A molecular survey of phenylketonuria in Lithuania: spectrum, frequency and phenotypical manifestation of *PAH* gene mutations

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Phenylketonuria (PKU), a relatively common inherited disorder of amino acid metabolism, is caused by a variety of mutations in the phenylalanine hydroxylase *(PAH)* gene. We report the spectrum of the *PAH* gene mutations in individuals with PKU residing in Lithuania. A total of 184 independent PKU chromosomes (92 unrelated patients with PKU) were investigated. All 13 exons of the *PAH* gene of all PKU probands were scanned for DNA alterations by denaturing gradient gel electrophoresis (DGGE) with subsequent identification of mutations by sequencing relevant exons. Twenty-one different *PAH* gene mutations were identified in Lithuania, resulting in an overall PKU mutation detection rate of 95.1%. Out of them, 16 were novel for Lithuania (*i.e.* identified in the course of this study). The most frequent *PAH* gene mutations in PKU patients from Lithuania were R408W (73.4% of PKU chromosomes) and R1580 (7.1% of PKU chromosomes). Relative frequencies of other mutations were less than 2%. Fourteen mutations were represented by single cases with a relative frequency of 0.5%. Such data point to a significant homogeneity of the Lithuanian population.

Key words: phenylketonuria, PKU, *PAH* gene mutations

INTRODUCTION

Phenylketonuria (PKU) is the commonest inborn error of amino acid metabolism in Europeans and one of the commonest autosomal recessive diseases worldwide. It is caused by mutations in the phenylalanine hydroxylase (*PAH*) gene. Mutations are responsible for a deficiency of the hepatic enzyme, phenylalanine hydroxylase (PheOH; EC 1.14.16.1). The spectrum of PheOH deficiency ranges from a severe hyperphenylalaninaemia (classical PKU), leading to a profound mental retardation unless the dietary intake of phenylalanine (Phe) is restricted [1], to mild hyperphenylalaninaemia (MHP) which does not require treatment. Since the identification of the *PAH* gene in 1986 [2], almost 400 different mutations and sequence polymorphisms have been identified and listed in the *PAH* mutation databalse [3]. Their phenotypic manifestation differs in residual enzyme activity, and the *PAH* genotype has recently been shown to be a good predictor of biochemical phenotype in the majority of patients [4].

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Over the last decade, comprehensive mutation data have become available for most European countries. There are marked differences in the spectrum of mutations and in the degree of heterogeneity among the regions. Southern European populations are heterogeneous, with the most common mutation IVS10–11g>a accounting for 10–20% of PKU chromosomes [5, 6], while northern and northern-western European populations have other relatively frequent mutations, such as IVS12+1g>a (37% of PKU chromosomes in Denmark [7]) or R408W (42% of PKU chromosomes in Ireland [8]). In Eastern Europe there is one predominant mutation, R408W, with a relative frequency 70–80% in the Baltic states [9–12].

The frequency of five *PAH* mutations (R408W, R158Q, R261Q, G272X and IVS10nt-11g>a) in Lithuania has already been reported [9–11]. Here we expand the analysis through the study of 92 unrelated PKU families from Lithuania, using the approach based on PCR amplification of the *PAH* gene exons, mutation scanning by denaturing gradient gel electrophoresis (DGGE), and mutation identification by direct automated DNA sequencing.

SUBJECTS, MATERIALS AND METHODS

Patients with PheOH-deficient hyperphenylalaninaemia from 92 independent families (88 probands with PKU and 4 probands with MHP) living in Lithuania were investigated.

Samples were collected at the Human Genetics Centre of Vilnius University Hospital. Paternal samples were available from 63 families. The nationality and place of birth of both sets of grandparents were recorded.

Genomic DNA was extracted from blood leukocytes of the patients and both parents (when available). Restriction enzyme digestion based testing for the most common R408W mutation was performed [14]. To identify other mutations, the whole *PAH* gene (all 13 exons) of each patient was scanned by standard [13] or multiplex [14] DGGE (DGGE C.B.S. Scientific Company, Inc., USA). The exons showing variant electrophoretic patterns were sequenced by an ABI PRISM™ 310 automated gene analyzer and Big Dye Terminator Sequencing protocol (Perkin Elmer Applied Biosystems, USA).

Maternal and paternal inheritance of *PAH* gene mutations was determined in all families where paternal DNA was available.

RESULTS AND DISCUSSION

Ninety-two unrelated patients with PKU (*i.e.* 184 unrelated PKU chromosomes) were available for molecular genetic testing in this study. Standard and/or multiplex DGGE was applied to screen for *PAH* gene mutations in 36 PKU probands with the *PAH* locus genotype Mut/? or ?/?; 20 DNA samples from the probands with the genotype R408W/R408W were screened for the presence of other DNA polymorphisms in the *PAH* gene. In the case of a DGGE pattern specific for the genotype, Mut/Norm, Mut/Mut or Mut_1/Mut_2 corresponding DNA fragments were sequenced to identify the mutation. Twenty-one different *PAH* gene mutations were identified in Lithuania (Table 1). Thus, implementation of multiplex DGGE and direct automated DNA sequencing increased the overall mutation detection rate to 95.1% *versus* 79.2% previously achieved by direct testing for a set of *PAH* gene mutations by PCR amplification of a definite *PAH* gene exon with subsequent digestion with a relevant restriction enzyme. It should be pointed out that the latter relatively high value was obtained due to the particular prevalence of the R408W mutation in Lithuania (see below). All 21 *PAH* gene mutations identified in Lithuania appeared to have already been registered in the *PAH* gene mutations database [3]. Out of them, 16 were novel for Lithuania (*i.e.* identified in the course of this study). The most frequent *PAH* gene mutations in PKU patients from Lithuania were R408W (73.4% of PKU chromosomes) and R158Q (7.1% of PKU chromosomes). Other mutations were rare and showed a relative frequency less than 2%. Of them, 14 mutations were represented by single cases with a relative frequency of 0.5%. Such data point to a significant homogeneity of the Lithuanian population. No disease-causing *PAH* gene mutation has been identified yet on nine PKU chromosomes (4.9%) using the PCR → DGGE → DNA-sequencing-based approach applied in the current

Table 1. **PAH gene mutations identified in 92 unrelated PKU patients residing in Lithuania**

Exon/intron	Mutation		Number of independent chromosomes	Frequency (%)
	Trivial name	Systematic name		
Exon 1	L15S16fsdelCT[b]	c.47–48delCT	1	0.5
Exon 2	DelF39[b]	c.115–117delTTC	1	0.5
	F55fs[b]	c.165delT	1	0.5
Intron 2	IVS2–13t>g[b]	c.169–13t>g	2	1.1
Exon 3	R111X[b]	c.311C>T	1	0.5
Exon 5	R158Q[a]	c.472G>A	13	7.1
Exon 6	R176X[b]	c.526C>T	1	0.5
	E221D222fsdelAG[b]	c.663–664delAG	1	0.5
Exon 7	V245A[b]	c.734T>C	1	0.5
	R261X[b]	c.781T>C	1	0.5
	R261Q[a]	c.782G>A	1	0.5
	G272X[a]	c.814G>T	3	1.6
	E280K[b]	c.838G>A	2	1.1
	P281L[b]	c.842C>T	2	1.1
Exon 9	1306V[b]	c.916A>G	1	0.5
	L311P[b]	c.932C>T	1	0.5
Intron 10	IVS10nt-1g>a[b]	c.1065+1g>a	1	0.5
	IVS10nt-11g>a[a]	c.1066–11g>a	1	0.5
Exon 12	A403V[b]	c.1208C>T	4	2.2
	R408W[a]	c.1222C>T	135	73.4
	Y414C[b]	c.1241A>G	1	0.5
Unidentified mutation[c]			9	4.9

[a] *PAH* gene mutations identified in Lithuania before and during this study.
[b] *PAH* gene mutations identified in Lithuania during this study.
[c] Mutations unidentifiable using PCR → DGGE → DNA-sequencing-based approach.

Table 2. **Genotype and phenotype correlation in PKU patients**

PAH locus genotype	No. of cases	Phenotype frequency (%)	Clinical phenotype
1. R408W/R408W	50	54.3	Severe PKU
2. R408W/158Q	10	10.9	Severe PKU
3. R408W/G272X	3	3.3	Severe PKU
4. R408W/IVS2nt-13t>g	1	1.1	Severe PKU
5. R408W/E280K	2	2.2	Severe PKU
6. delF39/IVS10nt-11g>a	1	1.1	Severe PKU
7. R408W/L311P	1	1.1	Severe PKU
8. R408W/P281L	1	1.1	Severe PKU
9. R408W/R176X	1	1.1	Severe PKU
10. R408W/L15S16fsdelCT	1	1.1	Severe PKU
11. R408W/R261X	1	1.1	Severe PKU
12. R408W/R261Q	1	1.1	Severe PKU
13. R408W/IVS10nt-1g>a	1	1.1	Severe PKU
14. R408W/E221D222delAG	1	1.1	Severe PKU
15. R408W/F55fs	1	1.1	Severe PKU
16. P281L/?	1	1.1	Severe PKU
17. R158Q/R158Q	1	1.1	Severe PKU
18. R158Q/IVS2nt-13t>g	1	1.1	Moderate PKU
19. R408W/?	4	4.2	Moderate PKU–1 Severe PKU–3
20. R408W/Y414C	1	1.1	Mild PKU
21. R408W/1306V	1	1.1	MHP
22. R408W/A403V	4	4.3	Mild PKU–3 MHP–1
23. R111X/V245A	1	1.1	Unclassified
24. ?/?	2	2.2	Severe PKU
Total	92	100	

investigation (*i.e*. DGGE patterns did not show any electrophoretic pattern characteristic of DNA alterations). This approach does not allow identification of large deletions spanning the whole exons or mutations in large introns. The mutation detection rate (95.1%) and methods for detection of *PAH* gene mutations are similar to the rate and methods of other relevant molecular diagnostic laboratories.

Data on the *PAH* locus genotypes identified in Lithuania are summarized in Table 2. The most frequent genotype in unrelated PKU patients was R408W/R408W (54.3%); 38% of patients harboured the R408W mutation with a different mutation on the other PKU chromosome.

The correlation of the *PAH* locus genotype and the clinical phenotype estimated in PKU patients from Lithuania corresponds to that observed by other investigators in a number of European populations, supporting the established assigment of *PAH* mutations to the metabolic phenotypes (according to the list presented in the article of P. Guldberg et al. [15]). In the case of severe PKU with one known severe mutation (P281L/?), the other unidentified mutation was most likely severe. In all cases with the genotype ?/? phenotypic features of the patients from Lithuania were typical of severe PKU with good response to the dietary treatment (low Phe diet). Therefore BH_4 deficiency was excluded, and mutations on both PKU chromosomes were most likely severe in these cases. The varying severity of hyperphenylalaninemia in the cases with the genotype R408W/? carrying a severe mutation R408W implies the unidentified mutations to be heterogeneous.

The homogeneity of PKU in Lithuania and other Baltic states is reflected by a particular prevalence of the R408W mutation and a reduced spectrum of other *PAH* gene mutations if compared to a number of European populations (Table 3). This mutation is still the most common one in individuals with PKU in Germany [4], while in Italy it is classified as rare [6]. Thus, a north to south frequency gradient is characteristic of the R408W mutation of the *PAH* gene in Europe. As regards the spectrum of other *PAH* gene mutations in European PKU populations, a combination of the particular prevalence of the R408W mutation and a

Table 3. **Features of PKU mutations in some European populations**

PKU population	Number of different *PAH* gene mutations identified	Frequency of the R408W mutation (%)	Number of PKU chromosomes investigated
Estonia[a]	6	84	68
Latvia[b]	10	77	96
Lithuania[c]	21	73.4	184
Germany[d]	91	22	546
Italy[e]	24	1	289

[a] [12]
[b] [17]
[c] Present study
[d] [4]
[e] [6]

relatively small number of indivuals with PKU available for investigation explain a relatively low variety of mutations identified in Lithuania, Latvia and Estonia.

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MOLEKULINĖ FENILKETONURIJOS APŽVALGA LIETUVOJE: *PAH* GENO MUTACIJŲ SPEKTRO IR DAŽNIO NUSTATYMAS IR JŲ FENOTIPINIS PASIREIŠKIMAS

S a n t r a u k a

Fenilketonurija (FKU) yra dažniausias aminorūgščių apykaitos sutrikimas, nulemtas mutacijų fenilalaninhidroksilazės (*PAH*) gene. Mes pateikiame sergančiųjų FKU Lietuvoje *PAH* geno mutacijų spektrą. Tyrimo metu analizuotos 184 FKU chromosomes (92 negimininių asmenų, sergančių FKU). Tiriamųjų DNR *PAH* geno visi 13 egzonų buvo tiriami denatūruojančio gradientinio gelio elektroforezės (DGGE) metodu ir nustatyta tiesioginė nukleotidų seka tų egzonų, kuriuose rasta pakitimų. Išaiškinta 21 skirtinga mutacija, 16 iš jų Lietuvoje nustatytos pirmą kartą. Mutacijos identifikuotos 95,1% chromosomų: 73,4% sudaro R408W, 7,1% – R158Q mutacija. Kitos mutacijos retos – jų dažnis svyruoja nuo 0,5 iki 2%. 14 mutacijų nustatyta tik vienoje chromosomoje (0,5%). *PAH* mutacijų įvairovės atžvilgiu lietuvių populiacija yra sąlyginai homogeniška.