대한 유전성 대사질환 학회지
제4권 제1호, pp.5-12, 2004

Mutational Analysis of Korean Patients with Phenylketonuria

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= **Abstract** =

Purpose Phenylketonuria is an inborn error of metabolism, which is inherited as an autosomal recessive trait. PKU is resulting from deficiency of phenylalanine hydroxylase. *PAH* gene spans about 90 kb on chromosome 12q and comprises 13 exons. In order to define the genetic basis of PKU and the frequencies and distribution of *PAH* mutations in the Korean population, we analyzed *PAH* gene in independent 80 patients with PKU.

Methods All 13 exons including exon-intron boundaries and 2 kb of 5' upstream region of the *PAH* gene were analyzed by PCR- direct sequencing methods.

Results *PAH* gene analysis revealed 39 different mutations including 10 novel mutations. The novel mutations consisted of 9 missense mutations (P69S, G103S, N207D, T278S, P281A, L293M, G332V, S391I and A447P) and a novel splice site variant (IVS10-3C>G). R243Q, IVS4-1G>A, and E6-96A>G were the most relevant mutations and they accounted in the whole for 38% of the mutant alleles identified in this study. We also observed that BH_4 responsibility was associated with genotype of R241C, R53H and R408Q.

Conclustion Our present study with 80 participants extends the previous results to more comprehensive understanding of *PAH* allele distribution and frequency in Koreans. Although Korean mutation profile of *PAH* is similar to those of the nearest oriental populations (Japanese, Chinese, and Taiwanese), several different characteristic features are revealed. The characterization of the genotype -phenotype relationship was also performed. Our data would be very useful information for diagnosis, genetic counseling and planning of dietary and therapeutic strategies in Korean PAH patients.

Key Words : Phenylketonuria, PKU, Phenylalanine hydroxylase, PAH, Mutation, Allele, Frequency, Korean

Introduction

Phenylketonuria (PKU; MIM 261600) is an inborn error of metabolism, which is inherited as an autosomal recessive trait. The incidence of PKU are variable among ethnic populations; 1/10,000 in Caucasian, 1/120,000 in Japanese and 1/18,000 in Chinese[1]. In Korea, it was estimated about 1/41,000 by the neonatal screening of PKU that was introduced in 1998.

PKU is resulting from deficiency of phenylalanine hydroxylase (PAH). *PAH* gene spans about 90 kb on chromosome 12q and comprises 13 exons. PAH is a hepatic enzyme, which catalyzes hydroxylation of phenylalanine to tyrosine using tetrahydrobiopterin (BH_4) as cofactor. It has three structural domains consisting of N-terminal regulatory domain, a catalytic domain, and a C-terminal tetramerization domain. The whole active PAH enzyme is composed of the four monomeric proteins. Recent studies of PAH crystal structure have provided the information on active site, binding sites of substrate and cofactor.

Mutation profile of *PAH* gene was not restricted to any one region but spreaded throughout the whole structural domains with enormous diversity. Until now, over 460 different mutations of *PAH* gene have been identified and recorded in the PAH Mutation Analysis Consortium Database (PAHdb)[2]. The severity of disease was also diverse from mild hyperphenylalaninemia to classical PKU, which are characterized by pretreatment blood-phenylalanine levels or dietary tolerance[3]. There were several studies to find the relationship between genotype and diverse phenotypic expression. Once the relationship between genotype and the phenotypic expressivity is revealed, it would be very useful information for planning dietary and therapeutic strategies.

Therefore, we analyzed *PAH* gene in 80 patients with PKU and their families to study genotype-phenotype relationship, and to help genetic counseling. Furthermore, we analyzed the mutation spectrum of *PAH* gene in Korean patients and compared them with those of other ethnic groups, including Japanese and Chinese.

Subjects and Methods

Subjects

This study was approved by institutional review board of NIH, Korea. This study included 80 unrelated families with PAH deficiency. Participants were recruited from the Korean PKU family support group. Most of them were primarily identified in neonatal screening and PAH deficiency was diagnosed by conventional biochemical methods. Informed consents for DNA analysis were obtained from the patients and their parents.

Mutation analysis

Genomic DNA was isolated from peripheral blood leukocytes using the QIAamp DNA blood kit following the manufacturer's

instruction (Qiagen, Hilden, Germany). All 13 exons including exon-intron boundaries and 2 kb of 5' upstream region of the *PAH* gene were amplified by PCR. PCR amplicons were extracted from an agarose gel using gel extraction kit (Qiagen). Direct sequencing was performed using a BigDye Terminator Cycle Sequencing Ready Reaction kit, version 2.0 (PE Applied Biosystems, Foster City, CA, USA) and analyzed with an ABI 3100 automated sequencer (PE Applied Biosystems) according to the standard methods. In addition, *PAH* genes in 50 normal individuals were analyzed to confirm that the novel sequence variations were not polymorphisms but real pathogenic mutations. Novel mutations were defined by exclusion from the PAHdb (http://www.pahdb. mcgill.ca) and previously reported mutations on Pubmed (http://www. ncbi.nlm.nih.gov/PubMed/).

Results and Discussion

PAH nucleotide sequence analysis of the 80 unrelated PKU probands revealed 39 different mutations (Tables 1 and 2). Among 80 patients, two mutation alleles were detected in 59 patients (74 %), either heterozygous or homozygous (52 and 7, respectively). Only one mutation allele was revealed in 19 patients, and no mutation was detected in 2 patients. Ten novel mutations were identified in this study. These novel mutations included 9 missense substitutions, P69S, G103S, N207D, T278S, P281A, L293M, G332V, S391I, and A447P. Glycine^{103} are conserved among human, mouse and rat. The other mutated amino acid residues are even more strictly conserved among human, mouse, rat and zebrafish. A novel splice site variant, IVS10-3C>G., was also detected. The -3 sequence of splicing acceptor site is a strictly conserved sequence and this substitution might result in the aberrant splicing products. No novel frameshift mutation or nonsense mutation was detected in this study.

The R243Q, IVS4-1G>A, and E6-96A>G were the most relevant mutations in Korean PKU patients (Table 3). They have been reported as one of the most frequent mutations in Asian populations[4] and accounted in the whole for 51 of the 136 mutant alleles (38 %) identified in this study.

It is well known that each ethnic group has its own distinctive and diverse *PAH* mutant allele series that includes one or a few prevalent founder alleles[5]. In comparison of *PAH* mutation data among ethnic groups, it shows the correlations between mutation and genetic history of investigated population. In Europe, there are several prevalent founder alleles including R408W, IVS10-11G>A, and Y414C, representing the result of expansion, migration, and genetic drift of European populations[5]. Especially, R408W mutation frequency covers 20 to 84 % in Eastern Europe and Germany. However, these mutations were rarely detected in oriental populations. In the previous study, Okano et al. reported the frequency and distribution of *PAH* gene mutations among Japanese, Korean and Chinese patients[4]. Since

the study was done in the early 1990s, it was restricted to screening for previously isolated mutations. Unidentified but relatively frequent allele such as R241C was not adjusted in that study. Only 10 Korean patients were subjected in that study, which was relatively small number to represent Korean allelic distribution. Our present study with 80 participants extends the previous results to more comprehensive understanding of *PAH* allele distribution and frequency in Koreans. Although some overlaps of mutant allele distribution were observed among Japanese, Chinese and Korean populations, there were several significant differences (Table 3). R243Q, E6-96A>G and IVS4-1G>A, the most frequent mutations in our study, are also frequently detected in Japanese, Chinese and Taiwanese. However, R111X is recurrent mutation in Japanese and Chinese patients but very rare genotype in Korean patients. R413P is the most prevalent allele in Japanese but very small proportion of probands has R413P allele in Korean and Taiwanese. IVS4-1G>A occupied relatively larger proportion in Korean mutant allele profile than in Japanese or Chinese. Although A259T was not detected at any other oriental population studies, it was identified at 9 different families in this study.

Interestingly, R241C homozygous patient showed mild hyperphenylalaninemia (MHP) and R241C heterozygous individuals (two with R241C/R243Q, and other two with R241C/A259T) showed BH_4 responsiveness. In the previous study, R241C was reported as a BH_4 responsive allele and 25 % of activity was remained *in vitro* experiment[6]. Guldberg et al. assigned the patient with R241C genotype to the MHP category[3]. Arginine[241] is located at near the cofactor binding region and does not directly interact with the cofactor, so the mutation may lead to relatively mild structural deformities[7]. Our data were consistent with these previous reports.

Patient 3 (genotyped with Y356X/R408Q) and patient 26 (genotyped with R53H/R243Q) also represented BH_4 responsiveness. The fact that Y356X is a null mutation and R408Q allele presents a mild phenotype in PAHdb, indicates that R408Q is a BH_4-responsible allele in patient 3. As R243Q is associated with classical PKU both in PAHdb and in our study, the R53H might introduce the response in patient 26. As the responsibility of R408Q or R243Q is not reported yet, our observation extended the BH_4-responsible allele series.

In summary, we screened the *PAH* gene from 80 Korean PKU affected families and identified 39 mutations including 10 novel mutations. Although Korean mutation profile of *PAH* is similar to those of the nearest oriental population, there are several different characteristic features. The relationship of genotype and phenotype, especially BH_4 responsiveness of some patients was also described. This study would contribute to the diagnosis, genetic counseling and planning of the dietary and therapeutic strategy in Korean PKU patients.

Table 1. Genotypes for mutations of *PAH* gene in 80 Korean PKU patients.

Patient No.	*PAH* allele 1	*PAH* allele 2	Patient No.	*PAH* allele 1	*PAH* allele 2
1	IVS4-1G/A	A259T	41	R243Q	V388M
2	P407S	R413P	42	P69S	R261Q
3*	Y356X	R408Q	43	S70[del]	IVS4-1G>A
4	S70[del]	L255S	44	IVS4-1G>A	?
5	N207D	Y325X	45	R413P	?
6	Y204C	?	46	Y204C	?
7	Y204C	?	47	W187X	Y356X
8	Y356X	?	48	IVS4-1G>A	R243Q
9	IVS4-1G/A	R243Q	49	R243Q	?
10	R241C	T278I	50*	R241C	A259T
11	R243Q	?	51	IVS4-1G>A	S310F
12	R243Q	?	52	Y204C	Y325X
13	A259T	T278I	53	R243Q	A345T
14	A345T	G332V	54	IVS4-1G>A	T278S
15	G103S	R413P	55	T278I	R413P
16	IVS4-1G>A	V388M	56	G332E	?
17	R243Q	R252Q	57	A259T	?
18	A259T	?	58	T278I	Y356X
19	R413P	Y325X	59	Y204C	R243Q
20	ivs2nt-2T>C	?	60*	R241C	A259T
21	Y204C	Y204C	61	IVS10-3C>G	IVS10-3C>G
22	Y325X	V388M	62	Y204C	P281L
23	Y204C	?	63	R53H	V388M
24	IVS4-1G>A	V388M	64	A447P	?
25**	R241C	R241C	65	R243Q	A345T
26*	R53H	R243Q	66	R176X	S391I
27	N207D	?	67	IVS4-1G>A	R261X
28	Y204C	Y204C	68*	R241C	R243Q
29	R111X	R243Q	69	IVS4-1G>A	IVS4-1G>A
30	A259T	?	70	R243Q	Y325X
31	R176X	A259T	71	Y356X	?
32	IVS4-1G>A	?	72	IVS4-1G>A	P281L
33	Y204C	Y356X	73	G239S	P281L
34	R158Q	R243Q	74	R243Q	Y356X
35	IVS4-1G>A	L293M	75	Y204C	Y204C
36	IVS4-1G>A	Y356X	76	R241C	R241C
37	R243Q	P281A	77	A259T	T278I
38	D84Y	Y356X	78	Y204C	R243Q
39*	R241C	R243Q	79	?	?
40	Y204C	?	80	?	?

* BH4-responsive type
** mild hyperphenylalaninemia

Table 2. Spectrum of *PAH* mutations detected in this study.

Amino acid change	Normal	Mutation	Location	Allele Frequency	Relative Frequency (%)	References
R53H	CGC	CAC	exon 2	2	1.3	1
IVS2-2T>C			intron 2	1	0.6	2
P69S	CCT	TCT	exon 3	1	0.6	novel
S70[del]	TCT	c.208-210delTCT	exon 3	2	1.3	1
D84Y	GAT	TAT	exon 3	1	0.6	1
G103S	GGT	AGT	exon 3	1	0.6	novel
R111X	CGA	TGA	exon 3	1	0.6	1
IVS4-1G>A	GT	AT	intron 4	16	10.0	1
R158Q	CGG	CAG	exon 5	1	0.6	1
R176X	CGA	TGA	exon 6	2	1.3	1
W187X	TGG	TAG	exon 6	1	0.6	1
E6-96A>G			exon 6	16	10.0	1
N207D	AAT	GAT	exon 6	2	1.3	novel
G239S	GGT	AGT	exon 7	1	0.6	1
R241C	CGC	TGC	exon 7	9	5.6	1
R243Q	CGA	CAA	exon 7	19	11.9	1
R252Q	CGG	CAG	exon 7	1	0.6	3
L255S	TTG	TCG	exon 7	1	0.6	1
A259T	GCC	ACC	exon 7	9	5.6	1
R261Q	CGA	CAA	exon 7	1	0.6	1
R261X	CGA	TGA	exon 7	1	0.6	1
T278I	ACC	ATC	exon 7	5	3.1	1
T278S	ACC	AGC	exon 7	1	0.6	novel
P281L	CCT	CTT	exon 7	3	1.9	1
P281A	CCT	GCT	exon 7	1	0.6	novel
L293M	TTG	ATG	exon 8	1	0.6	novel
S310F	TCT	TTT	exon 9	1	0.6	1
Y325X	TAC	TAG	exon 10	5	3.1	4
G332E	GGG	GAG	exon 10	1	0.6	1
G332V	GGG	GTG	exon 10	1	0.6	novel
A345T	GCT	ACT	exon 10	3	1.9	1
IVS10-3C>G			intron 10	2	1.3	novel
Y356X	TAC	TAA	exon 11	9	5.6	1
V388M	GTG	ATG	exon 11	5	3.1	1
S391I	AGT	ATT	exon 11	1	0.6	novel
P407S	CCT	TCT	exon 12	1	0.6	1
R408Q	CGG	CAG	exon 12	1	0.6	1
R413P	CGC	CCC	exon 12	5	3.1	1
A447P	GCC	CCC	exon 13	1	0.6	novel
Total				136	85	

1. Mutations are reported in the PAHdb.
2. This mutation is reported by Song et al.[8]
3. This mutation is reported by Chien et al.[9]
4. This mutation is reported by Park et al.[10]

Table 3. Relative frequencies of common PAH gene mutation found in oriental populations.

Mutation	Relative frequencies (%, allele frequency/total subject chromosome)			
	80 Korean	41 Japanese (Okano et al. 1998)	52 Chinese (Okano et al. 1992)	25 Taiwanese (Chien et al. 2004)
R243Q	11.9	7.3	18.3	6
IVS4-1G/A	10.0	7.3	7.7	2
Y204C	10.0	6.1	11.5	4
R241C	5.6	7.3	nd	32
A259T	5.6	0	nd	0
Y356X	5.6	4.9	6.7	0
T278I	3.1	7.3	nd	0
Y325X	3.1	0	nd	0
V388M	3.1	1.2	nd	0
R413P	3.1	30.5	8.7	4
R111X	0.6	3.7	10.7	0
R408Q	0.6	0	nd	14
Total detected	85.0	92.7	66.5	90.0

Acknowledgements

This study was supported by intramural research fund from National Institute of Health, Korea. The authors thank the members of Korean PKU family support group for the contribution and cooperation in this research. This study was supported by intramural research fund from National Institute of Health, Korea.

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