# Evaluation of electrospray-tandem mass spectrometry for the detection of phenylketonuria and other rare disorders

**Zoltan Lukacs and René Santer**

Department of Pediatrics, Hamburg University Medical Center, Hamburg, Germany

Since a few years ESI-MS/MS has been employed for the simultaneous detection of a wide range of inborn errors of metabolism. The screening center North at the Hamburg University Medical Center processes 40 – 50 000 samples *per* year. To assess current developments in neonatal screening, the Northern German Working Group on Neonatal Screening consisting of health care providers, metabolic centers, and screening laboratories was founded. Based on current literature and experience four categories of diseases were established. The first three categories were recommended for screening under constant scientific evaluation, while glutaric aciduria II, β-ketothiolase deficiency, short-chain acylCoA dehydrogenase deficiency, and homocystinuria were not included in the screening program. In contrast, general screening for phenylketonuria (PKU) remains undisputed and MS/MS screening reduced false positives by simultaneously detecting phenylalanine and tyrosine. Recently, (6R)-L-*erythro*-5,6,7,8-tetrahydrobiopterin ($BH_4$)-sensitive PKU has been discovered. We were able to demonstrate that $BH_4$ treatment without dietary restrictions may be sufficient for certain $BH_4$-responsive PKU patients. In general, MS/MS provides a potential to rapidly screen for a wide variety of rare metabolic disorders but a close cooperation between scientists and metabolic doctors is required to constantly evaluate results in terms of improving the outcome of patients.

**Keywords:** $BH_4$-responsive PKU / Electrospray-tandem mass spectrometry / Phenylketonuria

Received: October 12, 2005; revised: November 18, 2005; accepted: November 23, 2005

## 1 Introduction

Phenylalanine hydroxylase (EC 1.14.16.1), a nonheme iron(II)-containing enzyme, hydroxylates phenylalanine to tyrosine in the presence of the cofactor (6R)-L-*erythro*-5,6,7,8-tetrahydrobiopterin ($BH_4$, Fig. 1). This enzyme is functionally deficient in phenylketonuria (PKU), a frequent disorder of phenylalanine metabolism. The 50 kDa enzyme monomer consists of three domains, a regulatory (amino acid 1 – 142), a catalytic (amino acid 143 – 410), and a tetramerization domain (amino acid 411 – 452). The latter is crucial for the formation of a homotetramer displaying higher turnover than the dimer which, however, still possesses considerable enzymatic activity. More than 400 mutations are known in the phenylalanine hydroxylase gene [1, 2]. They are distributed throughout all three domains of each subunit with most of the pathologically significant mutations being located in the catalytic domain [3].

Classical PKU is characterized by reduced activity of the enzyme, while alternatively, a defect in the synthesis or recycling of the essential cofactor $BH_4$ also results in markedly elevated phenylalanine concentrations. The latter also affects the metabolism of neurotransmitters. When blood phenylalanine concentrations exceed 600 μmol/L, a phenylalanine-restricted diet is mandatory to allow normal development of the child [4, 5]. Dietary treatment was developed by H. Bickel in the late 1950s. However, diagnosis after clinical symptoms have already occurred does not allow efficient treatment because psychomotor deficiencies are usually not reversible. Therefore, Guthrie [6] has developed and introduced a method for neonatal screening of infants, which makes use of dried blood specimens. Those are exceptionally stable and can easily be mailed from health care providers to specialized screening laboratories. In the 1970s, bacterial inhibition assays have been employed for screening purposes. *Bacillus subtilis* spores

**Correspondence:** Dr. Zoltan Lukacs, Department of Pediatrics, Hamburg University Medical Center, Hamburg, Germany
**E-mail:** lukacs@uke.uni-hamburg.de
**Fax:** +49-40-428035984

**Abbreviations: Acid.,** aciduria; **$BH_4$**, (6R)-L-*erythro*-5,6,7,8-tetrahydrobiopterin; **CPT**, carnitin palmitoyl transferase; **HPA**, hyperphenylalaninemia; **MCADD**, medium-chain acylCoA dehdrogenase deficiency; **MRM**, multiple reaction monitoring; **PAH**, phenylalanine hydroxylase; **PKU**, phenylketonuria

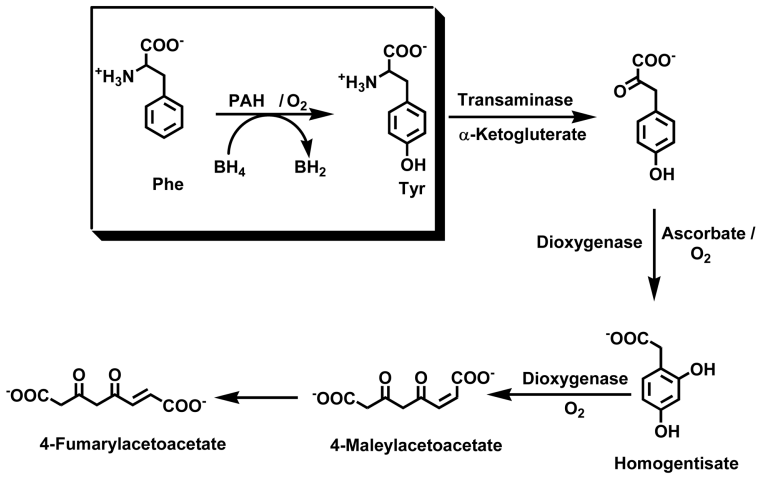


**Figure 1.** Degradation of phenylalanine (Phe) *in vivo*. Reaction is catalyzed by PAH which requires $BH_4$ as a cofactor. Further oxidation and cleavage processes result in the formation of 4-fumarylacetoacetate that can easily be fed into the citric acid cycle.

were plated on phenylalanine-free agar with β-2-thienylalanine as additional inhibitor. Standardized dried blood spots from neonates were put onto the surface of the plate. Bacterial growth was directly proportional to the phenylalanine content of the dried blood samples.

During the 1980s and 1990s new developments replaced the original method by Guthrie. Nowadays, many laboratories use ESI-MS/MS for neonatal screening. This paper will demonstrate the possibilities and limitations of the new technology. In addition, $BH_4$-therapy, a novel option in the treatment of some PKU patients, will be introduced.

## 2 Materials and methods

### 2.1 Patients

Hyperphenylalaninemia (HPA) was detected in newborns by routine screening from dried blood spots. To further differentiate the type of HPA, infants were admitted and an oral $BH_4$-loading test (described below) was performed. Currently, six unrelated individuals, born to nonconsanguinous parents, were identified as being responsive to $BH_4$-loading, while urinary pterins and blood dihydropterine reductase activity did not indicate a defect in $BH_4$-metabolism. The presence of phenylalanine hydroxylase (PAH) mutations was subsequently confirmed.

### 2.2 Neonatal screening at the Screening Center North

The Screening Center North located at the Department of Pediatrics, Hamburg University Medical Center processes approximately 40 – 50 000 samples *per* year from neonates born in Hamburg, Bremen, Schleswig-Holstein (Germany) and Quito, Ecuador. Blood is taken between 36 and 72 h of life and applied to standardized filter paper (Whatman 903, Dassel, Germany). The specimens are dried at room temperature and sent to the screening laboratory by regular or express mail.

### 2.3 Amino acid measurements

Neonatal screening for the patients in this study was performed on filter paper blood spots before MS/MS was introduced. For that purpose, the kit from PerkinElmer Life Sciences (Turku, Finland) was used. The assay was also adapted for plasma samples which were precipitated with TCA (20%) prior to analysis. Briefly, ninhydrine reacts with phenylalanine to yield the fluorescent hydrantine. The conditions used in this assay, especially the succinic acid buffer and addition of the dipeptide L-leucyl-L-alanine, ensure high selectivity for phenylalanine. Blood samples from the $BH_4$-loading test and from $BH_4$-optimization assays were analyzed for phenylalanine. In addition, samples were assayed by ion-exchange chromatography on a Biochrom 20 (Pharmacia, Freiburg, Germany) to measure tyrosine and other essential amino acids.

### 2.4 $BH_4$-loading test

Urine samples and blood specimens were taken immediately before oral application of 20 mg/kg body weight $BH_4$ (administered 30 min before a regular meal). Blood specimens were acquired 4 and 8 h after the $BH_4$-loading. Urine was collected between 4 and 8 h as well as 8 and 12 h after $BH_4$ administration. The phenylalanine and tyrosine concentrations of the collected blood samples were determined as indicated above. Furthermore, the pattern of urinary pterines was analyzed and dihydropterine reductase activity was determined in erythrocytes.

 

### 2.5 Preparation of butanolic HCl

All reagents were purchased from Sigma-Aldrich (Taufkirchen, Germany) or J. T. Baker (Griesheim, Germany). Hundred microliters of acetylchloride was added at a constant rate within 5 – 10 min to 900 mL of *n*-butanol under continuous stirring. The reaction vessel is cooled with ice. After completion of the reaction the product should remain colorless and pH should be around pH 1.

### 2.6 Preparation of blood samples for MS/MS measurement

Using an automated puncher system from PerkinElmer Life Sciences, 3 mm blood spots were punched from Whatman 903 filter paper specimens (Whatman) into 0.45 μm filter plates (Millipore, Schwalbach, Germany). Isotopically labeled amino acid and acylcarnitines were diluted with methanol or methanol/water (1 : 1) and mixed according to the manufacturer instructions (Cambridge Isotope Laboratories, Cambridge, USA). A 100 μL of the diluted standard mixture is given to each blood spot and analytes are eluted for 45 min under constant shaking. The eluate is transferred to a polypropylene 96-well plate (Greiner bio-one, Solingen-Wald, Germany) by centrifugation using an Eppendorf 5810R centrifuge (5 min, 3000 U, Eppendorf, Hamburg, Germany). This procedure ensures that fibers from the filter paper do not cause problems in the the HPLC system. Methanol is completely evaporated on a heating plate at 55°C under constant air flow for 28 min (Schrader, Nentershausen Germany). To ensure complete dryness the plate is placed for 1 min into a heating oven at 65°C. Then 60 μL of butanolic HCl is pipetted into each well and the plate is firmly sealed with a plastic lid (cap for Masterblock, Greiner, bio-one). The plate is placed into a heating oven at 65°C for 17 min and weights are placed on the lid to facilitate the reaction under pressure. Butanolic esters of amino acids and acylcarnitines are formed under these conditions. The acidic mixture would interfere with ESI ionization and, thus, has to be fully evaporated under the conditions described before. The plate is placed for another minute into a heating oven at 65°C and products are dissolved in 100 μL of ACN : $H_2O$ (80 : 20).

### 2.7 ESI-MS/MS (positive mode)

Samples (15 μL) are injected using a Waters 2796 HPLC system (Waters, Eschborn, Germany). The analytes are delivered into the steel-spray capillary with a flow of 200 μL/min acetronitrile : $H_2O$ (80:20). After 0.28 min the flow is decelerated to 10 μL/min (peak parking). Between 0.3 and 1.6 min the following experiments are run using a Quattro micro MS/MS system in positive ionization mode (Micromass, Eschborn, Germany): (a) multiple reaction monitoring (MRM) of 132.0 → 75.8 for glycine and 134.0 → 77.8 D2-glycine (dwell time: 0.05 s, cone voltage 20 V, collision energy 8 eV), (b) MRM of 231.2 → 69.9 for arginine and 236.2 → 74.9 for D5–arginine (dwell time 0.05 s, cone voltage 30 V, collision energy 25 eV), (c) neutral loss of *m/z* 102.2 for amino acids (scan time 1.5 s, range *m/z* 120 – 270, cone voltage 25 V, collision energy 14 eV), (d) neutral loss of *m/z* 119.2 for basic amino acids (Lys, Cit, and Orn – scan time 1 s, range *m/z* 160 – 270, cone voltage 20 V, collision energy 20 eV), and (e) parent (precursor) ion scan of *m/z* 85 for acylcarnitines (scan time 3.0 s, range *m/z* 200 – 505, cone voltage 35 V, collision energy 26 eV). General instrument settings were: capillary 3.40 kV, source temperature 120°C, desolvation temperature 250°C, desolvation (gas) flow 600 L/h, cone gas flow 100 L/h. The HPLC system is rinsed with eluent for 0.1 min at 700 μL/min flow. Then the flow rate is returned to 200 μL/min for another 0.5 min until the next sample is injected.

For acylcarnitines the LOD was < 0.06 μM except for free carnitine which showed an LOD of 0.69 μM. The LOD for amino acids varied between 1.8 and 9.1 μM and, therefore, is far lower than concentrations usually measured in neonatal screening (> 50 μM). Average within-run CVs were 9% for acylcarnitines and 5% for amino acids, as determined by 12 consecutive measurements of the same sample. Average between-run CVs were 22% for acylcarnitines and 14% for amino acids, as determined by 20 measurements of the same sample on separate days. CVs are usually higher close to the LOD and inhomogenous distribution of blood in dried blood samples may add significantly to scatter. For acylcarnitines we have obtained a minimum linear range from 1.6 to 25 μM, except for free carnitine which showed a linear range from 6 to 100 μM. Similarly, amino acids showed a linear range from 20 to 330 μM. Thus, the physiological range of these substances is fully covered.

## 3 Results and discussion

Recently, ESI-MS/MS replaced fluorometric and spectrophotometric methods for the measurement of phenylalanine in German neonatal screening laboratories. For PKU, the benefit of large-scale screening programs was undisputed [7]. However, MS/MS also allows the simultaneous measurement of acylcarnitines and amino acids in one analytical run. For that purpose, dried blood spots are eluted with isotope standards and directly injected into the MS/MS. A neutral loss experiment of *m/z* 102 is used to detect most amino acids. Basic amino acids lose an additional ammonia moiety, so that a neutral loss of *m/z* 119 has to be performed. For glycine and arginine sensitivity and reproducibility can be improved when MRM experiments are used instead of

 

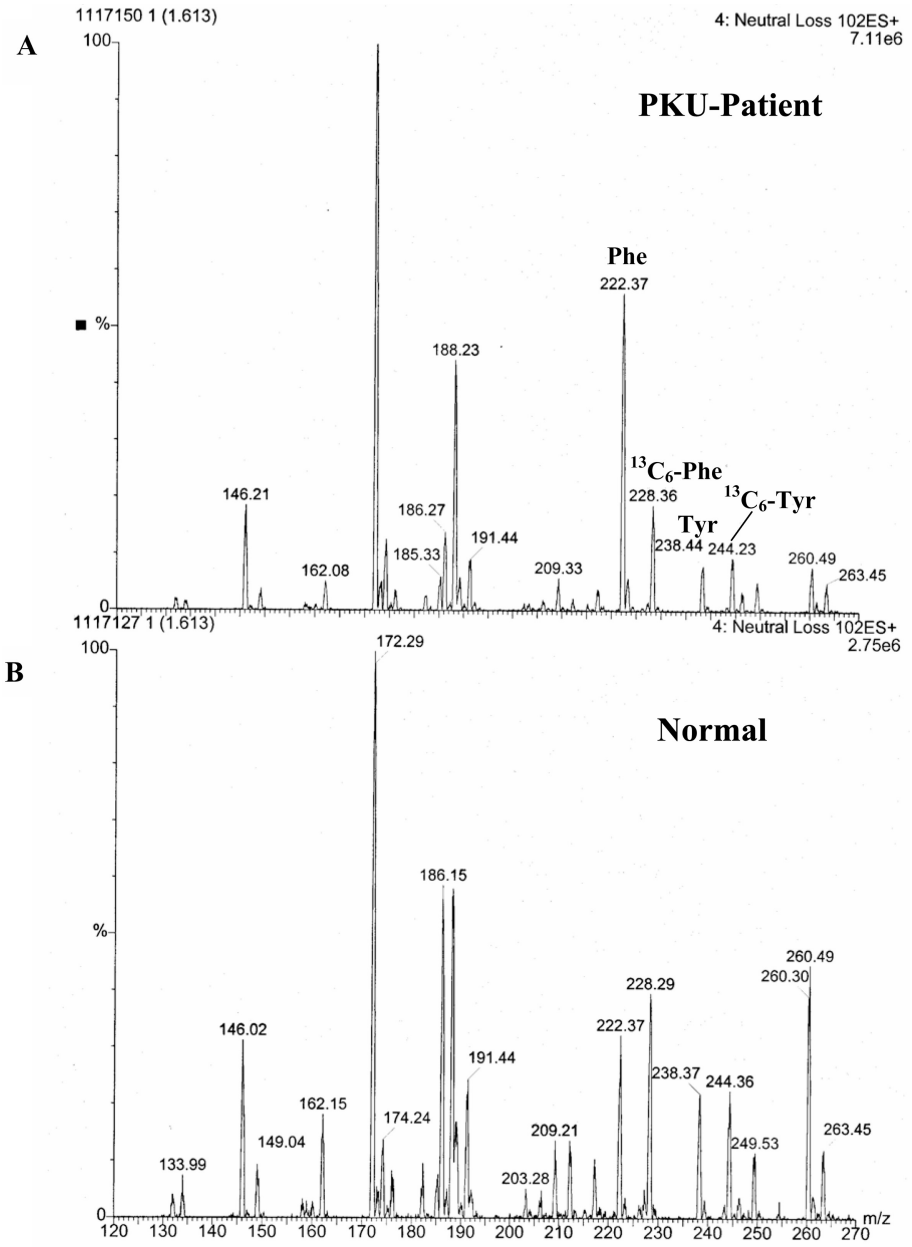


**Figure 2.** Typical spectra for the neutral loss experiments of *m/z* 102 which are used for the detection of most amino acids. Amino acids are derivatized prior to analysis to form butyl esters. Mass range from *m/z* 120 to 270 is scanned within 1.5 s. Spectra (A) from a PKU patient shows an elevated phenylalanine concentration (*m/z* 222.4) while tyrosine (*m/z* 238.4) remains as low as in the normal control (B). Other important amino acids and standards which can be determined from this experiment are: alanine (*m/z* 146.0), aspartic acid (*m/z* 246.2), $^2H_3$-aspartic acid (*m/z* 249.5), glutamic acid (*m/z* 260.5), $^2H_3$-glutamic acid (*m/z* 263.4), histidine (*m/z* 212.2), leucine/isoleucine/hydroxyproline (*m/z* 188.2), $^2H_3$-leucine (*m/z* 191.4), methionine (*m/z* 206.2), $^2H_3$-methionine (*m/z* 209.2), methyl-histidine (*m/z* 226.2), proline (*m/z* 172.3), serine (*m/z* 162.1), threonine (*m/z* 176.2), tryptophane (*m/z* 261.2), valine (*m/z* 174.2), and $^2H_8$-valine (*m/z* 182.2)

neutral loss monitoring. Acylcarnitines, which are derived from fatty acids can be easily detected when a parent ion scan of *m/z* 85 is used. All of the experiments are run within 1 – 1.5 min, so that a complete cycle time between injections of 2 – 2.5 min can be achieved. This certainly is a prerequisite for the use in high-throughput neonatal screening with an average workload of 50 000 samples *per* year and machine.

The simultaneous measurement of phenylalanine and tyrosine reduced false-positive samples due to postpartal liver damage or immaturity which usually are characterized by

 

**Table 1.** Comparison of the incidence of various disorders identified by MS/MS screening in Bavaria, Germany ($n = 323\,020$) and the New England States, USA ($n = 257\,000$). Additionally, the number of recalls for both screening centers is given (Liebl, 2001; Zytkovicz, 2001)

| Disorder | Incidence | | Recalls | |
|---|---|---|---|---|
| | Bavaria | New England | Bavaria | New England |
| HPA (total) classical PKU | 1:5 768<br>1:11 536 | 1:14 277<br>1:36 714 | 1:2 197 | 1:4 016 |
| Maple syrup urinary disease (MSUD) | 1:80 755 | 1:257 000 | 1:35 891 | 1:32 125 |
| Hypermethioninemia | 1:323 020 | 1:257 000 | 1:1 150 | 1:8 031 |
| Tyrosinemia | 1:161 510 | 0 | 1:771 | 1:3 905 |
| Urea cycle defects | Not included | 0 | – | 1:16 400 |
| Citrullinemia/ASS/ASL | Not included | 1:164 000 | – | 1:54 667 |
| Argininemia | Not included | 1:164 000 | – | 1:54 667 |
| PA/MMA | 1:96 000 | 1:82 000 | 1:841 | 1:4 556 |
| IVA | 1:323 020 | 0 | 1:1 318 | 1:4 686 |
| HMG-CoA Lyase-/methylcrotonyl CoA carboxylase (MCC)-deficieny | 1:46 156 | 1:164 000 | 1:2 291 | 1:3 154 |
| Glutaric acid. I + II | 1:107 673 | 0 | 1:3 296 | 1:5 125 |
| SCADD | 0 | 1:32 800 | 1:2 584[*)] | 1:4 970 |
| MCADD | 1:9 229 | 1:18 400 | 1:3 019 | 1:3 538 |
| VLCADD | 1:161 510 | 1:164 000 | 1:2 584[*)] | 1:41 000 |
| CPT I + II, CAT | 1:161 510 | 1:164 000 | n.a. | 1:82 000 |
| LCHAD | 1:323 020 | 0 | 1:2 584[a)] | 1:32 800 |

a) Recalls for all fatty-acid oxidation disorders, except MCADD, and CPT I, II, and CAT in Bavaria.

an elevation of both analytes while classical PKU shows relatively low tyrosine concentrations (Fig. 2).

However, the simultaneous identification of a number of metabolites also brought about new problems, primarily the interpretation of the large amount of data, the decision as to which diseases to screen for, and the communication of results to doctors who seldom can keep track of all the rare inherited metabolic disorders that could now be found [8]. In Germany, an evaluation of MS/MS screening was conducted in Bavaria from 1999 to 2001 [9, 10]. In addition, several further studies demonstrated the basic feasibility of large-scale newborn screening programs using MS/MS [11, 12]. The results for the Bavarian and New England studies are summarized in Table 1. In Australia, it was shown that more cases of inborn errors of metabolism were diagnosed with MS/MS than just clinically [13]. However, the impact and the necessity of such a diagnosis remain unclear. Also in our experience, a certain percentage of neonates with elevations in some biochemical parameters remained asymptomatic to date. This holds especially true for mild elevations of citrulline (about 100 μM, mild citrullinemia), moderately increased concentrations of hydroxyisovaleryl carnitine (approximately 1–2 μM, potentially heterozygeous for methylcrotonyl CoA carboxylase deficiency) and the rather frequent medium-chain AcylCoA dehydrogenase deficiency (MCADD).

Ideally, all diseases in a screening program should meet World Health Organization (WHO) criteria as established by Wilson and Jungner [14] (Fig. 3). However, many diseases which can be found by MS/MS are not preventable but the individual outcome may be positively influenced by early detection. To assess current developments in neonatal screening, the Northern German Working Group on Neonatal Screening (NANS – Norddeutsche Arbeitsgemeinschaft Neonatalscreening) consisting of health care providers, metabolic centers, and screening laboratories was founded in 2002. Based on current literature and experience, we have compiled a list of disorders which are categorized according to the benefit that can be expected from early detection (Table 2).

**Principles for Screening of Diseases**

1. Significance of the disease for the individual
2. Effective treatment is available
3. Prerequisites for recognition and treatment are met
4. The disease can be recognized when the patient is asymptomatic
5. Adequate assay available
6. Acceptable for the population
7. Pathogenesis is understood
8. Consensus about who should treat the disease
9. Cost efficiency

**Figure 3.** Principles of the WHO for screening of diseases according to Wilson and Jungner, 1968 [14].

 

**Table 2.** Recommendations for the neonatal screening of disorders that can be detected by MS/MS based on current literature[a] and experience. List has been compiled by the NANS in 2004

| Disorder | Category 1, beneficial | Category 2, partially beneficial | Category 3, unclear | Category 4, no benefit | Remarks |
|---|---|---|---|---|---|
| MSUD | | x | | | Rare but technically sound. Mild variants might be missed. |
| Citrullinemia | | | x | | Mild variants do not profit. Severe forms are probably diagnosed too late. |
| Propionic Acid | | | x | | Very rare with many recalls as unspecific elevations are frequent. Outcome unsatisfactory. |
| Methylmalonic Acid | | | x | | Mild cases might be missed. Severe forms may not benefit. |
| Glutaric Acid. Type 1 | | x | | | Early detection may improve outcome – only little experience until now. |
| Glutaric Acid. Type 2 | | | | x | No benefit – will be picked up with other disorders – facilitates diagnosis. |
| 3–HMGCoA-lyase-deficiency | | | x | | Only very few patients known after screening was introduced. May benefit. |
| SCAD-deficiency | | | | x | Diagnosis difficult, pathomechanism ill-understood, treatment not available. |
| β-Ketothiolase-deficiency | | | | x | Mild disease with only few known patients. Unclear if technically feasible. |
| 3–MCC-deficiency | | | x | | High proportion of nondisease. Only few symptomatic patients known. |
| MCAD-deficiency | x | | | | Successful prevention of metabolic crises and death. Problem: asymptomatic patients. |
| LCHAD-deficiency | | x | | | Treatment can reduce mortality but morbidity still remains high. |
| VLCAD-deficiency | | x | | | Basically treatable but symptoms will occur. Difficult to diagnose. |
| Homocystinuria | | | | x | Technically unsound. |
| Isovaleric Acid | | x | | | A benefit of early treatment can be seen. Asymptomatic cases also known. |
| Tyrosinemia Type I | | | x | | Screening can only be recommended with a second-tier assay, as neonatal hypertyrosinemia fairly frequent. |
| CPT I | | x | | | Benefit from early treatment. |
| CAT | | | x | | Very rare. |
| CPT II | | | x | | Very rare. |

[a] Scriver, C. R. *et al.* (Eds.), *The Metabolic and Molecular Basis of Interited Disease*, 8th Edition, McGraw-Hill, New York, 2001.

Disorders in category 1 fulfill the classical Wilson and Jungner criteria. Category 2 comprises diseases which will benefit from neonatal screening, but some children may not profit, because of the limited possibilities for treatment currently available. Both categories should be included in neonatal screening programs. Category 3 can be summarized as disorders that basically can be detected by MS/MS screening and indications of a potential benefit for the individual are present but current knowledge on the course of the disease under early treatment is still limited. Thus, these disorders should only be screened for within a scientific study that is continuously evaluated. A few disorders were not recommended for general screening (category 4). Either there is no indication of a clear-cut advantage for the patient (short-chain acylCoA dehydrogenase deficiency (SCADD), β-ketothiolase deficiency, and glutaric aciduria (Acid.) II) or it is technically unsound (homocystinuria). Glutaric Acid. II is recognized by elevations of glutarylcarnitine and other fatty-acid carnitine esters (similar to long-chain hydroxy-acylCoA dehydrogenase deficiency (LCHADD), MCADD, and very long-chain acylCoA dehydrogenase deficiency (VLCADD)). Therefore, it cannot be avoided that this disorder is picked up during routine neonatal screening. Due to the severity of the disease, early communication of the results to the intensive care unit may at least facilitate diagnosis, so that it should not be completely omitted. In case of homocystinuria, methionine seems to be an unreliable indicator. Frequently, hypermethioninemia is observed in the neonatal period due to general immaturity of the neonatal metabolism. In addition, breast-fed infants have low methionine intake so that even in cases of homocystinuria, methionine may not be elevated [15].

 

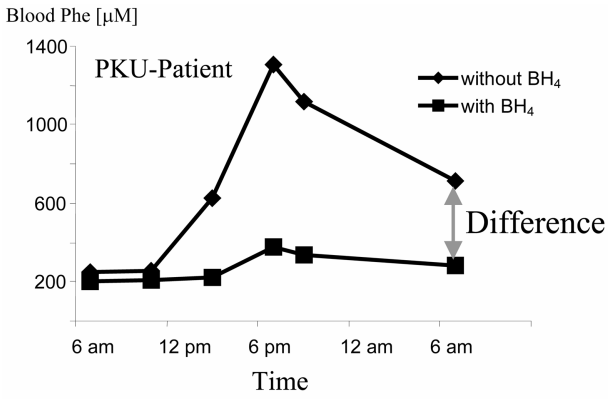


**Figure 4.** Blood phenylalanine concentrations of a PKU patient during a period of 24 h with and without $BH_4$ supplementation. It is important to note that patients with relatively high residual enzyme activity may show significant intraday variations in blood phenylalanine concentrations. Therefore, the difference between both curves should exceed 30% to qualify the patient as $BH_4$-responsive.

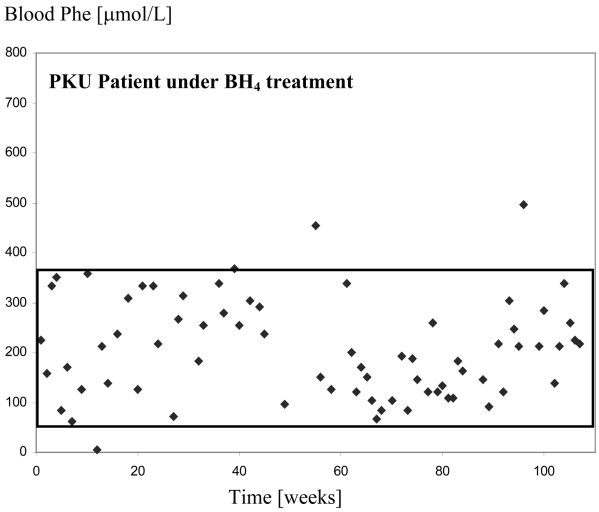


**Figure 5.** Blood phenylalanine concentrations of a PKU patient under $BH_4$ monotherapy. Phenylalanine concentrations remained in the desired range (area in the box) throughout the study period and did not exceed 500 μM during two infections.

In contrast, treatment of PKU with dietary restrictions prevents mental and psychomotor deterioration in early treated infants. More recently, it was found that treatment with a high dosage of $BH_4$ (5 – 20 mg/kg body weight × day) can increase phenylalanine turnover even in cases of PKU without defects in $BH_4$-metabolism [16]. The mechanism of the activation of the PAH enzyme is still not fully elucidated but there are indications that $BH_4$ can act as a molecular chaperone and correct misfolding of the protein [17]. As with many genetic disorders phenotype to genotype correlations are difficult and $BH_4$ sensitivity must be tested individually. For that purpose, a $BH_4$-loading test is performed with 20 mg/kg given in a single dose. Phenylalanine concentrations are assessed for a period of 24 h. Especially, in cases of high residual enzyme activity, it is important to determine phenylalanine concentrations for another 24 h without $BH_4$ supplementation. Then $BH_4$-responsiveness can be defined as the difference between both curves which has to be greater than 30% (Fig. 4). According to Muntau *et al*. [18], it is estimated that a large proportion of PKU patients with mutations causing a milder form of the disease can be expected to be $BH_4$-responsive. Certainly, a medical benefit of such a therapy has to be demonstrated individually. Currently, we have six patients which were $BH_4$-responsive, two of those are under $BH_4$-therapy for approximately 3 years without any additional dietary restrictions [19]. Figure 5 shows the phenylalanine concentrations of one patient under $BH_4$ treatment for a period of more than 2 years. Plasma phenylalanine concentrations remained in the desired range (60 – 360 μmol/L) throughout the 750-day study period and did not increase above 500 μmol/L during periods of infections.

Surprisingly, the causes for intellectual impairment in PKU are still not fully understood [20]. Especially, high phenylalanine concentrations during infancy and childhood lead to psychomotor retardation while later in life reversible neuropsychological deficits are observed. Morphologically, the number, length, and degree of arborization of the dendritic processes as well as synaptic spines were diminished, while cell packing density is increased with individual neurons being smaller than usual [21]. Among other causes, the effect of phenylalanine on the respiratory chain has been discussed in literature [22]. Preliminary studies on PAH knock-out mice in our laboratory showed normal activity of complex I-V in mice brain homogenates [23]. Thus, it appears that the reduced energy status described in literature is not caused by functionally impaired respiratory chain enzymes but may result from direct or indirect inhibition of energy production by phenylalanine.

## 4 Concluding remarks

In our experience, MS/MS provides a potential to rapidly screen for a wide variety of rare inherited disorders. In view of novel therapies (*e.g.*, enzyme replacement) further applications may be on the horizon. However, the cost, workload, additional anxiety of parents in cases of false-positive samples, and problems with heterozygote or asymptomatic children should not be underestimated. Therefore, responsible screening can only be performed with constant scientific evaluation and support of medical (metabolic) experts. The benefits of neonatal screening and early therapy are undisputed in PKU. In addition, for some PKU patients $BH_4$-therapy may be a viable option to replace or supplement traditional dietary treatment and thus increase their quality of life.

 

*We are grateful to our laboratory staff at the neonatal and metabolic laboratory, Hamburg, Germany for their accurate work. Furthermore, we want to thank all contributors to the NANS, especially, Dr. J. Hennermann (Charité, Berlin, Germany), Dr. J. Klein (Charité, Berlin, Germany), Dr. C. Müller (University Greifswald, Germany), Professor A. Kohlschütter (Hamburg University Medical Center, Germany), Dr. P. Huppke (University Göttingen, Germany), Professor Dr. Das (MH Hannover, Germany), and Professor Dr. J. Sander (Hannover, Germany).*

## 5 References

[1] Erlandsen, H., Stevens, R. C., *J. Inherit. Metab. Dis.* 2001, *24*, 213 – 230.

[2] Scriver, C. R., Waters, P. J., Sarkissian, C., Ryan, S., *et al.*, *Hum. Mutat.* 2000, *15*, 99 – 104.

[3] Zschocke, J., Hoffmann, G. F., *Hum. Genet.* 1999, *104*, 390 – 398.

[4] Schweitzer-Krantz, S., Burgard, P., *Eur. J. Pediatr.* 2000, *159 (Suppl. 2)*, S70 – S73.

[5] Walter, J. H., White, F. J., Hall, S. K., MacDonald, A., *et al*., *Lancet* 2002, *360*, 55 – 57.

[6] Guthrie, R., Susi, A., *Pediatrics* 1963, *32*, 338 – 343.

[7] Zabransky, S. (Ed.), *Screening auf Angeborene Endokrine und Metabole Störungen*, Springer, Vienna 2001.

[8] Lukacs, Z., Santer, R., Stehn, M., Kohlschütter, A., *Neugeborenenscreening mittels Tandemmassenspektrometrie (MS/MS) – Potenzial, Probleme und Perspektiven. Kinder- und Jugendmedizin* 2004, *4*, 222 – 225.

[9] Roscher, A., Liebl, B., Fingerhut, R., Olgemöller, B., *J. Inherit. Metab. Dis.* 2000, *23(suppl. 1)*, 4.

[10] Liebl, B., Nennstiel-Ratzel, U., von Kries, R., Roscher, A., *Abschlussbericht zum Modellprojekt zur Neuordnung des Neugeborenen-Screenings in Bayern* 2001, http://www.lgl.bayern.de/de/left/fachinformationen/gesundheit/_docs/_docs_screening/bericht.pdf.

[11] Zytkovicz, T. H., Fitzgerald, E. F., Marsden, D., Larson, C. A., *et al.*, *Clin. Chem.* 2001, *47*, 1945 – 1955.

[12] Schulze, A., Lindner, M., Kohlmüller, D., Olgemöller, K., *et al.*, *Pediatrics* 2003, *111*, 1399 – 1406.

[13] Wilcken, B., Wiley, V., Hammond, J., Carpenter, K., *New Engl. J. Med.* 2003, *348*, 2304 – 2312.

[14] Wilson, J. M. G., Jungner, G., *Principles of Screening for Disease*, World Health Organization, Geneva 1968.

[15] Lukacs, Z., *Homozystinurie*, in: Zabransky, S. (Ed.), *Screening auf Angeborene Endokrine und Metabole Störungen*, Springer, Vienna 2001, pp. 260 – 265.

[16] Kure, S., Hou, D. C., Ohura, T., Iwamoto, H., *et al.*, *J. Pediatr.* 1999, *135*, 375 – 378.

[17] Steinfeld, R., Kohlschütter, A., Ullrich, K., Lukacs, Z., *Amino Acids* 2003, *25*, 63 – 68.

[18] Muntau, A. C., Roschinger, W., Habich, M., *et al*., *New Engl. J. Med.* 2002, *347*, 2122 – 2132.

[19] Steinfeld, R., Kohlschütter, A., Ullrich, K., Lukacs, Z., *J. Inherit. Metab. Dis.* 2004, *27*, 449 – 453.

[20] Huttenlocher, P. R., *Eur. J. Pediatr.* 2000, *159 (Suppl. 2)*, S102 – S106.

[21] Baumann, M. L., Kemper, T. L., *Acta Neuropathol. (Berl.)* 1982, *58*, 55 – 63.

[22] Cielo Rech, V., Rosa Feksa, L., Dutra-Filho, C. S., de Souza Wyse, A. T., *et al.*, *Neurochem Res.* 2002, *27*, 353 – 357.

[23] Lukacs, Z., Matalon, R., Surendran, S., Ullrich, K., *J. Inherit. Metab. Dis.* 2004a, *27 (Suppl. 1)*, 34.

 