PROCEEDINGS OF THE LATVIAN ACADEMY OF SCIENCES. Section B, Vol. 65 (2011), No. 3/4 (674/675), pp. 73–79.
DOI: 10.2478/v10046-011-0021-5

ASSOCIATION BETWEEN MINIHAPLOTYPES AND MUTATIONS AT THE PHENYLALANINE HYDROXYLASE LOCUS IN LATVIAN PHENYLKETONURIA PATIENTS

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Communicated by Īzaks Rašals

Phenylketonuria (PKU) is an inherited metabolic disease caused by recessively inherited mutations in the PAH *gene that encodes the enzyme phenylalanine hydroxylase (PAH). Altogether, 20 diseases causing mutations were identified in Latvian PKU patients. R408W, the most common mutation, accounted for 73% of Latvian PKU chromosomes and was mostly observed in association with the VNTR3/STR238 minihaplotype. Minihaplotypes also were established for the other 19 mutations and one unknown PKU chromosome. Mutation E280K was almost exclusively associated with minihaplotype 9/250, and mutation IVS10–11G>A was strongly associated with the VNTR7/STR250 minihaplotype and was possibly of Mediterranean origin. It was found that minihaplotypes can be useful in studies concerning the origin and distribution of PAH mutations in human populations and for analysis of rare mutations in PAH gene and for prenatal diagnosis.*

Key words: *phenylalanine hydroxylase, phenylketonuria, minihaplotype, mutation, genetic heterogeneity,* PAH *gene.*

INTRODUCTION

Phenylketonuria (PKU; OMIM 261600) is one of the most common inborn errors of metabolism in Caucasians, with a frequency of 1 : 10 000 newborns in Europe. It is an autosomal recessive trait caused by a deficiency of hepatic phenylalanine hydroxylase (PAH; 1 phenylalanine 4-monooxygenase, EC 1.14.16.1), the main clinical signs of which are impaired cognitive development and function (Eisensmith *et al.,* 1995a; Scriver *et al.,* 1995). PAH catalyses the irreversible hydroxylation of phenylalanine (Phe) to tyrosine. Deficiency of this enzyme results in a high concentration of Phe and its metabolites, such as phenylpyruvate, phenyllactate, and phenylacetate, collectively known as phenylketones, which are neurotoxic, particularly during the first years of life (Nyhan *et al*., 2005; Albrecht *et al*., 2009). The neurotoxicity effect was suggested by a Norwegian physician and biochemist, Asbjörn Fölling, in 1934 and was later substantiated by George Jervis in 1947 (Jervis, 1947).

Chronic, untreated, severe hyperphenylalaninaemia in infants and children leads to seizures and mental retardation (Harding and Blau, 2010). Since the 1960s, it has been possible to prevent these complications by detection of through newborn screening, followed by dietary treatment consisting of restriction of Phe intake by a protein-restricted diet and supplementation of all amino acids except Phe (Hoedt *et al*., 2011).

There are four distinct phenylketonuria phenotypes, which are based on phenylalanine levels at diagnosis and dietary tolerance of phenylalanine: (1) classical phenylketonuria, (2) moderate phenylketonuria, (3) mild phenylketonuria, and (4) mild hyperphenylalaninemia (Williams *et al.,* 2008; Blau *et al.,* 2010). However, a remarkably wide variation of clinical manifestation is observed among phenylketonuria patients and PKU classification may differ by country according to each country guidelines or clinicians' experience.

The gene for PAH is located on chromosome 12 in humans. It is 79 277 bases long and encodes 452 amino acids (Anonymous, 2011). The phenylalanine hydroxylase (*PAH*) gene (OMIM 261600) (http://www.pahdb.mcgill.ca) was first cloned in 1983 (Woo *et al*., 1983). The cDNA sequence contains 13 exons that constitute approximately 2.9% of the genomic PAH sequence. The shortest and longest exons are 57 bp (exon 9) and 892 bp (exon 13), respectively; the mean exon size is 170 bp (Scriver *et. al*., 2008).

Over 560 mutations causing phenylketonuria have been reported in the *PAH* gene (OMIM 261600) (http://www.pahdb.mcgill.ca), most of them corresponding to point mutations causing missense changes. All disease-causing mutations fall into five classes: missense, 63%; small deletions, 13%; splice, 11%; putative silent, 7%; stop/nonsense, 5%; small insertions, 1%. Large deletions, once thought to be rare, probably account for 3% of PKU-

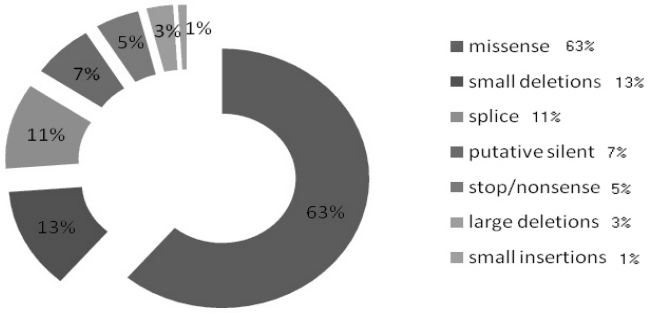

Fig. 1. PAH gene mutation types and relative frequencies (%).

causing mutations (Fig. 1) (Kozak *et al.,* 2006; Scriver, 2007).

There are marked differences in the spectrum of *PAH* mutations and the degree of heterogeneity between European countries. Common mutations include R408W on a haplotype 2 background in Eastern Europe, IVS10–11GA in the Mediterranean, IVS12+1GA in Denmark and England, Y414C in Scandinavia, I65T in Western Europe, and R408W on haplotype 1 in the British Isles (Zschocke, 2003).

Mutation R408W is found at relative allele frequencies as high as 84% in Europe (Zschocke, 2003). The R408W mutation (c.1222C > T; (DiLella *et al.,* 1987)), a C to T transition in exon 12 of the *PAH* gene, results in the substitution of tryptophan for arginine at amino-acid residue 408 and is a null mutation associated with 0.3% of normal activity and a severe PKU phenotype (Kayaalp *et al.,* 1997). This mutation involves a CpG dinucleotide in a so-called "hypermutable" codon, suggesting that c.1222C > T might be a recurrent allele following spontaneous methylation-mediated deamination of 5mC (Murphy *et al*., 2006).

In Europe, the R408W mutation is observed on chromosomes of two major haplotype backgrounds (Table 1). R408W-2.3 exhibits a west-to-east cline of relative frequency, reaching its maximum in the Balto-Slavic region. R408W-1.8 exhibits an east-to-west cline in north-western Europe, peaking in Connacht, the most westerly province of Ireland (Tighe *et al.,* 2003).

Multiallelic polymorphisms of *PAH* gene include a hypervariable sequence (variable number of tandem repeats (VNTRs)) of 30-bp cassettes that harbour at least ten alleles (differing by number of repeats) in a *Hind*III fragment 3 kb downstream from the last exon in *PAH* (Goltsov *et al.,* 1992; Latorra *et al.,* 1994), and a series of short tandem [tetranucleotide (TCTA)*n*] repeats (STRs) that include at least nine alleles in the third intron of *PAH* (Goltsov *et al.,* 1993; Zschocke *et al.,* 1994; Giannattasio *et al.,* 1997).

VNTR system is responsible for the three alleles of *Hin*dIII polymorphism of the human *PAH* gene, which are 4.0, 4.2, and 4.4 kb long (Woo *et al.,* 1983) and contain 3, 6–9 and 12 copies of VNTR, respectively (Goltsov *et al*., 1992). VNTR with 13 repeats was observed in two PKU families from south of Iran and was associated with normal alleles in both families (Kamkar *et al*., 2003).

The STRs are highly polymorphic and inherited stably. Tetrameric STR $(TCTA)_n$ has been described in the human phenylalanine hydroxylase gene, which harbours at least nine alleles (226bp to 258bp) in the third intron of *PAH* (Fig. 2). STR within the *PAH* gene has an average level of heterozygosity of about 75% in Orientals and about 80% in European Caucasian populations. This single marker is as informative as haplotype analysis in Europeans and nearly twice as informative as haplotype analysis in Orientals (Goltsov *et al.,* 1993).

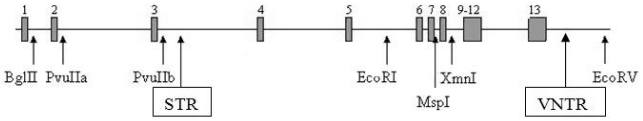

Fig. 2. Schematic map of the *PAH* locus indicating exons and sites of the RFLP, STR and VNTR polymorphisms (modified from Kidd and Kidd, 2005). Gene exons are marked by boxes.

In eastern European populations, the R408W mutation is strongly associated with RFLP haplotype 2, the three-copy VNTR allele (VNTR 3), and the 238-bp STR allele. In north-western European populations, it is strongly associated with RFLP haplotype 1, the VNTR allele containing eight repeats (VNTR 8), and the 242-bp STR allele. Examination of the linkage between the R408W mutation and highly polymorphic RFLP, VNTR, and STR haplotypes suggested that recurrence was the most likely mechanism accounting for the two different major haplotype associations of R408W in Europe (Eisensmith *et al.,* 1995b).

Since the VNTR is a highly polymorphic genetic marker and is inherited in a Mendelian fashion, it can be used to provide a risk estimation of linked defective alleles. In addition, this VNTR may prove useful in studies concerning the origins and distributions of *PAH* mutations in different human populations. The high degree of polymorphism and strong Mendelian segregation of the STR system makes it

Table 1

MUTATION R408W HAPLOTYPES AT THE HUMAN *PAH* LOCUS

Haplotype	BglII	PvuII(a)	PvuII(b)	EcoRI	MspI	XmnI	VNTR (HindIII)	EcoRV
1.8	-	+	-	-	+	-	8	-
2.3	-	+	-	-	+	-	3	+

Plus (+) and minus (-) indicate the presence or absence of a polymorphic restriction site, respectively.

useful for prenatal diagnosis and carrier screening determination in PKU families (Goltsov *et al.,* 1993).

The incidence of PKU in Latvia is about 1:8000 newborns (Purina *et al*., 1995). Our study group included all PKU patients registered in the Medical Genetics Clinic. According to the PKU classification, 91.4% (64/70) of patients were classified as having severe PKU, 5.7% (4/70) as having mild PKU and 2.9% (2/70) as having mild hyperphenylalaninaemia.

The aim of this study was to examine minihaplotype associations of the *PAH* gene mutations in Latvian PKU patients.

MATERIALS AND METHODS

A total of 70 PKU families (184 individuals, including 74 patients and 110 relatives) were included in the study. When families included more than one PKU patient, only one of them was randomly selected in the result processing.

DNA was extracted from whole peripheral blood based on selective detergent-mediated DNA precipitation from crude lysate ("Fermentas", Lithuania). Diagnostic identification of the most frequent mutation R408W for all PKU patients was based on the fact that it results in the formation of a new restriction enzyme *Sty*I site in exon 12, which can be identified using PCR – RFLP. Non-R408W chromosomes were screened for mutations by denaturing-gradient gel electrophoresis (DGGE; Guldberg and Guttler, 1994) of all 13 exons of the *PAH* gene (Guldberg and Güttler, 1994). Exons showing variant electrophoretic patterns were analysed by fluorescent automated sequencing using an ABI PRISM BigDye Terminator Cycle Sequencing kit v.3.1 on an ABI310 Genetic Analyser (Applied Biosystems, USA).

Intragenic VNTR and STR systems were analysed in compound heterozygote patients when parents were available, according to Goltsov *et al.* (1992) and Zschocke *et al.* (1994), respectively (Goltsov *et al.,* 1992; Zschocke *et al.* 1994). PCR fragments for the VNTR system were separated using high resolution agarose electrophoresis. Fluorescent PCR products for STR system were analysed on an ABI Prism 310 Genetic Analyser running GeneScan software. Results of VNTR and STR systems analysis were used to form *PAH* gene mutations' minihaplotypes.

RESULTS

PAH mutation profiles were obtained for 139 of 140 (99.3%) of Latvian PKU chromosomes (Table 2). The most common mutation was R408W, which accounted for 73% of mutant alleles. The second most common mutation E280K was present on 5.7% of PKU chromosomes. The frequency of the other five mutations (R261Q, R158Q, P281L, IVS10-11G>A and A104D) ranged from 1.4% to 3%. Twelve mutations were identified on a single chromosome only, corresponding to a frequency of 0.7%. Three mutations (P292T, K371E and IVS12-1G>A) had not been

Table 2

FREQUENCIES OF *PAH* GENE MUTATIONS IN LATVIAN PKU PATIENTS

No.	Mutation name	Systematic name	Location	Characters of mutation	No.	RF %
1	R408W	c.1222C>T	Ex 12	Missense	102	72.9
2	E280K	c.838G>A	Ex 7	Missense	8	5.7
3	R261Q	c.782G>A	Ex 7	Missense	4	3.0
4	R158Q	c.473G>A	Ex 5	Missense	4	3.0
5	P281L	c.842C>T	Ex 7	Missense	3	2.1
6	IVS10-11G>A	c.1066-11G>A	I10	Splice site	2	1.4
7	A104D	c.311C>A	Ex 3	Missense	2	1.4
8	A403V	c.1208C>T	Ex 12	Missense	1	0.7
9	E178G	c.533A>G	Ex 6	Missense	1	0.7
10	R111X	c.331C>T	Ex 3	Nonsense	1	0.7
11	R261X	c.781C>T	Ex 7	Nonsense	1	0.7
12	G272X	c.814G>T	Ex 7	Nonsense	1	0.7
13	I306V	c.916A>G	Ex 9	Missense	1	0.7
14	V230I	c.688G>A	Ex 6	Missense	1	0.7
15	L48S	c.143T>C	Ex 2	Missense	1	0.7
16	E221_D222>Efs	c.663_664delAG	Ex 6	Deletion	1	0.7
17	IVS12+1G>A	c.1315+1G>A	I12	Splice site	1	0.7
18	P292T*	c.874C>A	Ex 8	Missense	1	0.7
19	K371E*	c.1111A>G	Ex 11	Missense	1	0.7
20	IVS12-1G>A*	c.1316-1G>A	I12	Splice site	2	1.4
21	Unidentified	-	-	-	1	0.7
	Total				140	100

PKU, phenylketonuria. *Novel mutations identified in Latvian PKU chromosomes

previously identified; two of them were found only once, and the third was identified on two unrelated PKU chromosomes.

Mutation R408W was found to be homozygous in 36 (51.4%) of Latvian PKU families. Other patients in 34 families were compound heterozygous. No other *PAH* mutation was found in a homoallelic genotype.

None of the three previously unidentified mutations had been examined by *in vitro* expression analysis. A novel mutation was assumed to be disease-causing when (1) it was either non-silent or a potential splicing mutation, (2) no other mutation was identified in the coding region of the *PAH* gene, (3) the allele was inherited from the parent who did not carry the other PKU mutation, and (4) the mutation had not been previously identified on normal or mutant chromosomes.

One allele remained unidentified, despite repeated DGGE scanning of the whole coding region of the *PAH* gene and sequencing of all 13 exons.

PAH minihaplotypes (combination of *PAH* gene VNTR and STR alleles) for the mutant chromosomes were identified for compound heterozygous patients when parents were available. Their association with PKU mutations is reported in Table 3. Sixteen of the *PAH* gene minihaplotypes are associated with Latvian PKU chromosomes. Of these minihaplotypes, five (3/234, 2/238, 3/242, 7/242, 8/238 and 8/242) were associated with more than one mutation. On the other hand, more common PKU mutations, including the most common mutation R408W and mutations E280K, R261Q, R158Q and P281L, were associated to more than one minihaplotype.

Minihaplotype data were also obtained for 61 normal chromosomes from healthy family members of PKU patients. We found 20 alleles on normal chromosomes (Table 4). χ^2 analysis revealed highly significant differences between normal and mutant chromosomes in the spectrum of minihaplotype 3/238 ($P < 0.001$).

Table 3

ASSOCIATIONS WITH *PAH* MINIHAPLOTYPE OBSERVED FOR MUTATIONS IN THE LATVIAN POPULATION

No.	Mutation	Number of alleles	Minihaplotype	Alleles
1	R408W	34	3/238	28
			3/242	3
			3/234	2
			8/238	1
2	E280K	8	9/250	7
			9/246	1
3	R261Q	4	3/238	2
			8/238	2
4	R158Q	4	3/238	2
			3/234	1
			7/234	1
5	P281L	3	7/242	2
			8/242	1
6	IVS10-11G>A	2	7/250	2
7	A104D	2	8/242	2
8	A403V	1	8/246	1
9	E178G	1	7/242	1
10	R111X	1	8/250	1
11	R261X	1	7/238	1
12	G272X	1	9/234	1
13	I306V	1	3/234	1
14	V230I	1	3/246	1
15	L48S	1	3/234	1
16	E221_D222>Efs	1	3/242	1
17	IVS12+1G>A	1	8/242	1
18	P292T	1	8/226	1
19	K371E	1	3/238	1
20	IVS12-1G>A	2	7/242	2
21	Unidentified	1	3/234	1
Total alleles investigated		72	-	72

DISCUSSION

Phenylketonuria in Latvia is homogeneous. The most common mutation R408W accounts for 73% of Latvian PKU chromosomes. The same prevalence of this mutation is found in the Lithuanian population (Kasnauskiene *et al.,* 2003) and is even higher (84%) in the Estonian population (Ounap *et al.,* 1998).

51.4% of Latvian PKU families were found to be homozygous for mutation R408W, which explains the majority of patients with severe PKU. Patients in 30 (42.9%) families were compound heterozygous in combination with the R408W mutation. Non-R408W chromosomes harbouring other *PAH* mutations were found only in 4 (5.7%) compound heterozygous PKU patients.

The second most common mutation, E280K, which was present on 5.7% of all PKU chromosomes. In total, 20 mutations were identified in Latvian PKU patients; twelve mutations were identified on single chromosomes only, corresponding to a frequency of 0.7% each. The frequency of the other five mutations (R261Q, R158Q, P281L, IVS10-11G>A and A104D) ranged from 1.4% to 3%.

The distribution of *PAH* mutations in the Latvian PKU population simplifies routine genetic analysis, at least partly. In the first step in the analysis, screening for the most common PKU mutation R408W can potentially identify both alleles in 50% of patients and one allele also in almost 50%. Thus, about half of patients in our population require no further investigation. DGGE technology with further direct sequencing of *PAH* exons showing variant electrophoretic patterns can be used to detect the remaining *PAH* mutations causing PKU in Latvia. In some cases, the use of both of these methods is labour- and cost-efficient, especially, if both mutations are still unknown after the first step of analysis.

Table 4

FREQUENCIES OF MINIHAPLOTYPES ON NORMAL AND MUTANT *PAH* CHROMOSOMES

No.	Minihaplotype	Frequency	
		mutant alleles n = 72	normal alleles n = 61
1	3/234	0.0833	0.0492
2	3/238	0.4583	0.1147
3	3/242	0.0556	0.1475
4	3/246	0.0139	0.0164
5	7/230	—	0.0164
6	7/234	0.0139	0.0327
7	7/238	0.0139	0.0164
8	7/242	0.0694	0.0492
9	7/246	—	0.0492
10	7/250	0.0278	—
11	8/226	0.0139	0.0164
12	8/230	—	0.082
13	8/234	—	0.0492
14	8/238	0.0416	0.0984
15	8/242	0.0556	0.082
16	8/246	0.0139	0.0655
17	8/250	0.0139	0.0164
18	9/234	0.0139	0.0164
19	9/246	0.0139	0.0164
20	9/250	0.0972	0.0492
21	12/230	—	0.0164
	Total	1.0000	1.0000

Previous minihaplotypes study of the Latvian PKU population identified eight *PAH* gene minihaplotypes, which were associated with nine mutations in the PKU chromosomes (Pronina *et. al.,* 2003). The most frequent R408W mutation was mostly associated with minihaplotype 3/238. In that study, the results for other mutations were limited due to the chosen methods and due to the small number of samples analysed. The present study thus complements previous results.

Minihaplotypes (VNTR/STR) were determined for all 20 mutations found in Latvian PKU patients. Sixteen different minihaplotypes were found to be associated with PKU chromosomes. Most of the rare mutations were tightly linked to specific minihaplotypes, while the common mutations were associated with several minihaplotypes.

The most common minihaplotype was 3/238, due to the high frequency of mutation R408W. Mutation R408W was almost exclusively associated with minihaplotype 3/238, although it was found in association with four minihaplotypes: 3/238 (82.4%), 3/242 (8.8%), 3/234 (5.9%) and 8/238 (2.9%). Minihaplotype 3/238 is also the main minihaplotype for mutation R408W in Estonian, Polish and German populations (Ounap *et al.,* 1998; Zekanowski *et al.,* 2001; Zschocke *et al.,* 1999). It is also found in the Irish PKU population but in significantly lower frequencies (15%) compared with the minihaplotype 8/242, which is predominant (81.7%) minihaplotype for R408W (O'Donnell *et al.,* 2002). The frequency of minihaplotype 3/238 bp suggests a Balto-Slavic origin of R408W mutation.

Mutation E280K was associated with two minihaplotypes: minihaplotype 9/250 (87.5%) and minihaplotype 9/246 (12.5%). In other European populations E280K is found in association with other minihaplotypes – 7/246 in the German population, 8/238 and 8/246 in the Irish population and 9/234 in the Spanish population (Zschocke *et al.,* 1999; Zschocke *et al.,* 1995; Perez *et al.,* 1997). No data about other populations are available for E280K mutation, possibly, because it has not been found in significant frequencies.

R261Q mutation is found associated with minihaplotypes 3/238 and 8/238 in equal proportions. Minihaplotype 8/238 is predominant for R261Q in the Italian population (90.4%) and in the German population (90.9%) (Giannattasio *et al.,* 2001; Zschocke *et al.,* 1999). Other minor minihaplotypes associated with mutation R261Q in different populations are 3/246, 7/242, 8/234, 8/242 and 8/246, all of which are found in a few chromosomes.

Mutation R158Q was found in association with different minihaplotypes in Latvian PKU chromosomes: 3/238 (50%), 3/234 (25%) and 7/234 (25%). In the German population it is associated exclusively with minihaplotype 3/234 (Zschocke *et al.,* 1999). No data about other populations are available.

In our study, mutation P281L was found in association with two minihaplotypes: 7/242 and 8/242 at low frequencies, similarly as in German and Spanish populations (Zschocke *et al.,* 1999; Perez *et al.,* 1997). Minihaplotype 3/242 has been observed once in the Polish population (Zekanowski *et al.,* 2001).

The IVS10-11G>A mutation was found to be the most common in Mediterranean populations (> 30% in Turkey) and has been found on several distinct minihaplotypes, most commonly on minihaplotype 7/250. An east/west gradient of its relative frequency in the southern European population suggests that it originated in the Middle East and spread west and north during Neolithic migration (Cali *et al.,* 1997). Both Latvian PKU chromosomes harbouring mutation IVS10-11G>A were found in association with minihaplotype 7/250, which represents the ancestral background of this mutation.

The mild PKU mutation A104D was found in association with minihaplotype 8/242. The same minihaplotype for this mutation was observed in the German population (Zschocke *et al.,* 1999).

As the majority of the mutations in Latvian PKU chromosomes are rare, the adequate comparison of minihaplotype results for these mutations with other populations is uncertain. Also, minihaplotype analysis for the full spectrum of PKU mutations has been performed only in several populations but only the most common mutations for minihaplo-

type associations have been investigated in other populations.

Investigation of minihaplotypes for normal chromosomes obtained from PKU patients' parents revealed 20 different VNTR/STR combinations. The most common minihaplotype were 3/242 and 3/238 was less common. The majority of minihaplotypes were observed only once, likely because of the limited number of samples. Analysis of relative frequencies of the 3/238 minihaplotype in normal and mutant alleles showed statistically significant ($P < 0.001$) variations.

To be clinically useful in the management of neonates with PKU, routine genetic analysis should identify both mutations as rapidly as possible after a positive newborn screening. The analysis of minihaplotypes in all subjects subsequently allowed the identification of mutations likely to be on alleles with unknown mutations. Determining minihaplotypes proved very useful for the rapid identification of rare mutations. Having excluded the common mutation, the minihaplotypes of the allele under investigation usually implicate only one or two likely mutations, which can be confirmed by sequencing analysis. Minihaplotype analysis requires samples from the patient's parents, which may be a limitation in some cases. The knowledge of paternal or maternal inheritance is useful for carrier analyses in the extended family.

In comparison with conventional haplotypes, minihaplotypes are easier to obtain and are more informative for mutation analysis including prenatal diagnosis.

PKU mutations in Europe are shared between several populations. Mutation analysis can be helpful, since targeted screening for known rare mutations will identify the mutations from other European regions. Mutations spread with migrating peoples from founder populations to other regions and are frequently distributed over many countries. Analysis of minihaplotypes for as many mutations as possible, investigation of the origins of mutations, and study of the genetic history of different populations can thus further improve efficiency for diagnostic mutation analysis in PKU.

AKNOWLEDGEMENTS

The work was supported by grant No. 05.0023 from the Latvian Council of Science and European Social Foundation.

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Received 4 July 2011

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SAISTĪBA STARP MINIHAPLOTIPIEM UN MUTĀCIJĀM FENILALANĪNHIDROKSILĀZES LOKUSĀ FENILKETONŪRIJAS PACIENTIEM LATVIJĀ

Fenilketonūrija (FKU) ir iedzimta aminoskābju vielmaiņas slimība, ko izraisa mutācijas fenilalanīnhidroksilāzes gēnā (*FAH*), kas kodē fermentu fenilalanīnhidroksilāzi (FAH). Pētījumā tika veiktas analīzes 70 Latvijas fenilketonūrijas pacientiem un viņu vecākiem, ar mērķi noteikt pilnu mutāciju spektru *FAH* gēnā un noskaidrot šo mutāciju saistību ar minihaplotipiem. Latvijas pacientiem ar FKU ir atrastas 20 dažādas mutācijas. Biežākā gēna *FAH* mutācija ir R408W, kas ir atrasta 73% no visām FKU hromosomām un saistīta ar minihaplotipu VNTR3/STR238. Mutācijas E280K dominējošais minihaplotips ir VNTR9/STR250, bet mutācija IVS10-11G>A ir cieši saistīta ar VNTR7/STR250 minihaplotipu un, iespējams, ir cēlusies Vidusjūras reģionā. Minihaplotipu analīze ir noderīga pētījumos par mutāciju izcelsmi un izplatību dažādās populācijās, kā arī retu mutāciju noteikšanai un prenatālajā diagnostikā.