Phenylketonuria: optimizing care
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General introduction
Chapter 1
GENERAL INTRODUCTION

In 1950, the Nobel and Pulitzer prize winning author Pearl S. Buck published a personal memoir about the life of her daughter with severe cognitive impairment who was born in 1921, with the title: The child who never grew [1]. During the majority of her daughter’s life, the cause of her impaired mental functions remained unclear. She never received disease-specific medical treatment and spent most of her life institutionalized at the Vineland Training School in New Jersey [2]. In those days, having a child with cognitive impairment, especially as an accomplished upper class citizen, was never spoken about because the concept of eugenics, which was en vogue at that time, taught that ‘feeblemindedness’ was linked to the genetic pool of those less fortunate in the population [3]. It took Ms. Buck almost 30 years before she dared to come forward and write about her own daughter, intending to inform other parents of cognitively impaired children on how to deal with their ‘burden.’ She writes on the first two pages: “The final reason for setting down this story is that I want my child’s life to be of use in her generation. She is one who has never grown mentally beyond her early childhood, therefore she is forever a child, although in years she is old enough now to have been married and to have children of her own...”[1]. It was later discovered that PKU was the cause of her daughter’s severe cognitive impairment.

Phenylketonuria (PKU, ORPHA79254, MIM 261600) is an autosomal recessive inherited disorder of metabolism that arises due to mutations in the gene coding for the hepatic enzyme phenylalanine hydroxylase (PAH; EC 1.14.16.1). Because of this mutation, the essential amino acid phenylalanine (Phe) cannot be converted to tyrosine and accumulates in the blood and in the brain, causing severe cognitive impairment [4]. The incidence of PKU in European and Oriental Asian populations is approximately 1:10,000 [4,5]. PKU is therefore one of the most prevalent inborn errors of metabolism found in Europeans [6,7].

The book by Ms. Buck on the life of her daughter is an emotional tale of the difficulties of growing up with severe cognitive challenges; fortunately, the picture of a young, untreated patient with PKU is currently rare in developed countries. In 1934, Asbjörn Fölling, a Norwegian physician and biochemist, discovered that a disorder in the
biochemical pathway involved in the degradation of Phe was associated with severe 
cognitive impairment in several patients based on the identification of high levels of 
phenylpyruvic acid in the urine [8-11]. The disease was initially named ‘imbecillitas 
phenylpyruvica’ by Fölling in 1934. The name was later adapted to oligophrenia 
phenylpyruvica by Jervis in 1937 and it was finally changed to PKU in that same year by 
Penrose and Quastel in their work concerning one of the first Phe loading tests in 
untreated patients [4,9,11-13]. In 1951, treatment became available when the German 
physician Horst Bickel developed the first Phe free amino acid mixture to supplement all 
estential amino acids except for Phe in patients placed on a protein-restricted diet. This 
strategy has been corner stone of the treatment of PKU up to now [8]. The discovery of a 
screening test in dried blood spots by the American physician Robert Guthrie in the 
1960’s enabled the early introduction of dietary treatment in developed countries, 
resulting in near normalization of the cognitive outcomes for patients with PKU [14]. 
The enormous success of treatment has, however, led to new thresholds to overcome in 
the life-long treatment of PKU, and the road to optimizing care in PKU is open [14-21].
BIOCHEMISTRY

In patients with PKU, PAH is deficient and Phe catabolism is subsequently hampered, which causes increased levels of Phe throughout the body, including, most importantly, the brain. PAH is expressed in the liver [22], and it is a non-heme iron enzyme that catalyzes the hydroxylation of Phe into tyrosine using iron, molecular oxygen and tetrahydrobiopterin (BH4) as cofactors [23] (Figure 1). Phe is an essential amino acid and therefore must be ingested through dietary protein intake. In addition, protein catabolism contributes to the body’s free Phe pool.

The impaired degradation of Phe leads to an increase in the excretion of its metabolites in urine: phenylacetate, phenylpyruvate, and phenylethylamine. Of these metabolites phenylpyruvate is the most prominent and contains a ketone group, which is why the disease is called ‘phenylketonuria’ (Figure 1).
Figure 1. Degradation of phenylalanine
GENETIC SUBSTRATE

The PAH gene is located on the long arm of chromosome 12 (bands q22-q24). Almost 800 different mutations causing PKU have been identified in the PAH gene (the Human Gene Mutation Database; www.hgmd.org), with most patients being compound heterozygotes. The most frequently encountered allele is c.1222C>T, and the most frequent genotype is c.[1066-11G>A;[1066-11G>A][23,24]. Mutations in the catalytic domain, splice site variants and missense mutations (most prevalent) may be present, resulting in a full lack of enzyme activity, a truncated and non-functional protein or misfolded enzyme with low to absent activity, respectively [23,25]. Genotype based prediction of the phenotype is complex. However, several steps towards predicting phenotypic severity based on genetic changes have been taken [6,23,26,27]. Predicting clinical severity based on mutation analysis, in combination with the blood Phe concentration at diagnosis, may assist in selecting an optimal treatment approach for individual patients. Certain genotypes can predict responsivity to treatment with BH4 [28-30]. Personalized medicine based on known genotype-phenotype correlations is therefore rapidly becoming an option. Studies concerning the prediction of PAH activity and BH4 sensitivity based on genotype are ongoing [22], and several databases (such as the Human Gene Mutation Database) are used to collect new mutations of the PAH gene.
PATHOPHYSIOLOGY

The exact pathophysiology of the cognitive impairment in PKU is not fully known, and several hypotheses have been postulated. The following mechanisms are thought to play a role in causing brain damage in patients with PKU and will be discussed below: Phe toxicity, low concentrations in the brain of large neutral amino acids (LNAA) other than Phe, specific properties of the blood-brain barrier (BBB), impaired neuronal synthesis of neurotransmitters and impaired myelination [31-33].

*Phenylalanine Toxicity*

Higher levels of Phe in the blood are related to more extensive CNS damage than lower levels [34-36]. The most widely supported theory is that direct toxicity of Phe causes irreversible damage to brain cells and synapsis, leading to cognitive impairment [37,38]. Waisbren *et al.* performed a meta-analysis and found that for children up to the age of 12 years, an increase of mean lifetime blood Phe levels of 100 µmol/L (levels between 423 and 750 µmol/L) caused a decrease of 1.3 to 3.1 IQ points. [39]. Furthermore Jahja *et al.* showed in a preliminary study that executive functioning was better in pediatric patients with mean lifetime Phe levels below 240 µmol/L than in those with higher levels [40]. The effect of high Phe concentrations on the brain is age dependent, and Phe toxicity is more pronounced in the developing brain in utero and up to the age of 12 years than later in life [41-44].

*Large Neutral Amino Acids and the Blood-Brain Barrier*

Decreased LNAA concentrations in the brain may also play a role in the cerebral dysfunction in patients with PKU [32,45,46], but the precise role remains to be elucidated. Several studies have demonstrated that Phe competes with eight other LNAA (tyrosine, tryptophan, valine, isoleucine, leucine, threonine, methionine, and histidine) when crossing the BBB because they all use the same L-type amino acid transporter (LAT1, SLC7A5) [36,45,47-50]. Because the LAT1 transporter has a greater affinity to Phe than to the other LNAA, high levels of Phe in the blood lead to preferential
transport of Phe over the BBB, resulting in elevated Phe and diminished LNAA concentrations in the brain [48,51]. Furthermore, LNAA are deficient intra-cerebrally because high Phe concentrations lead to decreased protein synthesis. An indication that LNAA other than Phe play a role in brain pathology in patients with PKU is demonstrated by several authors describing improvement of symptoms and increased LNAA brain concentrations after supplementation with specific LNAA [45,46,52-54].

**Neurotransmitter Synthesis**

Neurotransmitter synthesis is decreased in patients with PKU [55], and this may consequently play a role in the pathophysiology of cerebral dysfunction in patients. Tyrosine is the precursor of dopamine and it is a degradation product of Phe [50,56]. Due to PAH deficiency, tyrosine is not properly formed, and it is hypothesized that PKU may lead to low levels of both tyrosine and dopamine. Small studies with patients on a natural protein-restricted diet reported that influx and efflux of tyrosine across the BBB is impaired [50,57-62]. Tryptophan, another LNAA in competition with Phe at the BBB, is a precursor of serotonin, and both have been found to be decreased in the brains of patients on dietary treatment [63,64]. The enzymes involved in the degradation of tyrosine and tryptophan (tyrosine- and tryptophan hydroxylase respectively) may be reduced in patients, not only leading to lowered neurotransmitters but also to decreased synaptic plasticity and decreased axonal growth. However, there is no proof of the role of deficiencies of these neurotransmitters and their precursors in the pathogenesis of CNS disease in PKU [32].

**Myelination**

White matter abnormalities have been observed in patients with PKU despite dietary treatment [65,65-71]. Two explanations have been suggested. First, decreased protein synthesis might lead to improperly formed myelin. Secondly, it is thought that white matter lesions occur due to intermyelenic edema [65]. Both hypothesis need to be further investigated.
Chapter 1

**DIAGNOSIS**

PKU is suspected when Phe is found to be elevated in dried bloodspots obtained through newborn screening in the first week of life. The diagnosis is subsequently confirmed by the detection of high plasma Phe levels and the exclusion of other genetic conditions causing hyperphenylalaninemia (Figure 2). Measuring PAH activity in patient blood is not effective because it is only detectible in hepatic tissue. Based on the level of Phe at diagnosis, disease severity is classified as either classic PKU ($\geq 1200 \, \mu\text{mol}/L$), moderate PKU (900–1200 $\mu\text{mol}/L$), mild PKU (600–900 $\mu\text{mol}/L$) or mild hyperphenylalaninemia (mHPA; 360–600 $\mu\text{mol}/L$) [16]. A Phe level below 120 $\mu\text{mol}/L$ is normal, and a level between 120–360 $\mu\text{mol}/L$ is classified as mHPA not in need of treatment. Another classification of PKU patients is based on their tolerance for natural protein or, more specifically, for Phe intake. According to this classification, patients with a daily tolerance of less than 500 mg Phe (which equals 10 grams of protein) are classified as severe patients, and those with a daily tolerance of 500 mg or more of Phe are classified as having a mild to moderate form of the disease [72,73].

Hyperphenylalaninemia may be found in disorders other than PKU. When investigating the cause of hyperphenylalaninemia, a disorder in BH4 synthesis should be ruled out as the cause of the elevated Phe levels [74,75]. The incidence of BH4 deficiencies is 1 to 2% of all patients with high Phe levels at birth [76]. For this reason, a blood or urine pterin profile (analysis of biopterin, neopterin and primapterin) should be obtained, and the activity of dihydropteridine reductase must be measured specifically in blood (Figure 2). If the results are abnormal, the patient should be tested for deficiencies of 1,6-pyruvoyl-tetrahydrobiopterin synthase, dihydropteridine reductase, pterin carbinolamine-$4\alpha$-dehydratase or GTP-cyclohydrolase [6,77]. In patients with 1,6-pyruvoyl-tetrahydrobiopterin synthase deficiency, urine biopterin is low with a decreased biopterin/neopterin ratio. In patients with GTP-cyclohydrolase deficiency, the urine pterin profile shows decreased levels of both biopterin and neopterin, and patients with pterin carbinolamine-$4\alpha$-dehydratase deficiency have primapterin (a specific pterin) in the urine. Because dihydropteridine reductase does not show a specific pterin profile in urine, the enzyme is measured in blood. The diagnosis is completed by evaluation of
neurotransmitters, pterin and folate levels in the cerebrospinal fluid, which show distinctive alterations based on the underlying genetic disorder [76,77].

Figure 2. Tetrahydrobiopterin (BH4) synthesis
THERAPY

The aim of dietary protein-restricted treatment is to ensure that patients have a safe level of Phe, as measured in plasma or bloodspots [78]. For children up to 12 years of age levels below 360 μmol/L have the best outcomes [39,79-81], and for adults the need for metabolic control is still under debate. However, a study by Okano et al. found evidence that Phe levels below 500 μmol/L are safest because below this value, the least disturbance in oxidative stress status and nitric oxide metabolism are found [82]. Reference values for patients under treatment vary worldwide [16], but in the Netherlands the consensus is as follows (based on the Dutch clinical pathway for PKU; see chapter 2 of this thesis): 0–12 years: 120–360 μmol/L, ≥ 12 years: 120–600 μmol/L, and during pregnancy: 120–240 μmol/L.

Diet

The corner stone in the treatment of PKU consists of a strict natural protein-restricted diet [6,14-16,83]. The main natural food sources restricted in the PKU diet are protein rich foods, such as meat, fish, dairy products, bread and pasta. Patients are allowed a limited intake of natural protein based on their individual Phe tolerance. To provide a safe and sufficient intake of protein, patients are supplemented with special fabricated amino acid supplements containing most amino acids except for Phe and variable amounts of micronutrients and essential fatty acids. Patients may also use modified low protein food products as an alternative to some natural high protein products, such as cheese, milk, pasta and bread. Without the fortified amino acid supplements, patients are prone to deficiencies [17,84,85]. For example, deficiencies of selenium [86,87], zinc [88,89], folate [90], vitamin B6, vitamin B12 (in patients not adherent to amino acid mixture intake) [91-96], carnitine [97,98], tryptophan, tyrosine [63], transferrin, ferritin [99,100] and specific essential poly-unsaturated fatty acids (eicosapentaenoic acid and docosahexaenoic acid) have been described in patients with PKU undergoing treatment [101,102]. To regularly assess the nutrient status of the patients, regular visits to a physician and dietician are important, with more frequent visits during childhood.
BH4

A novel treatment of PKU (available in the Netherlands since 2009) consists of oral supplementation with high doses of BH4, the co-factor of PAH, which may enhance the function of the PAH enzyme in responsive patients. Approximately 25–50% of patients are responsive to BH4 treatment [103-106]. Mostly mild patients benefit from treatment with BH4 because these patients more often have some residual PAH activity. Those responsive to treatment have an increased Phe tolerance and may somewhat relax their diet, with a minority of patients being able to go off diet entirely [6,107].

LNAA

LNAA have been studied as a treatment option for patients with PKU based on their ability to block the uptake of Phe from the intestine and at the BBB due to competition at the L-type amino acid transporter [45,46,54,64]. Studies that demonstrate a clear benefit are scarce, and the effectiveness of LNAA as a single treatment is debatable. Because LNAA do not lower Phe values satisfactorily, their use should be avoided in pregnant women.

PEG PAL

The most recent advance in the pharmacological therapy of PKU is that of polyethylene glycol-conjugated phenylalanine ammonia lyase (PEG-PAL) [108-110]. PEG-PAL is a bacterial enzyme that catalyzes the degradation of Phe to trans-cinnamic acid, which is cleared by the kidney and secreted in the urine. Trials to evaluate the efficacy of subcutaneously administered PEG PAL injections are ongoing. Because PEG-PAL degrades Phe by means of an enzyme other than PAH, it is applicable in all patients regardless of their genotype.
Pregnant women with PKU provide a number of special challenges for dietary treatment because moderately elevated levels of Phe may lead to adverse outcomes in the fetus [111]. Koch et al. investigated the effect of maternal blood Phe levels on neonatal outcomes and found that the optimum range for blood Phe values in pregnancy lies between 120 and 360 \( \mu \text{mol/L} \). It is advised to achieve this strict dietary control by 8 weeks before pregnancy to minimize the risk for fetal complications [112,113]. Elevated levels of blood Phe may lead to maternal PKU syndrome with mental retardation, microcephaly and congenital heart defects in the offspring [41,111,113-117]. The exact mechanisms causing the teratogenic effects of the heart are unknown [116].
COMPLICATIONS IN PKU

Patients with untreated PKU show severe intellectual impairment, motor problems, behavioral disturbances, epilepsy, eczema and relatively fair skin and fair hair due to melatonin deficiency [13,118]. Paine et al. in 1957 described in detail the variability in manifestations of untreated patients before early treatment was widely introduced and before details about the genotype/phenotype correlations and the role of the amount of residual enzyme activity became known [119].

Since the 1950’s, dietary treatment for patients with PKU has been available, and many advances in the care for patients have been made. Patients diagnosed through newborn screening and treated early and continuously have very good outcomes. Despite the significant achievements, treatment has also led to some new challenges in the care for patients with PKU. Although some of these new challenges are due to dietary restrictions, others are caused by the disease itself. These new challenges are: nutrient deficiencies [17,120], bone health impairment [102,121-124] and hypothesized deficiencies in neurotransmitters (serotonin and dopamine) [32,125-127]. Furthermore, hidden disabilities [128] are postulated to exist in patients with PKU, caused by executive functioning defects [40,129-134], social problems [130,135], possible impaired quality of life [53,79,104,135,136] and emotional difficulties. Finally, the dietary treatment of PKU is assumed to place a significant burden on the patient because the diet is demanding [137], and further attention to patients’ needs is required [138].
THESIS OUTLINE

Optimizing care in patients with PKU requires fine-tuning of the treatment itself as well as evaluation and management of the adverse outcomes of the treatment. This thesis focuses on several topics, and the outline is as follows:

Chapter 2 discusses the importance of establishing pathways to provide optimal care for patients with inborn errors of metabolism, within the line of national consensus. There is broad diversity in the management of care for patients with PKU (both nationally and internationally). To standardize and optimize care, clinicians need to reach consensus on what the best care is for their patients.

Chapter 3 presents the results of a study performed to assess the health-related quality of life (HRQoL) in PKU patients and the effect of the novel treatment with BH4 on this outcome. HRQoL is the ultimate outcome of provided care, and standardized questionnaires are available to assess the subjective wellbeing of patients in relation to pre-specified domains of life (such as work, school, and emotions).

Chapter 4 reports on a cross-sectional study investigating the burden of the time and costs of living with PKU for adult patients and caretakers of pediatric patients in the Netherlands. The strict dietary treatment places a significant burden on the patient. Therefore, the burden associated with disease and treatment management needs to be assessed to better comprehend how the patient experiences overall wellbeing.

Chapter 5 provides a systematic review and meta-analysis on bone health in patients with PKU. It has been postulated in several publications that bone mineral density in patients with PKU is diminished, possibly due to differences in nutrient intake or through direct effects of the disease itself. We have pooled available patient data and we have reviewed several topics known to affect bones in order to evaluate bone health in patients with PKU.

Finally, Chapter 6 reports the results of a multi-center study on the nutrient status and bone health in patients with PKU. Changes in nutrient status and a diminished bone condition are considered important complications of a natural protein-restricted diet.
REFERENCES


