General information

Venue
The symposium takes place at the:
**Rome Marriott Park Hotel**
Via Colonnello Tommaso Masala, 54
00148 Rome, Italy

Language
The official language of this symposium is English.

Scientific secretariat
Serono Symposia International Foundation
Salita di San Nicola da Tolentino, 1/b
00187 Rome, Italy
Senior Project Manager: Flaminia Masprone
Tel.: +39-06-420 413 206
Fax: +39-06-420 413 677
E-mail: flaminia.masprone@seronosymposia.org
Associate Project Manager: Dorina Monaco
Tel.: +39-06-420 413 314
Fax: +39-06-420 413 677
E-mail: dorina.monaco@seronosymposia.org
Serono Symposia International Foundation
is a Swiss Foundation with headquarters in
14, rue du Rhône, 1204 Geneva, Switzerland

Organizing secretariat
Meridiano Congress International
Via Mentana, 2/B | 00185 Rome, Italy
Congress coordinator: Sara Guglielmini
Tel.: +39 06 88595 211 | Fax: +39 06 88595 234
E-mail: s.guglielmini@meridiano.it
Aim of the symposium
While dietary restriction of phenylalanine still remains the mainstay of treatment, phenylketonuria (PKU) is an active area of research, and new treatment options are emerging that may reduce the burden of a difficult and limiting diet in these patients and in their families. Basic and clinical research is now focusing on the mechanisms of action of new treatment options, like sapropterin dihydrochloride (also BH₄), on monoamine neurotransmitters metabolism in the brain of PKU patients. Furthermore there’s an emerging need for evidence-based international guidelines to provide standardization in treatment initiation, in evaluation of the response to therapy with sapropterin dihydrochloride, and in blood phenylalanine concentration to target. A number of papers resulting from last year’s symposium reviewed the existing scientific evidences in several aspects of PKU, identifying the current positions in its management. The European Phenylketonuria Group (EPG) and the Serono Symposia International Foundation (SSIF) consider essential to continue their series of successful meetings on PKU, and have organized the 2012 Annual Symposium dedicated to that. The aims of this meeting are to review the most important outcomes of research in the field of PKU and to provide a forum for participants to discuss solutions that can optimize patient management in clinical practice.

Learning objectives
After attending this symposium, the participants will have up-to-date knowledge about:
- Brain pathology of PKU
- Blood-brain barrier and PKU
- CNS neurotransmitters in PKU
- Management of BH₄ deficiency
- Social and financial management of PKU
- Amino acid status in PKU

Target audience
Specialists in pediatric and adult inherited metabolic diseases, dietitians, nutritionists, clinical biochemists, experts in genetics, basic scientists, and professionals within the area of public health will benefit from this symposium.

Accreditation
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The conference 4th European Phenylketonuria Group (EPG) Symposium “Advances and Challenges in PKU” (March 23-24, 2012 - Rome, Italy) is designated for a maximum of 8 (eight) hours of European CME credits (ECMEC). Each medical specialist should claim only those credits that he/she actually spent in the educational activity. EACCME® credits are recognized by the American Medical Association (AMA) towards the Physician’s Recognition Award (PRA). To convert EACCME® credit to AMA PRA category 1 credit, please contact the AMA.

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• **Post-event**: three weeks after the event we will email you a short questionnaire which will give you the opportunity to tell us how much of what you learned has had an affect on your know-how and daily practice.
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Scientific organizer

Nenad Blau
Division of Inborn Metabolic Diseases
University Children’s Hospital
Department of General Pediatrics
Heidelberg, Germany

Scientific committee

Amaya Bélanger-Quintana
Unidad de Enfermedades Metabólicas
Servicio de Pediatría
Hospital Ramón y Cajal
Madrid, Spain

Nenad Blau
Division of Inborn Metabolic Diseases
University Children’s Hospital
Department of General Pediatrics
Heidelberg, Germany

Alberto Burlina
Division of Metabolic diseases
Department of Pediatrics
University Hospital Padua
Padua, Italy

Mübeccel Demirkol
Children’s Hospital
Division Nutrition and Metabolism
Faculty of Medicine Istanbul University
Istanbul, Turkey

François Feillet
Centre de Référence des
Maladies Héréditaires du Métabolisme
Hôpital d’Enfants, CHU Brabois
Vandoeuvre les Nancy, France

Anita MacDonald
Dietetics Department
Birmingham Children’s Hospital
Birmingham, UK

Ania C. Muntau
Departments of Inborn Errors of
Metabolism and of Molecular Pediatrics
Dr. von Hauner Children’s Hospital
Ludwig-Maximilians-University
Munich, Germany

Friedrich K. Trefz
Medical Centre Gammertingen
Kreiskliniken Reutlingen GmbH
Gammertingen, Germany

Francjan J. van Spronsen
Beatrix Children’s Hospital
University Medical Center of Groningen (UMCG)
Groningen, The Netherlands
List of Faculty members

Laurie Bernstein  
Clinical Genetics and Metabolism  
Children’s Hospital Colorado  
Aurora (CO), USA

Nenad Blau  
Division of Inborn Metabolic Diseases  
University Children’s Hospital  
Department of General Pediatrics  
Heidelberg, Germany

Alberto Burlina  
Division of Metabolic diseases  
Department of Pediatrics  
University Hospital Padua  
Padua, Italy

Maureen Clearly  
Metabolic Office  
Great Ormond Street Hospital NHS Trust  
London, UK

Mübeccel Demirkol  
Children’s Hospital  
Division Nutrition and Metabolism  
Faculty of Medicine Istanbul University  
Istanbul, Turkey

François Feillet  
Centre de Référence des Maladies Héréditaires du Métabolisme  
Hôpital d’Enfants, CHU Brabois  
Vandoeuvre les Nancy, France

Sören W. Gersting  
Departments of Molecular Pediatrics  
Dr. von Hauner Children’s Hospital  
Ludwig-Maximilians-University  
Munich, Germany

Marcello Giovannini  
Department of Pediatrics  
San Paolo Hospital  
University of Milan  
Milan, Italy

Berthold V. Koletzko  
Div. Metabolic and Nutritional Medicine  
Dr. von Hauner Children’s Hospital  
Munich, Germany

François Labarthe  
Médecine Pédiatrique  
Hôpital Clocheville, CHRU  
Tours, France

Vincenzo Leuzzi  
Paediatrics and Child Neurology and Psychiatry  
La Sapienza University of Rome  
Rome, Italy

Harvey L. Levy  
Medicine / Genetics  
Harvard Medical School  
Children’s Hospital Boston  
Boston (MA), USA

Anita MacDonald  
Dietetics Department  
Birmingham Children’s Hospital  
Birmingham, UK

Ania C. Muntau  
Departments of Inborn Errors of Metabolism and of Molecular Pediatrics  
Dr. von Hauner Children’s Hospital  
Ludwig-Maximilians-University  
Munich, Germany

Friedrich K. Trefz  
Medical Centre Gammertingen  
Kreiskliniken Reutlingen GmbH  
Gammertingen, Germany

Margreet van Rijn  
Section of Metabolic Diseases  
Beatrix Children’s Hospital  
University Medical Hospital Groningen  
Groningen, The Netherlands

Francjan J. van Sproonsen  
Beatrix Children’s Hospital  
University Medical Center of Groningen (UMCG)  
Groningen, The Netherlands
Scientific Program
March 23-24, 2012

Friday - March 23

8.40 Welcome to Rome
Vincenzo Leuzzi (Italy)

8.45 Welcome on behalf of EPG Group and SSIF
Nenad Blau (Germany)

Chair: Harvey L. Levy (USA)

9.00 PKU in Italy
Marcello Giovannini (Italy)

Session chairs: Ania C. Muntau (Germany) François Feillet (France)

9.30 L1: Mode of action of BH₄ beyond blood Phe control
Nenad Blau (Germany)

10.00 L2: Bioinformatic approaches in PKU research and implications for clinical management
Søren W. Gersting (Germany)

10.30 Discussion

10.45 Coffee break

Special lecture

Brain pathology and neurotransmitters metabolism in PKU

Session chairs: Ania C. Muntau (Germany) François Feillet (France)

9.30 L1: Mode of action of BH₄ beyond blood Phe control
Nenad Blau (Germany)

10.00 L2: Bioinformatic approaches in PKU research and implications for clinical management
Søren W. Gersting (Germany)

Session II Oral communications

Session chairs: Anita MacDonald [UK] Mübeccel Demirkol [Turkey]

11.15 Oral communications

OC1: Long-term BH₄ responsiveness, prediction with the 48-hour BH₄-loading test and genotype
Karen Anjema [The Netherlands]

OC2: GTP Cyclohydrolase I deficiency without hyperphenylalaninemia
Margarita Castro [Spain]

OC3: Molecular characterization and genotype/phenotype correlations in BH₄-responsive PKU
Caroline Heintz [Switzerland]

OC4: Clinical characterization of patients with early infantile onset of autosomal recessive GTP cyclohydrolase I deficiency without hyperphenylalaninemia
Thomas Opladen [Germany]

OC5: Influence of individual PAH mutations on sapropterin response in PKU patients: results of a single center study
Frank Rutsch (Germany)

OC6: Treatment of phenylketonuria (PKU) using minicircle-based naked-DNA gene transfer to murine liver
Hiu Man Viecelli [Switzerland]

OC7: Phenylketonuria: Evidence in favour of increased strictness in treatment during the first 12 years of life
Rianne Jahja [The Netherlands]

OC8: Phenylalanine concentrations versus Phenylalanine: Tyrosine ratios in predicting cognitive abilities of individuals with Phenylketonuria
Rianne Jahja [The Netherlands]

13.15 Lunch
Saturday - March 24

**Session III** Day to day experiences and treatment aspects in PKU

**Session chairs:** Francjan J. van Spronsen [Netherlands]
Alberto Burlina [Italy]

**8.30 L3:** Use of BH₄ in PKU patients below 4 years
François Labarthe [France]

**9.00 L4:** Diet and health in adult PKU: case observations
Berthold V. Koletzko [Germany]

**9.30 L5:** NIH PKU Conference report: State of the science and future research needs
Harvey L. Levy [USA]

**10.00 Discussion**

**10.15 Coffee break**

**Session IV** Workshops summaries

**Session chairs:** Maureen Clearly [UK]
Friedrich K. Trefz [Germany]

**10.40 Workshop summaries presentations**
Workshops chairmen

**Session V** Asbjørn Følling lecture and Award

**12.00 Introduction to Asbjørn Følling Lecture**
Nenad Blau [Germany]

**12.10 Asbjørn Følling Lecture**
Harvey L. Levy [USA]

**Session VI** SSIF PKU Award and PKU Academy fellowships

**Chair:** Ania C. Muntau [Germany]

**12.40 SSIF Award for the best oral communication**
**Chair:** Ania C. Muntau [Germany]

**12.50 PKU Academy fellowships presentation**
**Chair:** Francjan J. van Spronsen [Netherlands]

**13.00 Meeting closure**
Anita MacDonald [UK]
Francjan J. van Spronsen [Netherlands]

**13.10 Lunch**
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The following faculty provided information regarding significant commercial relationships and/or discussions of investigational or non-EMEA/FDA approved (off-label) uses of drugs:

Amaya Belanger-Quintana  Declared to be member of Merck Serono PKU European Scientific Advisory Board, European Nutritionist Expert Panel and Kamper Advisory Board.

Laurie Bernstein  Declared receipt of grants and contracts from Biomarin and Nutricia and of honoraria or consultation fees from Applied Nutrition and Nutricia.

Nenad Blau  Declared receipt of research grants from Merck Serono and Biomarin Pharmaceutical Inc. and to be member of Merck Serono SA PKU advisory board.

Alberto Burlina  Declared to be Kamper study Consultant.

Maureen Clearly  Declared to be member of the European PKU Group sponsored by Merck Serono.

Mübeccel Dermikol  Declared no potential conflict of interest.

François Feillet  Declared receipt of honoraria or consultation fees from Genzyme, Biomarin, Merck Serono and Nutricia, and to be member of Merck Serono and Nutricia advisory Board.

Søren W. Gersting  Declared receipt of grants and contracts from Merck Serono GmbH Darmstadt.

Bertold V. Koletzko  Declared receipt of research grants by European Commission in partnership with SHS Scientific Hospital Supplies.

François Labarthe  Declared receipt of honoraria or consultation fees as Member of the SPARK Steering Committee and that his presentation will include discussion on off-labeled use of BH₄.

Vincenzo Leuzzi  Declared no potential conflict of interest.

Harvey Levy  Declared receipt of grants and contracts from BioMarin Pharmaceuticals. He declared that his presentation will discuss current off-labeled uses of products.

Anita MacDonald  Declared receipt of research grants from Nutricia, Vitalfo, Cambrooke Foods and Merck, to be member of ENEP and Sapropterin Advisory Board, supported by Merck Serono and to be member of Nutricia ELEMENT (advisory board on IMD).

Ania C. Muntau  Declared receipt of grants and contracts, of honoraria or consultation fees from Merck Serono and to be member of a Merck Serono advisory board.

Friedrich K. Trefz  Declared receipt of honoraria or consultation fees from MerckSerono and to be member of the EPG and KAMPER advisory boards.

Margreet van Rijn  Declared receipt of grants and contract from Nutricia Netherlands, of honoraria or consultation fees from SHS Nutricia Global – Danone and Merck Serono and to be member of the European Nutritionist Expert Panel of Merck Serono.
Francjan J. Van Spronsen  Declared receipt of grants and contracts from Merck Serono, of honoraria or consultation fees from Merck Serono and Nutricia, to be member of Merck Serono and Nutricia advisory boards and participation in Merck Serono speaker’s bureau.

The following faculty have provided no information regarding significant relationship with commercial supporters and/or discussion of investigational or non-EMEA/FDA approved (off-label) uses of drugs as of March 12, 2012.

Marcello Giovannini
Lectures
Abstracts
Hyperphenylalaninemia (HPA) is an autosomal recessive inborn error of metabolism, caused by a mutated gene of the hepatic enzyme phenylalanine hydroxylase (PAH; OMIM 261600), which converts the amino acid phenylalanine (Phe) to other essential compounds in the body, such as tyrosine (Tyr).

To prevent mental retardation caused by elevated plasma Phe levels, early identification of affected subjects is mandatory. Neonatal screening for HPA is compulsory by law in Italy since 05/02/1992 but it has already been performed in Lombardy from 01/01/1977.

According to the latest Annual Technical Report (2010) compiled by the Italian Society for the Study of Hereditary Metabolic Diseases and Newborn Screening (SIMMESN), Italy’s incidence of newborns affected by any form of HPA is equal to 1:3,494 live births (1:8,681 affected by HPA forms requiring specific treatment), and in particular in three forms equal to:

- Type I = 1:17,905
- Type II = 1:17,362
- Type III = 1:5,907.

 Particularly in Lombardy, out of 98,690 live births submitted to neonatal screening in 2010, 31 patients were diagnosed, resulting in an overall incidence equal to 1:3,184, according to the Italian mean.

Our Clinical Department of Pediatrics, San Paolo Hospital, University of Milan, is Lombardy’s Regional Reference Center for the diagnosis and the treatment of patients affected by Hyperphenylalaninemia.

Up to the 1st of March 2012, the total number of patients diagnosed as affected by HPA at our Center is 660, 550 of which are actually followed (200 having more than 18 years). Their mean age is 14 years and 6 months (ranged between neonatal age and 50 years old). Of these, 93.8% are Europeans, 2.8% Asians, 2.9% Africans and 0.5% South Americans.

Since 2010 a National Registry for PKU patients has been activated in Italy.

Since the first attempt was to treat PKU patients with a low Phe diet, many steps forward have been made in PKU treatment. Many patients together with many specialists (pediatricians, nutritionists, biochemists, genetists, etc.) have traced natural history of PKU and evolution in its lifelong dietary treatment, with adequate supplementations.

After more than 50 years, we must be proud of the results obtained to prevent mental retardation, but also we must be aware of new challenges in the management of this metabolic disorder (optimal growth, nutritional status, compliance and patients quality of life).
The best documented functions of tetrahydrobiopterin (BH$_4$), beyond its cofactor role in the phenylalanine hydroxylating system and thus control of blood phenylalanine levels, are as a cofactor of the rate limiting enzymes in the biosynthesis of catecholamines and serotonin. BH$_4$ can cross the blood-brain barrier at certain blood concentrations and can affect functions of tyrosine and tryptophan hydroxylases in the central nervous system. It has been also shown that BH$_4$ plays an essential role for all isoforms of the nitric oxide synthase and that patients with inherited BH$_4$ deficiencies present with an impaired synthesis of nitric oxide in the brain. This may lead to generation of potentially neurotoxic peroxinitrite. BH$_4$ pathway is further tightly connected with the folic acid pathway and patients with one form of BH$_4$ deficiency (DHPR defect) present with cerebral folate deficiency.
The clinical phenotype of patients suffering from phenylketonuria results from a loss-of-function molecular phenotype of the enzyme phenylalanine hydroxylase, with the exception of rare cases of defects in tetrahydrobiopterin cofactor biosynthesis. From a clinician’s or patient’s point of view the most important parameter of phenylalanine hydroxylase function is the residual enzyme activity. However, this results from a multitude of different molecular and structural derangements caused by more than 600 known mutations in the phenylalanine hydroxylase gene. Bioinformatic approaches can help identify patterns in the plethora of data that provide mechanistic insight into the loss-of-function phenotypes and into the structural basis of responsiveness to a treatment with the cofactor drug sapropterin. Moreover, criteria beyond residual activity can be defined on a molecular level to distinguish between responders and non-responders to this pharmacological chaperone treatment. A deeper comprehension of the molecular basis of dysfunction and response to sapropterin will allow for improved clinical management as well as the development of next generation drugs that may expand to those patients that do not yet benefit from pharmacological treatment.
L3 - Use of BH₄ in PKU patients below 4 years

Oriane Leuret¹, Magalie Barth², Alice Kuster³, Didier Eyer⁴, Loïc de Parscau⁵, Sylvie Odent⁶, Brigitte Gilbert-Dussardier⁷, François Feillet⁸ and François Labarthe⁹

1 Médecine Pédiatrique & INSERM U921, CHRU de Tours, Université François Rabelais, Tours;  
2 Génétique, CHU Angers;  
3 Réanimation Pédiatrique, CHU Nantes;  
4 Pédiatrie, CHU Strasbourg;  
5 Pédiatrie et Génétique Médicale, CHU Brest;  
6 Génétique, CHU Rennes;  
7 Génétique, CHU Poitiers;  
8 Centre de référence des maladies héréditaires du métabolisme, CHU Nancy;  
9 Réseau Maladies Métaboliques Hôpitaux Universitaires du Grand Ouest, France.

Background
Sapropterin dihydrochloride, an EMEA-approved synthetic formulation of BH₄, has been available in Europe since 2009 for PKU patients older than four years, but its use with younger children is allowed in France based on an expert recommendation. We report the cases of 15 patients treated under the age of four years and demonstrate the safety and efficacy of this treatment for patients in this age group.

Patients and method
We report the use of BH₄ in 15 PKU patients treated before the age of four years.

Results
Fifteen patients were enrolled in this retrospective study. Mean phenylalaninemia at diagnosis was 542±164 µM and all patients had mild PKU (maximal phenylalaninemia: 600-1200µM). BH₄ responsiveness was assessed using a 24-hour BH₄ loading test (20mg/kg), performed during the neonatal period (n=11) or before 18 months of age (n=4). During the test, these patients exhibited an 80±12% decrease in phenylalaninemia. Long-term BH₄ therapy was initiated during the neonatal period (n=7) or at the age of 13±12 months (n=8). The median duration of treatment was 23 months [min 7; max 80]. BH₄ therapy drastically improved dietary phenylalanine tolerance (456±181 vs 1683±627 mg/day, p<0.0001) and allowed a phenylalanine-free amino acid mixture to be discontinued or not introduced in 14 patients. Additionally, in the eight patients treated after a few months of diet therapy, BH₄ treatment significantly decreased mean phenylalaninemia (352±85 vs 254±64µM, p<0.05), raised the percentage of phenylalaninemia tests within therapeutic targets [120-300µM] (35±16% vs 64±16%, p<0.05), and reduced phenylalaninemia variance (130±21 vs 93±27µM, p<0.05). No side effects were reported.

Conclusions
BH₄-therapy is efficient and safe before the age of four years in mild PKU, BH₄-responsive patients.
L4 - Diet and health in adult PKU: case observations

Juliana von Berlepsch, Berthold V. Koletzko
Div. Metabolic and Nutritional Medicine, Dr. von Hauner Children’s Hospital, Munich, Germany

Background
Patients with phenylketonuria (PKU) consume restricted, unusual diets from early childhood onwards that provide both macro- and micronutrient intakes deviating markedly from recommended intakes, along with often restricted eating behaviour. In PKU children, both growth faltering and increased overweight have been reported. Little is known on the consequences of treated PKU in adulthood with respect to weight and associated markers of health. We assessed obesity risk and physical activity level of adult PKU patients from a large treatment centre in Germany. Inclusion criteria were age ≥25 years, written informed consent, and for patients also a confirmed diagnosis of classical PKU.

Results
Included were 33 early PKU patients treated early infancy (mean age 34.5 yrs, 22 females) and 33 age matched healthy controls (usually family members or partners, mean age 34.6 yrs, 21 females) with similar levels of formal education. PKU patients had a higher mean BMI (kg/m²) (26.5 ± 4.5 vs. 22.5 ± 3.1, p<0.001) and a threefold higher prevalence of obesity (BMI >20 kg/m²) [6/33 vs. 2/33, p=0.011]. Eleven PKU patients but only three controls were overweight (BMI 25 to <30 kg/m²). Total energy expenditure measured over 3 days (Sense Wear) in PKU patients tended to be slightly lower (2791 ± 578 vs. 2896 ± 799 kcal/day, n.s.) with a markedly reduced active energy expenditure (595 ± 513 vs. 977 ± 659 kcal/day, p=0.013) and much lower duration of physical activity (115 ± 99 vs. 180 ± 89 min/day, p=0.009). Active energy expenditure was inversely correlated to BMI (r=-0.473, p<0.001). Physical activity duration was inversely correlated to BMI (r=-0.583, p<0.001), obesity (r=-0.455, p<0.001), and weight (r=-0.559, p<0.001). Weekly time spent doing sports correlated to active energy expenditure (r=0.349, p=0.005) and physical activity level (r=0.299, p=0.017), whereas reported duration of TV watching correlated inversely to physical activity per day (r=-0.0346, p=0.006) and to active energy expenditure (r=0.355, p=0.004).

Conclusions
Adult PKU patients have an increased obesity risk. BMI is predicted by physical activity level, which is lower in adult PKU patients than in controls and could be either cause or consequence of increased body weight. Potential reasons for the higher obesity risk will be discussed. Measures to achieve sustainable enhanced physical activity levels might be one useful strategy to reduce obesity risk in adult PKU patients.
In October 2000, the U.S. National Institutes of Health (NIH) published a Consensus Development Conference Statement for screening and management of PKU. Since then, new therapies have emerged, including dihydrochloride sapropterin, large neutral amino acids, and glycomacropeptide. To assist clinicians in making treatment decisions, NIH has revisited the 2000 guidelines via a work group process and concluding conference. Five working groups met over the course of one year to evaluate an expanded body of literature and their own clinical expertise in addressing questions related to treatment of PKU. Each working group was composed of 10-12 topical experts (including researchers and clinicians), public members (including those with PKU), and federal stakeholders, and met “virtually” in a series of 8-10 webinars. The working groups addressed the following broad questions:

1. **Long-Term Outcomes and Management across the Lifespan**: What evidence and practices should inform management of individuals with PKU over their lifespan?

2. **PKU and Pregnancy**: What are the considerations for management for women of reproductive age, focusing on preconception care, conception planning, pregnancy, and the postpartum period?

3. **Diet Control and Management**: Should the dietary recommendations that emerged from the 2000 Consensus Statement be changed? If so, what current knowledge would inform development of new recommendations?

4. **Pharmacologic Interventions**: What is the role of sapropterin dihydrochloride in individuals with PKU?

5. **Molecular Testing, New Technologies, and Epidemiologic Considerations**: Should there be any changes to the 2000 Consensus Conference Statement regarding newborn screening and molecular testing for PKU?

On February 22-23, 2012, a conference entitled “PKU Scientific Review Conference: State of the Science and Future Research Needs” was convened on the NIH campus in Bethesda, MD, USA. These working groups presented their findings and conclusions. As a parallel effort, an Evidence-based Practice Center (EPC) presented the results from its evidence review of the comparative effectiveness of treatments for PKU. Other speakers discussed newer treatments available for PKU, including PEG-PAL (PEGylated Phe ammonia lyase) and gene therapy; practice models for transitioning individuals with metabolic disorders to adult health care; and the Food and Drug Administration’s perspectives on the need for sound clinical trials to promote drug development for rare disorders. Two panels addressed industry and advocacy perspectives, and international practices, respectively. In addition, the conference participants identified critical research gaps, which included the need for more treatment options for those with classic forms of PKU, better knowledge of the effects of elevated phenylalanine levels on the brain, and improved understanding of social supports that facilitate access to treatments, minimize barriers to adherence, and promote the best clinical outcomes throughout the lifespan.
Heroes of PKU - A History

In recounting the history of PKU it is obligatory to mention the “big three” – Fölling, Bickel and Guthrie. This is as it should be, for they formed the core of PKU–discovery, diet, newborn screening. But all too often we end PKU history with them, and this is unfortunate because PKU today is the result of discoveries by many.

The first of these was Lionel Penrose of London. He coined the term “phenylketonuria,” recognized it as a Garrodian inborn error of metabolism, and examined phenylalanine metabolism for possible dietary treatment.

George Jervis came to the United States and embarked in a series of investigations into PKU. He published first extensive description of the clinical features, demonstrated the metabolic defect in the body, and showed the metabolic block in the liver.

Louis Woolf was instrumental in Bickel’s development of the diet for PKU. In London he informed Bickel about the process for extracting phenylalanine from casein hydrolysate and later in Vancouver was indirectly responsible for the cloning of the gene for phenylalanine hydroxylase. Savio Woo under Woolf learned how to isolate and purify phenylalanine hydroxylase, the critical process in cloning the gene.

Robert MacCready was immeasurably important in population-wide newborn screening for PKU. He began the screening in Massachusetts and published the newborn identifications in leading medical journals, which led to the early establishment of newborn screening in many states.

Seymour Kaufman in the United States stands alone in studies of the phenylalanine hydroxylase enzyme. His monumental discoveries include tetrahydrobiopterin (BH₄) as a required cofactor, the BH₄ deficiency syndromes, and the suggestion that BH₄ might be effective in the treatment of some individuals with PKU.

David McDonald is responsible for ingenuously developing a natural animal model for PKU, the “PKU mouse.” Where would PKU research be today without this mouse model for PKU?

No history of PKU should omit Charles Scriver of Montreal. He is a giant in so many ways, pointing out that PKU is the paradigm of biochemical genetic disorders, developing a method for assessing phenylalanine hydroxylase activity in the body, and cloning the gene for phenylalanine ammonia lyase (PAL) and examining this enzyme as a treatment for PKU are just a few of his important contributions.

I suspect that were Abjorn Følling with us today he would heartedly agree with all that I have said.
Oral Communications
Objective
To study the positive predictive value (PPV) of the 48h BH₄-loading test and genotype for true long-term BH₄ responsiveness (trueBH₄R).

Methods
Patients with ≥30% Phe reduction at ≥1 moment(s) during the 48h BH₄-loading test (Phase I, Anjema et al. 2011) were invited for Phase II: the BH₄ treatment-trial. TrueBH₄R was defined as ≥30% reduction in mean Phe concentration or >4g and/or ≥50% increase of natural protein intake.

Results
80/177 were Phase I responders. 67/80 completed Phase II. 58/67 were trueBH₄R. In trueBH₄R patients, natural protein tolerance increased [median 0.52; range 0.06–2.49 g/kg/day]. Genotype was complete in 65% of 177 patients. Patients with two favourable mutations (N=6, R261Q excluded) all were trueBH₄R. Of 39 patients with a functionally hemizygous genotype, 29 were trueBH₄R, 3 were Phase I non-responders, 1 proved to be not trueBH₄R (I65T/L311P), and 6 were Phase I responders but did not perform Phase II. 3/37 patients with two known putative null mutations were Phase I responsive (range 31.3 – 37.5% Phe reduction); one was a Phase II non-responder and two did not perform Phase II.

Conclusions
The 48h BH₄-loading test has a PPV of 87% according to predefined criteria. Genotypes described in the literature as favourable [including functionally hemizygous, R261Q excluded] showed a PPV of 97%. To further investigate the value of the BH₄-loading test and genotype, patients with 20-30% Phe decrease in Phase I and patients with a functionally hemizygous genotype but unexpected lack of response in the 48h BH₄-loading test, have to be studied further.

Conflict of interest
- Francjan J. van Spronsen is a member of the Scientific Advisory Boards of Merck-Serono and Danone, and has received grants for research purposes of both Merck Serono and Danone. Furthermore, he has received consultation fees and speaker relation fees for Merck Serono, Danone, and Swedish Orphan.
- M. van Rijn is a member of the European Nutritionis Expert Panel of Merck-Serono, and has received grants for research purposes of both Merck Serono and Nutricia. Furthermore, she has received consultation fees and speaker relation fees for Merck Serono and Danone.
GTP Cylohydrolase I deficiency (GTPCH, OMIM 600225) rate-limiting enzyme in the synthesis of tetrahydrobiopterin (BH4), results in decreased production of pterins and neurotransmitters. GTPCH deficiency occurs in autosomal recessive (AR) and autosomal dominant (AD) forms. While the AD form presents as Dopa-responsive dystonia (DRD) with absence of hyperphenylalaninemia (HPA), the AR form presents with complex neurological dysfunction with or without HPA.

We describe four patients with GTPCH deficiency without HPA: P1 (male, 12y), P2 (female, 7y), P3 (female, 3y), all presented marked diurnal fluctuation of symptoms and DRD. P4 (female, 5m), presented cephalic tremor, global hypotonia, oculogyric crises and progressive encephalopathy.

Analysis of biogenic amine neurotransmitters and pterins by HPLC in CSF in all patients revealed low levels of homovanillic acid, 5-hydroxyindolacetic acid, neopterin and biopterin, a pattern suggestive of GTPCH deficiency. Confirmation of the disease was made by molecular analysis of the GCH1 gene in three patients. In P2 only one mutation (p.W96X) was identified, which was present in the father. In P3, a novel mutation IVS5-6T>G also in one allele was detected, it was identified in the mother who also presented DRD. In P4 two different mutations (p.Q89X/p.K224R) were identified, which were present in heterozygous state in both parents.

Treatment with L-Dopa and Carbidopa has improved the clinical symptoms in all patients.

Conclusions
1) P2 and P3 had an AD form and P4 an AR form of GTPCH deficiency.
2) Disorders of BH4 metabolism should be considered in patients with neurological symptoms associated with extrapyramidal movement disorders.
3) We emphasize the importance of CSF neurotransmitters and pterins investigation.
Introduction
We have recently presented a genotype-based classification of tetrahydrobiopterin (BH$_4$)-responsiveness and documented the importance of residual PAH activity in a large cohort of Turkish PKU population. On the basis of these results, several mutations were characterized on molecular level to elucidate BH$_4$-response and explain inconsistent genotype/phenotype correlations. The goal is to document and catalog more genotype/phenotype data for easier prediction of patient’s outcome.

Material and Methods
A new PAH assay method has been developed by LC-ESI MS/MS with higher specificity for in vitro determination of PAH activities. The influence of p.T380M, p.F382L and IVS10-3 mutations on splicing mechanism of exon 11 was investigated. A number of mutations found among Turkish patients were characterized by transient expression and co-expression in eukaryotic cells and correlated with phenotype and available BH$_4$ loading test data.

Results
PAH exon 11 is a vulnerable exon, with regular splicing easily disturbed by mutations, leading to more severe effects on phenotype than predicted from amino acid change. Our expression system for the study of PAH alleles elucidates interallelic interactions and mutation dominance effects in previously identified genotypes combined with in vitro BH$_4$-responsiveness data.

Conclusion
Molecular, in vitro characterization of genotypes, i.e. the analysis of compound heterozygote mutations by co-expression, together with comparison to thoroughly cataloged patient data provides a sophisticated tool for BH$_4$-responsiveness testing in new PKU patients.
OC4 - Clinical characterization of patients with early infantile onset of autosomal recessive GTP cyclohydrolase I deficiency without hyperphenylalaninemia

Thomas Opladen
University Children’s Hospital Heidelberg, Division of Inborn Metabolic Diseases, Heidelberg, Germany

Introduction
Autosomal recessive guanosine triphosphate cyclohydrolase [GTPCH] type I deficiency is characterized by a complex neurological dysfunction. Patients are usually early diagnosed with hyperphenylalaninemia in newborn screening.

Patients
We describe two unrelated patients without hyperphenylalaninemia who presented during early infancy with severe motor retardation, hypokinesia and truncal hypotonia. Apart from the motor retardation both children appeared very alert and interested.

Results
A neuromuscular disease was excluded. Due to clearly decreased concentrations of CSF homovanillic acid and 5-hydroxyindoleacetic acid in combination with decreased tetrahydrobiopterin (BH4) and neopterin, GTPCH deficiency was suspected. Diagnosis was confirmed biochemically by reduced GTPCH enzyme activity in fibroblast. Analysis of the GCH1 gene revealed a novel homozygous mutation in one patient and a compound-heterozygous mutation in the other. Treatment with L-Dopa/Carbidopa resulted in striking clinical improvement, with ageappropriate development at the age of 6 years.

Conclusions
Autosomal recessive GTPCH deficiency should be considered in infants with severe truncal hypotonia even if hyperphenylalaninemia or classical extrapyramidal symptoms are missing. Neurotransmitter analysis followed by enzyme or mutation analysis can confirm the diagnosis, and L-dopa treatment should be started at high doses.

Conflict of interest
No financial support received for this work and no conflict of interest to declare.
It would be desirable to predict sapropterin response based on individual phenylalanine hydroxylase (PAH) mutations in PKU patients.

We investigated sapropterin response during two weeks of sapropterin therapy (1st week: 10 mg/kg per day, 2nd week: 20 mg/kg per day) in 101 PKU patients (age 4 to 44 years) in an outpatient setting. Response was defined as a reduction in serum phenylalanine levels of more than 30%. PAH was sequenced conventionally in all patients, and individual mutations were correlated with sapropterin response.

37 of 101 patients responded to sapropterin. The mutation Y414C was most often associated with a response to sapropterin (present on 13 alleles in 37 responders). In the 64 non-responders, the mutation R408W (33 alleles) and the mutation IVS12+1G>A (17 alleles) were detected most frequently. No mutation could clearly predict sapropterin response in our cohort, since most mutations were found in heterozygous state in non-responders and in responders. Homozygosity for the Y414C genotype, however, was always associated with sapropterin response. Response to sapropterin therapy cannot be predicted based on the presence of a single mutation on one allele alone. Instead, the complete PAH genotype should be taken into account.
OC6 - Treatment of phenylketonuria (PKU) using minicircle-based naked-DNA gene transfer to murine liver

Viecelli, H.M. 1; Harding, C.O. 2; Thöny, B. 1

1 - Department of Paediatrics, University of Zurich, Zurich, Switzerland;
2 - Departments of Molecular and Medical Genetics and Paediatrics, Oregon Health & Science University, Portland, Oregon, USA.

Introduction
We have previously reported long-term correction of hyperphenylalaninemia and hypopigmentation of the PKU mouse model, C57BL/6-Pahenu2, after liver-directed gene transfer with recombinant adeno-associated viral (AAV) vectors (Ding et al., Gene Ther, 2006, Ding et al., Mol Ther, 2008; Rebuffat et al., Hum Gene Ther, 2010). However, questions of expression stability, treatment toxicity, potential for insertional mutagenesis, and safety required for targeting newborn and paediatric patients for potential life-long treatment remain a risk even for AAV vector-dependent approaches. To potentially overcome these drawbacks, we are developing non-viral gene transfer methods for liver targeting.

Material and Methods
Here we report the successful use of naked-DNA/minicircle (MC) technology to treat murine PKU. Our MC-DNA vectors contain a de novo designed liver-specific hybrid promoter-enhancer fragment, the mouse phenylalanine hydroxylase gene (mPah), and the bovine growth hormone polyA signal. Delivery of MC vectors was mediated by hydrodynamic tail vein (HTV) injection as a liver-targeted approach.

Results
MC-Vector titration studies showed that the blood phenylalanine levels were normalized concomitant with reversion of hypopigmentation at optimal MC-DNA concentrations for up to two months without reapplication (ongoing experiment). In contrast, parallel injections of parental plasmids did not result in any Phe clearance. Upon scarifying MC-treated PKU mice, sustained transgene expression was confirmed by the presence of hepatic PAH enzyme activity.

Conclusions
MC technology offers a better safety profile and has the potential for gene-therapeutic treatment of liver diseases.
Introduction
Treatment of Phenylketonuria (PKU) focuses on keeping phenylalanine (Phe) levels below certain limits, as high Phe levels predict poor cognitive (and neurological) outcomes. Debate continues on which upper target levels should be aimed for in different age groups. Upper target Phe concentrations for the first 12 years of life vary between 240 and 360 μmol/l, the latter being used most frequently.

Objective: To examine cognitive outcomes in relation to different Phe upper target levels (240 μmol/l versus 360 μmol/l) in early- and continuously-treated children with PKU.

Material and methods
63 PKU patients (mean age 10.8 years, SD 2.3) and 73 controls (mean age 10.9, SD 2.2) performed tasks measuring inhibitory control, interference control, cognitive flexibility, and motor control. Lifetime Phe was determined by taking the mean of half-year median Phe levels.

Results
Mann-Whitney analyses showed that PKU patients with lifetime Phe ≤ 240 μmol/l outperformed patients with Phe > 240 μmol/l on cognitive flexibility and interference control. Compared to controls, patients with Phe ≤ 240 μmol/l performed comparable on all tests, which was not the case for patients with Phe between 240-360 μmol/l and with Phe ≥ 360 μmol/l.

Conclusions
When inhibitory control was required, either alone or in combination with working memory (i.e. cognitive flexibility), average Phe concentrations of ≤ 240 μmol/l appeared advantageous compared to ≤ 360 μmol/l, which may be (interpreted as) reason to further consider lowering the upper target Phe to 240 μmol/l during the first 12 years of life.

This study was funded with grants of Zorg Onderzoek Nederland, Stichting PKU Research, Fonds NutsOhra, and the University Medical Center Groningen, the Netherlands.

Conflict of interest
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- Stephan C. J. Huijbregts serves as a scientific consultant for Merck Serono S.A. Geneva, Switzerland.
- Leo M. J. de Sonneville declares receipt of consultation fees as consultant of Danone, FrieslandCampina, and Global Pharma Consultancy. He is also director of Sonares B.V., the firm that commercially distributes the ANT program, used in the study.
- Jaap J. van der Meere declared that he does not have any conflicts of interest.
- Rianne Jahja declared that she does not have any conflicts of interest.

All authors confirm independence from the sponsors; the content of this presentation has not been influenced by the sponsors.
Introduction
The value of the essential amino acid tyrosine (Tyr) in Phenylketonuria (PKU) treatment is unclear. Low tyrosine concentrations may interfere with adequate dopamine synthesis, which is important for cognitive functioning, and high Phe:Tyr ratios are suggested to interfere with neurocognitive outcome.

Objective: To determine the relative strength of lifetime and concurrent Phe concentrations, and lifetime and concurrent Phe:Tyr ratios in predicting neurocognitive outcome in early- and continuously-treated children with PKU.

Material and methods
63 PKU patients (mean age 10.8 years, SD 2.3) performed tasks measuring inhibitory control, cognitive flexibility, and motor control. Concurrent Phe and Tyr concentrations were determined from blood samples taken on the day of testing, whereas lifetime Phe and Tyr were calculated by taking the mean of half-year median levels.

Results
Inhibition of prepotent responding was significantly predicted by lifetime Phe:Tyr. Although both concurrent Phe levels and Phe:Tyr ratios were significantly correlated with cognitive flexibility, backward regression showed that only the Phe:Tyr ratios remained a significant predictor. Besides age, lifetime Phe predicted accuracy and stability on the motor control task.

Conclusions
Phe:Tyr ratios predicted cognitive functions, whereas Phe concentrations were a significant predictor of motor control. Phe:Tyr ratio was a predictor of performance on tasks measuring specific (dopamine-mediated) cognitive abilities, emphasizing the importance of tyrosine monitoring in PKU studies. As tyrosine levels are highly variable between measurements, great care should be paid to correct conditions of tyrosine measurement.

This study was funded with grants of Zorg Onderzoek Nederland, Stichting PKU Research, Fonds NutsOhra, and the University Medical Center Groningen, the Netherlands.

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- Jaap J. van der Meere declared that he does not have any conflicts of interest.
- Rianne Jahja declared that she does not have any conflicts of interest.
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