Editorial

It is now 76 years ago that the Norwegian doctor Asbjørn Følling noticed in 1934 that several mentally retarded patients had a strange odor. He figured out that it was from something called ‘phenylacetic acid.’ The patients’ urine also had a very high level of a chemical called ‘phenylketone’, from which the name of the disease was derived: phenylketonuria (PKU), coined by Lionel Sharpless Penrose. Fourteen years earlier Pearl Buck, best known for her Pulitzer-Prize-winning *The Good Earth*, delighted in the birth of her daughter Carol in 1920 and was heartbroken when she failed to develop normally. Buck was unable to discover what was wrong with Carol, who grew up mentally retarded as a result of PKU. She published *The Child Who Never Grew* in 1950 with the hope that her experience could help others. Only 1 year later, in 1951, Horst Bickel developed the first PKU formula while working in Birmingham, UK. The unflavored protein drink provided many amino acids without phenylalanine (Phe). For the first time, PKU patients could get most of the nutrition they needed while keeping the Phe blood levels low. The physician and microbiologist Robert Guthrie developed a simple screening test for newborns to determine elevated Phe in the blood using the world-known ‘Guthrie card’. By implementing this test Guthrie opened the door for international mass screening in the 1960s. So PKU has led the way to worldwide screening programs of metabolic diseases. The success of the mass screening of PKU and other metabolic diseases led to a different understanding of many metabolic disorders by early detection of a genetic disorder before the disease can establish its symptoms. The genotype can be assessed prior to the development of the PKU phenotype for the benefit of the patient, which is a fun-

damental shift from the disease as a clinical entity to a treatment which can avoid chronic brain damage.

The current knowledge about this first detected metabolic disorder, PKU, has increased over the last decades so much that it seemed appropriate to cover these results in an issue of *Annales Nestlé* and give an overview of today’s techniques and methods for the diagnosis and treatment modalities of PKU, as a great number of affected patients have reached their middle-aged adulthood now.

Johannes Zschocke from the Division of Human Genetics in Innsbruck, Austria, gives an overview of the molecular basis of PKU and its relation from genotype to clinical management. Worldwide, 556 mutations of the phenylalanine hydroxylase (PAH) gene have been discovered. The quality of PKU mutation analysis is very important because it became known that about 8% of all reports from laboratories that used comprehensive mutation detection methods, such as sequencing of all exons of the PAH gene, contained incorrect genotypes. There were also serious deficiencies in the interpretation of genotype data. In one instance, several laboratories misclassified a mild hyperphenylalaninemia mutation (associated with the mild form of PAH deficiency that does not require treatment), and some even offered prenatal diagnosis although an ‘affected’ child would have been completely healthy with regard to PKU. Zschocke concludes that molecular diagnostic services should only be provided by laboratories that are familiar with genotype-phenotype correlations in the respective disorders. Although mutation analyses are not absolutely essential in the management of patients with PKU, they help in understanding the molecular characteristics of PAH deficiency in the individual patient and assist in treatment

decisions. They are useful in the management of all patients with PKU provided that the necessary expertise to interpret the results correctly is available and the costs of DNA studies are not prohibitive.

Olaf Bodamer from the Institute of Inherited Metabolic Disease of the Paracelsus Medical University Salzburg, Austria, describes the current newborn screening procedures. After the worldwide success of the Guthrie test newborn screening has been recognized as an important public health measure with direct benefits for the affected individuals and their families and indirect advantages for society as a whole. Disorders that are screened for have to follow criteria that were developed in 1968 by Wilson and Jungner as part of a WHO initiative on the principles and practice of screening for disease. They stated: 'The central idea of early disease detection and treatment is essentially simple. However, the path to its successful achievement (on the one hand, bringing to treatment those with previously undetected disease and, on the other, avoiding harm not in need of treatment) is far from simple though sometimes it may appear deceptively easy.' These basic principles are still valid today, although they have been adapted to the requirements of modern newborn screening. Ideally, disorders that are screened for should be reasonably frequent and they should constitute a significant public health problem. The natural disease course should be understood, and there should be a form of treatment or intervention that alters the natural disease course to the benefit of the affected patient. In many ways, PKU has led the way and was the first disorder to fulfill all criteria, maybe with the exception of the countries where the birth incidence of PKU is low, such as Finland and Japan. Today the Guthrie test has been replaced by tandem mass spectrometry, which was introduced to newborn screening laboratories during the late 1990s. The main advantage of this technique is the simultaneous, fully automated analysis of different analytes such as amino acids including Phe and acylcarnitine species. The diagnostic sensitivity of tandem mass spectrometry for hyperphenylalaninemia/PKU is superior compared to other analytical techniques. The measurement of the Phe to tyrosine ratio may help in differentiating between false positives and cases of hyperphenylalaninemia/PKU.

In their article, Erin L. MacLeod and Denise M. Ney from the Department of Nutritional Sciences of the University of Wisconsin in Madison, USA, cover the nutritional management of PKU. The goal of nutritional management for those with PKU is to maintain plasma Phe concentrations that support optimal growth, develop-

ment and mental functioning while providing a nutritionally complete diet. Normal growth and development is the positive outcome noted with early diagnosis and treatment of infants with PKU with a nutritionally adequate, low-Phe diet. Evaluation of IQ scores in individuals with PKU treated shortly after birth through 12 years of age who maintained plasma Phe concentrations within the recommended treatment ranges show a mean IQ score of 101 ± 11 . The goal throughout the life cycle is continuous, uninterrupted diet adherence because once the PKU diet is discontinued, it is extremely difficult to reintroduce it. Compliance with the PKU diet and achievement of metabolic control is easily accomplished in infancy and early childhood before children start school. Not surprisingly, compliance becomes a significant problem during adolescence. Given the poor dietary compliance with the low-Phe diet after early childhood, new approaches are needed to reduce the Phe concentrations in plasma and brain with the potential to liberalize the diet by permitting a greater daily Phe allowance. Administration of H_4 -biopterin and large neutral amino acids are 2 new approaches to therapy that have recently been introduced and shown to be effective in selected individuals with PKU. The biggest challenge to living with PKU is lifelong adherence to a diet that allows only a small intake of natural foods and requires sufficient intake of amino acid formula to provide nutritional adequacy. New therapies which may help liberalize the strict diet for some with PKU include H_4 -biopterin drug therapy and supplementation of large neutral amino acids.

Ronen Eavri and Haya Lorberboum-Galski from the Picower Institute for Learning and Memory, MIT, Cambridge, Mass., USA, and the Department and Molecular Biology, Faculty of Medicine, IMRIC, Hebrew University, Jerusalem, Israel, focus on novel approaches to the therapy of PKU. They analyze the supplementation of H_4 -biopterin and large neutral amino acids to the standard PKU diet of PKU patients as well as the potential enzyme solution approach and the possibility of gene therapy. The enzyme replacement therapy approach turned out to be promising, using Phe-ammonia lyase (PAL) derived from fungus, plants and cyanobacteria. PAL has, however, stumbled over some obstacles on the way to its approval for PKU therapy due to the fact that the enzyme is either degraded in the intestine or attacked by the immune system, and thus is unable to lower blood Phe to the required levels. Efforts have been made to protect the enzyme from degradation by site-directed mutagenesis and chemical modification with polyethylene glycol while maintaining specific activity. These attempts have proven partly ben-

eficial to date. Recently, different variants of engineered polyethylene glycol-PAL were further assessed in a PKU mouse model showing a reduction in Phe blood levels over a 90-day trial period in mice treated with one of the tested variants. While these results are encouraging, further evaluation of this potential treatment will show if PAL could serve in the long-term treatment of PKU. Moreover, the use of fusion proteins of PAH bound to HIV trans-activator of transcription might be a promising way of bringing the missing enzyme into the liver. These proteins retain all the characteristics of the PAH enzyme such as the binding affinity to the Phe substrate, the dose-dependent conversion of Phe into tyrosine and the ability to form multimeric complexes. Yet, by fusing these enzymes to different targeting domains, they attain one additional important characteristic – the ability to be delivered into target cells. These proteins were proven to internalize into different cells, and, once inside, to reduce the Phe concentrations by converting this amino acid into tyrosine.

The observation of the neuropsychological functioning in early-treated PKU is described in detail by Louise H. Crossley and Peter J. Anderson from the Royal Children's Hospital in Parkville, Vic., Australia. Observations of specific cognitive skills, motor functioning, information processing, language as well as memory, learning, attention and behavior are important parameters for the neuropsychological assessment of PKU patients. This review has demonstrated that while children treated for PKU early and continuously escape severe intellectual impairment, they are at an increased risk for a range of neuropsychological impairments. While numerous neu-

ropsychological studies have been conducted, the findings have not been consistent. Executive functioning has attracted most interest, and while the bulk of studies have reported deficits in this domain, conflicting research also exists. Slowed processing speed appears to be a major area of concern for PKU patients, and this may have implications for memory and learning as well as executive functioning. Impairments in other domains have also been noted, including fine motor control, attention, memory and learning. Given these difficulties, it is not surprising that higher rates of academic and behavioral problems have been reported in PKU cohorts. In summary, the research to date would suggest that PKU is not associated with a specific pattern of deficits, although speed of processing and executive dysfunction are 2 areas of primary concern.

The discoveries in this single metabolic disease, PKU, over the last 76 years have led to fundamental understanding of a general neonatal mass screening in the world and by this the prevention of phenotypes of many metabolic diseases. In addition new drugs and compounds have been developed as supplements to the PKU diet and perhaps opened avenues to new treatment regimens that might overcome the burden of a lifelong, not very tasty diet.

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