RESEARCH NOTE

Mutation characteristics of the *PAH* gene in four nationality groups in Xinjiang of China

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Introduction

Xinjiang in northwest China was part of the ancient Silk Road, and previous studies have shown that there was an extensive genetic admixture in human population in the Silk Road region. Combined with historical records, studies on ethno-origin, migratory history, and marriage customs may contribute to an understanding of the role of different factors in shaping the genetic structure of different ethnic populations in this interesting region.

Phenylketonuria (PKU) is a common, autosomal recessive disorder of amino acid metabolism, caused by the deficiency of the hepatic enzyme phenylalanine hydroxylase (PAH) that controls L-phenylalanine catabolism. The incidence of PKU determined by international newborn mass screening is higher in Caucasian (northern Irish 1/4404, German 1/6971 and American 1/10059) (Bickel *et al*. 1981), than in Asian (Japanese 1/120000, Korean 1/41000) (Aoki and Wada 1988; Lee *et al*. 2004) populations. The average incidence of PKU in populations of Mainland China is 1/11188, which is higher than that in Taiwanese (1/55077) (Chien *et al*. 2004). Since the human *PAH* gene was cloned (Woo *et al*. 1983), more than 500 different alterations have been identified and listed in the *PAH* mutation database. In China, 70 different mutations were detected, with marked differences in the spectrum of mutations and in the degree of heterogeneity among different ethnic groups from diverse geographical origins (Song *et al*. 2005a). Xinjiang is a multinational region, an hinterland located between China and Europe. Mutation analysis of *PAH* in populations of Xinjiang could, therefore, be helpful for further understanding of the correlation between the genotype and phenotype, and for genetic counselling and prognostic evaluation of future cases of PKU in the region.

A previous study showed that the prevalent mutations and most of the novel mutations of *PAH* gene in Chinese were located in exons 3, 5, 6, 7, 11 and 12 (Song *et al*. 2007). In this study, the six exons and flanking sequence of *PAH* gene in 46 PKU patients from the Han, Uighur, Kazakh and Hui nationalities in Xinjiang were studied. A total of 20 different mutations were detected in 68 of 92 mutant alleles (73.9%). The most prevalent mutations *R243Q, Y204C, R111X, Y356X* and *V399V* were similar to the populations in northern China. The next most prevalent mutations *Fl61S, L255S, P281L* and *R413P*, however, were significantly different from the populations in other areas of China. For the first time in the three minority nationalities (Uighur, Kazakh and Hui) in Xinjiang 13 different mutations were identified. The mutation distribution of PKU in Xinjiang displayed a distinct ethnic pattern, suggesting that not only a distinct and conservative, but also a crossed and syncretic genetic pattern exists in Xinjiang.

Materials and methods

Subjects

A total of 46 patients (24 males and 22 females) with PAH deficiency, aged between 3 days and 27 years, in 46 independent families were used for this study. The patients were from 15 different towns, villages and cities in Xinjiang, including 34 Han, eight Uighur, three Hui and one Kazakh. Patients of tetrahydrobiopterin responsive phenylalanine hydroxylase deficiency were excluded from this study by tetrahydrobiopterin loading and phenylalanine tolerance test. Blood samples (5 ml) were collected from each patient, after obtaining informed consent from their parents

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or guardians. Parental samples were available for some of the families. There was no consanguinity among the families studied.

DNA preparation and PCR amplification

Genomic DNA was extracted from leucocytes of patients according to standard protocols. The six exons (3, 5, 6, 7, 11 and 12) and the exon-flanking intronic sequences of *PAH* gene were amplified by PCR. The sequences of primers for PCR were designed in accordance with the literature (primer data not shown), without the CG clamp at the 5′ end (Song *et al*. 2005a). Reaction mixtures were made up to the volume of 25 μl containing 0.5 μg of genomic DNA, 2 pmol/l of each primer, 0.25 mM of each dNTP, 1× reaction buffer and 1.5 U *Taq* DNA polymerase. The PCR conditions were 97°C, for 5 min for initial denaturing; followed by 30 cycles of 94°C, for 45 s, 55°C for 45 s, and 72°C for 45 s, and 72°C for an additional 8 min. The PCR product was electrophoresed on 2% agarose gel.

Single-strand conformation polymorphism analysis

Five μl of each PCR product was mixed with 5 μl of denaturation solution (95% formamide, 20 mM EDTA, 0.05% bromophenol blue and 0.05% xylene cyanol), heated for 5 min at 97°C, and then immediately cooled on ice. Samples were separated on 8% nondenaturing PAGE gel. For electrophoresis of the different fragments, the conditions were varied within the following ranges: 3.5–5.5 h, 10°C–28°C, 30–60 W, 550–600 V, for a 20 cm gel. The gel was stained with silver nitrate for visualizing the fragment migration. The samples showing abnormal SSCP migration patterns were sequenced to confirm the mutations. PCR products were purified and directly sequenced by ABI 377 DNA sequencer (Applied Biosystems, Foster City, USA).

Results

Identification of the PAH mutations

Mutations were identified for 68 of 92 alleles in the present study, representing a mutation detection rate of 73.9% (table 1). A total of 20 different mutations were identified, including 14 missense, three nonsense and three splice mutations. The most common mutations among the patients were *R243Q, Y204C, R111X, Y356X* and *V399V*, representing 21.7%, 10.9%, 5.4%, 4.4% and 4.4% of the 92 mutant alleles, respectively. The next most common mutations were *R413P, F161S, L255S* and *P281L*, each of them showing a relative frequency of 3.3%. Among the 20 mutations, it was the second time that *E280G* and *A434D* mutations were reported in the world. In China, *L255S, P281L, R261Q*, and *I65T* mutations were reported for the second time. In this series of cases, exon 7 and its flanking introns contain the greatest number of mutant alleles (32.6%). The other

Table 1. The *PAH* mutations identified in Xinjiang.

Trivial name (protein effect)	Systematic name (DNA level)	Location	Characters of mutation	No. of alleles	RF▲ (%)
I65T	c.194T>C	Exon 3	Missense	1	1.1
R111X	c.331C>T	Exon 3	Missense	5	5.4
IVS4-1G>A	c.442-1G>A	Exon 5	Splicing	2	2.2
R158Q	c.473G>A	Exon 5	Missense	1	1.1
F161S	c.482T>C	Exon 5	Missense	3	3.3
Y166X	c.498C>G	Exon 5	Nonsense	1	1.1
R176X	c.526C>T	Exon 6	Nonsense	2	2.2
Y204C	c.611A>G	Exon 6	Splicing	10	10.9
R243Q	c.728G>A	Exon 7	Missense	20	21.7
G247V	c.740G>T	Exon 7	Missense	1	1.1
L255S	c.764T>C	Exon 7	Missense	3	3.3
R261Q	c.782G>A	Exon 7	Missense	1	1.1
E280K	c.838G>A	Exon 7	Missense	1	1.1
E280G	c.839A>G	Exon 7	Missense	1	1.1
P281L	c.842C>T	Exon 7	Missense	3	3.3
Y356X	c.1068C>A	Exon 11	Nonsense	4	4.4
V399V	c.1197A>T	Exon 11	Splicing	4	4.4
R408W	c.1222C>T	Exon 12	Missense	3	3.3
R413P	c.1238G>C	Exon 12	Missense	1	1.1
A434D	c.1301C>A	Exon 12	Missense	1	1.1
Detected				68	73.9
Total				92	100

▲RF, relative frequency.

exons and their flanking intronic sequences with mutant alleles, listed from a high-to-low mutation frequencies are: exons 6 (13.0%), 11 (8.7%), 5 (7.6%), 3 (6.5%) and 12 (5.4%).

PAH mutations in three minority nationality PKU patients

Thirteen different mutations, including eight missense mutations, one splice mutation and three nonsense mutations, were identified in 18 of 24 mutant alleles (19.6%; table 2) in the three minority nationality of PKU patients. The *P281L, R111X, Y204C* and *R176X* were the most prevalent mutations. It was for the first time that 13 different mutations were detected in Uighur, Kazakh and Hui PKU patients.

Discussion

A previous study showed that the prevalent mutations and most of the novel mutations of *PAH* gene in Chinese were located in exons 3, 5, 6, 7, 11 and 12, with a combined relative frequency of 84.6% (Song *et al*. 2007). Therefore, we screened six exons and flanking sequences in *PAH* genes of Xinjiang PKU patients. Mutations were identified for 68 of 92 PKU alleles in the present study, representing a mutation detection rate of 73.9%, which was lower than that reported by Song L. *et al*. (2003) and Song *et al*. (2007), who detected all the 13 exons or most of the exons in *PHA* genes, but higher than that reported in other areas of China (Wang *et al*. 1999; Zhang *et al*. 2004). Among the six exons, E7 and its flanking introns contain the greatest number of mutant alleles (30/48, 62.5%). E6 and its flanking introns contain 12 mutant alleles with a relative frequency of 54.5% (12/22). These results suggest that these two exons should be first chosen in genetic counselling and prognostic evaluation of future cases of PKU in Xinjiang.

Among the 46 patients, four of them were homozygotes and 21 were compound heterozygotes. Eighteen patients had only one mutant allele detected. The result was consistent with the previous report that most PKU patients in China were compound heterozygotes (Song *et al*. 2005a). In four patients (compound heterozygotes, 4/46, 8.7%), the observed phenotypes were inconsistent with the expected genotype. Data on *PAH* mutation genotypes and corresponding metabolic phenotypes in 686 PKU patients referred to seven European centres showed that the proportion of patients for whom the observed phenotype did not match the predicted phenotype was 4%–23% (Guldberg *et al*. 1998). A potential cause of these inconsistencies may relate to the biological properties and functions of the mutant protein.

Among the 20 different mutations we found, the most prevalent mutations *R243Q, Y204C, R111X, Y356X* and *V399V* were similar to the populations in northern China. However, the next most prevalent mutations *F161S, L255S, P281L* and *R413P* were significantly different from the populations in other areas of China. The *E280G* and *A434D* mutations were identified for the first time in the world. In China, it was for the second time that *L255S, P281L, R261Q* and *I65T* mutations were found (Song F. *et al*. 2003, 2005b), but *L255S* and *P281L* were more prevalent in Xinjiang, compard to the other parts of China. Our results support the view that *R243Q, Y204C, R111X, Y356X, V399V, R413P* and *IVS4-1 A > G* detected in this study are common mutations distributed in Asia (Okano *et al*. 1998; Chien *et al*. 2004; Lee *et al*. 2004). The *P281L, R158Q, R408W, R261Q, I65T* and *E280K*, on the other hand, are common mutations distributed in Europe and Latin-America (Acosta *et al*. 2001; Yang *et al*. 2001; Zschocke *et al*. 2003). However, *L255S, F161S, Y166X, E280G, G247V* and *A434D* are Chinese-characteristic mutations (Zhang *et al*. 1995; Wang *et al*. 1999; Song *et al*. 2007) (table 3). The present study showed that there is not only a consanguineous relation but also a distinct difference in *PAH* characters of mutations between Xinjiang and the other parts of China, or the other countries of central Asia, suggesting that Xinjiang might be a special *PAH* gene distribution region.

Table 2. Distributions of the *PAH* gene mutations in three minority nationality PKU patients in Xinjiang.

Nationalities	Trivial name	Systematic name	Location	Characters of mutation	No. of alleles	RF▲ (%)
Uighur	*R111X*	c.331C>T	Exon 3	Missense	2	2.2
	R158Q	c.473G>A	Exon 5	Missense	1	1.1
	F161S	c.482T>C	Exon 5	Missense	1	1.1
	Y204C	c.611A>G	Exon 6	Splicing	2	2.2
	R243Q	c.728G>A	Exon 7	Missense	1	1.1
	P281L	c.842C>T	Exon 7	Missense	3	3.3
	R413P	c.1238G>C	Exon 12	Missense	1	1.1
Hui	*IVS4-1G>A*	c.442-1G>A	Exon 5	Splicing	1	1.1
	R176X	c.526C>T	Exon 6	Nonsense	2	2.2
	R243Q	c.728G>A	Exon 7	Missense	1	1.1
	R261Q	c.782G>A	Exon 7	Missense	1	1.1
Kazakh	*F161S*	c.482T>C	Exon 5	Missense	1	1.1
	Y204C	c.611A>G	Exon 6	Splicing	1	1.1

▲RF, relative frequency.

Table 3. Comparison of relative frequencies of *PAH* gene mutaions between Xinjiang and other areas of China and other countries*.

Characters of mutations	Xinjiang	Northern China	Southern China	Taiwan	Japan	Keuro	Icelander	Dutch	Czech	Irish	Belgian	German	Croazian	Lithuanian	Greek	Brazilian	US (Texas)
R243Q	21.7	21.7	9.5	6.0	7.0	12.0	-	-	3.0	3.0	2.0	1.9	-	-	-	1.3	-
EX6-96A>G	10.9	10.2	10.7	4.0	6.0	10.0	-	-	-	-	-	-	-	-	-	-	-
R111X	5.4	8.3	5.2	4.0	3.7	0.7	3.0	-	-	-	-	-	1.3	0.5	-	-	-
Y356X	4.4	6.1	7.7	-	5.0	6.0	-	-	-	-	-	-	-	-	-	-	-
R413P	3.3	6.5	7.1	4.0	31.0	3.0	-	-	-	-	-	-	-	-	-	-	-
IVS4-1G>A	2.2	3.5	-	2.0	7.0	10.0	-	-	-	-	-	-	-	-	-	-	-
V399V	4.4	4.1	7.7	2.0	-	-	-	-	-	-	-	-	-	-	-	-	-
P281L	3.3	0.4	-	-	-	2.0	19.0	1.0	1.0	1.0	6.0	3.6	11.0	1.0	10.0	2.1	-
R158Q	1.1	0.9	-	-	-	-	-	13.0	5.0	1.0	9.0	4.5	1.3	7.1	3.0	3.5	1.0
R408W	1.1	0.4	-	-	-	-	3.0	1.0	55.0	41.0	5.0	24.9	37.0	73.4	-	3.5	19.0
R261Q	1.1	0.7	-	-	-	1.0	-	18.0	2.0	1.0	4.0	6.0	9.0	0.5	-	12.2	-
I65T	1.1	0.4	-	-	-	-	-	-	1.0	20.0	2.0	2.0	-	-	-	3.5	1.0
E280K	1.1	0.7	-	-	-	-	-	4.0	-	1.0	1.0	-	-	1.0	5.0	-	3.0
R176X	2.2	1.1	-	-	-	-	-	-	-	-	-	1.1	-	0.5	-	1.7	-
Y166X	1.1	1.7	7.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-
G247V	1.1	1.1	3.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F161S	3.3	1.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L255S	3.3	0.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E280G	1.1	0.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A434D	1.1	0.9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

*Northern China Area comprises of Beijing, Hebei, Inner Mongolia, Jilin, Liaoning etc. Southern China area comprises of Shanghai, Jiangsu, Zhejiang, Anhui and Yunnan. References, Northern China (Song *et al*. 2007); Southern China (Zhang *et al*. 1995; Song *et al*. 2007); Taiwan (Chien *et al*. 2004); Japan (Okano *et al*. 1998); Keuro (Lee *et al*. 2004), Icelander, Dutch, Czech, Irish, Belgian, German, Croatian, Lithuanian and Greek (Zschocke *et al*. 2003), Brazilian (Acosta *et al*. 2001) and Texan (Yang *et al*. 2001).

Thirteen different mutations were found in Xinjiang minority nationality PKU patients with relative frequency of 19.6% (18/92). The mutations detected in the present study comprise not only Asian, European, Latin-American and Chinese-characteristic mutations, but also some interspersed mutants. The most prevalent mutation *P281L* was infrequent in China and other Asian countries, but was common in Europe and in Latin-America. It suggests a complex genetic context and distinct ethnical and regional characteristics exists in Xinjiang, which is in accord with our previous study on hemoglobinpathy and β-thalassemia in the Silk Road region (Yu *et al*. 1998, 2001).

Xinjiang, located in northwest China, is in the vicinity of and sometimes has been regarded as part of Central Asia. This region is famous for being home to part of the ancient Silk Road and has undergone unceasing migration events through present day, maintaining various cultures (Ge *et al*. 1997; Chen 1999). Previous studies have shown that there was an extensive genetic admixture in the Silk Road region. Many ethnic groups now live in central Asia as a result of migration of their ancestors to this region. Thus, Xinjiang could probably be an ideal genetic resource repertoire for studying diversity of gene mutations, heterogeneity of *PAH* gene, and human migration.

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