003). Moreover, the G46S mutation in the regulatory domain was associated with classical PKU and could be considered as one of the most frequent diseased mutations from the clinical point of view.

Because of the involvement of both genotype and environmental factors in the pathogenesis of the PKU disease, PKU has been suggested as a 'multifactorial disorder', (Scriver and Waters, 1999). Even individuals with the same mutation (PAH genotype) may have a different PKU phenotypes. The factors acting on these variations include: blood-brain barrier, intestinal absorption of phenylalanine, rates of phenylalanine transamination and decarboxylation, proteosome

degradation of defective Phe-OH and possible effects of PAH polymorphisms on gene transcription. Consequently, the same genotype may result in different clinical and metabolic phenotypes (Disilvestre *et al*., 1991; Madden, 2004). As a result, recent genotype-phenotype correlation studies revealed that no simple and direct relationship may always be apparent between genotype and clinical phenotype in PKU patients.

Clinical Symptoms: The most important symptom of PKU is mental retardation. Affected individuals with PKU differ significantly from controls on full-scale IQ, processing speed, attention and motor control. The IQ of patients is usually under 50. Cognitive problems, microcephaly, neuropsychological abnormalities and psychosocial problems have been reported frequently in the patients, in both on and off dietary treatment. The developmental delay in PKU patients does not appear for months after birth. Vomiting, eczematoid rash and irritability may be observed very early in the life. Some of clinical features of the disease includes: severe mental retardation, musty odor, epilepsy (50%), extrapyramidal manifestations (e.g. Parkinsonism), and eye abnormalities (e.g. hypopigmentation). Moreover, pigmentation symptoms correlated with skin consisting of fair colored skin and hair (most characteristic skin manifestation resulting from impairment of melanin synthesis), eczema (including atopic dermatitis), light sensitivity, increased incidence of pyogenic infections, increased incidence of ceratosis pilaris, decreased number of pigmented nevi and scleroderma like plaques. The patients may show limitation of mobility. However, hyperactivity is common in these patients. The stature of these individuals may be short. The minor malformations, including widely spaced incisor teeth, pes planus, partial syndactyly, and epicanthus were seen in these patients (Nyhan *et al*., 2005; Madden, 2004; Scriver and Kaufman, 2001; Huttenlocher, 2000; Pitt and Dansk, 1991; Verkerk *et al*., 1991).

Treatment and Management of PKU Disease: Since the 1960s, newborn screening for PKU has been performed in order to manage an early treatment of the disorder. The treatment of PKU consists of selectively restricting intake of Phe and supplementing tyrosine (the product of normal PAH enzyme activity). Moreover, special medical foods which are devoid of or low in Phe while providing enough additional protein, vitamins, and minerals to support normal growth are included. In earlier therapeutic protocols, treatment was only continued through the first few years of life, corresponding to the age at which brain myelination was completed (Committee on Genetics, 2008). In more recent studies, it has been shown that brain MRI abnormalities were observed in adults who were on unrestricted Phe intake. Therefore, it was recommended that the treatment strategy to be continued into adulthood (Committee on Genetics, 2008; Hahnel, 2008). Strict adherence to the PKU diet is especially important for women during their reproductive years because of the high risks to the fetus (Committee on Genetics, 2008).

Due to the difficulties in diet therapy for the treatment of PKU, efforts are directed toward alternative ways. There are some reports on gene therapy using different methods of gene delivery. The goal of gene therapy for PKU has been to restore the PAH enzyme activity permanently in the liver (Ding *et al*., 2004). The other proposed treatment for PKU disease was enzyme replacement therapy (ERT). Two enzyme systems were developed for treatment of PKU: the PAH enzyme and the Phe-degrading enzyme from plants named phenylalanine ammonia-lyase (PAL). There have been considerable advantages in ERT therapy to treat PKU; i) PAL therapy does not require any cofactors for degrading Phe. ii) The PAL product has a very low toxicity and no embryotoxic effects have been observed in laboratory animals. iii) The PAL product is converted to benzoic acid in the liver, excreting via the urine. iv) PAL is very stable under a wide temperature range (Hoskins and Gray, 1982; Kim *et al*., 2004).

Concluding Remarks: The incidence of PKU disease varies widely in different populations of the world. The incidence ranges from 1 in 4,500 births among the Ireland population to fewer than one in 100,000 births among the Finland population (Guldberg *et al*., 1995; DiLella *et al*., 1986 a). Nowadays, PKU diagnosis is based on the aberrant metabolic phenotype, determination of disease causing mutations and associated polymorphic haplotypes (Williams *et al*., 2008). It is now well documented that if the PKU disease is diagnosed soon after birth, the irreversible mental retardation could be prevented. This requires an active neonatal screening and carrier detection followed by on time prenatal diagnosis in all populations around the world (Vallian and Moeini, 2006). This must be considered as a national mandatory program especially in populations at risk, such as the Iranian population.

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