

REVIEW ARTICLE

Phenylketonuria: importance of neonatal screening, insights into genotype–phenotype correlations, and novel therapeutic approaches

✉ Maja Djordjevic Milosevic^{1, 2}, Bozica Kecman¹, Sanja Grkovic¹, Jelena Martić^{2, 3}, Kristel Klaassen⁴, Sara Stankovic⁴, Maja Stojiljkovic⁴

¹ Institute for Mother and Child Healthcare of Serbia, “Dr Vukan Cupic”, Metabolic Department, Belgrade, Serbia

² University of Belgrade, Faculty of Medicine, Belgrade, Serbia

³ Institute for Mother and Child Healthcare of Serbia, “Dr Vukan Cupic”, Department of Neonatology, Belgrade, Serbia

⁴ University of Belgrade, Institute of Molecular Genetics and Genetic Engineering, Belgrade, Serbia

Submitted: 08 February 2026

Revised: 10 March 2026

Accepted: 12 March 2026

Online First: 13 March 2026



Check for updates

Copyright: © 2026 Medicinska istraživanja

Licence:

This is an open access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

✉ Correspondence to:

Maja Djordjevic Milosevic

Institute for Mother and Child Healthcare of Serbia, “Dr Vukan Cupic”, Metabolic Department, 6-8 Radoja Dakica St., 11070, Belgrade, Serbia

University of Belgrade, Faculty of Medicine, 8 Dr Subotica starijeg St., 11000 Belgrade, Serbia

Email: maja.djordjevic@med.bg.ac.rs

Summary

Phenylketonuria (PKU) is the most common inborn error of metabolism (IEM) affecting amino acid metabolism among individuals of Caucasian origin, with an incidence ranging from 1:10,000 to 1:20,000 live births. It is inherited in an autosomal recessive manner and results from reduced activity of the enzyme phenylalanine hydroxylase (PAH), leading to phenylalanine accumulation and a deficiency of tyrosine. In untreated patients, the clinical phenotype is primarily a consequence of neurotoxicity and includes developmental delay, intellectual disability, and epilepsy. To date, more than 1,100 pathogenic variants of the PAH gene have been identified. The most frequently reported pathogenic variant in Serbia is p.Leu48Ser. Numerous studies have focused on elucidating genotype-phenotype correlations in PKU. Early detection through newborn screening (NBS) programs, based on the identification of elevated phenylalanine concentrations, and prompt initiation of treatment, remains the only effective strategy to prevent intellectual disability and epilepsy and to enable normal neurodevelopment. The introduction of tandem mass spectrometry has significantly expanded the number of IEMs detected by NBS programs across Europe and worldwide. PKU treatment continues on a phenylalanine-restricted diet, which is highly effective but demanding for patients and their families. Adjunct therapeutic approaches include the use of the PAH cofactor tetrahydrobiopterin (BH4) and enzyme-substitution therapy with pegvaliase, both aimed at liberalizing the diet. However, a major limitation of BH4 therapy is the lack of response in patients with severe phenotypes. The introduction of sepiapterin (BH2) offers promising prospects for increasing residual PAH activity across different phenotypic groups.

Keywords: phenylketonuria, newborn screening, genotype-phenotype correlation, pegvaliase, sepiapterin

INTRODUCTION

Phenylketonuria (PKU; OMIM #261600) is the most common inherited disorder of amino acid metabolism among individuals of Caucasian descent. It is inherited in an autosomal recessive manner and results from impaired hydroxylation of phenylalanine (phe) to tyrosine, leading to phe accumulation. This metabolic reaction requires the enzyme phenylalanine hydroxylase (PAH) and the cofactor tetrahydrobiopterin (BH4). In the majority of patients, mutations in the *PAH* gene cause the disease, whereas in only 1–2% of patients, elevated phe levels result from defects in BH4 metabolism. The main clinical manifestations of untreated PKU include intellectual disability and seizures; other neurological symptoms may also occur, along with symptoms of damage to other organ systems. The severity of the clinical presentation correlates with phe concentrations in the blood and cerebrospinal fluid, as well as with the duration of the metabolic disturbance. Implementation of a phe-restricted diet, initiated by the middle of the first month of life, prevents adverse outcomes and enables a good quality of life for affected individuals. A favorable disease outcome depends on early detection before the onset of clinical symptoms through neonatal screening (1-3).

Phenylketonuria was first described in 1934 by the Norwegian scientist Asbjørn Følling, following the detection of phenylpyruvic acid in the foul-smelling urine of two developmentally delayed brothers. He termed the condition phenylpyruvic idiocy and concluded that it is inherited in an autosomal recessive manner (4). Penrose and Quastel subsequently renamed the disease phenylketonuria (5). Jervis demonstrated reduced PAH enzyme activity in the livers of patients with this disorder (6,7). In 1954, Bickel showed that a phe-restricted diet can reduce phe concentrations and prevent the development of intellectual disability if dietary treatment is initiated early, before the onset of clinical symptoms (8). In the early 1960s, Guthrie and Susi established that phe concentration could be determined using a simple, inexpensive bacterial inhibition assay from a dried blood spot on filter paper (9), paving the way for neonatal screening. Screening for PKU in the United States began in 1962 (10,11). A pilot screening program in the territory of the former Yugoslavia was conducted in 1967 by Vulović and colleagues, while organized nationwide PKU screening in Serbia was introduced in 1983 (12).

The incidence of PKU in the Caucasian population is estimated to range from approximately 1:10,000 to 1:20,000 (13). A high incidence has been reported in Turkey (1:2,600), Iran (1:3,300), Ireland (1:4,500), Estonia (1:6,000), Tunisia (1:7,600), Latvia (1:8,700), and Hungary (1:9,000), whereas a low incidence has been observed in Finland (1:100,000), Japan (1:125,000), and Thailand (1:212,000) (1,14-16,17). Among the countries of the former Yugoslavia, Slovenia has the highest

incidence of PKU (1:8,000) (18). The incidence of PKU in Serbia ranges from 1:18,732 to 1:39,338, placing Serbia among countries with a moderate incidence of this disorder worldwide (3).

Reduced PAH activity, due to mutations in the *PAH* gene or disturbances in BH4 metabolism, invariably leads to an increase in blood phe concentrations above the normal upper limit of 120 µmol/L. This condition is referred to as hyperphenylalaninemia (HPA). Dietary therapy is initiated when phe concentrations reach 360 µmol/L or higher (19). The classification of HPA is presented in **Table 1** (17,19).

Table 1. Phenotypes of PAH deficiency according to blood phe level (17,19)

Type of hyperphenylalaninemia	Blood phe level (µmol/l)
Mild hyperphenylalaninaemia (MHP)	120 - 600
Mild phenylketonuria (mPKU)	600 - 1200
Classic phenylketonuria (cPKU)	> 1200

Only mild PKU (mPKU) and classic PKU (cPKU) require treatment after 12 years of age (19). In addition to blood phe levels, the concept of Phe tolerance is also used for classification. Phenylalanine tolerance refers to the maximum amount of phenylalanine that can be ingested through the diet while maintaining blood concentrations within acceptable limits (up to 240 in pregnancy or 360 µmol/L in children (19). More severe disease is associated with lower residual PAH activity, resulting in lower phe tolerance (17,19,20). There are additional approaches to define the phenotype of individuals with elevated phe concentrations. One approach is shown in **Table 2** (21).

Table 2. Interrelated phenotypes in HPA

Phenotypes	Explanation of the phenotype
Clinical	Comprises intellectual disability and other recognized clinical manifestations of the disease, as well as phenylalanine tolerance
Metabolic	Refers to blood phenylalanine concentrations in untreated patients or in patients before the initiation of therapy
Enzymatic	Denotes residual enzyme activity, which predicts the degree of PAH deficiency

Adapted from reference (21).

These phenotypes are interrelated: the enzymatic phenotype determines the metabolic phenotype, while the metabolic phenotype determines the clinical phenotype. All three phenotypes may be modified by therapy; the clinical and metabolic phenotypes through dietary treatment, and the enzymatic phenotype, in a subset of patients, through BH4 administration.

In patients with PKU, the brain is the primary and most severely affected organ. Multiple mechanisms are involved in neuronal damage, and not all of them are yet fully understood (22). Elevated phe concentration plays a

central role, as the most favorable therapeutic outcomes have been achieved through a phe-restricted diet. It is believed that L-phenylalanine directly depresses glutamatergic synaptic transmission by reducing the amplitude and frequency of N-methyl-D-aspartate (NMDA) and non-NMDA components of glutamate receptors (GluRs) (22,23). Furthermore, Mortell et al. suggested that phe directly affects the permeability of calcium channels in neuronal membranes, representing an important pathogenic mechanism (24). However, it should be noted that even in patients in whom dietary treatment is initiated early and good metabolic control is achieved, differences in cognitive function remain when compared with the general population (25-27). Amino acid transport across the blood-brain barrier is a dynamic process.

The transport of large neutral amino acids (LNAA), including phe, valine, methionine, isoleucine, leucine, tyrosine, histidine, tryptophan, and lysine, is mediated by a single transporter, designated LAT1. These amino acids compete for binding to this transporter. In conditions of phe excess, as observed in HPA, the transport of other LNAAs is reduced (28). Evidence supporting the role of LNAA deficiency in disease pathogenesis includes findings that oral LNAA supplementation reduces brain phe concentrations and improves electroencephalographic (EEG) patterns and neurophysiological findings (28). In healthy individuals, all LNAAs except tyrosine are essential amino acids; however, in patients with PKU, tyrosine also becomes essential and must be provided exogenously. Patients with PKU exhibit reduced concentrations of dopamine, catecholamines, serotonin, and their metabolites in both the brain and cerebrospinal fluid (29). This deficiency is most likely due to impaired synthesis resulting from LNAA deficiency, particularly tyrosine and tryptophan (28). Lower concentrations of these amino acids have been linked to reduced protein synthesis. Dopamine deficiency leads to basal ganglia dysfunction, and untreated PKU patients may develop chorea, tremor, and dystonia (22,30,31). Serotonin deficiency is associated with irritability and depression (32,33). It has been established that, in addition to alterations in neurotransmitter production, protein synthesis, oxidative stress, and energy homeostasis, white matter abnormalities also contribute to the neurological symptoms of PKU (22). However, some studies, including those in which we participated, demonstrate that in certain untreated PKU patients, elevated phe concentrations did not result in expected brain damage, as evidenced by the absence of intellectual disability (34-37). This phenomenon remains to be investigated.

Intellectual disability is the leading clinical manifestation in untreated PKU patients, and affected individuals may have an IQ below 35. It becomes evident within the first months of life, initially through the loss of previously acquired neurological functions. Later, marked behavioural changes and, in some cases, epilepsy may develop. Characteristic findings include stereotypic

movements, hyperactive deep tendon reflexes, and tremor. Most patients are hyperactive and present with microcephaly. The neurological changes are irreversible; therefore, initiating dietary treatment at a very early stage, ideally within the first month of life, is critical (1,38,39). Epilepsy occurs in approximately 25% of patients and may present as partial seizures, infantile spasms, or myoclonic seizures. EEG findings may show generalized slowing, epileptiform discharges, or hypsarrhythmia (40). However, in about 50% of patients, EEG abnormalities can be detected that do not necessarily correlate with clinical seizures. Both epilepsy and EEG abnormalities are reversible with a phe-restricted diet (1,41,42). Less frequently, but occasionally as the leading clinical manifestation, the disease may present with projectile vomiting (1). The excretion of phe and its metabolites, including ortho-hydroxyphenylacetic acid and phenylpyruvic acid, in urine and sweat results in a characteristic musty or "mousy" odour. Cutaneous manifestations include eczema or seborrheic dermatitis. Unlike neurological abnormalities, these skin changes are reversible following the initiation of a phe-restricted diet. Impaired tyrosine metabolism may also lead to hypopigmentation of the skin and hair (1,38). In addition to microcephaly, patients may present with maxillary prominence, widely spaced teeth, and enamel hypoplasia. Short stature and developmental delay may also be observed. Numerous studies have demonstrated a higher incidence of osteopenia in individuals with untreated PKU (43). Vitamin B12 deficiency observed in a subset of patients with HPA is most likely due to a low-protein diet. It is most commonly detected when patients discontinue phenylalanine-free amino acid mixtures while continuing protein restriction (44). Overall, the severity of the clinical phenotype depends on both the blood phenylalanine concentration and the duration of exposure to elevated phenylalanine levels.

The phenylalanine hydroxylase (PAH) gene is located on chromosome 12 at 12q23.2, spanning approximately 1.5 Mbp (45). In addition to the PAH gene, this region contains five other genes of unknown function (46). More than 1,100 distinct pathogenic variants in the PAH gene have been identified as causative of PKU, and the mutation spectrum varies across ethnic groups (47). Mutations in the PAH gene are predominantly missense (63%), resulting in amino acid substitutions within the protein sequence. Small deletions (13%) and splice-site mutations affecting intron processing (11%) are relatively common, whereas nonsense mutations leading to premature stop codons (5%) and small insertions (1%) are comparatively rare (48). Large deletions were previously considered very rare; however, with the introduction of multiplex ligation-dependent probe amplification (MLPA), their frequency has increased to approximately 3% of PAH mutations (49). In Serbia, we identified 38 distinct disease-causing variants in the PAH gene and classified them according to the American College of Medical

Genetics and Genomics (ACMG) guidelines. The most frequent variants are p.Leu48Ser (30.92%), followed by p.Arg408Trp (12.21%) and p.Ile306Val (8%) (50-54).

METHODS

This narrative review aims to summarize current knowledge on PKU, with particular focus on neonatal screening, genotype-phenotype correlations, and emerging therapeutic approaches. A literature search was conducted using major scientific databases, including PubMed, Scopus, and Google Scholar. Articles published in English between 1934 and 2025 were considered. The search included combinations of the following keywords: phenylketonuria, PKU, newborn screening, genotype-phenotype correlation, PAH mutations, tetrahydrobiopterin (BH4), sapropterin, pegvaliase, and novel therapies. Relevant original research articles, clinical studies, reviews, and international guidelines were considered for inclusion. In addition to the most relevant international publications, studies from Serbia that have contributed to advancing scientific knowledge on PKU were also included. In total, 13 references from Serbian authors were incorporated in this review. Articles focusing on unrelated metabolic disorders or lacking sufficient relevance to the topic were excluded. Findings from the literature were synthesized to provide an overview of current evidence and emerging perspectives in the field.

GENOTYPE-PHENOTYPE CORRELATION

Mutations in the PAH gene impair PAH activity; however, not all mutations have the same effect. Large deletions and splicing mutations that disrupt the processing of the primary mRNA transcript result in major alterations of the primary protein structure and completely abolish enzymatic activity. Small deletions and insertions whose length is not divisible by three, particularly when located at the beginning of the PAH gene, alter the reading frame and lead to the synthesis of a profoundly altered polypeptide chain that is unable to catalyze the hydroxylation of phenylalanine to tyrosine. Point mutations that introduce a premature stop codon prevent synthesis of the full-length polypeptide chain. These mutations are classified as functionally null mutations. In addition, certain missense mutations that have been shown in vitro to exhibit extremely low enzymatic activity (<10%) are also considered null mutations (55-57). Most severe forms of PKU, characterized by minimal or absent enzymatic activity, can be identified with a high degree of certainty. Other missense mutations impair the structure and/or catalytic properties of phenylalanine hydroxylase to varying degrees, resulting in reduced enzyme activity.

In vitro analyses have demonstrated that the activity of mutant PAH enzymes ranges from 0% to nearly 100% of normal PAH activity (58,59-60). For these reasons, it is difficult to predict the severity of the clinical phenotype associated with so-called non-null mutations; however, it is possible to identify patients who are likely to respond to BH4 therapy (61). We also demonstrated that our most common mutation, p.Leu48Ser, shows phenotypic inconsistency even in the homozygous state, and we predicted responsiveness to BH4 therapy based on genotype (3,50,51,54). Zschocke (2008) contributed to the understanding of genotype-phenotype correlations in inborn errors of metabolism, including reduced PAH function. He proposed that the interaction between mutations on both alleles also influences the enzymatic and metabolic phenotypes (55).

Each PAH mutation is assigned an allelic phenotype value (APV) from the BIOPKU database based on its severity in functionally hemizygous individuals (in combination with a null mutation) (56). An APV of 0 indicates severe disease (cPKU), while an APV of 10 indicates mild disease (MHP) associated with more than 70% residual enzyme activity. The genotypic phenotype value (GPV) is defined as the higher APV of the two mutations present. Based on the final GPV, patients are classified as having classical PKU (GPV 0.0–2.7), mild PKU (GPV 2.8–6.6), or MHP (GPV 6.7–10.0) (54,57,58). This system helps to predict the clinical phenotype. Studies have shown that this method predicts the phenotype in about 79% of cases. However, variability may arise from epigenetic factors or interallelic complementation (59,60). This approach may also help predict responsiveness to BH4 (sapropterin) (61).

NEWBORN SCREENING PROGRAM

Early diagnosis of PKU aims to detect the disease before the onset of neurological symptoms and to initiate a phe-restricted diet, as brain damage in these patients is irreversible. Newborn screening (NBS) programs (9, 17, 19), which identify elevated phe concentrations and other metabolic disorders, are now an integral part of mandatory national public health programs in many countries worldwide (62). In Serbia, neonatal screening currently includes only phenylketonuria among inborn errors of metabolism (IEM), whereas in countries across Europe, North America, Asia, and Australia, the number of IEM detected through neonatal screening ranges from a dozen to several dozen, and in some cases up to one hundred (62-67). Since newborn screening for IEM is based on biochemical and metabolic analyses, the blood sample must be collected 48 hours after birth. At the same time, feeding initiation is required for informative results. The blood sample is applied to special filter paper (dry blood spot, DBS) and sent to a reference laboratory. With the

introduction of tandem mass spectrometry (MS/MS) in the early 1990s, it became possible to analyze a large number of metabolites from a single blood sample (68). This approach allows not only the quantification of amino acids but also acylcarnitines and other compounds, enabling the diagnosis of a wide range of inborn errors of metabolism (IEMs). Today, this analytical method is routinely used for the early detection of PKU and other IEMs as part of NBS in almost all countries of Western Europe and the United States (17,19,67). In Serbia, the NBS for IEM uses a fluorometric method that measures only phenylalanine levels.

Today, there is considerable debate regarding the use of genetic testing as a basis for neonatal screening (genetic NBS) (69). However, in the context of IEM, biochemical and metabolic analyses remain the primary approach in newborn screening. Direct assessment of enzyme activity or metabolite accumulation enables the detection of clinically relevant abnormalities before the onset of symptoms, which is the primary focus of NBS. In addition to disease-causing genotypes, genetic testing may identify variants of uncertain significance (VUS) that do not always correlate with disease manifestation. Genomic NBS using WES from DBS is technically feasible and enables the early identification of actionable genetic conditions. Although the integration of genomic approaches into NBS represents a logical next step, significant ethical, logistical, and clinical challenges remain, and its implementation requires appropriate genetic counseling and long-term follow-up (69-71).

When elevated phe concentrations are detected through neonatal screening, disorders other than PAH gene mutations should also be considered. Although tetrahydrobiopterin (BH4) deficiency, resulting from defects in its synthesis or recycling, accounts for elevated phe levels in only approximately 2% of patients with HPA, any increase in phe concentration warrants further evaluation. This evaluation should include measurement of dihydropteridine reductase activity in erythrocytes and urinary pterin analysis in all children once elevated phenylalanine levels have been confirmed (17,19). Upon diagnosis of a disorder of BH4 metabolism, in addition to a phenylalanine-restricted diet, treatment with BH4 supplementation and neurotransmitter precursors should be initiated immediately (19,72). Another disorder, hyperphenylalaninemia due to DNAJC12 deficiency, also known as mild non-BH4-deficient hyperphenylalaninemia (HPA-NBH4), is excluded by genetic analysis (whole-exome sequencing, WES) in children with a positive newborn screening result for HPA. This is an inherited metabolic disorder characterized by elevated phenylalanine levels and deficiencies of neurotransmitters, including dopamine and serotonin, resulting from dysfunction of the DNAJC12 chaperone protein. Management of affected patients requires pharmacological therapy (73).

TREATMENT

Treatment of HPA is initiated when the blood phe level exceeds 360 $\mu\text{mol/L}$. Children with phe levels between 120 and 360 $\mu\text{mol/L}$ are monitored until age 6, and dietary treatment is considered if phe levels increase (19). Female patients with phe levels above 120 $\mu\text{mol/L}$, regardless of the dietary treatment, require long-term follow-up. It is particularly important to ensure that phe levels are maintained below 240 $\mu\text{mol/L}$ before conception and throughout pregnancy. When Phe levels fall between 360 and 600 $\mu\text{mol/L}$, dietary treatment is recommended at least until the age of 12 (19). Target phe levels during the first 12 years of life are 120-360 $\mu\text{mol/L}$. In treated patients with PKU aged 12 years and older, the recommended therapeutic phe range is 120-600 $\mu\text{mol/L}$ (3,19).

Phenylalanine-restricted diet

At present, dietary management is the primary, first-line therapeutic approach for the treatment of PKU. The diet is based on strict restriction of phe intake while ensuring adequate provision of other essential amino acids and sufficient total protein from amino acid mixtures to support normal growth, development, and brain maturation. The earliest attempts at dietary treatment were reported by Bickel and colleagues in 1953. They proposed removing phe from casein via acid hydrolysis, combined with oral administration of activated charcoal to eliminate excess phe (8). Contemporary dietary treatment relies on phe-free amino acid mixtures, with minimal amounts of phe supplied through natural protein sources. Evidence indicates that treatment outcomes in newborns and infants with PKU are superior when breast milk, rather than infant formula, is used as the source of natural protein (74). In later childhood and adulthood, dietary sources of phe primarily include fruits and vegetables, which have a lower protein content compared with meat, eggs, milk, dairy products, and cereals, which are almost excluded from diet. After the first year of life, low-protein commercially manufactured products, such as low-protein flour, milk substitutes, powdered egg products, and pasta, play an important role in dietary management. Current consensus guidelines recommend lifelong dietary treatment (19,7). Well-controlled studies have demonstrated significant differences in cognitive outcomes in children who discontinued the diet and those who maintained dietary treatment beyond adolescence (75). The official recommendation is that a lifelong dietary regimen should be maintained in all individuals with phe concentrations exceeding 600 $\mu\text{mol/L}$. Maintaining adequate metabolic control during pregnancy is particularly critical in women with PKU, as insufficient control may result in severe congenital anomalies in the fetus (19). The daily intake of phe is individual for each patient and is determined by the patient's tolerance to phe (20,57). Phenylalanine

tolerance implies a maximum daily intake of phe that maintains blood phe concentrations of 120-360 $\mu\text{mol/L}$. According to Van Spronsen (25), individual phe tolerance can be determined as early as age 2. A lower intake of protein or the most energetically active food components (such as fats and carbohydrates) leads to increased catabolism and endogenous production of phenylalanine. Catabolism happens during infections and traumas, and patients in these situations should consume less phenylalanine than usual and a higher intake of fats and carbohydrates. The plasma phe level also changes during the menstrual cycle in women, and phe is highest during the luteal phase (76).

Normal growth and development are indicators of adequate dietary management and effective metabolic control. Evaluation of developmental quotients (DQ) or intelligence quotients (IQ) serves as the most reliable measure of good metabolic control. In patients adhering to a well-managed diet, the expected IQ score is 101 ± 11 (19). Metabolic control through dietary treatment is fundamental for normal intellectual development in individuals with PKU. Lifelong dietary therapy is essential for maintaining optimal metabolic control and supporting normal growth. If a patient fails to consume sufficient amino acid mixtures or does not distribute intake appropriately throughout the day, there is not only a risk of increased blood phe concentrations due to enhanced catabolism, but also a risk of impaired growth resulting from energy-protein malnutrition. The required daily intake of amino acid formula depends on the allowed intake of natural protein, and sufficient energy intake must also be ensured. For children with PKU younger than 2 years, a protein intake of at least 3 g/kg/day is recommended, and after 2 years of age, 2 g/kg/day (74). The rationale for this higher protein intake compared with that of healthy children is the rapid absorption of free amino acids from formula, which results in slower protein synthesis than with natural protein alone (74,77).

Tetrahydrobiopterin (BH4)

In 1999, it was demonstrated for the first time that, in some patients with HPA due to PAH gene mutations, BH4 administration led to a significant reduction in blood phe levels while maintaining a normal diet. Until then, BH4 therapy had been limited to patients with BH4 deficiency. Following this and other reported cases, research focused on elucidating the mechanisms by which BH4 lowers phe concentrations in patients with PAH gene mutations (61).

BH4 has multiple functions in the human body. It serves as a cofactor for PAH and other enzymes, including tyrosine hydroxylase and tryptophan hydroxylase. In addition, BH4 acts as a negative regulator of PAH activity and as a chemical chaperone that stabilizes the structure of both normal and mutant PAH enzyme forms, thereby preventing proteolytic degradation of the mutant protein.

Owing to these functions, BH4 is the first pharmacological agent used in the treatment of patients with reduced PAH activity, provided that a positive response is demonstrated in the BH4 loading test (61,78,79). The generic name of the BH4-containing drug is sapropterin, while its proprietary name is Kuvan®. Sapropterin is a synthetic form of BH4. In a subset of patients with mPKU and MHP, sapropterin can reduce blood Phe concentrations by approximately 30%. Patients in whom BH4 lowers blood phe levels (commonly referred to as “responders”) may increase their daily phe intake by approximately 100-500 mg, or by about 18-40 mg/kg of body weight (80). The introduction of Kuvan in the treatment of mPKU represents a significant advancement in the management of patients with PKU. The major limitation of this therapy is the lack of therapeutic effect in patients with cPKU, who have minimal or absent PAH activity (80,81).

In Serbia, based on genotype analysis and allelic phenotype value (APV) and genotype-phenotype value (GPV), we estimated the proportion of patients with HPA who would be expected to respond to sapropterin therapy. However, treatment with this agent has not yet been implemented in Serbian patients with PAH gene mutations (3,51,54,72).

Enzyme therapy, Pegvaliase

The enzyme pegvaliase (phenylalanine ammonia lyase, PAL) converts phe into trans-cinnamic acid, which is subsequently metabolized to benzoic acid. Benzoic acid is non-toxic and is excreted in the urine. PAL is an autocatalytic enzyme and therefore does not require a cofactor for phenylalanine degradation (82). The efficacy of pegvaliase varies among individual patients and cannot be reliably predicted. Immunogenicity plays a significant role in the pharmacokinetics of pegvaliase, primarily through increased drug clearance during the early phase of treatment. In 2018, the U.S. Food and Drug Administration (FDA) approved the use of pegylated phenylalanine ammonia lyase (PEG-PAL, pegvaliase) as an enzyme substitution therapy for patients with PKU aged ≥ 18 years who have uncontrolled blood phe levels ($>600 \mu\text{mol/L}$) despite current treatment. In 2019, the European Medicines Agency (EMA) approved pegvaliase for individuals with PKU aged ≥ 16 years (19,83,84). The goal of pegvaliase therapy is to maintain blood phe concentrations within the recommended range while allowing dietary normalization, including unrestricted protein intake. Pegvaliase may be considered for all adult patients with PKU. The major limitations of enzyme replacement therapy are allergic reactions and an increased risk of anaphylaxis. To reduce the risk of hypersensitivity reactions, premedication with an H1-receptor antagonist, an H2-receptor antagonist, and/or an antipyretic should be considered (19,83,84).

Large neutral aminoacids and Glycomacropeptide

The discovery of competition between large neutral amino acids (LNAA) and phe for specific transporters required for absorption across the gastrointestinal mucosa into the bloodstream, as well as for transport across the blood-brain barrier, has opened new therapeutic possibilities for patients with phenylketonuria (PKU). This competition is concentration-dependent, and phenylalanine has a marked advantage in binding to these transporters in patients with elevated blood phenylalanine levels. Supplementation with other LNAAs in patients with PKU reduces phe transport across the blood-brain barrier, as demonstrated by quantitative brain nuclear magnetic resonance spectroscopy (85). This approach is particularly useful in patients who have difficulty adhering to or cannot tolerate a phe-restricted diet, who do not use phe-free amino acid mixtures, and who present with very high blood phe concentrations (exceeding 1000 $\mu\text{mol/L}$). This therapeutic strategy may also partially improve cognitive function in these patients (86). However, this treatment option is not widely used in current clinical practice.

Glycomacropeptide (GMP) is a protein naturally present in whey and is the only natural protein virtually free of phe. In addition, it contains high concentrations of non-toxic large neutral amino acids, including threonine, isoleucine, and valine. When supplemented with additional amino acids (arginine, histidine, leucine, and tyrosine), GMP can serve as a high-quality and sufficient protein source to support normal growth and development. Its advantages include its natural origin, favorable palatability, and high protein content (87). However, GMP has not yet been widely adopted in clinical practice.

Sepiapterin

Sepiapterin exerts its pharmacological effects through two distinct yet complementary pathways, both aimed at reducing blood phe concentrations. First, sepiapterin is actively transported into cells, leading to increased intracellular levels, where it is converted to BH₄ and shows greater bioavailability than sapropterin. At high intracellular concentrations, tetrahydrobiopterin acts, as well established, as an essential cofactor for PAH. Second, sepiapterin exerts a direct effect on PAH by functioning as a pharmacological chaperone, altering the enzyme's allosteric configuration and thereby enhancing its catalytic activity. These dual mechanisms of action are likely the main reason why sepiapterin

demonstrates superior therapeutic efficacy compared with sapropterin (88). Since 2025, sepiapterin has been approved by the European Medicines Agency (EMA) for the treatment of phenylketonuria (89). Clinical data indicate therapeutic benefit even in patients with cPKU. Furthermore, therapeutic response can, to some extent, be predicted based on genotype. The degree to which dietary phe restriction can be liberalized in patients receiving sepiapterin therapy remains to be determined (88,90). Treatment with sepiapterin has not yet been initiated in Serbia.

CONCLUSION

PKU is one of the most common IEMs. It results from reduced activity of the enzyme PAH, which converts phe to tyrosine with the aid of the cofactor BH₄. The clinical presentation of untreated PKU primarily includes intellectual disability and epilepsy. Since the introduction of NBS, the disease is diagnosed before the onset of clinical symptoms, and when treatment with a phe-restricted diet is initiated before the 15th day of life, the development of intellectual disability and epilepsy can be prevented. However, once intellectual disability has developed, the neurological damage is irreversible. Studies of genotype and genotype-phenotype correlations, to which we have also contributed through our own research, have significantly advanced the understanding of disease pathophysiology and facilitated the development of new therapeutic options. The use of phe ammonia lyase is currently limited to adult patients, and severe allergic reactions are a major limitation for the introduction of therapy. The use of BH₄ in the treatment of PKU caused by mutations in the PAH gene has enabled partial dietary liberalization. The most recent therapeutic agent, sepiapterin, lowers phe levels more efficiently as BH₄, and it is a treatment option even in a subset of patients with cPKU.

Acknowledgements: N/A

Funding information: This research received no external funding.

Conflicts of interest: The authors declare that there are no conflicts of interest related to this review.

Author contributions: All authors contributed to the conception and design of the review, literature search, and interpretation of the published data. All authors participated in drafting and critical revision of the manuscript and approved the final version for publication.

Ethical approval: N/A

Informed consent: N/A

Literature:

1. Scriver CR, Kaufman S. Hyperphenylalaninemia: phenylalanine hydroxylase deficiency. In: Scriver CR, Beaudet AL, Sly WS, Valle D, editors. *The Metabolic and Molecular Bases of Inherited Disease*. 8. New York: McGraw-Hill; 2001.p.1667-724.
2. Adams AD, Fiesco-Roa MÓ, Wong L, Jenkins GP, Malinowski J, Demarest OM, Rothberg PG, Hobert JA; ACMG Therapeutics Committee. Phenylalanine hydroxylase deficiency treatment and management: A systematic evidence review of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*. 2023; 25:100358. doi: 10.1016/j.gim.2022.12.005.
3. Đorđević M. Korelacija između genotipa i fenotipa bolesnika sa hiperfenilalaninemijom na teritoriji Srbije [doktorska disertacija]. Beograd: Univerzitet u Beogradu; 2013.
4. Folling A. Über Ausscheidung von Phenylbrenztraubensäure in den Harn als Stoffwechselanomalie in Verbindung mit Imbezillität. *Hoppe Seylers Z Physiol Chem* 1934; 277:169-76.
5. Penrose L, Quastel JH. Metabolic studies in phenylketonuria. *Biochem J* 1937; 31:266-74.
6. Jervis GA. Studies on phenylpyruvic oligophrenia: the position of the metabolic error. *J Biol Chem* 1947; 169:651-6.
7. Jervis GA. Phenylpyruvic oligophrenia deficiency of phenylalanine-oxidizing system. *Proc Soc Exp Biol Med* 1953; 82:514-5.
8. Bickel H, Gerrard J, Hickmans E. Influence of phenylalanine intake on phenylketonuria. *Lancet* 1953; 2:812. [https://doi.org/10.1016/s0140-6736\(53\)90473-5](https://doi.org/10.1016/s0140-6736(53)90473-5)
9. Guthrie R, Susi A. A simple Phenylalanine method for detecting Phenylketonuria in large populations of newborn infants. *Pediatrics* 1963; 32:338-43.
10. Dhondt JL. Neonatal screening from the 'Guthrie age' to 'genetic age'. *J Inherit Metab Dis* 2007; 30:418-22. <https://doi.org/10.1007/s10545-007-0624-9>
11. Tarini BA. The current revolution in newborn screening: new technology, old controversies. *Arch Pediatr Adolesc Med* 2007; 161:767-72. <https://doi.org/10.1001/archpedi.161.8.767>
12. Vulović D, Vilhar N, Banićević M, Šćević S, Hajduković R i Subotić Z. Rano otkrivanje naslednih mana u novorođenčeta. *Srpski arhiv* 1979; 3:269-81.
13. Erlandsen H, Stevens RC. The structural basis of phenylketonuria. *Mol Genet Metab* 1999; 68:103-25. doi: 10.1006/mgme.1999.2922.
14. DiLella AG, Kwok SCM, Ledley FD, Marvit J, Woo SLC. Molecular structure and polymorphic map of the human phenylalanine hydroxylase gene. *Biochemistry* 1986; 25:743-9. doi: 10.1021/bi00352a001.
15. Ozalp I, Coskun T, Ceyhan M, Tokol S, Oran O, Erdem G, et al. Incidence of phenylketonuria and hyperphenylalaninemia in a sample of the newborn population. *J Inher Metab Dis*. 1986; 9:237. <https://doi.org/10.1007/BF01799716>
16. Khemir S, Asmi M, Sanahaji H, Feki M, Jernna R, Tebib N et al. Phenylketonuria is still a major cause of mental retardation in Tunisia despite the possibility of treatment. *Clin Neurol Neurosurg* 2011; 113:727-30. doi: 10.1016/j.clineuro.2011.07.016.
17. Hillert A, Anikster Y, Belanger-Quintana A, Burlina A, Burton BK, Carducci C, et al. The Genetic Landscape and Epidemiology of Phenylketonuria. *Am J Hum Genet* 2020; 107:234-250. doi: 10.1016/j.ajhg.2020.06.006.
18. Groselj U, Zerjav Tansek M, Kovac J, Hovnik T, Trebusak Podkrajsek K, Battelino T. Five novel mutations and two large deletions in a population analysis of the phenylalanine hydroxylase gene. *Mol Genet Metab* 2012; 106:142-8. doi: 10.1016/j.ymgme.2012.03.015.
19. Van Wegberg AM, MacDonald A, Ahring K, Bélanger-Quintana A, Beblo S, Blau N et al. European guidelines on diagnosis and treatment of phenylketonuria: First revision. *Mol Genet Metab* 2025;145:109125. doi: 10.1016/j.ymgme.2025.109125.
20. Pinto A, Ilgaz F, Evans S, van Dam E, Rocha JC, Karabulut E et al. Phenylalanine Tolerance over Time in Phenylketonuria: A Systematic Review and Meta-Analysis. *Nutrients* 2023;15:3506. doi: 10.3390/nu15163506.
21. Zschocke J. Molecular basis of phenylketonuria: from genotype to clinical management. *Ann Nestle* 2010; 68:48-52.
22. Nulmans I, Lequeue S, Desmet L, Del'haye GG, Salvi NS, Rombaut M et al. Phenylketonuria: A guide through the complex maze of its neurological pathophysiology providing a new perspective on treatment strategies. *Biomed Pharmacother* 2025;191: 118522. doi: 10.1016/j.biopha.2025.118522.
23. Glushakov AV, Dennis DM, Sumners C, Seubert CN, Martynyuk AE. L-phenylalanine selectively depresses currents at glutamatergic excitatory synapses. *J Neurosci Res* 2003; 72:116-24. doi: 10.1002/jnr.10569.
24. Mortell KH, Anderson DJ, Lynch JJ 3rd, Nelson SL, Sarris K, McDonald H, et al. Structure-activity relationships of alpha-amino acid ligands for the alpha2delta subunit of voltage-gated calcium channels. *Bioorg Med Chem Lett* 2006; 16:1138-41. doi: 10.1016/j.bmcl.2005.11.108.
25. Van Spronsen FJ, Hoeksma M, Reijngoud DJ. Brain dysfunction in phenylketonuria: is phenylalanine toxicity the only possible cause? *J Inherit Metab Dis* 2009; 32:46-51. doi: 10.1007/s10545-008-0946-2.
26. Anderson PJ, Wood SJ, Francis DE, Coleman L, Anderson V, Boneh A. Are neuropsychological impairments in children with early-treated phenylketonuria (PKU) related to white matter abnormalities or elevated phenylalanine levels? *Dev Neuropsychol* 2007; 3:645-68. doi: 10.1080/87565640701375963.
27. Moyle JJ, Fox AM, Arthur M, Bynevelt M, Burnett JR. Meta-analysis of neuropsychological symptoms of adolescents and adults with PKU. *Neuropsychol Rev* 2007; 7:91-101. doi: 10.1007/s11065-007-9021-2.
28. Knudsen GM, Hasselbalch S, Toft PB, Christensen E, Paulson OB, Lou H. Blood-brain barrier transport of amino acids in healthy controls and in patients with phenylketonuria. *J Inherit Metab Dis*1995; 18:653-64. doi: 10.1007/BF02436753.
29. Pilotto A, Blau N, Leks E, Schulte C, Deuschl C, Zipser C et al. Cerebrospinal fluid biogenic amines depletion and brain atrophy in adult patients with phenylketonuria. *JIMD* 2019;42(3):398-406. doi: 10.1002/jimd.12049.
30. De Groot MJ, Hoeksma M, Blau N, Reijngoud DJ, van Sronsen FJ. Pathogenesis of cognitive dysfunction in phenylketonuria: Review of hypotheses. *Mol Genet Metab* 2010; 99:S86-9. doi: 10.1016/j.ymgme.2009.10.016.
31. Thompson AJ, Smith I, Brenton D, Youl BD, Rylance G, Davidson DC, et al. Neurological deterioration in young adults with phenylketonuria. *Lancet* 1990; 336:602-5. doi: 10.1016/0140-6736(90)93401-a.
32. Pietz J. Neurological aspects of adult phenylketonuria. *Curr Opin Neurol* 1998; 11:679-88. doi: 10.1097/00019052-199812000-00012.
33. Anderson PJ, Wood SJ, Francis DE, Coleman L, Anderson V, Boneh A. Are neuropsychological impairments in children with early-treated phenylketonuria (PKU) related to white matter abnormalities or elevated phenylalanine levels? *Dev Neuropsychol* 2007; 3:645-68. doi: 10.1080/87565640701375963.
34. Smith I, Knowles J. Behaviour in early treated phenylketonuria: a systematic review *Eur J Pediatr* 2000; 159:89-93. doi: 10.1007/pl00014392.
35. Van Vliet D, van Wegberg AMJ, Ahring K, Bik-Multanowski M, Blau N, Bulut FD, Casas K, Didycz B, Djordjevic M et al. Can untreated PKU patients escape from intellectual disability? A systematic review. *Orphanet J Rare Dis* 2018; 13:149. doi: 10.1186/s13023-018-0890-7.
36. Van Vliet D, van Wegberg AMJ, Ahring K, Bik-Multanowski M, Casas K, Didycz B, Djordjevic M et al. Untreated PKU Patients without Intellectual Disability: What Do They Teach Us? *Nutrients* 2019;11:2572. doi: 10.3390/nu11112572.
37. Klaassen K, Djordjevic M, Skakic A, Kecman B, Drmanac R, Pavlovic S, et al. Untreated PKU patients without intellectual disability: SHANK gene family as a candidate modifier. *Mol Genet Metab Rep* 2021; 29:100822. doi: 10.1016/j.ymgmr.2021.100822.

38. Stojanov Lj. Hiperfenilalaninemije. U: Stojanov Lj, Marjanović B, urednici. Nasledne neurometaboličke bolesti kod dece. Beograd: Zavod za udžbenike i nastavna sredstva; 2002.s. 61-82.
39. Weglage J, Oberwittler C, Marquardt T, Schellscheidt J, von Teefelen-Heithoff A, Koch G, et al. Neurological deterioration in adult phenylketonuria. *J Inherit Metab Dis* 2000; 23:83-4. doi: 10.1023/a:1005607115309.
40. Guida M, Pesaresi I, Fabbri S, Sartucci F, Cosottini M, Sean Giorgi F. Epilepsy and phenylketonuria: a case description and EEG-fMRI findings. *Funct Neurol*. 2014 ;29:75–79
41. Pietz J, Dunckelmann R, Rupp A, Rating D, Meinck HM, Schmidt H, Bremer HJ. Neurological outcome in adult patients with early-treated phenylketonuria. *Eur J Pediatr* 1998; 157:824-30. doi: 10.1007/s004310050945.
42. Pérez-Dueñas B, Valls-Solé J, Fernández-Alvarez E, Conill J, Vilaseca MA, Artuch R, Campistol J. Characterization of tremor in phenylketonuric patients. *J Neurol* 2005; 252:1328-34. doi: 10.1007/s00415-005-0860-6.
43. Modan-Moses D, Vered I, Schwartz G, Anikster Y, Abraham S, Segev R, Efrati O. Peak bone mass in patients with phenylketonuria. *J Inherit Metab Dis* 2007; 30:202-8. doi: 10.1007/s10545-007-0462-9.
44. Robinson M, White FJ, Cleary MA, Wraith E, Lam WK, Walter JH. Increased risk of vitamin B12 deficiency in patients with phenylketonuria on an unrestricted or relaxed diet. *J Pediatr* 2000; 136:545-7. doi: 10.1016/s0022-3476(00)90022-2.
45. GeneCards: The Human Gene Database. PAH Gene (Phenylalanine Hydroxylase) [Internet]. Rehovot (IL): Weizmann Institute of Science; [cited 2026 Mar 10]. Available from: <https://www.genecards.org/cgi-bin/carddisp.pl?gene=PAH>
46. Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, Sutton GG, et al. The sequence of the human genome. *Science* 2001; 291:1304-51. doi: 10.1126/science.1058040.
47. Jalali H, Zamanfar D, Amirzadegan, Ghadami MF, Mahdavi M, Mahdavi MR. Mutation Analysis of PAH Gene in Phenylketonuria Patients from the North of Iran: Identification of Three Novel Pathogenic Variants. *Int J Prev Med* 2024;15:22. doi: 10.4103/ijpvm.ijpvm_50_23.eCollection 2024.
48. Scriver CR. The PAH Gene, Phenylketonuria and a Paradigm Shift. *Hum Mutat* 2007; 28:831-45. doi: 10.1002/humu.20526.
49. Kozak L, Hrabincova E, Kintir J, Horky O, Zapletalova P, Blahakova I, et al. Identification and characterization of large deletions in the phenylalanine hydroxylase (PAH) gene by MLPA: evidence for both homologous and non-homologous mechanisms of rearrangement. *Mol Genet Metab* 2006; 89:300-9. doi: 10.1016/j.ymgme.2006.06.007.
50. Stojiljkovic M, Jovanovic J, Djordjevic M, Grkovic S, Cvorkov Drazic M, Petrucev B, et al. Molecular and phenotypic characteristics of patients with phenylketonuria in Serbia and Montenegro. *Clin Genet*. 2006; 70:151-5. doi: 10.1111/j.1399-0004.2006.00650.x.
51. Djordjevic M, Klaassen K, Sarajlija A, Tosic N, Zukic B, Kecman B, et al. Molecular Genetics and Genotype-Based Estimation of BH4-Responsiveness in Serbian PKU Patients: Spotlight on Phenotypic Implications of p.L48S. *JIMD Rep* 2013; 9:49-58. doi: 10.1007/97894_012_178.
52. Klaassen K, Stankovic B, Kotur N, Djordjevic M, Zukic B, Nikcevic G, et al. New PAH gene promoter KLF1 and 3'-region C/EBPalpha motifs influence transcription in vitro. *J Appl Genet* 2017; 58(1):79-85. doi: 10.1007/s13353-016-0359-0.
53. Klaassen K, Djordjevic M, Skakic A, Desviat LR, Pavlovic S, Perez B, et al. Functional Characterization of Novel Phenylalanine Hydroxylase p.Gln226Lys Mutation Revealed Its Non-responsiveness to Tetrahydrobiopterin Treatment in Hepatoma Cellular Model. *Biochem Genet*. 2018; 56:533-541. doi: 10.1007/s10528-018-9858-5.
54. Klaassen K, Kecman B, Stankovic S, Komazec J, Pavlovic S, Stojiljkovic M, et al. Genomic profiling, implications for genotype-based treatment of 131 patients with phenylketonuria and characterization of novel p.Pro416Leu PAH variant. *Sci Rep* 2025;15:19745. doi: 10.1038/s41598-025-04611-2.
55. Zschocke J. Dominant versus recessive molecular mechanisms in metabolic disease. *J Inherit Metab Dis* 2008; 31:599-618. doi: 10.1007/s10545-008-1016-5.
56. Wettstein S, Underhaug J, Perez B, Marsden BD, Yue WW, Martinez A, et al. Linking genotypes database with locus-specific database and genotype-phenotype correlation in phenylketonuria. *Eur J Hum Genet* 2014; 23:302–309. doi: 10.1038/ejhg.2014.114.
57. Guldberg P, Rey F, Zschocke J, Romano V, Francois B, Michiels L et al. A European multicenter study of phenylalanine hydroxylase deficiency: classification of 105 mutations and a general system for genotype-based prediction of metabolic phenotype. *Am J Hum Genet* 1998; 63:71-9. doi: 10.1086/301920.
58. Garbade S, Shen N, Himmelreich N, Haas D, Trefz FK, Hoffmann GF et al. Allelic phenotype values: a model for genotype-based phenotype prediction in phenylketonuria. *Genet Med* 2019;21:580-590. doi: 10.1038/s41436-018-0081-x.
59. Elhawary NA, AlJahdali IA, Abumansour IS, Elhawary EN, Gaboon N, Dandini M, et al. Genetic etiology and clinical challenges of phenylketonuria. *Mol Genet Genomic Med* 2019;7:e610.
60. Kayaalp E, Treacy E, Waters PJ, Byck S, Nowacki P, Scriver CR. Human phenylalanine hydroxylase mutations and hyperphenylalaninemia phenotypes: a metanalysis of genotype-phenotype correlations. *Am J Hum Genet* 1997; 61:1309-17. doi: 10.1086/301638.
61. Blau N, Hennermann J B, Langenbeck U, Lichter-Konecki U. Diagnosis, classification, and genetics of phenylketonuria and tetrahydrobiopterin (BH4) deficiencies. *Mol Genet Metab* 2011; 104:S2-S9. doi: 10.1016/j.ymgme.2011.08.017.
62. Loeber JG, Platis D, Zetterström RH , Almashanu S, Boemer F, Bonham JB et al. Neonatal Screening in Europe Revisited: An ISNS Perspective on the Current State and Developments Since 2010. *Int J Neonatal Screen* 2021;7:15. doi: 10.3390/ijns7010015.
63. Groselj U, Tansek MZ, Smon A, Angelkova N, Anton D, Baric I, et al. Newborn screening in southeastern Europe. *Mol Genet Metab* 2014;113:42-5. doi: 10.1016/j.ymgme.2014.07.020.
64. Zerjav Tansek M, Groselj U, Angelkova N, Anton D, Baric I, Djordjevic M et al. Phenylketonuria screening and management in southeastern Europe - survey results from 11 countries. *Orphanet J Rare Dis* 2015; 10:68. doi: 10.1186/s13023-015-0283-0.
65. Couce ML, Bóveda MD, Castiñeiras DE, Vázquez-Mosquera ME, Barbosa-Gouveia S, De Castro MJ et al. A newborn Screening Programme for Inborn errors of metabolism in Galicia: 22 years of evaluation and follow-up. *Orphanet J Rare Dis* 2024; 19:202. doi: 10.1186/s13023-024-03204-y.
66. Kanungo S, Patel DR, Neelakantan M, Ryali B. Newborn screening and changing face. of inborn errors of metabolism in the United States. *Ann Transl Med* 2018; 6(24):468. doi: 10.21037/atm.2018.11.68.
67. Koracin V, Mlinaric M, Baric I, Brincat I, Djordjevic M, Drole Torkar A et al. Current Status of Newborn Screening in Southeastern Europe. *Front Pediatr* 2021; 9:648939. doi: 10.3389/fped.2021.648939.
68. Schulze A, Lindner M, Kohlmüller D, Olgemöller K, Mayatepek E, Hoffmann GF. Expanded newborn screening for inborn errors of metabolism by electrospray ionization-tandem mass spectrometry: results, outcome, and implications. *Pediatrics* 2003; 111:1399-406. doi: 10.1542/peds.111.6.1399.
69. Sara S Ali I, Pankaj B Agrawal. Role of next generation sequencing in neonatal diagnosis and screening. *J Neuromuscul Dis* 2025;22143602251394481. doi: 10.1177/22143602251394481.
70. Reimers R, Bailey M, Brown C, Chan K, Defay T, Finkel T et al. Clinical utility and cost-effectiveness of Begin NGS newborn screening by genome sequencing and standard newborn screening for severe childhood genetic diseases: an adaptive, international and comparative clinical trial. *BMJ Open* 2025;15:e098609. doi: 10.1136/bmjopen-2024-098609.
71. Carli D, Quarello P, Porta F, Cagnazzo C, Zucchetti G, Proto CF, Gianasso R et al. Genomic Sequencing Approach to Newborn Mass Screening and Its Opportunities. *JAMA Network Open*. 2025; 8:e2538198. doi: 10.1001/jamanetworkopen.2025.38198.
72. Stojiljkovic M, Klaassen K, Djordjevic M, Sarajlija A, Kecman B, Ugrin M et al. Tetrahydrobiopterin deficiency among Serbian pa-

- tients presenting with hyperphenylalaninemia. *J Pediatr Endocrinol Metab* 2015; 28:477-80. doi: 10.1515/jpem-2014-0297.
73. Fino E, Barbato A, Scaturro GM, Procopio E, Balestrini S. DNA-JC12 deficiency: Mild hyperphenylalaninemia and neurological impairment in two siblings. *Mol Genet Metab Rep* 2023;37:10100. doi: 10.1016/j.ymgmr.2023.101008.
 74. Mac Donald A, Depondt E, Evans S, Daly A, Hendriksz C, Chakrapani A et al. Breast feeding in IMD. *J Inherit Metab Dis* 2006; 29:299-303. doi: 10.1007/s10545-006-0332-x.
 75. Acosta PB, Matalon KM. Nutrition management of patients with inherited disorders of aromatic amino acid metabolism. In: Acosta PB, editor. *Nutrition Management of Patients with Inherited Metabolic Disorders*. Boston: Jones and Bartlett Publishers; 2010. 119-74.
 76. Azen CG, Koch R, Friedman EG, Berlow S, Coldwell J, Krause W, et al. Intellectual development in 12-year-old children treated for phenylketonuria. *Am J Dis Child* 1991; 145:35-9. doi: 10.1001/arch-pedi.1991.02160010037012.
 77. Dangin M, Boirie Y, Garcia-Rodenas C, Gachon P, Fauquant J, Callier P, et al. The digestion rate of protein is an independent regulating factor of postprandial protein retention. *Am J Physiol Endocrinol Metab* 2001; 280:340-8. doi: 10.1152/ajpendo.2001.280.2.E340
 78. Burlina A, Blau N. Effect of BH(4) supplementation on phenylalanine tolerance. *J Inherit Metab Dis* 2009;32:40-5. doi: 10.1007/s10545-008-0947-1
 79. Blau N, Belanger-Quintana A, Demirkol M, Feillet F, Giovannini M, MacDonald A et al. Optimizing the use of sapropterin (BH4) in the management of phenylketonuria. *Mol Genet Metab* 2009;96:158-63. doi: 10.1016/j.ymgme.2009.01.002
 80. Pey AL, Perez B, Desvitat LR, Martinez MA, Aguado C, Erlandensen H et al. Mechanisms underlying responsiveness to tetrahydrobiopterin in mild phenylketonuria mutations. *Hum Mutat* 2004; 24:388-9. doi: 10.1002/humu.20097.
 81. Aguado C, Pérez B, Ugarte M, Desvitat LR. Analysis of the effect of tetrahydrobiopterin on PAH gene expression in hepatoma cells. *FEBS Letters* 2006; 580:1697-701. doi: 10.1016/j.febslet.2006.02.005
 82. Hyder T, Coppenrath VA. A Comprehensive Review of Pegvaliase, an Enzyme Substitution Therapy for the Treatment of Phenylketonuria. *Drug Target Insights* 2019;13:1177392819857089. doi: 10.1177/1177392819857089
 83. Harding CO, Longo N, Northrup H, Sacharow S, Singh R, Thomas JA et al. Pegvaliase for the treatment of phenylketonuria: Final results of a long-term phase 3 clinical trial program. *Mol Genet Metab Rep* 2018;39:101084. doi: 10.1016/j.ymgmr.2024.101084
 84. Burton BK, Longo N, Vockley J, Grange DK, Harding CO, Decker C, et al. Pegvaliase for the treatment of phenylketonuria: results of the phase 2 dose-finding studies with long-term follow-up. *Mol Genet Metab* 2020;130 (4):239-46. doi: 10.1016/j.ymgme.2020.06.006
 85. Schindeler S, Ghosh-Jerath S, Thompson S, Rocca Joy P, Kemp A et al. The effects of large neutral amino acid supplements in PKU: an MRS and neuropsychological study. *Mol Genet Metab* 2007;91:48-54. doi:10.1016/j.ymgme.2007.02.002
 86. Matalon R, Michals-Matalon K, Bhatia G, Burlina AB, Burlina, AP C Braga APC et al. Double blind placebo control trial of large neutral amino acids in treatment of PKU: effect on blood phenylalanine. *J Inherit Metab Dis* 2007; 30:153-8. DOI: 10.1007/s10545-007-0556-4.
 87. Ney DM, Gleason ST, van Calcar SC, MacLeod EL, Nelson KL, M Etzel et al. Nutritional management of PKU with glycomacropeptide from cheese whey. *J Inherit Metab Dis* 2009; 32:32-9. doi: 10.1007/s10545-008-0952-4.
 88. Muntau AC, Longo N, Ezgu F, Schwartz IVD, Lah M, Bratkovic D et al. Effects of oral sepiapterin on blood Phe concentration in a broad range of patients with phenylketonuria (APHENITY): results of an international, phase 3, randomised, double-blind, placebo-controlled trial. *Lancet* 2024;404:1333-45. doi: 10.1016/S0140-6736(24)01556-3.
 89. Lamb YN. Sepiapterin: First Approval. *Drugs* 2025;86(1):93-9. doi: 10.1007/s40265-025-02247-0
 90. Harding CO. Sepiapterin: a potential new therapy for phenylketonuria. *Lancet* 2024; 404:1284-6. doi: 10.1016/S0140-6736(24)01819-1.

FENILKETONURIJA: ZNAČAJ NEONATALNOG SKRININGA, GENOTIPSKO-FENOTIPSKA KORELACIJA I NOVINE U TERAPIJI

Maja Đorđević Milošević^{1,2}, Božica Kecman¹, Sanja Grković¹, Jelena Martić^{2,3}, Kristel Klaassen⁴, Sara Stanković⁴, Maja Stojiljković⁴

Sažetak

Fenilketonurija (PKU) je najčešća urođena bolest metabolizma (UBM) aminokiselina u populaciji bele rase, sa incidencijom od 1:10.000 do 1:20.000. Nasleđuje se autosomno recesivno i posledica je smanjene aktivnosti enzima fenilalanin-hidroksilaze (PAH), što dovodi do akumulacije fenilalanina i deficita tirozina. Kod nelečenih pacijenata, klinička slika je prvenstveno posledica neurotoksičnosti i obuhvata kašnjenje u razvoju, intelektualnu ometenost i epilepsiju. Do danas je identifikovano više od 1100 patogenih varijanti u PAH genu. Najčešća patogena varijanta u Srbiji je p.Leu48Ser. Brojna istraživanja fokusirana su na razjašnjavanje genotipsko-fenotipske korelacije kod bolesnika sa PKU. Rano otkrivanje bolesti putem neonatalnog skrininga (NS) zasnovanog na detekciji povišenih koncentracija fenilalanina, i blago-

vremeni početak lečenja dijetom, su preduslov prevencije intelektualne ometenosti i epilepsije. Uvođenjem tandemске masene spektrometrije (MS/MS) u NS u velikom broju zemalja Evrope i sveta, povećava se broj rano otkrivenih i lečenih UBM. Lečenje PKU se i dalje zasniva na dijeti sa restrikcijom fenilalanina, koja je dosta efikasna, ali zahtevna za pacijente i njihove porodice. Nove terapijske metode uključuju primenu kofaktora PAH kao što je tetrahydrobiopterin (BH4, sapropterin), i enzima pegvalijaze, sa ciljem liberalizacije dijetе. Ograničenje lečenjem preparatima BH4 je odsustvo terapijskog odgovora kod pacijenata sa teškim fenotipovima. Kako su pokazale kliničke studije primena BH2 (sepiapterina) u terapiji bolesnika sa PKU pokazani su dobri terapijski rezultati kod bolesnika sa različitim fenotipovima.

Ključne reči: fenilketonurija, neonatalni skrining, genotipsko-fenotipska korelacija, pegvalijaza, sepiapterin

Primljen: 08. 02. 2026. | **Revidiran:** 10. 03. 2026. | **Prihvaćen:** 12. 03. 2026. | **Online First:** 13. 03. 2026.

Medicinska istraživanja 2026