Original Article

The molecular epidemiology of hyperphenylalaninemia in Uygur population: incidence from newborn screening and mutational spectra

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Background: Neonatal hyperphenylalaninemia (HPA) screening did not begin until 2009 in the Uygur population because of poor medical and economic conditions. This study intended to investigate HPA incidence rate and characterize mutation spectrum of phenylalanine hydroxylase (*PAH*) gene within the Uygur population.

Methods: Cross-sectional data of National Direct Reporting System database from 2009 to 2016 were used to calculate incidence rate. All HPA positive newborns were diagnosed and confirmed by Sanger sequencing. A low Phe diet was implemented.

Results: A total of 580,608 Uygur neonates were screened, 111 were diagnosed with HPA with an incidence rate of 1:5,230, 58 different mutations in *PAH* gene were detected. Eight novel variants were found, including two nonsense mutations (L11*, L197*), two splicing mutations (IVS12-2A > C, IVS13-1G > A), one frameshift mutation (K115 > Hfs) and three missense mutations (E368K, E370G, D435V), distributing in twenty patients. A104D was the most frequent mutation in this study, and the other hot spot of R413P was found in 4 patients in a same Uygur village with a carrier rate of 1:2.1.

Conclusions: This is the first study to investigate HPA incidence rate in the Uygur population. Our study highlights regional differences in *PAH* genotypes and mutation rates.

Keywords: Phenylalanine hydroxylase (*PAH*); Uygur; neonatal screening; gene mutation; genotyping

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Introduction

Different from the Han Chinese, the Uygur population originated from the Dingling, Tiele and Uighur ethnic groups, who live in the south of Junggar Basin in Northwest China which is a part of the ancient Silk Road. The Uygur population harbors an extensive genetic admixture of the human population. Newborn screening for hyperphenylalaninemia (HPA) was initiated in 1964 and subsequently adopted in 1981 in mainland China (1). The incidence of HPA is approximately 1/15,000 in newborns

worldwide (2). The incidence varies among ethnic and geographical regions. For example, the incidence in Turkey is 1/2,600, Ireland is 1/4,500, China is 1/15,415 (http://zhibao2.xsesc.cn/Login.aspxl), Japan is 1/143,000, and Finland is 1/200,000 (3-6). In Uygur communities with poor economic and medical development, the HPA screening did not begin until 2009. Even today, the screening rate has not yet reached 100%, but a remarkable number of Uygur children have already been diagnosed with PKU in clinics.

HPA is a common autosomal recessive inborn error of amino acid metabolism that primarily results from mutations in the phenylalanine hydroxylase (*PAH*) gene. About 955 different *PAH* variants are recorded in database (http://www.biopku.org). The wide variability in the common mutations among ethnic groups and geographical areas makes PAH deficient with great allelic heterogeneity (7). PAH enzyme activity deficiencies result in the inability to convert phenylalanine (Phe) into tyrosine (Tyr), leading to an increased concentration of Phe in the blood and central nervous system. When the blood Phe levels rise above 360 μmol/L, a restrictive low Phe diet needs to be implemented immediately for patients in order to prevent progressively aggravated mental retardation, seizure disorders, and eczema (8). It has been recommended that blood Phe levels need to be controlled between 120 and 600 μmol/L for different age groups (9). So far, a wide array of new treatments such as cell directed therapy, gene therapy and enzyme therapy were performed to PKU patients.

Accordingly, our study aims to systematically analyse the incidence, *PAH* mutational spectrum, and the follow-up of the Uygur population from January 2010 to December 2016 who live in the southern Junggar Basin in Northwest China. Consequently, we uncovered geographical and ethnic differences in the HPA mutation profile between the places in Northwest China and some other regions and provided guidance for the molecular epidemiology diagnosis of patients with PKU in Uygur population.

Methods

Ethics statement

All procedures performed in this study involving human participants follows the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from individual participants included in the study.

Database and population

We used the National Direct Reporting System for Neonatal Disease Screening database to obtain neonatal birth and screening data from January 2010 to December 2016 (http://zhibao2.xsesc.cn/Login.aspxl). The Uygur screening population data from January 2013 to December 2016 was obtained from the Newborn Disease Screening System of the Xinjiang Uygur Autonomous Region (http://202.85.214.55:5888/login.screen). Also, we manually searched the Uygur screening population registry from January 2010 to December 2012. This study was reviewed and approved by the Human Ethics Committee of the People's Hospital of Xinjiang Uygur Autonomous Region (2017010).

Sample size calculation

We used the cross-sectional study method to calculate the annual gross incidence rate according to the following formula:

$$\text{Incidence rate} = \frac{\text{Number of individuals people who have a disease or condition}}{\text{Number of people at risk} \times \text{time period at risk}} \quad [1]$$

We assume that the word "people" above refers to a group with no relatives included because the number of relatives is negligible as the denominator is very large. Therefore we removed the patients' relatives from the numerator.

$$x = \frac{Z_{\frac{\alpha}{2}}^2 \pi(1-\pi)}{\delta^2} \quad [2]$$

N = number; Z = standard normal distribution boundary value; α=0.05, $Z_{\alpha/2}$=1.96; α=0.01, $Z_{\alpha/2}$=2.58; π= expected incidence rate; and δ = admissible error.

Subjects

The Phe levels on dried blood spots were initially quantified using the Guthrie test. The cut off level is 120 μmol/L. When the Phe values were >120 μmol/L at two times, the child would be recalled to our hospital. Tandem mass-spectrometry (TMS), urinary pterin analysis, and determination of DHPR activity were ordered. When the values of Phe at TMS were >120 μmol/L and Phe/Tyr ratio >2.00, it suggested HPA. Then we would determine whether the patients had BH4 deficiency and distinguish

the subgroups of them, based on the following indicators, the basic urinary neopterin whose normal value was 0.29–2.61 (mmol/mol Cr), biopterin whose normal value was 0.35–2.67 (mmol/mol Cr), and DHPR whose normal activity was 1.02–3.35 nmol/min/ (5 mmdisc). According to their pretreatment plasma Phe levels, all patients were assigned to one of the three phenotypic categories: Phe levels over 1,200 μmol/L were generally termed "classical Phenylketonuria (cPKU)", Phe levels of 360–1,200 μmol/L were termed "moderate PKU (mPKU)", and Phe levels of 120–360 μmol/L were termed "mild hyperphenylalaninemia (MHP)". Parents and siblings were also investigated to confirm their carrier status.

Treatment and follow-up

Patients with Phe levels above 360 μmol/L under uncontrolled protein intake, should be treated immediately via restricting dietary Phe, while ensuring sufficient calories and protein to meet the needs for children's growth. According to preliminary screening of Phe concentrations, infants were treated with specialized low Phe milk powder, with low Phe staple food provided after six months and low Phe protein powder could be given from birth to 10 years. We adjusted each child's diet according to their initial blood Phe concentration and monitored blood Phe for three days after each dietary adjustment. When achieving stable control of Phe levels, monitoring periods would be properly adjusted. A target blood Phe levels of: 120–240 μmol/L was safe for children less than one year old; 120–360 μmol/L was safe for children more than one year old (2,10,11).

Sanger sequencing and capture-based next-generation deep sequencing

Two mL blood samples were collected from patients and their parents. DNA isolation was performed using the QIAamp DNA Blood Mini Kit [250] (QIAGEN, Vienna, Austria). PCR primers were designed to amplify thirteen exons and ten base pairs boundary of the *PAH* gene. PCR products were sequenced, and data were analyzed using Mutation Survey or Software (SoftGenetics, State College, PA, USA) with the reference to *PAH* RefSeq NM_000277.1.

Genome DNA was captured using Agilent ClearSeq Inherited Disease kit and sequenced by Illumina XTen system by WuXi NextCODE Genomics Company, and an average coverage of 200× was obtained. Data analyses were performed by the bioinformatics team in our clinical genetic laboratory. Mutations and parental carriers were validated by Sanger sequencing using an ABI 3730 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

Results

Incidence of HPA within the Uygur population

From 2010 to 2016, the screening rate in Uygur population increased from an average of 79% to 83% (*Figure 1*). A total of 669,832 neonates were screened for HPA, including 580,608 Uygur neonates, 128 non-relative neonates were diagnosed with HPA, including 111 Uygur neonates. In 2010, only 1,969 individuals in Uygur were screened, no HPA patients were detected. From 2011 to 2016, yearly incidence rates varied widely, ranging from 1:1,917 to 1:9,522. The average HPA incidence rate within Uygur population was 1:5,230 (*Table 1*).

Demographics of the diagnosed patients

A total of 111 Uygur patients (54 males and 57 females) with HPA were detected. The age of diagnosis ranged from fifty-four days to eight years and the patients were classified based on plasma Phe levels before treatment. Among them, 34.23% (38/111) presented as classical PKU; 50.45% (57/111) had moderate PKU, and 14.41% (16/111) were categorized as MHP and 0.9% (1/111) was presented as BH4 deficiency.

PAH mutational spectrum

PAH variants were detected in 110 individuals among the 111 HPA patients by Sanger sequencing, reaching a 99.1% positive rate. Seven individuals had only one heterozygous PAH mutation been detected (6.37%). Among the seven patients, four have MHP and three have mPKU. Given the recessive inheritance pattern of PAH deficiency and the limited coverage of non-coding regions by current methods, we speculate that another allele mutation might locate in the deep introns or non-coding regions. The one *PAH* negative case then underwent capture-based next-generation deep sequencing for exploring other possible disease etiologies. Our analysis revealed that this individual has a homozygous mutation in *QDPR* gene, which is associated with tetrahydrobiopterin deficiency (BH4 deficiency): another manifestation of PKU. The clinical phenotype of BH4 deficiency is more serious than that of PAH deficiency. It's

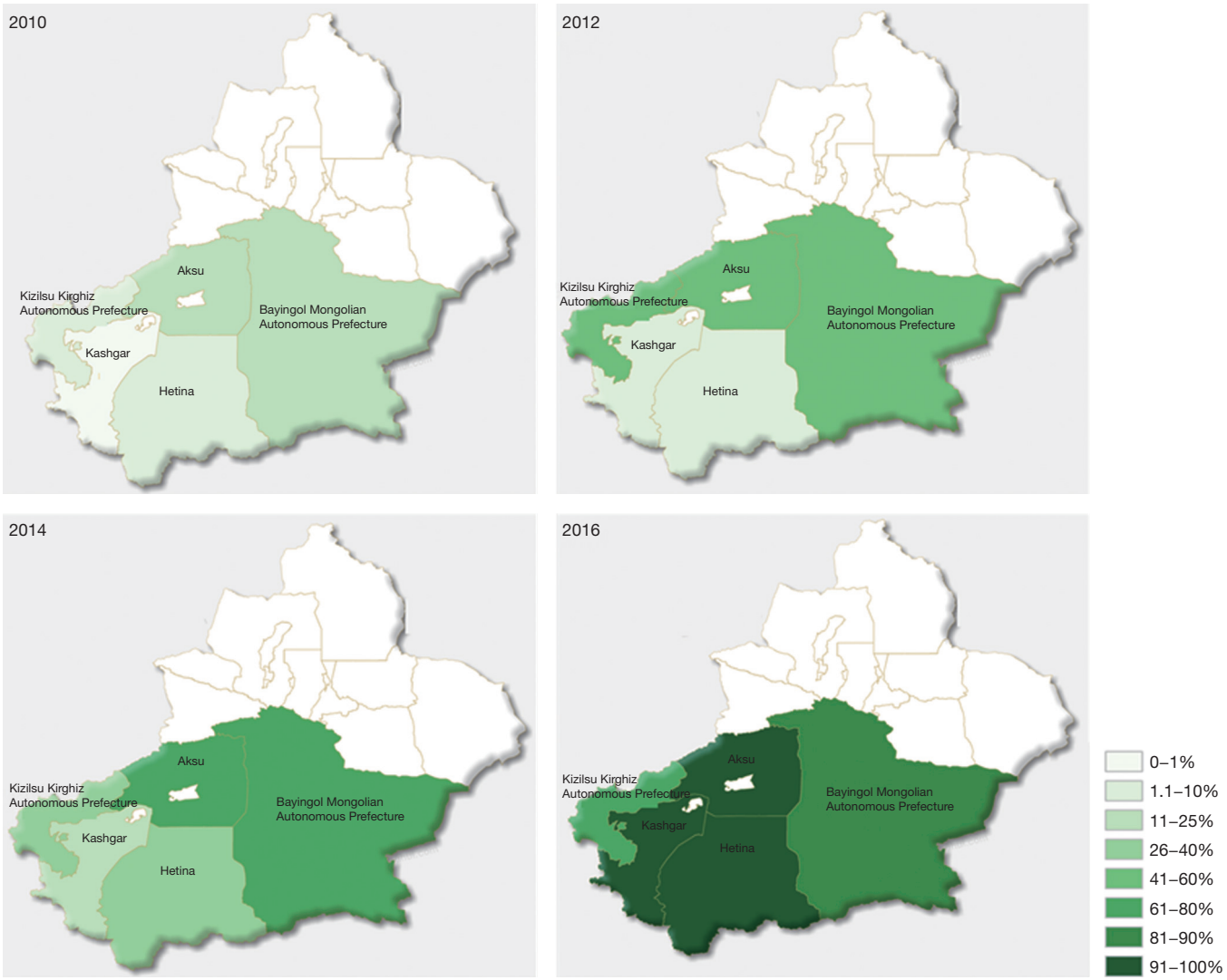

Figure 1 Screening rate in Uygur population. Newborn baby screening was performed, about 86.7% of the residents are Uygur population. From 2010 to 2016, the screening rate in Uygur population has gradually increased from an average of 79% to 83%. The color bar in the low-right side means the darker the more population screened.

congruent with the phenotypes of this patient, who showed hypotonia, seizures and mental retardation.

Overall, 58 unique *PAH* mutations were detected, including thirty-five missense mutations, nine splicing mutations, nine nonsense mutations, one inframe deletion, and one frameshift mutation. Mutations were distributed in all exons except exon 13. Exons 7 had the highest number of mutations (*Table 2*). A104D in exon 3 was the most prevalent hot spot mutation in the Uygur population (8.41%). Other hot-spot mutations with ten or more than ten alleles in our dataset were marked (*Table 2* with &). Comparison of these mutation frequencies based on published literatures (12-20), among countries is shown in *Figure 2*. R413P and R243Q are the hot spot mutations of Chinese and American population, IVS10-11G > A is the hot spot of Iranian and Turkish. However, R53H and A104D are specific in Uygur patients. Seven known *PAH* polymorphisms were also detected: IVS2+19T > C (33.3%), IVS4+47C > T (47.8%), IVS4-22C > T (42.4%), IVS9+43G > T (36.1%), p.Q232Q (64%), p.L385L (98.7%), and p.V245V (54.4%).

Eight novel PAH mutations that had not been previously reported or recorded in the PAHdb or HGMD databases

Table 1 HPA incidences in 2010–2016 in the Xinjiang Uygur population

Year	Total birth population	Screening population	Uygur screening population	Numbers of total cases diagnosed	Numbers of Uygur cases diagnosed	Total incidence[a]	Uygur incidence[a]
2010	37,935[b]	5,351	1,969	–	–	–	–
2011	212,011	17,570	9,215	5	4	1:3,514	1:2,304
2012	267,340	47,163	36,417	24	19	1:1,965	1:1,917
2013	284,668	95,387	82,313	18	16	1:5,299	1:5,145
2014	308,618	130,450	114,269	15	12	1:8,697	1:9,522
2015	307,150	192,633	173,822	31	31	1:6,214	1:5,607
2016	217,117	181,278	162,603	35	29	1:5,179	1:5,607
Total	1,634,839	669,832	580,608	128	111	1:5,233	1:5,230

[a], incidence = numbers/live births; [b], the number of live births was obtained from institutions that carried out screenings in 2010. This number does not reflect the total number of live births in Xinjiang in 2010. HPA, hyperphenylalaninemia.

Table 2 *PAH* mutations identified in Uygur HPA patients

No.	Base change	Amino acid	Mutation type	Exon	Homozygote/ heterozygote	Alleles (allele frequency %)	Allele in subclinical type		
							MHP	mPKU	cPKU
1	c.311C > A[&]	p.A104D[&]	Missense	E3	4/11	19 (8.41)	1	10	4
2	c.1238G > C[&]	p.R413P[&]	Missense	E12	3/12	18 (7.96)	1	6	8
3	c.728G > A[&]	p.R243Q[&]	Missense	E7	0/15	15 (6.64)	3	5	7
4	c.1066-11G > A[&]	IVS10-11G > A[&]	Splice	I10	3/8	14 (6.19)	0	6	5
5	c.158G > A[&]	p.R53H[&]	Missense	E2	2/8	12 (5.31)	4	5	1
6	c.1316-1G > A[#,&]	IVS13-2G > A[#,&]	Splice	I13	3/4	10 (4.42)	0	5	2
7	c.688G > A	p.V230I	Missense	E6	0/7	7 (3.10)	4	3	0
8	c.898G > T	p.A300S	Missense	E8	0/7	7 (3.10)	3	4	0
9	c.1200-2A > C[#]	IVS12-2A > C[#]	Splice	I12	0/7	7 (3.10)	0	2	5
10	c.1301C > A	p.A434D	Missense	E12	2/3	7 (3.10)	1	4	0
11	c.1199+1G > C	IVS11+1G > C	Splice	I11	1/4	6 (2.65)	0	2	3
12	c.781C > T	p.R261*	Nonsense	E7	1/4	6 (2.65)	0	1	4
13	c.611A > G	p.EX6-96A > G	Splice	E6	1/4	6 (2.65)	0	1	4
14	c.208_210delTCT	p.S70del	Deletion	E3	3/0	6 (2.65)	0	3	0
15	c.782G > A	p.R261Q	Missense	E7	3/0	6 (2.65)	0	2	1
16	c.331C > T	p.R111*	Nonsense	E3	1/2	4 (1.77)	0	2	1
17	c.1222C > T	p.R408W	Missense	E12	1/2	4 (1.77)	0	1	2
18	c.355C > T	p.P119S	Missense	E4	0/3	3 (1.33)	2	1	0
19	c.544G > A	p.E182K	Missense	E6	0/3	3 (1.33)	0	3	0
20	c.722G > A	p.R241H	Missense	E7	0/3	3 (1.33)	1	2	0
21	c.1169A > G	p.E390G	Missense	E11	0/3	3 (1.33)	1	2	0
22	c.1197A > T	p.V399V	Splice	E11	0/3	3 (1.33)	0	2	1

Table 2 (*continued*)

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No.	Base change	Amino acid	Mutation type	Exon	Homozygote/ heterozygote	Alleles (allele frequency %)	Allele in subclinical type		
							MHP	mPKU	cPKU
23	c.440C > T	p.P147L	Missense	E4	1/1	3 (1.33)	0	1	1
24	c.482T > C	p.F161S	Missense	E5	1/1	3 (1.33)	0	1	1
25	c.1042C > G	p.L348V	Missense	E10	1/1	3 (1.33)	0	0	2
26	c.1252A > C	p.T418P	Missense	E12	1/1	3 (1.33)	1	1	0
27	c.1289T > C	p.L430P	Missense	E12	1/1	3 (1.33)	0	2	0
28	c.838G > A	p.E280K	Missense	E7	0/2	2 (0.88)	0	2	0
29	c.842+2T > A	IVS7+2T > A	Splice	I7	0/2	2 (0.88)	1	1	0
30	c.1068C > A	p.Y356*	Nonsense	E11	0/2	2 (0.88)	0	1	1
31	c.727C > T	p.R243*	Nonsense	E7	0/2	2 (0.88)	0	1	1
32	c.441+5G > T	IVS4+5G > T	Splice	I4	1/0	2 (0.88)	0	0	1
33	c.143T > C	p.L48S	Missense	E2	1/0	2 (0.88)	0	1	0
34	c.498C > G	p.Y166*	Nonsense	E5	1/0	2 (0.88)	0	0	1
35	c.913-7A > G	IVS8-7A > G	Splice	I9	1/0	2 (0.88)	0	0	1
36	c.1262T > C	p.I421T	Missense	E12	1/0	2 (0.88)	1	0	0
37	c.265C > A	p.P89T	Missense	E3	1/0	2 (0.88)	0	1	0
38	c.800A > T	p.G267L	Missense	E7	1/0	2 (0.88)	0	1	0
39	c.308G > A	p.G103D	Missense	E3	0/1	1 (0.44)	0	0	1
40	c.809G > A	p.R270K	Missense	E7	0/1	1 (0.44)	0	1	0
41	c.32T > A[#]	p.L11*[#]	Nonsense	E1	0/1	1 (0.44)	0	0	1
42	c.346_347_ delGA[#]	p.K115 > Hfs[#]	Frameshift	E3	0/1	1 (0.44)	0	1	0
43	c.506G > A	p.R169H	Missense	E5	0/1	1 (0.44)	1	0	0
44	c.473G > A	p.R158Q	Missense	E5	0/1	1 (0.44)	0	0	1
45	c.574A > T	p.K192*	Nonsense	E6	0/1	1 (0.44)	0	0	1
46	c.590T > A[#]	p.L197*[#]	Nonsense	E6	0/1	1 (0.44)	0	1	0
47	c.694C > T	p.Q232*	Nonsense	E6	0/1	1 (0.44)	0	1	0
48	c.754C > T	p.R252W	Missense	E7	0/1	1 (0.44)	0	0	1
49	c.764T > C	p.L255S	Missense	E7	0/1	1 (0.44)	0	0	1
50	c.776C > T	p.A259V	Missense	E7	0/1	1 (0.44)	0	0	1
51	c.887A > G	p.D296G	Missense	E8	0/1	1 (0.44)	0	0	1
52	c.1102G > A[#]	p.E368K[#]	Missense	E11	0/1	1 (0.44)	0	1	0
53	c.1109A > G[#]	p.E370G[#]	Missense	E11	0/1	1 (0.44)	1	0	0
54	c.1139C > T	p.T380M	Missense	E11	0/1	1 (0.44)	1	0	0
55	c.1180G > C	p.D394H	Missense	E11	0/1	1 (0.44)	1	0	0
56	c.1223G > A	p.R408Q	Missense	E12	0/1	1 (0.44)	1	0	0
57	c.1304A > T[#]	p.D435V[#]	Missense	E12	0/1	1 (0.44)	1	0	0
58	c.842C > T	p.P281L	Missense	E7	0/1	1 (0.44)	0	0	1

[#,&], mentioned in main text. PAH, phenylalanine hydroxylase; HPA, hyperphenylalaninemia.

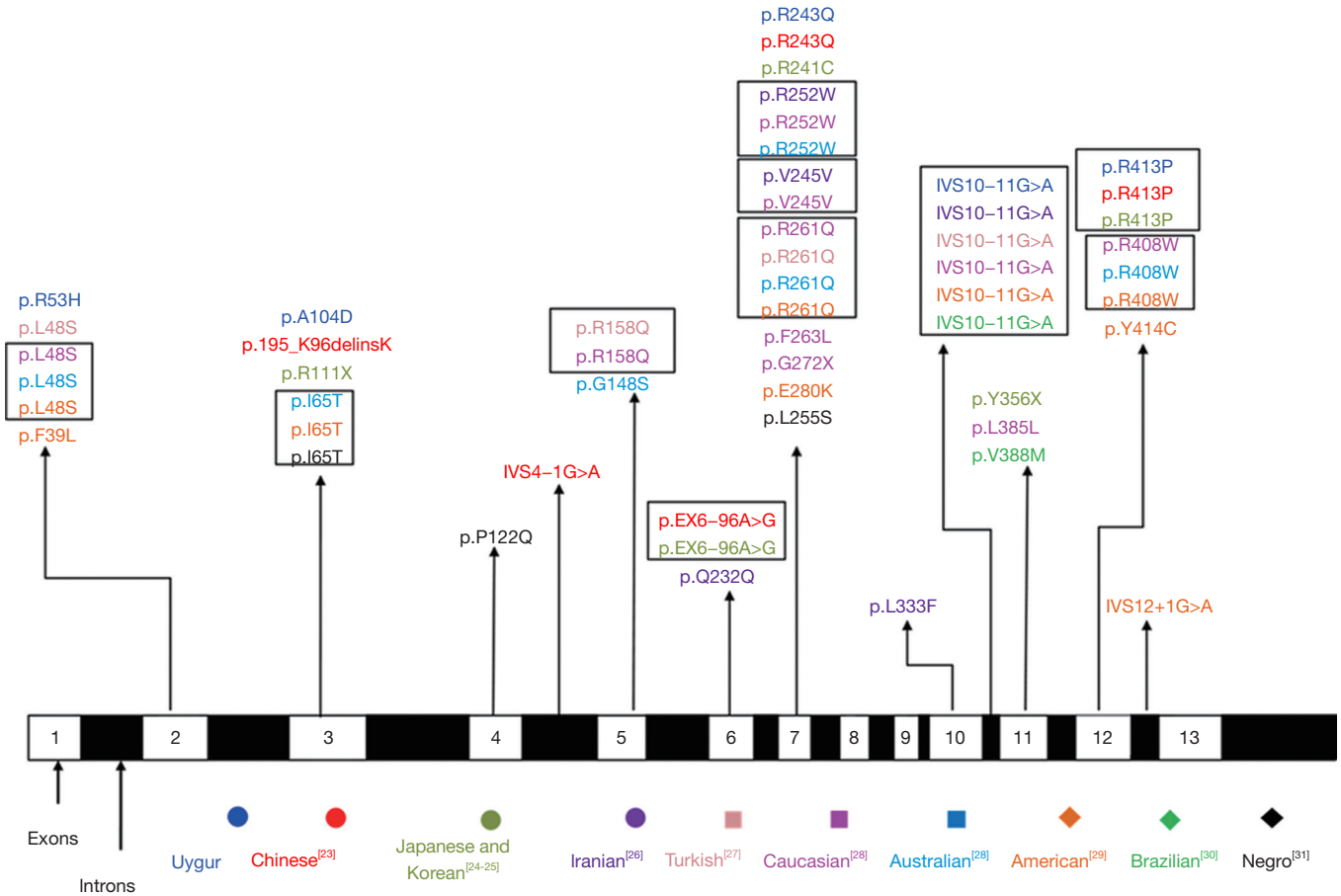

Figure 2 Hot spot mutation of *PAH* genes in different ethnicities Bright blue represents the Uygur population, red represents Chinese. R413P in exon 12 is the most prevalent mutation in the Uygur population, exons 7 and 12 had the highest number of mutations among the different ethnicities. *PAH*, phenylalanine hydroxylase.

were identified, including two nonsense mutations (c.32T > A, p.L11* and c.590T > A, p.L197*), three missense mutations (c.1102G > A, p.E368K; c.1109A > G, p.E370G; c.1304A > T, p.D435V), one frameshift mutation (c.346-347delGA, p.K115 > Hfs), each of them was detected in one allele; two splicing mutations (c.1200-2A > C, IVS12-2A > C; c.1316-1G > A, IVS13-1G > A), each of them was detected in seven alleles (*Table 2* with #), distributing in twenty patients (three patients were homozygous of IVS13-1G > A mutations, others were compound heterozygous mutations). Three missense mutations were all predicted to be pathogenic using SIFT, PolyPhen 2, and MutationTaster software. All of the IVS12-2A > C mutation was found in classical PKU and combination with IVS10-11G > A, A104D, P147L, which was mostly found in classical PKU. Two nonsense mutations (L11* and L197*) and missense mutations (E368K) were found in moderate PKU. Two missense mutations (E370G and D435V) were found in MHP.

Correlation between genotype and phenotype in Uygur HPA patients

A total of 110 Uygur patients carrying *PAH* mutations and one *QDPR* mutations constituted a spectrum of 58 different genotypic combinations (*Table S1*). The genotypes of *PAH* were divided into homozygous (n=36) and heterozygous (n=73). Variant A104D appeared in the most forms of homozygous. A total of 14 patients carried three variants, 7 of them were combination of 6 homozygous of A104, R413P, L430PC, IVS10-11G > A, IVS4+5G > T, I421T. A total of six patients carried only one variant, four of whom were in MHP and two were in mPKU.

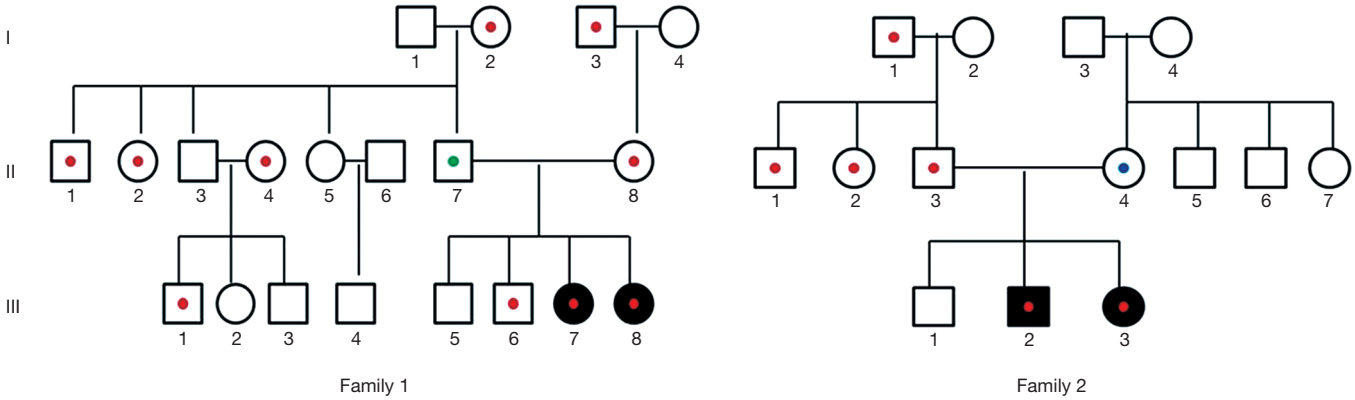

Figure 3 Hot spot of R413P mutation in two pedigrees in an Uygur village. R413P is identified in four patients from two families. The red dots represent R413P carriers, the green dot carried R261Q, and the blue dot carried R270K. 16 members carried R413P, with an alarming carrier rate of approximately 1:2.1.

Identification of the PAH gene mutation in patients from two families in a village

A Uygur village in Akesu has a population of about 2,000 with approximately 60 births every year. Villagers do not intermarry with other villages or other ethnic groups. The R413P mutation was identified in four patients from two families (*Figure 3*). The two families had no relationship but the family members were cousins, and all the patients had symptoms of PKU. As previously described, this mutation is a hot spot mutation within the Uygur population. Sixteen of 34 members of these two families carried R413P heterozygous mutations with an alarming carrier rate of approximately 1:2.1.

Discussion

Over the last 60 years, researches on the pathophysiology and treatment of PKU have progressed rapidly all over the world. However, similar analyses and investigation within the Uygur population have been lagging until now. Our data provide the first accurate assessment of PKU incidence within the Uygur population and highlight the genotypes and frequency of *PAH* mutations in this community.

The screening rates of HPA incidence fluctuated from 2011 to 2014. Such fluctuations could be affected by multiple factors, leading to variations in the incidence rates (21). In 2014, however, an unexpectedly low rate of HPA incidence was logged, likely due to a change in the screening system, from paper-based to electronic. We speculate that the reform was not so thoroughly executed at the beginning and consequently, some MHP patients were not recalled. HPA incidence rates within the Uygur population were relatively stable in 2015 and 2016, with an estimated rate of 1:5,230, which is consistent with the average rate across the entire period from 2010 to 2016. This rate is higher than that all around China (1:15,415).

Eight novel variants were identified in this study. Two variants were identified as splice variant IVS12-2A > C located in intron 12 and IVS13-1G > A located in intron 13. The bases at the junctions of the introns and exons are AG/GT. The mutation is located at the boundaries of the intron/exon and may cause abnormal RNA splicing. The remaining seven mutations were predicted to be pathogenic using mutation analysis software. The allele counts of top mutations are ten or more than ten (*Table 2* with &). Herein, the hot spot mutation of the R413Pand R243Q is also common in Chinese, Japanese, and American population, and IVS10-11G > A is also a common hot spot mutation among Iranian and Turkish. However, R53H and A104D are specific in Uygur patients.

The Uygur population originated from the Dingling, Tiele and Uighur ethnic groups and were socialized by Turk in 840–1212 Anno Domini (AD) with distinct culture characteristics driven by the ancient Silk Road, an important gallery that connected culture and trade among China, Asia, and Europe. At present, approximately 11.3 million of Uygur live in oasis of Tarim Basin, in the south of Tianshan mountains in Xinjiang (22). They have their own languages, eating habits, and national costumes. Uygur population community is on the region of Silk Road, and the significant genetic admixing may occur as a consequence of ancestral migration to this region.

The exclusivity marriage in this population makes a high incidence of some hereditary diseases. In a village with 2,000 people, four patients from two families were found symptoms of PKU. The mutation site is the hot spot of R413P. The carrier rate is approximately 1:2.1. Uygur have a relatively lower rate of DHPR deficiencies, compared with that in whole China, Turkey and Iran. Only one patient of QDPR mutation was detected. However, this study may miss another form of BH4 deficiency, GTP cyclohydrolase (GTPCH-deficient) by newborn screening when the blood Phe levels were not increased (23).

This is the first study to investigate the HPA incidence rate within a large Uygur population and the incidence rate is significantly high. The data also highlight the regional differences in *PAH* genotypes, suggesting not only a consanguineous relationship, but also distinct differences between Asian and Caucasian populations.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The study was approved by the Human Ethics Committee of the People's Hospital of Xinjiang Uygur Autonomous Region (2017010) and written informed consent was obtained from all patients.

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Table S1 The genotype and phenotype of 111 patients in Uygur HPA patients

Case No.	Variant_1	Zygosity	Variant_2	Zygosity	Variant_3	Zygosity	Age at diagnosis (month)	Gender	Blood Phe levels at diagnosis (mg/dL)	Diagnosis
1[a]	PAH c.311C > A, p.A104D	Hom	PAH c.158G > A, p.R53H	Het			2	Male	14.6	mPKU
2	PAH c.311C > A, p.A104D	Hom					2.5	Male	13.4	mPKU
3[a]	PAH c.311C > A, p.A104D	Hom	PAH c.728G > A, p.R243Q	Het			9	Male	13.9	mPKU
4	PAH c.311C > A, p.A104D	Hom					13	Female	14	mPKU
5	PAH c.782G > A, p.R261Q	Hom					2	Male	24.32	cPKU
6	PAH c.782G > A, p.R261Q	Hom					3	Female	16.63	mPKU
7	PAH c.782G > A, p.R261Q	Hom					2	Male	15.04	mPKU
8	PAH c.208_210delTCT, p.S70del	Hom					7.5	Female	11.51	mPKU
9	PAH c.208_210delTCT, p.S70del	Hom					2.5	Male	12.93	mPKU
10	PAH c.208_210delTCT, p.S70del	Hom					2	Male	14	mPKU
11	PAH c.1316-1G > A, IVS13-2G > A, IVS13-2G > A	Hom					3.5	Male	19.74	mPKU
12	PAH c.1316-1G > A, IVS13-2G > A, IVS13-2G > A	Hom					4	Male	18.5	mPKU
13	PAH c.1316-1G > A, IVS13-2G > A, IVS13-2G > A	Hom					3	Female	17.37	mPKU
14	PAH c.1238G > C, p.R413P	Hom					2	Female	23.87	cPKU
15	PAH c.1238G > C, p.R413P	Hom					15	Female	19.55	mPKU
16[a]	PAH c.1238G > C, p.R413P	Hom	PAH c.809G > A, p.R270K	Het	PAH c.838G > A, p.E280K	Het	2	Female	10.41	mPKU
17	PAH c.1066-11G > A, IVS10-11G > A	Hom					3	Female	27.74	cPKU
18	PAH c.1066-11G > A, IVS10-11G > A	Hom					2	Male	21.43	cPKU
19[a]	PAH c.1066-11G > A, IVS10-11G > A	Hom	PAH c.574A > T, p.K192*	Het			5	Female	30.63	cPKU
20	PAH c.1301C > A, p.A434D	Hom					5	Male	11.2	mPKU
21	PAH c.1301C > A, p.A434D	Hom					33	Male	14.35	mPKU
22	PAH c.913-7A > G, IVS8-7A > G	Hom					4.5	Male	31.71	cPKU
23	PAH c.800A > T, p.G267L	Hom					9	Female	6.94	mPKU
24	PAH c.781C > T, p.R261*	Hom					3	Female	24.38	cPKU
25	PAH c.611A > G, p.EX6-96A > G	Hom					2	Female	25.4	cPKU
26	PAH c.498C > G, p.Y166*	Hom					1.5	Female	28.24	cPKU
27	PAH c.482T > C, p.F161S	Hom					3	Male	30.9	cPKU
28[a]	PAH c.441+5G > T, IVS4+5G > T	Hom	PAH c.1199+1G > C, IVS11+1G > C	Het			7.5	Male	26.99	cPKU
29	PAH c.440C > T, p.P147L	Hom					4.5	Male	18.92	mPKU
30	PAH c.331C > T, p.R111*	Hom					6	Female	17.12	mPKU
31	PAH c.265C > A, p.P89T	Hom					4	Male	9.62	mPKU
32	PAH c.158G > A, p.R53H	Hom					4	Female	10.22	mPKU
33	PAH c.143T > C, p.L48S	Hom					9	Male	6.28	mPKU
34[a]	PAH c.1289T > C, p.L430P	Hom	PAH c.158G > A, p.R53H	Het			5	Male	6.36	mPKU
35[a]	PAH c.1262T > C, p.I421T	Hom	PAH c.158G > A, p.R53H	Hom			4	Female	4.95	MHP
36	PAH c.1252A > C, p.T418P	Hom					4	Male	7.79	mPKU
37	PAH c.1222C > T, p.R408W	Hom					4	Male	32.08	cPKU
38	PAH c.1199+1G > C, IVS11+1G > C	Hom					3	Female	26.46	cPKU
39	PAH c.1042C > G, p.L348V	Hom					2	Female	22	cPKU
40	PAH c.1238G > C, p.R413P	Het	PAH c.781C > T, p.R261*	Het			4	Male	26.54	cPKU
41[a]	PAH c.1238G > C, p.R413P	Het	PAH c.311C > A, p.A104D	Het	PAH c.688G > A, p.V230I	Het	5	Female	15.1	mPKU
42	PAH c.1238G > C, p.R413P	Het	PAH c.311C > A, p.A104D	Het			5	Female	26.18	cPKU
43[a]	PAH c.1238G > C, p.R413P	Het	PAH c.842+2T > A, IVS7+2T > A	Het	PAH c.158G > A, p.R53H	Het	5	Female	16.36	mPKU
44	PAH c.1238G > C, p.R413P	Het	PAH c.688G > A, p.V230I	Het			6	Female	3.22	MHP
45[a]	PAH c.1238G > C, p.R413P	Het	PAH c.781C > T, p.R261*	Het	PAH c.1068C > A, p.Y356*	Het	5	Female	34.34	cPKU
46	PAH c.1238G > C, p.R413P	Het	PAH c.1316-1G > A, IVS13-2G > A	Het			2	Male	22.52	cPKU
47	PAH c.1238G > C, p.R413P	Het	PAH c.781C > T, p.R261*	Het			5	Female	21.66	cPKU
48	PAH c.1238G > C, p.R413P	Het	PAH c.32T > A, p.L11*	Het			3	Male	25.25	cPKU
49	PAH c.1238G > C, p.R413P	Het	PAH c.728G > A, p.R243Q	Het			36	Male	23.12	cPKU
50	PAH c.1238G > C, p.R413P	Het	PAH c.838G > A, p.E280K	Het			3	Male	18.63	mPKU
51[b]	PAH c.1238G > C, p.R413P	Het					4	Male	11.79	mPKU
52	PAH c.728G > A, p.R243Q	Het	PAH c.1316-1G > A, IVS13-2G > A	Het			3	Male	29.86	cPKU
53	PAH c.728G > A, p.R243Q	Het	PAH c.544G > A, p.E182K	Het			2	Male	15.77	mPKU
54	PAH c.728G > A, p.R243Q	Het	PAH c.1066-11G > A, IVS10-11G > A	Het			4.5	Male	13.25	mPKU
55[b]	PAH c.728G > A, p.R243Q	Het					4.5	Male	7.24	mPKU
56	PAH c.728G > A, p.R243Q	Het	PAH c.1139C > T, p.T380M	Het			6	Female	3.5	MHP
57	PAH c.728G > A, p.R243Q	Het	PAH c.1200-2A > C, IVS12-2A > C	Het			2	Female	24.69	cPKU
58	PAH c.728G > A, p.R243Q	Het					9.5	Male	21.95	cPKU
59	PAH c.728G > A, p.R243Q	Het	PAH c.887A > G, p.D296G	Het			4	Female	29.07	cPKU
60	PAH c.728G > A, p.R243Q	Het	PAH c.1289T > C, p.L430P	Het			5	Male	16.5	mPKU
61	PAH c.311C > A, p.A104D	Het	PAH c.688G > A, p.V230I	Het			2.5	Female	11.2	mPKU
62[b]	PAH c.311C > A, p.A104D	Het					7	Male	4.16	MHP
63	PAH c.311C > A, p.A104D	Het	PAH c.754C > T, p.R252W	Het			5	Female	21.3	cPKU
64	PAH c.311C > A, p.A104D	Het	PAH c.1200-2A > C, IVS12-2A > C	Het			4	Male	28	cPKU
65	PAH c.311C > A, p.A104D	Het	PAH c.722G > A, p.R241H	Het			2.5	Female	11.03	mPKU
66	PAH c.311C > A, p.A104D	Het	PAH c.898G > T, p.A300S	Het			3	Female	6.6	mPKU
67	PAH c.311C > A, p.A104D	Het	PAH c.1301C > A, p.A434D	Het			6	Female	6.99	mPKU
68	PAH c.311C > A, p.A104D	Het	PAH c.1200-2A > C, IVS12-2A > C	Het			11	Male	10.5	mPKU
69	PAH c.1066-11G > A, IVS10-11G > A	Het	PAH c.898G > T, p.A300S	Het			12	Male	7.24	mPKU
70	PAH c.1066-11G > A, IVS10-11G > A	Het	PAH c.1200-2A > C, IVS12-2A > C	Het			8	Female	29.1	cPKU
71	PAH c.1066-11G > A, IVS10-11G > A	Het	PAH c.1102G > A, p.E368K	Het			7	Male	9.5	mPKU
72	PAH c.1066-11G > A, IVS10-11G > A	Het	PAH c.1301C > A, p.A434D	Het			3	Female	10.48	mPKU
73	PAH c.1066-11G > A, IVS10-11G > A	Het					11	Female	19.6	mPKU
74*	PAH c.158G > A, p.R53H	Het	PAH c.308G > A, p.G103D	Het	PAH c.1066-11G > A, IVS10-11G > A	Het	4	Female	29.32	cPKU
75	PAH c.158G > A, p.R53H	Het	PAH c.590T > A, p.L197*	Het			7	Male	17.16	mPKU
76	PAH c.158G > A, p.R53H	Het	PAH c.898G > T, p.A300S	Het			1.5	Male	4.23	MHP
77	PAH c.158G > A, p.R53H	Het	PAH c.728G > A, p.R243Q	Het			6	Male	3.7	MHP
78*	PAH c.158G > A, p.R53H	Het	PAH c.688G > A, p.V230I	Het	PAH c.842+2T > A, IVS7+2T > A	Het	11	Male	4.14	MHP
79	PAH c.688G > A, p.V230I	Het	PAH c.1223G > A, p.R408Q	Het			3	Female	4.54	MHP
80	PAH c.688G > A, p.V230I	Het	PAH c.1252A > C, p.T418P	Het			5	Male	3.19	MHP
81	PAH c.688G > A, p.V230I	Het	PAH c.1109A > G, p.E370G	Het			6	Male	6.75	mPKU
82	PAH c.898G > T, p.A300S	Het	PAH c.1066-11G > A, IVS10-11G > A	Het			3	Male	6.42	mPKU
83	PAH c.898G > T, p.A300S	Het	PAH c.1169A > G, p.E390G	Het			5.5	Male	4.24	MHP
84[b]	PAH c.898G > T, p.A300S	Het					17	Male	2.96	MHP
85	PAH c.1169A > G, p.E390G	Het	PAH c.346_347_delGA, p.K115 > Hfs	Het			12	Male	10.98	mPKU
86	PAH c.1169A > G, p.E390G	Het	PAH c.1197A > T, p.V399V	Het			5	Male	13.87	mPKU
87*	PAH c.1200-2A > C, IVS12-2A > C	Het	PAH c.311C > A, p.A104D	Het	PAH c.611A > G, p.EX6-96A > G	Het	5	Female	25.34	cPKU
88	PAH c.1200-2A > C, IVS12-2A > C	Het	PAH c.440C > T, p.P147L	Het			2	Female	21.83	cPKU
89	PAH c.1197A > T, p.V399V	Het	PAH c.1199+1G > C, IVS11+1G > C	Het			2	Male	16.73	mPKU
90	PAH c.1197A > T, p.V399V	Het	PAH c.728G > A, p.R243Q	Het			2	Female	37.85	cPKU
91	PAH c.1222C > T, p.R408W	Het	PAH c.355C > T, p.P119S	Het			10	Female	14.72	mPKU
92	PAH c.1222C > T, p.R408W	Het	PAH c.1199+1G > C, IVS11+1G > C	Het			5.5	Female	23.46	cPKU
93	PAH c.331C > T, p.R111*	Het	PAH c.1199+1G > C, IVS11+1G > C	Het			3.5	Male	17.92	mPKU
94	PAH c.331C > T, p.R111*	Het	PAH c.728G > A, p.R243Q	Het			3	Female	26.36	cPKU
95	PAH c.355C > T, p.P119S	Het	PAH c.1180G > C, p.D394H	Het			6	Female	3.11	MHP
96	PAH c.355C > T, p.P119S	Het	PAH c.728G > A, p.R243Q	Het			2	Male	4.77	MHP
97[b]	PAH c.722G > A, p.R241H	Het					6	Female	4.03	MHP
98	PAH c.722G > A, p.R241H	Het	PAH c.781C > T, p.R261*	Het			2	Male	7.26	mPKU
99	PAH c.727C > T, p.R243*	Het	PAH c.1200-2A > C, IVS12-2A > C	Het			7.5	Female	19.87	mPKU
100	PAH c.727C > T, p.R243*	Het	PAH c.842C > T, p.P281L	Het			6	Male	26.55	cPKU
101	PAH c.1316-1G > A, IVS13-2G > A	Het	PAH c.898G > T, p.A300S	Het			3	Female	7.99	mPKU
102	PAH c.482T > C, p.F161S	Het	PAH c.611A > G, p.EX6-96A > G	Het			1.5	Female	13	mPKU
103[b]	PAH c.506G > A, p.R169H	Het					7	Female	4.61	MHP
104	PAH c.544G > A, p.E182K	Het	PAH c.1316-1G > A, IVS13-2G > A	Het			8	Female	6.1	mPKU
105	PAH c.611A > G, p.EX6-96A > G	Het	PAH c.776C > T, p.A259V	Het			3	Female	31.9	cPKU
106	PAH c.1068C > A, p.Y356*	Het	PAH c.544G > A, p.E182K	Het			3	Female	7.4	mPKU
107	PAH c.473G > A, p.R158Q	Het	PAH c.611A > G, p.EX6-96A > G	Het			2.5	Male	37.2	cPKU
108	PAH c.764T > C, p.L255S	Het	PAH c.1042C > G, p.L348V	Het			2	Female	22.46	cPKU
109	PAH c.1301C > A, p.A434D	Het	PAH c.1304A > T, p.D435V	Het			2	Female	4.18	MHP
110[b]	PAH c.694C > T, p.Q232*	Het					3	Male	16.6	mPKU
111	QDPR c.508G > A, p.G170S	Hom					14	Female	13.41	DHPR

[a], detected three variants; [b], detected only one variant. HPA, hyperphenylalaninemia.