CHAPTER

General discussion and future perspectives
Chapter 8

GENERAL DISCUSSION AND FUTURE PERSPECTIVES

This thesis addresses the psychosocial consequences and neurocognitive sequelae, as well as various aspects of the management of patients with the inherited metabolic disorder phenylketonuria (PKU).

Treatment: diet for life?

There is universal consensus that all newborns with PKU need to start a phenylalanine (Phe) restricted diet as soon as possible, in order to prevent severe cognitive impairment caused by high blood Phe levels. However, there is much debate on the need for a strict dietary control during adulthood and on the safe upper limit of Phe levels (1;2). With a growing cohort of early and continuously treated adult patients with PKU, development of management guidelines for this group of patients is of great importance (3). Besides recommendations about Phe levels, such guidelines should address other aspects of the management as well, such as nutrition, psychosocial support and follow-up (4).

The dietary treatment in PKU is highly demanding, and maintaining plasma Phe levels within the advised range is generally difficult and challenging. Because the dietary treatment imposes a heavy burden and necessitates continuous education, family support and self-discipline are needed (5). In this light it is not surprising that non-compliance with the strict dietary regimen is common (2;6) and the difficulty to maintain dietary control is reflected in a high incidence of Phe levels above target ranges (6;7).

As the discussion on the necessity of maintaining a strict metabolic control throughout life is complex and often based on expert opinion, it is highly important to try to provide evidence based advise. During childhood, high Phe levels are associated with a reduction of the intelligence quotient (IQ) (8). In children as well as in adults, executive function deficits have been reported to be related to both concurrent as well as lifetime Phe levels (9). Executive functions are higher cognitive functions such as organization, planning, attention, working memory en inhibitory control (10). An impairment of these functions may affect daily life significantly.

An important result of the research reported in this thesis (chapter 4), is that elevated Phe levels are negatively associated with mood. Persistent negative changes in mood may ultimately impact stability in relationships and social interactions. Although the burden of a life long dietary regimen in PKU patients is substantial, in view of the existing evidence of the significant consequences of high Phe levels, we consider
it important to advise patients to maintain the diet throughout life. Further studies are necessary to investigate the effects of elevated Phe levels on behavior and quality of life.

**Outcome and follow-up**

Despite the early initiation of the dietary treatment, IQ levels of patients with PKU are frequently lower than those of family members and peers, although within the normal range (11). Moreover, patients experience hidden disabilities including subtle deficits in executive functioning, social difficulties and emotional problems, impairments which may remain unnoticed for years because of their delicateness (12; chapter 2). Executive functions play an important role in activities involved in the achievement of goals (10) and as these skills are embedded in many social and productive activities, it is highly plausible that there might be an effect on the daily life of PKU patients in case of an impairment of these abilities. These hidden disabilities may even contribute to the suboptimal intellectual outcome (13) and lower school achievement (14), reported in treated PKU patients. However, the exact correlation of the executive function deficits in PKU patients with clinical outcome is not known (9). More longitudinal research is needed to determine the clinical consequences of the neurocognitive deficits and the exact impact on quality of life.

Taken together the psychosocial difficulties, the burden of having a chronic disease and the necessity of compliance with a strict diet, it seems highly plausible that the health related quality of life (HRQoL) of PKU patients may be impaired. However, previous studies did not show a decreased HRQoL in children and adults with PKU (15;16), although a tendency of lower or delayed levels of autonomy and a lower rate of forming normal adult relationships compared to healthy adults, was reported (17). It might be that generic questionnaires used in these studies do not detect the obstacles and difficulties that affect the daily lives of patients with PKU. Therefore, the development of a questionnaire focussing on PKU-specific problems may result in more reliable assessment of HRQoL in PKU patients.

Emotional problems and disturbances of behavioural functioning have been reported in patients with PKU (chapter 1;18). PKU patients may demonstrate decreased autonomy and may suffer from low self esteem and might be more at risk to develop internalizing problems such as depression, anxiety and phobias (19;20). The underlying mechanisms that cause these psychosocial impairments are not clear. A direct relation to the increased Phe levels in PKU is possible, as elevated Phe levels may result in a disturbance of serotonin metabolism (21). However the
burden of living with a chronic condition may contribute as well to the psychosocial difficulties. Psychosocial support can aid patients with PKU and their parents to handle the difficulties of adherence to a strict and unpalatable diet and to cope with the legacy of living chronic medical condition (1). Furthermore longitudinal follow-up research is necessary to study neurological problems. In PKU patients minor neurological signs like intention tremor and brisk tendon reflexes are common (22). With aging PKU patients might develop other neurological impairments (23).

**Neuropathology and assessment of brain function**

The exact pathophysiological mechanisms by which Phe causes neurocognitive damage are still not elucidated. However, there are several hypotheses addressing possible causes of the neurotoxicity. It is highly likely that the neurocognitive damage in PKU is caused by different processes, all occurring together. The most important neurotoxic pathophysiological mechanisms are thought to be the direct effects of high Phe levels in combination with reduced levels of brain large neutral amino acids (LNAAAs) (24). Reduced LNAA concentrations are a consequence of the competition at the blood brain barrier (BBB) of Phe with other LNAAAs including tyrosine (Tyr) and tryptophan (Trp), as these amino acids all use the same transporter (the LNAA transporter) for transport across the BBB. The low levels of Tyr and Trp might contribute to the depletion of the neurotransmitters dopamine and serotonin. The dopamine depletion in patients with PKU, either due to a deficiency of its precursor Tyr (25) or secondary to hypomyelinisation (26), might play a prominent role in the development of the neurocognitive impairments. Since the prefrontal cortex is highly susceptible for changes in Tyr levels, the deficits established in PKU patients are frequently specific to the cognitive functioning of the prefrontal cortex (25;27). In addition, the decreased levels of other LNAAAs may be a cause of the inhibition of cerebral protein synthesis (28).

The mood disturbances in patients with PKU might be caused by a deficiency of brain serotonin levels (21). Possibly Phe interferes with the synthesis of serotonin by different mechanisms. The first mechanism is that as a result of the competition at the BBB, the availability of Trp is reduced and thereby the production of serotonin, which is synthesized from Trp via two subsequent enzymatic reactions, is limited. The other option is that elevated Phe levels might affect the synthesis of 5-hydroxytryptophan, the second rate-limiting factor in serotonin production (29). Finally, it may well be that both mechanisms occur together.
In order to study the pathophysiologic processes involved in the impaired cognitive functioning in PKU patients, several neuroimaging techniques and assessments of brain activity have been used. Brain white matter abnormalities, possibly caused by decreased myelinisation, have been described in MRI studies of PKU patients, and were found to be related to metabolic control. However, the functional implications of these white matter lesions are unclear (30). In future research it will be very interesting to perform longitudinal studies, examining the onset and course of the white matter alternations as well as the relationship with neurocognitive impairments. Transport of Phe across the BBB probably plays a crucial role in the neuropathogenesis of the cognitive impairments in patients with PKU. Therefore measurements of brain Phe concentrations and understanding of the main principles of the LNAA transport into the brain, are essential. Magnetic resonance spectrometry (MRS) studies have shown that the blood Phe/brain Phe ratios vary between 0.21 and 0.74 in adult patients with PKU (31). However, in other studies no clear correlation between the plasma Phe levels and the brain Phe levels could be established (32;33). Various reports suggest that interindividual variations exist in Phe transport across the BBB (34;35). This might explain the individual differences in vulnerability to elevated blood Phe levels, which is reflected by the 1% of the PKU patients in whom no cognitive damage occurs despite their deficient PAH enzyme (22). An important future research question is whether cognitive deficits are related to brain Phe levels and if brain Phe levels have a predictive value for future neuropsychological and behavioural problems. Future studies should integrate the MRS findings with clinical parameters including neuropsychological tests and mood questionnaires. In this thesis, magnetoencephalography (MEG) measurements were performed to investigate the relationship between blood Phe levels and brain activity (chapter 5). Elevated Phe levels appeared not to affect the power frequency spectrum of brain activity. In another disorder in which brain dopamine levels are disturbed, Parkinsons disease, it was suggested that dopamine has an effect on functional interactions between brain regions, but does not affect power (36). Possibly, the brain dopamine depletion in PKU patients might only affect functional connectivity. Analysis of the functional connectivity in the MEG recordings of these patients is warranted.

**Future management strategies**
An important goal in the management of patients with PKU will be to monitor and
follow-up the cognitive and emotional functioning in relation to different treatment strategies, in order to optimize the treatment. To this end large-scale multi-centre studies are needed to measure executive function deficits in early treated PKU patients, using a set of neuropsychological tests that can be performed in the clinical setting and can be repeated for the purpose of follow-up. In this way it should be possible to unravel which cognitive functions are affected most and should therefore be monitored throughout life.

Since PKU is a disorder which requires treatment for life, the responsibility for adherence to the dietary treatment gradually shifts from the parents to the patients. Therefore the management of patients needs to include teaching of self-management. Our study in which patients were provided with their own Phe levels via a personal web page, showed that this was a safe and highly appreciated approach (chapter 4). This approach, which we labelled “My PKU”, enables patients and/or their parents to make their own decisions about the frequency and timing of blood sampling and adjustments of their diet. Thereby “My PKU” provides patients more control on their own disease and less interference with their personal lifestyle. This might improve the quality of life like as was demonstrated in other chronic disorders (37;38), by enhancement of self-efficacy (39), confidence and control.

Novel alternative pharmacological approaches to the treatment of PKU may improve the long-term outcome of the disease and the HRQoL. Some of these options are currently being introduced while others are still under development.

Sapropterin is a synthetic formulation of the naturally occurring tetrahydrobiopterin (BH4), which is already used in the management of patients with PKU. This drug significantly lowers the blood Phe concentration in patients responsive to this treatment, particularly in patients with milder forms of PKU, by serving as a cofactor for PAH and consequently enhancing the residual enzyme activity (40). The exact pharmacologic mechanism is not fully elucidated, but there are several theories: 1) stimulation of the PAH activity; 2) improving the affinity of PAH for BH4; 3) a chaperone effect of Sapropterin thereby stabilizing PAH; and 4) effects on BH4 and PAH synthesis (41;42).

Another treatment strategy might be LNAA supplementation. LNAAAs have been shown to reduce brain Phe levels by competitive inhibition of the transport across the BBB (43). In the intestinal mucosa, a similar LNAA carrier protein exists as at the BBB and administration of a different LNAA formulation was reported to reduce the blood Phe concentration (44;45). A study evaluating the relationship between LNAA supplementation and cognitive outcome, showed that LNAA supplementation
resulted in better performance on executive functions particularly in verbal fluency and cognitive flexibility (32). More research is necessary to investigate the possible role of LNAA supplementation and its optimal dosage (46).

A possible alternative treatment for PKU, which is still under investigation, is enzyme substitution therapy with phenylalanine ammonia lyase (PAL; E.C.4.3.1.5). PAL degrades Phe into the metabolites trans-cinnamic acid and ammonia (47), which can be excreted in the urine. In the PKU mouse model, weekly subcutaneous enzyme injections effectively lowered the blood Phe levels (48). Clinical trials on the safety, tolerability and efficacy of subcutaneously and orally administered of PEGylated recombinant PAL (PEG-PAL) in PKU are currently performed (49).

Finally, gene therapy is a promising therapeutic option which has until now been studied in animal models. In a PKU mouse model, liver directed gene therapy has yielded a correction of hyperphenylalaninemia for several weeks (50). However a permanent correction of PAH deficiency is not attained through this method, because the genome of the vector is not integrated in the DNA of the hepatocyte and reinjection leads to an antibody-mediated immune response (49). An alternative target organ for gene therapy might be skeletal muscle, in which however besides the PAH enzyme, the expression of enzymes involved in the synthesis and recycling of BH4 are required as well (51).

In conclusion, despite over 50 years of experience with treatment of PKU with a Phe restricted diet, many questions are still unanswered. However, several promising treatment and follow-up strategies are now studied and these approaches might enhance the management of patients with PKU in the future, thus facilitating the daily life of patients with PKU.
REFERENCES


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