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Results of the comprehensive study in newborn babies with congenital phenylketonuria

Abstract. The timely detection of newborns with congenital phenylketonuria, in which the metabolism of the amino acid phenylalanine is disturbed due to the lack of the enzyme phenylalanine hydroxylase, remains an urgent issue. Increase in the level of phenylalanine and its toxic products in the cell leads to severe brain damage, which manifests itself in the form of mental retardation. Prompt diagnosis of phenylketonuria can prevent severe dementia and serious mental disorders. The aim of the work is a comprehensive study of newborns with congenital phenylketonuria for which enzymatic immunoassay, real-time PCR, tandem mass spectrometry were applied. Out of 5,293 newborns screened at the genetic laboratory of LPP "Center for Molecular Medicine" (Almaty, Kazakhstan) in 2019, two newborns were diagnosed with congenital phenylketonuria. The molecular genetic study in both of them indicated the presence of the *R408W* mutation in the heterozygous state in phenylalanine hydroxylase *PAH* gene. Upon application of the appropriate dietary therapy, the concentrations of phenylalanine in the blood reached 62.9 μM/L in the 1st and 173 μM/L in the 2nd newborn, which corresponds to its reference value. For the effective treatment of congenital phenylketonuria with a confirmed diagnosis based on molecular genetic studies (detection of a mutation in the *PAH* gene) in newborns, it is proposed to conduct additional biochemical studies for possible metabolic disorders.

Key words: newborn screening, congenital phenylketonuria, enzyme immunoassay, PCR, tandem mass spectrometry.

Introduction

Hereditary metabolic disorders are common worldwide and play an important role in human hereditary pathology. These diseases are characterized by severe and in many cases fatal symptoms. According to the statistics, 5% of children are born with genetic or congenital disorders [1].

Monogenic forms of metabolic disorders are characterized by impaired metabolism of amino acids and organic acids resulting in the accumulation of toxic metabolites in organs and tissues. Study of the molecular basics of monogenic disorders and their prevalence in different regions of the world is an urgent issue of medical human genetics.

One of the diseases detected by selective screening is congenital phenylketonuria (PKU), which is a severe autosomal recessive disease caused by a genetic deficiency of the enzyme phenylalanine hydroxylase (PAH). Without enzymatic activity, phenylalanine is not converted to tyrosine. Deficiency of the PAH enzyme leads to a marked increase in the level of phenylalanine (substrate), decrease in the level of tyrosine (product), appearance of phenylalanine metabolites, such as phenylpyruvic acid, phenyllactic acid and phenylacetic acid in the blood and urine, violation of the formation of tyrosine as well as adrenaline, norepinephrine and melanin synthesized from it, and a violation of tryptophan metabolism. A mutant gene in the PAH locus located on the long arm of chromosome 12 in the region q22-q24 [2] leads to the absence of PAH activity [3]. The protein coding sequence (cDNA) is approximately 90 kb long and consists of 13 exons. Most pathological changes in the *PAH* gene are single nucleotide substitutions, including missense (64%), splicing (13%) and nonsense (6%) mutations and neutral polymorphisms (6%) [4].

It was found that nearly 20 missense mutations affect highly mutagenic CpG dinucleotides. The remaining changes are the result of various deletions or insertions. Currently, more than 490 different variants of the *PAH* gene have been found with a high degree of variability and significant

interpopulation variance. The *R408W* missense mutation is the most common (major) *PAH* gene mutation in European populations. The *R408W* mutation leads to a severe form of PKU and reduces enzyme activity to 1–2.7% [5], while the genetic anomaly is associated with the replacement of C (cytosine) with T (thymine) in exon 12, which leads to the replacement of arginine with tryptophan at position 408 of the protein PAH [6].

In order to determine the prevalence of PKU in the Republic of Kazakhstan, the data on the mass newborn screening conducted in Almaty from 1989 to 1996 was analyzed, according to which the frequency of PKU was 1:6980, on average, with 80-90% of examined infants. State program for screening newborns for phenylketonuria and congenital hypothyroidism was initiated in 2007. According to the Republican Medical Genetic Consultation, the incidence of PKU in Kazakhstan is one case per 1:22 500 newborns (Public Foundation "Help Today"). According to the statistics of the public fund for 2019, almost 167 patients were registered with PKU in Kazakhstan [7].

The disease begins to develop as soon as phenylalanine enters the child's body with mother's milk. Symptoms of PKU appear in the first year of life, usually between 2 and 6 months of age. Children with PKU appear healthy at birth, despite the presence of such specific features as blond hair, blue eyes, and dry skin [8]. The child's lethargy, lack of interest in the environment, sometimes irritability, restlessness, regurgitation, problems with muscle tone (often muscle hypotension), seizures, and symptoms of allergic dermatitis are early signs of the condition. There is a characteristic "mouse" smell. Microcephaly (incomplete development of the brain or abnormal smallness of the brain), delayed static-motor and psychoverbal development are often observed [9].

An effective treatment for PKU is diet therapy based on food low in phenylalanine [10]. High degree of dementia and severe mental disorders develop in patients in the absence of a phenylalanine-free diet from the first days of life due to the accumulation and toxic effect of phenylalanine and its derivatives on tissues and, especially, brain cells. Without special therapy, the condition worsens slowly; mental retardation usually reaches a severe degree with 20 units on IQ test [10]. In the psychological state of patients, there is a lack of development of game and objective activity, illegibility of emotional reactions, lack of expressive and vivid speech. Motor stereotypes, aggressive movements, psychopathic or schizophrenic states are possible.

The diagnosis is based on a combination of genealogical data, clinical and biochemical findings:

- possible consanguineous marriage of the parents of a sick child;
- similar pathology in relatives;
- convulsions, impaired muscle tone; eczematous skin changes;
- hypopigmentation of hair, skin, iris;
- a peculiar "mouse" smell of urine;
- increase in the level of phenylalanine in the blood > 2.1 mg/dL [11; 12].

Symptoms of the disease are practically removed at the time of early detection and treatment. Such a study helps prevent high-grade dementia and severe mental disorders, as well as deaths due to long-term monitoring of congenital malformations.

The aim of the work is a comprehensive study of newborns with congenital phenylketonuria for which enzymatic immunoassay, real-time PCR, tandem mass spectrometry were applied.

Materials and methods

The object of research. Out of 5,293 newborns screened at the genetic laboratory of LPP "Center for Molecular Medicine" (Almaty, Kazakhstan) in 2019, two newborns were diagnosed with congenital phenylketonuria. They underwent clinical studies, including analysis of amino acids in blood, and assessment of the quantitative content of pathological metabolites of phenylalanine and tyrosine in biological fluids. The *PAH* gene mutation was analyzed by PCR and amino acid analysis, including the spectra of phenylalanine and acylcarnitine, by tandem mass spectrometry.

Methods of research. Neonatal screening was conducted using the "Delfia Neonatal Phenylalanine" kit (Perkin Elmer, Finland). Blood sampling for PKU was carried out 3 hours after feeding, in full-term newborns on the 2-3rd day of life (25-72 hours of life), in premature infants – on the 7-14th day of life. A few drops of blood were applied to a special filter paper (Whatman 903, GE Healthcare Life Sciences, Buckinghamshire, UK) used to collect biomaterial for study of hereditary metabolic diseases. For newborn blood spot screening, bloodstain samples in the form of discs were transferred to the wells of microplates with a V-shaped bottom, containing 15 mL of the prepared extraction solution (for which three parts of zinc sulfate were mixed with two parts of 90% ethyl alcohol), and left at room temperature. After incubation for 30-60 minutes on shaker (to ensure that all disks were soaked in), 40 μL of deionized water were consequently added to each well. Blood

spots were freed with a needle. 50 μL of PKU reagent (a bottle of dry PKU reagent was dissolved in 6.5 mL of PKU Reconstitution Buffer) were added and incubated at 60°C for 30-40 minutes. 200 μL of copper blue reagent solution were added and incubated at the room temperature for 35 minutes. The analysis was performed on an immunofluorescent analyzer (Victor2™ D, Perkin Elmer, Finland) with the "85PKU" program for automatic measurement and calculation of results [13].

Isolation of genomic DNA from the peripheral blood of the subjects was carried out using the method of phenol-chloroform extraction. To do this, distilled water was added to 100 μL of blood, mixed and left for 15 minutes. Next, the sample was centrifuged at 5,000 rpm for 10 minutes. The supernatant was discarded and the cell pellet was washed with saline-sodium citrate (SSC) buffer, centrifuged again and the supernatant was discarded. Then, 54 μL of 0.2 M sodium acetate and 6 μL of 10% SDS (sodium dodecyl sulfate) were added to the pellet. The cell pellet was thoroughly resuspended using a vortex and incubated for 30-60 min at 37°C to destroy the cells. Two volumes of Tris-EDTA (TE) buffer (single buffer of 10 mM Tris: 1 mM EDTA) were added and phenolic deproteinization of the sample was carried out with the aqueous phase (top layer) collected in a clean tube without capturing the precipitate. The same was repeated with a mixture of chloroform-isoamyl alcohol to remove residual phenol. The test sample was purified from proteins. To this 1/10 volume of solution III (3 M potassium acetate: 29 g potassium acetate, 11 mL glacial acetic acid and 60 mL distilled water, bring the volume to 100 mL) was added and stirred. DNA was precipitated by adding the two and a half volumes of cold 96% ethyl alcohol. Sample was placed in the freezer for 1-2 hours. The resulting sample was centrifuged for 10 minutes, then the supernatant was discarded. The precipitate was washed with 70% ethyl alcohol. The resulting DNA pellet was dried and dissolved in 20 μL of TE buffer. DNA isolated in this way was used for PCR analysis [14].

PCR-analysis of mutations in the *PAH* gene was carried out using commercial diagnostic kit PKU-8L manufactured by LLC "Center for Molecular Genetics" at the Moscow State Scientific Center of the Russian Academy of Medical Sciences, Russia. For the study, the diagnostic set of primers and restrictases (DNA technology, Russia) were used to determine common mutations in the *PAH* gene: *R408W, R261Q, R252W, IVS10-11, IVS12+1, R158Q, P281L, IVS14+5.* Amplification by PCR with addition of neonatal DNA was carried out according to the kit instructions [15].

Tandem mass-spectrometry for quantitative assessment of enzyme activity was conducted using the NeoBase kit (Perkin Elmer, Finland) that includes the NeoBase Flow solution and Extraction solution and NeoLSD™ kit. To identify congenital metabolic disorders, in particular, to detect elevated levels of the amino acid phenylalanine, acylcarnitines, and free carnitine with electrospray ionization tandem mass-spectrometry (QSight™ 210 MD Screening system, Perkin Elmer, Finland) was used. The material for the study was capillary blood collected on a special filter paper No. 903. This analysis allows the measurement of 75 metabolites for the simultaneous screening of 49 hereditary metabolic disorders [16; 19]. NeoBase™ amino acid assay kits and NeoLSD enzyme kits were used [20; 21].

Results and discussion

Peripheral blood was obtained from 5,293 children who passed newborn screening for phenylketonuria as part of the healthcare genetic consultation in January-December, 2019. The results of neonatal biochemical screening for PKU (Table 1).

Table 1 – The number of tests and the re-tests of newborns for congenital phenylketonuria

No.	Months of 2019	The number of tests for congenital phenylketonuria	The number of the re-tests	Detected PkU newborns
1	January	347	8	0
2	February	405	4	1
3	March	648	9	0
4	April	328	2	0
5	May	385	7	0
6	June	431	2	0
7	July	486	6	1
8	August	307	2	0
9	September	295	3	0
10	October	585	5	0
11	November	601	9	0
12	December	475	11	0
13	Total	5293	68	2

According to the results presented in Table 1, s, both false positive and false negative results are possible during the screening. In that case, the false

positive result determined in 68 children, and they were re-examined, thus the frequency of re-examination estimated 1.2%. Data shows that a false-positive diagnosis of congenital phenylketonuria is possible in about 2% of cases during neonatal screening. When a high level of phenylalanine was confirmed in the retest from the primary dry blood spot of the newborn, within 72 hours after receiving the first result, the dry blood spot of the newborn was retaken and delivered for re-analysis. Repeated dry blood spots were delivered to the laboratory in a separate envelope marked "Repeat PKU". A new blood test for a newborn baby was performed within 36 hours of receiving the blood sample. The increased content of the substance relative to the control with a double re-examination suggests the presence of pathology. As can be seen from Table 1, two newborns after a second examination revealed data corresponding to congenital phenylketonuria. According to the results, the probability of having children with congenital phenylketonuria per 10,000 was 2 cases, which was 10 times higher than the average for Kazakhstan (one in 22,500) [7].

During the neonatal screening in 2019, only two confirmed case of congenital phenylketonuria were detected in newborns. The results of enzyme immunoassay are presented in Table 2. Table 3 shows the reference values of the norm.

As can be seen from Table 2, the results of neonatal screening and retest exceed the reference values (Table 3) of the phenylalanine content in the first newborn by more than 3.5 and 12.4 times. These values for the second newborn were more than 6.3 and 10.8 times, respectively. PKU was diagnosed in newborns based on enzyme immunoassay, including clinical examinations.

Table 2 – The level of phenylalanine in the blood of two newborns

The level of phenylalanine in the blood	The result of 1 – newborn, mg/dL	The result of 2 – newborn, mg/dL
1 result	7.31	13.34
2 result (re-test)	26.20	22.86

Table 3 – Indicator level of congenital phenylketonuria [11; 12]

Meaning	The level of phenylalanine in the blood, mg/dL	The level of phenylalanine in the blood, μmol/L
Potentially negative result	< 2.1	< 127
Unclear zone	2.1 – 3.0	127 – 182
Potentially positive result	> 3.0	> 182
Conversion factor: 1 μmol/L to mg/dL equals 60		

When monitoring treatment and dietary therapy, the values of phenylalanine for the first and second newborn were 62.9 μM/L and 173 μM/L, respectively, presented in Table 4. The concentration of phenylalanine in two newborns varied within the normal range of 20-265 μM/L.

Table 4 – Amino acid values of blood of two babies

No.	Amino acids		Standard values, μM/L	The result of 1 – newborn, μM/L	The result of 2 – newborn, μM/L
1	Alanine	Ala	85 – 910	345	217
2	Arginine	Arg	2 – 125	63	26.7
3	ASA-total	ASA-total	–	0.54	0.19
4	Citrulline	Cit	4 – 80	19.9	23.4
5	Glutamine	Gin	–	50	555
6	Glutamate	Glu	62 – 615	338	127
7	Glycine	Gly	95 – 945	408	220
8	Leucine	Leu	35 – 380	191	93.5
9	Methionine	Met	6 – 155	33.1	9.55
10	Ornithine	Orn	22 – 405	118	64.1
11	Phenylalanine	Phe	20 – 265	62.9	173
12	Proline	Pro	30 – 490	167	131
13	Tyrosine	Tyr	15 – 235	76.9	64.1
14	Valine	Val	45 – 430	178	136

Subsequently, studies were carried out on the content of pathological metabolites of phenylalanine in biological fluids, the determination of *PAH* gene mutations using PCR methods and tandem mass spectrometry (determination of the spectrum of acylcarnitines, amino acids).

To clarify the clinical diagnosis of newborns, a molecular genetic study was performed for the presence of mutations in the *PAH* gene – phenylalanine hydroxylase among the eight most common mutations *R408W, R261Q, R252W, IVS10-11, IVS12+1, R158Q*, *P281L* and *IVS14+5*. The material for the study was DNA isolated from peripheral blood according to the standard method. Allele-specific ligation followed by amplification and registration of results in a polyacrylamide gel in both newborns revealed *R408W* mutations in the heterozygous state. It was discovered 93 base pairs fragment along with a fragment of 89 base pairs corresponding to the norm (Figure 1).

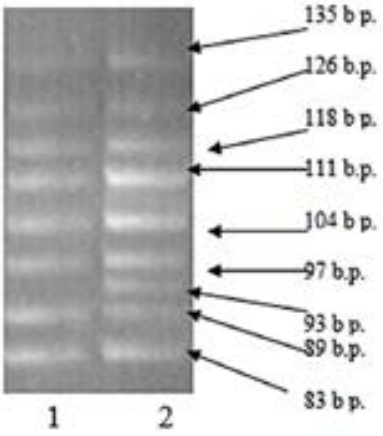

Figure 1 – Electrophoregram of two newborns.
Note: 1 – control DNA; 2-DNA of newborns;
b.p. stands for base pairs

It should be noted that, in order to detect congenital phenylketonuria and confirm the diagnosis, except mass screening and molecular genetic analysis, newborns with congenital phenylketonuria need to undergo an additional examination, since they are predisposed to diseases. An additional examination should include an analysis of the spectrum of amino acids and acylcarnitines by tandem mass spectrometry for hereditary metabolic diseases. When the possibility of a metabolic disorder is identified, certain key metabolic screening tests should be performed. These tests include plasma amino acid analysis. The amino acid profile may be indicative of a specific metabolic disorder causing neonatal disease, i.e., an increased or decreased amino acid content may indicate the presence of genetic diseases.

Based on the study of the spectrum of amino acids and acylcarnitines by tandem mass spectrometry, according to its multi-parametric nature, was made an analysis of the metabolism of amino acids, carnitines, organic acids to defects in ß-oxidation of fatty acids and the amount of enzymes that accompany Gaucher's disease (deficiency of 3-glycocerebrosidase), Niemann-pick (deficiency of sphingomyelinase), Krabbe (galactocerebrosidase deficiency), type 1 mucopolysaccharidosis (iduronidase deficiency), Fabry (alpha-galactosidase deficiency), Pompe caused by alpha-glucosidase enzyme deficiency [22].

Tandem mass spectrometry has the advantage of being able to determine the ratio of substrate concentration to its product concentration in a single sample, in this case the ratio of phenylalanine concentration to tyrosine concentration. The results of the study showed that the ratio of the concentration of phenylalanine to tyrosine was within the normal range and amounted to 0.82 μM/L in the 1st newborn, and 2.69 μM/L in the 2nd newborn (it is important to mention that the reference ratios of phenylalanine to tyrosine are 0.25 – 6.5 μM/L). The remaining indicators of the ratios of the components are shown in Table 5. Table 6 presents the data of the analysis of the metabolic test for carnitines in both newborns.

As can be seen from Table 6, the results of the metabolic test for carnitines in both newborns corresponded to the reference value. Along with free carnitines, observed the presence of several types of other carnitines, including acetyl, propionyl, malonyl, butyryl, methylmalonyl, isovalerylcarnitine, etc. Also, studied the breakdown of lysophospholipids, nucleosides, ketones, resulting in the formation of the energy, which is necessary for the life of the cell (Table 7).

Each step of the oxidation process is carried out under the action of specific enzymes. In the absence of one of the enzymes, the process is disrupted. The results of the study did not show data for hereditary aminoacidopathy, carnitines, organic aciduria and defects in mitochondrial ß-oxidation of fatty acids.

Data for impaired enzymatic activity for the following lysosomal storage diseases: Gaucher disease, Niemann-Pick disease, Pompe disease, Krabbe disease, Fabry disease and mucopolysaccharidosis type I were also not detected in two newborns (Figure 2).

Table 5 – The amino acid ratio indicators of two newborns

Ratio:	Standard values, μM/L	The result of 1 – newborn, μM/L	The result of 2 – newborn, μM/L
Phenylalanine/Tyrosine	0.25-6.50	0.82	2.69
Leucine/Phenylalanine	0 – 3.65	3.03	0.54
Methionine/Leucine	0.02 – 0.47	0.17	0.10
Methionine/ Phenylalanine	0.04 – 0.70	0.53	0.06
Tyrosine/Leucine	-	0.40	0.69
Tyrosine/Methionine	-	2.33	6.71
Citrulline/Arginine	0 – 5.56	0.32	0.88
Valine/ Phenylalanine	0 – 3.00	2.83	0.79
Citrulline/ Phenylalanine	0.10 – 0.67	0.32	0.14

Table 6 – The values of carnitines of two newborns

Types of carnitines:	Standard values, μM/L	1 – newborn, μM/L	2 – newborn, μM/L
Carnitine free C0	8.0 – 155.0	36.40	43.4
Acetylcarnitine C2	6.0 – 55.0	11.40	18.7
Propionylcarnitine C3	0.16 – 6.50	0.91	2.51
Malonylcarnitine C3D3	0 – 10.40	0.07	0.12
Butyrylcarnitine C4	0 – 1.10	0.23	0.44
Methylmalonyl C4DC	0 – 17.0	0.76	0.40
Isovalerycarnitne C5	0 – 0.70	0.14	0.12
Tiglylcarnitine C5:1	0 – 0.15	0.01	0.01
Glutarylcarnitine C5DC	0 – 6.99	0.12	0.08

Table 7 – The values of lysophospholipids, nucleosides, and ketones by TMS (MS/MS) of first and second newborns

Results:	1 – newborn, μM/L	2 – newborn, μM/L
Lysophospholipids:		
Lysophosphatidylcholine C20:0	0.65	0.63
Lysophosphatidylcholine C22:0	0.49	0.14
Lysophosphatidylcholine C24:0	0.55	0.26
Lysophosphatidylcholine C26:0	0.19	0.14
Nucleosides:		
Adenosine	0.35	0.3
2-deoxyadenosine	0.02	0.02
Ketones:		
Succinylacetone	0.39	0.18

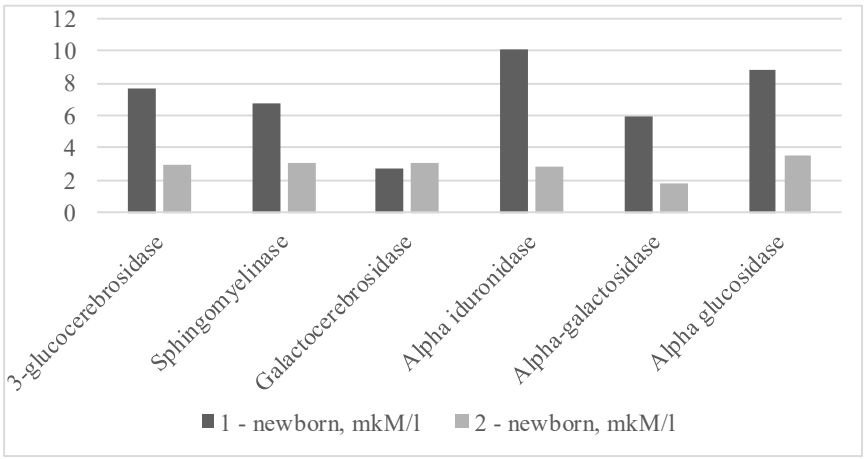

Figure 2 – The amount of specific enzymes in the body in two newborns

Blood donation for immunofluorescence analysis and genetics visits remained mandatory for both newborns once a month, regularly. Every three weeks they had to donate blood for examination. This prevented the complications of innate phenylalanine and allowed control over the amount of phenylalanine they had to consume. The normal amino acid content is 2.1 mg/dL. Values greater than 2.1 mg/dL represent an increased risk of developing phenylketonuria. Figures 3 and 4 show measurements of phenylalanine in a blood sample for two newborns, respectively. The content of phenylalanine in both newborns showed a steady but significant increase over this period.

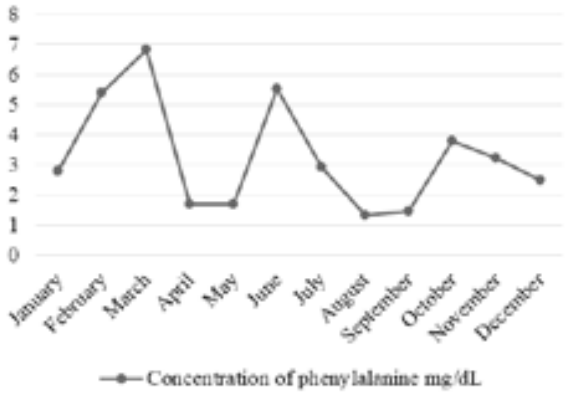

Figure 3 – Distribution of phenylalanine content in the 1st newborn for 2020

It should be noted that in the process of monitoring sharp fluctuations in content of phenylalanine in the process of development were observed in sick children.

According to the Order of the Ministry of Healthcare of the Republic of Kazakhstan No. 105 from March 14, 2018 “On amendments to the order of the Minister of Healthcare of the Republic of Kazakhstan No. 666 from August 29, 2017 “On approval of the List of medicines and medical devices to provide citizens within the guaranteed volume of free medical care and in the system of compulsory social health insurance, including certain categories of citizens with certain diseases (conditions) free

and(or) subsidized medicines, medical devices and specialized medical products at the outpatient level"" Phenylketonuria section includes therapeutic low-protein products and products with low phenylalanine content: Komida med PKU-formula + LCP (11.8 g protein per 100 g); Komida med PKU – B (31.1 g of protein per 100 g); Komida med PKU C – 45 (45 g of protein per 100 g); PKU-3 (69 g protein per 100 g); Komida med PKU C-75 (75 g of protein per 100 g); PKU-0 (13 g protein per 100 g); PKU-1 (20 g protein per 100 g); RAM-1 and RAM-2 (75 g of protein per 100 g); Isifen (16.8 g of protein in 1 pack) [23].

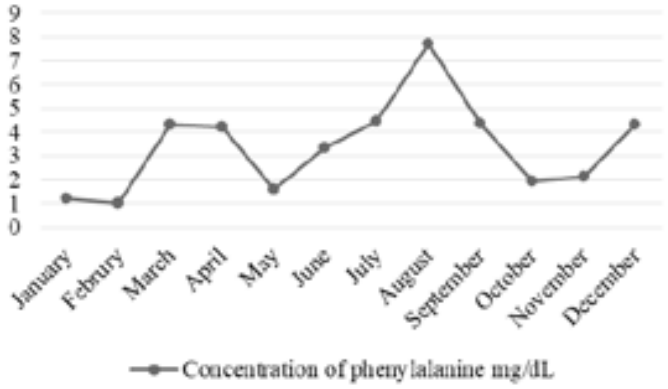

Figure 4 – Distribution of phenylalanine content in the 2nd newborn for 2020

Since good metabolic control in childhood is necessary to prevent cognitive impairment in PKU, it is recommended to treat patients with corrected phenylalanine concentration during the first 12 years [24]. Collaborative study for PKU revealed that discontinuation of diet control correlated with decreased school performance in children and increased behavioral and psychosocial problems in adults [25].

A comprehensive examination of newborns with congenital phenylketonuria will allow timely treatment of critical conditions and metabolic disorders, which will enable them to grow and develop healthy. However, they must be under constant medical supervision, and at the first suspicion of a developmental disorder, complex treatment is required, designed to reduce acute symptoms.

Since one of the main tasks of medicine is diagnosis of genetic pathologies in newborns, a lot of journals and articles have been written in this area, which have a specific purpose and a certain similarity. The significance of our work is a first time comprehensive study of phenylketonuria in newborns after mass neonatal screening. Also studying besides neonatal screening and *PAH* gene mutation, other number of enzymes, which are the causes of lysosomal storage diseases. Our data on the prevalence of PKU can be used to assess the genetic state of the Republic of Kazakhstan. The frequency of PKU according to our mass screening of newborns in Almaty is 2 cases per 10,000, and this is at the level of the average in Almaty 1 case per 6,980. In both studies, the most frequent mutations were *R408W*, which indicates the similarity of the results of our analysis.

The frequency of mutations in the phenylalanine hydroxylase gene varies in unrelated children diagnosed with PKU [26]. The *R408W* mutation is considered as more typical for Eastern European countries (47.9%) [27].

The average frequency of congenital phenylketonuria varies between 1:10,000 and 15,000 newborns. It varies from country to country, e.g. 1: 4,370 in Turkey and 1: 100,000 in Japan [28]. In addition, among cities of even one country the indices can differ: in Kazakhstan, the number equals to 1:22,500 and in Almaty is equal to 1:6,980 [7].

As mentioned by Levy et al. [15], despite the assumption of a normal newborn screen, it is vital to acknowledge the possibility that a child will may have metabolic illness or a related disorder. As a result, testing for all relevant metabolic diseases, including those covered by molecular-genetic analysis, should be included in the evaluation of newborns. In the next work [29] have been highlighted, that the second

sample should be taken if a high phenylalanine level is identified in a newborn screening sample. The latter has the advantage of being able to analyze all amino acids accurately and is preferred when the first phenylalanine is significantly elevated (e.g., above 2.1 mg/dL) [11; 12]. Catabolism can cause an increase in phenylalanine levels in premature and/or severely sick newborns. Under these conditions, it is advised that a full analysis of all amino acids be performed for a limited period of time

Our work emphasize the importance of expanding "Neonatal medicine" for the early detection of many inborn metabolic errors and making decisions to provide effective treatment and a good results at the end.

Conclusion

One of the main tasks of practical medicine is the diagnosis of hereditary pathologies. The study of the biochemical and molecular-genetic aspects of hereditary diseases is a comprehensive solution to the urgent problems of elucidating the etiology and pathogenesis of this group of diseases. In this regard, in particular, the timely detection of newborns with congenital phenylketonuria remains an urgent problem. As practice shows, a case of late diagnosis can be the cause of severe irreversible developmental disorders of the child. With timely effective treatment, children with this disease do not differ from their peers, develop according to age and study in regular schools. This is possible with neonatal screening, appropriate therapy and proper monitoring of the newborn.

The success of treatment is mainly determined by the extent to which the parents of a sick child have realized the importance of diet therapy, and how strictly they carry it out. The child needs to constantly monitor the content of phenylalanine in the blood, and, depending on laboratory parameters, adjust the composition of those products that, on the one hand, will not increase the level of phenylalanine, and on the other hand, ensure the normal growth and development of the child.

The most important aspect to consider in congenital phenylketonuria – is the psychological aspect. The transition to adulthood – is a period of high risk for patients with PKU. Difficulties are exacerbated by the need to adhere to dietary therapy, there is a risk of loss of metabolic control and a high risk of a complete loss of medical supervision. In this regard, it is necessary to organize psychological support for the families of patients, which should begin from the moment a sick child appears in the family. Without effective support, many of the benefits gained during early treatment may be lost in adulthood [30].

The results of the study allow us to draw the following conclusions:

1. Neonatal screening of 5,293 newborns revealed two newborns with congenital phenylketonuria: the concentration of phenylalanine in the dry blood spot in the 1st newborn was 7.31 mg/dL, in the retest 26.20 mg/dL, in the 2nd newborn the level of phenylalanine was 13.34 mg/dL in retest 22.86 mg/dL.

2. In these two newborns the *R408W* mutation in *PAH* gene was detected in the heterozygous state.

3. After appropriate dietary therapy, the concentrations of phenylalanine in the blood of the observed newborns decreased to their reference value – 62.9 μM/L in the 1st newborn and 173 μM/L in the 2nd newborn.

4. The ratio of phenylalanine to tyrosine was 0.82 μM/L in the 1st newborn, and 2.69 μM/L in the 2nd newborn.

5. The results of additional studies showed no data for hereditary aminoacidopathy, organic aciduria and defects in mitochondrial β-oxidation of fatty acids. In addition, there were no data for violations of enzymatic activity for the following lysosomal storage diseases: Gaucher disease, Niemann-Pick disease, Pompe disease, Krabbe disease, Fabry disease and mucopolysaccharidosis type I.

For early detection and effective treatment of congenital phenylketonuria, along with mass screening and molecular genetic studies to confirm the diagnosis, it is recommended to conduct additional studies on metabolic disorders in newborns with an established diagnosis due to their predisposition to disease.

References

1. Pechantnikova N.L., Bruhanova N.O., Potechin H.O., Vitkovskaya I.P., Petrikina E.E., Koltunov I.E. (2017) Selektivnyy skrining na nasledstvennyye bolezni obmena veshchestv: Metodicheskiye rekomendatsii no.16 /Departament zdravookhraneniya [Selective screening for hereditary metabolic diseases /Methodical recommendations no.6 /Department of Health]. Moskva. 24 p.

2. Pietro S., Daniela C. (2014) New Strategies for the Treatment of Phenylketonuria (PKU). pp. 1007-1017. http://doi.org/10.3390/metabo4041007

3. Hamid R.S., Ali A.H., Mohammad R.B., Zohre B. and Farzaneh Z. (2020) Global prevalence of classic phenylketonuria based on Neonatal Screening Program Data: systematic review and meta-analysis.

Clin Exp Pediatr., pp. 34-43. http://doi.org/10.3345/kjp.2019.00465

4. Bushueva T.V. (2010) Sovremennyy vzglyad na problemu fenilketonurii u detey: diagnostika, klinika, lecheniye. Voprosy sovremennoy pediatrii. [A modern view on the problem of phenylketonuria in children: diagnosis, clinic, treatment. Questions of modern pediatrics]. vol. 9, pp. 157-160

5. Hiu M.G., Gerald S., Cary O.H., Beat T. (2019) State-of-the-Art 2019 on Gene Therapy for Phenylketonuria. *Hum. Gene Ther.*, pp. 1274-1283 http://doi.org/10.1089/hum.2019.111

6. Blau N., Burton B.K., Thöny B. (2010) Phenylketonuria and BH4 Deficiencies. *J. Inherit. Metab. Dis*., 3rd edition, 112 p. ISBN 978-3-8374-1526-1

7. Orazgalieva M.G. (2005) Analiz gena fenilalaningidroksilazy u bol'nykh fenilketonuriyey i v populyatsiyakh Respubliki Kazakhstan [Analysis of the phenylalanine hydroxylase gene in patients with phenylketonuria and in the populations of the Republic of Kazakhstan]. Abstract. Ufa. http://www.dissercat.com/content/analiz-gena-fenilalaningidroksilazy-u-bilnykh-fenilketonuriei-i-v-populyatsiyakh-respubliki-#ixzz4w7GTBkrq

8. Coakley K.E., Douglas T.D., Goodman M., Ramakrishnan U., Dobrowolski S.F., Singh R.H. (2016) Modeling correlates of low bone mineral density in patients with phenylalanine hydroxylase deficiency. *J Inherit Metab Dis.*, pp. 363–372. http://doi.org/10.1007/s10545-015-9910-0

9. Regier D.S., Greene C.L. (2000) Phenylalanine Hydroxylase Deficiency. In: Adam M.P., Ardinger H.H., Pagon R.A., et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2022. Available from: https://www.ncbi.nlm.nih.gov/books/NBK1504/

10. Novikova P.B. (2007) Rukovodstvo po pediatrii. Vrozhdennyye i nasledstvennyye zabolevaniya [Guide to Pediatrics. Congenital and hereditary diseases]. Moskva. Izdatel'skiy dom «Dinastiya», pp. 61-70

11. Gladys H., John C. (2014) Phenylketonuria: translating research into novel therapies. *Transl Pediatr*, pp. 49-62 http://doi.org/10.3978/j.issn.2224-4336.2014.01.01

12. Serwet D., Katie E.C., Peter H.B., Carla E.M., Annet M.B., Rani H.S. (2015) Bone health in phenylketonuria: a systematic review and meta-analysis. *Orphanet J Rare Dis*, http://doi.org/10.1186/s13023-015-0232-y

13. Christopher J.F., Melissa L.M., Shanthi K., Mary L.L., Tyler R. (2013) Estimating the probability of IQ impairment from blood phenylalanine for phenylketonuria patients: a hierarchical meta-analysis. *J Inherit Metab Dis*., pp. 757-766, http://doi.org/10.1007/s10545-012-9564-0

14. Kaumov A.R., Gimadutdinov O.A. (2016) Praktikum po molekulyarnoy genetike. Uchebno-metodicheskoye posobiye [Workshop on molecular genetics. Teaching aid]. Kazan: KFU, p. 36

15. Levy H.L., Cornier A.S (1994) Current approaches to genetic metabolic screening in newborns. *Curr Opin Pediatr*, pp. 707-711 https://doi.org/10.1097/00008480-199412000-00017

16. Ugeskr L. (2002) Screening of newborns for inborn errors of metabolism by tandem mass spectrometry. *Ugeskr Laeger*, pp. 5607-5612

17. Wilcken B., Haas M., Joy P. (2009) Expanded newborn screening: outcome in screened and unscreened patients at age 6. *Pediatrics*, pp. 241-248 http://doi.org/10.1542/peds.2008-0586 p241–248

18. Daly A., Evans S., Chahal S., Santra S., Pinto A., Jackson R., Gingell C., Rocha J., Van F.J., MacDonald A. (2019) Glycomacropeptide: long-term use and impact on blood phenylalanine, growth and nutritional status in children with PKU. *Orphanet J Rare Dis*, 44 p. http://doi.org/10.1186/s13023-019-1011-y

19. Anne D., Sharon E., Alex P., Catherine A., Anita M. (2021) Protein Substitutes in PKU: their Historical Evolution. *Nutrients*, 484 p. http://doi.org/10.3390/nu13020484

20. NeoBase™2 Non-derivatized MSMS kit. Instructions for use. Perkin Elmer, Walac Oy. Finland. https://www.perkinelmer.com/product/neobase-non-derivatized-msms-kit-3040-001u

21. NeoLSD™2 Non-derivatized MSMS kit. Instructions for use. Perkin Elmer. Walac Oy. Finland https://www.perkinelmer.com/product/neolsd-msms-kit-3093-0020

22. Robin A.W., Cyril D.M., John R.B. (2008) Phenylketonuria: an inborn error of phenylalanine metabolism. *Clin. Biochem. Rev.*, pp. 31-41

23. Soglasno prikaza Ministra zdravookhraneniya Respubliki Kazakhstan ot 14 marta 2018 goda no.105 O vnesenii izmeneniy v prikaz Ministra zdravookhraneniya Respubliki Kazakhstan ot 29 avgusta 2017 goda no.666 "Ob utverzhdenii Perechnya lekarstvennykh sredstv i izdeliy meditsinskogo naznacheniya dlya obespecheniya grazhdan". Ekspertnoye zaklyucheniye na primeneniye novoy meditsinskoy tekhnologii [According to the Order of the Minister of Health of the Republic of Kazakhstan dated March 14, 2018 No. 105 On amendments to the order of the Minister of Health of the Republic of Kazakhstan dated August 29, 2017 No. 666 "On approval of the List of medicines and medical devices

for providing citizens". new medical technology]. https://adilet.zan.kz/rus/docs/V1700015856

24. Christopher J.F., Melissa L.M., Shanthi K., Mary L.L., Tyler R. (2013) Estimating the probability of IQ impairment from blood phenylalanine for phenylketonuria patients: a hierarchical meta-analysis. *J Inherit Metab Dis.*, pp. 757-766. http://doi.org/10.1007/s10545-012-9564-0

25. Naz A.H., John C. (2015) Phenylketonuria: a review of current and future treatments. *Transl Pediatr*, pp. 304–317. http://doi.org/10.3978/j.issn.2224-4336.2015.10.07

26. Nikiphorova A.I., Abramov D.D., Kadohnikova V.V., Zobkova G.U., Ogurscova K.A., Bruhanova N.O., Shestopalova E.A., Kochetkova T.O., Shubina E.S., Donnikov A.E., Trophimov D.U (2017) Opredeleniye chastoty vstrechayemosti mutatsiy v gene PAH s primeneniyem kombinatsii tekhnologiy PCR «V real'nom vremeni» i vysokoproizvoditel'nogo sekvenirovaniya u bol'nykh fenilketonuriyey Moskovskogo regiona [Determination of the frequency of occurrence of mutations in the PAH gene using a combination of real-time PCR technologies and high-throughput sequencing in patients with phenylketonuria in the Moscow region]. Vestnik RGMU, pp. 42-49

27. Johannes Z. (2003) Phenylketonuria mutations in Europe. *Hum Mutat*, pp. 345–356. http://doi.org/1002/humu.10192

28. Chastota fenilketonurii v stranakh mira [The frequency of phenylketonuria in the countries of the world]. Moskva. [Electronical resources]. http://ref-med.geiha.ru/data/30203.htm

29. Bodamer O.A. (2010) Screening for Phenylketonuria. Institute of Inherited Metabolic Diseases, Paracelsus Medical University. Salzburg, Austria, pp. 53-57. http://doi.org/10.1159/000312812

30. Matulevich S.A., Golihina T.A. (2014) Klinicheskiye rekomendatsii po diagnostike i lecheniyu fenilketonurii i narusheniy obmena tetragidrobioterapiya [Clinical guidelines for the diagnosis and treatment of phenylketonuria and metabolic disorders tetrahydrobiotherapy]. Moskva: Izdatel'skiy dom "Akademizdat", 52 p.