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BIOGENIC AMINES IN PHENYLKETONURIA: A NARRATIVE REVIEW

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ABSTRACT – Phenylketonuria (PKU) is a genetic disorder caused by a deficiency of the liver enzyme phenylalanine hydroxylase, resulting in the accumulation of neurotoxic phenylalanine (Phe) in the brain and body fluids. Although early studies highlighted reduced biogenic amines in untreated PKU patients, later research revealed that even early-treated individuals experience significant neurotransmitter imbalances, particularly affecting serotonin and dopamine. The most consistent findings across clinical studies are reduced levels of serotonin, dopamine, norepinephrine, and their metabolites in both peripheral (urine, plasma, platelets) and central (cerebrospinal fluid) biological samples.

Preclinical studies using PKU mouse models have confirmed persistent abnormalities in brain neurotransmitters, particularly serotonin, alongside elevated Phe levels. Additionally, serotonin precursor supplementation has been shown to improve behavioral alterations and the associated neuropathological background in the affected mouse model.

Two main hypotheses have been proposed to explain these imbalances. The first suggests that neurotransmitter depletion results from reduced brain levels of tryptophan (Trp) and tyrosine (Tyr) due to competition with Phe at the blood-brain barrier (BBB). Although a single, unconfirmed clinical study suggested that large neutral amino acid (LNAA) supplementation might reduce brain Phe uptake, this has not been confirmed in animal studies. Another mechanism suggests that high Phe interferes with Trp and Tyr hydroxylation, impairing neurotransmitter synthesis.

The clinical relevance of these neurotransmitter deficits remains unclear. A direct effect on IQ has not been established. Studies are lacking in early infancy, when brain neurotransmitter levels peak. In adults with early-treated PKU, serotonin metabolite depletion has been associated with specific neuropsychological impairments and brain structure alterations. Importantly, not all patients show reduced neurotransmitter levels, indicating inter-individual variability in susceptibility to Phe-related neurochemical changes.

Keywords: Phenylketonuria, Biogenic amine, Dopamine, Serotonin, Norepinephrine, Outcome.

List of abbreviations: 5-HIAA: 5-hydroxyindoleacetic acid; 5-HT: Serotonin; 5-HTP: 5-Hydroxytryptophan; AADC: Aromatic L-Amino Acid Decarboxylase; AMY: Amygdala; BBB: Blood-brain barrier; BTBR: Black and Tan Brachyury; Cg: Cingulate cortex; CNS: Central nervous system; CP: Caudate-putamen; DA: Dopamine; DAT: Dopamine Transporter; DOPAC: 3-4-Dihydroxyphenylacetic acid; ETPKU: Early-treated PKU; EP: Epinephrine; GM: Gray matter; GMP: Glycomacropeptide; HIP: Hippocampus; HPA: Hyperphenylalaninemia; HVA: Homovanillic acid; LNAA: Large neutral amino acids; MHPG: 3-Methoxy-4-hydroxyphenylglycol; NAc: Nucleus accumbens; NE: Norepinephrine; PAH: Phenylalanine hydroxylase; pFC: prefrontal cortex; PET: Positron emission tomography; Phe: Phenylalanine; PKU: Phenylketonuria; RT: Reaction time; SPECT: Single-photon emission computed tomography; TH: Tyrosine hydroxylase; TPH: Tryptophan hydroxylase; Trp: Tryptophan; Tyr: Tyrosine.

INTRODUCTION

Phenylketonuria (PKU; OMIM #261600) is an inborn error of amino acid metabolism due to a defect of *PAH*, encoding the hepatic enzyme phenylalanine hydroxylase (PAH) (EC 1.14.16.1), which converts phenylalanine (Phe) into tyrosine (Tyr). PAH enzyme belongs to the aromatic amino acid hydroxylase superfamily, which also includes tryptophan hydroxylase (TPH) (EC 1.14.16.4) and tyrosine hydroxylase (TH) (EC 1.14.16.2), limiting enzymes in the synthesis of serotonin (5-HT) and dopamine (DA), respectively (Figure 1). Based on treatment requirements, PAH deficiency is classified as either mild hyperphenylalaninemia (HPA), characterized by phenylalanine (Phe) concentrations of 120-360 µmol/L and typically not requiring treatment, or phenylketonuria (PKU), defined by Phe levels greater than 360 µmol/L and requiring treatment[1,2]. In the absence of treatment, PKU patients experience a severe clinical course, including intellectual impairment, microcephaly, seizures, motor defects, and behavioral abnormalities[1]. To prevent PKU encephalopathy, European guidelines[2] recommend strict metabolic control throughout life, adopting a Phe target of 120 to 360 µmol/L for children under the age of 12 and 120 to 600 µmol/L for adolescents and adults. Nevertheless, due to broad clinical variability, the predictive value of metabolic control (based on blood Phe levels) on the clinical outcome declines during adolescence and adulthood.

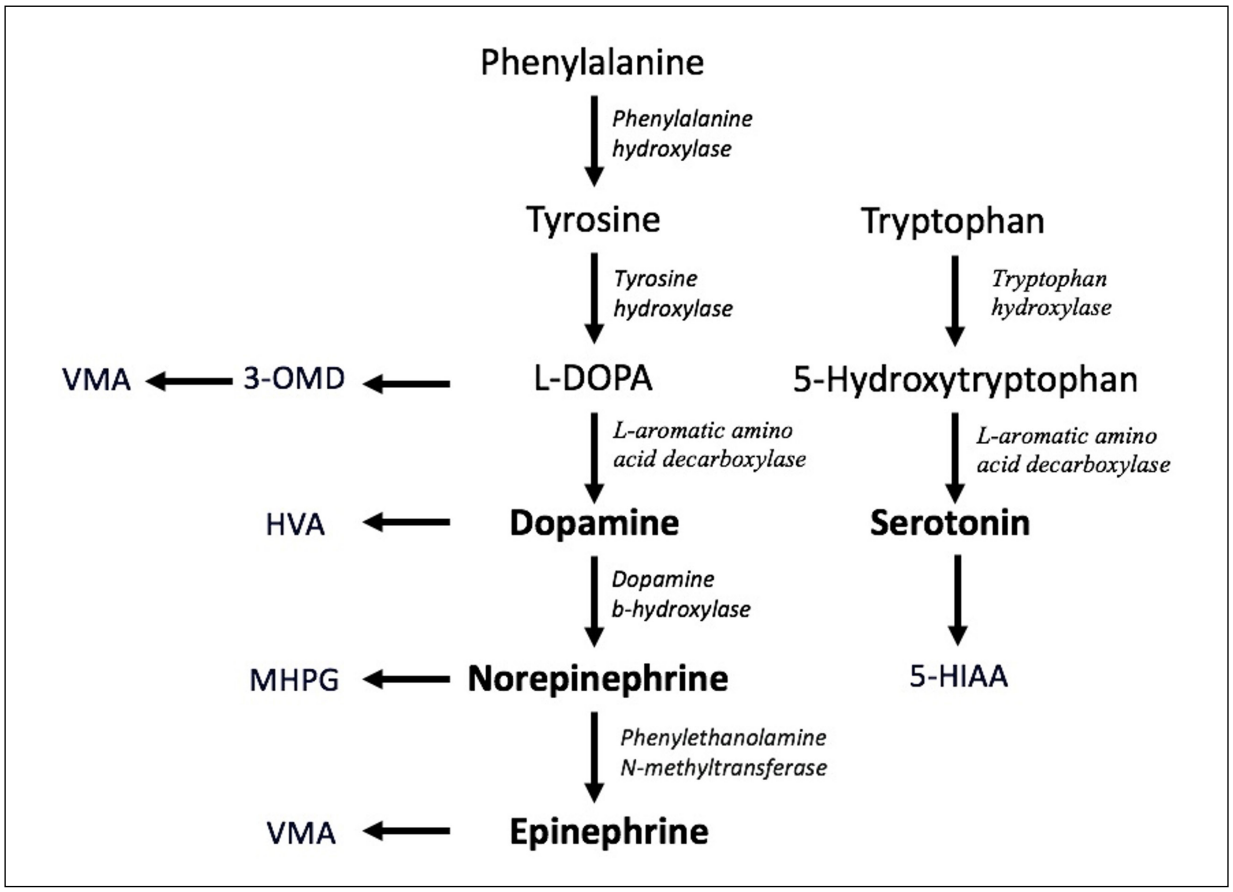

Figure 1. Pathways of serotonin and catecholamine synthesis. 3-OMD: 3-O-Methyldopa; 5-HIAA: 5-hydroxyindoleacetic acid; HVA: Homovanillic acid; MHPG: 3-Methoxy-4-hydroxyphenylglycol; VMA: Vanillylmandelic acid.

Apart from brain Phe elevation, the most consistent biochemical alteration detected in preclinical and clinical PKU brains[3-5] is the depletion of biogenic amines and their metabolites. A direct inhibitory effect of Phe on TPH and tyrosine hydroxylases (TH), the limiting enzymes in these neurotransmitter syntheses, has been suggested[3]. Furthermore, restoration of 5-HT by 5-hydroxytryptophan (5-HTP) supplementation[6,7] or DA and 5-HT levels by lowering blood Phe[8], has been shown to improve the anatomical and functional alterations in a mouse model of PKU.

Indeed, early-onset genetic disorders of 5-HT and DA synthesis cause neurodevelopmental disorders, including intellectual disability and movement disorders[9], disclosing the critical role of biogenic amines for normal brain development and functioning. How far these metabolic alterations, which have also been reported in PKU patients[4,10,11], may influence neurological outcomes in adulthood and elderly subjects remains to be established.

A literature search was conducted *via* PubMed up to the end of March 2025, using keyword combinations such as "phenylketonuria," "biogenic amines," "neurotransmitters," "serotonin," "dopamine," "epinephrine," "norepinephrine," and "outcome." The present narrative review aims to (a) compile data on neurotransmitter variations in untreated and early-treated patients with PKU (ETPKU) and (b) explore the pathophysiological mechanisms underlying alterations of brain amines in PKU, as well as their potential clinical and therapeutic implications.

BIOGENIC AMINES IMPAIRMENT IN PKU

Biological Fluids

Approximately 20 years after the discovery of the disease, abnormal indole metabolites were observed in PKU patients, demonstrating the presence of indolelactic acid and indoleacetic acid in the urine and reduced urinary excretion of 5-hydroxyindoleacetic acid (5-HIAA)[12]. Subsequently, Pare et al[13-15] found that blood 5-HT and its major urinary catabolite 5-HIAA, were in subnormal concentrations in untreated PKU patients, even when compared to other patients with a similar neurological phenotype. The same authors, analyzing serum and urine samples in seven PKU subjects before and during a low Phe diet, found a significant increase in the average serum 5-HT levels after the treatment. During the same period, subnormal blood concentrations and urinary excretion of epinephrine (EP) and norepinephrine (NE) and significantly reduced urinary excretion of DA were reported in untreated PKU patients[16,17].

Many researchers have since focused on biogenic amines (5-HT, DA, EP, NE) or their metabolites, such as 5-HIAA (5-HT metabolite), homovanillic acid (HVA, DA metabolite), and 3-methoxy-4-hydroxyphenylglycol (MHPG, NE metabolite) in both untreated and early-treated PKU patients. Most of these studies have confirmed depletion of one or more neurotransmitters and their metabolites in various biological fluids from PKU patients: urine[18,19], plasma[20,21], platelets[22,23], and cerebrospinal fluid (CSF)[4,11,24-29] (results summarized in Supplementary Table 1). In PKU patients, 5-HT and its metabolites seem more affected than DA or NE[11,26]. It has been shown that in patients with classic PKU, DA formation is inhibited by blood Phe concentrations above 1,500 µmol/L, while 5-HT formation is inhibited by blood Phe concentrations of 480 µmol/L[18]. Additionally, several studies[10,27,28] have reported low HVA and 5-HIAA levels in the CSF of PKU patients following the discontinuation of the Phe-restricted diet, with the 5-HT metabolite being affected to a greater extent.

By contrast, Krause et al[19], in a triple-blind crossover study, found an inverse relationship between plasma Phe levels and urinary DA excretion in late-treated PKU patients, but no direct correlation between Phe levels and 5-HT excretion. However, they explained this "atypical" result by considering that 5-HT is stored in many tissues and that its urinary excretion not only reflects biosynthesis in the central nervous system (CNS) but also peripheral metabolism.

Neurotransmitter abnormalities observed in PKU patients appear to be inversely correlated with Phe concentrations and may be reversible by reducing Phe levels[11,14,17-20,23-28]. However, Giovannini et al[22] (1988) demonstrated a decreased platelet concentration of 5-HT and NE not only in PKU subjects on unrestricted diet but also in patients with good metabolic control. Since platelet uptake of 5-HT was found to be normal in both PKU and HPA patients, the reduction in platelet 5-HT and NE was believed to be secondary to an impairment in their peripheral biosynthesis, suggesting that the negative effect of Phe occurs even with a slight increase.

Similar results were subsequently obtained by Ormazabal et al[23], who demonstrated a decrease in platelet 5-HT levels in PKU patients under a Phe-restricted diet and acceptable metabolic control, and by Burlina et al[4], who found low CSF 5-HIAA and HVA levels in a group of 14 ETPKU patients (9-20 years) with Phe levels in accordance with the German age-group recommendations[29] (40-240 µmol/L for patients <10 years, <900 µmol/L for patients 10-15 years, and <1,200 µmol/L for patients >15 years).

Although not associated with overt clinical consequences, the decrease in neurotransmitter levels in subjects with optimal metabolic control laid the groundwork for researching safer therapeutic targets for Phe levels and the possible long-term impacts of these alterations.

Brain

Only one study, conducted by McKean[24], assessed the impact of high Phe concentrations on the levels of 5-HT, catecholamines, and their amino acid precursors, tryptophan (Trp) and tyrosine (Tyr), in autopsied human PKU brain tissues. The results from four untreated PKU patients were compared with those from four non-PKU patients with intellectual disabilities. The PKU patients and the control group were similar in age and estimated post-mortem interval (3-6 hours between death and brain removal). Decreased concentrations of 5-HT, DA, and NE (30-40% of normal values), along with their amino acid precursors (40-50% of normal values), were found in the autopsied brains of untreated PKU patients: the most significant reduction in all three amine concentrations was observed in the caudate nuclei of PKU patients, displaying a 60-70% decrease; in the brainstem of these patients, concentrations of 5-HT and NE were similarly reduced, while DA levels remained stable; a similar pattern was noted in the occipital cortex, albeit with greater variability.

With a fivefold increase in Phe levels, cortical Trp and Tyr concentrations in the tissues of PKU patients were 50-60% lower compared to those of controls. In the CSF of PKU patients, however, Trp and Tyr levels were increased by 100% and 20%, respectively, compared to controls. The authors hypothesized that these results could be explained by either an increased outflow rate of amino acids into the extraneuronal CSF or a decreased inflow rate from the CSF compartment. However, these findings have never been replicated in human tissues, hindering further clarification of these hypotheses.

Pah^{enu2} Mouse

The development of the *Pah*^{enu2} (ENU2) genetic mouse model for PKU, created using two distinct background - C57BL/6 (B6) and Black and Tan Brachyury (BTBR) strains - has significantly enhanced the understanding of the mechanisms involved in PKU pathophysiology (results summarized in Supplementary Table 2). The *Pah*^{enu2} mouse model closely replicates the genetic, biochemical, and neurobiological characteristics of human PKU[30,31].

Puglisi-Allegra et al[3] found that the BTBR-*Pah*^{enu2} mouse brain exhibited a significant decrease in neurotransmitters and their metabolites across various brain regions. Specifically, levels of 5-HT and its metabolite 5-HIAA, as well as the NE metabolite MHPG, were markedly reduced in all examined brain areas [prefrontal cortex (pFC); nucleus accumbens (NAc); caudate putamen (CP); cingulate cortex (Cg); amygdala (AMY); hippocampus (HIP)]. Whereas NE levels were lower in most regions (pFC, Cg, AMY, and HIP), as well as DA and its metabolites (HVA and DOPAC) (pFC, AMY, NAc, and CP).

Pascucci et al[32] aimed to evaluate the availability of biogenic amines in the brain at various postnatal ages. They studied developing BTBR-*Pah*^{enu2} mice in comparison to BTBR-wild type (BTBR-WT) mice, collecting brain tissue at postnatal days P03, P05, P07, P14, P21, and P35. Their results revealed age-dependent deficits in brain amine availability between postnatal days 14 and 35. During this period, healthy mice exhibited developmental peak increases in amines (5-HT, DA, and NE) and their precursors [5-HTP and Levo-3,4-dihydroxyphenylalanine (L-DOPA)]. The most significant deficits were observed in 5-HT, with ENU2 mice showing a 50% reduction in 5-HT levels compared to BTBR-WT mice at all examined ages. NE levels decreased by 40% between postnatal days 14 and 21, while DA levels declined by 30% between days 7 and 35. Overall, BTBR-*Pah*^{enu2} mice had lower levels of L-DOPA and 5-HTP than BTBR-WT mice at nearly all time points, except for 5-HTP at P03, P05, and P21. These findings suggest a critical period around the third week of postnatal development, during which the brain is particularly vulnerable to disruptions in maturation.

Later, Pascucci et al[6,33] tested the hypothesis that Phe might interfere with aminergic neurotransmission in the adult BTBR-*Pah*^{enu2} pFC. Their study revealed that while restraint stress in BTBR-WT mice led to an increase in amine release in the pFC, this response was absent in PKU mice. The basal release of 5-HT and DA remained unchanged in BTBR-*Pah*^{enu2} mice, while the basal release of NE was reduced. Upon exposure to restraint stress, neither the serotonergic response nor DA outflow was observed. This indicated deficits in aminergic transmission in the pFC of PKU mice and suggested that compensatory mechanisms might help maintain basic physiological functions. Interestingly, the NE response to stress remained intact, though it was rapidly exhausted, suggesting a dynamic relationship between NE and DA in the pFC. During basal conditions, the availability of DA for NE synthesis appears to be reduced, but during stress, NE synthesis is favored. When ENU2 mice were exposed to a Phe-free diet without amino acid supplementation, their serotonergic response recovered, although DA transmission remained unaffected by Phe reduction, likely due to the absence of tyrosine supplementation in the diet.

Pascucci et al[5] further examined the behavioral and neurochemical profiles of several BTBR-Pah^{enu2}-based mouse models of HPA: c-PKU (classical PKU), m-PKU (mild PKU), and m-HPA (mild HPA). Their neurochemical analysis revealed that in c-PKU mice, deficits were observed in all amines and brain regions, with 5-HT being the most affected and DA the least. The pFC and AMY appeared to be the most vulnerable areas. Phe-induced impairment of the brain aminergic system was more severe in c-PKU compared to m-HPA, with m-PKU mice exhibiting a less severe deficit. m-HPA mice had only limited deficits in 5-HT, NE, and their metabolites in the pFC, AMY, and Cg. The study also noted that increasing Phe levels in the blood were associated with more severe behavioral symptoms.

Sawin et al[34] compared C57BL/6-*Pah^{enu2}* mice with C57BL/6-WT mice and found a 10% reduction in absolute brain mass, as well as a defective concentration of catecholamines (NE, EP, DOPAC, DA, HVA) and 5-HT. Behavioral assessments revealed that PKU mice exhibited reduced digging behavior, hyperactivity, and impaired vertical activity and episode counts. Mice treated with a low-Phe diet supplemented with an amino acid formula or low-Phe glycomacropeptide (GMP) diets showed improvements in brain mass, neurotransmitter levels, and behavioral symptoms compared to those on a high-Phe casein diet. However, anxiety behaviors did not differ between C57BL/6-*Pah^{enu2}* and C57BL/6-WT mice. A sex-based difference was also observed in response to HPA. Female mice exhibited greater ambulatory and horizontal activity than male mice, regardless of genotype or diet. This difference may be explained by variations in neurotransmitter concentrations and the effects of estrogen, which influences catecholamine and 5-HT levels in specific brain regions. Additionally, WT female mice on a casein diet had higher brain 5-HT concentrations than male mice on the same diet. However, female PKU mice displayed a more severe response to a high-Phe diet compared to male mice, as evidenced by reduced vertical activity in the open field test. Interestingly, the GMP diet led to a greater increase in relative brain mass in female PKU mice compared to male PKU mice.

Winn et al[8] also described behavioral changes and cognitive disorders related to CNS 5-HT and DA deficiencies in *Pah^{enu2}* mice on a C57BL/6 background. These authors reported altered exploratory activity in the open field and fear conditioning, as well as deficits in nesting and learning behaviors in the water maze. As noted previously, treatment with a Phe-reduced diet or liver-directed gene therapy initiated during adulthood improved brain amine content and nesting behavior but had no effect on severe cognitive deficits. Despite improvements in neurotransmitters, the reduced protein abundance and activity of TH and TPH in the brains of PKU mice, compared to WT mice, were not influenced by treatment. This suggests that Phe reduction treatments in adulthood do not fully correct the PKU neuropathology or the cognitive deficits that develop during early development.

Bruinenberg et al[35] found reduced levels of NE and 5-HT, but not DA, in whole brain homogenates of BTBR-*Pah^{enu2}* mice. Moreover, 5-HT turnover was decreased in PKU mice compared to WT controls. Long-term low-Phe dietary treatment normalized neurotransmitter levels but did not affect 5-HT turnover. A combination of specific nutrients designed to improve brain function had a positive effect on memory in the novel object recognition test, but did not influence neurotransmitter levels.

Similarly, Dijkstra et al[36] found that in 114 *Pah^{enu2}* mice (both BTBR and B6), plasma (and brain) Phe concentrations were strongly correlated with brain 5-HT and NE levels, but only weakly correlated with DA levels. The stronger relationship between plasma Phe and brain Phe, compared to plasma Phe and neurotransmitters, suggests that neurotransmitter depletion is not solely due to Phe toxicity but may also result from Phe's inhibition of neurotransmitter precursor influx across the blood-brain barrier (BBB) or from direct inhibition of TH and TPH activities.

Bregalda et al[37] confirm a persistent impairment of monoaminergic neurotransmitters throughout the lifespan of untreated BTBR-*Pah^{enu2}* mice, with significant reductions in 5-HT, 5-HTP, 5-HIAA, and 3-OMD, particularly between postnatal day (PND) 14 and 60. Notably, while 5-HT levels showed a significant reduction only from PND 60 onward, its precursor 5-HTP was consistently lower across all time points, suggesting an early and sustained disruption in 5-HT biosynthesis. Likewise, 5-HT and L-DOPA catabolites (5-HIAA and 3-OMD, respectively) were significantly reduced at PND 14 and 60 but later converged with control values, indicating a transient, yet functionally relevant, metabolic imbalance. Unlike previous studies[34,36], brain amino acid concentrations were not significantly altered in ENU2 mice. These findings reinforce the hypothesis that PKU-related neurotransmitter deficits originate upstream of the final enzymatic step catalyzed by AADC, likely involving impaired TH and TPH activity. As previously reported[38], this study confirms a reduction in myelin basic protein (MBP) during postnatal development, followed by a spontaneous restoration of myelin synthesis and function, while brain neurotransmitter depletion remains a permanent biochemical consequence of PKU. Despite the recovery of myelin integrity and conduction velocity after PND 60, locomotor defi-

cits persisted, reinforcing the hypothesis that neurotransmitter imbalances play a central role in the long-term neurological consequences of PKU. Finally, brain amino acids play a minor role, if any, in the pathogenesis of the disease in this preclinical model, suggesting the need to reconsider their actual impact on PKU cerebral outcomes.

MECHANISMS OF NEUROTRANSMITTERS ALTERATION

Since PAH belongs to the DA synthesis pathway, the effect of Phe levels on DA synthesis seems straightforward to understand. However, it is less clear how the excess of Phe affects the 5-HT synthesis pathway.

Currently, two main hypotheses have been proposed to explain the effect of elevated blood Phe levels on biogenic amine synthesis (results summarized in Supplementary Table 3).

The first hypothesis, known as the BBB hypothesis, is based on the finding that large neutral amino acids (LNAAs) – valine, isoleucine, leucine, methionine, threonine, tryptophan, tyrosine, histidine, and phenylalanine – share the same transporter, LAT1 (*SLC7A5*), and therefore compete for transport across the BBB[39-41]. Phenylalanine binds to the LAT1 transporter more strongly than other LNAAs, as it has the lowest Km value[42]. Thus, high plasma Phe concentrations could compromise the cerebral absorption of other LNAAs, limiting the transport of Tyr and Trp and consequently reducing their availability for neurotransmitter synthesis[40,43-49].

Using positron emission tomography (PET) and adopting L-[1-^{11}C]-Tyr as a tracer, Paans et al[50] detected a reduced protein synthesis rate (PSR) in the brains of PKU patients with plasma Phe concentrations greater than 700 µmol/L, compared to healthy controls and patients with lower blood Phe levels. This finding was interpreted as resulting from decreased Tyr availability for neurotransmitter synthesis, leading to reduced intracerebral catecholamine concentrations. However, the study does not prove that elevated plasma Phe concentrations limit Tyr transport across the BBB.

Krause et al[19] employed a triple-blind, multiple-trial, crossover protocol to quantify renal tubular transport of amino acids in eight patients with PKU. Each patient was tested at the conclusion of three one-week periods of high or low Phe intake. The biochemical tests included plasma amino acid analysis along with measurements of urinary amino acids, DA, and 5-HT. They found no evidence that Phe significantly affects the absorption of Tyr and Trp in the proximal renal tubule, despite a decreased urinary excretion of DA and 5-HT. However, as noted by the authors, the Km values for Phe, Tyr, and Trp transport differ between the brain and kidneys. Furthermore, while the BBB is saturated at normal plasma amino acid concentrations, the renal tubular epithelium is not.

Contrary to the BBB hypothesis, data from several studies suggest that: (1) CSF concentrations of Tyr and Trp do not consistently decrease after cessation of dietary treatment[10,27,28,51] and (2) the CSF and plasma Phe:Tyr ratio remains virtually identical in control and PKU patients[52]. However, cerebrospinal fluid concentrations of Tyr and Trp may not necessarily reflect their actual intracerebral levels in PKU. Experimental evidence for this comes from studies using membrane vesicles from rat brain synaptosomes[53,54]. Transport studies[39] across the BBB have produced similar kinetic results in different species, suggesting that carrier-mediated transport follows a common pattern among mammals. It has been found that L-Phe inhibits the transport of aromatic amino acids, such as Tyr and Trp, at concentrations comparable to those observed in PKU patients[53,54]. This finding aligns with earlier research by McKean[24], which found reduced Tyr content in the post-mortem cerebral cortex of PKU patients.

The second hypothesis (not necessarily in alternative to the first), known as the hydroxylation hypothesis, suggests that high cerebral Phe levels compete with Tyr and Trp for hydroxylation by TH[18,55,56] and TPH[57-59]. This theory is in line with the indication that all three enzymes (PAH, TH and TPH) have evolved from a common ancestral hydroxylase, sharing several similarities in structure and function[60].

To investigate the neurochemical mechanism of HPA-induced 5-HT depletion, several *in vivo* studies[6,32,61] were conducted using *Pah^{enu2}* mouse genetic model. The results showed a significant reduction in brain 5-HTP levels, whereas the decrease in brain Trp, dependent on BBB transport, was relatively limited. These findings support the hypothesis that Phe inhibits TPH, reducing the conversion of Trp to 5-HTP, and thus causing 5-HT deficiencies in HPA.

According to this hypothesis, administering 5-HTP to ENU2 mice restored 5-HT levels and improved specific cognitive functions, as evaluated through the object recognition and spatial novelty tests. It also enhanced the morphological maturation of pyramidal neuron dendritic spines in the pFC. However, the persistence of locomotor deficits suggests they are primarily linked to the dopaminergic pathway[7].

Phenylalanine was also demonstrated to be able to inhibit the activity of human TPH (and less consistently human TH) expressed in *E. coli*[62].

Further research has explored additional mechanisms of Phe toxicity, particularly its impact on DA. A PET imaging study[63] of 6-[^{18}F]Fluoro-L-dopamine (FDOPA) in seven young adults with PKU and seven age-matched controls revealed: (1) reduced FDOPA uptake across the BBB, likely due to competitive inhibition of the LNAA transporter by Phe; and (2) a 41% decrease in striatal FDOPA utilization, suggesting reduced DOPA decarboxylase activity. However, plasma Phe concentration, Phe/Tyr ratio, and cumulative LNAA levels did not significantly correlate with striatal AADC activity.

Similar to what was made for 5-HT, changes in L-DOPA levels in the developing brain of a PKU mouse model closely corresponded to changes in DA levels, supporting the hypothesis that Phe-mediated inhibition of TH activity is a probable mechanism for reduced DA synthesis[32]. However, the debate on the inhibition of TH by high brain Phe concentrations continues, as Phe appears to have a lower affinity for TH than for TPH, as previously reported[62,64].

As a hypothetical treatment approach, Harding et al[65] tested the pharmacological inhibition of Tyr degradation in PKU mice through the oral administration of Nitisinone (NTBC), a drug used for the treatment of human tyrosinemia type 1. NTBC treatment led to sustained increases in blood and brain Tyr, a decrease in blood and brain Phe, and, consequently, a 36% increase in brain DA content. There was also a similar increase in markers of DA turnover, suggesting Phe-mediated TH inhibition.

The expression of TH in dopaminergic neurons was studied in two primary dopaminergic pathways: the nigrostriatal pathway, which controls motor function, and the tuberoinfundibular pathway, which regulates prolactin secretion from the pituitary gland. Chronically elevated Phe levels in ENU2 mice resulted in a dramatic and early-onset downregulation of TH expression in both pathways. Treatment with pegylated recombinant phenylalanine ammonia lyase (pegvaliase), which significantly reduced plasma Phe levels, led to a notable increase in TH-positive neurons compared to placebo-treated ENU2 mice, although not to WT levels[66].

Finally, Szigetvari et al[67] exposed a DA-producing cell line (PC12) to varying concentrations of Phe in culture media and observed that increasing extracellular Phe levels depleted cellular Tyr and DA (up to 61.8%). In the same PC12 cell line, exposure to both low and high concentrations of Tyr reduced cellular DA, TH protein levels, and their phosphorylation status. Proteomic analysis identified multiple altered proteins, biological processes, and pathways in response to changes in extracellular Tyr, including dysregulation of amino acid transporters and widespread modifications in intracellular amino acid and monoamine metabolism.

CLINICAL CONSEQUENCES OF NEUROTRANSMITTER ALTERATIONS

Biogenic amines, besides their role as neurotransmitters, are crucial for brain development, synapse formation and maintenance, and the refinement of synaptic connectivity during postnatal development[68,69].

This suggests that deficiencies in biogenic amines during critical periods of neurodevelopment may explain the brain damage and progressive neuropsychiatric impairment observed in adult PKU patients. Dysfunction in the dopaminergic and serotonergic neuronal systems, especially in the pFC, seems to play a key role in the emotional symptoms and executive function impairments associated with poorly treated PKU[42,70,71]. Additionally, the movement disorders seen in untreated PKU patients (e.g., chorea, tremors, and dystonia) are likely due to DA deficiency in the basal ganglia[72].

To understand the potential pathogenic role of neurotransmitter disruption in PKU, some authors have attempted to correlate PKU patients' cognitive performance with levels of dopaminergic and/or serotonergic metabolites, but the results have been inconclusive (results summarized in Supplementary Table 4).

The hypothesis that IQ reduction might be linked to 5-HT deficiency was not confirmed by two earlier studies conducted by Pare et al[13,15]. The authors demonstrated that, although PKU patients exhibited significantly lower serum 5-HT levels and reduced urinary 5-HIAA concentrations compared to non-PKU patients with a similar phenotype, these values were not correlated with IQ. Subsequently, Giovannini et al[22] found normal psychomotor development and an absence of neurological signs in PKU subjects with normal Phe levels but diminished peripheral 5-HT and NE synthesis.

Lou et al[10] assessed reaction time (RT) in four adolescent or young adult PKU patients (ages 16-23) under varying Phe levels, both before and after the discontinuation of dietary treatment. Performance

results were correlated with biogenic amine metabolites in the CSF. In three out of four patients, stopping the Phe-restricted diet resulted in approximately a twofold increase in plasma and CSF Phe levels, which was associated with a significant decrease in CSF HVA and, more consistently, in CSF 5-HIAA. These patients also exhibited greater variability in RT. However, in one patient with a mild form of PKU, diet discontinuation did not significantly impact plasma AA levels, CSF HVA and 5-HIAA levels, or RT variability. In all patients, RT and CSF 5-HIAA levels followed a linear relationship, with median RT tending to increase as CSF HVA and CSF 5-HIAA concentrations decreased.

The same authors[28,73] replicated this study in seven ETPKU patients. The results confirmed variability in individual sensitivity to Phe. In one group (n=4), RT performance was linked to Phe levels and CSF biogenic amine concentrations (5-HIAA and HVA), with performance worsening following the discontinuation of the diet. In another subgroup (n=3), no differences were observed in either performance or biogenic amine concentrations in the CSF, despite changes in Phe levels. Based on these findings, the authors proposed that adult PKU patients can be divided into two groups: those vulnerable to high Phe levels and those who are not.

In a subsequent study[74], 6/7 patients who had abnormally long reaction times while on a free diet showed improved vigilance after switching to a Phe-poor diet. Tyrosine supplementation (160 mg/kg/day) in free-diet patients increased the CSF HVA/5-HIAA ratio and enhanced vigilance, particularly in those with RT values longer than the normal average.

The possible consequences of DA deficiency were evaluated by Diamond[75]. Contrast sensitivity, which depends on retinal dopaminergic neurons, was studied in 47 children aged 5.4 to 9.8 years: 12 with PKU, 6 unaffected siblings, and 29 healthy controls, all with binocular acuity of 20/20 (with correction if needed). The authors found that children with ETPKU exhibited significantly lower visual contrast sensitivity than both age-matched controls and their siblings across the entire spatial frequency range of 1.5 to 18.0 cycles per degree of visual angle. Contrast sensitivity was not significantly related to IQ. During the first month of life, mean Phe levels differentiated PKU children with lower contrast sensitivity at the two highest spatial frequencies from those who performed similarly to controls. This could be due to greater fluctuations in Phe levels during this early period or the irreversible negative impact of elevated Phe levels on the visual system.

Evans et al[76] later reported a case of a PKU patient who was diagnosed late and developed levodopa-responsive Parkinsonism at age 33, approximately 20 years after discontinuing dietary treatment. CSF analysis indicated normal HVA levels but reduced 5-HIAA levels. However, at the time of testing, the patient was undergoing L-DOPA therapy. SPECT imaging using [^{123}I]-FP-CIT demonstrated normal striatal dopamine transporter (DAT) levels, while iodobenzamide (IBZM) scans revealed reduced caudate-over-putamen binding, suggesting a possible upregulation of postsynaptic D2 receptors indicative of DA depletion.

Boot et al[21], who investigated striatal DA receptor availability as a marker of DA deficiency, found higher availability in PKU than in the control group, suggesting DA depletion (i.e., the more receptors that bind to their agonist, the lower the amount of agonist remaining). The PKU group had lower plasma levels of HVA and MHPG, the predominant metabolites of DA and NE, respectively. Self-reported impulsivity levels were significantly higher in the PKU group compared with healthy controls. Within the PKU group, D2/3R availability showed a positive correlation with both impulsivity and the error rate during a cognitive flexibility task.

Pilotto et al[11] examined correlations between grey matter (GM) atrophy and CSF biogenic amine levels in 10 adults with ETPKU (mean age: 38.2 years; mean plasma Phe: 782±483 µmol/L) and 15 age-matched controls. CSF analysis revealed lower levels of 5-HIAA and 5-HTP in PKU patients, but not of HVA or L-DOPA. CSF levels of 5-HIAA, HVA, and 5-HTP showed significant negative correlations with Phe levels. Interestingly, brain volumetric MRI analysis revealed that lower 5-HIAA levels were associated with reduced volumes of the right frontal lobe, left precuneus, and cingulate (BA 24) cortex. Lower 5-HTP and HVA levels correlated with reduced volumes of the right inferior frontal gyrus and right temporo-occipital area, respectively. No correlation was found between L-DOPA levels and GM atrophy.

In a group of PKU subjects treated with pegvaliase, no correlation was found between the change in plasma HVA and symptoms of inattention; however, a change in plasma MHPG was correlated with improvement in the inattention subscale domain of the Attention Deficit Hyperactivity Disorder Rating Scale IV scores[77].

Gassiò et al[78] investigated sleep disorders in 32 children and adolescents with PKU and 32 controls, finding no significant differences between the groups. However, 43.3% of PKU patients exhibited low melatonin excretion, and 43.8% showed low 5-HT levels. No clear link was established between sleep disorders and these biochemical markers, suggesting that longer exposure or more severe neurotransmitter deficiencies may be necessary to manifest sleep issues.

Finally, Manti et al[79] in a cross-sectional study explored the prognostic value of peripheral biogenic amines in ETPKU. Using an Ultra Performance Liquid Chromatography - Tandem Mass Spectrometry (UPLC-ESI-MS/MS) method, levels of 5-HTP, 5-HT, 5-HIAA, and 3-OMD were measured in different blood-derived matrices: whole blood, plasma, and platelet-rich plasma. ETPKU patients showed significantly reduced levels of 5-HTP, 5-HIAA, and 3-OMD compared to controls. 5-HIAA and 3-OMD were inversely correlated with plasma Phe levels. Importantly, certain peripheral biogenic amines levels were associated with executive function outcomes independently of concurrent Phe levels: 5-HIAA with the ability to control and manage emotions, and 3-OMD with the capacity to generate ideas and problem-solving strategies, suggesting their potential role as novel biomarkers for clinical monitoring in adult ETPKU patients.

BIOGENIC AMINE AND PKU THERAPEUTIC OPTIONS

The recommended treatment for PKU patients is a Phe-restricted diet, which should be maintained for as long as possible, combined with an amino acid formula supplemented with trace elements[2].

LNAAs represent approximately 35-50% of the total L-amino acid supplements that do not contain Phe. The use of additional non-Phe LNAA supplements aimed at reducing brain Phe and increasing non-Phe LNAA levels and brain neurotransmitter concentrations is not currently supported by existing guidelines[2].

In PKU mice[80], LNAA supplementation without a Phe-restricted diet led to a significant reduction in blood and brain Phe concentrations after six weeks, an increase in brain levels of certain supplemented LNAAs (including Trp), and a notable rise in brain 5-HT and NE concentrations, although DA levels remained unchanged. In PKU patients, LNAA supplementation, particularly with Tyr and Trp, resulted in elevated CSF concentrations of the previously deficient metabolites HVA and 5-HIAA[27,28]. A prospective, double-blind study[81] indicated that while LNAA supplementation tended to lower plasma Phe levels and specifically impacted executive functions, its additional benefits were limited for individuals already following a Phe-restricted diet with PKU medical products.

In 2013, Yano et al[82] conducted a randomized, double-blind, placebo-controlled crossover study with 10 adults with PKU and 10 healthy controls to investigate whether blood and urine melatonin levels could serve as a peripheral biomarker of brain 5-HT synthesis in PKU patients. Melatonin, a metabolite of 5-HT in the pineal gland, is released into the blood during the night and subsequently excreted in the urine as 6-sulfatoxymelatonin. In subjects with PKU, researchers observed significantly lower concentrations of serum melatonin, urinary 6-sulfatoxymelatonin, and DA compared to controls, which were associated with markedly lower Trp/LNAA and Tyr/LNAA ratios. Furthermore, in the same PKU patients, the neurotransmitter metabolites and the mean plasma Trp/LNAA and Tyr/LNAA ratios significantly increased after LNAA supplementation compared to the placebo. Additionally, urinary 6-sulfatoxymelatonin and urinary DA were positively correlated with Trp/LNAA and Tyr/LNAA ratios, respectively.

The additional supplementation of isolated Tyr already present in Phe-free L-amino acid supplements (approximately 100 mg/g protein equivalent) is not recommended in routine clinical practice due to insufficient evidence of improvements in neurological outcomes[2,83]. However, as previously reported, Tyr supplementation in patients on an unrestricted diet resulted in enhanced DA biosynthesis and visual RT[74].

Pascucci et al[6], by administering a *per se* ineffective dose of the hydroxylation product of tryptophan (5-HTP) to ENU2 mice, documented an increase in 5-HT outflow in the pFC in response to stress. Similarly, Andolina et al[7] reported that treatment with 5-HTP in ENU2 mice during the early postnatal period temporarily restored physiological brain levels of 5-HT, reversing cognitive deficits in adult mice in spatial and object recognition tests and promoting the maturation of pFC pyramidal neuron spines. Pascucci et al[33] demonstrated that PKU mice exhibited reduced blood and brain Tyr levels, alongside a deficiency in TH levels and protein activity within the medial pFC. Low doses of L-DOPA restored prefrontal DA transmission (but not NE release) to levels observed in WT mice, even in the presence of high blood and brain Phe levels. Thus, it was suggested that L-DOPA administration, rather than Tyr supplementation, could have a positive therapeutic effect on DA neurotransmission.

Several studies suggest that BH_4 therapy, a crucial cofactor of TH and TPH, can stimulate synaptic neurotransmitter release and the subsequent metabolism of monoamines. In PKU patients treated with BH_4 (n=11), Ormazabal et al[23] demonstrated a significant increase in platelet 5-HT concentration after one and six months of therapy compared to baseline values, with no changes in Phe and Trp observed throughout the study. Similarly, Van Vliet et al[84] reported that in a group of 9 BH_4-responsive PKU males, blood prolactin concentration (a parameter of brain DA availability) was lower compared to

baseline, and an inverse correlation was found between prolactin levels and BH_4 dosage. Median blood Phe concentrations in BH_4-treated individuals tended to be lower, although not significantly different from pre-treatment baseline values.

Subsequently, Yano et al[85] conducted a study in which they combined LNAA supplementation with BH_4 treatment to evaluate their effects on peripheral markers of serotonergic and dopaminergic metabolism. Their study involved 9 adults with PKU undergoing four-week phases: (1) LNAA supplementation, (2) washout, (3) sapropterin (20 mg/kg/day), and (4) BH_4 + LNAA. After each phase, plasma amino acids, serum melatonin, 6-sulfatoxymelatonin, and DA were measured using an overnight protocol. Three out of nine PKU patients responded to BH_4, showing decreased blood Phe levels and a significant increase in blood melatonin levels. No statistically significant differences were observed in urinary 6-sulfatoxymelatonin or DA with BH_4 supplementation. In BH_4-responsive patients, a synergistic effect was observed in serum melatonin after combined LNAA and BH_4 supplementation.

The potential effects of BH_4 therapy on neurotransmission in PKU were also studied in PKU mouse models. Enteral BH_4 treatment at 50 mg/kg/day in ENU1/2 mice (with partial PAH deficiency) resulted in normalization of brain Phe levels, but only partial recovery of TPH2 protein and activity, as well as 5-HT and 5HIAA concentrations in the brain[86].

In hyperphenylalaninemic C57BL/6-*Pah*^{enu2} mice, treatment with high doses of BH_4 (100 mg/kg/day) led to an increase in total brain biopterin content and elevated HVA and 5-HIAA levels. In contrast, brain DA, 5-HT, and blood and brain Phe remained unchanged. This suggests that BH_4 therapy may influence neurotransmitter release and metabolism rather than their synthesis[87]. Although further investigation is needed, current findings indicate potential benefits of BH_4 treatment[88].

Recently, the effects of pegvaliase on brain monoamine neurotransmitters were investigated. In ENU2 mice, pegvaliase treatment resulted in a complete restoration of DA, NE, and 5-HT levels, coinciding with a reduction in Phe levels[77,89]. Tyr supplementation in pegvaliase-treated PKU mice had no effect on brain DA levels but did increase NE levels[77]. Although neurotransmitter metabolites also rose after pegvaliase treatment, a mild deficiency in HVA and 5-HIAA suggested a persistent slight impairment in monoamine metabolic pathways[89]. When pegvaliase treatment was discontinued, neurotransmitter abnormalities increased, highlighting the strong correlation between brain DA/5-HT levels and blood and brain Phe concentrations, rather than Tyr or Trp levels[89]. Additionally, pegvaliase treatment significantly increased TH-positive neurons in ENU2 mice after five weeks, with sustained effects throughout the treatment duration[66].

A study[77] involving 23 PKU patients with baseline Phe levels greater than 900 µmol/L confirmed findings from animal models. After 12 months of pegvaliase treatment, participants were divided into two groups: those maintaining Phe levels above 900 µmol/L and those reducing Phe to below 360 µmol/L. In the low-Phe group (less than 360 µmol/L), plasma HVA and MHPG normalized, while 5-HIAA approached levels seen in healthy controls. In the high-Phe group (greater than 900 µmol/L), plasma HVA and 5-HIAA remained unchanged and lower than in controls, while MHPG increased. Plasma 5-HIAA levels correlated with Phe levels in both groups but showed a stronger correlation in the low-Phe group. HVA and MHPG correlated with Phe levels only in patients with Phe levels below 360 µmol/L. This suggests that a greater reduction in Phe is necessary to affect DA and NE metabolism.

Finally, liver-targeted gene therapy in ENU2 mice reversed previously observed nigrostriatal neurodegenerative changes. At eight weeks of age, gene therapy improved DA, NE, and 5-HT levels and reduced blood and brain Phe levels[8,90,91] (results summarized in Supplementary Table 5).

CONCLUSIONS

This narrative review synthesizes evidence from clinical studies, autopsy data, and animal models, highlighting the complex and multifactorial effects of elevated Phe levels on neurotransmitter metabolism in PKU. Elevated Phe disrupts neurotransmitter synthesis — particularly 5-HT — *via* several mechanisms.

However, the precise pathophysiological mechanisms remain unclear. Two main hypotheses have been proposed to explain these neurotransmitter imbalances: competition for LNAA transport across the BBB, and direct inhibition of key enzymes such as TPH and TH. However, there is more substantial evidence supporting only the latter hypothesis. Indeed, a recent study[37] in PKU mice found that, contrary to previous reports[34,36], brain amino acid concentrations were not significantly altered. Furthermore, although a disruption in neurotransmitter levels is observed in PKU, the link between these alterations and cognitive deficits remains uncertain and underscores the complexity of the condition.

Several important questions remain unresolved: (1) How do alterations in neurotransmitter levels contribute to the suboptimal outcomes observed in adult ETPKU patients? (2) Can neurotransmitter levels serve as predictive biomarkers for brain vulnerability to Phe, given the considerable interindividual variability observed among adult patients?

In the context of personalized medicine, answering these questions is crucial for refining treatment strategies and ultimately improving the quality of life for individuals with PKU. Future research should aim to clarify these mechanisms and optimize therapeutic interventions to ensure more effective and lasting neurological protection.

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