# DETERMINING THE FREQUENCY OF *PAH* MUTATIONS IN MOSCOW REGION RESIDENTS WITH PHENYLKETONURIA USING A COMBINATION OF REAL-TIME PCR AND NEXT-GENERATION SEQUENCING

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The present study aimed to determine frequencies of mutations in the phenylalanine hydroxylase gene (*PAH*) in unrelated children (n = 71) diagnosed with phenylketonuria, who presented to Morozovskaya Children's City Clinical hospital (Moscow) over the period from 2015 to 2016. The patients were tested for the most common *PAH* mutations using the original real-time PCR-based technique for the identification of nucleotide variants; additionally, next generation sequencing (NGS) was performed on the unidentified genotypes. The original PCR-based technique allowed us to effectively identify 83 % of the pathogenic allelic variants in the sample. Using the combination approach (real-time PCR + NGS), we found mutations in both alleles of *PAH* in 66 of total 71 patients. Altogether, 26 pathogenic *PAH* mutations were identified, the most common being p.R408W (47.9 %) and p.R261Q (9.9 %). Frequencies of mutations common for the Russian population, such as IVS10nt546, IVS12+1G>A, p.R158Q, p.Y414C, and IVS4+5G>T, ranged from 4.2 to 2.8 %. Half of the identified variants accounted for the total frequency of < 10 %. Sequencing of *PAH* revealed a few functional mutations previously unreported for Moscow region residents, including p.D222Terfs, p.R111Ter, p.F161S, p.G188D, p.R270K, p.L311P, p.F55L, p.F55Leufs, IVS1+5G>T, and IVS8-7A>G. It could be reasonable to include mutations p.D222Terfs and p.R111Ter (carrier frequency of 2.1 %) in PCR testing panels. The data obtained in our study can also be used in the development of genetic tests for phenylketonuria.

**Keywords:** phenylketonuria, phenylalanine hydroxylase gene, *PAH*, real-time PCR genotyping

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# ОПРЕДЕЛЕНИЕ ЧАСТОТЫ ВСТРЕЧАЕМОСТИ МУТАЦИЙ В ГЕНЕ *PAH* С ПРИМЕНЕНИЕМ КОМБИНАЦИИ ТЕХНОЛОГИЙ ПЦР «В РЕАЛЬНОМ ВРЕМЕНИ» И ВЫСОКОПРОИЗВОДИТЕЛЬНОГО СЕКВЕНИРОВАНИЯ У БОЛЬНЫХ ФЕНИЛКЕТОНУРИЕЙ МОСКОВСКОГО РЕГИОНА

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Определена частота встречаемости мутаций в гене фенилаланингидроксилазы (*PAH*) у неродственных детей (n = 71) с диагнозом «фенилкетонурия», наблюдавшихся в Морозовской детской городской клинической больнице (г. Москва) в 2015–2016 гг. Для выявления частых вариантов мутаций в гене *PAH* была применена оригинальная технология определения нуклеотидных замен на основе ПЦР в режиме «реального времени» (real-time PCR), выполнено дополнительное исследование гена методом целевого секвенирования нового поколения (NGS). Эффективность диагностирования методом ПЦР при выявлении носительства патогенного аллеля в выборке составила 83 %. При проведении комбинированной диагностики мутации в двух аллелях были выявлены в 66 случаях из 71. Всего определено 26 патогенных мутаций в гене *PAH*, наиболее часто представлены мутации p.R408W (47,9 %) и p.R261Q (9,9 %). Распространенные в России IVS10nt546, IVS12+1G>A, p.R158Q, p.Y414C, IVS4+5G>T выявлены с частотами от 4,2 до 2,8 %. Суммарная частота встречаемости половины определенных вариантов мутаций составила менее 10 %. По итогам секвенирования гена *PAH* обнаружен ряд ранее не описанных для Московского региона мутаций различного функционального типа: p.D222Terfs, p.R111Ter, p.F161S, p.G188D, p.R270K, p.L311P, p.F55L, p.F55Leufs, IVS1+5G>T, IVS8-7A>G. Мутации p.D222Terfs и p.R111Ter (с частотами 2,1 % каждая) являются потенциальными кандидатами на включение в состав скрининговой панели. Полученные данные могут быть использованы для разработки схем генодиагностики фенилкетонурии.

**Ключевые слова:** фенилкетонурия, ген фенилаланингидроксилазы, *PAH*, генетическая диагностика

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Deleterious mutations in the gene coding for phenylalanine hydroxylase cause a disabling disease called phenylketonuria (classical PAH-dependent PKU, or type I PKU). This disease is inherited in an autosomal-recessive manner. WHO recommends including it into newborn screening. In Russia PKU occurs in 1 in 7,000 individuals [1]. The disorder is associated with deficient activity of phenylalanine hydroxylase, the hepatic enzyme that converts phenylalanine (PA) into tyrosine. Because of the compromised enzyme activity, the levels of PA and its derivatives go up while tyrosine concentrations decrease; PAH deficiency also affects metabolism of other amino acids [1, 2]. Untreated babies show signs of damage to the central nervous system within first six months after birth. But tragic consequences of PKU can be avoided by timely diagnosis and adequate treatment.

In Russia, blood levels of phenylalanine are measured in all neonates shortly after birth to facilitate early diagnosis [1, 2]. If PA concentrations exceed 2 mg/mol (0.12 mmol/l), i. e. indicate hyperphenylalaninemia (HPA), the test if repeated; other tests are taken to differentiate between different types of the disease. To verify the clinical diagnosis of PKU and to identify the *PAH* genotype, genetic testing may be advised. *PAH* mutations affect properties of the synthesized enzyme differently depending on their location and functional type [1, 3–6]. Severe forms of the disease are caused by alterations in the nucleotide sequence of the gene that disrupt protein synthesis or result in the production of an enzyme with zero residual activity. The mutant variant p.R408W\c.1222C>T is the most prevalent in the Russian population [1, 3, 6–10] and also the most severe. In its homozygous state it results in the production of the protein with minimal residual activity. Recently it has been found that synthetic analogs of tetrahydrobiopterin (the natural coenzyme of PAH called HB4) used in the treatment of $HB_4$-dependent forms of HPA bring down PA blood levels in patients with classical PKU given that the residual activity of the enzyme is retained. In this case medications help to alleviate clinical symptoms and relax a patient's diet. Therefore, genetic testing is a basis for an adequate choice of treatment strategy in patients with PKU.

Approaches to genetic screening may vary. For example, the most common *PAH* mutations can be detected by various types of selective PCR or PCR-RFLP (restriction fragment length polymorphisms) assays [6–8]. Also, great promise is held by multiplex ligation-dependent probe amplification (MLPA) [9] and real-time PCR based on the use of adjacent probes [10]. These approaches allow identification of dozens of sequence variants in parallel. However, these mutation-selective diagnostic techniques are only 70–80 % effective [7, 8]. Rare (with <1 % frequency) or previously undescribed mutant variants can be effectively detected by targeted sequencing techniques [3, 4, 6, 11] ensuring a wealth of information on the studied sequence. Currently, in Russia there is a need for domestic diagnostic solutions for PKU or other types of hyperphenylalaninemia based on next generation sequencing (NGS).

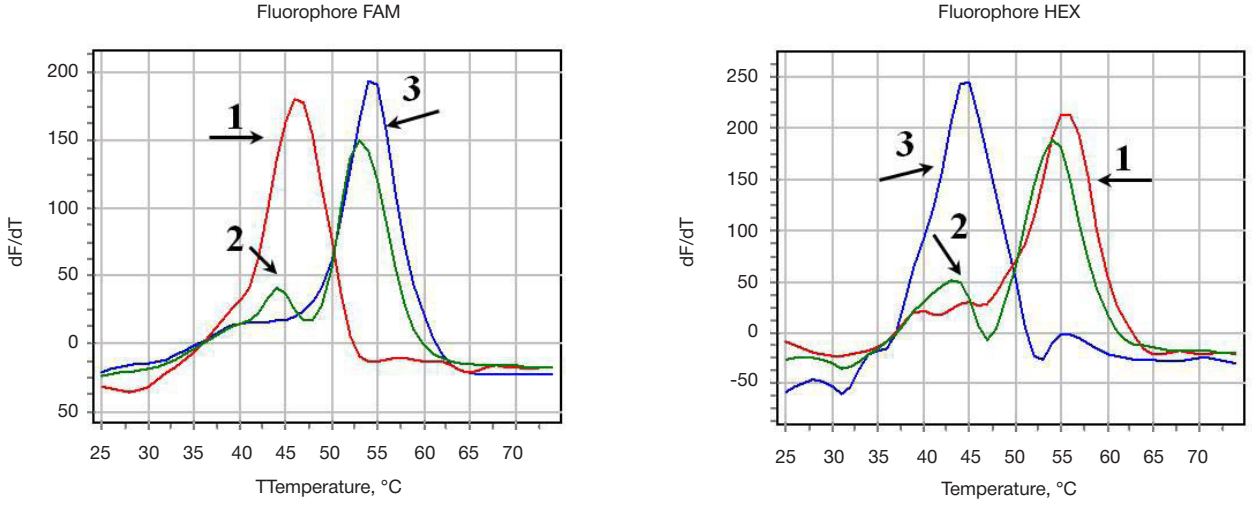
The aim of this study was to conduct screening for *PAH* mutations in 71 children (residents of the Moscow region) diagnosed with classical phenylketonuria or hyperphenylalaninemia. Screening for mutations commonly observed in this gene was performed using the original technique that allows detection of nucleotide substitutions and employs real-time PCR and the analysis of melting curves; rare mutations and those overlooked by the analysis were detected using targeted NGS.

## METHODS

The study involved 71 children diagnosed with either classical phenylketonuria or hyperphenylalaninemia (69 and 2 patients, respectively) who had been undergoing treatment in Morozovskaya Children's City Clinical hospital (Moscow) in 2015—2016. Diagnosis was established based on the clinical symptoms and results of the blood chemistry test. The patients were unrelated. At the time of study the patients were residing in the Moscow region. Ethnically, 85 % of the patients were Russians; about 15 % were of different origin (South Caucasus, Central Asia, and East Asia: one of the patients was Chinese). The study was conducted in full compliance with the Declaration of Helsinki. Parents gave their informed consent.

Genomic DNA was isolated from venous whole blood of the patients using the reagent kit Proba-GS-Genetics by DNA-Technology, Russia. The obtained DNA samples were either immediately genotyped or stored at −20 °C for later genotyping.

PCR-genotyping used in our study is a modification of the method based on the use of adjacent (kissing) probes [12]. It employs two types of sequence-specific oligonucleotide probes that hybridize to the DNA template at low temperatures in close proximity to each other. One of the probes (a reporter) carries a source of fluorescence, another one carries a quencher. To increase the reliability of the results, two variations




Melting curves representing different allelic variants generated by the mutation p.R408W\c.1222C>T. Curve 1 represents a homozygous variant; curve 2 represents a heterozygous variant (note the two peaks on the curves); curve 3 represents a homozygous wild type

of reporter probes are used labeled with different fluorophores and complementary to the studied polymorphic regions. After the targeted DNA sequence is amplified, the reaction mix is cooled down, and the probes hybridize to the PCR product. Genotyping is performed during temperature denaturation of oligoprobe–amplicon duplexes by measuring fluorescence in real time. The figure below shows how melting curves represent certain genotypes. A detailed description of the used genotyping technique is available in the article by Sergeev et al. [13]

In our study we used pre-tested primers and probes for the following set of 16 *PAH* mutations: p.R408W, p.R261Q, p.R158Q, IVS10nt546\c.1066-11G>A, IVS12+1G>A, p.Y414C, IVS4+5G>T, p.R252W, p.L48S, p.R261Ter, p.P281L, p.G188D, p.E280K, p.F331S, p.P279L, and IVS2+5G>C. This list contains 8 variants most common for the Russian population and recommended for inclusion into newborn screening programs [1]. PCR was performed using the detection thermocycler DTprime (DNA-Technology) as described in [10]. Melting temperatures were determined using the same PCR machine. The entire PCR-genotyping procedure took 1.5 hours.

DNA samples of patients whose genotype had not been identified in the course of PCR-genotyping were analyzed on the Ion Torrent targeted next-generation sequencing platform (Thermo Fisher Scientific, USA). The sequencing panel covered exon regions (100 % coverage of the coding sequence), exon-intron border regions, and untranslated regulatory regions of the gene (partial coverage). In total, 3,337 b. p. of the *PAH* gene were covered. Targeted sequences were amplified by multiplex PCR. For amplification >10 ng of the genomic DNA were used. Adaptor sequences were ligated to amplicons with T4 DNA ligase (Thermo Fisher Scientific) as described in the manufacturer's ligation protocol. Quality control of DNA libraries for NGS was performed on the Agilent 2100 Bioanalyzer using the Agilent High Sensitivity DNA Kit (Agilent Technologies, USA). Samples were sequenced on the Ion PGM System for Next-Generation Sequencing (Thermo Fisher Scientific.) using the Ion PGM Template OT2 400 Kit (Thermo Fisher Scientific).

The obtained data were first processed using Torrent Server 4.4.3. Reads were aligned to the reference genome GRCh37/hg19 by TMAP; variant calling was performed using Torrent Variant Caller 4.4 (all software by Thermo Fisher Scientific). Further analysis was conducted using the original software developed by the authors of this work. In average, the number of reads per targeted sequence was 7,300; the minimal number of reads was 590 reads. The average number of reads per sample was 95,500. Pathogenicity of mutant variants was inferred based on the analysis of data from dbSNP Build 147, PAHvdb, and BIOPKUdb [14] and data available in the literature. Selective Sanger validation of NGS results was performed on the ABI PRISM® 310 Genetic Analyzer (Applied Biosystems, USA), with the reaction kits supplied by the manufacturer in strict adherence to the protocol. All applied genotyping techniques yielded the same results.

## RESULTS

In the first part of our study we screened the patients for 16 most common *PAH* mutations using real-time PCR. Genotyping revealed the presence of 13 mutant variants: p.R408W, p.R261Q, p.R158Q, p.L48S, p.G188D, p.Y414C, p.R252W, IVS4+5G>T, p.R261Ter, IVS10nt546/c.1066-11G>A, p.E280K, IVS12+1G>A, and p.P281L (Table 1). In 70.4 % of cases both alleles were affected; 25.4 % of patients had mutations in one of the two alleles. The rest 4.2 % of patients had no deleterious mutations.

In the second part of the study, NGS was applied to sequence clinically significant *PAH* regions in 21 samples with unidentified genotype. The results allowed us to considerably extend the list of pathogenic *PAH* variants, comprising now p.D222Terfs, p.R111Ter, IVS11+1G>C, p.F161S, p.E390G, p.A300S, p.F55L, p.F55Leufs, p.R176Ter, p.L311P, p.R270K, IVS1+5G>T, and IVS8-7A>G (Table 1). These mutations were previously described in the literature and are listed in PAHdb as deleterious. Subsequent Sanger sequencing supported our findings.

The combination approach to genetic screening yielded good results: 2 deleterious *PAH* mutations were found in 66 patients (93 %); 4 patients (5.6 %) were found to have only one mutation. One patient (1.4 %) did not have any mutations in the *PAH* gene.

Frequencies of 26 pathogenic variants identified in the studied sample are presented in Table 1. The most frequent mutations were p.R408W and p.R261Q (found in 54 and 12 patients, respectively, in homo- or heterozygous state). Relatively frequent were IVS10nt546\c.1066-11G>A, IVS12+1G>A, and p.R158Q, all heterozygous, with individual allele frequencies ranging between 4.2 and 3.5 %. Half of the pathogenic variants identified in our sample had a total frequency <10 %. Based on the study results, we described 34 allelic variants of *PAH*; 21 patients had mutations in one or two alleles that resulted in the production of phenylalanine hydroxylase retaining >10 % of its residual activity (Table 2).

## DISCUSSION

The frequency of p.R408W, the most common mutant variant of *PAH* found in the Russian population, was as high as 47.9 % in the studied sample of patients with PKU, which is close to the regional average [9], but significantly lower than frequencies reported in the Rostov region [15], Kemerovo region [11], Novosibirsk region [3] and the Russian Far East [7, 9]. Another mutation, p.R261Q, was the second most frequent mutation in the sample. It is considered to be among the most common mutant variants found in the Russian population [1, 3, 6–8, 15]. It is prevalent in the Karachay-Cherkess Republic [16]. Both p.R408W and p.R261Q often occur in the European population, p.R408W being more widespread in the Eastern Europe and p.R261Q being frequently found across the South of Europe, the Netherlands and Switzerland [5]. Unlike p.R408W, p.R261Q is a mild mutant variant of *PAH*.

The following mutations were relatively frequent in the studied sample: IVS10nt546, IVS12+1G>A, p.R158Q, p.Y414C, IVS4+5G>T, p.L48S, and p.R252W (individual allele frequencies ranged from 4.2 to 2.1 %). The heterozygous p.P281L was identified in 1 patient of Russian origin. In some Russian regions this mutation is reported to be one of the most common [3, 15, 17].

The compound p.D222Terfs and p.R111Ter were identified in 3 genotypes each (allele frequency of 2.1 %). The mutant variant p.D222Terfs is a two-nucleotide deletion (GA) spanning positions 664–665. The deletion causes a frame shift and results in the synthesis of a shortened protein. This mutation was previously reported in Europe [18]. Another mutant variant p.R111Ter is a stop-mutation also resulting in the synthesis of a shortened phenylalanine hydroxylase molecule. It is rarely found across the European population [5], but often occurs in Chinese patients with PKU [19].

Frequencies of p.R261Ter and IVS11+1G>C in the studied sample were >1 %. The p.R261Ter mutation was previously reported in different regions of Russia [3, 11]. The splicing-disrupting IVS11+1G>C mutation, which is generally rare for the Russian population, was previously reported in patients with PKU from Kemerovo [11] and Rostov [15] regions.

The rest 12 mutant variants of *PAH* were heterozygous and were detected in only one patient each. The missense mutations p.E280K, p.E390G and p.A300S and the stop-mutation p.R176Ter were previously registered in two Russian regions [3, 11]. The missense mutation p.R270K was previously reported in Tatarstan [20]. The p.F161S mutations was first reported in the North of China [21] but is now rarely found in Chinese patients with PKU [19]. The mutant variants p.L311P, p.F55L, p.F55Leufs, IVS1+5G>T, and IVS8-7A>G are observed in European populations [18, 22–25]. The rare p.G188D mutation was previously reported in China [26].

The wide variety of *PAH* allelic variants revealed by targeted sequencing is comparable to that reported by the literature on Rostov [15], Novosibirsk [3] and Kemerovo [11] regions. Our study shows that allele frequencies of severe and mild mutations are 73.8 and 20.4 %, respectively. Frequency of mild mutations is consistent with the data provided by Gundorova et al. obtained in 2017 from the patients residing in Moscow and the Moscow region [9] and exceeds the regional average.

In this study we piloted the application of a modified real-time PCR technique designed for detecting nucleotide substitutions and based on the use of adjacent probes to screening for frequent mutations in the *PAH* gene in the sample of Moscow region residents suffering from PKU. The proposed technique is quite simple. The same PCR machine can be used for both chemical reactions and fluorescence signal registration, making it possible to test the sample for a variety of mutant variants in parallel within a relatively short time. This promising technique could be used for both scientific research and routine diagnostic screening. The diagnostic effectiveness of the method exceeds 80 % with respect to mutation carriership. The list of 16 *PAH* mutations included into the screening panel is not complete, but can be considerably extended using the proposed PCR technique which allows almost immediate addition of new variants to the panel.

Low-frequency mutations cannot be identified by methods of selective genetic screening. The range of rare variants in a given population can be relatively wide. So far over 800 mutant variants have been described for *PAH*, of which only a few occur at a 1 % frequency. In our study next generation sequencing performed in addition to the main technique

**Table 1.** *PAH* mutations and their frequencies in the patients of Morozovskaya Children's City Clinical hospital (n = 71)

| Deleterious mutation | | Location | *PAH* domain | Frequency, % |
|---|---|---|---|---|
| *CDS* | AA | | | |
| c.1222C>T | p.R408W | exon 12 | CAT | 47.9 |
| c.782G>A | p.R261Q | exon 7 | CAT | 9.9 |
| c.1066-11G>A | IVS10nt546 | intron 10 | – | 4.2 |
| c.1315+1G>A | IVS12+1G>A | intron 12 | – | 4.2 |
| c.473G>A | p.R158Q | exon 5 | CAT | 3.5 |
| c.1241A>G | p.Y414C | exon 12 | TET | 2.8 |
| c.441+5G>T | IVS4+5G>T | intron 4 | – | 2.8 |
| c.143T>C | p.L48S | exon 2 | REG | 2.1 |
| c.754C>T | p.R252W | exon 7 | CAT | 2.1 |
| c.664_665delGA | p.D222Terfs | exon 6 | CAT | 2.1 |
| c.331C>T | p.R111Ter | exon 3 | CAT | 2.1 |
| c.781C>T | p.R261Ter | exon 7 | CAT | 1.4 |
| c.1199+1G>C | IVS11+1G>C | intron 11 | – | 1.4 |
| c.563G>A | p.G188D | exon 6 | CAT | 0.7 |
| c.838G>A | p.E280K | exon 7 | CAT | 0.7 |
| c.842C>T | p.P281L | exon 7 | CAT | 0.7 |
| c.482T>C | p.F161S | exon 5 | CAT | 0.7 |
| c.1169A>G | p.E390G | exon 11 | CAT | 0.7 |
| c.898G>T | p.A300S | exon 8 | CAT | 0.7 |
| c.165T>G | p.F55L | exon 2 | CAT | 0.7 |
| c.165delT | p.F55Leufs | exon 2 | CAT | 0.7 |
| c.526C>T | p.R176Ter | exon 6 | CAT | 0.7 |
| c.932T>C | p.L311P | exon 7 | CAT | 0.7 |
| c.809G>A | p.R270K | exon 7 | CAT | 0.7 |
| c.60+5G>T | IVS1+5G>T | intron 1 | – | 0.7 |
| c.913-7A>G | IVS8-7A>G | intron 8 | – | 0.7 |
| Unidentified | – | – | – | 4.2 |

**Note.** CAT is a catalytic domain; REG is a regulatory domain; TET is a tetramerization domain.

revealed the presence of 12.6 % of pathogenic alleles. A number of mutations were detected that had not been described previously for the Russian population: p.D222Terfs, p.R111Ter, p.F161S, p.G188D, p.L311P, p.F55L, p.F55Leufs, IVS1+5G>T, and IVS8-7A>G. Noteworthy, p.D222Terfs and p.R111Ter are potential candidates for inclusion into PCR screening panels for genotyping the Moscow region population ( these mutations were discovered in 3 Russian individuals). In 7 % of cases we failed to detect pathogenic mutations in any of the *PAH* alleles. These cases require additional genetic tests, a more in-depth analysis of *PAH* sequences and a differential diagnosis for PAH-independent forms of PKU that account for 2–3 % of cases [1, 4].

## CONCLUSIONS

The study of unrelated patients with phenylketonuria presented to Morozovskaya Children's City Clinical hospital (Moscow) in 2015–2016 revealed a wide variety of deleterious mutations and different *PAH* genotypes. The use of PCR for detecting nucleotide substitutions in the *PAH* gene with relation to 16 mutations allowed us to successfully identify 83 % of pathogenic alleles in the sample. The diagnostic potential of real-time PCR encourages its application to routine screening for frequent/pathogenic *PAH* mutations in patients with PKU. The mutation p.R408W was prevalent in the sample; the obtained allelic frequency for this mutation is consistent with

**Table 2.** Genotypes of the patients of Morozovskaya Children's City Clinical hospital (n = 71)

| Genotype | | Number of carriers | Residual activity of PAH*, % | |
|---|---|---|---|---|
| allele 1 | allele 2 | | mutation 1 | mutation 2 |
| p.R408W | p.R408W | 14 | 2 | 2 |
| p.R158Q | p.R408W | 4 | 10 | 2 |
| IVS10nt546 | p.R408W | 3 | 5 | 2 |
| IVS12+1G>A | p.R408W | 3 | 0 | 2 |
| X | p.R408W | 3 | – | 2 |
| p.Y414C | p.R408W | 2 | 57 | 2 |
| IVS4+5G>T | p.R408W | 2 | 0 | 2 |
| p.L48S | p.R408W | 2 | 39 | 2 |
| p.R252W | p.R408W | 2 | 0 | 2 |
| p.R261Ter | p.R408W | 2 | 0 | 2 |
| p.R111Ter | p.R408W | 2 | 0 | 2 |
| p.D222Terfs | p.R408W | 1 | 0 | 2 |
| p.G188D | p.R408W | 1 | N/A | 2 |
| p.E280K | p.R408W | 1 | 2 | 2 |
| p.F55Leufs | p.R408W | 1 | 0 | 2 |
| p.L311P | p.R408W | 1 | 1 | 2 |
| p.R270K | p.R408W | 1 | 11 | 2 |
| IVS1+5G>T | p.R408W | 1 | 0 | 2 |
| IVS8-7A>G | p.R408W | 1 | 0 | 2 |
| p.R261Q | p.R408W | 7 | 44 | 2 |
| p.R261Q | p.R261Q | 2 | 44 | 44 |
| IVS10nt546 | p.Y414C | 1 | 5 | 57 |
| IVS10nt546 | IVS4+5G>T | 1 | 5 | 0 |
| IVS10nt546 | p.L48S | 1 | 5 | 39 |
| IVS12+1G>A | p.R111Ter | 1 | 0 | 0 |
| IVS12+1G>A | p.R158Q | 1 | 0 | 10 |
| IVS12+1G>A | IVS4+5G>T | 1 | 0 | 0 |
| IVS11+1G>C | p.F161S | 1 | 0 | 7 |
| IVS11+1G>C | p.R261Q | 1 | 0 | 44 |
| p.D222Terfs | p.Y414C | 1 | 0 | 57 |
| p.D222Terfs | p.R252W | 1 | 0 | 0 |
| p.R261Q | p.F55L | 1 | 44 | N/A |
| p.R261Q | p.R176Ter | 1 | 44 | 0 |
| p.E390G | p.A300S | 1 | 62 | 31 |
| p.P281L | X | 1 | 2 | – |
| X | X | 1 | – | – |

**Note.** X — unidentified pathogenic variant.

* — according to BIOPKUdb [14].

the up-to-date data for Moscow and the Moscow region. The range of frequent mutations found in the studied sample is corroborated by the literature data on the Russian population. The number of mild mutations observed in the sample exceeds the average across the country. Mutations p.D222Terfs and p.R111Ter identified in a few patients are potential candidates for inclusion into PCR panels for screening Moscow region residents. Next generation sequencing detected a number of functionally different mutations previously unregistered in Moscow region, including p.D222Terfs, p.R111Ter, p.F161S, p.G188D, p.R270K, p.L311P, p.F55L, p.F55Leufs, IVS1+5G>T, and IVS8-7A>G.

**References**


1. Klinicheskie rekomendatsii po diagnostike i lecheniyu fenilketonurii i narusheniy obmena tetragidrobiopterina. Moscow: Akademizdat; 2014. 70 p. Russian.
2. Bushuyeva TV. [Phenylketonuria in children: diagnostics, clinic, treatment]. Voprosy sovremennoy pediatrii. 2010; 9 (1): 157–60. Russian.
3. Baturina OA, Tupikin AE, Lukjanova TV, Sosnitskaya SV, Morozov IV. PAH and QDPR Deficiency Associated Mutations in the Novosibirsk Region of the Russian Federation: Correlation of Mutation Type with Disease Manifestation and Severity. J Med Biochem. 2014; 33 (4): 333–40.
4. Blau N, Shen N, Carducci C. Molecular genetics and diagnosis of phenylketonuria: state of the art. Expert Rev Mol Diagn. 2014 Jul; 14 (6): 655–71.
5. Zschocke J. Phenylketonuria mutations in Europe. Hum Mutat. 2003 Apr; 21 (4): 345–56.
6. Amelina MA, Zinchenko RA, Stepanova AA, Gundorova P, Polyakov AV, Amelina SS. [Examine the relationship genotypes (PAH) and phenotype in patients with phenylketonuria Rostov region]. Meditsinskaya genetika. 2016; 15 (6): 3–10. Russian.
7. Kasimov DA, Sikora NV, Checheva NN. [Incidence and structure of phenylketonuria in children of Khabarovsk territory]. Zdravookhranenie Dal'nego Vostoka. 2014; 3 (61): 26–8. Russian.
8. Anichkina AA, Gavriliuc AP, Tverskaya SM, Polyakov AV. [Analysis of most prevalent mutations of PAH gene in phenilketonuria patients]. Meditsinskaya genetika. 2003; 2 (4): 175–81. Russian.
9. Gundorova P, Stepanova AA, Bushueva TV, Belyashova EYu, Zinchenko RA, Amelina SS, et al. Genotyping of Patients with Phenylketonuria from Different Regions of Russia for Determining BH4 Responsiveness. Russ J Genet. 2017; 53 (6): 712–8.
10. Abramov DD, Kadochnikova VV, Yakimova EG, Belousova MV, Maerle AV, Sergeev IV, et al. [High carrier frequency of CFTR gene mutations associated with cystic fibrosis, and PAH gene mutations associated with phenylketonuria in Russian population]. Bulletin of RSMU. 2015; (4): 32–5. Russian.
11. Baturina OA, Bondar AA, Tupikin AE, Zhabin SG, Morozov IV. Analysis of phenylalanine hydroxylase gene mutations in phenylketonuria patients from Kemerovo oblast and the Sakha Republic. Cytol Genet. 2012 Jul; 46 (4): 227–32. DOI: 10.3103/S0095452712040032.
12. Kofiadi IA, Rebrikov DV. Methods for detecting single nucleotide polymorphisms: Allele-specific PCR and hybridization with oligonucleotide probe. Russ J Genet. 2006 Jan; 42 (1): 16–26.
13. Sergeev IV, Khaitov MR, Trofimov DYu, Abramov DD, Grudakova EG, Goncharova EV, et al. Razrabotka metodov dlya provedeniya shirokomasshtabnykh issledovaniy polimorfizma genov, reguliruyushchikh razlichnye komponenty immunnogo otveta. Fiziologiya i patologiya immunnoy sistemy. 2009; 13 (4): 21–6. Russian.
14. Blau N, Yue W, Perez B, database managers. PAHvdb: Phenylalanine Hydroxylase Gene Locus-Specific Database [Internet]. [cited 2017 Aug 14]. Available from: http://www.biopku.org/pah/home.asp
15. Amelina MA, Stepanova AA, Polyakov AV, Amelina SS, Zinchenko RA. [Spectrum and frequency of mutations in RAH gene in patients with phenylketonuria from Rostov region]. Meditsinskaya genetika. 2015; 14 (8): 30–6. Russian.
16. Gundorova P, Stepanova AA, Makaov AKh, Zinchenko RA, Abaykhanova ZM, Polyakov AV. Mutation spectrum of the PAH gene in phenylketonuria patients in the Karachay-Cherkess Republic (Russia). Russ J Genet. 2016; 52 (12): 1282–90.
17. Zinchenko LN, Matulevich SA, Kucher AN. [Territorial and ethnic specificity of mutations in a gene phenylalanin-hydroxilase in Krasnodar territory]. Kubanskiy nauchnyy meditsinskiy vestnik. 2006; (3–4): 39–42. Russian.
18. Guldberg P, Henriksen KF, Güttler F. Molecular analysis of phenylketonuria in Denmark: 99 % of the mutations detected by denaturing gradient gel electrophoresis. Genomics. 1993 Jul; 17 (1): 141–6.
19. Liu N, Kong XD, Zhao DH, Wu QH, Li XL, Guo HF, et al. Prenatal diagnosis of Chinese families with phenylketonuria. Genet Mol Res. 2015 Nov 19; 14 (4): 14615–28.
20. Kuzmin AI, Eisensmith RC, Goltsov AA, Sergeeva NA, Schwartz EI, Woo SL. Complete spectrum of PAH mutations in Tataria: presence of Slavic, Turkic and Scandinavian mutations. Eur J Hum Genet. 1995; 3 (4): 246–55.
21. Li J, Eisensmith RC, Wang T, Lo WH, Huang SZ, Zeng YT, et al. Identification of three novel missense PKU mutations among Chinese. Genomics. 1992 Jul; 13 (3): 894–5.
22. Zekanowski C, Cabalska B, Borsuk P, Bal J. Mutations of the phenylalanine hydroxylase gene in mild hyperphenylalaninemia: a novel mutation in exon 3. Hum Mutat. 1997; 10 (3): 258–9.
23. Eigel A, Dworniczak B, Kalaydjieva L, Horst J. A frameshift mutation in exon 2 of the phenylalanine hydroxylase gene linked to RFLP haplotype 1. Hum Genet. 1991 Oct; 87 (6): 739–41.
24. Lichter-Konecki U, Konecki DS, DiLella AG, Brayton K, Marvit J, Hahn TM, et al. Phenylalanine hydroxylase deficiency caused by a single base substitution in an exon of the human phenylalanine hydroxylase gene. Biochemistry. 1988 Apr 19; 27 (8): 2881–5.
25. Pérez B, Desviat LR, Ugarte M. Analysis of the phenylalanine hydroxylase gene in the Spanish population: mutation profile and association with intragenic polymorphic markers. Am J Hum Genet. 1997 Jan; 60 (1): 95–102.
26. Song F, Qu YJ, Yang YL, Jin YW, Zhang YM, Wang H, et al. [The mutant spectrum of phenylalanine hydroxylase gene in Northern Chinese]. Zhonghua Yi Xue Yi Chuan Xue Za Zhi. 2007 Jun; 24 (3): 241–6. Chinese.


**Литература**


1. Клинические рекомендации по диагностике и лечению фенилкетонурии и нарушений обмена тетрагидробиоптерина. М.: Академиздат; 2014. 70 с.
2. Бушуева Т. В. Современный взгляд на проблему фенилкетонурии у детей: диагностика, клиника, лечение. Вопр. соврем. педиатр. 2010; 9 (1): 157–60.
3. Baturina OA, Tupikin AE, Lukjanova TV, Sosnitskaya SV, Morozov IV. PAH and QDPR Deficiency Associated Mutations in the Novosibirsk Region of the Russian Federation: Correlation of Mutation Type with Disease Manifestation and Severity. J Med Biochem. 2014; 33 (4): 333–40.
4. Blau N, Shen N, Carducci C. Molecular genetics and diagnosis of phenylketonuria: state of the art. Expert Rev Mol Diagn. 2014 Jul; 14 (6): 655–71.

5. Zschocke J. Phenylketonuria mutations in Europe. Hum Mutat. 2003 Apr; 21 (4): 345–56.
6. Амелина М. А., Зинченко Р. А., Степанова А. А., Гундорова П., Поляков А. В., Амелина С. С. Изучение взаимосвязи генотипов (РАН) и фенотипов у больных фенилкетонурией Ростовской области. Мед. ген. 2016; 15 (6): 3–10.
7. Касимов Д. А., Сикора Н. В., Чешева Н. Н. Результаты исследования детей на фенилкетонурию в Хабаровском крае. Здравоохранение Дальнего Востока. 2014; 3 (61): 26–8.
8. Аничкина А. А., Гаврилюк А. П., Тверская С. М., Поляков А. В. Анализ наиболее часто встречающихся мутаций в гене фенилаланингидроксилазы у больных фенилкетонурией. Мед. ген. 2003; 2 (4): 175–81.
9. Гундорова П., Степанова А. А., Бушуева Т. В., Беляшова Е. Ю., Зинченко Р. А., Амелина С. С. и др. Генотипирование больных фенилкетонурией из различных регионов Российской Федерации с целью определения чувствительности к препаратам BH4. Генетика. 2017; 53 (6): 732–9.
10. Абрамов Д. Д., Кадочникова В. В., Якимова Е. Г., Белоусова М. В., Маерле А. В., Сергеев И. В. и др. Высокая частота носительства в российской популяции мутаций гена CFTR, ассоциированных с муковисцидозом, и мутаций гена РАН, ассоциированных с фенилкетонурией. Вестн. РГМУ. 2015; (4): 32–5.
11. Батурина О. А., Бондарь А. А., Тупикин А. Е., Жабин С. Г., Морозов И. В. Анализ мутаций гена фенилаланингидроксилазы у больных фенилкетонурией Кемеровской области и Республики Саха. Цитол. и ген. 2012; 46 (4): 40–7.
12. Кофиади И. А., Ребриков Д. В. Методы детекции однонуклеотидных полиморфизмов: аллель-специфичная ПЦР и гибридизация с олигонуклеотидной пробой. Генетика. 2006; 42 (1): 22–32.
13. Сергеев И. В., Хаитов М. Р., Трофимов Д. Ю., Абрамов Д. Д., Грудакова Е. Г., Гончарова Е. В. и др. Разработка методов для проведения широкомасштабных исследований полиморфизма генов, регулирующих различные компоненты иммунного ответа. Физиол. и патол. иммун. системы. 2009; 13 (4): 21–6.
14. Blau N, Yue W, Perez B, database managers. PAHvdb: Phenylalanine Hydroxylase Gene Locus-Specific Database [Интернет]. [Дата обращения: 14 августа 2017 г.]. Доступно по: http://www.biopku.org/pah/home.asp
15. Амелина М. А., Степанова А. А., Поляков А. В., Амелина С. С., Зинченко Р. А. Спектр и частота встречаемости мутаций в гене РАН у больных фенилкетонурией Ростовской области. Мед. ген. 2015; 14 (8): 30–6.
16. Гундорова П., Степанова А. А., Макаов А. Х., Зинченко Р. А., Абайханова З. М., Поляков А. В. Особенности спектра мутаций в гене РАН у больных фенилкетонурией из Карачаево-Черкесской Республики. Генетика. 2016; 52 (12): 1448–57.
17. Зинченко Л. В., Матулевич С. А., Кучер А. Н. Территориальная распространенность и этническое разнообразие мутаций гена фенилаланингидроксилазы в Краснодарском крае. Кубанск. науч. мед. вестн. 2006; (3–4): 39–42.
18. Guldberg P, Henriksen KF, Güttler F. Molecular analysis of phenylketonuria in Denmark: 99 % of the mutations detected by denaturing gradient gel electrophoresis. Genomics. 1993 Jul; 17 (1): 141–6.
19. Liu N, Kong XD, Zhao DH, Wu QH, Li XL, Guo HF, et al. Prenatal diagnosis of Chinese families with phenylketonuria. Genet Mol Res. 2015 Nov 19; 14 (4): 14615–28.
20. Kuzmin AI, Eisensmith RC, Goltsov AA, Sergeeva NA, Schwartz EI, Woo SL. Complete spectrum of PAH mutations in Tataria: presence of Slavic, Turkic and Scandinavian mutations. Eur J Hum Genet. 1995; 3 (4): 246–55.
21. Li J, Eisensmith RC, Wang T, Lo WH, Huang SZ, Zeng YT, et al. Identification of three novel missense PKU mutations among Chinese. Genomics. 1992 Jul; 13 (3): 894–5.
22. Zekanowski C, Cabalska B, Borsuk P, Bal J. Mutations of the phenylalanine hydroxylase gene in mild hyperphenylalaninemia: a novel mutation in exon 3. Hum Mutat. 1997; 10 (3): 258–9.
23. Eigel A, Dworniczak B, Kalaydjieva L, Horst J. A frameshift mutation in exon 2 of the phenylalanine hydroxylase gene linked to RFLP haplotype 1. Hum Genet. 1991 Oct; 87 (6): 739–41.
24. Lichter-Konecki U, Konecki DS, DiLella AG, Brayton K, Marvit J, Hahn TM, et al. Phenylalanine hydroxylase deficiency caused by a single base substitution in an exon of the human phenylalanine hydroxylase gene. Biochemistry. 1988 Apr 19; 27 (8): 2881–5.
25. Pérez B, Desviat LR, Ugarte M. Analysis of the phenylalanine hydroxylase gene in the Spanish population: mutation profile and association with intragenic polymorphic markers. Am J Hum Genet. 1997 Jan; 60 (1): 95–102.
26. Song F, Qu YJ, Yang YL, Jin YW, Zhang YM, Wang H, et al. [The mutant spectrum of phenylalanine hydroxylase gene in Northern Chinese]. Zhonghua Yi Xue Yi Chuan Xue Za Zhi. 2007 Jun; 24 (3): 241–6. Chinese.