



SHORT COMMUNICATION

Analysis of EX5del4232ins268 and EX5del955 *PAH* gene mutations in Ukrainian patients with phenylketonuria



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Abstract Phenylketonuria (PKU) is an autosomal recessive metabolic disorder caused by deficiency of phenylalanine hydroxylase (PAH). The major molecular defects causing PKU are missense mutations of *PAH* gene. Large deletions of exon 5 (EX5del955 and EX5del4232ins) were first reported by the Czech study and were later found also in the Polish, Slovak, Slovenian and Italian PKU-patients. These observations demonstrate the existence of a common subset of this mutation predominantly among Central European populations of Slavic descent. That is why we suggest that EX5del955 and EX5del4232ins268 mutations might be frequent causes of PKU in Ukrainian patients. EX5del955 and EX5del4232ins268 mutations were analyzed in 106 unrelated PKU patients negative for *PAH* gene mutations on one or both alleles from our previous analysis. The simultaneous detection of EX5del4232ins268 and EX5del955 mutations was performed by PCR amplification of mutant alleles. EX5del955 mutation was not detected in the Ukrainian patients. This relative alleles frequency of EX5del4232ins268 mutation in the Ukrainian PKU population was determined as 1,66%. Our findings can be the one more evidence of Central European Slavic origin of EX5del4232ins268 mutation, suggested previously. This finding is important for the improvement of DNA diagnosis necessary for the management of PKU patients from Ukraine.

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Introduction

Phenylketonuria (PKU; MIM #261600) is an inherited metabolic disease caused by impaired levels, or absence, of an enzyme needed for the conversion of the amino acid phenylalanine into the amino acid tyrosine. It is characterized by mild to severe mental disability in untreated patients. PKU is inherited in an autosomal-recessive manner. The prevalence of PKU shows considerable geographic variation. It is estimated to be 1/10,000 live births in Europe with a higher rate in some countries (Ireland, Italy, Turkey).¹ In Ukraine this value is estimated by State Expert Center MoH as 1/7000 (<http://www.dec.gov.ua>).

PKU is caused by mutations in the gene encoding the phenylalanine hydroxylase (PAH) enzyme. The *PAH* gene is 90 kb in length (about 171 kb if flanking regions are included) with 13 exons.²

Locus-specific databases, such as PAHdb (<http://www.pahdb.mcgill.ca>) and BIOPKU (<http://www.biopku.org>), have been an important resource for understanding the nature, prevalence, and impact on PAH deficiency. The open-access BIOPKU reports 955 variants of this gene (May 23, 2016). An analysis of this database showed that 60% of *PAH* variants are missense mutations, with other common variants being splice variants and deletions (14% each).³

The *PAH* gene mutations frequency varies among different ethnic and regional groups. The large number of phenylalanine hydroxylase gene mutations, the high incidence of compound heterozygosity, and the variability in distribution of common mutations between ethnic groups and geographical areas makes phenylketonuria a genetic disease with pronounced allelic heterogeneity. Large deletions of exon 5 (EX5del955 and EX5del4232ins268) were first reported by the Czech study and were later found also in the Polish, Slovak, Slovenian and Italian PKU patients.^{4–8} These observations demonstrate the existence of a common subset of this mutation predominantly among Central European populations of Slavic descent. That is why we suggest that EX5del955 and EX5del4232ins268 mutations might be frequent causes of PKU in Ukrainian patients.

Materials and methods

The investigated cohort consisted of 241 unrelated PKU patients from different regions of Ukraine previously analyzed by PCR/RFLP method for following *PAH* gene mutations: R408W, Y414C, R158Q, Ivs10nt546, Ivs12nt1, R252W, R261Q, R261X, G272X, S273F, P281L. In 135 of 241 patients mutations on both alleles were found. In the rest PKU-patients which were negative for above mentioned *PAH* gene mutations on one (86 patients) or both (20 patients) alleles the analysis of exon 5 deletions in the *PAH* gene was performed.

The detection of EX5del4232ins268 and EX5del955 mutations was performed by PCR amplification of using a protocol describe previously by M. Bik-Multanowski et al.⁵

with our modifications. The reaction mix contained primers for simultaneous detection of both mutations. The following primer sequences were used: forward EX5del955: 5'/GCACATTGGAATCCACAGCAAGG3'; forward EX5del4232ins268 5'/TCACTGACCCATTCCAGAGTACA3'; reverse Common 5'/AATCCCGACAACCCAGAAGTAA3'. In the case of EX5del4232ins268 mutation presence, the 1425 bp fragment was detected, in the case of EX5del955 mutation – 735 bp fragment. If the case of the normal allele 1690 bp fragment was identified. This fragment is absent in the case of EX5del4232ins268 and EX5del955 mutation homozygous or their compound state.

Results and discussions

In our previous studies 11 mutations: R408W, Y414C, R158Q, Ivs10nt546, Ivs12nt1, R252W, R261Q, R261X, G272X, S273F, P281L were analyzed by PCR/RFLP method in 241 PKU patients from different regions of Ukraine.^{9,10} In 135 of 241 patients above mentioned mutations were identified on both alleles. In present study EX5del955 mutation, previously identified in Czech, Polish, Slovak, Slovenian, Italian PKU patients,^{4–8} was not detected in the Ukrainian PKU cohort of 241 PKU patients. At the same time EX5del4232ins268 mutation was found in seven PKU patients negative for 11 above mentioned *PAH* gene mutations on one or both alleles. Six of them were carriers of EX5del4232ins268 mutation in compound heterozygous state and in one patient this mutation was detected in homozygous state.

In present study we have added our data taking into account the results of EX5del4232ins268 mutation analysis and obtained the following distribution on analyzed *PAH* gene in Ukrainian PKU population. The obtained of relative frequency detected mutant alleles are: R408W (51,5%), P281L (3,7%), R158Q (3,3%), Y414C (2,7%), R252W (2,7%), R261Q (2,5%), Ivs10nt546 (2,1%), EX5del4232ins268 (1,66%), Ivs12nt1 (1,5%). It is important to note that the frequency of PKU allele with EX5del4232ins268 mutation in the Ukrainian PKU population (1,66%), is similar to data obtained in previous studies concerning of PKU patients of Slavic origin 1,63% of the PKU alleles in the Czech population⁴; 1,1% in the Polish patients,⁵ 1,93% in the Slovak PKU patients (Table 1).⁶ EX5del4232ins268 mutation in our patients also shared the same intragenic VNTR (variable number tandem repeat) allele #3 as it was shown in Czech PKU patients.⁴ Such findings can be the one more evidence of Central European Slavic origin of EX5del4232ins268 mutation, suggested previously by L. Kozak et al.⁴

Conclusion

The EX5del955 mutations was not found study group of PKU patients from Ukraine. The relative frequency of mutant allele bearing EX5del4232ins268 mutation in the Ukrainian PKU population is 1,66%. This finding important for improvement of DNA diagnosis necessary for management of PKU patients from Ukraine.

Table 1 Frequency of the EX5del4232ins268 mutation in some European populations.

| Pku population | Frequency of the EX5del4232ins268 mutation (%) | Number of PKU chromosomes investigated | Reference |
|----------------|--|--|-------------------|
| Czech | 1,63 | 1042 | 4 |
| Polish | 1,1 | 540 | 5 |
| Slovak | 1,93 | 414 | 6 |
| Ukrainian | 1,66 | 482 | Present study |

Conflicts of interest

There is no conflict of interest.

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