General information

Venue
The symposium takes place at the:
Hilton Istanbul Hotel
Cumhuriyet Caddesi Harbiye
Istanbul, Turkey

Language
The official language of this symposium is English.

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Aims of the symposium
Phenylketonuria (PKU) is an active field of investigation, and new diagnostic and treatment options are emerging that may reduce the burden of a life-long and limiting dietary management for these patients, their families and the society. Basic and clinical research is today focusing on brain metabolism in PKU patients and its repercussion on neurological functions, on a better clinical characterization by biochemical and genetic analysis, and also on developing new therapeutic strategies or implementing the use of those already available, such as diet and sapropterin dihydrochloride. Furthermore, evidence-based international guidelines are being developed to provide standardization in PKU management, including appropriate biomolecular diagnosis, treatment's choice, evaluation of response to therapy, and achievement of targeted blood phenylalanine levels, also considering the social and economical impact of this disease. The European Phenylketonuria Group (EPG) together with Serono Symposia International Foundation (SSIF) consider essential to continue their series of successful meetings on PKU, and are organizing the 2013 annual symposium dedicated to that. Aims of this meeting are to review the most important achievements of research in the field of PKU and to provide participants with solutions for optimizing patient management in their daily clinical practice.

Learning objectives
After attending the symposium, participants will better understand the importance of and will be able to improve their clinical practice on the following aspects of PKU:

- New therapy options for PKU
- Genetic modifiers and metabolomics in PKU
- Management of BH₄ deficiencies
- Neuropsychological and executive function assessment in PKU
- Development of EU guidelines for PKU management

Target audience
Specialists in pediatric and adult inherited metabolic disorders, dietitians, nutritionists, clinical biochemists and genetists, basic scientists, psychologists, nurses and all health care professionals dealing with PKU.

Accreditation
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The conference 5th European Phenylketonuria Group (EPG) Symposium “Advances and Challenges in PKU” (15-16 March 2013 - Istanbul, Turkey) is designated for a maximum of 9 (nine) 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.
Learning effectiveness project

The world of CME is changing with many different live and online formats, and Serono Symposia International Foundation (SSIF) is continually trying to improve its CME activities.

With your participation in a structured series of evaluations, SSIF can provide cutting-edge learning activities designed to give you the greatest value from the time you invest.

SSIF is running the learning effectiveness project for this meeting.

During the conference you will be asked to answer a real time survey to evaluate your knowledge and opinions on the specific topics that will be covered in these two days.

We also kindly ask you to assess the program in various domains such as whether you were satisfied with the meeting, whether it met the stated learning objectives, whether the contents were neutral and will be applicable to your daily practice.

After the event, you will be involved in one additional step:

• Post-event: sixty days 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.

We will collate and analyse your responses and use the results to improve and develop our ongoing programs.

Of course, we commit to maintaining the confidentiality of the information you provide and we will inform you about the results of the process regarding the activity that you attended.

Thank you very much for participating in this project!
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Scientific program
15-16 March 2013

Friday - 15 March

8.45 Serono Symposia International Foundation (SSIF)
   Opening
   Robert Fischer [Germany]

8.50 Welcome
   Mübeccel Demirkol [Turkey]

8.55 European Phenylketonuria Group (EPG) Introduction
   Nenad Blau [Switzerland]

Session I  Advances in PKU research

Session chair: François Feillet [France]

Real time survey

9.00 L1: Novel mechanisms of PKU brain pathology
   Ehud Gazit [Israel]

9.30 L2: Importance of gene modifiers in PKU: what we can learn from other diseases?
   Niels Gregersen [Denmark]

10.00 L3: Interallelic complementation in PKU
   Ania C. Muntau [Germany]

Real time survey

10.30 Discussion

10.45 Coffee break

Session II  Free oral communications

Session chairs: Maria Gizewska [Poland]
                Turgay Coskun [Turkey]

11.15 OC1: Structure-function relationship of the pharmacological chaperone
tetrahydrobiopterin stabilizing phenylalanine hydroxylase
   Marta K. Danecka [Germany]

11.30 OC2: Man made disease: effects of low phenylalanine levels in an inadequately treated phenylketonuria patient and mouse model
   Ben Pode-Shakked [Israel]

11.45 OC3: Living with PKU: quality of life and the effect of tetrahydrobiopterin (BH4): a prospective multi-center cohort study
   Serwet Demirdas [The Netherlands]

12.00 OC4: Cost and time burden of living with phenylketonuria in the Netherlands
   Serwet Demirdas [The Netherlands]

12.15 OC5: Testing neonatal BH4 responsiveness: treat first, diagnose later
   Karen Anjema [The Netherlands]

12.30 OC6: Executive functioning and behaviour problems in adult phenylketonuria patients: preliminary results
   Rianne Jahja [The Netherlands]

12.45 OC7: Long-term treatment with tetrahydrobiopterin in BH4-responsive PKU patients: effect on quality of life and cognitive function
   Chiara Cazzorla [Italy]

13.00 OC8: Undiagnosed phenylketonuria in parents of phenylketonuria patients, is it worthy to be checked?
   Arnaud Wiedemann-Fodé [France]

13.15 Lunch
Scientific program
15-16 March 2013

Session III  Parallel workshop sessions

14.15 Workshop Sessions 1-4

1. European guidelines for PKU
   Francjan J. van Spronsen (The Netherlands)
   François Maillot (France)

2. How to test executive function in PKU
   Stephan Hujibregts (The Netherlands)
   Jaime Campistol Plana (Spain)

3. Overweight in PKU
   Anita MacDonald (UK)
   Júlio César Rocha (Portugal)
   Friedrich K. Trefz (Germany)

4. Pitfalls in the management of BH₄ deficiencies
   Nenad Blau (Germany/Switzerland)
   Alberto Burlina (Italy)

15.45 Coffee break and rotation of workshop

Session IV  Poster presentations

17.45 Poster presentations

18.00 End of Day 1
### Saturday - 16 March

**Session V: Advances in PKU management**

**Session chair:** Maureen Clearly (UK)

- **8.30 L4:** Clinical trials with the PEG-PAL enzyme substitution  
  Cary O. Harding (USA)

- **9.00 L5:** Novel chemical and pharmacological therapies for PKU  
  K. Michael Gibson (USA)

- **9.30 L6:** Neuropsychological assessment in PKU  
  Josef Weglage (Germany)

- **10.00 L7:** Metabolomics in patients with phenylketonuria  
  Uta Ceglarek (Germany)

- **10.30** Discussion

- **10.45** Coffee break

**Session VI: Workshops summaries**

**Session chair:** Anita MacDonald (UK)

- **11.00** Workshop summaries presentations and discussion

**Session VII: Asbjørn Følling lecture and Award**

- **12.00** Introduction to Asbjørn Følling Lecture  
  Friedrich K Trefz (Germany)

- **12.10** Asbjørn Følling Lecture  
  Ursula Wachtel (Germany)

**Session VIII: SSIF Award in PKU**

**Session chair:** Ania C. Muntau (Germany)

- **12.40** SSIF Award for best oral presentation in PKU

**Session IX: PKU Academy fellowships**

**Session chair:** François Feillet (France)

- **12.50** PKU Academy 2012 fellowship update  
  Priscila Mazzola (Brazil)

- **13.00** PKU Academy 2013 fellowship presentation  
  Francjan J. van Spronsen (The Netherlands)

- **13.10** Closing remarks  
  Francjan J. van Spronsen (The Netherlands)

- **13.20** Lunch
Disclosure of faculty relationships

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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 no potential conflict of interest.
- **Nenad Blau** Declared to be member of Merck Serono Germany advisory board.
- **Alberto Burlina** Declared to be member of the SAB Kamper Study.
- **Jaime Campistol Plana** Declared no potential conflict of interest.
- **Chiara Cazzorla** Declared no potential conflict of interest.
- **Uta Ceglarek** Declared no potential conflict of interest.
- **Maureen Clearly** Declared receipt of honoraria or consultation fees related to the EPG Committee work and to be member of the EPG Group.
- **Turgay Coskun** Declared no potential conflict of interest.
- **Serwet Demirdas** Declared no potential conflict of interest.
- **Mübeccel Demirkol** Declared no potential conflict of interest.
- **François Feillet** Declared receipt of honoraria or consultation fees from Merck Serono and Nutricia and to be member of Merck Serono, Shire and Nutricia advisory boards.
- **Ehud Gazit** Declared no potential conflict of interest.
- **K. Michael Gibson** Declared no potential conflict of interest.
- **Niels Gregersen** Declared no potential conflict of interest.
- **Cary O. Harding** Declared receipt of grants and contracts, honoraria or consultation fees from BioMarin Corp. Novato, CA and that his presentation will include discussion on off-labeled or otherwise non-approved uses of products, i.e. progress on clinical trials of an investigational drug.
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Ursula Wachtel  Declared no potential conflict of interest.

Josef Weglage  Declared no potential conflict of interest.

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 28 February 2013.

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Maria Gizewska
Rianne Jahja
François Maillot
Priscila Mazzola
Ben Pode-Shakked
D.D. Reiss
Friedrich K. Trefz
Arnaud Wiedemann-Fodé
Lectures
Abstracts
We had studied the molecular self-assembly of the phenylalanine amino acid as part of our studies of the association of peptides and proteins at the nano-scale. We demonstrate that at pathological concentrations, phenylalanine self-assembles into fibrils with amyloid-like morphology and distinct electron diffraction. In order to study the process and outcome of phenylalanine fibril formation, we have applied a series of biophysical and biological assays. Transmission electron microscopy analysis of phenylalanine at millimolar concentrations range indicated the occurrence of well-ordered and elongated assemblies. Scanning electron microscopy (SEM) was also used in order to study the three dimensional structures of the fibrils and environmental SEM was used in order to study fibrillar structures in a humid environment. Microscopy had demonstrated that the assemblies are relatively homogeneous and are evidently discrete entities with persistence length in the order of few micrometers. These assemblies are specifically recognized by antibodies, show cytotoxicity that can be neutralized by the antibodies and are present in the hippocampus of model mice and in parietal cortex brain tissue from individuals with PKU. This is, to our knowledge, the first demonstration that a single amino acid can form amyloid-like deposits, suggesting a new amyloidosis-like etiology for PKU.

References:
During the last two decades the realization has emerged that the phenotype of the majority of inherited genetic diseases, including inborn errors of metabolism, cannot be predicted by the genotype identified in patients. This is particularly true when the disease-associated gene variations are of the missense type, giving rise to misfolding proteins with varying residual function and which exert cytotoxicity, in many cases creating oxidative stress. Factors, either genetic, which may modify the protein quality systems, such as varying expression of chaperones and proteases, or varying amounts of cofactors, such as BH4 or FAD (Riboflavin), which may function as chemical chaperones, may modify the amount of misfolding. Further, varying amounts/efficiency of antioxidant systems may alleviate or aggravate the damaging effects of misfolding proteins. In addition to the cytotoxic effects of misfolding proteins, accumulated substrates may also damage cell functions, as has been shown both for phenylalanine in PKU and fatty acid in fatty acid oxidation defects.

We have been interested in the pathophysiology of fatty acid oxidation (FAO) defects, and especially the folding efficiency and creation of oxidative stress in model and patient cells carrying missense variations in the short-chain acyl-CoA dehydrogenase (SCAD) and electron transfer flavoprotein dehydrogenase (ETFDH) genes, giving rise to SCAD deficiency and multiple acyl-CoA dehydrogenation deficiency (MAD) deficiency, respectively. By traditional molecular biological methods and advanced protein mass spectrometric (proteomic) techniques, in combination with current knowledge from other studies, we conclude that patients with these diseases suffer from chronic oxidative stress due to the cumulative effects of protein misfolding and oxidative damage as well as of the toxic effects of metabolites. We propose that further environmental stress, such as heat (fever) and metabolic stress (fasting) may aggravate the cellular dysfunction and worsen the conditions. We ask the question whether the current knowledge is sufficient to institute treatment experiments with folding enhancing chemicals, such as chemical chaperones, and/or with antioxidants, or it is necessary to know more about the creation of the observed oxidative stress in order to suggest treatment. The discussion of this question may be relevant for other protein misfolding/conformational disorders, including PKU.
Phenylketonuria (PKU; MIM 261600) is the most frequent inborn error of amino acid metabolism. It is caused by autosomal recessively transmitted deficiency of phenylalanine hydroxylase (PAH; EC 1.14.16.1). Early phenotype prediction concerning residual enzyme activity and dietary phenylalanine tolerance would be very helpful in daily clinical care to plan therapeutic procedures. However, liver biopsies for enzyme activity determination are not performed in PKU patients and genetic heterogeneity is considerable with more than 600 mutations in the PAH gene described to date. This is further enhanced by the fact that more than 80% of all PKU patients are compound heterozygous carrying two different mutations on the maternal and the paternal allele. Thus, genotype-phenotype correlation in PKU patients often is weak.

The PAH enzyme is composed of a homotetramer, a dimer of dimers. At a molecular level, it was recently shown that PAH missense mutations associated with residual PAH enzyme activity lead to protein misfolding with destabilization and early degradation of the PAH protein. It has to be hypothesized that in compound heterozygote patients PAH heterooligomers derived from both mutated alleles are formed and that there may be an interaction between the two protein species (interallelic complementation). Interallelic complementation refers to the change in the properties of the multimeric protein as a consequence of the interaction of subunits coded by the different mutant alleles. Thus in PKU, the mixed protein (heterotetramer) may exhibit more activity (positive interallelic complementation) or less activity (negative interallelic complementation) than the homotetramer.

To enable early phenotype prediction in individual compound heterozygote PKU patients, we exploited the bioluminescence resonance energy transfer (BRET) technology and classical biochemical PAH assays to analyze PAH interallelic complementation in eukaryotic cells. This allowed to gain insights into (i) composition of PAH oligomers, (ii) protein stability (PAH protein amount), and (iii) protein function (PAH enzyme activity) in the presence of two different mutations.

We observed both, positive and negative interallelic complementation. The protein amount and function as endpoints of interallelic complementation in oligomeric proteins not only depend on residual activity and stability but also on the composition of heterooligomers and this can be modulated by the genotype specific differences in subunit’s stability and affinity.

In conclusion, our results support the hypothesis that the PAH tetramer in vivo consists of subunits arising from both alleles and proteins derived from one allele can have an impact on protein conformation and stability of the protein derived from the other allele leading to negative or positive interallelic complementation. The combined approach of in vivo techniques may aid in translating molecular findings into the patient’s phenotype in PAH deficiency and by this support genotype-phenotype prediction at an early stage of clinical care.
Enzyme substitution is a novel approach to the treatment of PKU. Phenylalanine ammonia lyase (PAL), an enzyme found in many plants and fungi but not mammals, catalyzes the conversion of phenylalanine to two harmless products, trans-cinnamic acid and ammonia, and repetitive subcutaneous injection of PAL into PKU mice led to sustained decrease in blood phenylalanine to the normal range. Coating the PAL molecules with polyethylene glycol (PEG) helps protect the enzyme from destruction by the immune system. PEGylated recombinant PAL from the blue-green algae Anabaena variabilis has been developed into a clinical grade pharmaceutical [rAvPAL-PEG], and evaluation of its effects in humans with PKU has now been completed in multiple Phase I and Phase II clinical trials carried out at 14 centers in the US. To date, a total of 56 adults with PKU have received repetitive rAvPAL-PEG injections for up to 2.5 years. Adverse reactions have included transient skin rashes and joint pain that have been manageable with antihistamines. All subjects tolerated rAvPAL-PEG injections in the long term. Over time, all subjects who complete the dosing schedule have achieved control of blood phenylalanine. Enzyme substitution with rAvPAL-PEG is a promising novel approach to the treatment of PKU; planning is underway for a Phase 3 clinical trial to begin in 2013.
Background
Dietary therapy in patients with phenylketonuria (PKU) represents the gold-standard of treatment, yet cognitive deficits persist in treated patients, over-restriction of amino acid intake may have untoward effects, and lifelong adherence to dietary restriction is challenging. We have begun to outline a small-molecule pharmacotherapeutic approach employing non-physiological amino acids [NPAs] to lower brain phenylalanine [PHE] levels in the murine model of PKU, Pahenu2 mice. Our objective is to maximally restrict PHE accretion into brain while minimally impacting other large neutral amino acids [LNAAs].

Hypothesis
LNAAs transport across the blood brain barrier (BBB) is facilitated by the L-type amino acid transporter, LAT1. We predicted that differing K_m values of LNAAs for LAT-1 would facilitate identification of NPAs that selectively lower PHE transport into brain. We formed the hypothesis that selected LNAAs [D,L-norleucine [NL], 2-aminonorbornane [NB; 2-aminobicyclo-(2,1,1)-heptane-2-carboxylic acid], 2-aminoisobutyrate [AIB], and N-methyl-aminoisobutyrate [MAIB]], acting as competitive inhibitors of LAT-1, could reduce brain PHE in Pahenu2 mice, and we implemented dietary studies in Pahenu2 mice to evaluate this hypothesis.

Approach, Results
Feeding of 5% NL, 5% AIB, 0.5% NB and 0.3% MAIB (w/w chow, 18% protein) to Pahenu2 mice for 3 weeks reduced brain PHE by 56% (p<0.01), -1% (p=NS), 27% (p<0.05) and 14% (p<0.01), respectively, compared to untreated subjects. Significant effects on other LNAAs (tyrosine, methionine, branched chain amino acids [BCAAs]) were also observed, however, with MAIB displaying the mildest effects 1. Combinatorial application of 1% D,L-NL and 0.1% NB lowered phe 32% (p<0.001), but still revealed a dramatic lowering of other LNAAs (tyrosine, BCAAs).

Discussion, conclusions
MAIB purportedly represents an inhibitor of the system A (alanine) transporter that primarily traffics small amino acids. Since AIB was ineffective at PHE reduction, yet MAIB was effective and specific, we have further hypothesized that 2- (methylaminol)alkanoic acid analogues of MAIB [one and two methylene groups larger] will selectively retard PHE transport into brain of Pahenu2 mice. To insure uptake and facilitate dose-response analyses, we will evaluate intraperitoneal administration. Further, we are embarking upon in silico modeling of LAT-1 in order to identify optimal ligand inhibitors for future evaluation in Pahenu2 mice. Our pilot studies have demonstrated the feasibility of NPAA intervention in Pahenu2 mice that targets restriction of brain PHE accumulation, and our novel chemico-pharmacological approach may provide new tools for PKU treatment that, even if it does not replace dietary restriction, could ultimately loosen dietary restriction in PKU patients. [Funding from the National PKU Alliance is gratefully acknowledged].

References:
The long-term prognosis of treated phenylketonuria (PKU) and the need for a life-long treatment are still controversial issues in adult PKU patients. A controlled long term study was done to assess the neurological and neuropsychological performance in adult patients with PKU.

We investigated 57 well characterized patients with early treated classical PKU aged 19-41 years (mean age: 31) and 46 healthy controls, matched for age and socioeconomic status. Patients and controls were assessed for their IQ and information processing abilities. MRI was performed in patients for investigation of cerebral white matter. The study program was repeated at a five year follow up.

The longitudinal study revealed stable assessment results for both patients and healthy controls in the five year interval, especially no decrease in neuropsychological functioning in PKU patients. In detail the full scale IQ was significantly lowered in patients compared to controls. Information processing and attention were normal in young patients and in healthy controls. Older patients, however showed poorer information processing and attention related to poorer dietary control during early adolescence. There were no significant correlations of MRI abnormalities with IQ, information processing, and attention. IQ and information processing, however, were significantly correlated to blood Phe levels in childhood and adolescence.

Neuropsychological assessment in adults with early treated PKU revealed neurocognitive impairment particularly in older patients, but there is no decrease in Performances over a 5 year period. This seems not to refer to MRI-abnormalities in adulthood, but to an early relaxation to diet that still was recommended when the older patients were adolescents. Results strongly indicate dietary control during adolescence. Further longitudinal studies are needed to clarify whether low blood Phe levels (by dietary control or BH₄ treatment) are indicated in adulthood.
Patients with phenylketonuria (PKU) have to follow a lifelong phenylalanine restricted diet. This type of diet is accompanied by a reduced intake of saturated and unsaturated fatty acids especially long chain polyunsaturated fatty acids (LC PUFA). In our study we investigated whether the long-term fatty acid restriction influence mitochondrial fatty acid oxidation, sphingosine, cholesterol and PUFA metabolism.

12 children with PKU and 8 healthy controls were included in our study. Activated fatty acids [acylcarnitines C6-C18] in dried blood and cholesterol metabolites [phytosterols, lanosterol, oxyesterols, cholesterol esterification] and sphingolipids in serum were analyzed by liquid chromatographic tandem mass spectrometry (LC-MS/MS). Fatty acid composition of plasma glycerophospholipids was determined by gas chromatography. PUFA metabolites were analyzed by LC-MS/MS before and after platelet activation and aggregation using a standardized protocol.

Patients with PKU had significantly lower free carnitine and lower activated fatty acid concentration in whole blood compared to controls. Cholesterol resorption, synthesis and esterification were not influenced by the dietary fatty acid restriction. Fatty acid composition in glycerophospholipids and sphingolipid concentrations were comparable to that of healthy controls. However, patients with PKU showed significantly increased concentrations of α-linolenic acid (C18:3n-6) a precursor of arachidonic acid. In the PKU patients significantly higher platelet counts were observed. After activation with collagen platelet aggregation and thromboxane B2 and thromboxane B3 release did not differ from that of healthy controls.

Conclusion/Significance: Long-term dietary fatty acid restriction influenced energy production by mitochondrial beta-oxidation. No functional influence on unsaturated fatty acid metabolism and platelet aggregation in patients with PKU was detected [1].

References:

There is no doubt about the tremendous progress which has been made in medicine as well as in all disciplines of natural sciences since Følling’s discovery in 1934. And it is also a fact that phenylketonuria – the first known defect in amino acid metabolism, had as a unique example large impacts on the research in various fields of medicine and natural sciences.

However, taking into consideration about 80 years of scientific progress and gain of experience, we are far away from an optimal care for the about 14,800 PKU patients across Europe. Some important gaps in care will briefly be discussed.

A European evidence based guideline is urgently requested, including greater consistency in both treatment goals and outcome measures. This will give patients and carers greater certainty of receiving optimal care and help to achieve good compliance to life-long treatment.

The need for a consensus paper will be discussed in more details.
Oral communications
Background
Sapropterin dihydrochloride, the synthetic form of tetrahydrobiopterin (BH$_4$), is an approved drug for treatment of phenylalanine hydroxylase (PAH) deficiency causing phenylketonuria. The compound was shown to correct misfolding of the PAH protein but structural mechanistic insight into the pharmacological chaperone effect is still scarce. Lack of BH$_4$ responsiveness for a significant share of PAH missense mutations and unfavorable pharmacokinetic properties of the small molecule substantiate the need for alternative compounds.

Aims
To gain mechanistic insight into the pharmacological chaperone effect and to identify essential structural elements for PAH stabilization we aimed to elucidate the BH$_4$ structure-function relationship.

Material and methods
The individual contributions of the molecule’s different structural moieties, the pyrimidopyrazin ring system and the dihydroxypropyl side-chain, were dissected by comparing ligand-binding induced effects of BH$_4$ and its derivatives BH$_2$ and sepiapterin as well as of the synthetic cofactor 6-MPH$_4$ on biochemical and biophysical properties of the PAH protein.

Results
The structural moieties of tetrahydrobiopterin have distinct impact on conformation of the target protein. Two partly countervailing molecular movements were observed where induction of a compact t-state-like conformation was governed by the pyrimidopyrazin ring system and reduced solvent accessibility of hydrophobic groups was triggered by the dihydroxypropyl side-chain. In addition, the magnitude of these movements was influenced by the redox status.

Conclusions
This study provides insight into the relationship of tetrahydrobiopterin structural moieties to the molecule’s function as a pharmacological chaperone. Both parts of the molecule are of relevance for stabilization of the PAH protein. This knowledge may assist future structure aided drug design to develop optimized therapeutic compounds. Moreover, our results are in line with the notion that structural alterations induced by different mutations in the PAH gene may require different pharmacological correction. This may pave the way for the development of small molecules with improved efficacy in the context of personalized medicine strategies for phenylketonuria patients.
Introduction
Phenylalanine (Phe) deficiency and its clinical manifestations had been previously described mostly as sporadic case reports dating back to the 1960’s and 1970’s. In these reports, low plasma Phe levels were associated with listlessness, eczematous eruptions and failure to gain weight, most often in infants in their first year of life.

Case Report
Herein we describe a 9 month old patient with known phenylketonuria, who presented with an unusual constellation of symptoms, including severe erythema and desquamation, alopecia, keratomalacia, corneal perforation, failure to thrive and prolonged diarrhea. The diagnostic possibilities of acrodermatitis enteropathica and vitamin deficiencies were ruled out, and further investigation into her medical history led to the conclusion that during the weeks preceding the hospitalization, the patient’s diet consisted of the phenylalanine-free medical formula alone, without addition of a standard infant formula or food as recommended.

Objective
Following this experience, and due to the relative paucity of data regarding the clinical manifestations of low serum phenylalanine levels in humans and their putative pathogenetic mechanisms, we sought to further investigate the effects of a phenylalanine-free diet in a mouse model.

Materials and methods
For this purpose, twenty mice were randomly allocated to receive either a Phenylalanine-deficient diet (n=10) or a normal diet (n=10). Laboratory tests were obtained including complete blood count and electrolyte studies. Finally, necropsies and histopathological examinations of different tissues were performed, either early after diet initiation (n=4), late after diet initiation (n=8) or following reintroduction of normal diets (n=8).

Results
Gross lesions noted on necropsy in the Phe-deficient mice included scruffy coat, tendency toward weight loss, a reduction in thymic mass, and most notably severe gastric dilation, all of which were not seen in the controls. Histologic findings included thymic depletion, hepatocellular vacuolation, and in 2 of 6 mice exocrine pancreatic atrophy. No histopathological lesions were evident in the brain, nor were significant lesions in the eyes.

Conclusions
Diagnosis of the iatrogenic condition of phenylalanine deficiency, which manifests in gastrointestinal, dermatological and ocular findings, requires a high index of suspicion. Mice fed a phenylalanine-deficient diet display to some extent similar organ involvement, although no eye abnormalities were evident.
Introduction
Mental retardation in Phenylketonuria (PKU) is prevented by treatment with a strict, unpalatable and socially demanding phenylalanine restricted diet with amino acid supplementation. Residual phenylalanine hydroxylase activity enhancement with its co-factor tetrahydrobiopterin (BH₄) is a novel treatment that increases dietary tolerance for phenylalanine in some patients and thus permits some dietary relaxation. Relaxation of diet may improve patients’ health related quality of life (HRQoL). This prospective cohort study aims to evaluate the HRQoL of PKU patients and the effects of BH₄ treatment on the HRQoL of BH₄ responsive patients.

Methods
Patients aged 4 years and older, diagnosed through newborn screening and treated early and continuously, were recruited from eight metabolic centres. Pediatric patients, their mothers and adult patients completed validated web-based HRQoL questionnaires aimed at the general population (PedsQL, TA AQOL) and at people with chronic disorders (DISABKIDS). Questionnaires were completed twice: before testing for BH₄ responsivity (T1) and after a period of at least 17 months (T2).

Results
Questionnaires were completed by 69 patients (aged 4-44 years) at T1 and again by 45 patients at T2. Our complete cohort of patients demonstrated a normal HRQoL when compared to the general population. However, analysis by domain demonstrated some differences: Children aged 13-18 years old reported significantly higher scores on PedsQL domains “total and psychosocial functioning”. Adult patients reported significantly lower scores on the TAAQOL domain for “cognitive function”. Of our cohort ten patients proved BH₄ responsive. All 10 patients could relax their diet, with full normalisation of diet in 4. HRQoL scores of the BH₄ responsive patients during BH₄ treatment did not demonstrate a significant difference compared to their scores at T1 nor compared to the scores of the unresponsive patient group at T2.

Discussion and conclusion
We demonstrated a normal HRQoL in a Dutch cohort of 69 patients with PKU. The small group of BH₄ responsive patients already had a normal HRQoL at baseline measurement and we were not able to demonstrate an improved HRQoL in these patients during BH₄ treatment. This is in contrast with clinical observation of treating professionals. Important limiting factors of the study are that the generic and chronic-health-conditions questionnaires used may not be sensitive enough to demonstrate more disease-specific consequences of PKU, and the small sample size due to the rarity of the disease.
Introduction
The treatment of phenylketonuria (PKU) constitutes a strict and socially demanding phenylalanine (Phe) restricted diet with amino acid supplementation. Management of PKU is time-consuming and presents an economic burden. This cross-sectional study aims to evaluate out-of-pocket-costs (OOPC) and time burden of living with PKU for adult patients and caregivers of pediatric patients in the Netherlands.

Methods
A web based questionnaire was developed based on the Short Form Health & Labour Questionnaire, literature review and expert opinion. Early and continuously treated adult patients and caregivers of early and continuously treated pediatric patients aged four years and older from seven metabolic centers were invited to complete the questionnaire online. The questionnaire related to OOPC and time burden resulting from medical treatment, regular blood testing, organizing and preparing the diet, obtaining dietary supplements, potential impact on productivity, as well as restrictions on patient and caregiver social lives.

Results
Caregivers of 24 children with PKU and 22 adult patients participated. OOPC are considerable with an annual median of € 604 per patient (interquartile range, the distance between the first and third quartile, [IQR] 28 – 1206). Because amino acid supplements are reimbursed in the Netherlands, OOPC constituted mainly expenditure of low-protein food products. Caregivers of severe patients had significant higher annual OOPC than those of mild patients (median € 1309 [IQR 1054-1793] versus € 5 [IQR 0-486]). Adequately controlled patients (Phe levels within target range >70% of the time) had significant lower annual OOPC than inadequately controlled patients (median € 300 [IQR 5-614] versus € 1132 [IQR 285-1686]).

The time burden for patients and caregivers includes time spent on dietary management (mainly) and Phenylalanine blood testing, and is considerable with a median of 265 [IQR 129–588] hours/year. Caregiver time burden was significantly higher than that for adult patients: up to a median of 679 [IQR 391–927] hours annually, of which 13% is spent on supervising amino acid intake of their child. Time burden was approximately two times higher for severe versus mild patients. Adequately controlled patients had a significantly lower time burden than inadequately controlled patients (median 169 [107–326] versus 454 [IQR 219–826] hours/year, respectively).

Conclusion
PKU management is associated with considerable cost and time burden for the adult patient and the caregiver of the pediatric patient. Both severity of disease and inadequate control lead to higher costs and time burden.

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OC5 - Testing neonatal BH$_4$ responsiveness: treat first, diagnose later

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Introduction
It is unknown whether the 8- or 24-hour neonatal BH$_4$ loading test, intended to distinguish BH$_4$ deficiencies from PAH deficiency, is also adequate to diagnose long-term BH$_4$ responsiveness in PAH deficiency.

Aims
To compare the predictive value of the neonatal (T=8/T=24) versus the 48-hour BH$_4$ loading test and long-term BH$_4$ responsiveness.

Methods
Data on the neonatal BH$_4$ -loading test (≥1991, 20 mg/kg) at T=8 (n=86) and T=24 (n=5) were collected and compared with the 48-hour BH$_4$ -loading test and long-term BH$_4$ responsiveness at later age. In both test ≥30% was used as cut-off.

Results
The median (Q1 – Q3) age at diagnosis (hospital) was 9 (7 – 11) days. The median (Q1 – Q3) age at the 48-hour BH$_4$ -loading test was 11.8 (6.6 – 13.8) years. Of 86 patients at T=8, 60 patients had the same negative result (n=41 48h test <30% and of 19 remaining patients, n=2 no long-term treatment, n=6 not long-term responsive and n=11 long-term responsive), eight the same positive result (all long-term responsive), eleven patients had positive present with negative neonatal results (n=10 long-term responsive, n=1 no long-term treatment) and seven had negative present and positive neonatal results (n=1 48h test <30%, n=1 no long-term treatment and n=5 long-term responsive). Of 5 patients at T=24, two patients had the same negative result (both 48h test <30%), one the same positive result (long-term responsive) and two had positive present and negative neonatal results (n=1 not long-term responsive, n=1 long-term response). Three patients had 20-30% Phe decrease at T=8 neonatally, all of them showed long-term BH$_4$ responsiveness.

Conclusions
Conclusions: Positive 8- and 24-hour neonatal BH$_4$ -loading tests are good predictors for long term BH$_4$ -responsiveness, but more data are needed to determine the optimal threshold. A negative neonatal test of eight hours and even 24 hours does not rule out long-term BH$_4$ -responsiveness. Longer delays in dietary treatment are disputable, therefore, other diagnostic possibilities should be developed, e.g. the neonatal BH$_4$ withdrawal test: starting treatment with diet and BH$_4$, withdrawing BH$_4$ some weeks later, to diagnose BH$_4$ responsiveness.
Introduction
Treatm ent of Phenylketonuria (PKU) focuses on keeping phenylalanine (Phe) levels below certain limits, as high Phe levels predict poor neurocognitive outcomes. It is still unclear whether relaxation of dietary control throughout adolescence and during adulthood adversely affects such outcomes after the possibly protective effects of a strict diet throughout childhood. Evidence is also mixed on the presence of behaviour problems and whether cognitive difficulties might predict such problems.

Aim
To examine executive functions and behavioural problems in daily life settings in a group of adults with PKU, who had been treated early- and continuously according to Dutch guidelines [which also incorporated a less strict diet from adolescence onwards].

Material and methods
Seventeen adult PKU-patients (10 female, mean age 28.2, SD 6.7) and 8 controls (5 female, mean age 27.2 years, SD 6.6) filled out questionnaires measuring executive functions (Behavior Rating Inventory of Executive Functions – Adult version, BRIEF-A) and behavioural problems [Adult Self-Report, ASR]. Controls and PKU patients were compared on the BRIEF- and ASR-dimensions using independent-samples t-tests, and associations between BRIEF-dimensions and ASR-dimensions were examined using Pearson correlations.

Results
PKU-patients had mean concurrent Phe levels of 749 µmol/l (SD 468, min. 106, max. 1760). Compared to controls, PKU-adults reported more “Physical complaints” (p = .022), “Aggressive behaviour” (p = .047), “Internalizing behaviour” (p = .021), and had a higher total problem score on the ASR (p = .041). There was a trend for the “Anxious/depressed”-dimension (p = .090). There were no significant group differences on the BRIEF. For the PKU-group, many BRIEF- and ASR-dimensions were significantly related (significant correlations ranged between .50 and .93), whereas for controls fewer (and weaker) associations were observed.

Conclusions
Despite strict treatment and monitoring from infancy onwards, adults with PKU differed from controls regarding behavioural problems. This was not only the case for internalizing behaviour problems but for externalizing behaviour problems as well. Strict treatment throughout childhood and adolescence may have resulted in relatively good executive function development, as no group differences were observed in that respect. Still, the associations between executive functions and behaviour, which were particularly evident for PKU-patients, suggest that their behaviour problems could be influenced by cognitive abilities. Therefore, it may be concluded that monitoring of executive functioning should continue throughout life in PKU-patients as they might present a means to affect behaviour problems. Taking into account the small sample size, these results should be considered preliminary.

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All authors confirm independence from the sponsors; the content of this presentation has not been influenced by the sponsors.
Introduction
In the past few years, the introduction of tetrahydrobiopterin (BH₄) as therapeutic alternative to diet restriction for the treatment of PKU, has allowed a significant reduction of phenylalanine blood levels in patients, with a consequent improvement of the metabolic control and an increased freedom from the dietary regime. The aim of the study is to evaluate the quality of life (QoL) and the cognitive profile including attentive and executive abilities in a group of BH₄-responsive PKU patients on long-term treatment with BH₄.

Materials and Methods
Twenty-two BH₄-responsive PKU patients (8 adults, age range 18-35, and 14 pediatric, age range 4-18) on BH₄ therapy (Tetrahydrobiopterin and Sapropterin, dose 10 mg/Kg/die) for more than 12 months were enrolled. The mean value of Phe and Tyr at the time of the visit were 386 μmol/L (S.D. +/- 222) and 48 μmol/L ( +/- 6) respectively. In particular, the paediatric patients group had a mean value of Phe and Tyr of 314 μmol/L (+/- 127) and Ty 49 μmol/L (+/- 7) respectively; in the past year, the mean annual level of Phe was 296 μmol/L (+/-117) and Tyr 55 μmol/L (+/-13). The adult patient group had a mean value of Phe and Tyr at the time of the visit of 540 μmol/L (+/-306) and 46 μmol/L (+/-6) respectively. In the past year the mean value of Phe was 483 μmol/L (+/-198), and Tyr was 57 μmol/L (+/-8). QoL has been evaluated using different scales depending on the patient’s age: PedsQL (both from the patient’s and the parent’s point of view) and WHOQoL-100. The cognitive functions were assessed using the WAIS-R, WISC-III, GMDS-ER and an evaluation of the attentive abilities.

Results and Conclusions
In every scale of the instruments for QoL, it emerges a generally good quality of life for all patients on long-term treatment with BH₄. The evaluation of the aspects deemed to constitute QoL that we examined highlighted: a) adult patients showed a generally good satisfaction profile, better for specific aspects linked to sleep/food/free time and social well-being b) pediatric patients perceive a better physical well-being and underline the importance of the emotional aspect; in one patient we observed slight anxiety. From the parents’ point of view, the perception of general QoL of the child is good, however, in two parents we observed anxiety and in one parent slight depression. As far as the executive functions are concerned, the total of the patients, both in the adult and in the pediatric group, showed a performance within the normal range. In particular we have shown that during the administration of the executive functions battery, the percentage of adult subjects with the max score was 71 % (6/8). In conclusion, QoL was perceived to be good in all patients in treatment with BH4, especially adult patients long-term treatment with BH₄. In the case of paediatric patients, it is important to consider the perception of the quality of life not just of the patient, but also of the parents.
Among more than 120 patients in our PKU cohort, we report two cases of PKU diagnosis in two mothers of our PKU patients screened in the neonatal period. The first diagnosis was done in a mother of a PKU child who presented microcephaly and developmental delay despite excellent metabolic control. Considering maternal PKU as a possible etiology of the child syndrome, atypical PKU was diagnosed in this mother born before the implementation of the neonatal screening in France. The second case concerns a woman diagnosed at birth with mild Hyperphenylalaninemia (HPA) and who was lost of follow up. Her first child was screened for PKU and the mild HPA of the mother was re discovered at this occasion. Fortunately, the Phe levels did not reach 600µm/L under normal diet and her child is strictly normal.

On 197 different mutations observed in the French cohort (697 patients genotyped), the residual activity (RA) is known for 49% of them. 14.2% and 27% of these mutations induce respectively a RA between 1 and 10% and above 10%. Consecutively, these mutations can induce silent hyperphenylalaninemia or very mild clinical presentation allowing normal social and familial life.

As the frequency of PKU in France is about 1/16 000 individuals, the carrier frequency is about 1/200 individuals. As mild mutations can be not clinically expressed, there is a risk of undiagnosed PKU in mothers and fathers of screened PKU patients. Based on Hardy-Weinberg law, we can calculate the proportion of patients born from parents of whom one is homozygous or compound heterozygous for two mutations. Depending upon the ratio of moderate versus severe mutations, the percentage of patients born from at least one HPA parent may vary from 0.7 to 1.2%. Considering the rate of mutations with some residual activity around 40%, the real risk to have a (more or less) silent second mutation may vary from 0.28 to 0.58%. This average risk of 0.4 % justifies the systematic screening in parents of patients with HPA. In fact, this risk of undiagnosed PKU is very low in countries where the neonatal screening is implemented for more than 40 years, but it could be quite high in countries like Turkey in which PKU prevalence is very high (PKU: 1/4000 and carrier frequency of 1/30) and where the systematic neonatal screening is implemented for less than 30 years.
Poster presentations
PP1 - Body composition and phase angle by bioelectrical impedance analysis in patients with phenylketonuria

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Background
Phenylketonuria (PKU) is caused by the enzyme deficiency of phenylalanine hydroxylase (PAH) resulting in high blood and tissue concentrations of phenylalanine (Phe). Treatment means a Phe restricted diet supplemented with a Phe-free L-amino acid mixture. PKU and/or the diet may impair normal growth and body composition. The bioelectrical impedance analysis (BIA) determines the body composition in general and phase angle (PA). PA is considered an indicator of nutritional status and predictive of impaired clinical outcome and mortality in several disease states. Higher values reflect better cell function.

Objectives
To investigate body composition and PA in PKU patients compared to healthy controls.

Material and Methods
Body composition and PA were assessed by BIA in 21 PKU patients who were treated in Hospital de Clínicas de Porto Alegre (HCPA) and in 13 healthy age and gender-matched controls. Correlations were studied between PA and, weight, length, basal metabolic rate (BMR), Phe concentrations and body composition.

Results
No differences were found in body composition of PKU patients compared to controls. No significant difference in PA was found in PKU patients compared to healthy controls (6.38±0.92 vs 6.97±0.94; p=0.087). In both groups, strong positive correlations were observed between PA and weight, basal metabolic rate (BMR) and fat free mass in kg, whereas PA and length moderately correlated. In PKU-patients, PA and Phe concentrations were significantly correlated.

Conclusions
PA does not differ between patients and controls, but is correlated with Phe concentrations. Our study was limited by the small sample size and lack of standard PA values. Further research is needed to enhance the sample size and create cutoff points to determine the potential of PA upon clinical assessment.
6-pyruvoyl-tetrahydropterin synthase (PTPS) deficiency is an autosomal recessive disorder with the presentation of hyperphenylalaninemia. Those babies who were born by a hyperphenylalanemia mother would have multiple anomalies. By using tetrahydrobiopterin (BH$_4$), levodopa (L-dopa), and 5-hydroxytryptophan (5-HTP), we can achieve a satisfactory serum phenyalanine (Phe) control in PTPS deficiency patient. In this presentation, we present a severe form PTPS deficiency female patient who was diagnosed in her infancy and received medical treatment since 1 month-old. Her serum Phe was within acceptable result under the management of BH$_4$, L-dopa/carbidopa and 5-HTP. However, because of being afraid of the side effect, she stopped taking 5-HTP since her 5 years old. At the age of 19 and 21 year-old, she experienced twice successful pregnancies while serum Phe level was controlled by BH$_4$ and L-dopa/carbidopa without diet restriction. She gave birth to two babies who have no congenital anomaly. The developmental evaluation of her children at the age of 21 month-old and 3 year-old revealed normal respectively. Our satisfactory results encourage PTPS deficiency female patients to be pregnant and have their own normal babies.
Background
Phenylketonuria (PKU) treatment, although focused in nutritional recommendations – restriction in natural protein and phenylalanine (Phe) [1] – cannot be dissociate of food aspects. Therefore, the dietetic treatment consists in a semi-synthetic low-phenylalanine diet including Phe-free amino acids mixtures and a wide range of dietetic low-protein products, as well as strictly controlled amounts of natural foods, such as fruits and vegetables. If on one hand it is considered to be a protein restricted diet, on the other, this type of therapy assumes characteristics of a vegan diet, with all the concerns and nutritional implications arising from this dietary pattern, as a result from the restriction of proteins of animal origin [2]. It is of utmost importance to know in detail the nutritional composition of foods, natural or processed, in order to facilitate the nutritional and dietetic prescription, as well as a good metabolic control of the patient.

Aims
Our goal was to study the composition of some recipes specifically planned for PKU patients obtained almost from Portuguese books [3,4], with particular focus in its protein and phenylalanine contents.

Materials & methods
We selected and cooked 10 recipes using common ingredients and low-protein dietetic products, following the instructions for each recipe. Every sample was analyzed by the Kjeldahl method and by High Performance Liquid Chromatography, to quantify the protein and Phe contents, respectively. Analytical results were then compared with protein and Phe contents calculated by a specific software tool methodology [5], using several Food Composition Databases (FCD).

Results
The protein content of the studied recipes varied between 0.67 and 3.15 g/100 g, and Phe content between 3.89 e 158.51 mg/100 g. When comparing analytical results of protein and Phe contents with calculated values obtained by a specific software tool methodology, the latter tends to overestimates both protein and Phe content.

Conclusions
FCD are essential tools to plan PKU diets. Nevertheless, considering our results and the deviations observed, it is necessary to use these tools with some precaution and carefully adapting it to each reality.

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References:
Background
Phenylketonuria (PKU; OMIM 261600) dietary treatment consists in a semi-synthetic low-phenylalanine diet, as well as strictly controlled amounts of low protein natural foods, including fruits and vegetables, as well as dietetic low protein products. The type of foods included in the diet gives it the features of a vegan food pattern [1]. One of the concerns associated with the food pattern of PKU patients, also recognizable in vegan diets, is the deficiency of some trace elements, like iron and zinc.

Aims
In this work, our goal was to study the content of iron and zinc of 10 recipes, including low protein recipes [2,3] specifically planned for PKU patients, as well as natural daily basic cooked foods.

Materials & methods
The recipes were cooked following its instructions and the determination of the trace elements was performed by atomic absorption spectrometry (AAS), after acid microwave assisted digestion [4] of the homogenized samples.

Results
The mean values for the recipes iron content varied between 0.10 and 2.82 mg/100 g sample, and, for zinc content, between 0.08 and 0.48 mg/100 g sample. Analytical results from the recipes were then compared with the calculated iron and zinc contents obtained with a specific software tool [5], using 3 different Food Composition Databases (FCD).

Conclusions
We concluded that the later methodology overestimates both trace elements content. FCD are essential tools, but its use has to be precautious.

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References:
The mainstay of PKU treatment is a phe-restricted diet; however, new studies show that the diet alone may not prevent significant problems in some PKU individuals including poor myelination of the brain, white matter anomalies and lower bone density, even when good metabolic control is maintained. Compounding the issue, diet compliance is a very real problem.

Advances in PKU treatments are being introduced and studied around the world. Parents of PKU babies are assured of “good” outcomes but are looking for access to sapropterin and hopeful for future treatments such as Peg-Pal. In North America, treating PKU children under 4 years of age with sapropterin is becoming more and more common.

This case review looks at R. Pallone and how sapropterin helped with management of her PKU from 20 months to 5 years of age. At birth a BH4 load resulted in a drop in her blood-phe level. Patient Pallone at 20 months had phe levels that were stable but occasionally fell outside the clinical target (120-360 μmol/L). She was enrolled in the PKU-015 study in Vancouver, BC and sapropterin has lowered her blood-phe levels so that they are consistently between 120-240 μmol/L while at the same time doubling her daily phe tolerance. The presentation will show the effect sapropterin had on her mean blood-phe level, the standard deviation of blood-phe levels, the phe tolerance of the patient as well as the neuro-cognitive outcomes.

A significant concern of her parents was how to manage the increased phe tolerance without normalizing her diet - a potential disaster given the current lack of government funding in Canada and the high price of sapropterin. Even without the benefit of diet normalization, this case review clearly shows the benefits of treating responding PKU children under four with sapropterin.
Improving the patient’s life through medical education

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