Phenylketonuria: impact and implications

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CHAPTER

GENERAL INTRODUCTION
Chapter 1

HISTORY OF PKU

“He is barely able to sit upright when supported. His head is inclined to drop to one side. He is unable to talk, expels some inarticulate sounds, cries and smiles, wants to play and to be entertained. He is unable to eat without assistance or to chew solid food and must accordingly be nourished on a liquid diet. He was never clean in his habits. He is unable to focus his eyes and there is a pronounced horizontal nystagmus” (1). This is a description of one of the two siblings in which the Norwegian biochemist and physician Asbjørn Følling discovered a new inborn error of metabolism responsible for their severe mental retardation, in 1934 (2;3;4). Dr Asbjørn Følling identified high levels of the phenylketone phenylpyruvic acid in the urine of these patients and he described the disorder as *imbecillitas phenylpyruvica*, thereafter renamed as *phenylketonuria* (2;3). Dr Følling’s initial theory was that the high amount of phenylpyruvic acid was the result of an inability to metabolize the amino acid phenylalanine (Phe), a hypothesis that was confirmed a few years later (3). The following two decades researchers discovered that the activity of the hepatic enzyme phenylalanine hydroxylase (PAH) is deficient in patients with phenylketonuria (PKU) (5;6;7).

Untreated PKU results in progressive mental deterioration with additional symptoms like motor deficits, epilepsy, behavioural and psychiatric disorders, a musty body odour and eczema. In the 1950s, Dr Horst Bickel developed a treatment: a phenylalanine restricted diet, and recommended that in order to prevent the known symptoms, treatment should be started in the newborn period (8). Early treatment became a possibility after the implementation of PKU newborn screening programs with the development of the Guthrie bacterial inhibition assay (9). Nowadays, with early initiation of the dietary treatment the severe cognitive disabilities and neurological sequelae can be prevented.

GENETICS AND BIOCHEMISTRY

Phenylketonuria (PKU; MIM 261600) is an autosomal recessive inborn error of metabolism caused by a deficiency of the enzyme phenylalanine hydroxylase (PAH; EC 1.14.16.1). The PAH gene is located on the long arm of chromosome 12 in the band region q22-q24 and until now, over 500 different mutations have been identified (www.pahdb.mcgill.ca). With an average incidence of 1:10.000, PAH deficiency is
the most common inborn error of metabolism in Europeans (10). As a result of the defect in the hepatic enzyme PAH, the essential aromatic amino acid phenylalanine (Phe) is not converted to tyrosine (Tyr), accumulates in the body and causes hyperphenylalaninemia (HPA) (11). PAH deficiency is a heterogeneous trait with a broad spectrum of phenotypes, spanning from very mild hyperphenylalaninemia, to the classical form of PKU in which there is no or hardly any residual enzyme activity (12). Genotypes correspond well with biochemical phenotypes like pre-treatment Phe levels and Phe tolerance (13).

Phe is derived from dietary protein and turnover of endogenous pools. Disposal of Phe is by hydroxylation to Tyr, incorporation into bound (polypeptide) pools, transamination and decarboxylation (11) (Table 1). At physiological levels, hydroxylation to Tyr accounts for 75%, and incorporation into protein for 25% of the total Phe disposal. In PKU, PAH is deficient, hydroxylation to Tyr is blocked (pathway 1) and consequently plasma Phe levels will rise. At elevated plasma Phe levels and when pathway 1 is blocked, pathway 3 becomes functionally significant and conversion to phenylpyruvic acid occurs (14). Products of the transamination are excreted in the urine. Pathway 2 is not an important alternative pathway not even in case of high Phe levels (15). The hydroxylation of Phe to Tyr, catalyzed by PAH, is dependent on tetrahydrobiopterin (BH4), as a cofactor, plus molecular oxygen, and iron.

Table 1: Phenylalanine metabolism

<table>
<thead>
<tr>
<th>Pathway</th>
<th>Reaction</th>
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<tbody>
<tr>
<td>1</td>
<td>Hydroxylation of phenylalanine to tyrosine</td>
</tr>
<tr>
<td>2</td>
<td>Decarboxylation of phenylalanine to Phenylethylamine</td>
</tr>
<tr>
<td>3</td>
<td>Transamination of phenylalanine to Phenylpyruvate/Phenylpyruvic acid</td>
</tr>
</tbody>
</table>

1. Hydroxylation of phenylalanine to tyrosine
2. Decarboxylation of phenylalanine to Phenylethylamine
3. Transamination of phenylalanine to Phenylpyruvate/Phenylpyruvic acid


**PATHOGENESIS AND NEUROTOXICITY**

The major effect of hyperphenylalaninemia (HPA) in PKU patients is on the brain function and development. Although the exact pathophysiologic mechanism by which HPA causes the neurocognitive damage is still not completely dissolved, there are several hypotheses supported with evidence, which address the different possible causes of the neurotoxicity secondary to elevated blood Phe levels. Discussed below are the following possible aspects: the effect on the transport of large neutral amino acids (LNAAs) across the blood brain barrier (BBB), the effect on brain white matter and myelinisation and the effect on neurotransmitters and protein synthesis.

**Large Neutral Amino Acids in the brain**

Phe transport into the brain is mediated by the large neutral amino acid type 1 (LAT1) transporter at the BBB, which selectively binds to, and transports, the large neutral amino acids (phenylalanine, tryptophan, tyrosine, histidine, valine, methionine, threonine, leucine and isoleucine) (16). Because Phe has the lowest $K_m$, it is most effectively transported by the LAT1 transporter in comparison with other LNAAs (16;17). In vivo studies (18;19) demonstrated competition at the BBB between Phe and other LNAAs (methionine, tyrosine and tryptophan). The expected result of this competitive transport is that in case of high blood Phe levels, brain Phe levels are increased and the uptake of the other LNAAs will be reduced. Elevated brain Phe levels have been established in PKU patients (20;21). Moreover, several studies performed in a PKU mouse model, showed reduced levels of LNAAs in the brain (22;16).

**Protein synthesis**

In animal studies it has been proven that elevated plasma and brain Phe levels resulted in decreased LNAAs in the brain (22;16) This deficiency of LNAAs has been hypothesized to cause the inhibition of protein synthesis in case of acute and chronic hyperphenylalaninemia (23;24). In the PKU mouse this decreased cerebral protein synthesis was confirmed (17). In PKU patients a negative relationship has been established between the plasma Phe concentration and cerebral protein synthesis (25;26).

**White matter abnormalities**

Reduced cerebral protein synthesis might explain the white matter changes in the brain of PKU patients (27), which have been established in several studies (28;29).
Different types of white matter abnormalities have been described in pathological, animal and magnetic resonance imaging (MRI) studies. The most frequently described phenomenon is myelin disturbance. In untreated patients there might be a lack of myelin formation, in early treated patients it is hypothesized that the white matter alteration might be a result of intramyelinic edema (30).

**Dopamine and serotonin**

Impaired neuronal synthesis of amine neurotransmitters, including dopamine, is likely to play an important role in the neurotoxicity of elevated brain Phe levels. Two distinct hypotheses have been proposed to explain the decreased dopamine levels. The first theory proposes that this phenomenon is due to the low levels of its precursor Tyr, caused by competition of high Phe levels with the uptake of Tyr across the BBB as both amino acids are transported by the same neutral amino acid transporter (31). As dopamine synthesis in dopaminergic neurons projecting on the prefrontal cortex, is highly sensitive to even small decreases in Tyr (32), the low tyrosine levels in the brain might cause dopamine depletion and thereby cognitive impairment (33). The second hypothesis focuses on the findings of decreased myelinisation in the brain of treated PKU patients which may cause down-regulation of neurotransmitter production as has been considered in a PKU mouse model (34).

Synthesis of serotonin occurs by hydroxylation of tryptophan by tryptophan hydroxylase. Like dopamine, tryptophan competes with Phe across the BBB (35). At elevated plasma Phe concentrations, brain tryptophan concentrations, and consequently brain serotonin levels, might be reduced. Indeed, in the cerebrospinal fluid of treated patients, reduced serotonin levels have been demonstrated (36). Furthermore it is hypothesized that high Phe levels might reduce tryptophan hydroxylase activity (37).

**DIAGNOSIS**

Since the late 1960’s, following the development of the Guthrie bacterial inhibition assay, PAH deficiency has been implemented in newborn screening programs in many countries.

Newborn screening is based on the detection of hyperphenylalaninemia in blood spots, as Phe is the marker for PAH deficiency. Phe levels in blood spots can be measured by several possible methods, but currently tandem mass spectrometry is the routine method of analysis (11;38).
TREATMENT

Dietary therapy
The mainstay of the treatment is the early initiation of a strict and often unpalatable protein restricted diet, severely limiting the Phe intake from natural protein (11). Protein substitutes are provided by commercially available special medical formulas containing all amino acids as well as vitamins and minerals, which are advised to be taken three times a day. Aim of the treatment is to maintain plasma Phe levels within safe limits and thereby prevent neurological damage. Regular blood Phe monitoring is performed to evaluate dietary adherence and to adjust therapy. Adherence to the diet is arduous and socially difficult (39) and maintaining Phe levels within the advised ranges is highly demanding. Therefore intensive dietary education, family involvement, promotion of self-reliance and self-efficacy are required (40).

BH4 therapy
Recently a new therapeutic option for PKU patients has been developed, sapropterin dichloride. Sapropterin is a synthetic formulation of the naturally occurring BH4. It has been shown that 20-40% of the PKU patients respond to sapropterin by a reduction in blood Phe concentration (41;42). The exact mechanism of action of sapropterin is not fully elucidated but several possible mechanisms have been proposed: increased BH4 levels may enhance the residual activity of the PAH enzyme; a chaperone effect which stabilizes PAH; improving affinity of PAH for BH4; and effects on BH4 and PAH synthesis (41;43;44). In responsive patients, Phe concentration is lowered significantly and thereby allows patients to relax the onerous dietary restrictions (45;46).

Guidelines
Guidelines for the treatment of patients with PKU vary widely between countries with respect to treatment criteria, recommended ranges of Phe levels and follow-up for all ages. However, this inconsistency is most prominent for the treatment of adults with PKU (47;48). Most treatment centers recommend a ‘diet for life’ and there is consensus about the necessity of monitoring Phe levels throughout life, however there is no consensus about the required dietary stringency in adulthood and there is limited evidence on the upper target Phe concentrations (49).
Future therapies
Although the dietary treatment of PKU is relatively effective, it is a diet not easy to comply with. The past years new treatment strategies and additional therapeutic options have been investigated (50).
A treatment approach under investigation is the administration of the enzyme phenylalanine ammonia lyase (PAL). PAL degrades Phe into the metabolites trans-cinnamic acid and ammonia (51). A different treatment strategy which is being developed, is LNAA supplementation. As Phe crosses the BBB via a transporter shared with other LNAAAs (17), LNAA supplementation has shown to reduce brain Phe levels by competition at this transporter and thereby decreasing the influx of Phe in the brain (52). Moreover a similar LNAA carrier protein exists in the intestine and administration of a different LNAA formulation was reported to reduce the blood Phe concentration significantly (53).
Another promising future treatment strategy which is being explored in animal models, is somatic gene therapy (54).

OUTCOME
Early initiation of the dietary therapy in PKU patients prevents severe neurological and cognitive damage. However, despite adequate treatment, subtle intellectual and cognitive sequelae in early and continuously treated patients have been reported (55-57). In spite of having an intelligent quotient within the normal range, PKU patients have slightly but significantly lower scores compared to control groups (58;59). Furthermore they show neuropsychological deficits, including higher-order cognitive abilities like planning, organization, working memory and inhibitory control, conceptualized as executive functions (60). All these deficits are associated with quality of the dietary control; high Phe levels during childhood and elevated lifetime Phe level results, are negatively related to IQ scores (55); impairments of executive functions have been reported to be related to concurrent and lifetime Phe levels (61-63).
Furthermore in some studies early treated PKU patients show more school and behavioural problems than do healthy controls. Patients with PKU more often repeat classes, need more global tutoring (64) and exhibit more task-orientated problems (65). In addition, in patients with PKU disturbances of emotional and behavioural functioning have been reported (47;66). Patients may demonstrate
decreased autonomy, suffer from low self esteem and might be more prone to develop internalizing problems as depression, anxiety and phobias (67;68). Despite these reported problems, findings on quality of life are contradictory: Weglage et al (69) reported patients with PKU to experience their social situation as being restricted, feeling less satisfaction with life and less carefreeness compared to the norm. A decreased quality of life in children with PKU was established recently (70), whereas in other studies adult patients with PKU reported a quality of life comparable to the normal population (71;72).

OUTLINE OF THE THESIS

Although phenylketonuria (PKU) is one of the first known inborn errors of metabolism and has been studied now for over 60 years, there are still many issues which remain to be clarified.

In this thesis several aspects of PKU, in children and in adults, are presented. It focuses on the psychosocial consequences and neurocognitive sequelae as well as on different aspects of dietary treatment.

Early detection and treatment of PKU is effective in preventing severe neurological impairments and developmental disabilities. However, patients can suffer from mild cognitive and neuropsychological deficits and sometimes experience social difficulties. A concise overview of these psychosocial deficits, associated with early-treated PKU, is given in chapter 2. In the treatment of children with PKU, parents play an important role. As the mental health of these parents influences the health, development and adjustment of their children, it is important to gain insight in the parental mental wellbeing. Therefore in chapter 3 the health-related quality of life of parents of children with PKU or galactosemia is studied.

The treatment of adult patients is still under discussion. Although there is consensus about a diet for life, treatment guidelines vary widely. Since dietary relaxation results in elevated Phe levels, it is of importance to further elucidate the consequences. The results of a placebo controlled trial, in which the effects of high Phe levels on neuropsychological functioning and mood are investigated, are presented in chapter 4. In addition it was attempted to contribute to the understanding of the effects of high Phe levels on the cognitive processes in the brain, with the help of magnetoencephalography (chapter 5).
Measurement of blood Phe levels to evaluate dietary adherence and to adjust therapy, is the cornerstone of the management of patients with PKU. However, maintaining Phe levels within the advised range is onerous. We hypothesized that enhanced self-management might encourage PKU patients and parents of children with PKU, to take more control of their diet or the diet of their child, in relationship to the Phe levels. For that reason, we performed a trial to evaluate the safety and feasibility of online availability of Phe levels (chapter 6). Since several methods are available to measure Phe levels for the purpose of monitoring, in chapter 7 the results are described of a comparative study of the different analysis methods.
REFERENCES


