الجمهورية الجزائرية الديمقراطية الشعبية
People's Democratic Republic of Algeria
وزارة التعليم العالي والبحث العلمي
Ministry of Higher Education and Scientific Research
جامعة محمد البشير الإبراهيمي برج بوعريريج
University Mohamed El Bachir El Ibrahimi - BBA
كلية علوم الطبيعة والحياة وعلوم الأرض والكون
Faculty of Nature and Life Sciences and Earth and Universe Sciences
قسم العلوم البيولوجية
Department of Biological Sciences

# Thesis

**In Partial Fulfillment of the Requirements for a Master's Degree**
**Domain: Nature and Life Sciences**
**Field: Biological Sciences**
**Speciality: Biochemistry**

## Title

# Bibliographical Study of Phenylketonuria

**Presented by:**
AGUIDA Rim and ASLI Djouhara

**Defended on 30 June 2024 Before The Board of Examiners:**

| | | |
|---|---|---|
| **President: Mr. ZIAD Abdelaziz** | **Asst.Lect.** | **University of B.B.Arreridj** |
| **Supervisor: Ms. MEZITI Asma** | **Sr.Lect.** | **University of B.B.Arreridj** |
| **Examiner: Mr.BECHAMI Sofiane** | **Assoc.Lect.** | **University of B.B.Arreridj** |

**Academic Year: 2023-2024**

# Acknowledgments

Words cannot express our gratitude and immense appreciation to our professor Ms. MEZITI Asma for her invaluable patience since the beginning of our research process. This endeavor would not have been possible without her generous support, provided knowledge and expertise. Our special thanks go to both Mr.ZIAD and Mr. BECHAMI for their energy and time generously spent while reading and examining this scientific work.

We are also extremely grateful to all our professors in the Department of Biochemistry for being such a source of motivation, moral support and intellectual growth, and without whom, we would have never been capable of reaching this point and handling this right all these years.

Lastly, we would be remiss in not mentioning our families, especially our parents, who always kept believing in our potentiel in delivering nothing but the best. Thank you for the trust and all the emotional support.

# *Dedication*

This work is dedicated to our beloved parents, the most inexhaustible source of faith, inspiration, and happiness in our lives. The compass that has always provided us with emotional, moral, spiritual, and financial support under every condition.

A big « Thanks » goes to our friends as well, who shared our moments of smiles and tears and for being a comforting shoulder to rely on.

Thank you to all our teachers too, who opened our eyes to new horizons and introduced us to curiosity and the magic of science.

Finally, all this work is made for and in His name God Almighty, our eternal guide, strength, and protection. To you, we offer this.

## TABLE OF CONTENTS

# ABREVIATION LIST

**AA :** Arachidonic acid
**AAV:** Adeno-associated virus
**AR :** Aldose reductase
**BBB :** Blood-brain barrier
**BH4 :** Tetrahydrobiopterin
**CNS:** Central nervous system
**CR :** Carbonyl reductase
**CSF :** cerebrospinal fluid
**DBS:** Dried blood spots
**DHA:** Docosahexaenoic acid
**DHPR :**Dihydropteridinereductase
**DNA:** Deoxyribonucleic Acid
**DNAJC12:** DnaJ heat shock protein hydratase family (Hsp40) member C12
**E-Coli :** Escherichia coli
**G-418:** Geneticin
**GFAP :** Glial Fibrillary Acid Protein
**GMP :**Glycomacropeptide
**GTP :** Guanosine triphosphate
**GTPCH :** Guanosine triphosphate cyclohydrolase 1
**HNF1α :** Hepatocyte nuclear factor 1α
**HPA:** Hyperphenylalaninemia
**HSDH2 :** 3-α-hydroxysteroiddehydrogenase 2
**IQ:** Intelligence Quotient
**LAT1 / SLC7A5:** large neutral amino acid transporter
**LC-PUFAs :** Long-chain polyunsaturated fatty acids
**LNAA :** Large neutral amino acide
**LV :** Lentivirus vectors
**MBP:** Myelin Basic Protein
**mHPA/MHP:** Mild hyperphenylalaninemia
**MPKU :** Maternal PKU
**mPKU:** Mild phenylketonuria
**NOS:** Nitric oxide synthase
**NT:** neurotransmitter
**OPCs:** Oligodendrocyte precursor cells
**PAL :** Phenylalanine ammonia-lyase
**PAH :** Phenylalanine Hydroxylase
**PCD / DCoH1:** Pterin-4a-carbinolamine
**PEG:** Polyethylene glycol
**Phe :** Phenylalanine
**PKU :** Phenylketonuria
**PKUc :** Classical phenylketonuria
**PTPS :** 6-pyruvoyltetrahydropterin synthase
**Q10 :** 1,4-benzoquinone / Ubidecarenone
**ROS :** Reactive oxygen species
**RNS :** Reactive nitrogen species
**SR :**Sepiapterinreductase
**TH:** Tyrosine hydroxylase
**TMS :** Tandem mass-spectrometry
**Trp :** Tryptophane
**TPH:** Tryptophan hydroxylases
**TYR :** Tyrosinase
**Tyr :**Tyrosin
**L-DOPA** : Levodopa / dihydroxyphenylalanine

# LIST OF FIGURES

# Introduction

## Introduction

Inborn errors of metabolism encompass a group of genetic disorders characterized by defects in the body's ability to process specific substances. These conditions arise from mutations in genes encoding enzymes or proteins essential for metabolic pathways. While individually rare, collectively they represent a significant category of inherited disorders responsible for various health challenges, particularly in children **(Ferreira et *al*., 2018).**

Clinical signs and symptoms might result from a toxic substrate accumulation, a product insufficiency, or both also result from the deficiency or the abnormality of an enzyme, its cofactor or a transporter. The beginning of the clinical picture might range from the neonatal stage to maturity, depending on the residual activity of the defective enzyme **(Ezgu, 2016)**.

Phenylketonuria (PKU) stands as a prominent example within this spectrum of metabolic disorders. It is an autosomal recessive condition caused by a mutation in the gene encoding phenylalanine hydroxylase (PAH), an enzyme crucial for the breakdown of phenylalanine (Phe) into tyrosine (Tyr). The disorder affects the metabolism of phenylalanine and causes neurological dysfunction, particularly at high phenylalanine concentrations. This brain malfunction causes significant intellectual impairment, epilepsy, and behavioral issues if left untreated **(Van Spronsen et *al*., 2021).**

In the early years, Phenylketonuria (PKU) was seen as a condition leading to mental retardation with no effective treatment. However, by the mid-1950s, a specialized diet had been developed, enabling successful prevention of PKU-related mental impairment in infants if started early in infancy. Early detection and dietary intervention have since become standard practice, preventing mental retardation in hundreds of infants annually in the US alone. The progress in PKU detection and treatment owes much to the dedication of numerous researchers and clinicians worldwide over several decades **(Centerwall SA and Centerwall, 2000).**

In this bibliographical research on Phenylketonuria (PKU), we provide an overview of the disease by exploring its key points and delving into its fundamental aspects. Understanding these points is essential for comprehending the intricate details of PKU, which serves as a renowned model for inherited metabolic disorders. The research is organized into three chapters.

The first chapter, "Generalities" delves into the historical context of PKU's discovery, analyzing the epidemiology associated with the disease, and discussing the metabolism of PKU and its mode of inheritance.

The second chapter, "Physiopathology," focuses on the pathophysiological mechanisms underlying PKU, classifying PKU based on relevant criteria, and examining the evolutionary aspects of the disease.

The final chapter, "Diagnosis and Treatment," outlines established diagnostic methods for PKU and explores the available and evolving treatment options.

# Chapter 1:
# GENERALITIES

# 1 Generalities

## 1.1 History of PKU

Phenylketonuria is associated with the name of Asbjorn FOLLING, a pediatrician from western Norway. in 1934 when he first discovered two children from a Norwegian family with mental retardation whose mother had for chance insisted on following their health condition in order to discover the origin of their mental retardation, thus beginning the story of the disease **(Woolf and Adams, 2020).**

Dr. Folling analyzed the children's urine and identified the presence of previously unknown substances, which were ketone bodies. Using ferric chloride to test the urine, he detected a mystery chromogen that he accurately identified as phenylpyruvic acid—a substance not previously found in nature. Recognizing the significance of his discovery, Følling named the condition "oligophrenia phenylpyruvica." and concluded that it was produced by dietary phenylalanine **(Woolf and Adams, 2020).**

Additional investigation during the 1930s, particularly by Penrose (1935) in the UK, resulted in the introduction of the term "phenylketonuria" and the recognition of its autosomal-recessive genetic inheritance. Despite Penrose's unsuccessful dietary intervention efforts, George Jervis (USA) and Horst Bickel (UK) established the groundwork for dietary management of PKU in the 1950s **(Bickel *et al.*, 1953)**

The development of the first screening test in the 1960s by Robert Guthrie enabled early detection. The cloning of the PAH gene in the 1980s and subsequent genomic sequencing laid the foundation for understanding PKU genetics **(Blau , 2016)**

## 1.2 Epidemiology

The occurrence of phenylketonuria exhibits significant variations across different regions of the world. In Europe, the prevalence stands at approximately one case per population. The occurrence of persistent hyperphenylalaninaemia is relatively rare, affecting approximately one in every 4000 births in Turkey and certain regions of Europe, such as Northern Ireland. This higher prevalence can be attributed to the increased consanguinity within these populations. In general, the rate of occurrence is around 1 in 10,000 live births, but certain areas experience a slightly higher incidence In Europe, Finland boasts the lowest prevalence rate with only one case per 100,000 individuals. On the other hand, the prevalence rate in the United States is significantly higher at one case per 15,000

individuals. In Latin America, the prevalence rate varies across different regions. This condition is found in roughly one out of every 50,000 to 25,000 births. In southern Latin America, the prevalence tends to be higher compared to other parts of the region. Estimations regarding the prevalence have been made. The occurrence of phenylketonuria in Asia exhibits a wide range, with rates ranging from approximately one per 15,000 to one per 100,500 births in certain areas of China. In Thailand, the prevalence is less than one per 200,000, while in Japan, it stands at around one per 70,000. On the other hand, Africa demonstrates a notably low frequency of this condition. Spain exhibits a notably high prevalence of mild hyperphenylalaninemia, along with the presence of ketonuria **(Blau *et al.*, 2010) (Figure 01).**

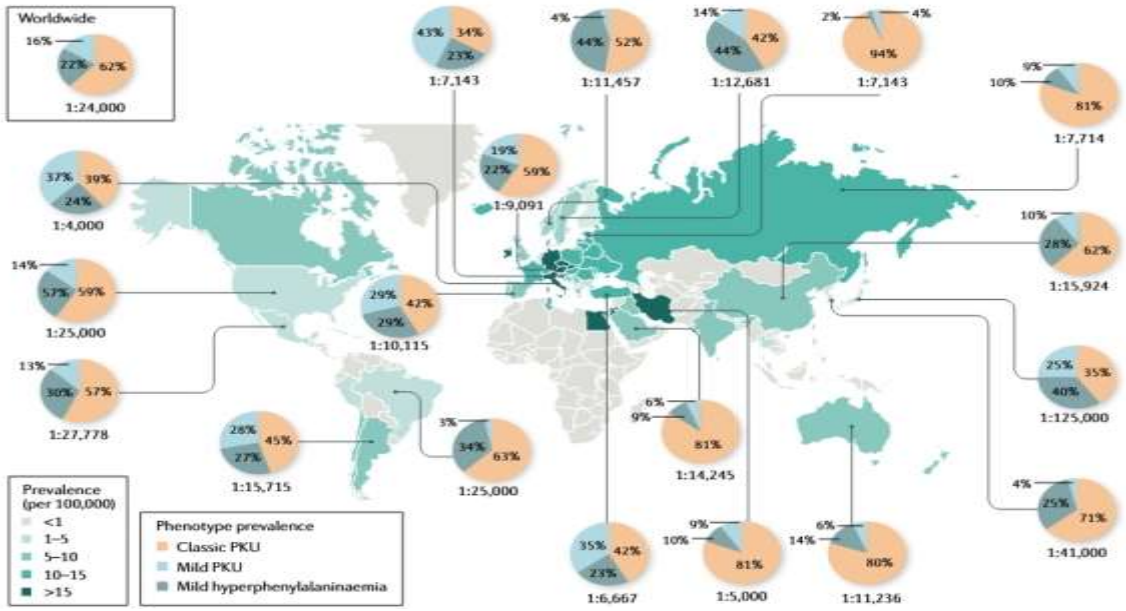


**Figure 01.** PKU worldwide distribution **(Van Spronsen *et al.*, 2021).**

## 1.3 Metabolism

### 1.3.1 Phenylalanine

Phenylalanine (Phe) is an essential amino acid that the body cannot produce on its own, so the food is the only source. It plays vital roles such as converting to -tyrosine and synthesize neurotransmitters like dopamine and catecholamines, a building blocks of the proteins necessary within the body. Phe is very important in neurological function and brain health and also involving in pigmentation throw the production of melanin.

Like many other metabolites, Phe concentration is regulated to equilibrium levels by dynamic input and output fluxes. Continuous disturbances in flow eventually lead to changes in steady-state concentration. Dietary intake of Phe and endogenous recycling of amino acid

stores are the major sources of Phe, whereas utilization or loss of Phe occurs through incorporation into proteins, oxidation to Tyr, or conversion to other metabolites **(Williams *et al.*, 2008).**

#### 1.3.1.1 Sources

Phenylalanine is a component of many protein-rich foods, particularly high-protein foods such as meat, milk, eggs, nuts, soy, and fish **(Figure 02).** It can also be found in synthetic sources like aspartame, an artificial sweetener composed of L-phenylalanine and L-aspartate **(Czarnecka *et al.*, 2021)**. The exact daily requirement for phenylalanine is not precisely known, but earlier 24-hour tracer studies have indicated that the requirement is between 30 and 40 mg per kg of body weight **(Kurpad *et al.*, 2006).**


**Figure 02.** Phenylalanine food sources **(Whitbread, 2024)**

### 1.3.2 Metabolic pathway

The conversion of Phe to Tyr occurs via a hydroxylation system that includes PAH, the unbound pterin cofactor tetrahydrobiopterin (BH4), and enzymes used to regenerate BH4, namely dihydropteridinereductase and 4α-carbinolaminedehydratase **(Figure 03).**

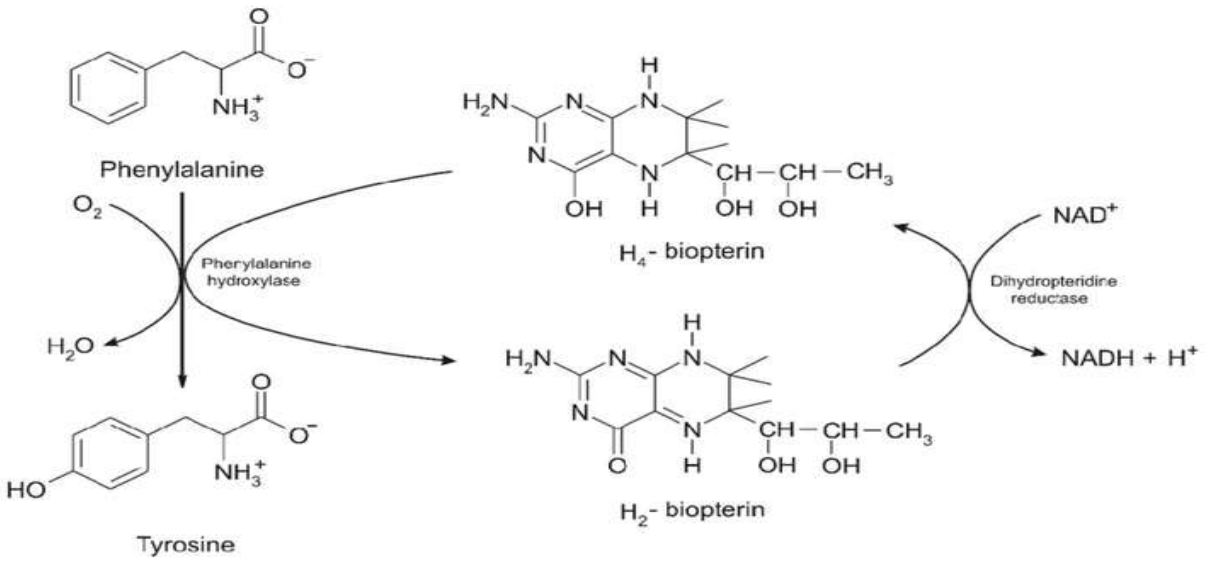


**Figure 03.**Conversion of Phe to Tyr by PAH **(Dinu and Apetrei, 2020).**

The conversion of Phe to Tyr has two results. First, it drives endogenous production of the non-essential amino acid tyrosine. Second, the hydroxylation reaction is the rate-limiting step for the complete oxidation of Phe to CO2 and H2O and contributes to the pooling of glucose and 2-carbon metabolites**.**Many rare associated diseases due to defects in the BH4 regenerative system may also affect Phe homeostasis and catecholamine and serotonin biosynthesis, as this cofactor is a common cofactor for the hydroxylases Phe, Tyr, and tryptophan (Trp). Although p-hydroxylation of Phe is essential for cleavage of the benzene ring, it is not required for further metabolism of the alanine side chain. This alternative pathway of transamination and decarboxylation results in the formation of metabolites such as phenyl pyruvate, phenyl lactate, and o-hydroxyphenylacetate, which are excreted in the urine **(Figure 04)**.

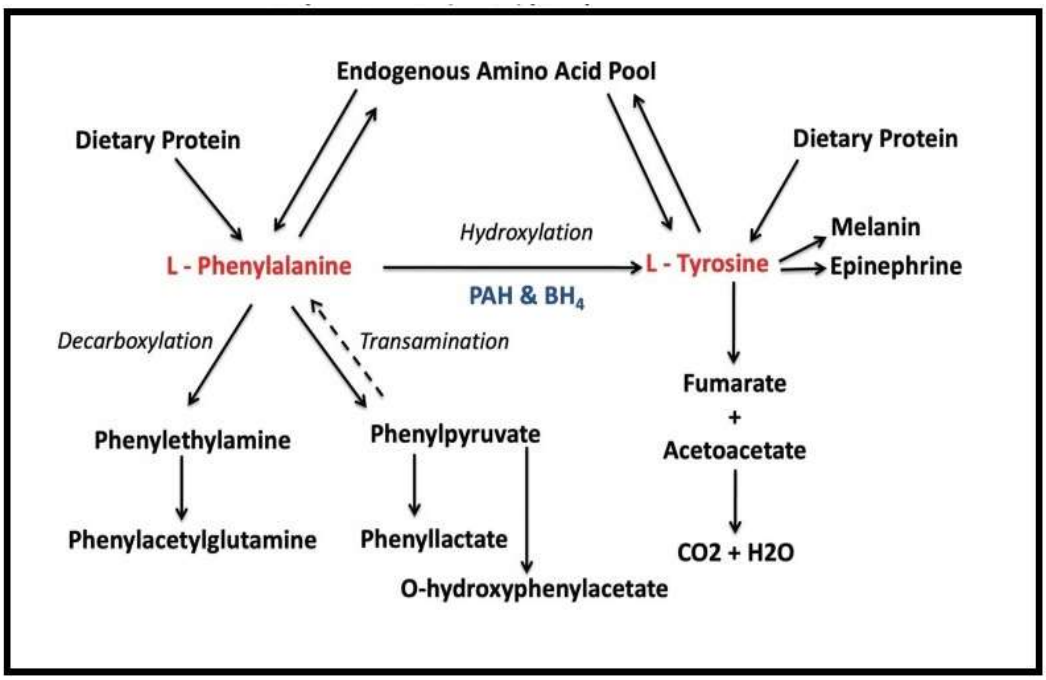


**Figure 04.** Phenylalanine metabolic pathway **(Giżewska, 2015)**

### 1.3.3 Phenylalanine hydroxylase

PAH catalyze the stereospecific hydroxylation of L-Phe, a key step in the degradation of this amino acid. Phe catabolism and PAH activity were mainly associated with the liver **.** In humans, PAH enzymes exist as tetramers. the monomer is approximately 50 kDa in size and consists of 452 amino acids **(Figure 05)**. PAH enzymes require BH4 as a cofactor and molecular oxygen to exert their activity. they can be divided into multiple functional domains. The regulatory domain contains a serine residue that is thought to be involved in phosphorylation activation. The catalytic domain contains a 26 or 27 amino acid motif responsible for cofactor and iron binding. The C-terminal domain is thought to be involved in binding between subunits (**Flydal and Martinez, 2013).**

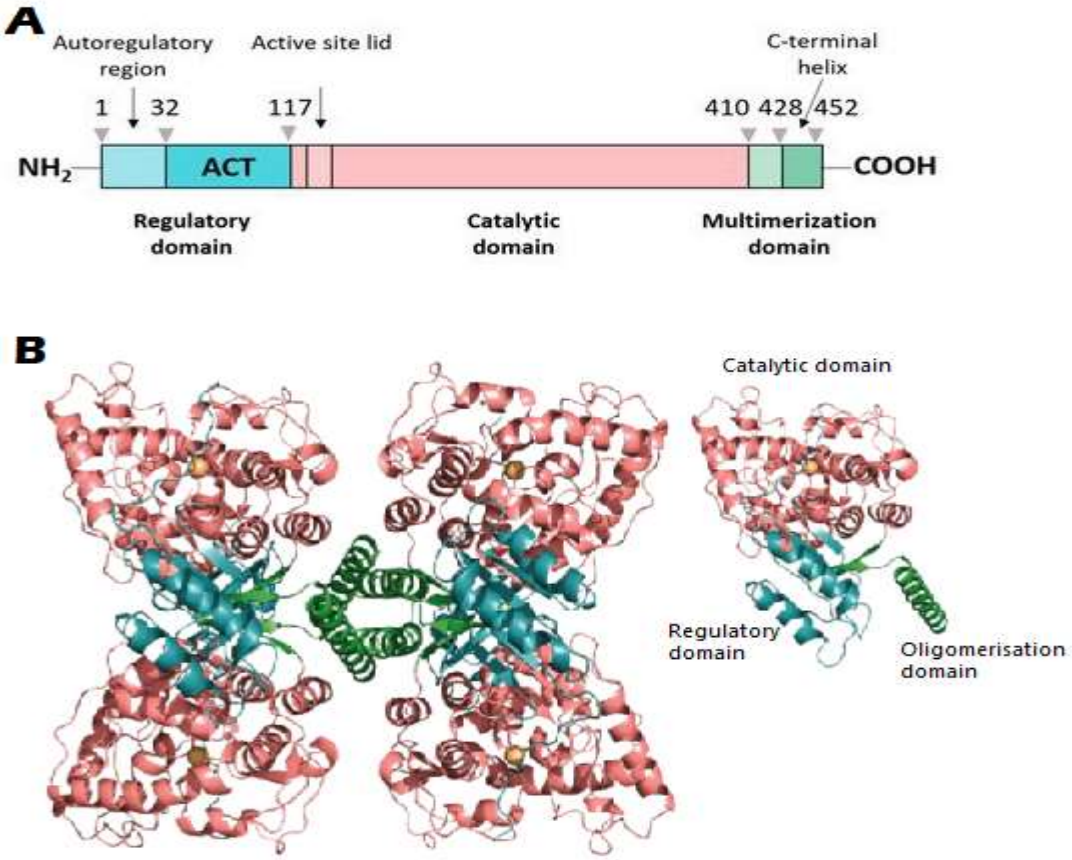


**Figure 05.** Structure of phenylalanine hydroxylase **. A :** PAH open structure of one monomere.**B :**Composite model of full-length tetrameric human PAH (**Flydal and Martinez, 2013 ; Conde-Giménez and Sancho, 2021).**

The hydroxylation mediated by PAH is the critical step in the intermediary metabolism of L-Phenylalanine. Despite advancements in research techniques, the precise amount of Phe utilized for net protein metabolism remains uncertain. Based on differences in Phe requirements between healthy individuals and those with Phenylketonuria, it is estimated that approximately 10-20% of typical dietary Phe intake contributes to routine protein turnover, while the remainder is converted to Tyr by PAH. Tyr serves various metabolic functions (**Figure 06)**, including neurotransmitter synthesis (dopamine, adrenaline, and norepinephrine), conversion to thyroxine in the thyroid gland, melanin synthesis in melanocytes, and complete breakdown into acetoacetate (a ketone) and fumarate (an intermediate in the Krebs cycle) for energy production. Deficiency in PAH activity, whether inherited or functional, results in hyperphenylalaninemia (HPA), mild Tyr deficiency, and, in severe cases, the urinary excretion of phenylpyruvate (a product of spontaneous Phe deamination) and other phenylketone bodies **(Van spronsen *et al.*, 2021).**

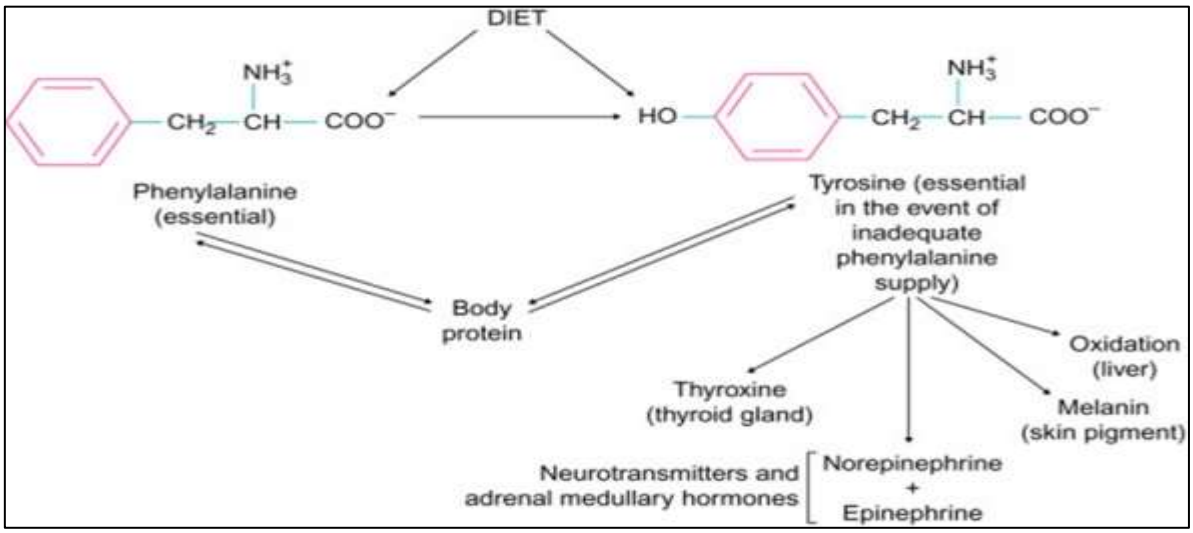


**Figure 06:** Overview of the metabolism of phenylalanine and tyrosine **(Bhagavan and Ha, 2015)**.

### 1.3.4 Impact of phenylalanine regulation on hormone synthesis

Disruption in the regulation and metabolism of phenylalanine, such as in phenylketonuria (PKU), can have implications for hormone synthesis due to decreased availability of tyrosine. In PKU, mutations in the PAH gene lead to reduced or absent PAH activity, resulting in elevated phenylalanine levels and decreased tyrosine production. As a consequence:

#### 1.3.4.1 Thyroid hormone production

Reduced tyrosine availability can impair the synthesis of thyroid hormones, potentially leading to hypothyroidism and its associated symptoms.

#### 1.3.4.2 Catecholamine synthesis

Insufficient tyrosine can limit the production of catecholamines, affecting neurotransmitter balance and potentially leading to neurological symptoms associated with PKU, such as cognitive impairment and behavioral issues **(Giżewska, 2015).**

### 1.3.5 Tetrahydrobiopterin (BH4)

#### 1.3.5.1 BH4 metabolic pathways synthesis and regeneration

Cofactor of phenylalanine hydroxylase, BH4 also acts as a chaperone protein in some patients who are deficient in phenylalanine hydroxylase. BH4 is a cofactor of tyrosine and tryptophan hydroxylase and has a role in the conversion of L-arginine to nitric oxide by nitric

oxide synthases **(Van spronsen *et al.*, 2017).** PAH enzymatic activity relies on several cofactors for optimal function. Alongside BH4, ferrous iron (Fe2+) acts as a core component, assisting in the activation of molecular oxygen during the hydroxylation process. BH4 is synthesized *de novo*, primarily involving enzymes like GTP cyclohydrolase 1 (GTPCH), 6-pyruvoyltetrahydropterin synthase (PTPS), and sepiapterinreductase (SR). These enzymes facilitate the biosynthesis of BH4 from guanosine triphosphate (GTP), ultimately aiding in phenylalanine metabolism. Additionally, the final reduction steps in BH4 biosynthesis can also occur through alternative pathways known as salvage pathways, where enzymes like aldose reductase (AR), carbonyl reductase (CR), and 3-α-hydroxysteroid dehydrogenase 2 (HSDH2) participate. These alternative pathways, predominant in liver and peripheral organs, contribute to BH4 recycling and regeneration. Furthermore, BH4 regeneration requires the enzymatic activities of pterin-4a-carbinolamine hydratase (PCD) and dihydropteridinereductase (DHPR). PCD, also known as DCoH1, possesses a dual function, acting not only as an enzyme but also as a dimerization cofactor of hepatocyte nuclear factor 1α (HNF1α). These cofactors collectively play a pivotal role in maintaining PAH activity and phenylalanine metabolism, thereby influencing PKU management strategies **(Figure 07).**

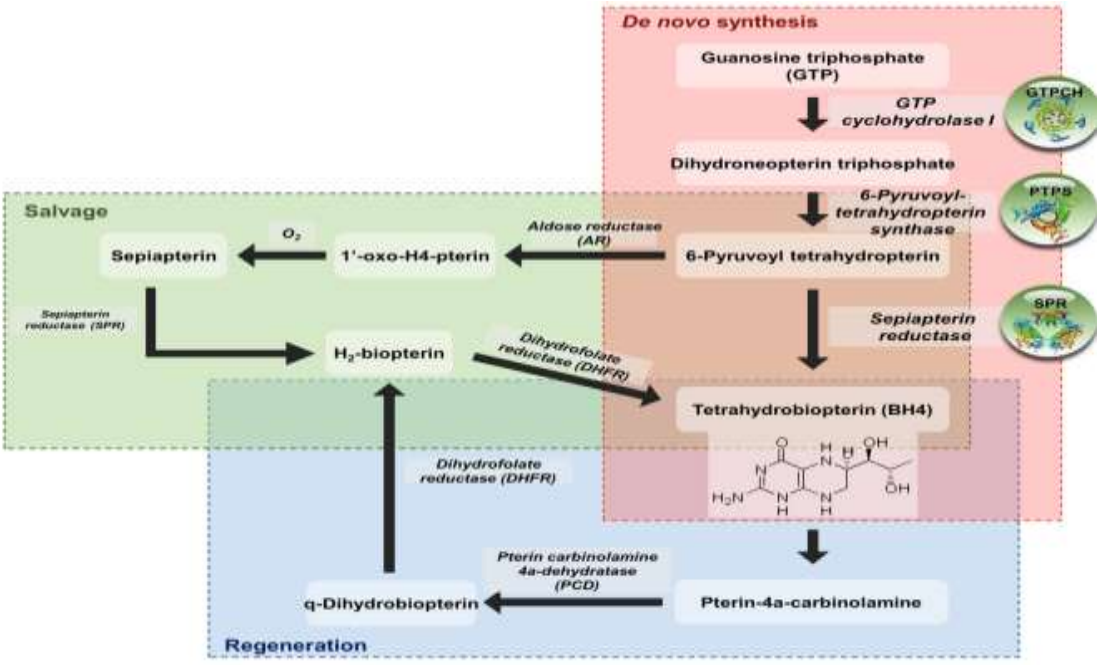


**Figure 07.** BH4 Synthesis and regenerations **(Kim and Han, 2020).**

#### 1.3.5.2 BH4 deficiencies

Biosynthesis and regeneration defects in tetrahydrobiopterin (BH4) metabolism lead to a deficiency of this crucial co-factor, impacting various biological functions and systems. BH4 plays a pivotal role as a co-factor for several enzymes involved in the synthesis of essential biomolecules such as nitric oxide (NO), dopamine, tyrosine, and serotonin. These molecules are integral to numerous biological processes and systems, including the cardiovascular, nervous, and immune systems. BH4 serves as a co-factor for enzymes such as nitric oxide synthase (NOS), tyrosine hydroxylase (TH), tryptophan hydroxylases (TPH), and phenylalanine hydroxylase (PAH).**(Van spronsen *et al.,* 2017)**.

## 1.4 Genetic causes of hyperphenylalaninemia (HPA)

Phenylalanine hydroxylase (PAH) deficiency accounts for 98% of PKU cases, while the remaining 2% involve deficiencies in the synthesis or recycling of the enzyme cofactor, BH4, or deficits in proteins such as DNAJC12, the PAH chaperone protein, as well as tyrosine and tryptophan hydroxylases. These deficiencies result in hyperphenylalaninemia often accompanied by deficiencies in neurotransmitters, originally termed "malignant phenylketonuria" due to the neurological deterioration that dietary interventions failed to prevent **(Blau *et al.,* 2011; Wiedemann *et al.,* 2020).**

### 1.4.1 PAH gene mutation

An autosomal recessive variant in the PAH gene on chromosome 12 is the main cause of HPA. As a result, the production of PAH defective monomers with low activity or completely inactivated (non-functional), or the complete absence of the protein. **(Wiedemann *et al.,* 2020). (Figure 08).**

There are 13 exons and 12 introns, in total covering 100 kb of genetic data. A 452 amino acid polypeptide is encoded by it with a molecular weight of ~52 kDa. More than 950 mutations in the gene encoding PAH obtained from BIOPKU database, are known to be associated with PAH deficiency. The PAH gene has variations in several domains, the most affected of which is the core catalytic domain. Most of the mutations are missense, usually resulting in protein misfolding or impairment of catalytic functions **(Elhawary *et al.,* 2022; VanSpronsen *et al.,* 2017).** The PAH gene comprises three domains: N-terminal regulatory domain, central catalytic domain, and C-terminal oligomerization domain **(Figure 09).** Most

variants in the PAH gene are in the central domain (59.2%), followed by the N-terminal and C-terminal regions (17.5% and 5.4%, respectively) **(Elhawary *et al.*, 2022).**

Over 1,180 different bi-allelic mutations within the phenylalanine hydroxylase (PAH) gene, situated on chromosome 12q22–24.1 have been discovered, over 2,600 genotypes have been identified as PKU-causing. **(Blau *et al.*, 2010).** In-frame missense amino acid changes account for 58.3% of all PAH variations; synonymous substitutions (4.9%), splice variants (13.1%), frameshift variants (13.9%), and nonsense variants (6.9%) are less common **(Van spronsen *et al.*, 2021)**.

A study on the mutations discovered in the PAH gene within the Italian PKU community is depicted in **figure (08).**The mutations were distributed throughout the PAH gene, from exons 1 to 13, except the exons 9 and 10. The exon 7 seemed to be a hot spot for mutations. It exhibits the greatest number of different mutations (5) and is affected in 28.7% (19/66) of the total mutant alleles. Therefore, the majority of mutations (n = 18) were distributed along the catalytic domain (54.6%), 8 mutations (24.3%) belonged to the regulatory domain, 1 (3.1%) to the tetramerization domain and 6 (18.2%) are intronic **(Trunzo *et al.*, 2014).**

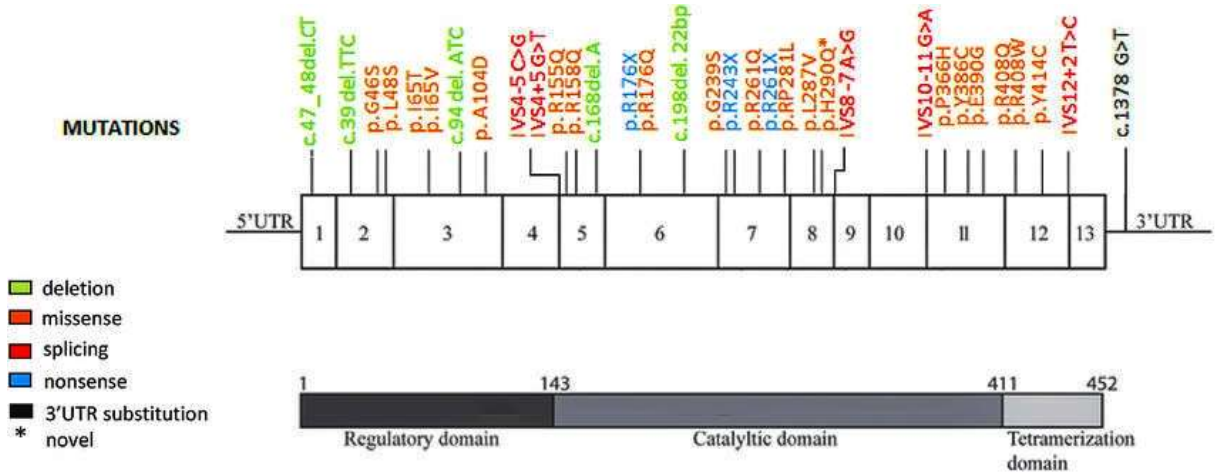


**Figure 08.** Schematic representation of the PAH mutations detected in PKU population **(Trunzo *et al.*, 2014).**

### 1.4.2 BH4 deficiency

In certain instances, HPA can also results to deficiencies in tetrahydrobiopterin (BH4), which is necessary for PAH function, caused by inherited defects in biopterin synthesis (GTP cyclohydrolase 1 (GTPCH) or 6-pyruvoyl-tetrahydropterin synthase (PTPS) deficiencies) or BH4 recycling (dihydropteridinereductase (DHPR) or pterin-4a-carbinolamine dehydratase (PCD) deficiencies).Additionally, forms of GTPCH deficiency and sepiapterinreductase (SR) deficiency, though not typically resulting in hyperphenylalaninemia (HPA), can still lower PAH activity **(Van spronsen *et al.,* 2021)**. Variants in these genes impair BH4 metabolism and can result in mild to severe forms of HPA, depending on the specific genetic defect **(Elhawary *et al.,* 2022).**

### 1.4.3 Lack of the chaperone DNAJC12

Notably, another genetic etiology of PKU with significant clinical diversity can arise from the lack of the chaperone DNAJC12, which can interfere with the folding and assembly of functional PAH monomers **(Van spronsen *et al.,* 2021).**

## 1.5 Transmission of pku

This anomaly is transmitted within a family in what is called "autosomal recessive" manner. The term "autosomal" means that the gene involved in the disease is not located on the sex chromosomes (X and Y chromosomes), but on one of the other chromosomes, called "autosomes." The disease can therefore occur in both boys and girls. Each individual carries two copies of each gene (including the PAH gene involved in phenylketonuria): one copy is inherited from their mother and the other from their father. The term "recessive" means that both copies of the gene must be altered for the disease to appear. Thus, the parents of a child with phenylketonuria are not sick themselves, but they both carry one normal copy of the PAH gene and one mutated copy **(Figure 09).**

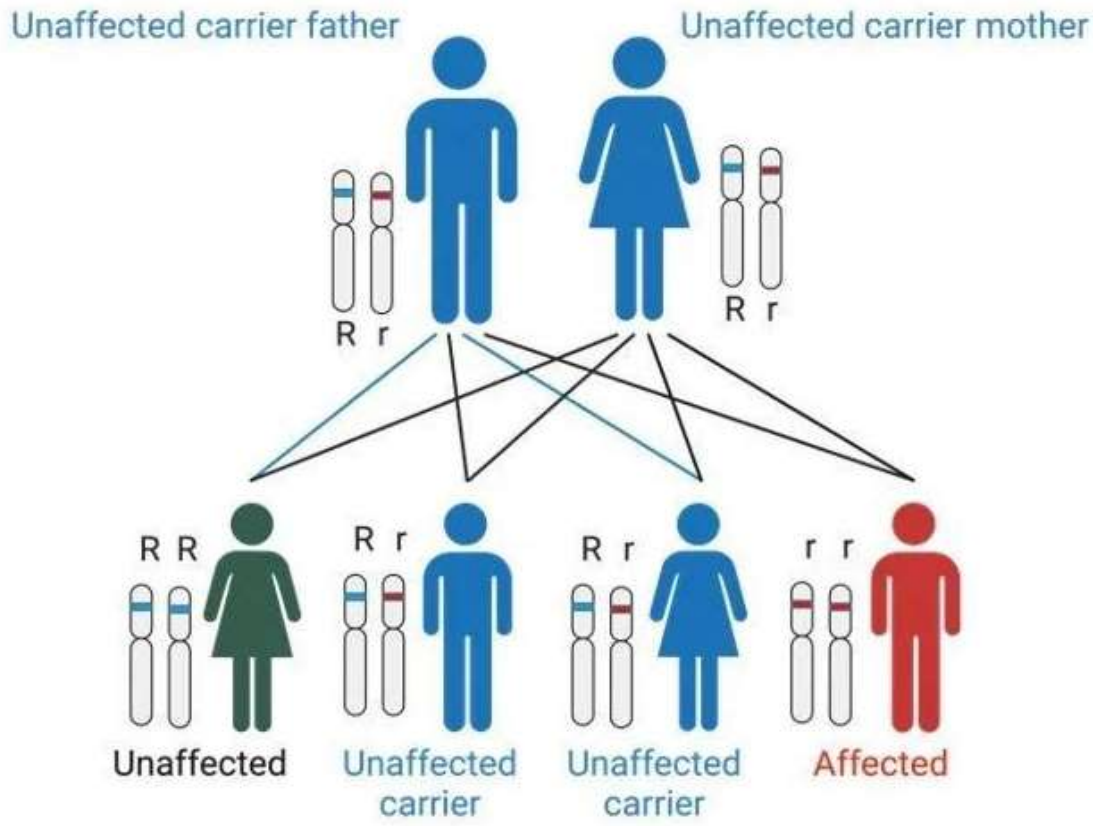


**Figure 09.**Autosomal recessive transmission of PKU **(Website 1)**

Given this situation, any kid born to the pair has the following odds: 25% will inherit two mutated copies of the gene and become a PKU carrier, 50% will inherit one mutated copy and become a carrier like their parents, and 25% will inherit two normal copies of the gene and remain PKU-free **(Figure09).** It is significant to highlight those carriers of PKU usually do not exhibit symptoms of the disorder since they are able to metabolize phenylalanine normally due to having one functional copy of the PAH gene. They are able to pass on the mutant gene to their progeny, nevertheless **(NICHD, 2000).**

# Chapter 02: Physiopathology

# 2 Physiopathology

## 2.1 Physiopathology of PKU

The pathophysiology of PKU involves several issues that lead to a range of symptoms **(Figure10),** especially its severe impact on neurological function, which is the main symptom.

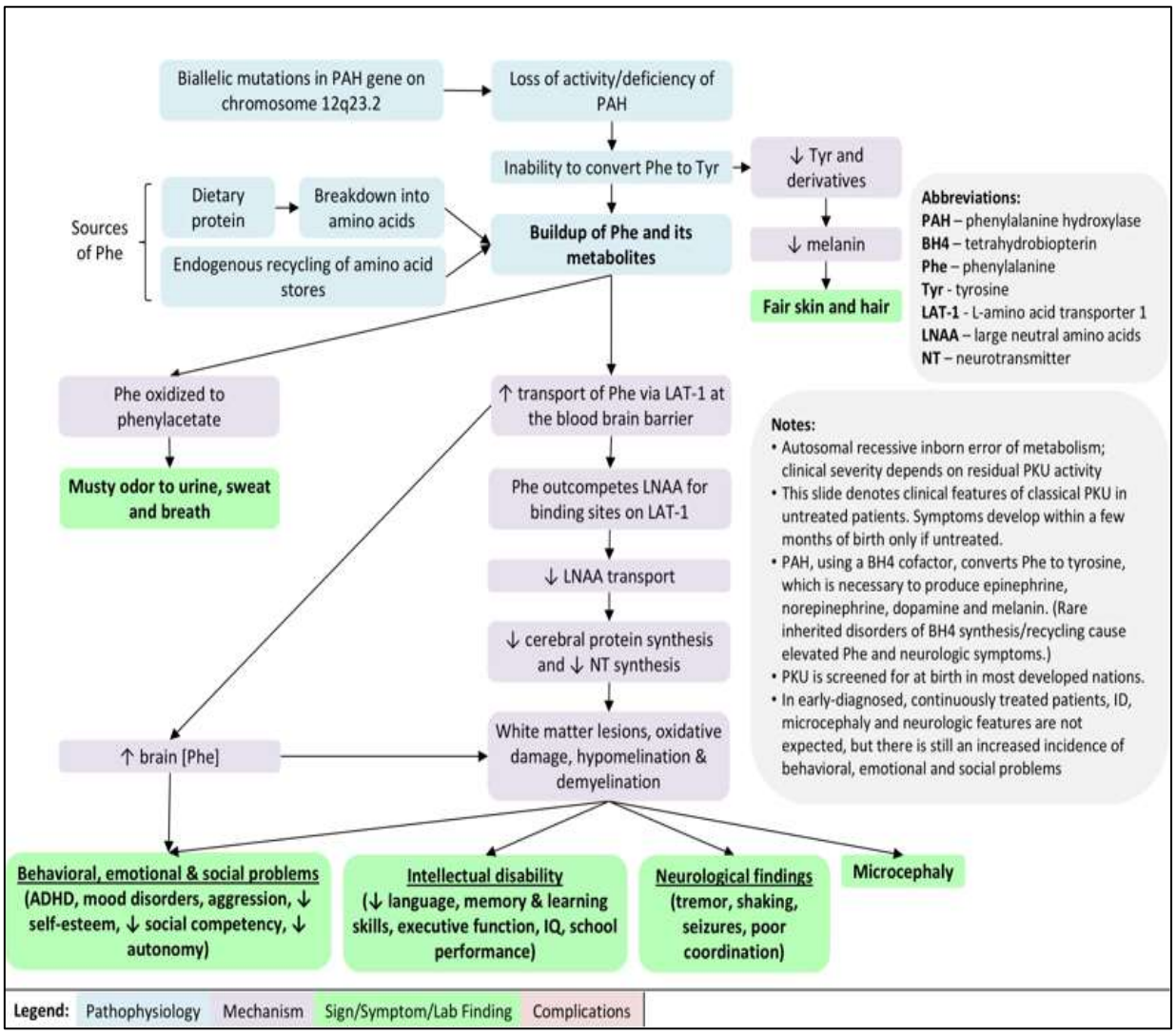


**Figure 10.** Pathophysiology and clinical finding of PKU **(Hamma, 2018).**

### 2.1.1 Impact of PKU on nervous system:

The impact of PKU on the nervous system involves several mechanisms such as competitive mechanisms at the transporter level, impairment of neurotransmitters and protein synthesis, demyelination and oxidative brain damages.

#### 2.1.1.1 Competitive mechanism

Numerous studies have been conducted using various animal models to induce acute and chronic hyperphenylalaninemia (HPA) at different life stages. These studies have corroborated the findings that elevated blood phenylalanine (Phe) levels lead to a predominance of its absorption over the absorption of other large neutral amino acids into the brain due to competition at the blood-brain barrier (BBB) transporter.

This phenomenon is more pronounced in humans than in rodents due to the high affinity of the large neutral amino acid carrier for Phe in humans**.** Recent studies employing advanced imaging techniques in humans have shown similar results in PKU patients, further supporting the hypothesis of competition for the blood-brain barrier LNAA (large neutral amino acide) transporters and its role in the cascade of neurological effects of HPA **(Surtees and Blau, 2000)** This excess Phe can cross into the brain through the blood-brain barrier (BBB) via a transporter called LAT1 **(SLC7A5) (Feillet *et al.,* 2010).** Phenylalanine and neutral amino acids (tyrosine, tryptophan, threonine, methionine, valine, isoleucine, leucine, histidine) reach the brain via the same common transporter (LAT1, large neutral amino acid transporter) in a competitive manner **(Wiedemann *et al.,* 2020 ;Vanspronsen *et al.,* 2021 ).**

The depletion of tyrosine (Tyr) in the brain is exacerbated by a competitive process in which phenylalanine (Phe) and Tyr compete to cross the blood-brain barrier. This barrier regulates the passage of large neutral amino acids (LNAA) through a single transport system characterized by high affinity but low capacity meaning that it can only transport a limited number of amino acids at a given time. When plasma levels of one amino acid rise, the transport system becomes saturated, blocking the entry of other LNAA, thereby causing its depletion in the brain **(Figure 11)**. Consequently, increased plasma levels of Phe impede the influx of Tyr into the cerebral tissue leading to imbalances in brain chemistry **(De Groot *et al.,* 2013).** This flood of Phe soon reaches dangerously toxic levels, creating serious health issues**.**

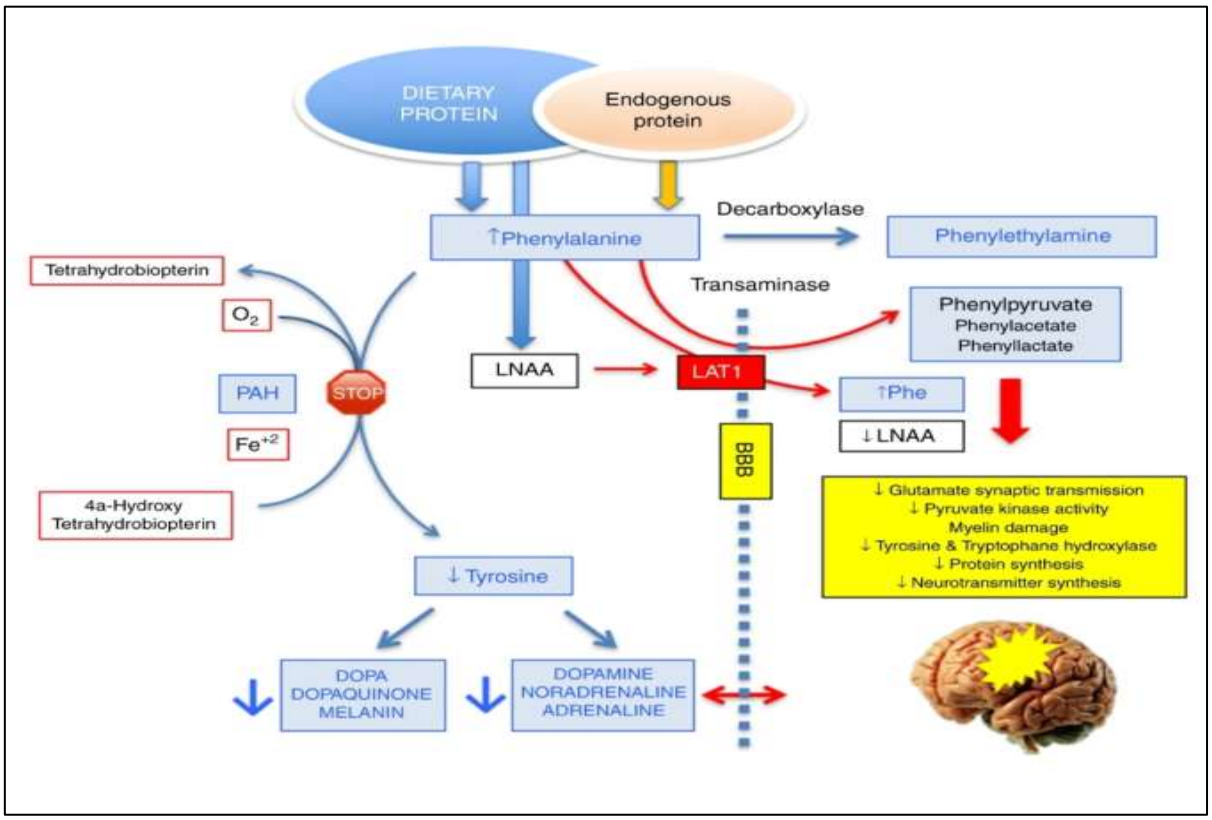


**Figure 11.** Competitive transport mechanism between Phe and LNAA **(Rausell *et al.*, 2019).**

### 2.1.1.2 Alteration of neurotransmitters

The metabolic imbalance caused by PKU affects neurotransmitter levels and neuroendocrine system. Tyrosine and tryptophan are two amino acids precusors of the biosynthesis of the neurotransmitter monoamines dopamine, noradrenaline and serotonin. The production of the neurotransmitters depends on the abundance of their precursors tyrosine and tryptophan in the brain, alongside normal levels of phenylalanine **(Surtees and Blau, 2000)**. Individuals with phenylketonuria (PKU) experience a reduced levels of neurotransmitters such as dopamine, noradrenaline, and serotonin, alongside lower amounts of tyrosine and tryptophan due to elevated phenylalanine (Phe) in the brain, resulting in Research conducted on PKU patients has revealed decreased production of neurotransmitter metabolites in both urine and cerebrospinal fluid (CSF). Lowering plasma Phe concentrations has been observed to elevate neurotransmitter metabolites. These neurotransmitters play crucial roles in executive brain functions and the initiation of movement **(Surtees and Blau, 2000).** Phe competitively inhibits the activities of Tyr hydroxylase (TH) and tryptophan hydroxylase (TPH) in the brain, leading to deficiencies in dopamine and serotonin. This mechanism is probably related to the high prevalence of anxiety and mood disorders in affected individuals

**(Van spronsen *et al.*, 2021).**Dopamine plays a critical role in the functioning of prefrontal pyramidal neurons associated with tasks such as working memory and inhibitory control **(Anderson and Leuzzi, 2010).** Even with treatment, there is evidence that dopamine deficiency can lead to impairments in executive functions of the brain. More specifically, the dorsolateral prefrontal cortex **(figure12),** which receives an important dopamine projection, is responsible for these executive functions and it shown a clear effect with dopamine levels. Children with early treated PKU and moderate hyperphenylalaninemia (HPA) show impairments in these functions compared to those of the same age group, and the severity of damage proportional to the severity of HPA **(Surtees and Blau, 2000).**

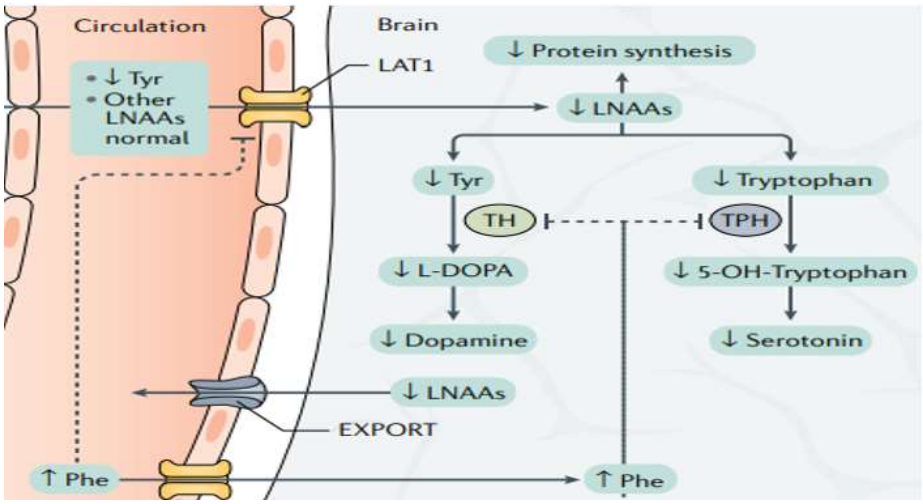


**Figure 12.** Alteration of neurotransmitter synthesis in PKU **(Van spronsen *et al.*, 2021).**

### 2.1.1.3 Alteration of protein synthesis

PKU also affects protein synthesis in the brain, causing a decrease in brain weight and leading to microcephaly due to "cellular undernutrition." The competition among amino acids reduces the flow of neutral amino acids entering the brain, prioritizing phenylalanine (Phe). Both acute and chronic hyperphenylalaninemia (HPA) decrease the integration of amino acids into brain protein **(Surtees and Blau, 2000).**

### 2.1.1.4 Demyelination

A deficiency in myelination has been discovered. There are various mechanisms responsible of demyelination **(Figure13),** (including the abnormalities of oxidative stress, a deficiency in Coenzyme Q10, activation of genes within the brain by Phenylalanine, and notably, abnormalities in neurotransmitters associated with deficits in the serotonin,

catecholamine, and glutamate systems **(HAS, 2018).** Phenylketonuria (PKU) can have a negative impact on myelination. A deficiency in myelination arises when myelinating oligodendrocytes, in the presence of phenylalanine (Phe), exhibit a non-myelinating characteristic by excessively expressing GFAP (Glial Fibrillary Acid Protein) **(chopin, 2013).**

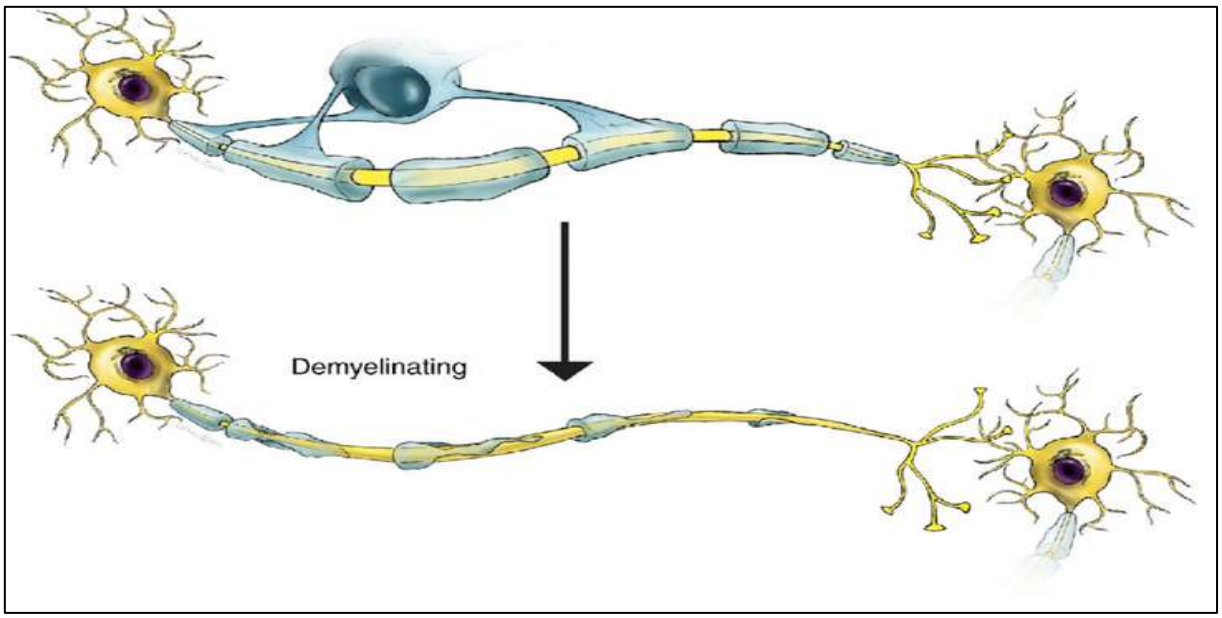


**Figure13.** Axonal demyelination **(Miller *et al.*, 2018)**

Phenylketonuria (PKU) profoundly affects myelination by many ways:

**a)-Delayed myelination**

Elevated levels of phenylalanine in individuals with PKU can interfere with myelination process, that they may experience delays in the formation of myelin sheaths surrounding nerve fibers. This delay can affect the transmission of nerve signals and contribute to developmental delays and neurological deficits observed in individuals with untreated PKU.

**b)- White matter abnormalities**

The brain's white matter can be adversely affected by elevated levels of phenylalanine and its metabolites. Itcomprises nerve fibers encased in myelin, which are crucial for interregional brain communication. Disruption of white matter integrity due to PKU can impair cognitive function, motor skills, and other neurological processes. PKU-related abnormalities in myelination may contribute to intellectual disability and cognitive

impairment. Adequate myelination is vital for efficient neural communication, learning, and memory. In individuals with PKU, disrupted myelination can hinder cognitive function and lead to learning difficulties.

In severe cases of untreated PKU, myelination abnormalities and white matter damage can manifest as neurological symptoms such as seizures, tremors, and movement difficulties. Continued exposure to elevated phenylalanine levels during childhood and adolescence can lead to declines in neurocognitive performance, attention deficits, and changes in IQ. These effects are linked to disruptions in dendritic pruning, synaptic remodeling, and crucial myelination processes necessary for proper brain function **(Nave and Werner, 2014; Thau-Zuchman *et al.*, 2022).**

Understanding demyelination, or the loss of myelin, in PKU requires an understanding of the stability and interaction of Myelin Basic Protein (MBP) with myelin membranes. The MBPis a key component of the myelin sheath, contributing to its structure, stability, and function. It is essential for efficient nerve impulse transmission and overall neurological health.

Phe toxicity may have an impact on oligodendrocyte precursor cells (OPCs), which are involved in myelin repair, but it does not cause remyelination. High Phe levels may hinder OPC proliferation and their capacity to differentiate into myelinating oligodendrocytes, further hindering myelin repair and maintenance. It is unclear if OPCs can return to a myelinating phenotype after Phe levels are corrected. **(Thau-Zuchman *et al.*, 2022).**

#### 2.1.1.5 Oxidative brain damage

The accumulation of potentially toxic metabolites of Phe in the blood does not seem to be sufficient to cause brain injury on its own but there are other parts of the puzzle under investigation. indeed. High levels of phenylalanine can disrupt various metabolic pathways and may contribute to oxidative stress by increasing the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS).

Even with controlled phenylalanine (Phe) levels, patients may still encounter neurocognitive issues, suggesting other contributing factors. Metabolomic studies have identified disruptions in protein synthesis, energy metabolism, and oxidative stress as significant factors. Elevated Phe levels also impact the epigenome in both blood and brain tissue by altering the methylation patterns of certain genes and microRNAs. Additionally,

evidence supports heightened oxidative stress in PKU, as evidenced by increased lipid peroxidation and microglial activation observed in mouse models **(Van Spronsen *et al.*, 2021).**

The proposed mechanisms underlying the physiopathology of PKU involve changes in brain metabolism, mitochondrial dysfunction, and increased oxidative stress, which are further exacerbated by dietary variables and weakened antioxidant defenses. This oxidative stress triggers cellular damage, particularly harmful to sensitive cerebral tissue. It increased the severity of manifestations in PKU patients, often resulting in diminished myelin sheath integrity, disrupted axonal conduction, and impaired synaptic transmission velocity, thereby compounding cerebral impairment **(Huttenlocher, 2000).** Despite strict adherence to dietary regimens, oxidative stress remains a formidable force in perpetuating cerebral deterioration in PKU, emphasizing the necessity for effective management strategies. Subsequent research demonstrates that high concentrations of Phe induce oxidative damage to proteins and lipids in specific brain areas **(Rausell *et al.*, 2019) (Figure 14).**

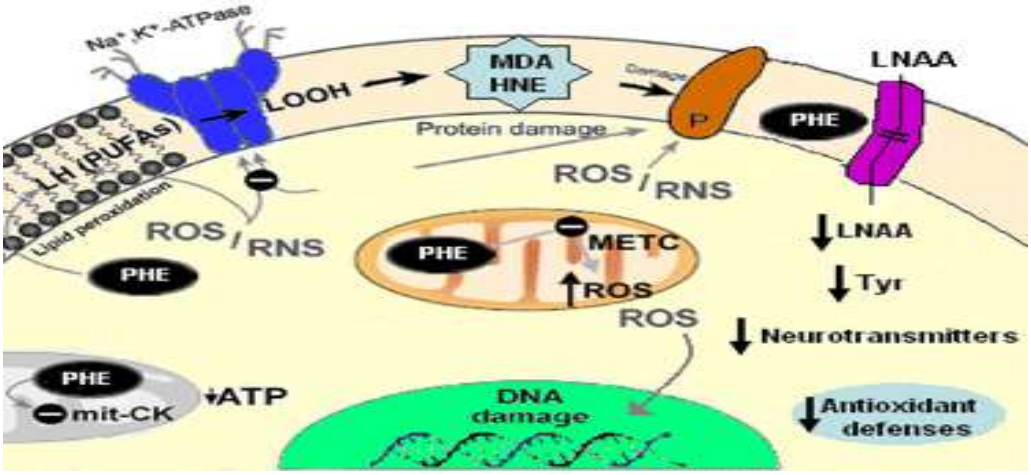


**Figure 14.** Suggested pathomechanisms for neurological damage in PKU **(Ribas *et al*., 2011)**

According to studies on Wistar rats, high Phe concentrations suggest the inhibition of key enzymes necessary for brain energy metabolism **(Rech,V.C *et al* ., 2002).** Experiments using astrocyte cultures show that Phe stimulates the generation of free radicals and disrupts mitochondrial function, leading to impaired cellular function or death **(Dobrowolski *et al.*, 2022).**It has been demonstrated that one early-identified feature of PKU disease is decreased brain metabolism. Legacy data from the late 1960s to the mid-1970s found that phenylpyruvate, a byproduct of phenylalanine metabolism, impedes mitochondrial pyruvate transport **(Figure15)**. This finding may be linked to a reduction in energy metabolism in the

brain. Respirometry results were also obtained with studies on the mitochondria in the brains of PKU patients, revealing reduced induction of respiratory chain complex 1 in response to pyruvate substrate, suggesting impaired energy transfer. affecting energy production and overall brain function. PKU may also be involved in the formation of amyloid plaques in the brain, related with neurodegenerative disease like Alzheimer's disease, and it is worth noting the increase in oxidative stress and its damage **(Dobrowolski *et al.*, 2022).**

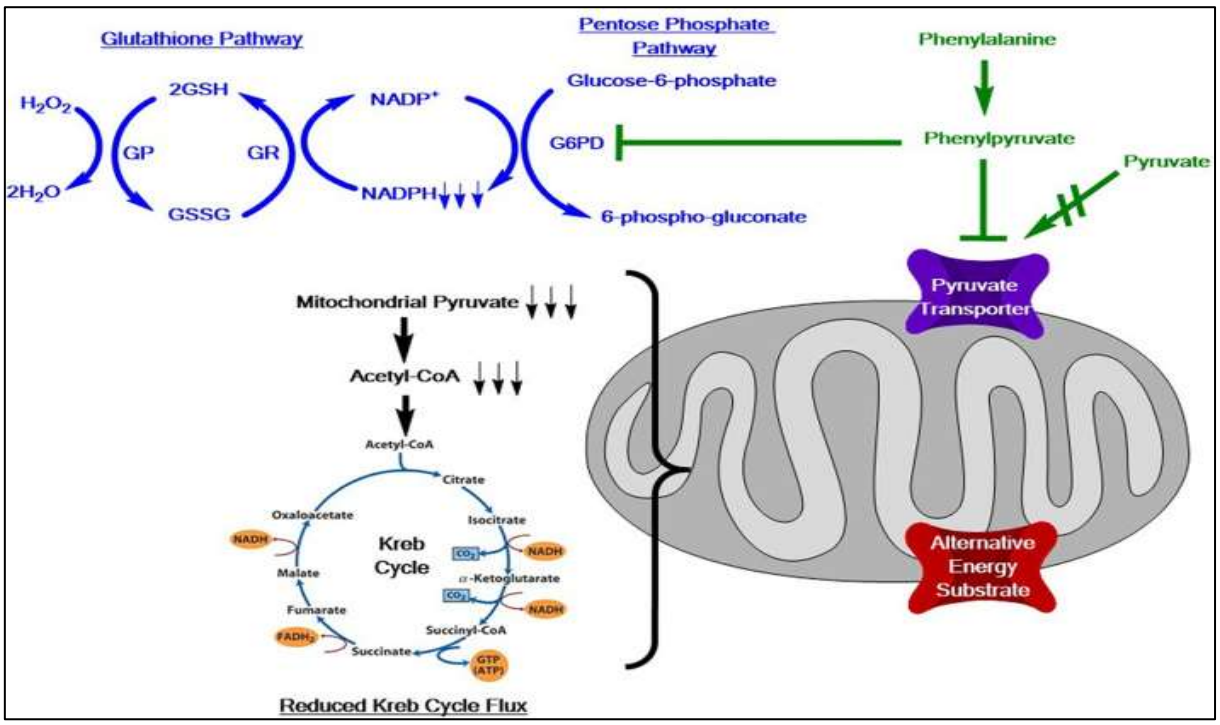


**Figure15.** PKU and Energy dysregulation **(Dobrowolski *et al.*, 2022).**

Chemically induced hyperphenylalaninemia may cause dysfunction by reducing the activity of the mitochondrial electron transport chain. The increased phenylalanine levels may cause deficits in coenzyme (Q10) and downregulate genes linked to oxidative phosphorylation. This promotes access to the cytosol and aids in oxidative damage to mitochondrial DNA. **(Bortoluzzi *et al.*, 2021).**

### 2.1.2 Dermatological impact of PKU

It is relevant to mention that although PKU primarily affects the brain, hypopigmentation constitutes the only non-neurological symptom associated with the disease noting the role of tyrosinase (TYR) in hypopigmentation status **(Van Spronsen *et al.*, 2021; Wiedemann *et al.*, 2020).**

As well as skin discoloration, lighter skin and hair and eye color, also skin disorders like eczema. These dermatological problems arise from insufficient production of melanin, a secondary metabolite of tyrosine metabolism, the first two steps of metabolism are catalyzed by tyrosinase, in which L-tyrosin converts to L-DOPA and then L-DOPA changes to a quinone which spontaneously polymerizes to produce melanin pigments **(Van Wegberg *et al.*,2017) (Figure16) .**

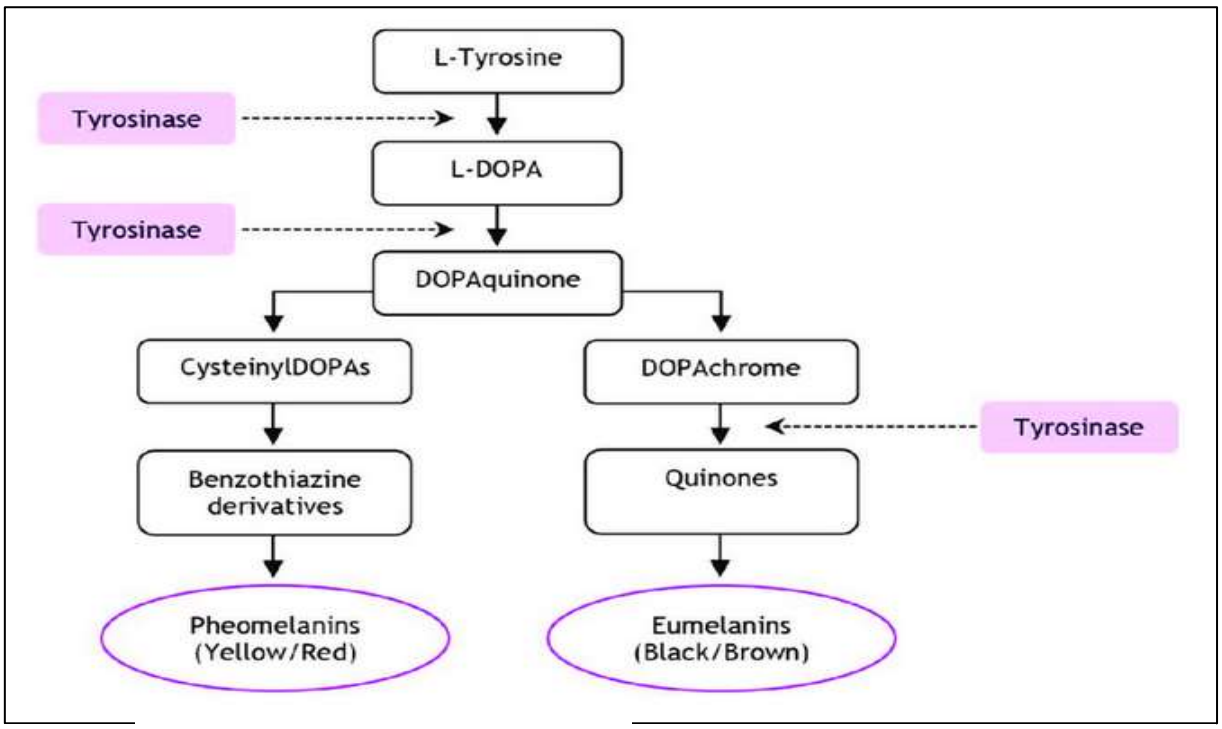


**Figure 16.** Melanin synthesis Pathway **(Raoufinejad and Rajabi, 2021).**

Phenylketonuria (PKU) is linked to several dermatological issues, often termed pseudo-scleroderma, stemming from the accumulation of phenylalanine and phenylpyruvic acid in circulation and skin tissues. These include cellulitis due to immune cell dysregulation and persistent skin bleeding, inflammatory skin conditions like psoriasis and parapsoriasis, and bleeding problems contributing to skin distortions. PKU patients may develop benign skin tumors and have an elevated risk of skin cancer, particularly melanoma. Additionally, they may experience dermatitis, eczema, and vitiligo, though the latter is not categorized as pseudo-scleroderma **(Asare and Opoku, 2018).**

### 2.1.3 Characteristic urine odor

In addition to the impact of PKU on the nervous system and skin, a distinctive feature of PKU is the characteristic "mousy" or "musty" odor in the urine,breath and sweat of affected

individuals. This odor is the result of phenylacetic acid, a by-product of phenylalanine metabolism. Phenylacetic acid is produced when phenylalanine accumulates in the body and is not properly converted into tyrosine. This specific odor can be a crucial diagnostic clue for healthcare providers **(Scriver and Kaufman, 2001).**

## 2.2 Classification of PKU

The severity of phenylketonuria (PKU) is determined by the individual's daily tolerance for phenylalanine (Phe). The typical classification method relies on the blood Phe concentration before treatment and the daily dietary allowance of Phe. Patients were classified into three phenotypes: cPKU, mPKU, and MHP Based on pretreatment plasma Phe levels. The majority of patients had compound heterozygous genotypes, with cPKU being the most common phenotype observed:

- **Type 1**: **Classical phenylketonuria cPKU**

The severest form of pku, characterized by Phe levels more than 1200 μmol/L.

- **Type 2: Mild phenylketonuria mPKU**

**Also nown as** also atypical involved a spontaneous decline in Phe levels from above 1200 μmol/L after day 2 to 1200–600 μmol/L.

- **Type 3: Mild hyperphenylalaninemia (mHPA)**

showed fluctuating Phe levels approximately 600 μmol/L **(Blau *et al.*, 2011; Li *et al.*, 2018;Wiedemann *et al.*, 2020).**

The classification of patient phenotypes is shown in **(Figire17),** among the 1104 patients, 217 were classified as having MHP (19,66%), 301 as having mPKU (27,26%), and 561 as having cPKU (50,82%); information about the clinical phenotype of the remaining 26 patients (2.26%) was not available **(Li *et al.*, 2018).**

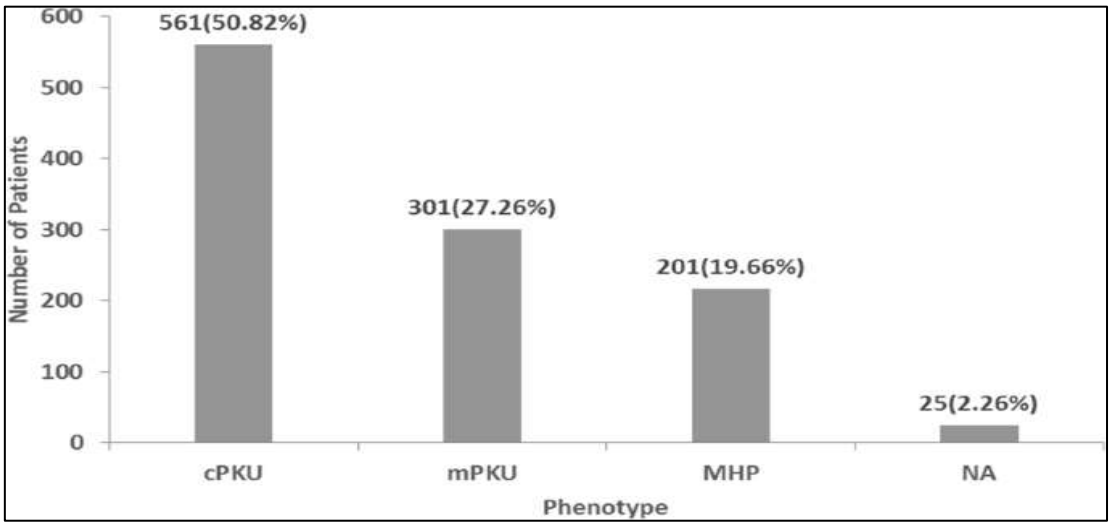


**Figure 17.** PKU phenotypesclassification **(Li *et al.*, 2018).**

### 2.3 Evolution of the disease

The evolution of the disease is largely depending on the severity of the first occurrence of PKU,Complications vary with age and adherence to treatment. Childhood outcomes are usually favorable with proper diet and treatment compliance. However, cognitive outcomes are largely influenced by metabolic control during the first decade of life. Later relaxation of treatment can lead to executive function abnormalities and behavioral issues. Severe neurological impairments have been observed in patients who have neglected treatment **(Van Spronsen *et al.*, 2017).**

Early detection through newborn screening is crucial to prevent the cascade of symptoms that can emerge if left untreated. Without intervention, PKU can inflict serious neurological and developmental damage, impacting not only infancy but extending into adolescence and adulthood.

#### 2.3.1 Infancy and early childhood

During the newborn period, PKU might not immediately manifest obvious symptoms, but subtle signs like feeding difficulties and a distinct musty odor can be early indicators. If undetected and untreated, the disorder progresses, potentially leading to significant developmental delays and behavioral abnormalities in early childhood. These may include failure to meet developmental milestones and exhibiting hyperactivity or seizures **(van Spronsen *et al.*, 2021; Wiedemann *et al.*, 2020).**

Figure 18 depicts two children with PKU: The first one (A) shows a child with subtle signs like an eczematoid rash, brown eyes (highlighting not all patients have blue eyes),

epicanthal folds, and left internal strabismus. The second one(B) diagnosed with PKU at 1 month old, characterized by blue eyes, fair skin, and blond hair.

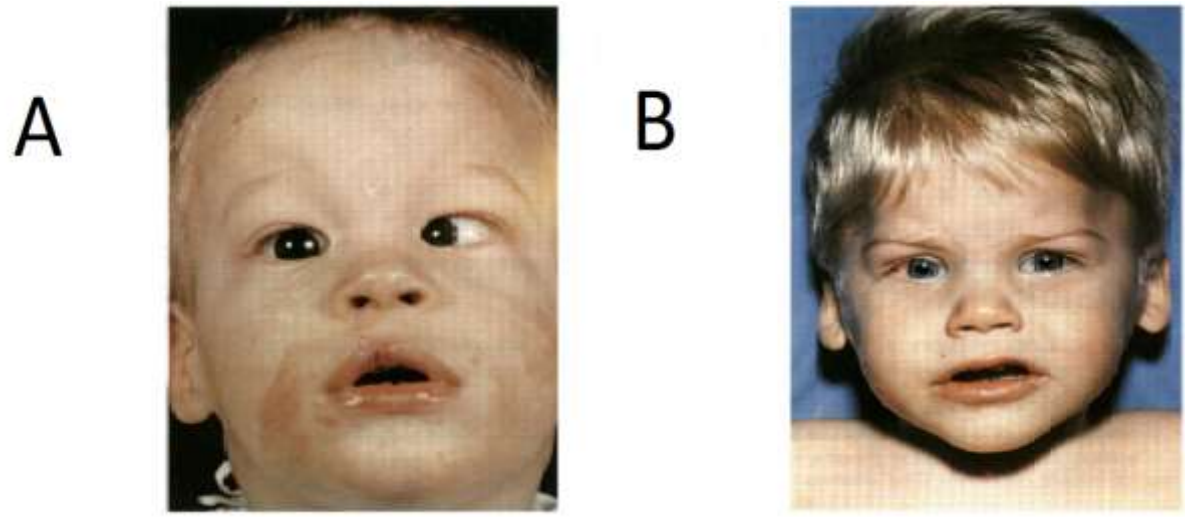


**Figure 18.** Children with phenylketonuria **(Potrous *et al.*, 2019).**

### 2.3.2 Adolescence and adulthood

As individuals with PKU enter adolescence and adulthood, the untreated symptoms can exacerbate, with cognitive impairments affecting academic and occupational pursuits. Behavioral and psychiatric issues, such as depression and anxiety, may emerge alongside neurological problems like tremors and seizures. Adverse effects may emerge due to higher Phe concentrations during adolescence and adulthood, affecting attention, mood, memory, and executive function **(van Spronsen *et al.*, 2021; Wiedemann *et al.*, 2020).**

Adults with PKU may develop lower extremity spasticity, cerebellar ataxia, tremor, encephalopathy, and visual abnormalities. Dementia has been described in adults with PKU. Immediate dietary treatment after birth prevents major cognitive and neurological deficits. Incidence of attention-deficit–hyperactivity disorder and specific learning disabilities might remain higher in well-treated patients **(Wiedemann *et al.*, 2020; van Spronsen *et al.*, 2021).**

# CHAPITER 03: Diagnosis And Treatment

# 3 Diagnosis and treatment

## 3.1 Diagnosis of phenylketonuria

PKU diagnosis indeed involves various methods, from the Guthrie test, which detects elevated phenylalanine levels in blood, to genetic testing (genotyping) that identifies specific genetic mutations associated with PKU. These methods play crucial roles in early detection and management of the condition.

### 3.1.1 Newborn screening

Robert Guthrie developed an effective test for using a bacterial inhibition assay based on Bacillus subtilis, which relies on phenylalanine for growth **(Guthrie and Sus, 1963).** The Guthrie test is a semi-quantitative assay designed to detect elevated blood levels of the amino acid phenylalanine, using the ability of phenylalanine to facilitate bacterial growth in a culture medium with an inhibitor. A drop of blood is usually obtained by pricking the heel of a newborn infant on the second or third day of life. The blood is collected on a piece of filter paper (Guthrie card). The dried blood spot (DBS) on the filter paper is punched out into small disks (**Figure19)** and placed on an agar gel plate containing Bacillus subtilis and β-2-thienylalanine. The agar gel supports bacterial growth, but the β-2-thienylalanine inhibits bacterial growth. However, in the presence of extra phenylalanine leached from the impregnated filter paper disk, the inhibition is overcome and the bacteria grow. Within a day, the bacterial growth surrounding the paper disk is visible to the eye **(Figure 20).** The amount of growth, measured as the diameter of the colony, is roughly proportional to the amount of phenylalanine in the serum. The result is read by comparing the diameter of each sample disk's colony to the colonies of a series of reference disks with standard phenylalanine content included on each large plate **(Dave and Das, 2010).**

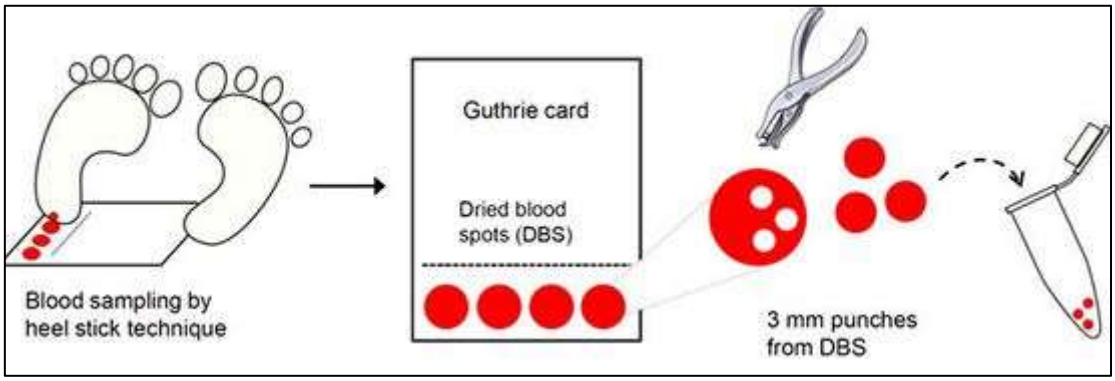


**Figure 19.**Blood Spot Sample Preparation **(Sharer, 2021).**

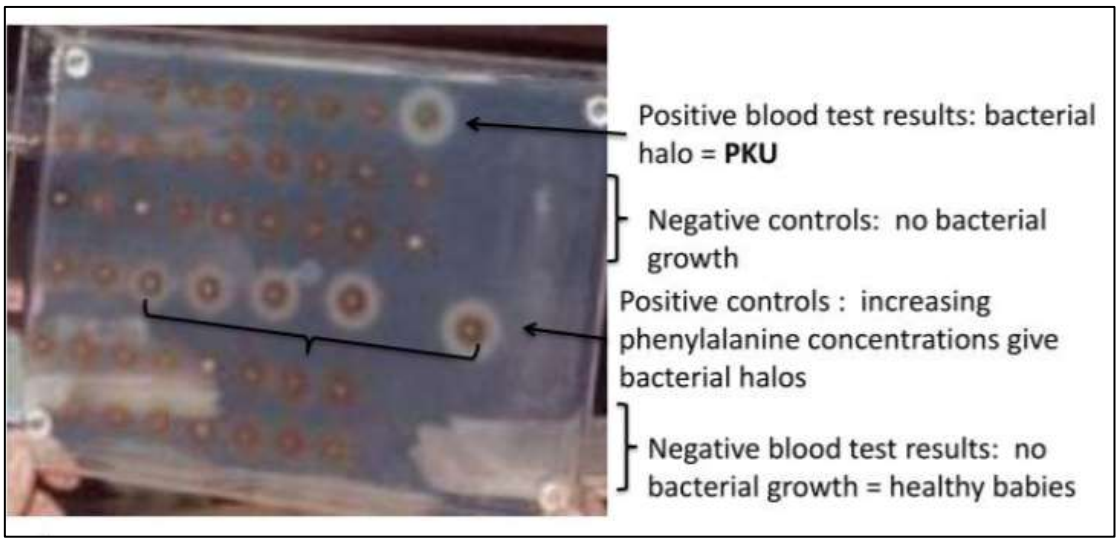


**Figure 20.**Bacterial plate with newborn blood samples **(Dave and Das, 2010).**

Tandem mass-spectrometry (TMS) emerged as a rapid method for accurately measuring amino acid concentrations in dried blood spots **(Figure 21)**, reducing false positives by assessing both phenylalanine (Phe) and tyrosine (Tyr) levels **(Figure 22),** and identifying other metabolic disorders simultaneously **(Blau *et al.*, 2014).**

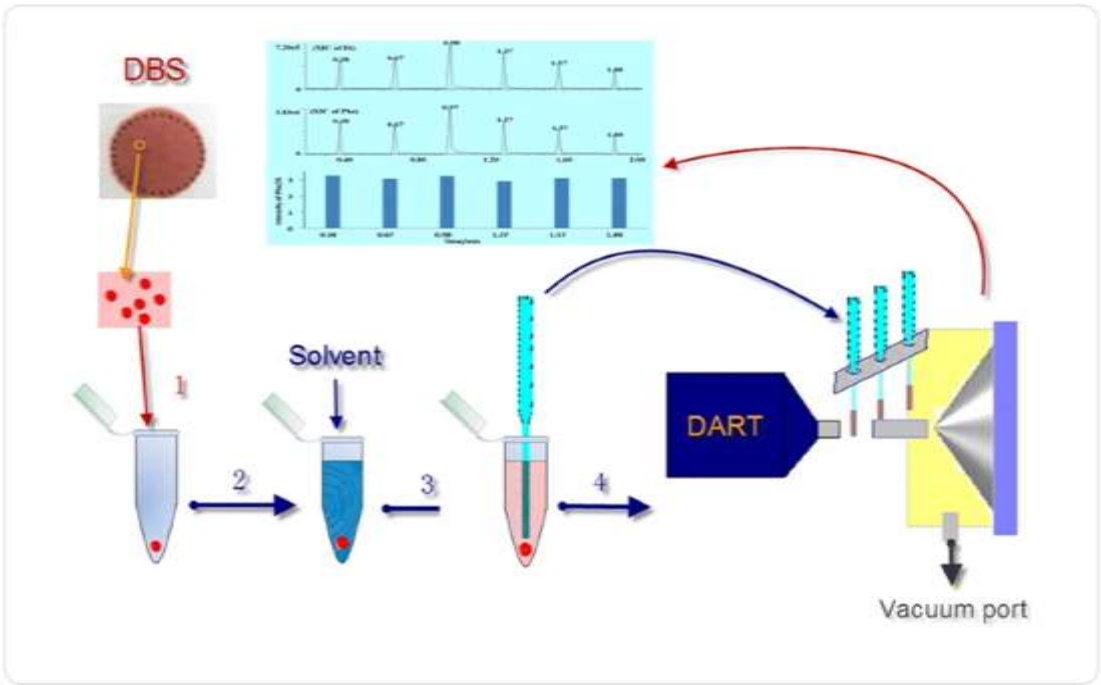


**Figure 21.**rapid screening of PKU by Tandem mass-spectrometry (**Wang *et al,* 2013).**

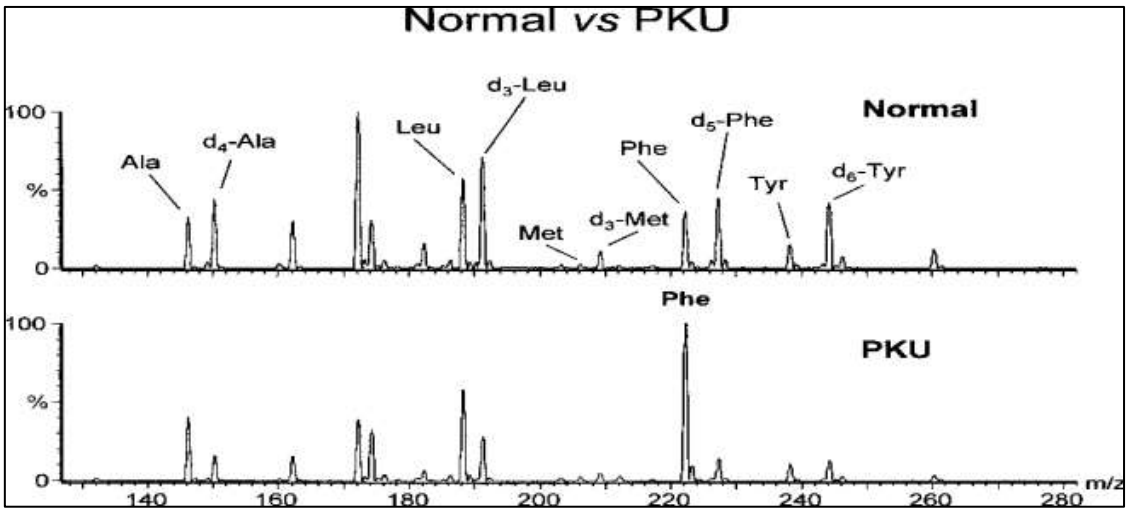


**Figure 22.** Amino Acid profile generated by TMS **(Banta-Wright and Steiner, 2004).**

Early PKU screening, typically conducted between days 2 and 7 of life, aims to enable timely dietary interventions to prevent neurological damage. However, concerns arise regarding screening too early, potentially leading to false negatives due to inadequate phenylalanine buildup. Current consensus suggests sufficient sensitivity for screening within 24 hours, especially when incorporating Phe/Tyr ratios. Yet, new cutoffs are needed for classifying PKU phenotypes when measuring Phe levels so early.

Premature infants may exhibit transiently elevated blood Phe levels due to immature enzyme systems, potentially yielding positive PKU screening results. Interpretation of early PKU screens should be cautious in sick or parenterally fed neonates, with second screenings recommended if initial results are unclear regarding protein intake **(Banta-Wright and Steiner, 2004).**

### 3.1.2 Differential diagnosis

This refers to the process of distinguishing between PKU and other conditions that may present with similar symptoms. As mentioned earlier, conditions such as biopterin deficiency, hypothyroidism, maple syrup urine disease, tyrosinemia, nonketotichyperglycinemia,,methylmalonicacidemia, Hartnup disease, and others may need to be considered in the differential diagnosis of PKU. Differential diagnosis typically involves a comprehensive evaluation of clinical symptoms, biochemical tests, genetic analysis, and sometimes imaging studies to rule out other possible causes. Roughly 2% of instances where newborn screening detects heightened blood phenylalanine (Phe) levels result from disorders in tetrahydrobiopterin (BH4) metabolism, underscoring the importance of consistently

considering a wide range of diagnoses for even slightly elevated Phe levels. BH4 deficiency occurrence is more prevalent in certain countries where consanguineous marriages contribute to the perpetuation of genetic disorders within families **(Blau *et al.*, 2010)**. BH4 deficiencies pose more severe challenges compared to PKU regarding response to therapy, necessitating distinct treatment approaches. Implementing a low-Phe diet is ineffective, while early substitution with dopamine, serotonin precursors, and synthetic BH4 (sapropterindihydrochloride) is crucial for favorable outcomes.

Analyzing dried blood spots (DBS) or urine for neopterin and biopterin, along with measuring dihydropteridinereductase (DHPR) activity in DBS, is pivotal for accurate diagnosis and should be conducted as soon as possible. Additionally, BH4 loading tests and evaluating neurotransmitter metabolites, pterins, and folates in cerebrospinal fluid provide further valuable insights into disease severity **(Longo, 2009)**.

In individuals with BH4 deficiency, pterin patterns remain consistent across blood, urine, and cerebrospinal fluid (CSF). Although utilizing DBS on filter paper (Guthrie card) is more convenient and enables the measurement of pterins, DHPR activity, and amino acids from a single sample, it's essential to note that classic PKU patients excrete higher pterin levels in urine compared to healthy individuals, directly correlating with blood Phe levels. Conditions that trigger immune system activation (elevated neopterin) or therapies like methotrexate for cancer or rheumatic diseases (DHPR inhibition) may affect analytical procedures. Some patients with DHPR deficiency may exhibit a normal blood or urinary neopterin and biopterin profile, emphasizing the necessity of DHPR activity measurement in all patients with hyperphenylalaninemia (HPA), regardless of pterin measurements **(Opladen *et al.*, 2011)**.

### 3.1.3 BH4 loading test

Originally, the BH4 loading test served to distinguish between individuals with elevated phenylalanine (Phe) levels due to phenylalanine hydroxylase (PAH) deficiency and those with elevated Phe levels due to BH4 deficiency (enzyme defects in BH4 cofactor biosynthesis or regeneration). Thus, this test served as an additional valuable tool for early detection of BH4 deficiencies and was utilized in Europe for nearly three decades. Furthermore, this test identifies PKU patients responsive to BH4 administration. Identifying BH4-responsive PKU patients is significant because some PKU patients experience a decrease or normalization of blood Phe levels under pharmacological therapy with BH4

(sapropterindihydrochloride) **(Figure23).** The concept of BH4-sensitive PKU was initially observed in Japanese patients and later confirmed through extensive studies with large patient groups. BH4 challenge procedures vary, ranging from a 24-hour test with a single BH4 administration (10–20 mg/kg) to multiple weeks of administration with regular blood Phe level monitoring **(Anjema *et al.*, 2016)**. It is generally agreed that a blood Phe reduction of at least 30% following BH4 loading indicates a clinically relevant effect, though some centers may establish lower cut-off values for individual patients or opt not to use specific cut-off values. BH4 responsiveness is most prevalent in patients with mild (non-PKU) hyperphenylalaninemia or mild PKU resulting from PAH mutations allowing residual enzyme activity. Conversely, the response rate is very low among patients with classic PKU (minimal or no residual PAH activity). Several PAH mutations associated with BH4 responsiveness have been identified, and genotyping serves as a useful additional tool for predicting responsiveness **(Van Spronsen *et al.*, 2021).**

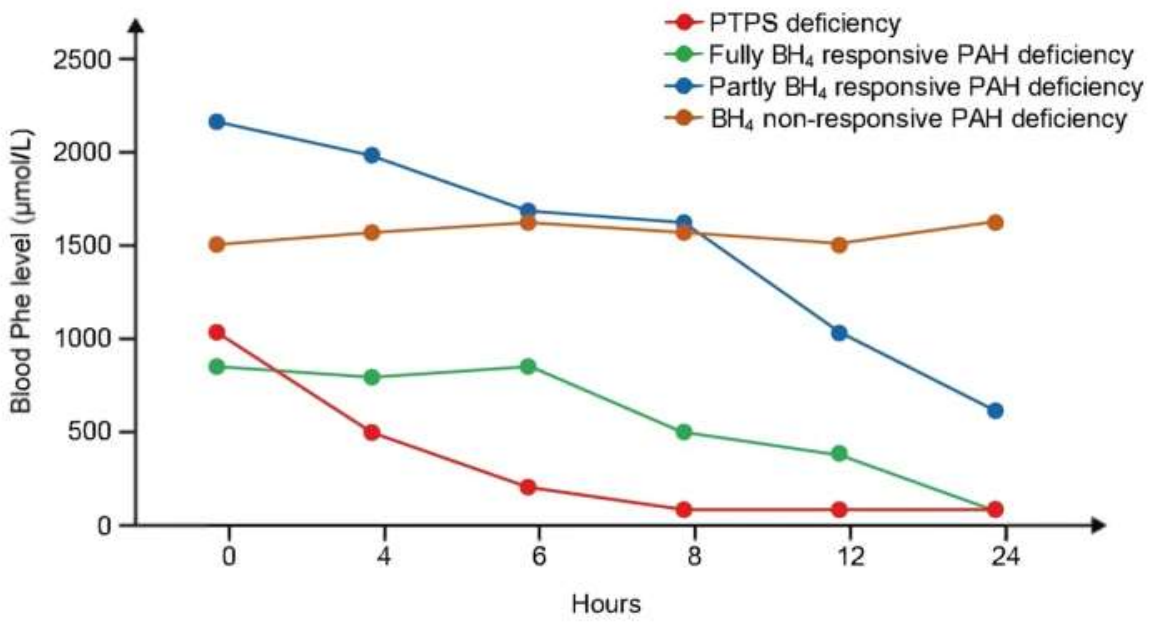


**Figure 23.** Examples of neonatal $BH_4$ loading test outcomes **(Muntau *et al.*, 2018) .**

In newborns, the test should be conducted before initiating the low-Phe diet and at elevated blood Phe levels (≥400 µmol/L). For infant or adult PKU patients on a Phe-restricted diet, protein intake should be adjusted by incorporating sources like egg or milk powder before and during the test.

while the BH4 loading test focuses on assessing responsiveness to BH4 therapy in PKU patients, the process of conducting a differential diagnosis aims to identify the underlying cause of elevated Phe levels by considering a broad range of potential disorders.

### 3.1.4 Cerebrospinal fluid investigation

Deficiency in tetrahydrobiopterin (BH4) affects the production of catecholamines, serotonin, and nitric oxide within the central nervous system (CNS), and evaluating their metabolites in cerebrospinal fluid (CSF) is crucial for distinguishing between various forms (severe versus mild) of BH4 deficiencies. The measurement of not only the absolute levels of 5-hydroxyindoleacetic acid and homovanillic acid in CSF but also differences in the ratios of neurotransmitter levels offers valuable diagnostic insights into the severity and prognosis of BH4 deficiency **(Blau *et al.*, 2011)**.

### 3.1.5 Genotyping

Patient genotyping isn't mandatory for diagnosing phenylketonuria, it can provide insight into the extent of protein dysfunction, residual PAH activity, and consequently, the metabolic phenotype. Classifying PAH genotypes may aid in predicting biochemical and metabolic phenotypes for various genotypes, potentially assisting in managing hyperphenylalaninemia in newborns. Additionally, the patient's genotype may offer some indication of BH4 responsiveness, with variants associated with higher residual enzyme activity more likely to respond to BH4 treatment. The BIOPKU database lists alleles known to be responsive to BH4 treatment. Patients with genotypes indicating non-responsiveness to BH4 treatment should avoid BH4 testing, while those with genotypes featuring two responsive variations may opt for a treatment trial instead of a BH4 loading test. BH4 loading should be considered for all other patients. Prenatal diagnosis for PKU is feasible, but genetic counseling considerations encompass various factors, including ethical, religious, and legal aspects specific to each country **(Van Wegberg *et al.*, 2017).**

## 3.2 Treatment

### 3.2.1 Therapy initiation

Treatment for PKU should commence at the earliest opportunity, ideally within the first week after birth, aiming to achieve blood Phe levels within the therapeutic range within the initial two weeks of life. Upon diagnosis, efforts should be made to promptly adjust blood PHE levels to the desired therapeutic range. Depending on the initial blood PHE levels, PHE

exclusion from the diet may be necessary until levels approach the therapeutic range, followed by the introduction and adjustment of a Phe-restricted diet. Breastfeeding can often be continued alongside medical formula. Early treatment initiation necessitates timely newborn screening, regular follow-up, and diagnostic assessments, along with transparent communication between the family and the primary healthcare provider, as well as access to appropriate specialized care **(Lichter-Konecki and Vockley, 2019).**

Infants with blood Phe levels exceeding 600 µmol/l require treatment. While many treatment centers in North America now begin treatment at a Phe level of 360 µmol/l or higher, the evidence regarding clinical outcomes in untreated patients with blood Phe levels between 360 and 600 µmol/l is varied, with some studies indicating normal outcomes and others indicating subtle neurocognitive deficits. Further research is needed to guide decisions regarding the treatment of individuals with Phe levels in this range. Given the potential risk of neurocognitive consequences, it is advisable to treat infants with sustained blood Phe levels >360 µmol/l following appropriate discussion with parents about the controversy. Although a definitive threshold for the adverse effects of elevated blood Phe has not been established, treatment for infants with Phe levels between 120 and 360 µmol/l is not recommended. However, these individuals should be monitored for at least the first two years of life to ensure that levels do not rise with increased protein intake. If treatment is not deemed necessary before two years of age, monitoring on an annual or biennial basis is sufficient for subsequent evaluation **(Vockley *et al*., 2014).**

### 3.2.2 Dietary treatment

Treatment options for phenylketonuria still have room for improvement, leading to the search for innovative alternatives. New dietary approaches involve more appealing formulas with enhanced caloric content to enhance adherence. Generally, PKU diets lack sufficient taurine and other essential micronutrients found in animal products, as well as long-chain polyunsaturated fatty acids (LC-PUFAs) like arachidonic acid (AA) and docosahexaenoic acid (DHA). Studies have shown that adding LC-PUFAs to formulas can enhance visual system development and motor skills in PKU patients.

While synthetic amino acids are the main protein source in PKU management, di-tripeptides are absorbed more efficiently. Glycomacropeptide (GMP), derived from cheese whey, is low in phenylalanine and improves diet taste, variety, satiety, and compliance, thus enhancing metabolic control and quality of life for patients. However, financial constraints

hinder some families' ability to afford formula and low protein foods. Efforts to enhance adherence to dietary treatment include protein restriction, with supplementation of Phe-free medical formulas, and the development of a wider range of medical foods, some containing GMP. Monitoring dietary treatment involves frequent measurement of plasma/blood phenylalanine levels. Although a portable Phe monitoring device could boost adherence by providing real-time Phe levels, such a device has not been developed yet. Iontophoretic extraction of Phe from the skin is correlated with high plasma Phe levels but lacks sensitivity to detect low phenylalanine levels **(MacDonald *et al*., 2020).**

### 3.2.3 Large neutral amino acid supplementation

Alternative therapeutic approaches can be classified based on their mode of action or target organ, which includes enteral, systemic, and liver-directed methods. An example of an enteral approach is dietary restriction of phenylalanine (Phe) intake. Alternatively, Large neutral amino acids (LNAAs) can be utilized.

LNAAs compete with Phe for the same transporter across the gastrointestinal tract and blood-brain barrier, thereby reducing Phe absorption and entry into the brain. Research, including a double-blind, placebo-controlled study, suggests a significant reduction in blood Phe levels after LNAA treatment in patients with PKU for two weeks, indicating competition with Phe transport in the gastrointestinal tract. Oral LNAA supplementation has shown promise in reducing brain Phe concentrations and improving neuropsychological function, though outcomes may vary based on composition, dosing, and duration of supplementation **(Burlina *et al*., 2020).**

Studies in PKU mice indicate that restoring LNAA levels in the brain may enhance cognitive outcomes. Additionally, new medical foods fortified with higher LNAA concentrations, vitamins, and antioxidants like lutein have been developed to support brain development. Research in PKU mouse models suggests that non-physiological amino acids can act as competitive inhibitors of brain transporters, reducing brain Phe concentrations while minimally affecting other downstream intermediates **(Figure24).** However, evidence supporting the efficacy of LNAA supplementation in significantly reducing blood Phe levels in PKU patients is still limited. Studies have typically been short-term, with variable dosages and formulations of LNAA used. Positive effects on executive functions have been reported in some randomized controlled trials, particularly in patients with high Phe levels **(Strisciuglio and Concolino, 2014).**

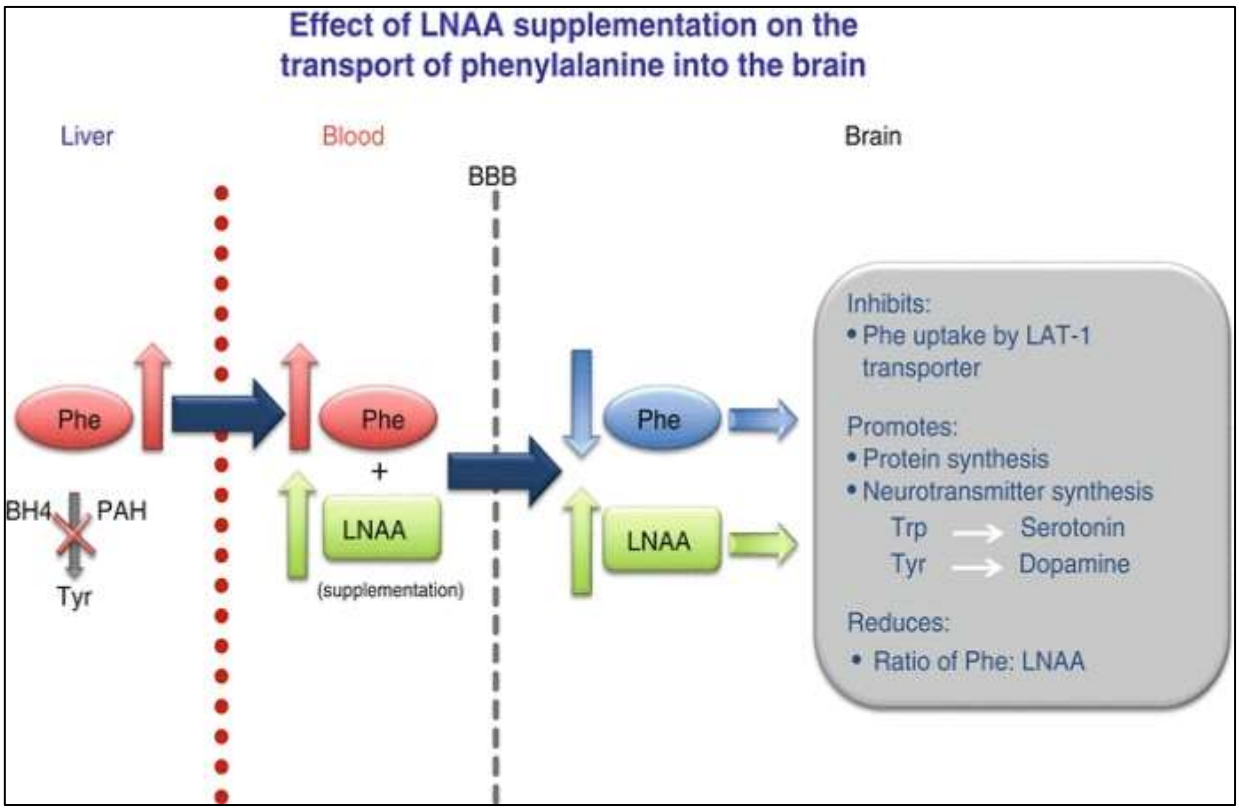


**Figure 24.** Effects of LNAA therapy on the transport of phenylalanine into the brain **(Yannicelli, 2015)**

Overall, while LNAA supplementation, either alone or combined with a low-Phe diet, has shown promise in improving health outcomes for individuals unable to adhere to the low-Phe diet, long-term studies assessing efficacy and safety are warranted.

### 3.2.4 Tetrahydropterin as enzyme enhancement therapy for PKU:

Some individuals with phenylketonuria exhibit a positive response to therapeutic doses of tetrahydropterin (BH4), as initially demonstrated in 1999. At these therapeutic doses, sapropterin hydrochloride serves as a molecular chaperone, aiding in the proper folding and stability of the PAH enzyme **(Kure *et al*., 1999).**

Guidelines regarding the assessment of BH4 responsiveness in patients with hyperphenylalaninemia are evolving. It is recommended that all patients with phenylalanine levels exceeding 360 µmol/L undergo testing for sapropterin responsiveness (at a dose of 20 mg/kg/day). Multiple phenylalanine measurements should be taken both before and after initiating BH4 treatment to accommodate normal fluctuations in phenylalanine levels. The efficacy of BH4 is assessed over short-term (up to 48 hours) and long-term (up to several

weeks) periods to ensure consistent reduction of phenylalanine levels compared to baseline. A reduction of at least 30% in blood phenylalanine from baseline indicates a positive response to sapropterin therapy. Typically, patients with milder phenotypes are more likely to respond to treatment. Long-term administration of sapropterin to responsive PKU patients enhances phenylalanine tolerance and may even allow some individuals to discontinue restrictive diets **(Evers *et al.*, 2019).**

The utilization of pharmacological chaperones to stabilize or facilitate the correct folding of mutant proteins presents a promising approach in treating various genetic disorders characterized by protein misfolding. Besides tetrahydrobiopterin, other proteins and small molecules may act as chaperones to assist in PAH folding.

### 3.2.5 Enzyme Therapy

Enzyme therapy presents a viable option for addressing phenylketonuria (PKU), wherein elevatedPhe levels can be mitigated through the introduction of Phe-metabolizing enzymes, altering the metabolic profile of PKU, regardless of the genotype. This therapy can be achieved via either enzyme replacement using phenylalanine hydroxylase or enzyme substitution with phenylalanine ammonia-lyase (PAL). Since Phe metabolism predominantly occurs in the liver, orthotopic liver transplantation can correct the metabolic profile. However, liver transplantation is not a standard treatment option for PKU, except for rare cases where patients require a liver transplant for conditions like cirrhosis, largely due to the significant therapy burden post-transplantation. In murine models, enzyme replacement with PAH-fusion proteins shows promise **(Spécola and Chiesa, 2017).**

Enzyme substitution therapy using phenylalanine ammonia-lyase appears more encouraging. PAL can serve as a stand-in for deficient PAH, converting excess systemic Phe into trans-cinnamic acid and ammonia. Both pharmacological and physiological efficacy has been demonstrated with PAL administration orally or via injection in PKU mouse models. Oral administration is complicated by enzyme proteolysis, yet the enzyme remains active within the gastrointestinal tract. Injected PAL can induce immunogenic responses; however, conjugation with polyethylene glycol (PEG-PAL) has shown success in reducing immune reactions **(Levy *et al*., 2018).**

Phenylalanine ammonia lyase is an alternative enzyme that can substitute for PAH by reducing the phenylalanine concentration in PKU (**Figure 25).** As a lyase (or deaminase) PAL

removes the amine (NH2) and a proton (H+) from phenylalanine to form ammonia (NH3) leaving a deaminated and desaturated phenylalanine (transcinnamic acid). The trans-cinnamic acid is converted to benzoic acid which is conjugated with glycine in the liver and excreted as hippuric acid (benzoylglycine) while the ammonia is metabolized via the urea cycle and largely excreted as urea. PAL is a non-mammalian protein, so has been PEGylated (PAL-PEG) to reduce its immunogenicity

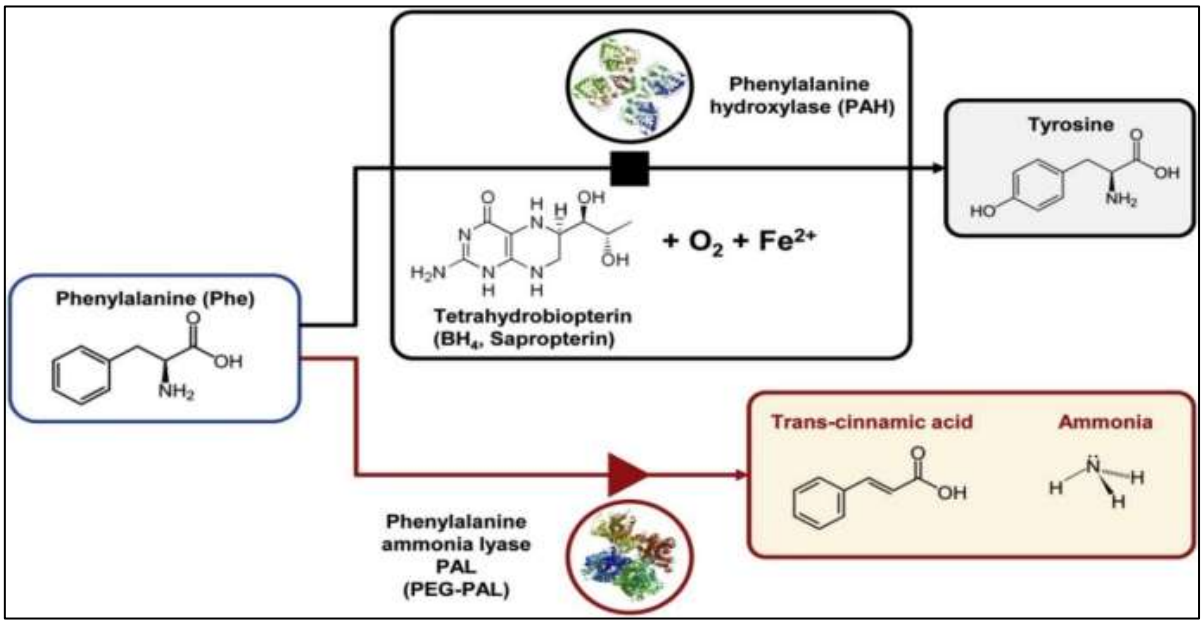


**Figure 25.** Phenylalanine ammonia lyase as a cure for PKU **(Levy *et al.*, 2018).**

Clinical trials assessing the safety and efficacy of repetitive PEG-PAL injections have been conducted. Subcutaneous PAL-PEG administration was well tolerated, safe, and demonstrated effectiveness in reducing blood Phe levels in participants, with peak efficacy occurring approximately six days post-injection, and an inverse correlation observed between drug and Phe concentrations in plasma. Efforts to modify oral PEG-PAL to prevent degradation by digestive enzymes are underway, aiming to develop effective oral therapies.

### 3.2.6 Cell Directed Therapy:

Another therapeutic avenue being explored involves replenishing the liver with cells that express phenylalanine hydroxylase following hepatocyte or hematopoietic stem-cell transplantation. Hepatocyte transplantation is being investigated because donor cells must possess a specific growth advantage over native hepatocytes to be effective. Studies have conducted hepatocyte transplantation in both animal models and humans with metabolic disorders such as urea cycle defects or glycogen storage disorders. This cellular approach

holds promise for providing a long-term solution for PKU if donor hepatocytes can attain a growth advantage. Successful outcomes have been reported in animal models where donor cells exhibited selective advantages. However, future directions may involve cell-based therapies utilizing stem cells or more specialized progenitor cells for treating metabolic liver diseases like PKU **(Harding *et al*., 2019).**

### 3.2.7 Gene Therapy

Gene therapy has garnered significant attention for treating PKU in the past two decades, with various research groups focusing on this approach. In mouse models of PKU, notable advancements have been achieved through the use of an adenovirus-related gene targeting the liver.

However, liver-directed gene therapy does not result in a permanent correction of PAH activity. The vector's genome does not integrate into hepatocyte DNA, and episomal adeno-associated virus (AAV) vector genomes are eliminated as hepatocytes regenerate. Additionally, reinjection of the same vector serotype leads to its destruction by antibody-mediated immune reactions. Studies on PKU mouse models have also demonstrated the successful delivery of gene therapy to non-hepatic tissues like muscle. Inserting vectors containing PAH and tetrahydrobiopterin synthesis genes into muscle cells has created a system capable of converting phenylalanine into tyrosine, simulating hepatic phenylalanine metabolism. Advancements in viral vector design have led to human gene therapy trials for other metabolic disorders such as α1 antitrypsin deficiency and Canavan disease. Ongoing research aims to optimize the direct muscle approach and enhance the sustainability of liver-directed gene therapy, potentially paving the way for human trials in PKU in the coming years **(Grisch-Chan *et al.*, 2019).**

#### 3.2.7.1 Lentivirus Vectors for PKU Therapy

Lentivirus vectors (LV) are proposed as a promising gene therapy approach for PKU due to their lack of pre-existing immunity, efficient targeting to the liver, and ability to integrate the transgene into chromosomal DNA for long-lasting effects (**Figure26)**. LV therapy is seen as particularly suitable for adults and infants with PKU, offering safety, durability, and cost-effectiveness advantages over other vector types like AAV **(Vockley, 2024).**

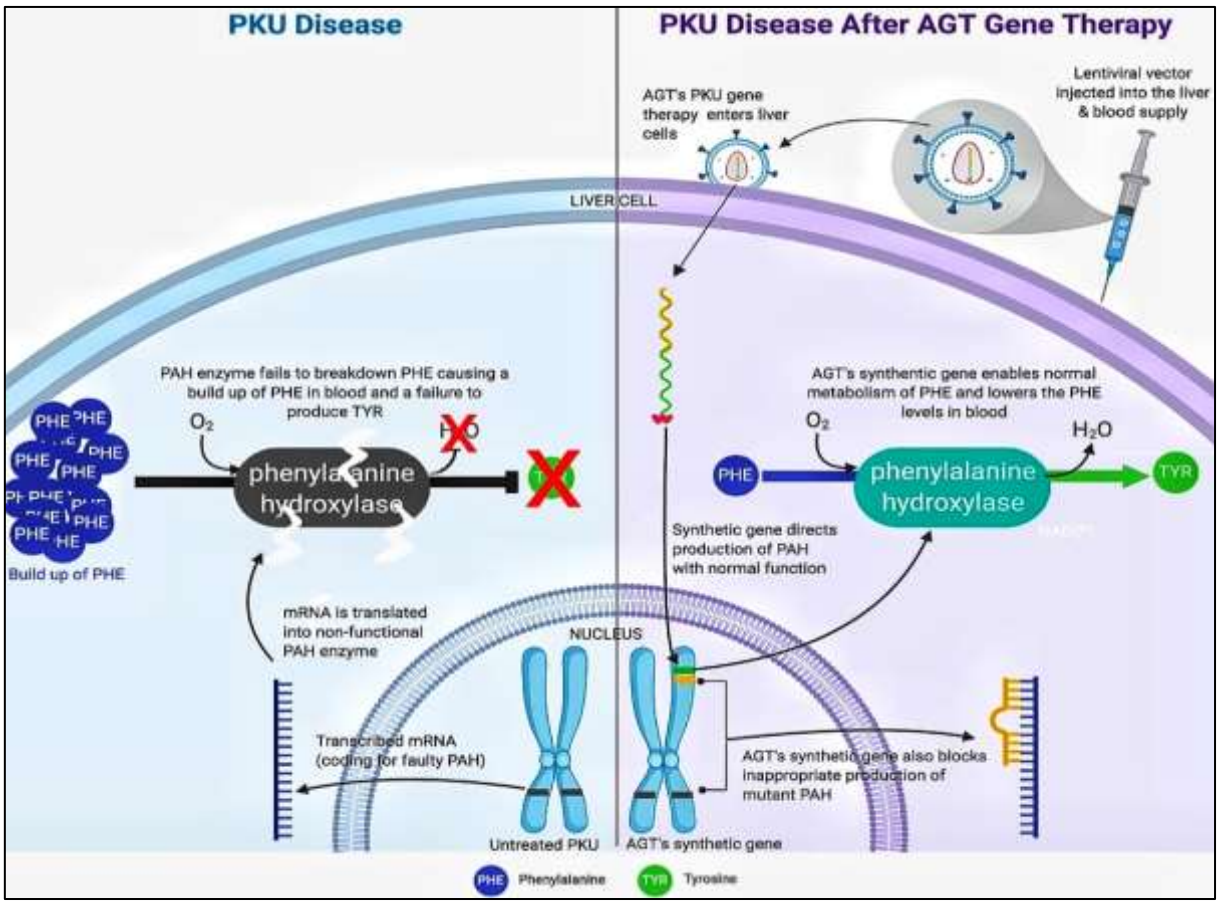


**Figure 26.** PKU Genetherepy **(Vockley, 2024).**

Another approach involves *in vitro* read-through of PAH nonsense mutations using aminoglycosides. About 10% of PKU patients carry a nonsense mutation, resulting in premature stop codon insertion. Aminoglycoside antibiotics like gentamicin and G-418 can promote read-through of stop codons, leading to protein production. Studies in mammalian cell lines have shown that read-through PAH products exhibit enzymatic activity similar to moderate PKU levels. However, the efficacy of this approach in PKU therapy requires further investigation, particularly regarding its impact on restoring Phe tolerance.

### 3.2.8 Genetically modified probiotic

Metabolic deficiencies can cause harmful metabolite accumulation, prompting research into genetically modified probiotics expressing metabolic enzymes to address this issue. Examples include *E. coli* expressing *Klebsiella aerogenes* urease, which reduced plasma levels in rats with renal failure, and *L. lactis* expressing S*taphylococcus hyicus* lipase, which improved lipid digestion in pigs with pancreatic insufficiency. Additionally, probiotics delivering the PAL enzyme could benefit conditions like phenylketonuria (**Figure 27)**, with

studies showing reductions in phenylalanine levels in animal models. However, concerns exist about the safety of using *E. coli* due to potential interactions and alterations in gut microbiota. Further research, particularly on models resembling human PKU, is needed to assess the safety and efficacy of genetically modified probiotics in both short and long-term applications **(Al Hafid Christodoulou, 2015).**

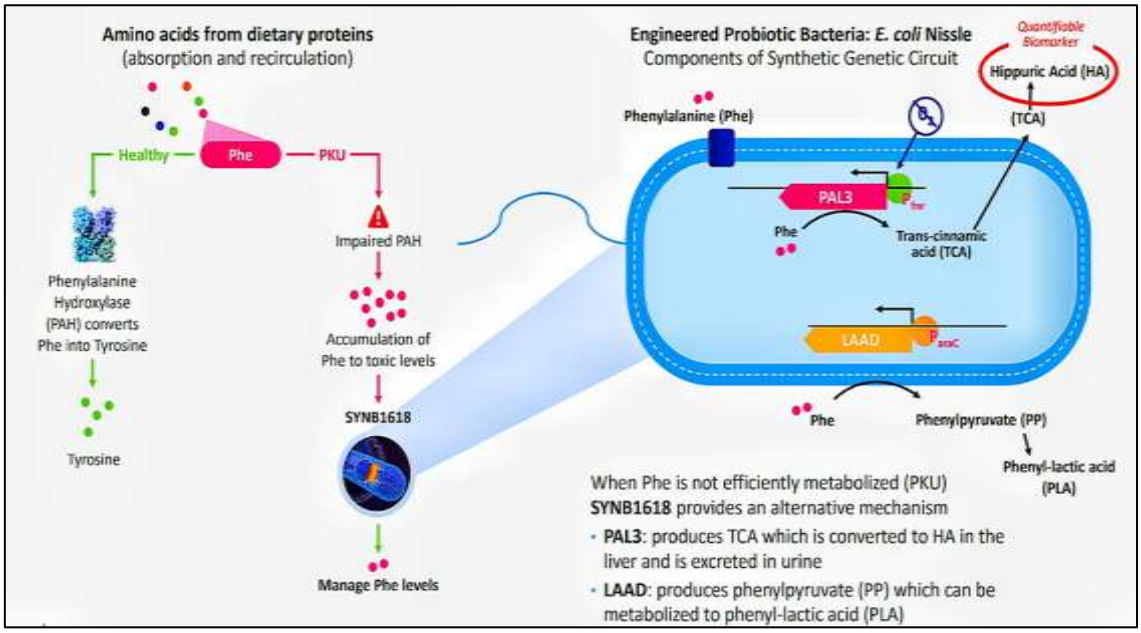


**Figure 27.** Synthetic evolution of E. *coli* Nissle for phenylalanine reduction **(Jones, 2020).**

# Conclusion

## Conclusion

Phenylketonuria (PKU) stands as a pivotal condition in the realm of inherited metabolic disorders and is the first successfully treated inborn error of metabolism, characterized by heightened phenylalanine levels due to mutations in the gene encoding phenylalanine hydroxylase. Left untreated, PKU precipitates severe neurological deficits, including mental retardation, epilepsy, and behavioral challenges. However, with rigorous dietary management encompassing strict protein restriction supplemented by manufactured substitutes significant strides have been made in enhancing patients' quality of life and mitigating associated health risks.

Despite these advancements and several studies, the precise mechanism by which abnormal Phe metabolism causes intellectual impairment remains unclear. Challenges persist, particularly in addressing neurological impairments, underscoring the crucial role of the blood-brain barrier in PKU pathophysiology. While conventional treatment methods have historically relied on dietary interventions, ongoing research has unveiled promising therapeutic avenues, including gene therapy and chaperone treatments. Furthermore, emerging modalities such as large neutral amino acids and tetrahydrobiopterin offer hope for refining treatment protocols and reducing dependency on dietary restrictions.

Looking forward, the future of PKU research holds the potential for groundbreaking developments. Continued exploration into the molecular mechanisms underlying PKU could illuminate new therapeutic targets. Advances in genetic engineering, personalized medicine, and neuroprotective strategies may offer transformative solutions, paving the way for more effective and sustainable approaches in the years to come.

# Bibliographic references

# Bibliographic References


**A**

Al Hafid, N., & Christodoulou, J. (2015). Phenylketonuria: a review of current and future treatments.

*Translational Pediatrics, 4*(4), 304-317.

Anderson, P. J., & Leuzzi, V. (2010). White matter pathology in phenylketonuria. *Molecular Genetics and Metabolism*, 99(Suppl 1), S3-S9.

https://doi.org/10.1016/j.ymgme.2009.10.005

Anjema, K., et al. (2016). The neonatal tetrahydrobiopterin loading test in phenylketonuria: What is the predictive value? Orphanet Journal of Rare Diseases, 11(1), 10.

Asare, K. K., Afrifa, J., & Opoku, Y. K. (2021). Mechanisms Underlying Induced Pseudo-Scleroderma among Patients with Phenylketonuria Metabolic Disorder. *Global Journal of Dermatology & Venereology, 9*, 1-81. https://doi.org/10.12970/2310-998X.2021.09.01

**B**

Banta-Wright, S. A., & Steiner, R. (2004). Tandem Mass Spectrometry in Newborn Screening. *Chemistry, Medicine*. Retrieved from Semantic Scholar website: https://www.semanticscholar.org/paper/Tandem-Mass-Spectrometry-in-Newborn-Screening-Banta-Wright-Steiner/a9eb63aacd9171b18c62141fc2c161a35552dd1e

Bhagavan, N. V., & Ha, C.-E. (2015). Protein and Amino Acid Metabolism. In Essentials of Medical Biochemistry: With Clinical Cases (pp. 227-268). Elsevier.

Bickel, H., Gerrard, J., &Hickmans, E. M. (1953). Influence of phenylalanine intake on phenylketonuria. The Lancet, 265(6791), 812-813.

Blau, N. (2016). Genetics of phenylketonuria: Then and now. *Human Mutation*, 37(6), 508-515. https://doi.org/10.1002/humu.22980

Blau, N., Hennermann, J.B., Langenbeck, U., & Lichter-Konecki, U. (2011). Diagnosis, classification, and genetics of phenylketonuria and tetrahydrobiopterin (BH4) deficiencies. Mol Genet Metab, 104(Suppl), S2-9.

Blau, N., Shen, N., & Carducci, C. (2014). Molecular genetics and diagnosis of phenylketonuria: state of the art. Expert Review of Molecular Diagnostics, 14(1), 1–17.

https://doi.org/10.1586/14737159.2014.923760

Blau, N., van Spronsen, F. J., & Levy, H. L. (2010). Phenylketonuria. The Lancet, 376(9750), 1417–1427.

Bortoluzzi, V. T., Dutra Filho, C. S., &Wannmacher, C. M. D. (2021). Oxidative stress in phenylketonuria-evidence from human studies and animal models, and possible implications for redox signaling. *Metabolic Brain Disease*, 36(4), 523-543. https://doi.org/10.1007/s11011-021-00676-w

Burlina, A. P., Cazzorla, C., Massa, P., Loro, C., Gueraldi, D., &Burlina, A. B. (2020). The Impact of a Slow-Release Large Neutral Amino Acids Supplement on Treatment Adherence in Adult Patients with Phenylketonuria.

**C**

Centerwall, S. A., &Centerwall, W. R. (2000). The discovery of phenylketonuria: The story of a young couple, two retarded children, and a scientist. Pediatrics, 105(1 Pt 1), 89-103. https://doi.org/10.1542/peds.105.1.89

Chopin, A. (2013). Phénylcétonurie et grossesse. Gynécologie et obstétrique. HAL Id: dumas-00872317. Retrieved from https://dumas.ccsd.cnrs.fr/dumas-00872317. Submitted on 11 Oct 2013

Conde-Giménez, M., & Sancho, J. (2021). Unravelling the Complex Denaturant and Thermal-Induced Unfolding Equilibria of Human Phenylalanine Hydroxylase. *International Journal of Molecular Sciences, 22*(12), 6539. https://doi.org/10.3390/ijms22126539

Czarnecka, K., Pilarz, A., Rogut, A., Maj, P., Szymańska, J., Olejnik, Ł., & Szymański, P. (2021). Aspartame-True or False? Narrative Review of Safety Analysis of General Use in Products. Nutrients, 13(6), 1957.

**D**

Dave, U. P., & Das, B. R. (2010). Newborn screening – From 'Guthrie age to genomic age. Journal of Obstetrics and Gynecology of India, 60(3), 210-220

De Groot, M. J., Hoeksma, M., Reijngoud, D. J., et al. (2013). Phenylketonuria: reduced tyrosine brain influx relates to reduced cerebral protein synthesis. *Orphanet Journal of Rare Diseases*, 8, 133.(https://doi.org/10.1186/1750-1172-8-133)

Dinu, A., Apetrei, C. (2020). A Review on Electrochemical Sensors and Biosensors Used in Phenylalanine Electroanalysis. *Sensors*, *20*(9), 2624. https://doi.org/10.3390/s20092624

Dobrowolski, S.F., Phua, Y.L., Vockley, J., Goetzman, E., & Blair, H.C. (2022). Phenylketonuria oxidative stress and energy dysregulation: Emerging pathophysiological elements provide interventional opportunity. Molecular Genetics and Metabolism, 136(2), 111-117.

**E**

Elhawary, N.A., AlJahdali, I.A., Abumansour, I.S., Elhawary, E.N., Gaboon, N., Dandini, M., ... Kensara, O.A. (2022). Genetic etiology and clinical challenges of phenylketonuria. Human Genomics, 16(22).

Evers, R. A. F., Vliet, D., &Spronsen, F. J. (2019). *Tetrahydrobiopterin treatment in phenylketonuria: a repurposing approach. Journal of Inherited Metabolic Disease.*

Ezgu, F. (2016). Inborn errors of metabolism. Advances in Clinical Chemistry, 73, 195-250. https://doi.org/10.1016/bs.acc.2015.12.001

**F**

Feillet, F., van Spronsen, F.J., MacDonald, A., et al. (2010). Challenges and pitfalls in the management of phenylketonuria. Pediatrics, 126, 333–341.

Ferreira, C. R., van Karnebeek, C. D. M., Vockley, J., & Blau, N. (2018). A proposed nosology of inborn errors of metabolism. *Genetics in Medicine*, 21(1). https://doi.org/10.1038/s41436-018-0022-8

Flydal, M. I., & Martinez, A. (2013). Phenylalanine hydroxylase: Function, structure, and regulation. IUBMB Life, 65(4), 341–349

**G**

Giżewska, M. (2015). Phenylketonuria: Phenylalanine Neurotoxicity. In Bernstein, L., Rohr, F., & Helm, J. (Eds.), Nutrition Management of Inherited Metabolic Diseases (pp. 89–99). Springer, Cham. https://doi.org/10.1007/978-3-319-14621-8_9

Grisch-Chan, H. M., Schwank, G., Harding, C. O., & Thony, B. (2019). *State-of-the-Art 2019 on Gene therapy for Phenylketonuria. Human Gene Therapy.*

Guthrie, R., & Susi, A. (1963). A simple phenylalanine method for detecting phenylketonuria in large populations of newborn infants. Pediatrics, 32, 338–343.

**H**

Hamma, T. (2018, November 19). Phenylketonuria (PKU): Pathogenesis and Clinical Findings. Retrieved from http://www.thecalgaryguide.com

Harding, C. O. (2019). Prospects for Cell-Directed Curative Therapy of Phenylketonuria (PKU). MolecularFrontiers Journal, 03(02), 110–121.

Huttenlocher, P.R. (2000). The neuropathology of phenylketonuria: human and animal studies. Eur J Pediatr, 159, S102–S106.

**J**

Jones, A. (2020, May 12). Synlogic: A Clinical-Stage Biopharma With A Good Risk/Reward Ratio. Retrieved from https://seekingalpha.com/article/4346633-synlogic-clinical-stage-biopharma-good-risk-reward-ratio

**K**

Kim, H.K., & Han, J. (2020). Tetrahydrobiopterin in energy metabolism and metabolic diseases. Pharmacological Research, 104827.

Kure, S., Hou, D. C., Ohura, T., Iwamoto, H., Suzuki, S., Sugiyama, N., ... Narisawa, K. (1999). Tetrahydrobiopterin-responsive phenylalanine hydroxylase deficiency. *The Journal of Pediatrics*, 135, 375–378.

Kurpad, A.V., Regan, M.M., Raj, T.D., Rao, V.N., Gnanou, J., & Young, V.R. (2006). The daily phenylalanine requirement of healthy Indian adults. Am J Clin Nutr, 83(6), 1331-6.

**L**

Levy, H. L., Sarkissian, C. N., & Scriver, C. R. (2018). *Phenylalanine ammonia lyase (PAL): From discovery to enzyme substitution therapy for phenylketonuria. Molecular Genetics and Metabolism, 124(4), 223–229.*https://www.sciencedirect.com/science/article/abs/pii/S1096719218302920

Li, N., He, C., Li, J., Tao, J., Liu, Z., Zhang, C., Yuan, Y., Jiang, H., Zhu, J., Deng, Y., Guo, Y., Li, Q., Yu, P.,

& Wang, Y. (2018). Analysis of the genotype-phenotype correlation in patients with phenylketonuria in mainland China. Scientific Reports, 8(1), 11251. https://doi.org/10.1038/s41598-018-29409-1

Longo, N, Disorders of biopterin metabolism, J. Inherit. Metab. Dis. 32 (2009)

**M**

MacDonald, A., van Wegberg, A. M. J., Ahring, K., Beblo, S., Bélanger-Quintana, A., Burlina, A., … van Spronsen, F. J. (2020). PKU dietary handbook to accompany PKU guidelines. Orphanet Journal of Rare Diseases, 15(1).

Miller, R. H., Fyffe-Maricich, S., &Caprariello, A. C. (2018). Animal Models for the Study of Multiple Sclerosis. George Washington University, Washington, DC, United States; University of Pittsburgh, Rangos Research Center, Pittsburgh, PA, United States; University of Calgary, Hotchkiss Brain Institute, Canada.

Muntau, A. C., du Moulin, M., &Feillet, F. (2018). Diagnostic and therapeutic recommendations for the treatment of hyperphenylalaninemia in patients 0-4 years of age. Orphanet Journal of Rare Diseases, 13(1),

173. https://doi.org/10.1186/s13023-018-0911-6

**N**

National Institutes of Health Consensus Development Conference Statement Phenylketonuria: Screening and Management (2000). NICHD. Retrieved from https://web.archive.org/web/20161005140234/https://www.nichd.nih.gov/publications/pubs/PKU_screening.htm

Nave, K.A., & Werner, H.B. (2014). Myelination of the nervous system: mechanisms and functions. Annu Rev Cell Dev Biol, 30, 503-33.

**O**

Opladen, T., Abu Seda, B., Rassi, A., Thöny, B., Hoffmann, G. F., & Blau, N. (2011). Diagnosis of tetrahydrobiopterin deficiency using filter paper blood spots: Further development of the method and 5 years experience. *Journal of Inherited Metabolic Disease*, 34, 819–826.

**R**

Raoufinejad, Kosar & Rajabi, Mehdi. (2021). Licorice in the Treatment of Acne Vulgaris and Postinflammatory Hyperpigmentation: A Review. Journal of Pharmaceutical Care, 8. Retrieved from https://doi.org/10.18502/jpc.v8i4.5242

Rausell, D., García-Blanco, A., Correcher, P., Vitoria, I., Vento, M., &Cháfer-Pericás, C. (2019). Newly validated biomarkers of brain damage may shed light into the role of **oxidative** stress in the pathophysiology of neurocognitive impairment in dietary restricted phenylketonuria patients. Pediatric Research, 85(2),

Rech, V. C. et al. Inhibition of the mitochondrial respiratory chain by phenylalanine in rat cerebral cortex. Neurochem Res. 27, 353–357 (2002).

Ribas, G. S., Sitta, A., Wajner, M., & Vargas, C. R. (2011). Oxidative Stress in Phenylketonuria: What is the Evidence? *Cellular and Molecular Neurobiology, 31*(4), 653–662.

https://doi.org/10.1007/s10571-011-9693-2

**S**

Scriver, C. R., Kaufman, S. (2001). The Hyperphenylalaninemias and Phenylketonuria. In The Metabolic and Molecular

Bases of Inherited Disease (8th ed., pp.1667-1724). New York: McGraw-Hill..

Spécola, N., & Chiesa, A. (2017). *Alternative Therapies for PKU. Journal of Inborn Errors of Metabolism and Screening, 5, 232640981668573.*

Strisciuglio, P., &Concolino, D. (2014). New Strategies for the Treatment of Phenylketonuria (PKU). Metabolites, 4(4), 1007-1017.

Surtees, R., & Blau, N. (2000). The neurochemistry of phenylketonuria. *European Journal of Pediatrics*, 159(Suppl 2), S109-S113.(https://doi.org/10.1007/pl00014370)

**T**

Thau-Zuchman, O., Pallier, P. N., Savelkoul, P. J. M., Kuipers, A. A. M., Verkuyl, J. M., & Michael-Titus, A. T. (2022). High phenylalanine concentrations induce demyelination and microglial activation in mouse cerebellar organotypic slices. *Frontiers in Neuroscience*, 16, 926023. https://doi.org/10.3389/fnins.2022.926023

Trunzo, R., Santacroce, R., D'Andrea, G., Longo, V., De Girolamo, G., Dimatteo, C., Leccese, A., Bafunno, V., Lillo, V., Papadia, F., &Margaglione, M. (2015). Phenylalanine hydroxylase deficiency in south Italy: Genotype-

phenotype correlations, identification of a novel mutant PAH allele and prediction of BH4 responsiveness.

*Clinical Chimica Acta*, *450*, 51-55. https://doi.org/10.1016/j.cca.2015.07.014

**V**

van Spronsen, F. J., Blau, N., Harding, C., Burlina, A., Longo, N., & Bosch, A. M. (2021). Phenylketonuria. Nature Reviews. Disease Primers, 7(1), 36. https://doi.org/10.1038/s41572-021-00267-0

van Spronsen, F. J., van Wegberg, A. M., Ahring, K., Bélanger-Quintana, A., Blau, N., Bosch, A. M., ... MacDonald, A. (2017). Key European guidelines for the diagnosis and management of patients with phenylketonuria. *The Lancet Diabetes & Endocrinology*, 5(9), 743-756. https://doi.org/10.1016/S2213- 8587(16)30320-5

Van Wegberg, A. M. J., MacDonald, A., Ahring, K., Bélanger-Quintana, A., Blau, N., Bosch, A. M., … van Spronsen, F. J. (2017). *The complete European guidelines on phenylketonuria: diagnosis and treatment.*

*Orphanet Journal of Rare Diseases, 12(1).*

Vockley, J., Andersson, H. C., Antshel, K. M., Braverman, N. E., Burton, B. K., Frazier, D. M., Mitchell, J., Smith, W. E., Thompson, B. H., & Berry, S. A. (2014). Phenylalanine hydroxylase deficiency: Diagnosis and management guideline. Genetics in Medicine, 16(2), 188–200.

Vockley, J., M.D., Ph.D. (2024). University of Pittsburgh: Cleveland Family Endowed Chair in Pediatric Research | Professor of Human Genetics. Retrieved from https://www.americangene.com/pipeline/phenylketonuria-pku/

**W**

Wang, C., Zhu, H., Cai, Z. *et al.* Newborn screening of phenylketonuria using direct analysis in real time (DART) mass spectrometry. *Anal BioanalChem* 405, 3159–3164 (2013). https://doi.org/10.1007/s00216-013-6713-8

Wiedemann, A., Oussalah, A., Jeannesson, E., Guéant, J.-L., &Feillet, F. (2020). La phénylcétonurie: De la diététique à la thérapie génique [Phenylketonuria, fromdiet to genetherapy]. Med Sci (Paris), 36(8-9), 725-734. https://doi.org/10.1051/medsci/2020127

Williams, R. A., Mamotte, C. D. S., & Burnett, J. R. (2008). Phenylketonuria: An inborn error of phenylalaninemetabolism. Clinical Biochemistry Reviews, 29(1), 31–41.https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2423317/

Whitbread, D. (2024). Top 10 Foods Highest in Phenylalanine.MyFoodData.Reviewed by Dr. Patricia Shelton. Retrieved May 19, 2024, from https://www.myfooddata.com/articles/high-phenylalanine-foods.php

Woolf, L. I., & Adams, J. (2020). The Early History of PKU. *International Journal of Neonatal Screening*, 6(3), 59. https://doi.org/10.3390/ijns6030059)

**Y**

Yannicelli, S. (2015). Effects of LNAA therapy on the transport of phenylalanine into the brain. In L.E. Bernstein et al. (Eds.), *Nutrition Management of Inherited Metabolic Diseases: Lessons from Metabolic University* (Chapter 11). Springer International Publishing.

**Website**

Website 1 : *https://researchtweet.com/wp-content/uploads/2021/08/Phenylketonuria-research-tweet-1.jpg*

Haute Autorité de Santé. (2018). Protocole national de diagnostic et de soins (PNDS) : Phénylcétonurie [PDF]. Retrieved from https://www.has-sante.fr/upload/docs/application/pdf/2018-06/phenylcetonurie_-_pnds.pdf

Lichter-Konecki, U., &Vockley, J. (2019). *Phenylketonuria: Current Treatments and Future Developments. Drugs.*

Potrous, F., Muthanna, F., & Saad, M. (2019). Phenylketonuria (PKU). Retrieved from https://www.uomosul.edu.iq/public/files/datafolder_55/oldUo_20190816_010123_50_177.pdf

Sharer, J. D. (2020, January 28). *Population scale metabolomics: Newborn screening*. Lecture presented in *GBSC 724 Advanced Special Topics in Metabolomics*, University of Alabama at Birmingham. Retrieved from https://www.uab.edu/proteomics/pdf_files/2020/GBSC724%20NBS%20Lecture%202020_student.pdf

**Abstract :**

Phenylketonuria (PKU) is a metabolic disorder characterized by the body'sinability to properly metabolize phenylalanine (Phe), resulting in its accumulation and subsequentneurological damage. This condition stems from a deficiency in phenylalanine hydroxylase (PAH), the enzyme responsible for converting Phe to tyrosine. The global prevalence of PKU varies around the world withhigher rates in Europe and the United States. PAH activityis essential for maintaining phenylalanine homeostasis and preventinghyperphenylalaninemia, with BH4 serving as a crucial cofactor for PAH function. PKU is an autosomal recessive inborn error of phenylalanine metabolism, primarily caused by mutations in the PAH gene. Hyperphenylalaninemia disruptsbrainfunctionprimarily by competitive lyinhibiting the blood-brainbarrier, leading to deficiencies in criticalbrainaminoacids like tyrosine. This imbalance impairs neurotransmittersynthesis, notably dopamine and serotonin, affecting cognitive and motorfunctions. Advances in PKU care have transformedthroughearlydetection via newborn screening and personalized interventions, including techniques like the Guthrie test and geneticanalysis for precise identification. Treatmentmodalities, including BH4 supplementation and emergingtherapies like PAL enzyme therapy, reflect an evolvinglandscapeencompassingdietaryregulation, large neutral aminoacidsupplementation, enzyme replacement, and genetherapy.

**Key Words :**

Phenylketonuria, Neurological damage, Phenylalanine hydroxylase, PAH gene mutations, Hyperphenylalaninemia, , Newborn screening, Tetrahydrobiopterin

**Résumé :**

La phénylcétonurie (PCU) est un trouble métabolique caractérisé par l'incapacité de l'organisme à métaboliser correctement la phénylalanine (Phe), entraînant son accumulation et des dommages neurologiques ultérieurs. Cette condition découle d'un déficit en phénylalanine hydroxylase (PAH), l'enzyme responsable de la conversion du Phe en tyrosine. La prévalence mondiale de la PCU varie selon les régions du monde, avec des taux plus élevés en Europe et aux États-Unis. L'activité des HAP est essentielle au maintien de l'homéostasie de la phénylalanine et à la prévention de l'hyperphénylalaninémie, le BH4 servant de cofacteur crucial pour la fonction des HAP. La PCU est une erreur innée autosomique récessive du métabolisme de la phénylalanine, principalement causée par des mutations du gène PAH. L'hyperphénylalaninémie perturbe les fonctions cérébrales principalement en inhibant de manière compétitive la barrière hémato-encéphalique, entraînant des carences en acides aminés essentiels au cerveau comme la tyrosine. Ce déséquilibre altère la synthèse des neurotransmetteurs, notamment la dopamine et la sérotonine, affectant les fonctions cognitives et motrices. Les progrès dans les soins de la PCU se sont transformés grâce à une détection précoce via le dépistage néonatal et des interventions personnalisées, notamment des techniques telles que le test de Guthrie et l'analyse génétique pour une identification précise. Les modalités de traitement, y compris la supplémentation en BH4 et les thérapies émergentes comme la thérapie enzymatique PAL, reflètent un paysage évolutif englobant la régulation alimentaire, la supplémentation en acides aminés neutres importants, le remplacement enzymatique et la thérapie génique.

**Mots clés :**

Phénylcétonurie, Dommages neurologiques, Phénylalanine hydroxylase, mutations du gène PAH, Hyperphénylalaninémie, Dépistage néonatal, Tétrahydrobioptérine.

**الملخص:**

الفينيل كيتونيوريا (PKU)هي اضطراب ايضي يتميز بعدم قدرة الجسم على استقالب الفينيل النين (Phe) بشكل صحيح، مما يؤدي إلى تراكمه وتلف عصبي لاحق. تنبع هذه الحالة من نقص في هيدروكسيلز الفينيل النين (PAH)، وهو الانزيم المسؤول عن تحويل Phe إلى تيروزين. يخقلف معدل الانتشار العالمي لمرض الفينيل كيتونيوريا حول العالم، حيث توجد معدلات اعلى في اوروبا والوليات المتحدة. يعد نشاط PAH ضروريا للحفاظ على توازن الفينيل النين ومنع فرط فينيل النين الدم، حيث يعمل BH4 كعامل مساعد حاسم لوظيفة هيدروكسيلز الفينيل النينPKU . (PAH)هو خطأ وراثي جسمي متنحي في استقالب الفينيل النين، وينتج في المقام الاول عن طفرات في جين .PAH يؤدي فرط فينيل النين الدم إلى تعطيل وظائف المخ بشكل رئيسيعن طريق تثبيط حاجز الدم في الدماغ بطريقة تنافسية، مما يؤدي إلى تناقص في الاحماض الامينية المهمة في الدماغ مثل التيروزين. يؤدي هذا الخلل إلى إضعاف تخليق الناقلات العصبية، وخاصة الدوبامين والسيروتونين، مما يؤثر على الوظائف المعرفية والحركية. ان التحول القادم في رعاية الفينيل كيتونوريا من خلل الكشف المبكر عن طريق فحص حديثي الولادة والتدخلات الشخصية، بما في ذلك تقنيات مثل اختبار جوثري والتحليل الجيني لتحديد الهوية بدقة. تعكس طرائق العلاج، بما في ذلك مكملت BH4والعلاجات الناشئة مثل العلاج بإنزيمPAL ، مشهدا متطورا يشمل التنظيم الغذائي ومكملات الاحماض الامينية المحايدة الكبيرة واستبدال الانزيم والعلاج الجيني.

**الكلمات المفتاحية:**

الفينيل كيتونوريا - الضرر العصبي فينيل النين هيدروكسيلز - طفرات جين فرط فينيل النين الدم - فحص حديثي الولادة - رباعي هيدروبيوبترين