Chapter 1

General Introduction

Published as Mol. Genet. Metab. 2010; 99 Suppl 1: S86-S89

A brief history of phenylketonuria

In 1930, Mr. and Mrs. Egeland were in a state of deep concern. At the age of three years, their daughter Liv had still not begun to speak. Now, their several month old son Dag also seemed to be developmentally delayed. At first, Dag had developed normally. However, during the last few months, he showed decreasing interest for his surroundings and was increasingly difficult to feed. In addition to these developmental symptoms, the parents had noted that their children's urine had a particular, musty odor. After visiting several doctors to no avail, the Egelands turned to Asbjørn Følling, a physician and chemist studying metabolic diseases.

Puzzled by the developmental symptoms combined with the particular urine smell, Følling started analyzing urine samples. He discovered that these samples contained phenylpyruvic acid, a ketonic breakdown product of the amino acid phenylalanine (Phe; the particular odor was related to phenylacetate, a metabolite of phenylpyruvic acid). Følling proceeded to test urinary samples of mentally retarded children in institutions, and found that phenylpyruvic acid was present in several of those samples. In 1934, approximately six months after being approached by the Egelands, Følling published his findings, terming the newly discovered condition "imbecillitas phenylpyruvica" (1). A year later, the English geneticist Lionel Penrose changed the disorder's name to phenylketonuria (PKU) (2), reflecting the presence of phenylketones in the urine associated with the disease. Although PKU could now be diagnosed, treatment options were still unavailable.

It would not be until the 1950s that further steps in the characterization and treatment of PKU were made. In 1951, Louis Woolf and David Vulliamy reported the effects of treating PKU with glutamic acid, theorizing that reducing blood concentrations of Phe and/or its metabolites could improve cognitive development. After observing that glutamic acid did not achieve the desired effects, the authors suggested that a dietary restriction of Phe might be an effective treatment form (3). In 1953, George Jervis showed that PKU results from a deficiency of phenylalanine hydroxylase (PAH), the enzyme converting Phe into tyrosine (Tyr) (4). In the same year, Horst Bickel and colleagues, in cooperation with Louis Woolf, published the first report on the effects of a Phe-restricted diet in PKU. In a severely affected two-year-old girl, this diet not only reduced blood Phe concentrations, but also improved development and behavior (5). To this day, a dietary Phe-restriction treatment, achieved by limiting natural protein intake while supplementing non-Phe amino acids, remains the cornerstone of PKU therapy (6,7).

Could early diagnosis and treatment of PKU lead to a better outcome? In 1963, Robert Guthrie and Ada Susi developed a blood test to detect elevated concentrations of Phe, allowing for population-wide screening of neonates (8). In

subsequent years, continuous treatment of early-diagnosed patients proved to prevent the severe mental retardation associated with untreated PKU. Unfortunately, the diet could not normalize development once severe mental retardation had occurred (as in the Egeland children). Throughout the following decades, the genetic and enzymatic abnormalities associated with PKU were characterized in detail, and dietary treatment was further developed, e.g. by improving taste and applicability of the amino acid supplementations. In recent years, PKU treatment was additionally optimized, following the discovery that certain PKU patients benefit from treatment with tetrahydrobiopterin (BH4), the naturally occurring cofactor of PAH (6,9). In these so-called BH4-responsive patients, treatment with the cofactor reduces blood Phe concentrations, thus improving metabolic control and/or allowing for a relaxation of the strict dietary treatment.

Despite the advances in the PKU field, several pathophysiological and treatment-related questions remain today. For example, although the strong relationship between elevated blood Phe concentrations and disturbed cognitive development has been well-established (10,11), the mechanisms mediating this relationship are only partially known (12,13). Moreover, current PKU treatment may be further optimized, as early and continuously treated patients still show impaired mental functioning, manifested as mild reductions in intelligence quotient (14-16), executive function deficits (17-20), and possibly an increased risk for psychiatric disorders (21). Several new treatment modalities have been suggested to further improve outcome in PKU (6,7).

This thesis investigates several topics associated with these pathophysiological and treatment-related questions. The remainder of this introduction provides a background to these topics. First, current knowledge on blood-brain barrier (BBB) transport of large neutral amino acids (LNAAs), cerebral LNAA and neurotransmitter metabolism, and cerebral protein synthesis (CPS) is reviewed in the context of PKU. Second, the molecular regulation of CPS in relation to cognitive functioning is addressed. Third, the presently identified phenotypes of existing genetic PKU mouse models are discussed, followed by two examples of how pathophysiological insights may improve PKU treatment.

LNAA transport across the blood–brain barrier

In the pathophysiology of mental dysfunction in PKU, amino acid transport across the BBB is considered to be important for two reasons. First, PKU symptomatology almost exclusively concerns the brain (22). Second, some untreated PKU subjects show normal intelligence despite having the blood biochemical characteristics of untreated PKU (23-25).

Amino acid transport across the BBB is a dynamic process, currently known to be mediated by ten amino acid transport systems (26). One of these transport systems involves the large neutral amino acid type 1 (LAT1)-transporter, which selectively binds to the LNAAs (valine, isoleucine, leucine, methionine, threonine, tryptophan, Tyr, histidine, and Phe) (27,28). Binding of LNAAs to the LAT1-transporter is a competitive process (27-29). Moreover, the LAT1-transporter likely acts as a counter-transporter, excreting one LNAA for each LNAA taken up into the brain (30).

At physiological LNAA concentrations, the LAT1-transporter is almost fully saturated (27-29). It has different affinities and k_m-values (the k_m-value is the substrate concentration at which the reaction rate is 50% of its maximum value) for each LNAA, and Phe has the lowest k_m-value, indicating that it binds the LAT1-transporter more strongly than other LNAA (27,28,30). Therefore, elevated blood Phe concentrations in PKU are believed to markedly increase uptake of Phe from blood to brain and to reduce uptake of non-Phe LNAA by two mechanisms. First, non-Phe LNAA uptake into the brain is reduced because of competitive inhibition by Phe. Second, non-Phe LNAA export from the brain in exchange for blood Phe is increased. These processes likely continue until a new equilibrium is reached and Phe is continually transported across the BBB, resulting in a net Phe transport of zero. Several clinical studies support the notion that elevated blood Phe concentrations reduce blood-to-brain transport of non-Phe LNAAs. In nine late-treated mentally retarded PKU patients with blood Phe concentrations mostly >1000 µmol/L, blood-to-brain transport of ^{75}Se-selenomethionine was reduced compared to non-PKU mentally retarded subjects (31). Similarly, Landvogt et al. (32) reported reduced uptake of F-dihydroxyphenylalanine (F-DOPA) in PKU patients compared to healthy controls. Similar to LNAA uptake, F-DOPA uptake from blood to brain is mediated by the LAT1-transporter (32). In addition, in healthy volunteers consuming a single dose of 100 mg Phe/kg of body weight, uptake of the artificial LNAA ^{11}C-amino-cyclohexanecarboxylate was reduced in the presence of markedly elevated plasma Phe concentrations (33).

If elevated plasma Phe concentrations disturb LNAA uptake from blood to brain, one would expect elevated brain Phe concentrations and reduced brain non-Phe LNAA concentrations in PKU. Indeed, elevated brain Phe concentrations have been observed in PKU patients, as measured by magnetic resonance spectroscopy (34-40), and in the Pah-enu2 PKU mouse model (41-48). Moreover, reduced brain concentrations of valine, isoleucine, leucine, methionine, and Tyr have been reported in the Pah-enu2 PKU mouse model (41,42,45,46,48). In autopsied brains of PKU patients, brain concentrations of Tyr and tryptophan were reduced (49). It is not yet technically feasible to measure brain non-Phe LNAA concentrations non-invasively

in vivo.

Even in the relatively mild supraphysiological range of 200–600 µmol/L, blood Phe concentrations are negatively associated with CPS (27,50). Thus, even in early- and continuously-treated patients with blood Phe concentrations within the currently recommended treatment range, LNAA transport across the BBB may be disrupted.

Based on the concept that disturbed LNAA transport is central in PKU pathogenesis, studies using oral LNAA supplementation as a PKU treatment were conducted. These studies showed that oral LNAA supplementation lowered brain Phe concentrations (36,38,51), mitigated electroencephalography (EEG) abnormalities (36), and improved neuropsychological performance (40).

In healthy individuals, all LNAA except Tyr are essential amino acids (EAAs; i.e. they cannot be biosynthesized in man). In PKU, Tyr synthesis is reduced, so that Tyr may also function as an EAA, in particular in untreated PKU. Reduced non-Phe LNAA transport across the BBB in PKU may thus result in cerebral EAA deficiencies, possibly impairing cerebral neurotransmitter and/or protein synthesis, leading to the mental retardation and other cognitive and neurological abnormalities observed in PKU. Thus, reduced brain non-Phe LNAA concentrations, rather than elevated brain Phe concentrations, might be considered of paramount importance in the pathogenesis of PKU (13). This theory will be discussed in more detail below.

Neurochemical findings in PKU

In the Pah-enu2 PKU mouse model, concentrations of catecholamines, serotonin, and their associated metabolites are reduced in homogenized brain (42,45,47,52-55) and in different brain regions, including the prefrontal cortex, amygdala, hippocampus, and striatum (46,52,56-59). Embury et al. (58,60) also reported reductions in dopaminergic cell body density in the substantia nigra and nigrostriatum, another finding possibly consistent with decreased dopamine synthesis. In PKU patients, reduced concentrations of catecholamines, serotonin, and associated metabolites have been reported that are similar to those reported in the PKU mouse brain, both in brain tissue (49) and in cerebrospinal fluid (61-63). Dietary treatment restores neurotransmitter metabolite concentrations in cerebrospinal fluid (61,63), as do Tyr and tryptophan supplementation (64). Taken together, these findings suggest that reduced neurotransmitter concentrations in PKU are caused by reduced neurotransmitter synthesis rather than increased neurotransmitter degradation.

Synthesis of catecholamines occurs via hydroxylation of Tyr to L-dihydroxyphenylalanine (L-DOPA) by tyrosine hydroxylase. L-DOPA is subsequently converted to dopamine, which is next metabolized to noradrenalin and adrenalin.

Reduced catecholamine synthesis in PKU may be caused by competition between brain Phe and Tyr for hydroxylation by tyrosine hydroxylase (45,46,54,56,57). Other explanations for reduced brain catecholamine synthesis in PKU include reduced synthesis and/or availability of tyrosine hydroxylase, which has been reported in the Pah-enu2 PKU mouse model (46,57,58), and reduced BBB transport of Tyr. This latter theory is supported by the reduced brain Tyr concentrations reported in PKU mice (41,45,46,57) and reduced brain Tyr concentrations in PKU patients (49).

Synthesis of serotonin occurs via hydroxylation of tryptophan to 5-hydroxytryptophan by tryptophan hydroxylase. Subsequently, 5-hydroxytryptophan is converted to serotonin (5-hydroxytryptamine). Little is known about the cause of the probable reduction of brain serotonin synthesis in PKU. Reduced serotonin synthesis may be the result of reduced tryptophan brain concentrations caused by reduced BBB transport of tryptophan at elevated plasma Phe concentrations (13). Although brain tryptophan concentrations of PKU mice are comparable to those found in heterozygous or wild type mice (42,45,52,55), reduced brain tryptophan concentrations have been identified in PKU patients (49). Alternatively, reduced brain serotonin synthesis may be caused by reduced tryptophan hydroxylase activity at elevated brain Phe concentrations. In accordance with this idea, Pascucci et al. (52) reported reduced hydroxylation of tryptophan to 5-hydroxytryptophan in PKU mice compared to non-PKU controls while the amount of tryptophan hydroxylase was unaltered, suggesting reduced tryptophan hydroxylase activity. Interestingly, tryptophan hydroxylase activity was restored after treatment with Phe-restriction without amino acid supplements (52). This *in vivo* work supports the *in vitro* finding of an inhibitory effect of Phe on tryptophan hydroxylase activity (65).

The clinical significance of reduced brain catecholamine and serotonin concentrations in PKU patients has not been fully elucidated. Of these neurotransmitters, dopamine has been studied most extensively. Reduced dopamine availability may be particularly problematic for prefrontal cortex neurons, which have a higher dopamine turnover than neurons elsewhere in the brain (14,66,67). Dopamine availability in the dorsolateral prefrontal cortex is important for executive functioning, and thus may explain the reduced neuropsychological performance observed in PKU patients (14,17,18). Moreover, untreated PKU patients may occasionally develop chorea, tremors, and dystonia (68,69), symptoms possibly caused by dopamine deficiency in the basal ganglia. Cerebral serotonin deficiency may explain the increased occurrence of anxiety and depression disorders in PKU patients (21).

However, although both dopamine and serotonin are likely to be involved in postnatal brain development and maturation, severe mental retardation is not

the most characteristic feature of inborn deficiencies of these neurotransmitters in humans (70). Therefore, while neurotransmitter deficiencies likely underlie certain cognitive deficiencies in PKU, they do not seem to fully explain its clinical presentation.

Cerebral protein synthesis in PKU

Reduced brain non-Phe LNAA concentrations rather than elevated brain Phe concentrations may be the main pathophysiological mechanism of cognitive dysfunction in PKU. Reduced brain non-Phe LNAA concentrations may underlie the impairments of CPS reported by several authors in PKU animal models. Increases in inactive monoribosomes, reductions in polyribosomes, and reductions in polypeptide elongation have been reported in pharmacologically induced chronic hyperphenylalaninemia (HPA) in mice (71,72) and after a single Phe injection (73). LNAA supplementation restored brain non-Phe LNAA concentrations (72), and restored polyribosome formation and polypeptide elongation either partially (73) or completely (71,72). Interestingly, brain Phe concentrations were unaltered, suggesting that CPS may be more affected by reduced brain non-Phe LNAA concentrations than by elevated brain Phe concentrations (71,72).

In hyperphenylalaninemic rats, reduced incorporation of ^{3}H-leucine and ^{3}H-lysine into cerebral proteins has been reported (27,74). Likewise, reduced incorporation of ^{14}C-leucine into cerebral protein has been reported in the Pah-enu2 PKU mouse model (41). Studies in early and continuously treated PKU patients demonstrated that CPS decreased as blood Phe concentrations increased (50,75). These data show that in PKU, CPS is negatively associated with increased blood Phe concentrations, both in patients and in animal models.

Reduced CPS in PKU might underlie a variety of neuroanatomical findings reported in PKU patients, in the Pah-enu2 PKU mouse model, and in the pharmacologically induced HPA rat model. Bauman and Kemper reported reduced myelination and reduced dendritic arborization of brain structures in three adults with untreated PKU at post-mortem investigation, a possible consequence of reduced CPS (76). Impaired dendritic arborization has similarly been reported in the Pah-enu2 PKU mouse model (53,77). Moreover, abnormalities of periventricular and subcortical white matter have been reported in PKU patients (78-80). In the PKU mouse model, reduced myelin staining in forebrain structures has been reported (57,81,82). Oligodendroglia, cells that synthesize myelin under healthy conditions, seemed to have adapted to a non-myelinating phenotype in the PKU mouse brain (57). Berger et al. (74) found myelin proteins to be reduced by 50% compared to controls in a HPA rat model. Dyer et al. (81) reported altered isoform expression

of myelin basic protein in PKU mice, and reduced concentrations of myelin basic protein were later reported in untreated PKU mice, which were restored upon dietary Phe-restriction (57).

Regulation of CPS is essential for brain development and function, as it forms the molecular basis of synaptic plasticity, long-term potentiation, and cognition (83,84). Deficiencies in the regulation of CPS may cause mental retardation syndromes in man (83-86) and therefore could be associated with mental retardation in patients with PKU.

Molecular regulation of cerebral protein synthesis

In the field of neurobiology, a wide array of studies have contributed to knowledge of the molecular regulation of CPS, many of which relate CPS to learning and memory formation. An extensive review of the molecular processes regulating learning and memory is beyond the scope of this thesis. Therefore, this section focuses on one particularly important molecular regulator of CPS.

This key molecular regulator is the cAMP responsive element binding (CREB) protein (87-90). CREB activity is mediated by phosphorylation at different sites, which can be induced by a wide variety of stimuli and associated kinases. In the regulation of CREB activity, its most important phosphorylation site is serine-133 (Ser-133) (88,91). When phosphorylated at Ser-133, CREB binds to a specific promotor region called the cAMP responsive element (CRE) motif (88,90-92). Following CREB binding to the CRE motif, transcriptional coactivators are recruited (including CREB-binding protein), after which RNA polymerase II binds to the transcription complex and transcription is initiated (88,90,91). By this process, CREB regulates the transcription of many genes involved in the formation of learning and memory, such as those encoding transcription factors, growth factors, and neurotransmitter receptor subunits (90,92). The translation of mRNA derived from these genes into cerebral proteins underlies the cellular acquisition and maintenance of memory (90,92,93).

CREB regulates protein synthesis in a wide variety of organisms, ranging from sea snails to humans (87,88,90). Increased CREB phosphorylation at Ser-133 underlies learning and memory in many paradigms and associated brain regions (88,90,92). Along these lines, impaired CREB Ser-133 phosphorylation disturbs the formation of learning and memory in several tasks (88,90). Together, these findings show the importance of CREB phosphorylation at Ser-133 in regulating CPS and the associated formation of learning and memory.

From fundamental neurobiology to cognitive functioning

One may wonder to which extent the above neurobiological findings are relevant for PKU, and whether learning and memory performance in animal models translates to cognitive functioning in humans. Several arguments support the validity of extending these fundamental neurobiological findings to humans. First, the role of CPS in healthy development and cognitive functioning has been well-established (85,86), and several mental retardation syndromes in humans are associated with reduced CPS (84-86). Second, comparing CREB-mediated effects across different species shows that CREB regulates increasingly complex processes as species complexity increases (88,90), suggesting that CREB-mediated CPS could underlie processes as complex as human cognitive functioning. Clinically, the relevance of CREB and associated regulatory factors for cognitive functioning in humans is evidenced by two genetic mental retardation syndromes affecting CREB-mediated signaling, i.e. the Rubinstein-Taybi syndrome and the Coffin-Lowry syndrome. In the Rubinstein-Taybi syndrome, mutations in the gene encoding CREB binding protein prevent CREB from mediating its regulatory effects (94). In the Coffin-Lowry syndrome, reduced activity of ribosomal S6 kinase 2 (RSK2) prevents phosphorylation of several of its downstream targets, one of which is CREB (95). Both syndromes have severe mental retardation as one of their clinical hallmarks, signifying the importance of well-regulated CREB activity for normal cognitive development. This importance is further underlined by the observation that in patients with Coffin-Lowry syndrome, the residual ability of RSK2 to phosphorylate CREB at Ser-133 correlated with cognitive outcome (96). Third, in several studies investigating human cognitive deficits, learning and memory performance in animal models have contributed to pathophysiological understanding and treatment development (85,86,88,90). Combined, the above findings illustrate the translational value of neurobiological experiments assessing CREB-mediated signaling, CPS, and learning and memory for the study of cognitive functioning in humans.

The development of the Pah-enu2 PKU mouse models

For the study of neurobiological processes associated with PKU, the development of the genetic Pah-enu2 PKU mouse model has been crucial. Previous *in vitro* and *in vivo* PKU models showed several characteristics limiting their translational value, such as biochemical alterations not observed in PKU patients and inhibition of enzymes other than PAH (97). First described in 1993 (98), the Pah-enu2 PKU model was developed by exposing mice of the BTBR strain to *N*-ethyl-*N*-nitrosourea (enu), a chemical which randomly induces gene mutations. By crossing and backcrossing several generations, mice displaying hyperphenylalaninemia were

obtained. In the so-called enu2 mice, the hyperphenylalaninemia proved to result from PAH deficiency, thus reflecting the enzymatic deficiency underlying PKU. In 1997, the PAH deficiency in enu2 mice was shown to result from homozygosity for a point mutation in the associated gene, validating that the enu2 mouse line genetically modelled PKU (99). In subsequent years, the BTBR Pah-enu2 PKU mouse model was further characterized on a biochemical and behavioral level, and was shown to display corresponding phenotypes reflecting PKU in humans (41,42,45,100,101). In 2006, the Pah-enu2 mutation was bred into the C57Bl/6 mouse strain to increase breeding efficacy (102). Although studies investigating blood and brain biochemical phenotypes of C57Bl/6 PKU mice have been limited, existing data suggest that the blood and brain biochemical phenotypes of these mice are similar to those observed in BTBR PKU mice (47,48,55,103). Contrary to these biochemical phenotypes, learning and memory phenotypes of C57Bl/6 PKU mice have not been reported. The absence of such reports matters, as in contrast to the BTBR strain, the C57Bl/6 strain is widely used in neurobiological studies to investigate learning and memory performance (104-106). Moreover, thus far, no studies have investigated neuromolecular pathways in the C57Bl/6 PKU mouse model. In conclusion, while several studies have investigated behavioral, biochemical and molecular phenotypes in the BTBR PKU mouse model, the C57Bl/6 PKU mouse model has scarcely been characterized, in particular regarding learning and memory deficits and associated molecular pathways.

New treatment modalities in PKU

Pathophysiological knowledge obtained in PKU mouse models may serve to optimize current PKU therapy, regarding both treatment burden and treatment outcome. This paragraph addresses two treatment modalities that may improve current PKU treatment using pathophysiological considerations as a starting point, i.e. LNAA supplementation and BH4 treatment.

LNAA supplementation refers to increasing intake of non-Phe LNAAs in order to achieve specific treatment aims, which include 1) reducing blood Phe concentrations and increasing blood concentrations of non-Phe LNAAs by influencing gastro-intestinal LNAA uptake, 2) reducing brain Phe concentrations, 3) increasing brain concentrations of tyrosine and tryptophan, aiming to increase synthesis of associated neurotransmitters, and 4) increasing brain concentrations of all non-Phe LNAAs, aiming to increase CPS. The concept of LNAA supplementation therapy in PKU has been suggested as early as 1948 (107). In the past decades, several studies have investigated LNAA supplementation in relation to the above treatment aims, both fundamentally and clinically. Still, LNAA supplementation is currently not

used in PKU treatment. Thus, the question arises what the current state of evidence is for this therapeutic modality, and which issues should be clarified before LNAA supplementation can be applied clinically.

A second treatment modality of interest to this thesis is BH4 treatment. The decrease of blood Phe concentrations occurring in certain BH4-treated PKU patients is mediated by increased activity of mutated PAH. BH4 acts as a cofactor for not only PAH, but also for tyrosine hydroxylase and tryptophan hydroxylase. Considering that BH4 is able to increase activity of mutated PAH, it could be conceived that BH4 treatment similarly increases activity of the tyrosine and tryptophan hydroxylases. Thus, BH4 treatment could increase synthesis of the associated downstream neurotransmitters and improve mental functioning. If so, BH4 treatment might be beneficial to all PKU patients, regardless of its effect on blood Phe concentrations.

Thesis outline

This thesis focuses on the pathophysiology of reduced CPS and cognitive dysfunction in PKU, combining data obtained in PKU patients and in the C57Bl/6 PKU mouse model. In addition, the relevance of several pathophysiological insights for clinical practice is addressed, by reviewing the current state of evidence for LNAA supplementation, and by investigating possible new therapeutic targets in BH4 treatment.

As discussed above, CPS has been shown to play a pivotal role in cognitive development, and impairments of this process result in cognitive deficits. Reduced CPS has been described in PKU in both patients and rodent models (27,41,50,75). We recently showed that CPS decreases as blood Phe concentrations increase (50). However, many aspects of the biochemical and molecular pathways mediating the relationships between blood Phe concentration, CPS and cognitive dysfunction in PKU remain to be clarified. To this aim, this thesis addresses the following research questions:

1. To which extent is the association between CPS and blood Phe concentrations in PKU patients related to alterations in blood-brain barrier LNAA transport?
2. Are elevated brain Phe concentrations related to CPS in PKU patients?
3. Do untreated C57Bl/6 PKU mice show behavioral phenotypes of learning and memory deficits?
4. Is CREB phosphorylation at Ser-133 reduced in relation to behavioral phenotypes of learning and memory deficits in C57Bl/6 PKU mice?
5. Do untreated C57Bl/6 PKU mice display behavioral phenotypes of impaired mood and motor deficits?

In current PKU treatment, the stringent restriction of protein intake profoundly impacts everyday life, while treatment outcome appears to be suboptimal. The current treatment burden and outcome raise the question whether new therapeutic modalities, aiming to influence known pathophysiological targets, could improve current PKU treatment. In this context, this thesis addresses the following research questions:

6. What is the current state of evidence for LNAA supplementation in PKU in relation to treatment aims?
7. Does BH4 treatment impact brain concentrations of catecholamines, serotonin, and LNAAs in C57Bl/6 PKU mice?

The final chapter of this thesis provides an overview of the findings answering these questions, provides additional experimental avenues and associated data, and outlines the relevance of the results for today and tomorrow.

References

1. Følling A (1934) Über Ausscheidung von Phenylbrenztraubensäure in den Harn als Stoffwechselanomalie in Verbindung mit Imbezillität. Hoppe-Seylers Ztschr. Physiol. Chem. 227: 169-176.
2. Penrose LS (1935) Inheritance of phenylpyruvic amentia (phenylketonuria). Lancet 2: 192-194.
3. Woolf LI, Vulliamy DG (1951) Phenylketonuria with a study of the effect upon it of glutamic acid. Arch. Dis. Child. 26: 487-494.
4. Jervis GA (1953) Phenylpyruvic oligophrenia deficiency of phenylalanine-oxidizing system. Proc. Soc. Exp. Biol. Med. 82: 514-515.
5. Bickel H, Gerrard J, Hickmans EM (1953) Influence of phenylalanine intake on phenylketonuria. Lancet 265: 812-813.
6. Blau N, van Spronsen FJ, Levy HL (2010) Phenylketonuria. Lancet 376: 1417-1427.
7. van Spronsen FJ (2010) Phenylketonuria: a 21st century perspective. Nat. Rev. Endocrinol. 6: 509-514.
8. Guthrie R, Susi A (1963) A simple phenylalanine method for detecting phenylketonuria in large populations of newborn infants. Pediatrics 32: 338-343.
9. Kure S, Hou DC, Ohura T, et al. (1999) Tetrahydrobiopterin-responsive phenylalanine hydroxylase deficiency. J. Pediatr. 135: 375-378.
10. Smith I, Beasley MG, Ades AE (1990) Intelligence and quality of dietary treatment in phenylketonuria. Arch. Dis. Child. 65: 472-478.
11. Waisbren SE, Noel K, Fahrbach K, Cella C, Frame D, Dorenbaum A, Levy H (2007) Phenylalanine blood levels and clinical outcomes in phenylketonuria: a systematic literature review and meta-analysis. Mol. Genet. Metab. 92: 63-70.
12. Surtees R, Blau N (2000) The neurochemistry of phenylketonuria. Eur. J. Pediatr. 159 Suppl 2: S109-S113.
13. van Spronsen FJ, Hoeksma M, Reijngoud DJ (2009) Brain dysfunction in phenylketonuria: is phenylalanine toxicity the only possible cause? J. Inherit. Metab. Dis. 32: 46-51.
14. Anderson PJ, Wood SJ, Francis DE, Coleman L, Anderson V, Boneh A (2007) Are neuropsychological impairments in children with early-treated phenylketonuria (PKU) related to white matter abnormalities or elevated phenylalanine levels? Dev. Neuropsychol. 32: 645-668.
15. Moyle JJ, Fox AM, Arthur M, Bynevelt M, Burnett JR (2007) Meta-analysis of neuropsychological symptoms of adolescents and adults with PKU. Neuropsychol. Rev. 17: 91-101.
16. DeRoche K, Welsh M (2008) Twenty-five years of research on neurocognitive outcomes in early-treated phenylketonuria: intelligence and executive function. Dev. Neuropsychol. 33: 474-504.
17. Huijbregts SC, de Sonneville LM, Licht R, van Spronsen FJ, Verkerk PH, Sergeant JA (2002) Sustained attention and inhibition of cognitive interference in treated phenylketonuria: associations with concurrent and lifetime phenylalanine concentrations. Neuropsychologia 40: 7-15.
18. Huijbregts SC, de Sonneville LM, van Spronsen FJ, Licht R, Sergeant JA (2002) The neuropsychological profile of early and continuously treated phenylketonuria: orienting, vigilance, and maintenance versus manipulation-functions of working memory. Neurosci. Biobehav. Rev. 26: 697-712.

19. Channon S, German E, Cassina C, Lee P (2004) Executive functioning, memory, and learning in phenylketonuria. Neuropsychology 18: 613-620.

20. Channon S, Mockler C, Lee P (2005) Executive functioning and speed of processing in phenylketonuria. Neuropsychology 19: 679-686.

21. Smith I, Knowles J (2000) Behaviour in early treated phenylketonuria: a systematic review. Eur. J. Pediatr. 159 Suppl 2: S89-93.

22. Scriver CR, Kaufman S (2001) In: The metabolic and molecular bases of inherited disease. Hyperphenylalaninemia: phenylalanine hydroxylase deficiency. Scriver CR, Beaudet AL, Sly WS, Valle D, eds. New York: McGraw-Hill, 1667-1724.

23. Primrose DA (1983) Phenylketonuria with normal intelligence. J. Ment. Defic. Res. 27 (Pt 4): 239-246.

24. Ramus SJ, Forrest SM, Pitt DD, Cotton RG (1999) Genotype and intellectual phenotype in untreated phenylketonuria patients. Pediatr. Res. 45: 474-481.

25. Möller HE, Weglage J, Wiedermann D, Ullrich K (1998) Blood-brain barrier phenylalanine transport and individual vulnerability in phenylketonuria. J. Cereb. Blood Flow Metab. 18: 1184-1191.

26. Valdovinos-Flores C, Gonsebatt ME (2012) The role of amino acid transporters in GSH synthesis in the blood-brain barrier and central nervous system. Neurochem. Int. 61: 405-414.

27. Pardridge WM (1998) Blood-brain barrier carrier-mediated transport and brain metabolism of amino acids. Neurochem. Res. 23: 635-644.

28. Smith QR (2000) Transport of glutamate and other amino acids at the blood-brain barrier. J. Nutr. 130: S1016-1022.

29. Boado RJ, Li JY, Nagaya M, Zhang C, Pardridge WM (1999) Selective expression of the large neutral amino acid transporter at the blood-brain barrier. Proc. Natl. Acad. Sci. U. S. A. 96: 12079-12084.

30. Zielke HR, Zielke CL, Baab PJ, Collins RM (2002) Large neutral amino acids auto exchange when infused by microdialysis into the rat brain: implication for maple syrup urine disease and phenylketonuria. Neurochem. Int. 40: 347-354.

31. Oldendorf WH, Sisson BW, Silverstein A (1971) Brain uptake of selenomethionine Se 75. II. Reduced brain uptake of selenomethionine Se 75 in phenylketonuria. Arch. Neurol. 24: 524-528.

32. Landvogt C, Mengel E, Bartenstein P, et al. (2008) Reduced cerebral fluoro-L-dopamine uptake in adult patients suffering from phenylketonuria. J. Cereb. Blood Flow Metab. 28: 824-831.

33. Shulkin BL, Betz AL, Koeppe RA, Agranoff BW (1995) Inhibition of neutral amino acid transport across the human blood-brain barrier by phenylalanine. J. Neurochem. 64: 1252-1257.

34. Novotny EJ,Jr, Avison MJ, Herschkowitz N, Petroff OA, Prichard JW, Seashore MR, Rothman DL (1995) In vivo measurement of phenylalanine in human brain by proton nuclear magnetic resonance spectroscopy. Pediatr. Res. 37: 244-249.

35. Kreis R, Pietz J, Penzien J, Herschkowitz N, Boesch C (1995) Identification and quantitation of phenylalanine in the brain of patients with phenylketonuria by means of localized in vivo 1H magnetic-resonance spectroscopy. J. Magn. Reson. B 107: 242-251.

36. Pietz J, Kreis R, Rupp A, Mayatepek E, Rating D, Boesch C, Bremer HJ (1999) Large neutral amino acids block phenylalanine transport into brain tissue in patients with phenylketonuria. J. Clin. Invest. 103: 1169-1178.

37. Koch R, Moseley KD, Yano S, Nelson M,Jr, Moats RA (2003) Large neutral amino acid therapy and phenylketonuria: a promising approach to treatment. Mol. Genet. Metab. 79: 110-113.

38. Moats RA, Moseley KD, Koch R, Nelson M,Jr (2003) Brain phenylalanine concentrations in phenylketonuria: research and treatment of adults. Pediatrics 112: 1575-1579.

39. Sijens PE, Oudkerk M, Reijngoud DJ, Leenders KL, de Valk HW, van Spronsen FJ (2004) 1H MR chemical shift imaging detection of phenylalanine in patients suffering from phenylketonuria (PKU). Eur. Radiol. 14: 1895-1900.

40. Schindeler S, Ghosh-Jerath S, Thompson S, et al. (2007) The effects of large neutral amino acid supplements in PKU: an MRS and neuropsychological study. Mol. Genet. Metab. 91: 48-54.

41. Smith CB, Kang J (2000) Cerebral protein synthesis in a genetic mouse model of phenylketonuria. Proc. Natl. Acad. Sci. U. S. A. 97: 11014-11019.

42. Pascucci T, Ventura R, Puglisi-Allegra S, Cabib S (2002) Deficits in brain serotonin synthesis in a genetic mouse model of phenylketonuria. Neuroreport 13: 2561-2564.

43. Matalon R, Surendran S, Matalon KM, et al. (2003) Future role of large neutral amino acids in transport of phenylalanine into the brain. Pediatrics 112: 1570-1574.

44. Glushakov AV, Glushakova O, Varshney M, et al. (2005) Long-term changes in glutamatergic synaptic transmission in phenylketonuria. Brain 128: 300-307.

45. Pascucci T, Andolina D, Ventura R, Puglisi-Allegra S, Cabib S (2008) Reduced availability of brain amines during critical phases of postnatal development in a genetic mouse model of cognitive delay. Brain Res. 1217: 232-238.

46. Pascucci T, Giacovazzo G, Andolina D, Conversi D, Cruciani F, Cabib S, Puglisi-Allegra S (2012) In vivo catecholaminergic metabolism in the medial prefrontal cortex of ENU2 mice: an investigation of the cortical dopamine deficit in phenylketonuria. J. Inherit. Metab. Dis. 35: 1001-1009.

47. Vogel KR, Arning E, Wasek BL, Bottiglieri T, Gibson KM (2013) Non-physiological amino acid (NPAA) therapy targeting brain phenylalanine reduction: pilot studies in Pah-enu2 mice. J. Inherit. Metab. Dis. 36: 513-523.

48. Vogel KR, Arning E, Wasek BL, Bottiglieri T, Gibson KM (2013) Characterization of 2-(methylamino) alkanoic acid capacity to restrict blood-brain phenylalanine transport in Pah-enu2 mice: preliminary findings. Mol. Genet. Metab. 110 Suppl: S71-S78.

49. McKean CM (1972) The effects of high phenylalanine concentrations on serotonin and catecholamine metabolism in the human brain. Brain Res. 47: 469-476.

50. Hoeksma M, Reijngoud DJ, Pruim J, de Valk HW, Paans AM, van Spronsen FJ (2009) Phenylketonuria: High plasma phenylalanine decreases cerebral protein synthesis. Mol. Genet. Metab. 96: 177-182.

51. Koch R, Moats R, Guttler F, Guldberg P, Nelson M,Jr (2000) Blood-brain phenylalanine relationships in persons with phenylketonuria. Pediatrics 106: 1093-1096.

52. Pascucci T, Andolina D, Mela IL, et al. (2009) 5-Hydroxytryptophan rescues serotonin response to stress in prefrontal cortex of hyperphenylalaninaemic mice. Int. J. Neuropsychopharmacol. 12: 1067-1079.

53. Andolina D, Conversi D, Cabib S, Trabalza A, Ventura R, Puglisi-Allegra S, Pascucci T (2011) 5-Hydroxytryptophan during critical postnatal period improves cognitive performances and promotes dendritic spine maturation in genetic mouse model of phenylketonuria. Int. J. Neuropsychopharmacol. 14: 479-489.

54. Harding CO, Winn SR, Gibson KM, Arning E, Bottiglieri T, Grompe M (2014) Pharmacologic inhibition of L-tyrosine degradation ameliorates cerebral dopamine deficiency in murine phenylketonuria (PKU). J. Inherit. Metab. Dis.: doi:10.1007/s10545-013-9675-2.

55. Sawin EA, Murali SG, Ney DM (2014) Differential effects of low-phenylalanine protein sources on brain neurotransmitters and behavior in C57Bl/6 Pah-enu2 mice. Mol. Genet. Metab. 111: doi: 10.1016/j.ymgme.2014.01.015.

56. Puglisi-Allegra S, Cabib S, Pascucci T, Ventura R, Cali F, Romano V (2000) Dramatic brain aminergic deficit in a genetic mouse model of phenylketonuria. Neuroreport 11: 1361-1364.

57. Joseph B, Dyer CA (2003) Relationship between myelin production and dopamine synthesis in the PKU mouse brain. J. Neurochem. 86: 615-626.

58. Embury JE, Charron CE, Martynyuk A, et al. (2007) PKU is a reversible neurodegenerative process within the nigrostriatum that begins as early as 4 weeks of age in Pah(enu2) mice. Brain Res. 1127: 136-150.

59. Pascucci T, Giacovazzo G, Andolina D, et al. (2013) Behavioral and neurochemical characterization of new mouse model of hyperphenylalaninemia. PLoS One 8: e84697.

60. Embury JE, Reep RR, Laipis PJ (2005) Pathologic and immunohistochemical findings in hypothalamic and mesencephalic regions in the pah(enu2) mouse model for phenylketonuria. Pediatr. Res. 58: 283-287.

61. Butler IJ, O'Flynn ME, Seifert WE,Jr, Howell RR (1981) Neurotransmitter defects and treatment of disorders of hyperphenylalaninemia. J. Pediatr. 98: 729-733.

62. Lou HC, Guttler F, Lykkelund C, Bruhn P, Niederwieser A (1985) Decreased vigilance and neurotransmitter synthesis after discontinuation of dietary treatment for phenylketonuria in adolescents. Eur. J. Pediatr. 144: 17-20.

63. Burlina AB, Bonafe L, Ferrari V, Suppiej A, Zacchello F, Burlina AP (2000) Measurement of neurotransmitter metabolites in the cerebrospinal fluid of phenylketonuric patients under dietary treatment. J. Inherit. Metab. Dis. 23: 313-316.

64. Lou H (1985) Large doses of tryptophan and tyrosine as potential therapeutic alternative to dietary phenylalanine restriction in phenylketonuria. Lancet 2: 150-151.

65. Ogawa S, Ichinose H (2006) Effect of metals and phenylalanine on the activity of human tryptophan hydroxylase-2: comparison with that on tyrosine hydroxylase activity. Neurosci. Lett. 401: 261-265.

66. Diamond A, Ciaramitaro V, Donner E, Djali S, Robinson MB (1994) An animal model of early-treated PKU. J. Neurosci. 14: 3072-3082.

67. Diamond A (1996) Evidence for the importance of dopamine for prefrontal cortex functions early in life. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 351: 1483-1493.

68. Thompson AJ, Smith I, Brenton D, et al. (1990) Neurological deterioration in young adults with phenylketonuria. Lancet 336: 602-605.

69. Pietz J (1998) Neurological aspects of adult phenylketonuria. Curr. Opin. Neurol. 11: 679-688.

70. Pearl PL, Capp PK, Novotny EJ, Gibson KM (2005) Inherited disorders of neurotransmitters in children and adults. Clin. Biochem. 38: 1051-1058.

71. Binek PA, Johnson TC, Kelly CJ (1981) Effect of alpha-methylphenylalanine and phenylalanine on brain polyribosomes and protein synthesis. J. Neurochem. 36: 1476-1484.

72. Binek-Singer P, Johnson TC (1982) The effects of chronic hyperphenylalaninaemia on mouse brain protein synthesis can be prevented by other amino acids. Biochem. J. 206: 407-414.

73. Hughes JV, Johnson TC (1978) Experimentally induced and natural recovery from the effects of phenylalanine on brain protein synthesis. Biochim. Biophys. Acta 517: 473-485.

74. Berger R, Springer J, Hommes FA (1980) Brain protein and myelin metabolism in hyperphenylalaninemic rats. Cell. Mol. Biol. Incl Cyto Enzymol. 26: 31-36.

75. Paans AM, Pruim J, Smit GP, Visser G, Willemsen AT, Ullrich K (1996) Neurotransmitter positron emission tomographic-studies in adults with phenylketonuria, a pilot study. Eur. J. Pediatr. 155 Suppl 1: S78-S81.

76. Bauman ML, Kemper TL (1982) Morphologic and histoanatomic observations of the brain in untreated human phenylketonuria. Acta Neuropathol. 58: 55-63.

77. Liang L, Gu X, Lu L, Li D, Zhang X (2011) Phenylketonuria-related synaptic changes in a BTBR Pah-enu2 mouse model. Neuroreport 22: 617-622.

78. Bick U, Fahrendorf G, Ludolph AC, Vassallo P, Weglage J, Ullrich K (1991) Disturbed myelination in patients with treated hyperphenylalaninaemia: evaluation with magnetic resonance imaging. Eur. J. Pediatr. 150: 185-189.

79. Thompson AJ, Tillotson S, Smith I, Kendall B, Moore SG, Brenton DP (1993) Brain MRI changes in phenylketonuria. Associations with dietary status. Brain 116: 811-821.

80. Huttenlocher PR (2000) The neuropathology of phenylketonuria: human and animal studies. Eur. J. Pediatr. 159 Suppl 2: S102-6.

81. Dyer CA, Kendler A, Philibotte T, Gardiner P, Cruz J, Levy HL (1996) Evidence for central nervous system glial cell plasticity in phenylketonuria. J. Neuropathol. Exp. Neurol. 55: 795-814.

82. Shefer S, Tint GS, Jean-Guillaume D, et al. (2000) Is there a relationship between 3-hydroxy-3-methylglutaryl coenzyme a reductase activity and forebrain pathology in the PKU mouse? J. Neurosci. Res. 61: 549-563.

83. Ramakers GJ (2002) Rho proteins, mental retardation and the cellular basis of cognition. Trends Neurosci. 25: 191-199.

84. Johnston MV (2003) Brain plasticity in paediatric neurology. Eur. J. Paediatr. Neurol. 7: 105-113.

85. Greer PL, Greenberg ME (2008) From synapse to nucleus: calcium-dependent gene transcription in the control of synapse development and function. Neuron 59: 846-860.

86. Vaillend C, Poirier R, Laroche S (2008) Genes, plasticity and mental retardation. Behav. Brain Res. 192: 88-105.

87. Cardin JA, Abel T (1999) Memory suppressor genes: enhancing the relationship between synaptic plasticity and memory storage. J. Neurosci. Res. 58: 10-23.

88. Josselyn SA, Nguyen PV (2005) CREB, synapses and memory disorders: past progress and future challenges. Curr. Drug Targets CNS Neurol. Disord. 4: 481-497.

89. Wu H, Zhou Y, Xiong ZQ (2007) Transducer of regulated CREB and late phase long-term synaptic potentiation. FEBS J. 274: 3218-3223.

90. Sakamoto K, Karelina K, Obrietan K (2011) CREB: a multifaceted regulator of neuronal plasticity and protection. J. Neurochem. 116: 1-9.

91. Johannessen M, Delghandi MP, Moens U (2004) What turns CREB on? Cell. Signal. 16: 1211-1227.

92. Carlezon WA,Jr, Duman RS, Nestler EJ (2005) The many faces of CREB. Trends Neurosci. 28: 436-445.

93. Costa-Mattioli M, Sossin WS, Klann E, Sonenberg N (2009) Translational control of long-lasting synaptic plasticity and memory. Neuron 61: 10-26.

94. Roelfsema JH, Peters DJ (2007) Rubinstein-Taybi syndrome: clinical and molecular overview. Expert Rev. Mol. Med. 9: 1-16.

95. Pereira PM, Schneider A, Pannetier S, Heron D, Hanauer A (2010) Coffin-Lowry syndrome. Eur. J. Hum. Genet. 18: 627-633.

96. Harum KH, Alemi L, Johnston MV (2001) Cognitive impairment in Coffin-Lowry syndrome correlates with reduced RSK2 activation. Neurology 56: 207-214.

97. Martynyuk AE, van Spronsen FJ, van der Zee EA (2010) Animal models of brain dysfunction in phenylketonuria. Mol. Genet. Metab. 99 Suppl 1: S100-S105.

98. Shedlovsky A, McDonald JD, Symula D, Dove WF (1993) Mouse models of human phenylketonuria. Genetics 134: 1205-1210.

99. McDonald JD, Charlton CK (1997) Characterization of mutations at the mouse phenylalanine hydroxylase locus. Genomics 39: 402-405.

100. Zagreda L, Goodman J, Druin DP, McDonald D, Diamond A (1999) Cognitive deficits in a genetic mouse model of the most common biochemical cause of human mental retardation. J. Neurosci. 19: 6175-6182.

101. Cabib S, Pascucci T, Ventura R, Romano V, Puglisi-Allegra S (2003) The behavioral profile of sevwere mental retardation in a genetic mouse model of phenylketonuria. Behav. Genet. 33: 301-310.

102. Ding Z, Georgiev P, Thony B (2006) Administration-route and gender-independent long-term therapeutic correction of phenylketonuria (PKU) in a mouse model by recombinant adeno-associated virus 8 pseudotyped vector-mediated gene transfer. Gene Ther. 13: 587-593.

103. Arning E, Bottiglieri T, Sun Q, et al. (2009) Metabolic profiling in phenylalanine hydroxylase deficient (Pah-/-) mouse brain reveals decreased amino acid neurotransmitters and preferential alterations of the serotoninergic system. Mol. Genet. Metab. 98: 21.

104. Crawley JN, Belknap JK, Collins A, et al. (1997) Behavioral phenotypes of inbred mouse strains: implications and recommendations for molecular studies. Psychopharmacology (Berl) 132: 107-124.

105. Holmes A, Wrenn CC, Harris AP, Thayer KE, Crawley JN (2002) Behavioral profiles of inbred strains on novel olfactory, spatial and emotional tests for reference memory in mice. Genes Brain Behav. 1: 55-69.

106. Mishina M, Sakimura K (2007) Conditional gene targeting on the pure C57BL/6 genetic background. Neurosci. Res. 58: 105-112.

107. Christensen HN, Streicher JA, Elbinger RL (1948) Effects of feeding individual amino acids upon the distribution of other amino acids between cells and extracellular fluid. J. Biol. Chem. 172: 515-524.