



Meeting Report

# 23<sup>rd</sup> Annual Meeting of the German Society for Newborn Screening (Deutsche Gesellschaft für Neugeborenen-Screening, DGNS)

Gwendolyn Gramer \*, Jürgen G. Okun and Georg F. Hoffmann

Centre for Paediatric and Adolescent Medicine, Division of Neuropaediatrics and Metabolic Medicine, Im Neuenheimer Feld 430, University Hospital Heidelberg, 69120 Heidelberg, Germany; Juergen.Okun@med.uni-heidelberg.de (J.G.O.); georg.hoffmann@med.uni-heidelberg.de (G.F.H.)

\* Correspondence: Gwendolyn.Gramer@med.uni-heidelberg.de

Academic Editor: Ralph Fingerhut

Received: 7 July 2016; Accepted: 24 August 2016; Published: 29 August 2016

**Abstract:** From 3–4 June, 2016, the 23<sup>rd</sup> Annual Meeting of the German Society for Newborn Screening (Deutsche Gesellschaft für Neugeborenen-Screening, DGNS) was held at the University Hospital Heidelberg. The meeting was organized by PD Dr. med. Gwendolyn Gramer (conference president) from the Newborn Screening Centre at the University Hospital Heidelberg, Centre for Paediatric and Adolescent Medicine. Prof. Dr. med. Prof. h.c. mult. (RCH) Georg F. Hoffmann, PD Dr. phil. nat. Jürgen G. Okun and PD Dr. med. Gwendolyn Gramer formed the scientific board for the selection of presentations. Abstracts of plenary lectures, oral communications, and posters presented during the meeting are collected in this report.

**Keywords:** newborn screening; target disorders; cystic fibrosis; pilot projects; second-tier strategies; organic acidurias; tyrosinaemia type I; sickle cell disease; next generation sequencing

## 1. Aim and Scope of the Meeting

The main topic of the meeting was “New target disorders for newborn screening”. This included a session on newborn screening for cystic fibrosis, which will become part of the nationwide screening program in Germany in 2016. In addition, results from pilot projects on newborn screening for e.g., methylmalonic and propionic aciduria, tyrosinaemia type I, classical homocystinuria, remethylation disorders, and creatine synthesis disorders were presented. The national newborn screening report 2014 for Germany was presented by the president of the DGNS, Dr. med. Uta Nennstiel-Ratzel. The meeting ended with a plenary lecture on massive parallel sequencing as a potential future perspective in newborn screening.

## 2. Lectures

### *L-01. Newborn Screening for Creatine Synthesis Defects*

Sylvia Stockler-Ipsiroglu

Division of Biochemical Genetics, Department of Pediatrics, University of British Columbia, Canada

GAMT (Guanidinoacetate Methyltransferase) Deficiency is one of the three known inborn errors of creatine metabolism, affecting creatine synthesis (GAMT and AGAT deficiency) and transport (SLC6A8 deficiency).

Less than 100 patients have been diagnosed worldwide with GAMT deficiency. Clinical features include a spectrum of intellectual disability (ID), autistic behavior, epilepsy, and

spasticity/dystonia/ataxia. Treatment with creatine and ornithine supplements, and/or arginine restricted diet, results in partial correction of cerebral creatine deficiency and in reduction of guanidinoacetate accumulation. Elevated guanidinoacetate concentration in body fluids is a specific diagnostic marker. Based on evidence that early recognition prevents ID and neurologic manifestations, GAMT deficiency is a candidate for newborn screening. GAA is reliably measured in blood spots via a two-tier test protocol, integrating a standard tandem mass spectrometry flow injection (FIA-MS/MS) method (as used for acylcarnitines/amino acids) as a first-tier assay, and an LC-MS/MS quantification step as a second tier. GAMT gene sequencing in positive samples is a third tier option.

AGAT deficiency presents with a spectrum of ID and myopathy. Less than 20 patients have been described worldwide. Based on observations in early treated patients, treatment with creatine supplement prevents clinical manifestations. In the absence of a specific biomarker, newborn screening for AGAT deficiency has not yet been implemented. SLC6A8 deficiency might be another candidate for newborn screening, once effective therapies have been developed.

## Reference

1. Stockler-Ipsiroglu, S.; Apatean, D.; Battini, R.; DeBrosse, S.; Dessoffy, K.; Edvardson, S.; Eichler, F.; Johnston, K.; Koeller, D.M.; Nouioua, S.; et al. Arginine:glycine amidinotransferase (AGAT) deficiency: Clinical features and long term outcomes in 16 patients diagnosed worldwide. *Mol. Genet. Metab.* **2015**, *116*, 252–259.
2. Stockler-Ipsiroglu, S.; van Karnebeek, C.; Longo, N.; Korenke, G.C.; Mercimek-Mahmutoglu, S.; Marquart, I.; Barshop, B.; Grolik, C.; Schlune, A.; Angle, B.; et al. Guanidinoacetate methyltransferase (GAMT) deficiency: outcomes in 48 individuals and recommendations for diagnosis, treatment and monitoring. *Mol. Genet. Metab.* **2014**, *111*, 16–25.
3. Pasquali, M.; Schwarz, E.; Jensen, M.; Yuzyuk, T.; DeBiase, I.; Randall, H.; Longo, N. Feasibility of newborn screening for guanidinoacetate methyltransferase (GAMT) deficiency. *J. Inher. Metab. Dis.* **2014**, *37*, 231–236.
4. Sinclair, G.B.; van Karnebeek, C.D.; Ester, M.; Boyd, F.; Nelson, T.; Stockler-Ipsiroglu, S.; Vallance, H. A three-tier algorithm for guanidinoacetate methyltransferase (GAMT) deficiency newborn screening. *Mol. Genet. Metab.* **2016**, *118*, 173–177.
5. Health Council of the Netherlands. *Neonatal Screening: New Recommendations*; Health Council of the Netherlands: The Hague, The Netherlands, 2015; publication no. 2015/08E.
6. Utah Department of Health. Utah Newborn Screening. Available online: <http://www.health.utah.gov/newbornscreening> (accessed on 1 December 2015).

L-02. *International Disease Registries for Organic Acidurias and Urea Cycle Disorders—Understanding the Impact of Newborn Screening on the Long-Term Outcome*

Stefan Kölker, Roland Posset, Nikolas Boy, Corinna Bürger, Florian Gleich, Gisela Haege, Jana Heringer, Ulrike Mütze, Christian Staufner, Matthias Zielonka, Peter Burgard; for the E-IMD consortium

University Hospital Heidelberg, Centre for Pediatrics and Adolescent Medicine, Division of Neuropediatrics and Metabolic Medicine, Heidelberg, Germany

In 2011, the EU-funded project “European registry and network for intoxication type metabolic diseases (E-IMD)” started. Since then, 456 patients with urea cycle disorders (UCDs) and 567 patients with organic acidurias (OADs) have been registered. Among them, 178 patients with OADs, but only 21 with UCDs, were identified by newborn screening (NBS).

In OAD patients, the overall median age at diagnosis was 21 days (interquartile range (IR) 6–300 days). It was dependent on the mode of diagnosis and the proportion of patients with early (EO) and late disease onset (LO). Across all OADs, the age at diagnosis was consistently lower in the NBS group than in the LO group, whereas, in the EO group, an analogous effect was only observed for patients with isovaleric aciduria and glutaric aciduria type I. This indicates that some OAD

patients ( $n = 77$ ) already presented with symptoms before NBS results were available. For patients with glutaric aciduria type I and for cobalamin-nonresponsive forms of methylmalonic aciduria, we found a significant improvement of motor function compared to patients diagnosed after the onset of symptoms. This effect was not confirmed for other OADs. For cobalamin-nonresponsive patients, we identified a statistical trend towards a lower probability of renal failure, and, for patients with propionic aciduria, a lower risk of cardiac manifestation with increasing age.

In UCDs, the age at diagnosis in the NBS group was significantly reduced for argininosuccinate lyase deficiency only. To increase statistical power, we also included patients from high-risk families who had been diagnosed during the newborn period while being asymptomatic. Overall, odds ratios showed a trend towards a decreased frequency of movement disorder and improved motor milestones in the NBS group (argininosuccinate synthase and lyase deficiency, argininemia). The initial peak ammonium level (except for argininemia) and age of disease onset had a strong influence on the neurological outcome. They are important non-interventional variables that should be included in each analysis aiming to evaluate the benefit of NBS.

In conclusion, long-term outcome studies like E-IMD are required to elucidate the beneficial effect of NBS (if any) on the disease course. Multiple clinical endpoints and relevant non-interventional variables should be included in the analysis.

#### *L-03. To Screen or not to Screen: Sickle Cell Disease in Germany*

Stephan Lobitz<sup>1</sup>, Claudia Frömmel<sup>2</sup>, Oliver Blankenstein<sup>3</sup>, Annemarie Brose<sup>3</sup> and Jeannette Klein<sup>3</sup>

<sup>1</sup> Charité—Universitätsmedizin Berlin, Klinik für Pädiatrie m.S. Onkologie/Hämatologie/SZT

<sup>2</sup> Labor Berlin GmbH

<sup>3</sup> Charité—Universitätsmedizin Berlin, Neugeborenencreeninglabor

Sickle cell disease (SCD) is an autosomal-recessively inherited disorder of hemoglobin and is associated with both high morbidity and mortality. However, simple prophylactic measures may prevent early death and facilitate inclusion into an appropriate comprehensive care program.

Epidemiological data on SCD in Germany is sparse. However, within the last three years, three pilot studies on universal newborn screening for SCD have been published. Taken together, the authors investigated nearly 90,000 babies born in the areas of the screening laboratories in Berlin, Hamburg, and Heidelberg, representing births in both rural and urban regions. The cumulative incidence was on an order of 1 in 6000. Thus, SCD is probably the second most common disease (after hypothyroidism) in the German newborn screening program that covers 14 disorders to date. There is now a broad consensus within the medical community that Germany requires a newborn screening for SCD. It is now up to politicians to make a decision.

In terms of methodology, the situation is not as clear. High performance liquid chromatography (HPLC) is the international gold standard. However, capillary electrophoresis (CE) provides a similar quality of testing at a similar cost. More recent publications suggest that mass spectrometry (MS), in particular tandem mass spectrometry (MS/MS), is a cost-efficient option in the long term.

We have analyzed 9173 consecutive dried blood spot samples from children born in Berlin and Brandenburg in a prospective setting with MS/MS and CE and did not observe any discordant result between both methods. We have identified three babies suffering from sickle cell disease, 34 carriers of the HbS mutation and 23 carriers of various other hemoglobin variants. The study will be continued until we have investigated 35,000 newborns and hopefully provide a basis for the decision on the appropriate method for newborn screening for SCD in Germany.

This talk will give an update on the status of concerted efforts of several bodies to get SCD into the German newborn screening program.

*L-04. Combining IRT/PAP+SN with DNA Analysis for the Best CF Newborn Screening Strategy in Germany*

Olaf Sommerburg<sup>1,2</sup>, Mirjam Stahl<sup>1,2</sup>, Martina Muckenthaler<sup>1,2</sup>, Dirk Kohlmüller<sup>3</sup>, Margit Happich<sup>1</sup>, Andreas E. Kulozik<sup>1</sup>, Jürgen G. Okun<sup>3</sup>, Marcus A. Mall<sup>1,2,4</sup> and Georg F. Hoffmann<sup>2,3</sup>

<sup>1</sup> Division of Pediatric Pulmonology & Allergy and Cystic Fibrosis Center, Department of Pediatrics III, University of Heidelberg, Im Neuenheimer Feld 430, D-69120 Heidelberg, Germany

<sup>2</sup> Translational Lung Research Center Heidelberg (TLRC), Member of the German Center for Lung Research (DZL), Im Neuenheimer Feld 350, D-69120 Heidelberg, Germany

<sup>3</sup> Division of Metabolic Diseases and Newborn Screening Center, Department of Paediatrics I, Children's Hospital, University of Heidelberg, Im Neuenheimer Feld 430, D-69120 Heidelberg, Germany

<sup>4</sup> Department of Translational Pulmonology, University of Heidelberg, Im Neuenheimer Feld 350, D-69120 Heidelberg, Germany

**Introduction:** Evidence from recent European studies suggests that protocols using immunoreactive trypsin (IRT) and pancreatitis associated protein (PAP) for cystic fibrosis (CF) newborn screening (NBS) may be successfully used as a purely biochemical NBS strategy that does not require genetic screening. Pure IRT/PAP protocols can reach acceptable sensitivities and specificities, but this comes at the expense of a relatively low positive predictive value (PPV). The Dutch CHOPIN study (Vernooij-van Langen 2012) has reported that using a three-tier strategy (IRT/PAP + extended gene analysis) results in a better PPV.

**Objective:** Assessment of the performance of two different IRT/PAP/DNA CF NBS protocols (projected German CF NBS protocol by the Federal Joint Committee (GBA) vs. an alternative CF NBS protocol suggested by the Heidelberg CF NBS study group (HD)) using different safety net strategies.

**Methods:** Recent data available from the cohort of the Heidelberg CF NBS study (2008–2015, 372,906 newborns) were evaluated in a post hoc analysis. Both IRT/PAP/DNA protocols used one IRT-independent PAP-cut-off and a safety net (SN) strategy (CF-NBS positive, if IRT  $\geq$  99.9<sup>th</sup> IRT percentile) to reach a sufficient sensitivity. SN-positive newborns of the GBA-protocol are immediately considered CF NBS positive, while SN-positive newborns of the HD-protocol circumvent PAP measurement and will directly receive DNA analysis.

**Findings:** Both combined protocols (IRT/PAP + SN/DNA) achieved sensitivity, which is similar to that of the IRT/PAP + SN protocol. Both strategies keep main advantages of IRT/PAP, such as the detection of considerably fewer carriers and fewer newborns with CFSPID when compared with current IRT/DNA screening strategies. PPV of both IRT/PAP + SN/DNA protocols is higher than that of the IRT/PAP + SN protocol. However, PPV reached by the HD-protocol is four times higher than that of the GBA protocol. This goes along with a much higher number of falsely positive detected newborns, which would lead to higher costs for confirmatory diagnosis, and might create psychological burden and anxiety in the parents of these infants.

**Conclusions:** Our results support the use of an IRT/PAP + SN/DNA protocol. However, the projected German CF NBS protocol results in a higher number of falsely positive detected newborns than an alternative IRT/PAP + SN/DNA protocol.

*L-05. Newborn Screening for Cystic Fibrosis in Switzerland—Experiences after 5 Years*

Jürg Barben, MD, A/Prof.

President Task Force Neonatal CF Screening, Swiss Working Group for Cystic Fibrosis (SWGCF); Head Division of Paediatric Pulmonology and CF Center, Children's Hospital of Eastern Switzerland, CH-9006 St. Gallen

In Switzerland, newborn screening (NBS) for cystic fibrosis (CF) started in January 2011, after application to the Ministry of Health and thorough evaluation of the screening protocol (IRT/DNA/IRT). One part of the approval was the obligation of yearly evaluation and a parental survey.

All heel prick tests (taken on day 4) are sent to the centralized Swiss National Screening Laboratory (SNSL). If IRT-1 is above the cut-off (P 99.2), a DNA screening is performed. The genetic kit included the seven most common CFTR mutations in Switzerland. Since 2013, 18 mutations have been tested, because the components of the initial in-house kit were no longer available and a commercial kit was used. If no mutation is found, a second IRT is performed if IRT-1 was  $\geq 60$  ng/mL (safety loop). All positively screened children are notified from the SNSL to the nearest CF-center. A CF-specialist invites the parents by phone call for diagnostic evaluation (sweat test) on the next day. If a sweat test is positive or borderline a diagnostic DNA analysis is performed. Due to the law on human genetics, the SNSL is not allowed to report details of CFTR mutations, as the Swiss NBS program requires only an oral informed consent (with a possibility for parents to refuse) but not a written consent.

Within five years, out of 430,351 births, 447 children were screened positive and referred to a CF-center. A total of 123 (28%) children were diagnosed with CF, 18 (4%) had an inconclusive result (CFSPID), 300 (67%) children were CF negative, and six (1.3%) were lost to follow up. Furthermore, seven children with negative screening result were later diagnosed with CF (5.4% false negatives = 7/130). The sensitivity was 94.6% (123/130) and the PPV was 27.5% (123/447); or 31.5% (141/447) when CFSPID cases were included. The specificity (429,897/430,221) and NPV (429,897/429,904) reached almost 100%.

The questionnaire survey of all parents of positively screened children revealed great satisfaction with the screening. The information provided by the CF-center is essential to relieve the anxiety of parents and underlines the importance of a short delay between the information about the screening result and the first visit in the CF-center.

After five years, CF-NBS remains successful in detecting nearly all children with CF and avoiding inconclusive cases. Regular evaluation has resulted in improvement of CF-NBS. In order to improve the PPV, pancreatitis-associated protein (PAP) measurement as a further technique is now under evaluation.

## Literature

1. Newborn screening for CF in Switzerland—Consequences after analysis of a 4 months pilot study. *J. Cyst. Fibros.* **2013**, *6*, 667–674.
2. One-year evaluation of a neonatal screening program for cystic fibrosis in Switzerland. *Dtsch. Arztebl. Int.* **2013**, *110*, 356–363.
3. Newborn screening for cystic fibrosis—The parent perspective. *J. Cyst. Fibros.* **2016**, *15*, 443–451.
4. Measurement of fecal elastase improves performance of newborn screening for CF. *J. Cyst. Fibros.* **2016**, *15*, 313–317.

### *L-06. Newborn Screening for Vitamin B6 Non-Responsive Classical Homocystinuria—Systematical Evaluation of a Two-Tier Strategy*

Jürgen G. Okun, Gwendolyn Gramer, Junmin Fang-Hoffmann, Kathrin V. Schmidt, Peter Burgard and Georg F. Hoffmann

University Children's Hospital Heidelberg, Division of Neuropediatrics and Metabolic Medicine, Dietmar-Hopp-Stoffwechszentrum, Im Neuenheimer Feld 669, 69120 Heidelberg, Germany

**Background:** In classical homocystinuria (HCU, MIM# 236200), due to deficiency of cystathionine  $\beta$ -synthase (EC 4.2.1.22), there is clear evidence for the success of early treatment. The aim of this study was to develop and evaluate a two-tier strategy for HCU newborn screening.

**Patients/Methods/Results:** We reevaluated data from our newborn screening program for Qatar, in a total number of 125,047 neonates, including 30 confirmed HCU patients. Our hitherto existing screening strategy involves homocysteine (Hcy) measurements in every child, resulting in a unique dataset for evaluation of two-tier strategies. Reevaluation included methionine (Met) levels, Met to phenylalanine (Phe) ratio, and Hcy. Four HCU cases identified after database closure were also

included in the evaluation. In addition, dried blood spot samples selected by Met values  $>P97$  in the newborn screening programs in Austria, Australia, The Netherlands, and Taiwan were analyzed for Hcy. Met to Phe ratio was found to be more effective as a first sieve than Met, sorting out nearly 90% of normal samples. Only 10% of the samples would have to be processed by second-tier measurement of Hcy in dried blood spots. As no additional patients with HCU were found neither in the samples investigated for HCU, nor by clinical diagnosis in the other countries, the generalization of our two-tier strategy could only be tested indirectly.

**Conclusions:** The presented two-tier algorithm using Met to Phe ratio as first and Hcy as second-tier requires 10% first-tier positives to be transferred to Hcy measurement, resulting in 100% sensitivity and specificity in HCU newborn screening.

#### *L-07. Newborn Screening for Remethylation Disorders*

Gwendolyn Gramer, Jürgen G. Okun and Georg F. Hoffmann

University Hospital Heidelberg, Centre for Paediatric and Adolescent Medicine, Division of Neuropaediatrics and Metabolic Medicine, Im Neuenheimer Feld 430, 69120 Heidelberg, Germany

**Background:** Newborn screening is a precondition for early diagnosis and successful treatment of remethylation disorders. The German newborn screening panel does not currently include remethylation disorders. Recent improvements in diagnostic and therapeutic options suggest an extension of the newborn screening panel.

**Patients/Methods/Results:** On the basis of newborn screening for classical homocystinuria, using total homocysteine measurement in dried blood spots, we developed and evaluated a new optimized newborn screening strategy for remethylation disorders for newborns in Qatar, using total homocysteine measurement as a first-tier and methionine, ratio methionine/phenylalanine and propionylcarnitine as second-tiers. Proposed cut-offs were also retrospectively evaluated in newborn screening samples of patients with remethylation disorders and vitamin B12 deficiency from Qatar and Germany. An adapted strategy for Germany is proposed using homocysteine determination as a second-tier test. From summer 2016, the Heidelberg newborn screening center will be performing a pilot study to evaluate newborn screening for 21 additional metabolic disorders for the German newborn screening panel, including remethylation disorders, classical homocystinuria, and vitamin B12 deficiency. Whether the extension of the newborn screening panel fulfills the criteria for a population based screening program, especially concerning technical feasibility, process quality, and medical benefit will be prospectively evaluated. As an example, the clinical histories of two patients from Germany, diagnosed symptomatically with one of the new target disorders (combined cobalamin deficiency and severe maternal vitamin B12 deficiency), are presented and their newborn screening results are retrospectively evaluated.

**Conclusions:** We expect that a considerable number of children will benefit from screening for additional target disorders, including remethylation disorders, in the course of the pilot study and in the case of a future comprehensive extension of the newborn screening panel for Germany.

#### *L-08. Diagnosing Hepatorenal Tyrosinaemia: Newborn Mass Screening versus Selective Screening*

Anibh M. Das

Department of Paediatrics and Centre for Rare Diseases, Hannover Medical School, Carl Neuberg Str. 1, D-30625 Hannover, Germany; Tel.: +49-511-532-3220; das.anibh@mh-hannover.de

Hepatorenal tyrosinaemia (HT1) is a serious condition that used to be fatal before the advent of nitisinone (NTBC, Orfadin<sup>®</sup>) as a therapeutic option. There are still “unmet needs” regarding, diagnostics, clinical course, treatment, monitoring, and outcome, as identified in a recent retrospective multicenter, multinational cross-sectional study by our group (Mayorandan, S. et al. *Orphanet. J. Rare Dis.* 2014, 9, 107).

We have shown that selective screening is inadequate as initial clinical symptoms are often uncharacteristic, which leads to a considerable delay in diagnosis and treatment. There is a clinically latent phase in many patients. This delay in treatment has a negative impact on morbidity and mortality, as well as long-term outcome. For example, the odds ratio to develop hepatocellular carcinoma is 12.7 when treatment is initiated after the 1<sup>st</sup> birthday, compared to start of treatment in the neonatal period. Timely diagnosis is only possible when neonatal mass screening is operational. HT1 meets all the criteria for neonatal mass screening at a clinical and analytical level.

The natural course of the disease is well known, clinically there is a latent phase in most patients when pre-symptomatic treatment can be initiated. There are no mild phenotypes which do not require treatment. Using succinylacetone as the screening parameter, a highly specific and sensitive test is available with acceptable financial burden. Neonatal mass screening for HT1 is acceptable to the target population, as it can be performed simultaneously with the already existing screening tests in dried blood; there are no false negative and false positive cases and the financial burden to the health system is moderate. An efficient treatment is available with nitisinone and protein-reduced diet supplemented with special amino acid mixtures.

Despite compelling evidence in favor of a neonatal mass screening for HT1, only 57% of European centers taking part in our recent cross-sectional study have included HT1 in their newborn screening programs. In Germany, neonatal mass screening for HT1 is currently in the process of evaluation by regulatory bodies.

#### *L-09. Massive Parallel Sequencing and Newborn Screening*

Johannes Zschocke

Division of Human Genetics, Medical University Innsbruck, Innsbruck, Austria

Newborn screening may be regarded as a predictive genetic test for the pre-symptomatic diagnosis of treatable inherited diseases. Massive parallel (next generation) sequencing methods allow the analysis of many genes, or the whole genome, at relatively little cost and will also have an increasing relevance in predictive settings, such as newborn screening. The major challenge of this approach is the correct interpretation of genetic variants. In principle, it is possible to identify coding variants in all genes; however, the clinical relevance of the majority of human genes is poorly understood, and the functional impact of identified variants may be difficult to determine. In line with established screening criteria, it is necessary to restrict analyses to specific disease genes for which the genotype-phenotype correlation is well understood, the natural disease course is known, and the balance between benefits and harms of the test has been favourably assessed. There are plenty of examples from the past and present, where these criteria have not been fulfilled in newborn screening programs. Even for diseases with well-established screening indications, the clinical impact of attenuated forms or rare and novel genotypes may not be known. For some genes, it may be prudent restrict the analyses to known, well-characterized mutations. The choice of method is based on technical and cost considerations rather than analytical necessities, and includes panel, exome, and genome sequencing strategies. Major challenges include empowerment of the (parents of) tested individuals to decide which disease genes are analyzed, correct bioinformatics assessment and interpretation of identified variants, adequate transfer of the genetic results into clinical practice, and adequate counselling structures for patient families.

### **3. Oral Presentations**

#### *O-01. High Resolution Accurate Mass Spectrometry Enables Simultaneous Analysis of Amino Acids, Acylcarnitines and Organic Acids from Dried Blood Spots*

Thomas F. Metz, Mariam Pircher-Sanou, Dorothea Möslinger, Vassiliki Konstantopoulou, Susanne Greber-Platzer and Maximilian Zeyda

Austria Newborn Screening, Department of Pediatrics and Adolescent Medicine, Medical University of Vienna

**Introduction:** Determination of organic acids (OA) from plasma and urine is a common method for confirmation or differential diagnosis for children found positive for various inborn errors of metabolism in newborn screening. Mass spectrometers as typically used for newborn screening are not capable of OA analysis directly from dried blood spots (DBS). The aim of this study was to set up a method, which is suitable to simultaneously measure amino acids, acyl carnitines, as well as OA, and to evaluate its applicability for screening for organic acidemias and disorders of fatty acid oxidation.

**Method:** Using a heated electrospray ionization high resolution mass spectrometer a quantitative screening panel was created, which in addition to the established amino acid and acyl carnitine profile includes 28 OA. Amino acid and acylcarnitines could be measured with satisfying intra- and inter-day variance in 578 samples of healthy newborns and proved to be satisfactory for our screening laboratory. DBS from 41 affected children (12 propionic acidemia, four methylmalonic acidemia, eight Very Long- und eight Medium-Chain Acyl-CoA Dehydrogenase-Deficient (VLCADD and MCADD), 11 Long-Chain 3-Hydroxy Acyl-CoA Dehydrogenase-Deficient (LCHADD), and six Glutaric acidemia type I (GA I)) were compared to DBS from healthy newborns.

**Result:** We could show that the additional measurement of propionylglycine und methylmalonic acid is suitable to confirm elevated propionylcarnitine results in the same sample. Additionally, it was possible to distinguish between propionic acidemia and methylmalonic acidemia. We observed several OA concentrations in DBS from patients affected with VLCADD, MCADD and LCHADD to significantly differ to those from healthy controls. Furthermore, the ratio of 3-OH-glutaric acid to glutaconic acid was highly significantly altered in DBS from GA1 patients compared to healthy controls.

**Conclusions:** We showed that it is possible to determine OA from the first DBS within a normal newborn screening routine. This could lead to a reduction of false positive findings, in particular for the relatively unreliable propionylcarnitine. This method could also provide a direct differential diagnosis between propionic acidemia and methylmalonic acidemia.

#### *O-02. Newborn Screening for Sickle Cell Disease by FIA-MS/MS*

Jeannette Klein <sup>1</sup>, Annemarie Brose <sup>1</sup>, Claudia Frömmel <sup>2</sup>, Oliver Blankenstein <sup>1</sup> and Stephan Lobitz <sup>3</sup>

<sup>1</sup> Newborn Screening Laboratory, Charité—Universitätsmedizin Berlin, Berlin, Germany

<sup>2</sup> Labor Berlin GmbH, FB Labormedizin, Berlin, Germany

<sup>3</sup> Department of Pediatric Oncology/Hematology/BMT, Charité—Universitätsmedizin Berlin, Berlin, Germany

Sickle cell disease (SCD) is an inborn error of hemoglobin synthesis characterized by the formation of the Hb variant S (HbS) instead of normal Hb (HbA). Production of HbS in combination with a lack of HbA is, therefore, pathognomonic for SCD. Techniques to detect Hb variants, such as HPLC, isoelectric focusing, or capillary electrophoresis, are well established, and are adapted to the needs of a neonatal screening lab.

However, the implementation of these methods into existing programs requires additional technical equipment and expertise. Moreover, chromatograms or spherograms have to be manually revised by a technician, and heterozygous states and clinically silent variants are detected and reported by the instrument. Tandem mass spectrometry with flow injection (FIA-MS/MS) is a fast and specific method being used for screening of inherited metabolic diseases for about 20 years. Recently, the application of MS/MS for SCD screening with automated analysis and reporting has been presented, excluding the detection of heterozygous states (Moat 2014).

Our protocol evaluating MS/MS was adopted from Daniel et al. (2005). Tryptic digestion of the globin chains leads to a series of well-characterized peptides with known amino acid sequences. Their doubly-charged ions, formed under ESI+ conditions, have m/z ratios of between 200 and 1000 amu and are well suited for analysis with standard triple quad mass spectrometers. Since the characteristic

peptides of HbA and Hb variants differ by an altered amino acid sequence the resulting mass shifts allow a highly specific detection of Hb variants.

Up to now, 10,000 samples have been analyzed in parallel with capillary electrophoresis (Perkin Elmer/Sebia), resulting in the detection of two HbSS, and one HbSC affected newborns.

Even by simple visual inspection of raw intensity data, their profiles are clearly distinguished from normal and carrier samples. Thus, detection of carriers can be prevented by setting appropriate cut-offs. No false positives/false negatives were observed with MS/MS.

FIA-MS/MS shows promising results with regard to the rapid and specific detection of SCD in the context of newborn screening and the requirements in Germany not to report clinically insignificant results for children.

1. Moat, S.J.; Rees, D.; King, L.; Ifederu, A.; Harvey, K.; Hall, K.; Lloyd, G.; Morrell, C.; Hillier, S. Newborn blood spot screening for sickle cell disease by using tandem mass spectrometry: Implementation of a protocol to identify only the disease states of sickle cell disease. *Clin. Chem.* **2014**, *60*, 373–380.
2. Rapid and specific detection of clinically significant haemoglobinopathies using electrospray mass spectrometry–mass spectrometry. Daniel, Y.A.; Turner, C.; Haynes, R.M.; Hunt, B.J.; Dalton, R.N. *Br. J. Haematol.* **2005**, *130*, 635–643.

#### O-03. A Novel Method for Inclusion of all Urea Cycle Disorders into Newborn Screening

Ralph Fingerhut<sup>1,2,\*</sup>, Susanna Sluka<sup>1,2</sup>, Johannes Häberle<sup>2,3</sup>, Mia Halme<sup>4</sup> and Géraldine Carrard<sup>4</sup>

<sup>1</sup> Swiss Newborn Screening Laboratory, University Children's Hospital, Zurich, Switzerland

<sup>2</sup> Children's Research Center, Zurich, Switzerland

<sup>3</sup> Division of Metabolism, University Children's Hospital, Zurich, Switzerland

<sup>4</sup> Labsystems Diagnostics Oy, Vantaa, Finland

\* Correspondence: [Ralph.Fingerhut@kispi.uzh.ch](mailto:Ralph.Fingerhut@kispi.uzh.ch); Tel.: +41-44-266-7732; Fax: +41-44-266-8110

The inclusion of urea cycle disorders (UCD) detection into newborn screening (NBS) is highly desirable; however, it is hampered by the lack of a specific marker for most of these disorders (exceptions to this are citrulline and argininosuccinate for detection of citrullinemia and argininosuccinic aciduria, respectively). Thus far, the common feature of all (proximal- and distal-) UCDs, hyperammonemia, is not directly detectable in dried blood spots (DBS). The quantification of secondary elevations of glutamine seemed, thus far, not feasible, based on the assumption of the instability of glutamine in DBS. We describe here a reliable method for the simultaneous detection of lysine and glutamine from DBS in multiple reaction monitoring (MRM) with a second-tier UPLC-method for the separation and specific quantification of glutamine. We combined this newly developed method with the measurement of all specific amino acids (arginine, argininosuccinic acid, citrulline, ornithine, and proline), *N*-acetyl-glutamate, and orotic acid. This combination proved to be a reliable and sensitive method for the detection of all UCDs using tandem-mass spectrometry NBS. The next step will be a prospective study with dried blood samples from patients with hyperammonemia, allowing further testing and evaluation of the method in practice.

#### 4. Poster Presentations

##### P-01. Upcoming Nationwide CF Implementation in Germany- Chances and Potential Pitfall

Theresa Winter<sup>1</sup>, Cornelia Müller<sup>1</sup>, Sebastian M. Schmidt<sup>2</sup> and Matthias Nauck<sup>1</sup>

<sup>1</sup> Institute for Clinical Chemistry and Laboratory Medicine, University Medicine Greifswald, Greifswald, Germany

<sup>2</sup> Clinic for Pediatrics, University Medicine Greifswald, Greifswald, Germany

**Objectives:** In August 2015, the G-BA voted for the nationwide implementation of the Cystic fibrosis newborn screening (CF-NBS) in Germany. Until its final realization in 2016, Mecklenburg-Vorpommern (MV) is the only German state, with an CF-NBS for all newborn in due course of an EU founded Interreg IVa project. The experience, gained since 2012 is a valuable pool of information concerning the optimization of processes in accordance to the proposed CF-NBS screening protocol and for analytical drawbacks.

**Methods:** The nationwide proposed CF-NBS protocol is three staged. The first two steps are conventional laboratory analysis of (1) immunoreactive trypsinogen (IRT) and of (2) pancreatitis associated protein (PAP). As a third step, a molecular genetic analysis follows. CF-NBS will be positive in case of an elevated IRT > 99.9<sup>th</sup> percentile or with at least one mutated CFTR-gene. In contrast, a two-step strategy was used since 2012 in MV—including the analysis of IRT and PAP.

**Results:** Despite the missing molecular analysis, a profound data base with more than 55,000 screened newborns was established. The acceptance of the CF-NBS reached soon after its introduction more the 95%. The mean time between positive CF-NBS was significantly reduced to 33 days in 2015—ranging now in European cystic fibrosis society standards of care recommendations. Furthermore, the applied cut-offs for IRT and PAP detected all seven positive CF-NBS newborns.

**Conclusions:** The challenges for nationwide implementation are, nevertheless, diverse. The inclusion of the third analytical step, the resulting tight time schedule until the sweat test and the safe use of the altered IRT cut-off to reduce the false positives will be challenging. Furthermore, reimbursement concerning the sweat tests, the additional laboratory analytics, and the tracking, have not yet been solved.

#### *P-02. Dried Blood Spot Contamination—An Underestimate Risk in Newborn Screening*

Cornelia Müller<sup>1</sup>, Theresa Winter<sup>1</sup>, Anja Lange<sup>2</sup> and Matthias Nauck<sup>1</sup>

<sup>1</sup> Institut for Clinical Chemistry and Laboratory Medicine, University Medicine Greifswald, Germany

<sup>2</sup> Hospital for Paediatric and Adolescent Medicine, University Medicine Greifswald, Germany

**Objectives:** Filter paper with dried blood is the standard specimen used for newborn screening all around the world. Convenient transportation via regular mail and the reasonable stability of metabolites of interest support its use. The heel pick procedure, as the method of sample acquisition, is well standardized. The use of native blood without anticoagulants (e.g., EDTA) is required since they are known to interfere with screening laboratory methods.

However, other invisible contamination of the filter paper, prior, during, or after sample collection are often not visually detectable and do have, nevertheless, a significant influence on the screening results. In order to emphasize the correct pre-analytical phase within newborn screening, eight different contamination sources, which are present in our neonatal ward, were evaluated in terms of their impact on the screening results.

**Methods:** Capillary blood was obtained from 10 volunteers and applied to screening filter papers. Afterwards, separate spots were intentionally contaminated with either (1) disinfectant; (2) feces; (3) urine; (4) baby cream; (5) baby food; (6) ultrasonic gel; (7) breast milk; and (8) baby wipes. One spot was not contaminated for control purposes.

TSH, 17-OHP, GALT, Biotinidase, IRT, amino acids and acylcarnitines were analyzed.

**Results:** All contaminants affected in some way the analysis. Most pronounced were the false positive effects of feces on IRT, of urine on the Glutaric acidemia type I analyte C5DC and baby cream on GALT activity.

**Conclusions:** Mishandling of filter papers in the pre-analytical phase is a source of false positive and false negative screening results and underlines the importance of regular training of the medical staff involved in the screening process.

*P-03. Why to Choose a CF Reference Center for Sweat Testing in NBS Positive Babies?*

Jutta Bend <sup>1</sup>; Project group Newborn Screening

<sup>1</sup> Mukoviszidose e.V., Bonn

Project group Newborn Screening:

Prof. Dr. med. Manfred Ballmann, Prof. Dr. med. Helmut Ellemunter, Dr. med. Jutta Hammermann, Dr. med. Hans-Eberhard Heuer, Dr. med. Lutz Nährlich, PD Dr. med. Thomas Nüßlein, PD Dr. med. Sebastian Schmidt, Dr. Ulrike Smrekar, PD Dr. med. Olaf Sommerburg

Cystic fibrosis (CF) is one of the most common life-shortening inherited diseases. In Germany, about 8000 people are affected. This gene defect results in the production of thick mucus in the internal organs, causing inflammation, frequent lung infections, and digestive problems.

According to the German CF Registry, the median age of diagnosis is currently around 0.6 years and medium survival is predicted to be 40 years, although patients are still dying with 31.5 years on average.\* The Joint Federal Committee (G-BA) has decided to start a Newborn Screening (NBS) Program for Cystic fibrosis in Germany. From several international and local projects and programs, it is known that newborn screening for CF enables early diagnosis followed by early treatment, which eventually leads to improved survival.

Gold standard for the diagnosis is the sweat test (Pilocarpin iontophorese), which is also used as a confirmation test for screening positive babies. In patients with CF, movement of salt and water in and out the cells is defective. This leads to a higher concentration of chloride in the sweat, which can be measured safely and reliably with the above-mentioned test. Sweat test can be performed from the 3<sup>rd</sup> day of life, optimally from 14<sup>th</sup> day of life, in newborns with weight above 3000 g and age of  $\geq 36^{\text{th}}$  week of gestation. A German guideline defines the requirements for a high-quality sweat test \*\*: The performing site has to be experienced with the test, i.e., at least 50 sweat tests should be performed per year.

The German cystic fibrosis association, Mukoviszidose e.V., has established a certification system, which is accredited by the scientific societies GPP (Gesellschaft für Pädiatrische Pneumologie) and DGP (Deutsche Gesellschaft für Pneumologie). Certified CF centers conduct sweat tests according to the guideline. There are also well established procedures to handle a screening positive baby, including timing of sweat test, communication of the test result, and if CF is diagnosed, starting a state of the art treatment. The certified sites are additionally very committed to the CF newborn screening program and will forward the sweat test results to the screening laboratories where data for screening reports will be collected and evaluated. Thus, it is crucial for the success of this new newborn screening program that parents choose to go to a certified CF reference center for confirmation diagnostics via sweat test.

Regularly-updated address listings of certified CF centers can be received by Mukoviszidose e.V.

\* Qualitätsbericht 2012 des deutschen Mukoviszidose Registers/Mukoviszidose e.V.

\*\* AWMF-Leitlinie: Diagnose bei Mukoviszidose: <http://www.awmf.org/leitlinien/detail/ll/026-023.html>

*P-04. Benefit Assessment of Newborn Screening for Tyrosinaemia Type I—A Systematic Review*

Lina Rodenhäuser <sup>1</sup>, Michael Borte <sup>2</sup>, Ulrike Lampert <sup>1</sup>, Tim Mathes <sup>3</sup>, Anne Rummer <sup>1</sup> and Stefan Sauerland <sup>1</sup>

<sup>1</sup> Institute for Quality and Efficiency in Health Care (IQWiG)

<sup>2</sup> Klinik für Kinder- und Jugendmedizin, Klinikum Sankt Georg, Leipzig

<sup>3</sup> Institut für Forschung in der Operativen Medizin (IFOM), Universität Witten/Herdecke

Background: Tyrosinaemia type I is a rare hereditary metabolic disease. There are different tests to diagnose tyrosinaemia type I in newborn screening: The conventional approach is to measure tyrosine; however, this is known to produce false results. Another approach is to measure

succinylacetone in dried blood spots. Both approaches are commonly used in screening programs. The German Federal Joint Committee (G-BA) is considering including tyrosinaemia type I in an expanded newborn screening program and commissioned IQWiG to assess the benefits of newborn screening for tyrosinaemia type I versus no screening.

**Methods:** IQWiG is conducting a systematic literature review in MEDLINE, Embase, and Cochrane. The primary aim is to identify intervention studies on the whole screening chain. The investigated screening test consists of using dried blood spots and measuring succinylacetone levels via tandem mass spectrometry. Ideally, prospective comparative intervention studies can be identified, where the participants are randomly allocated to a strategy, with or without application of the screening test (randomized controlled trials, RCTs). If no RCTs are identified, studies of a lower evidence level, such as non-randomized comparative studies or comparative cohort studies (either with retrospective or historical comparators), are also included. Regardless of the type of intervention study, patient-relevant outcomes are analyzed, including mortality, morbidity, hospitalization, developmental disorders, adverse events, and health-related quality of life. If evidence from the above studies is insufficient, assessment of single components of the screening chain is considered. Therefore, intervention studies comparing an earlier and later start of therapy, as well as test accuracy studies, are needed. Because of the rarity of the disease and the assumption that newborn screening shows a dramatic effect, retrospective comparative cohort studies and historically controlled studies are included as intervention studies. In test accuracy studies, positive test results have to be confirmed by genetic analysis and follow-up for negative test results has to be reported. Otherwise—in the case of verification of only positive testers—no information on sensitivity and specificity can be given. If feasible and useful, single study results are summarized in meta-analyses.

**Results:** Preliminary results of the benefit assessment are expected to be published in summer 2016.

*P-05. A Simple Method to Overcome the “Floating Disc Problem” Using the GALT-Assay on the PerkinElmer GSP*

Ralph Fingerhut<sup>1,2,\*</sup> and Susanna Sluka<sup>1,2</sup>

<sup>1</sup> Swiss Newborn Screening Laboratory, University Children’s Hospital, Zurich, Switzerland

<sup>2</sup> Children’s Research Center, Zurich, Switzerland

\* Correspondence: Ralph.Fingerhut@kispi.uzh.ch; Tel.: +41-44-266-7732; Fax: +41-44-266-8110

The Perkin Elmer Genetic Screening Processor (GSP)<sup>TM</sup> is a fully automated system for the processing of immunoassays for TSH, 17-OHP, IRT, biotinidase, and total T4, as well as enzymatic assays for total galactose and galactose-1-phosphate uridyltransferase (GALT) from dried blood spots (DBS). The system, however, has one draw back: It can not transfer samples from one microtiter plate to another one. While this is not a problem with immunoassays, it makes enzymatic assays more problematic, because dried blot spots have to remain in the wells and can cause significant signal quenching, thereby reducing sensitivity, or they can increase fluorescence intensity, when the extracted spots are floating on the surface of the reacting mixture. The latter can cause false negative results, when GALT activity is measured for galactosaemia screening. To overcome this problem, GSP includes an additional measurement step to check for floating disks, leading to prevention of the affected measurements. However, this causes a secondary problem in this totally closed system. We detected floating disk signals in approx. 0.5% of all screening, as well as quality control samples, which had to be repeated. In some cases even a second sample had to be requested, because floating disks were detected again in the 2<sup>nd</sup>, 3<sup>rd</sup>, and 4<sup>th</sup> repeat assays. To overcome this problem, we developed a method of employing a second-tier measurement on a victor fluorescence reader. Using this second-tier measurement made all repeat measurements unnecessary.

*P-06. Interim Results of a Pilot Newborn Screening Program in Nepal*

Ralph Fingerhut<sup>1,2,\*</sup> and Arti Sharma Pandey<sup>3</sup>

<sup>1</sup> Swiss Newborn Screening Laboratory, University Children's Hospital, Zurich, Switzerland

<sup>2</sup> Children's Research Center, Zurich, Switzerland

<sup>3</sup> Kathmandu Medical College and Teaching Hospital, Kathmandu, Nepal

\* Correspondence: Ralph.Fingerhut@kispi.uzh.ch; Tel.: +41-44-266-7732; Fax: +41-44-266-8110

Over the last few years, there were numerous countries that celebrated 50 years of newborn screening (NBS), and, within the next few years, more will follow. The International Society for Neonatal Screening (ISNS) will also celebrate at the conference in The Hague: "ISNS silver jubilee—25 years of sharing knowledge globally". Additionally, while Switzerland celebrated its 50 years NBS anniversary, the Kathmandu Medical College and Teaching Hospital and the Children's Hospital in Zurich started their collaboration with a pilot NBS program for Nepal. This program is supported Nepal Health Research Council (NHRC), and will be comprised of 5000 dried blood samples from Nepal in the first phase. We can present the interim results of the first year, from March 2015 to March 2016, of this pilot study. Until now, 3786 samples have been tested. Within this cohort, we could detect one case with congenital hypothyroidism and one case with Cystic fibrosis. At the moment, we are planning to extend the pilot to a total number of 10,000 samples, and explore the possibility to further transfer knowledge and methodology to Nepal.

*P-07. Assessing Performances and Usability of the NS2400 Automated Platform from LabSystems Diagnostics*

Ralph Fingerhut <sup>1,2,\*</sup>, Susanna Sluka <sup>1,2</sup>, Lotta Virtanen <sup>3</sup> and Géraldine Carrard <sup>3</sup>

<sup>1</sup> Swiss Newborn Screening Laboratory, University Children's Hospital, Zurich, Switzerland

<sup>2</sup> Children's Research Center, Zurich, Switzerland

<sup>3</sup> LabSystems Diagnostics Oy, Vantaa, Finland

\* Correspondence: Ralph.Fingerhut@kispi.uzh.ch; Tel.: +41-44-266-7732; Fax: +41-44-266-8110

Newborn screening, in most countries, is highly centralized, with daily sample loads of 300 to over 2000 in a few cases. Therefore, totally automated systems are highly desirable. The main advantage of these systems is less hands-on-time for laboratory personnel, better traceability of the working steps, and less danger of mistakes and false screening results. The NS2400 from LabSystems Diagnostics combines total automation by replacing exactly the technician processed without altering the product design. The same kit reagents can be used manually and/or be integrated in the NS2400 platform, ensuring continuity of measurement in the case of downtime. The automate's modules can also be used as standalone, providing further flexibility of use. We tested the system thoroughly with all, thus far, available tests (TSH, 17-OHP, IRT, Biotinidase, total Galactose, G-6-PDH, and PKU) and comparisons with routine tests are reported.

*P-08. MCAD Deficiency with Severe Neonatal Onset, Fatal Outcome, and Normal Acylcarnitine Profile*

Ralph Fingerhut <sup>1,2,\*</sup>, Pascal Joset <sup>3</sup>, Susanna Sluka <sup>1,2</sup>, Theresia Herget <sup>3</sup>, Silvia Miranda Azzarello-Burri <sup>3</sup>, Anita Rauch <sup>3</sup> and Matthias Baumgartner <sup>2,4</sup>

<sup>1</sup> Swiss Newborn Screening Laboratory, University Children's Hospital, Zurich, Switzerland

<sup>2</sup> Children's Research Center, Zurich, Switzerland

<sup>3</sup> University of Zurich, Institute for Medical Genetics, Zurich, Switzerland

<sup>4</sup> Division of Metabolism, University Children's Hospital, Zurich, Switzerland

\* Correspondence: Ralph.Fingerhut@kispi.uzh.ch; Tel.: +41-44-266-7732; Fax: +41-44-266-8110

Medium-chain acyl-CoA dehydrogenase deficiency (MCADD) is an autosomal recessively-inherited disorder of fatty acid oxidation with potentially fatal outcomes in undiagnosed patients. Introduction of tandem mass spectrometry into newborn screening (NBS) has led to the inclusion of MCADD into NBS in many countries, which has resulted in a significant reduction of morbidity and mortality. We report a child with MCADD presenting neonatally with apnoea and heart arrest. Despite intensive efforts to rescue the child, including reanimation for 90 minutes, the child died at the 2<sup>nd</sup>

day of life. Autopsy revealed fatty liver and also fat storage in heart muscle, which was suggestive for a fatty acid oxidation defect. However, acylcarnitines determined from stored EDTA blood were not suggestive for MCAD deficiency. Nevertheless, a subsequent whole exome sequencing analysis revealed homozygosity for the ACADM gene c.1084A>G/p.Lys362Glu mutation.

#### *P-09. Classical PKU with Unusual Neonatal Presentation*

Ralph Fingerhut <sup>1,2,\*</sup>, Nina Lehnherr <sup>3</sup>, Viktoria Pfeifle <sup>3</sup>, Stefan Holland-Cunz <sup>3</sup>, Susanna Sluka <sup>1,2</sup>, Gabor Szinnai <sup>3</sup>, Martina Huemer <sup>2,3,4</sup> and Marianne Rohrbach <sup>2,4</sup>

<sup>1</sup> Swiss Newborn Screening Laboratory, University Children's Hospital, Zurich, Switzerland

<sup>2</sup> Children's Research Center, Zurich, Switzerland

<sup>3</sup> University Children's Hospital, Basel, Switzerland

<sup>4</sup> Division of Metabolism, University Children's Hospital, Zurich, Switzerland

\* Correspondence: Ralph.Fingerhut@kispi.uzh.ch; Tel.: +41-44-266-7732; Fax: +41-44-266-8110

Newborn screening (NBS) for phenylketonuria (PKU) started in Switzerland in 1965. Since 2005, NBS for PKU, using tandem mass spectrometry, has been performed at the University Children's Hospital in Zurich. Phenylalanine (Phe) and the phenylalanine/tyrosine (Phe/Tyr) ratio are sensitive and specific tests to detect phenylketonuria (PKU). The main clinical features of untreated PKU are developmental delay and intellectual disability, which become evident from the first weeks of life.

We describe a boy born at term to non-consanguineous Swiss parents with tetrahydrobiopterine (BH<sub>4</sub>) sensitive PKU with unusual neonatal presentation. The child presented with floppiness, irritability, recurrent bilious vomiting and failure to pass meconium until 32 h after birth, resulting in the clinical suspicion of an intoxication type metabolic disease, such as maple syrup urine disease (MSUD) rather than PKU. Ketonuria or hyperammonaemia were not observed.

Newborn screening performed on a dried blood sample from the 4<sup>th</sup> day of life revealed elevated Phe of 650 µmol/L, low Tyr of 30 µmol/L, and a Phe/Tyr ratio of 22. However, slightly elevated branched-chain amino acids (leucine/isoleucine 306 µmol/L; valine 299 µmol/L) initially supported the clinical suspicion of MSUD, but alloisoleucine was not detectable. Morbus Hirschsprung was suspected due to dilated intestinal loops and lack of intestinal gas in the anorectal region. On day 8, branched-chain amino acids had normalised; A BH<sub>4</sub>-test resulted in a significant decrease of Phe from 1011 to 437 µmol/L within 24 h with normal pterin measured in urine; supporting the diagnosis of BH<sub>4</sub> sensitive PKU. Dietary restriction of Phe was initiated immediately. However, oral feeding turned out difficult due to the intestinal motility disorder.

#### **Conclusions**

The poster prize of the DGNS was awarded to Lina Rodenhäuser from the Institute for Quality and Efficiency in Health Care (IQWiG) for her poster entitled "Benefit assessment of newborn screening for tyrosinaemia type I—a systematic review".

In 2017, the Annual Meeting of the DGNS will be held in Hamburg, Germany.



© 2016 by the authors; licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC-BY) license (<http://creativecommons.org/licenses/by/4.0/>).