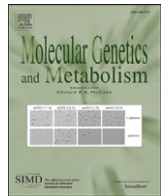




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Conference Proceedings

Phenylketonuria Scientific Review Conference: State of the science and future research needs<sup>☆</sup>

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**Abbreviations:** AAV, adeno-associated virus; ACMG, American College of Medical Genetics and Genomics; AHRQ, Agency for Healthcare Research and Quality; BH<sub>4</sub>, tetrahydrobiopterin; BMD, bone mineral density; CDE, Common Data Element; CHD, congenital heart disease; CNS, central nervous system; COA, clinical outcome assessment; DXA, dual-energy X-ray absorptiometry; DHA, docosahexaenoic acid; DHP, dihydropteridine reductase; DRI, Dietary Reference Intake; EPC, Evidence-based Practice Center; FDA, U.S. Food and Drug Administration; GMDI, Genetic Metabolic Diagnostics International; GMP, glycomacropeptide; HPA, hyperphenylalaninemia; IEM, inborn errors of metabolism; IQ, intelligence quotient; LNAA, large neutral amino acids; MPKCUS, Maternal PKU Collaborative Study; MPKUS, maternal PKU syndrome; MRI, magnetic resonance imaging; NBSTRN, Newborn Screening Translational Research Network; NDSI, nutrition and dietary supplement interventions; NICHD, Eunice Kennedy Shriver National Institute of Child Health and Human Development; NIH, National Institutes of Health; ODS, Office of Dietary Supplements; ORDR, Office of Rare Diseases Research; PAH, phenylalanine hydroxylase; PAL, phenylalanine ammonia lyase; PEG, polyethylene glycol; Phe, phenylalanine; PKU, phenylketonuria; PKUDOS, Phenylketonuria [PKU] Demographic, Outcomes, and Safety Registry; RUSP, Recommended Uniform Screening Panel; SIMD, Society for Inherited Metabolic Disorders; SOE, strength of evidence; START, Sapropterin Therapy Actual Response Test; Tyr, tyrosine.

<sup>☆</sup> The findings and conclusions of this report are those of the authors and do not necessarily represent the views of the National Institutes of Health, the U.S. Food and Drug Administration, the Centers for Disease Control and Prevention, the Centers for Medicare & Medicaid Services, the Agency for Healthcare Research and Quality, or the U.S. Department of Health and Human Services.

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## ABSTRACT

New developments in the treatment and management of phenylketonuria (PKU) as well as advances in molecular testing have emerged since the National Institutes of Health 2000 PKU Consensus Statement was released. An NIH State-of-the-Science Conference was convened in 2012 to address new findings, particularly the use of the medication sapropterin to treat some individuals with PKU, and to develop a research agenda. Prior to the 2012 conference, five working groups of experts and public members met over a 1-year period. The working groups addressed the following: long-term outcomes and management across the lifespan; PKU and pregnancy; diet control and management; pharmacologic interventions; and molecular testing, new technologies, and epidemiologic considerations. In a parallel and independent activity, an Evidence-based Practice Center supported by the Agency for Healthcare Research and Quality conducted a systematic review of adjuvant treatments for PKU; its conclusions were presented at the conference. The conference included the findings of the working groups, panel discussions from industry and international perspectives, and presentations on topics such as emerging treatments for PKU, transitioning to adult care, and the U.S. Food and Drug Administration regulatory perspective. Over 85 experts participated in the conference through information gathering and/or as presenters during the conference, and they reached several important conclusions. The most serious neurological impairments in PKU are preventable with current dietary treatment approaches. However, a variety of more subtle physical, cognitive, and behavioral consequences of even well-controlled PKU are now recognized. The best outcomes in maternal PKU occur when blood phenylalanine (Phe) concentrations are maintained between 120 and 360  $\mu\text{mol/L}$  before and during pregnancy. The dietary management treatment goal for individuals with PKU is a blood Phe concentration between 120 and 360  $\mu\text{mol/L}$ . The use of genotype information in the newborn period may yield valuable insights about the severity of the condition for infants diagnosed before maximal Phe levels are achieved. While emerging and established genotype-phenotype correlations may transform our understanding of PKU, establishing correlations with intellectual outcomes is more challenging. Regarding the use of sapropterin in PKU, there are significant gaps in predicting response to treatment; at least half of those with PKU will have either minimal or no response. A coordinated approach to PKU treatment improves long-term outcomes for those with PKU and facilitates the conduct of research to improve diagnosis and treatment. New drugs that are safe, efficacious, and impact a larger proportion of individuals with PKU are needed. However, it is imperative that treatment guidelines and the decision processes for determining access to treatments be tied to a solid evidence base with rigorous standards for robust and consistent data collection. The process that preceded the PKU State-of-the-Science Conference, the conference itself, and the identification of a research agenda have facilitated the development of clinical practice guidelines by professional organizations and serve as a model for other inborn errors of metabolism.

## 1. Introduction

The purpose of this paper is to provide a synthesis of the activities leading up to and the findings garnered from a conference that was held at the National Institutes of Health (NIH) on scientific advances in the diagnosis and treatment of phenylketonuria (PKU) (see Table 1 for definitions of key terms used in this paper). At the conclusion of the conference, a research agenda was proposed that outlined the needs, priorities, and next steps to be taken to improve outcomes for individuals with PKU.

### 1.1. Background of the initiative

In October 2000, NIH convened a Consensus Development Conference and issued an NIH Consensus Statement titled: "Phenylketonuria (PKU): Screening and Management" [1]. The 2000 conference and the subsequent statement arose from the NIH Consensus Development Program (<http://consensus.nih.gov/>) under which experts convened to evaluate available scientific information and develop a statement that would advance understanding of the issues in question and be useful to health professionals and the public at large. Hence, consensus statements were considered independent reports of the consensus panel and not a policy statement of NIH or the federal government. The 2000 PKU Consensus Statement was prepared by a nonfederal panel of experts based on presentations given by investigators during the conference, questions and statements from conference attendees, and closed deliberations by the panel. The 2000 NIH Consensus Development Statement on PKU included the following highlights:

- Metabolic control is necessary across the lifespan of individuals with PKU.
- A comprehensive, multidisciplinary, integrated system is required for the delivery of care to individuals with PKU.
- Consistency and coordination are needed among screening, treatment, data collection, and patient support programs.
- It is recommended that phenylalanine (Phe) levels below 360  $\mu\text{mol/L}$  be achieved at least 3 months before conception.
- Research on nondietary alternatives to treatment of PKU is strongly encouraged.

- All persons with PKU should have mutation analysis for initial diagnosis, genetic and management counseling, follow-up, and long-term prognosis.

Since the 2000 statement's release, a number of important developments in the identification and treatment of PKU have emerged, including new therapeutic and management modalities, advances in molecular testing, and new findings from over a decade of clinical and epidemiological research. To understand and synthesize this new body of research, inform clinical treatment decisions, and develop a future research agenda based on gaps in current knowledge, NIH held a Scientific Review Conference from February 22 to 23, 2012, sponsored by the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), the Office of Rare Diseases Research (ORDR; now in the National Center for Advancing Translational Sciences), and the Office of Dietary Supplements (ODS; in the Office of the Director of NIH). In contrast to the condensed nature of the work that was done for the 2000 NIH Consensus Development Conference, the 2012 State-of-the-Science Conference was preceded by a year-long working group process. Five working groups, each composed of 8–12 topical experts, public members, and federal partners, met regularly over the year via teleconference calls to address important questions regarding diagnosis, treatment, and long-term outcomes in PKU (Supplementary Table 1). The working groups were organized more specifically around the following topics:

- Long-term outcomes and management across the lifespan
- PKU and pregnancy
- Diet control and management
- Pharmacologic interventions
- Molecular testing, new technologies, and epidemiologic considerations.

In a parallel and independent activity, an Evidence-based Practice Center (EPC) supported by the Agency for Healthcare Research and Quality (AHRQ) conducted a systematic review of the use of adjuvant treatments for PKU during the time the working groups were addressing their topics. These adjuvant treatments included sapropterin dihydrochloride (sapropterin) and large neutral amino acids (LNAA). Sapropterin is a synthetic form of tetrahydrobiopterin ( $\text{BH}_4$ ), the naturally occurring co-factor for phenylalanine hydroxylase (PAH), the

**Table 1**  
Definitions of key terms used in this paper.

Term	Definition
$\text{BH}_4$ responsiveness	The lowering of blood Phe concentrations in response to a trial of sapropterin/ $\text{BH}_4$ . At the time of approval of sapropterin by the FDA, a reduction in blood Phe concentration of $\geq 30\%$ defined responsiveness. Some groups have defined $\text{BH}_4$ -responsiveness as a smaller drop in blood Phe concentration.
Hyperphenylalaninemia (HPA)	Elevated concentrations of phenylalanine in the blood.
Maternal PKU syndrome (MPKUS)	A constellation of fetal abnormalities due to the teratogenicity of excessive Phe from the mother with PKU. These findings in the offspring of a woman with elevated blood Phe levels include microcephaly, congenital heart disease, and fetal growth restriction [76].
Phenylalanine hydroxylase (PAH) deficiency	PAH deficiency is inherited in an autosomal recessive manner and results in a deficiency of phenylalanine hydroxylase, an enzyme that catalyzes the formation of Tyr from the essential amino acid Phe [331]. PAH requires the co-factor, 6R-tetrahydrobiopterin ( $\text{BH}_4$ ). PAH deficiency encompasses a spectrum of disorders that includes classic PKU, mild PKU, and mild hyperphenylalaninemia [266]. While the term phenylketonuria (PKU) has historically been used, PAH deficiency more accurately encompasses the variable clinical phenotypes.
Medical food	A medical food, as defined by the Orphan Drug Act, is "a food which is formulated to be consumed or administered enterally under the supervision of a physician and which is intended for the specific dietary management of a disease or condition for which distinctive nutritional requirements, based on recognized scientific principles, are established by medical evaluation." <a href="http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/MedicalFoods/default.htm">http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/MedicalFoods/default.htm</a>
Phenylketonuria (PKU)	Describes the presence of phenylketones in the urine. This term is also used to describe the condition that affects a patient with phenylalanine hydroxylase deficiency.
Phe challenge	An individual with PAH deficiency can be given a known quantity of dietary Phe followed by obtaining blood Phe concentrations in order to ascertain the degree of rise in blood Phe levels. Phe challenges are no longer used in the newborn period to categorize the severity of PKU, but they are used in sapropterin-responsive individuals to determine the extent to which the dietary Phe restriction can be liberalized [162,163].
Phe tolerance	The amount of dietary Phe an individual can tolerate while maintaining blood Phe concentrations within the accepted treatment range [188].
Sapropterin dihydrochloride	Synthetic form of tetrahydrobiopterin marketed as Kuvan®.
Tetrahydrobiopterin ( $\text{BH}_4$ )	Co-factor essential for a number of enzyme activities, including PAH. Also known as 6R- $\text{BH}_4$ .

enzyme deficient in individuals with PKU. LNAA act by competing with Phe at both the gut and the blood-brain barrier and thereby theoretically decrease the brain concentration of Phe. The conclusions from the EPC activity were also presented at the conference.

### 1.2. Conference agenda (see Supplementary Fig. 1)

The first day of the conference began with opening remarks from Melissa Parisi, M.D., Ph.D. (NICHD), Alan Guttmacher, M.D. (NICHD), Stephen C. Groft, Pharm.D. (ORDR), and Paul M. Coates, Ph.D. (ODS). Dr. Parisi stated that the conference was open to all, including clinicians, researchers, individuals with PKU and their families, industry representatives, and advocacy organizations. Dr. Guttmacher, director of NICHD and a pediatric medical geneticist, remarked that the science had come far in improving outcomes for PKU, as he related the story of a late-diagnosed patient born a few years before newborn screening became available. He also acknowledged how much further there is to go to answer the truly important questions that will improve care for patients with PKU and their families. Dr. Groft, director of ORDR, commented that disorders such as PKU require collaborative efforts across many offices and individuals. He explained that the outcomes of this conference were to identify and develop research questions; encourage investigators to answer those questions through research; and, ultimately, support the development of new clinical practice guidelines. Through these efforts, the goal of providing optimal care for those with PKU will be achieved. Dr. Coates, director of ODS, explained that ODS had provided nutritional expertise in inborn errors of metabolism (IEM) to the conference because many of the products used in the management of these conditions are dietary supplements.

Keynote speaker R. Rodney Howell, M.D., gave a brief history of PKU (see Section 1.3 below and Supplementary Fig. 2) from its discovery to treatment development to current NIH studies. He presented an award to Jean Koch in honor of the contributions made by her late husband, Richard Koch, M.D., to advance treatments for individuals with rare metabolic disorders, including PKU.

Following the keynote address, the results from the EPC report (see Section 2) were presented. An overview of the NIH working group process and definitions for PAH deficiency and other forms of PKU was given. The five working groups presented their findings (see Section 3), followed by a presentation on new treatments for PKU given by Cary O. Harding, M.D. (see Section 4.1).

The second day began with two panel discussions — perspectives from industry and advocacy communities and perspectives from the international community (see Sections 4.2 and 4.3). Sandra Sirrs, M.D., gave an overview of issues pertaining to transitioning adolescents with PKU into the adult health care system (see Section 4.4). Breakout sessions for each working group topic gave conference participants an opportunity to provide input, which was then reported to the entire audience by a representative from each of the original working groups. Anne R. Pariser, M.D., from the U.S. Food and Drug Administration (FDA) Office of New Drugs, provided the FDA perspective on the need for sound clinical studies (see Section 4.5). The conference concluded with the development of a research agenda that included needs, priorities, and next steps to improve outcomes for patients with PKU (see Section 5). The conference webcast is available at: [https://www.team-share.net/Phenylketonuria\\_Scientific\\_Review\\_Conference/Overview.aspx](https://www.team-share.net/Phenylketonuria_Scientific_Review_Conference/Overview.aspx).

### 1.3. History of discovery and treatment of PKU (R. Rodney Howell, M.D.)

Eighty years ago, Dr. Asbjørn Følling identified children with profound developmental delay who were excreting large amounts of phenylpyruvic acid in their urine [2], secondary to the build-up of Phe in the blood. Because Phe was known to be an essential amino acid, treating these children with a diet deficient in this amino acid was proposed. In 1951, Drs. Bickel, Gerrard, and Hickmans conducted a

trial of a Phe-deficient diet on a 2-year-old patient with elevated urinary phenylketones; although the diet proved helpful, Dr. Bickel suggested that a better result might be achieved if the diet was initiated in infancy [3]. Over time, the diet for PKU has been refined and currently consists of restricting dietary Phe from natural protein to the amount needed to maintain blood Phe concentrations in the desired treatment range while providing the amount needed for body growth and development and maintenance of biological functions. The bulk of nutrient needs are then met through the use of medical foods [4].

Because PKU is inherited in an autosomal recessive fashion, identifying infants without a family history required the development of an inexpensive and reliable test that could be applied to the entire population. In the early 1960s, Dr. Robert Guthrie developed a protocol to test blood Phe in infants using blood spots dried on filter paper, combined with a bacterial inhibition assay [5]. This elegantly simple test proved to be extremely useful in identifying newborn babies with elevated Phe. The detection of newborns with PKU using this screening test, combined with early treatment, mitigated the cognitive delays associated with PKU [6]. The observation that newborns with PKU could be detected in this fashion was quickly recognized as crucially important. By 1968, 45 states in the United States had legislative mandates for newborn screening with all states and the District of Columbia on board by 1985 [7]. Over time, state public health departments assumed responsibility for newborn screening, providing uniform access and social equity to this important screening program. Other congenital conditions known to cause developmental delay or cause other serious medical consequences, and that could be effectively treated in the newborn period were later added to the newborn screening panel, currently known as the Recommended Uniform Screening Panel (RUSP) (<http://www.hrsa.gov/advisorycommittees/mchbadvisory/heritabledisorders/recommendedpanel/>) [8].

Most of the metabolic disorders on the RUSP utilize medical foods and/or nutrients that have become conditionally essential, function as co-factors in metabolic processes, or remove toxic compounds. Expertise is required to modify the diet to prevent either a deficiency or great excess of a given metabolite. In addition, collaborative research projects largely funded by NIH have shown that a lifespan approach to management of these disorders is essential. Individuals with PKU who discontinued a Phe-restricted diet by age 10 years had much lower college graduation success than did individuals who remained on a Phe-restricted diet [9]. Moreover, the infants of adult women with PKU who were off a Phe-restricted diet had poor medical and cognitive outcomes, while pregnant women with PKU who were treated during pregnancy had healthy babies [10].

The FDA approval of sapropterin for reduction of blood Phe levels in patients with hyperphenylalaninemia (HPA) due to BH<sub>4</sub>-responsive PKU in 2007 has provided additional treatment options for some patients. Sapropterin lowers blood Phe concentrations in a proportion of individuals with PKU with BH<sub>4</sub>-responsive PKU. However, at the present time, diet modification remains the key treatment element.

The NIH Consensus Development Conference in 2000 and this PKU State-of-the-Science Conference in 2012 have summarized our current understanding of PKU as well as the significant gaps that remain in our knowledge of PKU. In a complementary activity, ODS and ORDR have sponsored an initiative to identify research gaps in the safety and effectiveness of nutrition and dietary supplement interventions (NDSI) used to manage persons with IEM, creating a framework to conduct evidence-based research with partnerships involving key stakeholders [11]. All of these activities have been designed to improve long-term outcomes for those with IEM, including PKU, which is the newborn metabolic condition about which we know the most.

### 1.4. Classification of Phe-related disorders

In an effort to use consistent terminology and shared definitions for the various forms of HPA and PKU, a subset of members of the working

groups met to attempt to develop consensus on the categories used to describe these entities. These efforts are summarized in Table 2, which compares different classification schemes for Phe-related disorders. Of note, the use of the term “Mild-HyperPhe-gray zone” was proposed to describe blood Phe levels between 360 and 600  $\mu\text{mol/L}$ . There are inconsistent data about whether levels in this range impact cognitive and executive function and require treatment [12,13], which is discussed further in Section 3.4.5.1. The use of this table to make a specific diagnosis on the subtype of PKU based on a single value obtained from newborn screening is limited because the value may not reflect peak untreated blood Phe concentrations; moreover, classifying a newly diagnosed individual takes time and may need to account for Phe tolerance.

## 2. The Vanderbilt EPC Report on Adjuvant Treatment for PKU (Melissa McPheeters, Ph.D., M.P.H.)

### 2.1. Summary of results

The EPC conducted systematic reviews on the association of Phe levels and intelligence quotient (IQ) and on the effectiveness of two adjuvant therapies, BH<sub>4</sub>/sapropterin and LNAA, for reducing cognitive decline. The report, including detailed methods, is available on the AHRQ Effective Health Care website (<http://www.effectivehealthcare.ahrq.gov/search-for-guides-reviews-and-reports/?pageaction=displayproduct&productid=957>) [14,15]. Five databases were searched for relevant literature: MEDLINE® via the PubMed interface, PsycINFO, EMBASE Drugs and Pharmacology, the Cumulative Index of Nursing and Allied Health Literature database, and the National Agricultural Library database. Studies were included based on predetermined criteria, reviewed by two experts, and assessed for scientific rigor. Data were compiled in evidence tables from April to June of 2011.

The relationship between Phe level and IQ was measured using a Bayesian hierarchical mixed-effects model, employing Markov chain and Monte Carlo methods. Measurements of Phe could be concurrent (taken within 6 weeks) with IQ testing or historical (taken more than 1 year prior), or both [16]. Measurements taken before age 6 years were considered to be in the critical period. Two models were separately estimated, one for each type of Phe measurement (concurrent and historical). Both were used to predict the probability of an IQ below 85 at varying levels of blood Phe. Treatment data were analyzed qualitatively using evidence tables and summaries because of the paucity of studies.

The strength of evidence (SOE) for a particular outcome can be regarded as insufficient, low, moderate, or high, and is a measure of confidence that the observed effect will not change with future research. Methods of assessing SOE were developed by AHRQ's EPC Program and are detailed in the *Methods Guide for Effectiveness and Comparative Effectiveness Reviews* [17] developed by AHRQ's EPC Program. SOE was assessed in this report for key outcomes identified by the clinical investigators to be most clinically important: cognitive outcomes including IQ and executive function, nutritional outcomes, quality of life, and liberalization of diet. Secondary outcomes included changes in blood Phe levels, Phe variability, and Phe tolerance.

Of 2469 titles and abstracts originally identified through the broad search, 69 (comprising 46 unique studies) were found to be relevant and met the inclusion criteria. We included all study designs and required that papers be published in English. Of these, 17 studies (reported in 21 publications — see references [60–79] in The Vanderbilt EPC Report on Adjuvant Treatment for PKU [14]) could be used to assess the relationship of Phe levels and IQ. These studies included a total of 432 individuals with PKU and varied in their requirements that participants adhere to dietary control. Nineteen studies (reported in 26 papers — see references [67,68,70,75–78,82–99] in The Vanderbilt EPC Report on Adjuvant Treatment for PKU [14]) assessed the relationship of Phe levels with other measures of executive function. Meta-analysis was not possible with this set of studies, and although Phe levels correlate with various measures of executive function, the degree

of correlation on individual measures is inconsistent. The SOE for the association of Phe and measures of executive function is insufficient.

One longitudinal study provided evidence for increased risk of poor cognitive outcomes in the offspring of women with high maternal blood Phe. The relationship is not linear, and there is a marked threshold of approximately 360  $\mu\text{mol/L}$  above which impairment is observed [18,19]. The research on maternal PKU supported the recommendation that appropriate blood Phe levels should occur as early as possible in pregnancy and ideally preconceptionally.

The meta-analysis supported the commonly used blood Phe target of 120 to 360  $\mu\text{mol/L}$  in the nonpregnant PKU population and provided evidence that cognitive effects accumulate over time such that concurrent measures (where blood Phe levels are measured within 6 weeks of the time of cognitive assessment) are poor predictors of outcomes. The SOE is moderate for a threshold effect of a Phe level of >400  $\mu\text{mol/L}$  associated with an IQ <85. Of note, in developing the model for this estimate, the Phe levels used were those available in the included studies.

Ten studies were available to assess the effectiveness of sapropterin (see references [112–121] in The Vanderbilt EPC Report on Adjuvant Treatment for [14]), and three studies were available on LNAA (see references [16,124,125] in The Vanderbilt EPC Report on Adjuvant Treatment for PKU [14]). The studies on sapropterin included two randomized controlled trials, two uncontrolled open-label trials, one prospective cohort study, and several case series. Data were available for a total of 284 individuals. Definitions of a positive response to the drug differed among the studies and are described in detail in the full report. Ten studies provided data on harms of medical treatment. Sapropterin demonstrated statistically significant greater effectiveness over placebo at reducing Phe levels in some patients and may be helpful in supporting patients in achieving their clinical goals. The review included two randomized controlled trials and two open label trials in which sapropterin reduced Phe levels in some patients over the short term. However, none of the existing studies could be used to identify which patients were likely to have a positive response, in part because the trials only included patients who were shown in a loading phase to be initially responsive. No data are yet available to provide evidence about the effects on longer-term clinically important outcomes, including cognition, executive function, or quality of life, but additional studies continue to be conducted. The review thus concluded that there was moderate SOE for a large positive effect of sapropterin on reducing Phe levels over the short term in patients who show initial responsiveness. Data for longer-term clinical outcomes had low or insufficient SOE and were based on indirect associations, including the meta-analysis described above. There was insufficient evidence from a small body of mostly poor-quality studies on the effectiveness of LNAA [20].

### 2.2. Need for rigor in IEM research with humans

Both substantive and methodological gaps currently exist in the literature. Research is challenging, in part due to the rarity of the condition, making participant accrual difficult. In addition, most research on treatment of PKU with sapropterin has been supported by the pharmaceutical industry, raising questions of conflicts of interest. The EPC suggested that a multicollaborative process that crosses public and private lines would greatly benefit the field. In particular, continued studies that include adequate numbers of participants should be conducted in both tightly controlled and nonadherent populations, and among different age groups, for both types of adjuvant therapies. In addition, data on effectiveness in various groups of patients outside the clinical trial setting are needed, including data on those individuals with variability in adherence. Registries have been established and will provide important data, as will ongoing studies that measure additional outcomes, including behavioral and psychiatric measures. Data are not currently available to understand potential modifiers of treatment effectiveness, including genotype. Moreover, the variability in responsiveness to BH<sub>4</sub>

**Table 2**  
Comparison of classification schemes for Phe-related disorders.<sup>a</sup>

Phe-related disorder	Classification schemes						
	Pretreatment Phe level (traditional-modified) <sup>b</sup>	Phe and Phe:Tyr ratio in newborn period <sup>c</sup> (NBS/TMS)	Phe tolerance <sup>d</sup>			PAH genotype <sup>e</sup>	Likelihood of responding to BH <sub>4</sub> <sup>f</sup>
			Infants <1 year (mg/kg/day) <sup>g</sup>	2 to 5 years old to maintain Phe 300 μmol/L <sup>h</sup>	>Age 5 years to maintain Phe 120–360 μmol/L <sup>i</sup>		
Tetrahydrobiopterin deficiencies	Normal to elevated	Phe 2–35 mg/dL (120–2120 μmol/L) (some normal)	Variable	Variable	Variable	N/A	Very high <sup>l</sup>
<i>PAH deficiency requiring treatment</i>							
Classical PKU	>1200 μmol/L (>20 mg/dL)	Phe ≥7 mg/dL (≥420 μmol/L); Phe:Tyr >5	25–45 mg/kg (130–330 mg/day) <sup>k</sup>	<20 mg/kg/day 250–350 mg/day	<12 mg/kg/day	2 classic mutations (often null)	Low
Moderate PKU	900–1200 μmol/L (15–20 mg/dL)	Limited data	45–50 mg/kg	20–25 mg/kg/day 350–400 mg/day	12–18 mg/kg/day	Classic + moderate or 2 moderate mutations	Low
Mild PKU	600–900 μmol/L (10–15 mg/dL)	Limited data	55 mg/kg	25–50 mg/kg/day 400–600 mg/day	>18 mg/kg/day	Classic, moderate, or mild mutation + 1 mild HPA mutation	Medium
Mild HPA-gray zone <sup>b</sup>	360–600 μmol/L (6–10 mg/dL)	Limited data	70 mg/kg	>50 mg/kg/day	No data	Classic, moderate, or mild mutation + 1 mild HPA mutation	High
<i>PAH deficiency not requiring treatment</i>							
Mild HPA-NT <sup>b</sup>	120–360 μmol/L (2–6 mg/dL)	Phe 151–360 μmol/L (avg 244); Phe:Tyr 0.8–8.25 (avg 3.3)	Unrestricted diet	Unrestricted diet	Unrestricted diet	Classic, moderate, or mild mutation + 1 mild HPA mutation	N/A

BH<sub>4</sub>, tetrahydrobiopterin; HPA, hyperphenylalaninemia; N/A, not applicable; NBS, newborn screening; PAH, phenylalanine hydroxylase deficiency; TMS, tandem mass spectrometry.

<sup>a</sup> This table is an attempt to integrate the different schemes used to classify individuals with Phe-related disorders, but is neither exhaustive nor complete. Clinical and laboratory correlation is essential for any diagnostic algorithm or classification scheme. A single blood Phe level is not sufficient for classification, especially in the first year of life, nor is a single NBS test result without confirmatory testing. For a summary of many of the issues associated with classification of these disorders, see Blau et al. [188].

<sup>b</sup> The traditional classification scheme cited for PKU severity based on pre-treatment Phe levels [196,332] has been modified and consists of the creation of two new disorder categories highlighted in light gray: (1) “mild HPA-gray zone” to reflect baseline Phe levels between 360 and 600 μmol/L, for which there is disagreement about the need to treat and there are conflicting data on the effects on cognition and executive function [12]; and (2) “mild HPA-NT” to reflect baseline Phe levels between 120 and 360 μmol/L, for which there is general consensus that no treatment is required (however, before conception and during pregnancy, target Phe should be 120–360 μmol/L to reduce risk of maternal PKU syndrome). The normal range of blood Phe for an individual without HPA or PKU is 58 ± 14 (SD) μmol/L [333]. Babies identified by NBS may have treatment initiated before the Phe level would equilibrate if on an unrestricted diet; therefore, reliance on an early pre-treatment level(s) may not lead to accurate classification.

<sup>c</sup> Data from NBS using TMS must be used with caution in making a specific categorization of PKU severity because a single Phe concentration obtained in the neonatal period from TMS is unlikely to reflect peak untreated levels, neonates vary in their dietary exposure to Phe, and early treatment often precludes obtaining more definitive Phe concentrations used historically to classify individuals. After NBS, a baby requires additional testing to confirm if there is a disturbance of phenylalanine metabolism and if confirmed, to differentiate PAH from tetrahydrobiopterin deficiency disorders. Consider transient HPA in premature infants, those on parenteral nutrition, infants of mothers with HPA, and those with liver dysfunction [334]. The values in this table for BH<sub>4</sub> deficiency are derived from Opladen et al. [258]. Tetrahydrobiopterin deficiency due to a defect in GTP cyclohydrolase (GTPCH) may present with normal blood Phe concentrations on NBS. The values for PAH deficiencies are derived from Marsden & Levy [334] using a Phe cutoff of 139 μmol/L and Phe:Tyr ratio of 1.5 from infants whose first specimen was collected between 1 and 5 days of life. The Phe:Tyr ratio has been shown to be helpful in discriminating between PKU, false positives, and mild HPA [335]; this group used a Phe cutoff of 180 μmol/L and Phe:Tyr of 2.5 on TMS to identify both classic and “variant” PKU in samples collected within 24 h post-delivery. Cutoff values are NBS laboratory-dependent, but analysis tools using Phe and Phe:Tyr ratios across multiple international NBS laboratories [336] and enhanced interpretation using multivariate pattern-recognition software without the need for specific analyte cutoff values have also been developed [337].

<sup>d</sup> Phe tolerance can be determined using different algorithms; the ranges cited here may not be applicable for all individuals with PKU and do not apply to those being treated with sapropterin.

<sup>e</sup> These categories are derived from the assessment of 686 individuals with PKU, with assignment of mutation severity based on those with functionally hemizygous genotypes; however, up to 21% of individuals still had discordant phenotypes despite predictions based on PAH mutation severity assignment [196]. The combination of the two mutant alleles is important to predict the residual PAH enzyme activity given that it is a homotetramer. Thus, disease severity in most cases is determined by the least severe of the two PAH mutations, and two mutations with similar severity can confer a milder phenotype than either of the mutations would do if it acted alone [196]. In addition, the ability to predict BH<sub>4</sub> responsiveness from genotype is not always reliable [188,273]. See Section 3.5.3 for discussion of genotype-phenotype correlations, and these specific databases of complete genotypes and disease phenotypes, some of which include BH<sub>4</sub> responsiveness: the Phenylalanine Hydroxylase Locus Knowledgebase ([www.pahdb.mcgill.ca/](http://www.pahdb.mcgill.ca/)) and the BIOPKU database ([www.biopku.org/home/biopku.asp](http://www.biopku.org/home/biopku.asp)).

<sup>f</sup> Reports of BH<sub>4</sub> responsiveness rarely provide classification of severity based on pretreatment Phe levels. BH<sub>4</sub> responsiveness testing typically reports baseline Phe levels (the level at the initiation of the BH<sub>4</sub> load), and that value cannot be used to assign a degree of PKU severity. Hence, this column classifies the likelihood of response to BH<sub>4</sub> within degrees of disease severity using broad, qualitative descriptors. In general, the degree of severity of PAH deficiency is inversely proportional to the likelihood of response to BH<sub>4</sub>. These descriptions are based on Blau et al. [198] and derived from Bernegger and Blau [340], Fiege and Blau [205] and Trefz et al. [165].

<sup>g</sup> Data derived from Acosta and Yannicelli, Ross Metabolic Formula System: Nutrition Support Protocols, 3rd Ed. 1997 [338]. Breastfeeding to provide dietary Phe is allowable in addition to the use of medical food. Phe requirements in the first month of life may be high and these ranges may underestimate actual needs.

<sup>h</sup> These data reflect Phe tolerance to maintain Phe at 300 μmol/L for children between the ages of 2 and 5 years [189,196,332]. Some authors suggest that Phe tolerance is relatively stable between the age of 2 years through 10 years [190]. Growth spurts and intercurrent illness may modify Phe tolerance; use ideal body weight when doing calculations [338].

<sup>i</sup> These data reflect Phe tolerance per kg body weight per day given as natural protein during nongrowth periods later in life (usually after age 5 years) [197]. Another study recommends reassessment of Phe tolerance with a metabolic dietitian in adulthood to meet the adult Phe requirements of 9.1 mg Phe/kg ideal body weight/day; several subjects in this limited study had higher Phe tolerance than that calculated at age 5 years due to changes in body mass and other factors [150].

<sup>j</sup> Many BH<sub>4</sub> deficiencies also require supplementation with neurotransmitter precursors [188].

<sup>k</sup> An additional source [339] recommends dietary Phe per day rather than based on body weight of the infant.

is not completely understood. Finally, data on strategies that improve adherence to both drug and diet treatments could suggest approaches to optimize positive outcomes.

### 3. Working group reports

#### 3.1. Long-term outcomes and management across the lifespan

##### 3.1.1. Scope of work

The Long-Term Outcomes and Management across the Lifespan Working Group was charged with exploring the evidence and practices that would inform management of individuals with PKU across their lifespan, with an emphasis on adulthood. Dietary management and issues pertaining to pregnancy were excluded as two other working groups addressed these topics. Key areas of investigation included:

- The domains of impairment that can occur in PKU
- Current tools to screen for and measure the domains of cognitive, physical, emotional, behavioral, and social impairments
- Current approaches that deliver interdisciplinary care to support individuals with PKU across the lifespan.

##### 3.1.2. Introduction

This working group affirmed the 2000 NIH Consensus Development Statement, which called for a comprehensive, multidisciplinary approach to lifelong care for people with PKU. The working group built on this statement and added that particular attention is necessary in the transition from adolescence to adulthood.

Serious neurological impairments are now preventable in treated individuals with PKU. However, there is increasing recognition of more subtle physical, cognitive, and behavioral findings in these individuals. Current treatment approaches for PKU often do not adequately address the clinical and metabolic intricacies of this complex disorder. There are inconsistencies in treatment schemes for adolescents and adults and a general lack of evidence to support many common treatment approaches currently in use.

##### 3.1.3. Domains of impairment that can occur in PKU

**3.1.3.1. Medical.** Although medical care for individuals with PKU includes the routine health maintenance required for all individuals, there are specific areas of focus for those with PKU, especially children. These include careful monitoring of growth and development and neurological findings and assessment of bone mineral density and neurocognitive functioning. Medical history assessments should gather information about diet and dietary compliance as well as the ability to access treatments and receive community support.

Peak bone mass is reduced in individuals with PKU from childhood [21,22], but it is unclear whether this is the consequence of the disease itself and/or its dietary treatment. Imbalances in bone formation and bone resorption as measured by biomarkers, decreases in bone mineral density measured by dual-energy X-ray absorptiometry (DXA), and alterations in the appearance of permanent teeth have been reported in early-diet-treated individuals with PKU [23].

Osteopenia and osteoporosis have been reported in adults and children with PKU [21,24] and may be due to long-standing dietary deficiencies in natural protein, calcium, vitamin D, and/or trace elements, or a primary defect in bone turnover inherent to the disease itself [25]. It is not clear what the clinical significance is of this deficit and whether it is progressive in nature [24]. Other dietary nutrients positively correlated with bone health include docosahexaenoic acid (DHA), eicosapentaenoic acid, and total omega-3 fatty acids [26]. Individuals with PKU have significantly diminished plasma levels of these nutrients compared with controls [27].

Neurological investigations of treated adults with PKU revealed minor neurological signs such as tremor, brisk deep tendon reflexes,

and awkward motor coordination, indicating the possibility of a specific neurological syndrome in these individuals. These symptoms are not known to have a clear clinical relevance, but data relating metabolic control to neurological signs are scarce [28]. A small number of adolescents and adults with PKU who typically had poor metabolic control in childhood have developed neurological disease such as seizures, loss of balance, hallucinations, and lower limb paralysis, which usually improved upon returning to dietary treatment [29,30].

Brain magnetic resonance imaging (MRI) has revealed white-matter abnormalities characterized by hyperintense parieto-occipital lesions on T2 images or by periventricular signal abnormalities in some individuals with PKU with and without neurological impairment [31]. Early-treated, late-treated, and untreated patients differ in the extent of myelin changes observed by brain MRI [32,33]; however, this pathology has been associated with poor metabolic control and may be reversible after a minimum of 2 months of strict dietary treatment [30,33].

**3.1.3.2. Neurocognitive outcomes.** Although the reasons are unknown, there are still individuals with PKU who exhibit some degree of neuropsychological dysfunction, even though they have had careful nutritional management [34]. Two meta-analyses show a relationship between mean lifetime [35] or concurrent blood Phe level and cognitive functioning. In the analysis of concurrent blood Phe levels, effects were more pronounced in children and adolescents than in adults. Further analysis suggested an upper threshold for Phe concentrations of 320  $\mu\text{mol/L}$  for children (ages 7–12) and 570  $\mu\text{mol/L}$  for adolescents (ages 13–18) that allowed for normal cognitive function. In adults, the negative effects remained the same between Phe concentrations of 750–1500  $\mu\text{mol/L}$ , which was the range described in the meta-analysis [36]. The measurement of average variability of Phe levels over time may have a better prognostic significance as reported by a retrospective study of 45 children with PKU [37].

A meta-analysis of neuropsychological studies also showed that adolescents and adults with PKU perform significantly more poorly than matched controls without PKU on tests of attention, inhibition, motor control, and processing speed, with processing speed having the largest effect size [38]; however, this analysis did not include blood Phe concentrations. Moreover, reductions in executive function, nonexecutive functions (e.g., processing speed, fine motor skills, perception/visual spatial abilities), and increased hyperactivity and impulsivity are among the neuropsychological impairments described in individuals with PKU, even in those treated early [39–41].

**3.1.3.2.1. Educational achievement.** Children with PKU span the full range of educational achievement but often perform less well at school than children without PKU, especially when metabolic control is suboptimal [42]. Poor performance in arithmetic/mathematics [43] and impaired ability to copy letters and geometrical designs, for example, are common. These impairments may be misdiagnosed as behavioral problems, unless adequate neuropsychological testing is conducted.

**3.1.3.2.2. Behavioral, emotional, and social disturbances.** Behavioral impairments such as hyperactivity, tantrums, anxiety, low self-esteem, and social withdrawal have been noted in children with PKU. However, without evidence of correlations with Phe levels, causality remains unexplained. In one study, these behaviors were hypothesized to be a consequence of living with a chronic condition rather than a biological effect of increased Phe levels [44]. However, with respect to hyperactivity, Arnold et al. reported significantly higher stimulant use in children with PKU versus age-matched controls with type 1 diabetes, and those children with PKU on stimulants had significantly higher blood Phe concentrations compared with children with PKU not on these medications [45].

**3.1.3.2.3. Psychiatric disorders.** Psychiatric symptoms are well documented across the lifespan of individuals with PKU, even those treated from infancy. Depressed mood, generalized anxiety, phobias, decreased positive emotions, social immaturity, agoraphobia, panic attacks, and social isolation have been reported especially during adulthood [46].

The correlation between degree of metabolic control and severity of symptoms suggests a biological basis of psychiatric dysfunction. Studies in Germany have reported rates of psychiatric issues among patients with PKU that were up to twice that of the general population [47,48]. Screening for psychiatric distress is an important ongoing component of PKU management [49].

3.1.4. Current screening tools

A variety of screening tools and measures are available to assess medical, nutritional, metabolic, neurologic, cognitive, and emotional, behavioral, and social domains of health. These measures were selected to provide a starting point to screen, evaluate, and monitor individuals with PKU (Table 3). Many of these screening tools may be used in the metabolic or pediatric clinic. If an in-depth evaluation is warranted, the patient is then referred for a neuropsychological assessment. The noted measures are not intended to represent the full extent of assessment that may be necessary; rather, they represent the most basic components of evaluations and provide consistent follow-up procedures, permitting multisite retrospective research studies or meta-analyses in the future.

For psychological status, screening instruments are available that can be completed by parents or self-administered by adults and provide cutoff scores indicating risk for behavioral, neuropsychological, and mood disorders [50]. Screening instruments provide justification for insurance coverage for more formal neuropsychological testing and

access to early intervention and special education evaluations and services, as well as to identify individuals with impairing psychiatric symptoms (i.e., depression, anxiety, inattention, psychosis) that may merit consultation with a psychiatrist who specializes in neurodevelopmental disorders.

3.1.5. Current approaches to deliver interdisciplinary care to support individuals with PKU across the lifespan

Emotional health, absence of neuropsychological deficits, and a satisfying quality of life signify successful treatment for PKU as much as blood Phe concentrations in the treatment range and a “normal” IQ. Achieving these outcomes requires an interdisciplinary approach utilizing a medical home framework. Geneticists and dietitians who are specifically trained to treat IEM provide care in most metabolic clinics and often serve as the medical home for young pediatric patients, while coordinating care with the pediatric primary care provider. Genetic counselors, psychologists, nurse practitioners, and social workers also may participate in interdisciplinary care. Communication with laboratory personnel, early intervention specialists, teachers, school nurses, and other health care providers is critical.

3.1.5.1. Transitioning into adulthood. The foci of discussions should change as individuals with PKU age, necessitating appropriate health care provider input. For example, as girls enter puberty, it is important to begin discussions of pregnancy planning and maternal PKU. Strong

**Table 3**  
Medical, cognitive, and psychological management across the lifespan.

DOMAIN	Infant-Toddler 3 mo–5 yr	School Age 6–11 yr	Adolescent/ Transition 12–17 yr	Early Adulthood 18–25 yr	Middle Adulthood 26–49 yr	Later Adulthood ≥50 yr
Medical	Issue: General health					
	Measure: Medical examination; medical history			Measure: Medical examination; medical history; bone density		
Nutritional	See Table 6. Nutritional management across the lifespan					
Metabolic	Issue: Phe level					
	Measure: Serum Phe					
Neurological	Issue: Tremor; gait; strength; reflexes					
	Measure: Neurological examination					
Cognitive	Issue 1: General cognition					
	Measure 1a: Bayley III Measure 1b: WPPSI-III		Measure 1: WASI			
	Issue 2: Executive abilities					
	Measure 2: BRIEF-P		Measure 2: BRIEF	Measure 2: BRIEF		Measure 2: BRIEF -A
	Issue 3: Academic achievement					
	Measure 3: Discuss early skills		Measure 3: Standard scores; discuss progress			Measure 3: Highest education level attained
Behavioral, Emotional, and Social	Issue 1: Behavioral, emotional, social					
	Measure 1: BASC-II or CBCL			Measure 1: BSI or BDI -II/BAI		
	Issue 2: Adaptive function					
	Measure 2: ABAS-II		Measure 2: ABAS - II; discuss pregnancy with teens	Measure 2: ABAS -II; discuss pregnancy	Measure 2: ABAS - II; discuss pregnancy; discuss social issues	Measure 2: ABAS-II; discuss social issues

Abbreviations: Bayley-III: Bayley Scales of Infant and Toddler Development – Third Edition [341]; WPPSI-III: Wechsler Preschool and Primary Scale of Intelligence [342]; WASI: Wechsler Abbreviated Scale of Intelligence [343]; BRIEF-P, BRIEF, BRIEF-A: Behavior Rating Inventory of Executive Function; BASC-II: Behavior Assessment System for Children – Second Edition; CBCL: Child Behavior Checklist; BSI: Brief Symptoms Inventory; BDI-II: Beck Depression Inventory – II; BAI: Beck Anxiety Inventory; ABAS-II: Adaptive Behavior Assessment System – Second Edition (see Waisbren and White [50] for detailed descriptions of these instruments).



social supports are an important element in aiding young women with PKU who are “off diet” to return to treatment and prevent unplanned pregnancies [51,52]. In these transition years, issues such as educational and vocational planning emerge. Young adults often need assistance in understanding and navigating issues related to health insurance, especially in the context of a chronic health condition. In early and middle adulthood, discussions of pregnancy planning and maternal PKU should continue, along with discussions regarding social issues such as living situation, public assistance programs, marriage, and child rearing. In later adulthood, it is important to continue discussions of the social issues that were considered during early and middle adulthood; as more individuals age into this demographic, other issues such as neurological deterioration may emerge [53,54]. For late-treated individuals who cannot live independently, it is important to have discussions with their parents as they age about long-term care for the person with PKU.

An interdisciplinary approach is required to recognize when additional referrals are needed, establish communication processes to relay concerns and evaluation results to other service providers, and ensure successful transition from pediatric to adult care. Summer camps, teen conferences, parent networks, and maternal PKU workshops are helpful to provide social support, which improves adherence to medical recommendations [55]. See Table 4 for a listing of social support resources and those available to assist in transitioning from pediatric to adult care.

### 3.1.6. Breakout session input

Breakout session participants affirmed the domains of impairment that can occur in PKU that were identified by the working group (Table 3) and suggested that approaches for periodic assessment of these domains need to be practical, comprehensive, and covered by health care payers. In the medical domain, questions were raised

about how often and at what age bone mineral density should be evaluated, and participants suggested that adolescence may be too late for girls. In the neurological domain, the assessment of tremor in PKU needs to be distinguished from essential tremor and correlated with blood Phe levels and other neuropsychological outcomes. In the neuropsychological domain, participants voiced concern about how the results of cognitive measures would affect treatment, and they suggested that formal evaluations versus screening tools are more successfully covered by health care payers. Regarding measuring executive function, specific instruments must be used and questions remain regarding whether IQ or executive functioning best predicts neuropsychological outcome, when intellectual functioning falls within the normal range. Participants suggested the identification or development of screening tools that are feasible to administer within clinics and those that families can utilize directly.

### 3.1.7. Summary and key points

The most serious neurological impairments in PKU are preventable with current dietary treatment approaches. However, a variety of subtle physical, neuropsychological, and behavioral impairments have been recognized, even in individuals with well-controlled PKU. These outcomes stem from a complex array of factors, including the severity of PKU, the timing and adherence to treatment, and a variety of other genetic and environmental factors.

Optimal patient outcomes will depend upon how health care services are delivered. Full access to medical foods and foods modified to be low in protein, the coordination of pediatric, social work, psychological, psychiatric, nutritional, nursing, and genetic services, as well as a qualified biochemical laboratory with the ability to provide timely results, facilitates good care of individuals with PKU. This coordinated approach also allows for research to improve diagnosis and treatment.

**Table 4**  
Selected resources.

Patient and Family Organizations	Registries/Databases	Research Networks	Pediatric to Adult Care Transitioning
National PKU Alliance: <a href="http://www.npkua.org">www.npkua.org</a>	International Database of Patients and Genotypes Causing HPA/PKU: <a href="http://www.biopku.org/home/biopku.asp">http://www.biopku.org/home/biopku.asp</a>	Canadian Inherited Metabolic Diseases Research Network: <a href="http://www.cimdm.ca/">http://www.cimdm.ca/</a>	The Action Sheets (ACMG Transition ACT Sheets): <a href="http://www.acmg.net">http://www.acmg.net</a>
The World of PKU: A PKU Knowledgebase: <a href="http://www.pkuworld.org">http://www.pkuworld.org</a>	PAH locus-specific database: <a href="http://www.biopku.org/home/pah.asp">www.biopku.org/home/pah.asp</a>	NICHD-funded Newborn Screening Translational Research Network (NBSTRN): <a href="https://www.nbstrn.org/">https://www.nbstrn.org/</a>	Center for Health Care Transition Improvement: <a href="http://www.gottransition.org/">http://www.gottransition.org/</a>
Emory University Email List Service Phenylketonuria Support Group: <a href="http://listserv.cc.emory.edu/cgi-bin/wa?A0=PKU-SUPPORT-L">http://listserv.cc.emory.edu/cgi-bin/wa?A0=PKU-SUPPORT-L</a>	PKU Demographic, Outcomes, and Safety Registry (PKUDOS): <a href="http://clinicaltrials.gov/show/NCT00778206">http://clinicaltrials.gov/show/NCT00778206</a>	The ORDR-funded Rare Disease Clinical Research Network: <a href="http://rarediseases.info.nih.gov/research/pages/41/rare-diseases-clinical-research-network">http://rarediseases.info.nih.gov/research/pages/41/rare-diseases-clinical-research-network</a>	New England Consortium of Metabolic Programs: <a href="http://www.newenglandconsortium.org">www.newenglandconsortium.org</a>
National PKU News: <a href="http://www.pkunews.org/index.htm">http://www.pkunews.org/index.htm</a>	Common Data Elements (CDEs): <a href="https://grdr.ncats.nih.gov/">https://grdr.ncats.nih.gov/</a>		
	Common Data Element (CDE) Resource Portal <a href="http://www.nlm.nih.gov/cde/">http://www.nlm.nih.gov/cde/</a>		

Abbreviations: ACMG, American College of Medical Genetics and Genomics; NICHD, Eunice Kennedy Shriver National Institute of Child Health and Human Development; ORDR, Office of Rare Diseases Research.

### 3.2. Pregnancy and PKU

#### 3.2.1. Scope of work

The Pregnancy and PKU Working Group was charged with investigating whether the current management recommendations for women with PKU who become pregnant should be modified. Key areas of investigation by the working group included:

- Identifying and addressing barriers to metabolic control in the preconception period
- Whether current dietary recommendations for pregnant women with PKU should be modified
- Whether sapropterin is safe and effective during pregnancy, and what considerations should guide its use
- Metabolic control in the postpartum period and during lactation.

#### 3.2.2. Introduction

High levels of Phe in the intrauterine environment are considered teratogenic; thus, the importance of maintaining metabolic control before and during pregnancy cannot be overemphasized. In uncontrolled maternal PKU, excess maternal plasma Phe is actively transported into the fetal circulation leading to possible microcephaly, congenital heart disease (CHD), fetal growth restriction, and other less common malformations; this is known as the maternal PKU syndrome (MPKUS) [56]. The 2000 NIH Consensus Development Statement [1] recommended that women with PKU maintain plasma Phe levels between 120 and 360  $\mu\text{mol/L}$  before and throughout pregnancy to prevent adverse outcomes associated with untreated maternal PKU. Numerous studies that further examined the relationship between nutrient intake, maternal blood Phe levels, and pregnancy and neonatal outcomes have supported the need for good control [10,57–59]. In the international Maternal PKU Collaborative Study (MPKUCS), Koch and colleagues showed that the best outcomes were in offspring of women who had plasma Phe concentrations in the range of 120–360  $\mu\text{mol/L}$  before conception and that developmental outcome declined when average plasma Phe exposure during pregnancy exceeded 360  $\mu\text{mol/L}$  [10]. A plasma Phe range between 100 and 300  $\mu\text{mol/L}$  was recommended in 2005 as a result of analysis of the British Maternal PKU Registry [57].

Children born to mothers who were in metabolic control prior to and during pregnancy have been shown to attain normal IQs [19]. Studies also have shown that better control of maternal plasma Phe levels correlates with a lower incidence of microcephaly, normal growth measurements, and higher IQ in offspring [57,58,60,61]. Severe CHD has been reported in offspring of several women who had high untreated plasma Phe concentrations and did not begin a Phe-restricted diet until 7 to 18 weeks gestation [58], whereas there was no increased risk of CHD in infants born to women whose plasma Phe was between 120 and 360  $\mu\text{mol/L}$  during the first 8 weeks of gestation [62,63]. Greater fluctuation of maternal blood Phe levels during pregnancy is also associated with poorer outcomes in offspring [58]. This greater variability or fluctuation may be a marker for the more severe forms of PKU [19].

A lower limit of maternal plasma Phe of 120  $\mu\text{mol/L}$  was set by the MPKUCS to prevent hypophenylalaninemia in the developing fetus. However, it is not known whether outcomes would be better if maternal blood Phe concentrations were maintained closer to physiologically normal concentrations of 60–120  $\mu\text{mol/L}$ . A retrospective review of medical and dietary records of all known pregnancies in women with PKU in France over a 5-year period found that intrauterine growth retardation was significantly more common in newborns where blood Phe concentrations were more often above 300  $\mu\text{mol/L}$  or below 120  $\mu\text{mol/L}$  during the pregnancy [64], suggesting, in the latter case that insufficient Phe was available for anabolism in the fetus.

#### 3.2.3. Barriers to achieving preconceptional control

The proportion of women who are on a Phe-restricted diet at conception has steadily increased [63]. However, the majority of women

of child-bearing age still do not maintain their prescribed diet [65,66] despite understanding the importance of doing so [67]. Studies have shown that, in general, as individuals with PKU age, adherence to dietary recommendations decreases [67,68]. Barriers to continuation of a Phe-restricted diet include the unpalatability of the diet, perception of peer rejection, inadequate insurance coverage for medical foods and foods modified to be low in protein, and limited access to care by qualified metabolic specialists and other health care professionals experienced in the treatment of adults with PKU [67]. Factors that are correlated with better blood Phe control before and during pregnancy include higher maternal age, IQ, and socioeconomic status [19]; in addition, women who have planned pregnancies have an increased understanding of the need for metabolic control and the importance of a Phe-restricted diet.

#### 3.2.4. Dietary considerations

Adequate protein and energy intakes are needed to support fetal growth and development and to prevent catabolism. Protein needs increase by 50% during pregnancy from 46 to 71 g/day in the general population (Dietary Reference Intakes [DRIs]). Inadequate protein or energy intake in any individual with PKU leads to elevated blood Phe concentrations due to muscle catabolism and can be a risk factor during pregnancy [60]. Because natural protein is limited in the traditional maternal PKU diet, protein requirements must be met through the use of amino acid-based medical foods. These products are costly, often unpalatable, and may not be covered by health care payers. Women who consume less than 50% of recommended protein intake have a higher incidence of congenital heart disease in their offspring [69].

Other nutrients in the maternal diet have effects on fetal development. Dietary total fat and essential fatty acids are low in individuals with PKU, especially when the medical food contains no fat [70–72]. Dietary cholesterol is almost nonexistent in the PKU diet, resulting in low serum cholesterol concentrations, which have been associated with spontaneous abortions [72]. Additional concerns include inadequate intakes of copper and niacin, which are associated with birth defects in the offspring of pregnant women without PKU. Folic acid and vitamin B<sub>12</sub> deficiencies have been associated with risk of spontaneous abortion, birth defects, CHD, and preterm birth in infants of women without PKU [73], and a higher incidence of CHD has been documented in the offspring of women with PKU with low intakes of vitamin B<sub>12</sub> [61].

Thus, the maternal PKU diet must be evaluated for adequacy of energy, protein, total fat and essential fatty acids, vitamins, and minerals. Supplemental fat, vitamins, and minerals may be needed, especially if the medical food used is devoid of these nutrients. Biomarkers of nutritional status should be routinely monitored, and additional monitoring performed if the woman has high blood Phe, is not gaining weight well, has inadequate nutrient intake, or has hyperemesis gravidarum.

Two other dietary interventions used in PKU, glycomacropeptide (GMP) and LNAA, are discussed in detail in Section 3.3.3.1 (see also Table 5) and deserve mention here. GMP, a protein that is derived from whey and is naturally low in Phe [74], is available as the protein source in some medical food products for PKU but has not yet been studied in pregnant women with PKU. LNAA are contraindicated in pregnancy because LNAA do not sufficiently reduce circulating blood Phe concentrations to prevent MPKUS [75].

#### 3.2.5. The postpartum period and lactation

PKU is an autosomal recessive disorder with a carrier frequency in the general population of 1:60 in the United States, thus approximately 1:120 infants born to mothers with PKU will have PKU and, as such, will require treatment for this condition after birth. All other offspring will be carriers and are at an increased risk for having affected children when they reproduce [76].

**Table 5**  
Additional treatment options.

	Sapropterin	LNAA <sup>a</sup>	GMP <sup>b</sup>
Criteria for use	No age restriction Greater response if residual enzyme activity, but also in some individuals with classical PKU	Adolescents and adults not on optimal treatment	>1 year of age
How used	With Phe restriction and medical foods, but range of liberalization possible	With medical foods if patient is already on; without if not	With Phe-restricted diet Replaces medical foods
Benefits	May increase Phe tolerance and intake of natural protein, and improve nutritional status Neurocognitive effects still under study	May be an acceptable treatment when not on Phe-restricted diet May increase brain neurotransmitter synthesis	Natural protein Palatable Increases medical food protein options
Cautions/risks	Response to sapropterin varies depending on the severity of PKU (more frequent in milder phenotypes)	Not in pregnancy or in young children Caution with psychotropic medications Some products do not contain minerals and vitamins	Contains some Phe that needs to be counted Some products do not contain minerals and vitamins
Research needs	Pregnancy and young children Effects on neurocognitive function	More controlled trials on effectiveness, nutrition status, growth	Long-term safety, efficacy, and effects on nutrition status

<sup>a</sup> LNAA – large neutral amino acids (LNAA) include Phe, Tyr, tryptophan, threonine, methionine, valine, isoleucine, leucine, and histidine. With the exception of Tyr, they are essential amino acids. They share a common transport system to enter the brain [182]. With elevated blood Phe levels as seen in PAH deficiency, commercial mixtures of these amino acids that do not contain Phe are used to decrease the transport of Phe into the brain [171,172,174]. Some LNAA products have been reported to reduce blood Phe concentrations [177].

<sup>b</sup> GMP – glycomacropeptide is a 64-amino acid glycoposphopeptide produced during cheese making. Commercially available GMP contains minimal Phe and also limiting amounts of histidine, leucine, tryptophan, and tyrosine. When supplemented with the limiting amino acids, it is an alternative to amino acid-based medical foods for use in individuals with PKU [184].

There is insufficient information to guide dietary management during the postpartum period and lactation. High energy and protein demands during lactation coupled with dietary restriction of Phe present challenges to maintaining an adequately controlled diet during lactation, and supplementation of nutrients such as vitamins, minerals, fats, cholesterol, and DHA may be required.

Although higher total Phe content was found in the breast milk of women with PKU compared with women without PKU [77], blood Phe concentrations in breast-fed infants of mothers with PKU were normal [78]. It is recommended that mothers with PKU breastfeed their infants who do not have a diagnosis of PKU. Even infants with PKU may be breast-fed by mothers with PKU as long as breastfeeding is used in combination with medical food for both mother and baby and the infant's blood Phe is carefully monitored [79].

During the postpartum period and throughout lactation, attention to maternal and infant nutritional status, psychosocial risk factors, and the home environment are important for optimal management. In addition, an awareness of cultural beliefs and rituals in the postpartum period is required for culturally competent provision of care [80]. Inadequate dietary control and high Phe concentrations in the mother with PKU may be associated with maternal emotional difficulties, depression, or anxiety that can compromise parenting abilities and result in less stimulation in the home environment, leading to an increased risk for a low IQ in the infant [65].

### 3.2.6. Use of sapropterin in pregnancy and during the postpartum period

**3.2.6.1. Pregnancy.** Sapropterin has been used successfully in conjunction with dietary Phe restriction and Phe-free medical food to decrease blood Phe levels in a subset of sapropterin-responsive individuals with PKU. Because of the high probability of fetal damage in uncontrolled maternal PKU [56] and the challenges in maintaining dietary control throughout pregnancy, use of sapropterin in responsive pregnant individuals with PKU has the potential to prevent miscarriages and MPKUS, and ultimately improve outcomes.

FDA has classified sapropterin use in pregnancy as risk category C [81]. Category C denotes drugs with insufficient research data to prove safety, and drugs in this category should be used after careful consideration and only if the benefit outweighs any risk. There are several case reports [82,83], one case with dosing at 20 mg/kg ideal body weight throughout pregnancy [84], indicating that sapropterin can be used by sapropterin-responsive mothers during pregnancy with good short-term outcomes in their newborns (i.e., normal weight, length, and head circumference with no congenital abnormalities). Some adverse reactions reported with sapropterin use, such as vomiting and abdominal pain, are also common in pregnancies of women without PKU,

making attribution and management of symptoms challenging. Dosing in pregnancy has not been established; however, case reports of pregnant women include doses up to 20 mg/kg/day.

There is no evidence of teratogenicity in rats on oral doses of sapropterin up to 400 mg/kg/day or in rabbits on doses up to 600 mg/kg/day. No human studies are available at a comparable dosage. Studies in mice showed no evidence of carcinogenetic effect, but the study was not ideal due to its duration of 78 weeks instead of 104 weeks; studies with rats showed an increase in benign pheochromocytoma on twice the recommended human dose [81]. The drug did not seem to have an effect on fertility and reproductive function in rats [85].

**3.2.6.2. Use of sapropterin in the postpartum period.** Although there is a paucity of information on safety and efficacy of sapropterin use during the postpartum period, its cautious use as an adjuvant to dietary Phe restriction in women with high Phe levels may be considered during the postpartum period for sapropterin-responsive mothers (NIH PKU Working Group on Pregnancy, personal communication). Although sapropterin was excreted in the milk of lactating rats when the sapropterin was administered intravenously but not orally, the excretion of sapropterin or its metabolites in human breast milk is not known. Effects of sapropterin from breast milk on a nursing infant also are essentially unknown. Of note, human breast milk contains biopterins, including BH<sub>4</sub> [86].

### 3.2.7. Breakout session discussion points

Breakout session participants pointed to the need for transition plans to address specific needs across the lifespan of an individual, particularly during pregnancy. Coordination of care by relaying concerns and evaluation results to other service providers (e.g., obstetricians) is essential.

Another essential activity identified is a lifelong approach to education of individuals with PKU, their families, and health care providers. Informational websites and social media groups are abundant (see <http://www.npkua.org>) and were suggested as a means to keep female adolescents engaged and aware of the importance of preconception diet control. Engagement of adult care providers and obstetricians in the monitoring of women with PKU who may become pregnant or are already pregnant is critical to prevent MPKUS and its devastating consequences.

Although the 2000 NIH Consensus Development Statement indicated that women with PKU should maintain blood Phe concentrations between 120 and 360 μmol/L before conception and throughout pregnancy to prevent MPKUS, participants reinforced the need to evaluate whether maternal blood Phe concentrations should be maintained

closer to the normal physiological range of 60–120  $\mu\text{mol/L}$ . Additional research questions included whether optimal Phe concentrations are different in pregnancy and how the maternal Phe concentration is reflected in the fetus.

### 3.2.8. Summary and key points

The best outcomes in maternal PKU occur when blood Phe is maintained between 120 and 360  $\mu\text{mol/L}$  before and during pregnancy which confirms prior recommendations [1]. Poor nutrient intake and inadequate monitoring of diet during pregnancy pose a nutritional risk to the mother and fetus. Maintaining good metabolic control after pregnancy is best for the health and well-being of both mother and infant. New therapies, including sapropterin and medical foods using GMP, hold promise but require further study. In contrast, the use of LNAA is contraindicated in pregnancy. Education and support for women with PKU is needed to improve adherence to diet, prevent unplanned pregnancies, and overcome barriers to treatment.

### 3.3. Diet control and management

#### 3.3.1. Scope of work

The Diet Control and Management Working Group sought to identify and describe current knowledge and clinical practice standards for nutritional treatment of PKU that have emerged since the NIH 2000 Consensus Statement was released. Key areas of investigation included:

- The outcomes (endpoints) important to nutrition management and the parameters that should be used to monitor these outcomes
- In patients with PKU who are found to be responsive to sapropterin, the criteria to identify patients appropriate for diet liberalization, the approach to altering nutrition therapy, and the best practices for making modifications
- Criteria for identifying patients in whom LNAA and GMP would be beneficial and the approach to altering treatment when using these products
- Individual patient variation and the influence on management
- Treatment considerations for persons with PKU who were never treated or had inadequate treatment.

#### 3.3.2. Introduction

There is general consensus that the current standard of care and primary treatment for PKU is dietary and that this treatment should be continued throughout an individual's lifetime [1,14,87,88] to prevent adverse clinical outcomes and cerebral MRI changes, and to promote normal cognitive development [9,89]. The 2011 AHRQ report supports typical target blood Phe levels of 120–360  $\mu\text{mol/L}$ . There is no evidence that blood Phe control in adolescents and adults can be relaxed [14], especially for women of childbearing age. The overall goals of dietary management for PKU are to prevent complications associated with untreated PKU and promote normal growth and development and adequate nutritional status.

Dietary treatment for PKU consists of a restricted intake of Phe-containing natural protein and consumption of medical foods. Medical foods include Phe-free amino acid-based formulas that also contain energy, vitamins, and minerals; products that use GMP (see Section 3.3.5.2) as the protein source; and specialized products that have been modified to be low in protein. Products modified to be low in protein are necessary to provide adequate energy, satiety, and variety beyond that provided by amino acid-based medical foods [4].

Although early and continuous dietary treatment prevents severe intellectual disability in individuals with PKU, almost 60 years of experience using dietary treatment has enabled assessment of its impact on other long-term outcomes. Varying levels of dietary adherence success, a risk for suboptimal nutritional status, neuropsychological and psychosocial deficits, and compromised quality of life have all been observed in individuals with PKU, even when early and well treated. Negative

outcomes have been reported when dietary treatment is withdrawn or discontinued and/or relaxed during childhood [89–96] and early adolescence [35,43], and even in adulthood following early and continuous treatment [9,35]. A longitudinal study of children with PKU followed into adulthood reported that among those who discontinued diet, asthma, recurrent headache, eczema, neurological signs, hyperactivity, lethargy, phobias, and depression were found [9]. The majority of physical signs and symptoms of PKU resolve when blood Phe concentrations return to within the treatment range. Skin findings (e.g., scleroderma, atrophoderma) also have been shown to resolve with treatment [97–100]. Thus, the multisystem negative manifestations of elevated Phe levels in those with PKU support the need for careful and continuous dietary management throughout the lifespan.

#### 3.3.3. Outcomes (endpoints) important to dietary management and how to monitor them

**3.3.3.1. Blood amino acids.** Blood Phe is the most widely used measure to monitor metabolic status and has been shown to predict clinical outcomes [35]. The optimal time to obtain blood amino acid levels relative to food intake is influenced by when protein-containing medical food is consumed [101]. Individuals' blood samples should be collected at the same general time of day, and not right after eating or consuming medical foods [102]. Because low plasma tyrosine (Tyr) concentrations have been noted among individuals with PKU [103], Tyr levels are also routinely measured [104] and should be between 50 and 100  $\mu\text{mol/L}$  or within the normal range established by the laboratory [105]. Other amino acid concentrations should also be in the normal range [106] and monitored periodically and/or when there is concern about dietary adequacy.

Blood Phe:Tyr ratios may also be a useful monitoring tool for those with PKU [104], including individuals receiving sapropterin [107]. Infants identified by newborn screening with blood Phe levels consistent with "mild HPA" but with elevated Phe:Tyr >2.5 (normal Phe:Tyr = 1:1 [108]) at the time of newborn screening eventually required dietary treatment to maintain Phe levels <360  $\mu\text{mol/L}$  [109]. In addition, there are reports of impairments in executive function among individuals with high Phe:Tyr [110,111]. At this time, the clinical relevance of Phe:Tyr as an outcome measure is unknown.

**3.3.3.2. Growth in infants and children and weight in adults.** With adequate nutrient intake, individuals with PKU should have normal growth. Growth is dynamic, and expectations are relative to the age of the individual. Studies that have described growth below age-appropriate expectations either did not evaluate nutrient intake or documented nutrient deficiencies in those individuals [112,113]. When growth measurements of children with PKU were compared with established standards, no significant differences were observed [114–118].

In adults with PKU, weight should remain stable (unless weight loss or gain is desirable for an individual who is over- or underweight or is pregnant) and should not be affected by PKU.

Overweight and obesity have increased steadily in the United States [119]. Although obesity has been observed in children with PKU [115], the rates of overweight and obesity in children with PKU are not different from the general population [120,121]. A recent report found that children with PKU had a higher percentage of body fat than age-matched controls [122], and overweight was more common among children [123] and women [124] with higher blood Phe levels. More research is needed to document the trends in overweight and obesity among individuals with PKU, understand the long-term implications on development of other chronic diseases, and identify strategies to prevent excessive weight gain.

**3.3.3.3. Nutritional status and nutrient requirements.** Management of PKU primarily involves manipulation of the diet; thus careful attention to

dietary intake and nutritional status is necessary. Individuals with well-managed PKU should have adequate nutritional status. Nutrient adequacy is assessed by monitoring growth (Table 6), quantifying dietary intake using diet intake records, and measuring biomarkers. Skinfold measurements also provide useful information on fat mass [125], and reference data are available [126]. A computer-based diet analysis tool for use by metabolic dietitians is available (GMDI.org), which can be used in conjunction with blood Phe levels to adjust Phe and Tyr intake, macro- and micronutrients, fluids, and meal pattern.

Individuals with PKU need to be individually assessed to provide recommendations to meet total energy needs. Adequate energy must be provided for individuals with PKU during illness to limit catabolism and resulting elevated blood Phe concentrations [127].

Protein status needs to be carefully monitored, because treatment for PKU involves altering protein intake and mild protein insufficiency has been associated with decreased linear growth [113]. Normal protein status is achievable when adequate protein is provided [106,115]. Prealbumin is a useful measure of protein status in individuals with PKU [128]. Tyr becomes conditionally essential in PKU and must be supplemented in the diet; however, medical foods generally contain large amounts of Tyr. Recommendations for protein intake have been published and generally exceed age- and sex-specific recommendations [88,115,116,127,129,130] due to decreased protein utilization when amino acids are the primary source of protein. Published recommendations for protein intake include 120–140% of the Recommended Dietary Allowance for age [116] and 2–3 g/kg/day [88,129,130].

Iron deficiency and iron deficiency anemia have been reported among individuals with PKU, and routine evaluation of iron status is recommended [114,131,132]. Other mineral and vitamin deficiencies have been reported among individuals with treated and untreated PKU [114,133,134]. Even though these reports highlight the importance of these micronutrients (selenium, zinc, copper, iron, vitamin A), it is not clear that PKU, in and of itself, leads to a specific nutrient deficiency (except Tyr). Normal mineral status is expected for individuals with treated PKU who consume adequate amounts of medical foods containing the full complement of nutrients. Nutrient blood levels may require monitoring when there is a question about adequate intake [135].

Decreased bone mineral density (BMD), generally indicated by DXA, has been noted among individuals with PKU [136–138] and has been associated with reduced compliance with therapy [22,138]. Despite significantly higher intakes of calcium, phosphorus, and magnesium, decreased BMD has been reported in children with PKU [136]; however, normal bone density has also been documented in individuals with well-controlled PAH deficiency [22]. Therefore, it is important to monitor the bone health of individuals with PKU due to potential effects of elevated blood Phe levels on bone density. Further research is warranted to examine the effects of PKU itself, PKU management, and nutrient intake on bone density.

Essential fatty acid status should be normal when total fat intake is adequate [139]. Long-chain fatty acid supplements may improve fatty acid status [140–143]. Individuals with PKU are more likely to have inadequate fatty acid levels when fat-free medical food is consumed [144].

Regarding cognitive and behavioral functions, see Section 3.1. Many nutrients have roles in mood, cognition, and behavior. For example, vitamin D has many roles related to brain function, including neuroprotection and neurotransmission, and the B vitamins are involved in energy metabolism and neurotransmitter synthesis [145]. Selenium status has been linked to cognitive function in individuals with PKU [146]. Future research into the neuropsychological effects of nutrients that are often low among individuals with PKU is needed.

As information emerges about the effects of other nutrients (e.g., prebiotics, probiotics, other functional components) on nutritional and overall health, research into implications for individuals with PKU should continue [147].

3.3.3.4. *Use of medical foods.* With the basic requirements for medical food formulation well established, recent product design has focused on improving palatability, packaging, and product type (e.g., “sport drinks,” bars, puddings). Products with lower volume or caloric density modular products using amino acids without added fat, carbohydrates, or micronutrients, and newer products utilizing intact protein sources naturally low in Phe have been developed. Although these products address adherence issues and changing stage-of-life needs, some are nutritionally incomplete, which could compromise nutritional status if not correctly utilized. Medical foods should always be chosen to meet age-appropriate nutritional and adherence needs while considering cost and health care payer reimbursement. When nutritionally incomplete medical foods are components of therapy, regular monitoring of growth and nutritional status is essential. In addition, vitamin and mineral supplementation, including calcium, is required when using modular medical foods.

The ideal timing of medical food and dietary Phe consumption on Phe tolerance has been studied [127,148,149]. Frequent consumption of amino acid-containing medical food throughout the day increased Phe tolerance [150], and timing of protein substitute versus total energy intake or excess natural protein predicted plasma Phe concentrations [101]. To enhance nutrient utilization and minimize blood Phe fluctuations, medical foods should be consumed throughout the day.

3.3.4. *Criteria to identify patients who are found to be responsive to sapropterin who are appropriate for diet liberalization; how nutrition therapy should be altered; and best practices for making modifications*

Two categories of individuals with PKU who are BH<sub>4</sub> responsive may realize improved quality of life and nutritional benefits. First, in some patients who are unable to adhere to dietary therapy or unable to maintain a level of dietary restriction and medical food intake that sufficiently controls blood Phe, sapropterin may lower blood Phe to an acceptable range without further dietary modification. Second, patients whose dietary therapy and adherence already maintain blood Phe levels within a therapeutic range may be able to increase intake of natural protein, positively impacting nutritional status [151]. Quality of life is further enhanced with the ability to eat more normal sources of protein [152–154].

3.3.4.1. *Criteria to identify patients appropriate for diet liberalization.* Criteria to identify individuals for which a trial of sapropterin therapy may be appropriate vary widely and range from including all patients, prioritizing patients by clinical need or adherence history, or limiting therapy to a known mutation or enzyme activity. Protocols to determine responsiveness to BH<sub>4</sub> also differ, especially in dosage used (5–20 mg/kg/day), length of time to determine response (24 h to >4 weeks), and definition of response. Some programs follow parameters set by clinical trials in the United States to define response, generally >30% decrease in blood Phe [155,156], whereas others consider a response to be anything clinically beneficial to an individual patient. Most centers consider a sustained 20–30% or greater decrease in blood Phe to represent a response, while others also include an increase in dietary Phe tolerance as a marker for response [157–159].

Current label indications for use of sapropterin in the United States are limited to patients who exhibit a decrease in blood Phe. However, a recognized benefit to patients is the potential to liberalize dietary restrictions.

Reports differ on the value of using the magnitude of blood Phe response as the criterion for identifying patients who should be evaluated for increased Phe tolerance. Studies describing blood Phe response as predictive of increased Phe tolerance included only those who demonstrated an early and clear decrease in blood Phe (>30%) with sapropterin use [157,160,161]. When individuals with wider variance in response were included, the magnitude of blood Phe decrease did not predict which patients should be evaluated for increased Phe tolerance. Most reports state that any patient exhibiting a response to sapropterin that

**Table 6**  
Nutritional management across the lifespan.

Dietary mgt outcomes	Infant Newborn–1 year	Preschool Ages 1 year–<4 years	School age Ages 4–<11 years	Adolescent/transition Ages 11–<15 years	Early adulthood Ages 19–<25 years	Adulthood Ages 25–51 + years	Pregnancy
<i>Domains</i>							
Growth	With appropriate management, growth as expected for infant/child/adolescent without PKU [116,117,127,130]						
Head circumference (HC)	HC for age plotted on CDC or WHO growth charts; following growth curve with appropriate management [117]		Normal for age				
Length/height	Length for age plotted on WHO or CDC growth charts, following growth curve, and consistent with family [116,117,127]	Length or height for age plotted on WHO or CDC growth charts, following growth curve, and consistent with family [116,117,127]	Height for age plotted on CDC growth charts, following growth curve, and consistent with family [116,117,127]	Height for age plotted on CDC growth charts up to age 20 years, following growth curve, and consistent with family [115,116]	N/A		
Weight	Rate of weight gain as expected for age (WHO/CDC/growth velocity charts) with appropriate PKU management [116,117,127]		Rate of weight gain as expected for age (CDC/growth velocity charts) with appropriate PKU management [115–117,127]			Stable weight with appropriate management	Weight gain consistent with WHO guidelines
Weight for length/BMI	Weight for length “in channel” and appropriate (5th–95th percentiles, CDC or 2nd–98th percentiles, WHO growth charts) [116]	Weight/length (<24 months) or BMI/age (>24 months) appropriate (5–95th percentiles, CDC or 2nd–98th percentiles, WHO growth charts) [115,116]	Appropriate BMI for age (<95th percentile, “in channel,” or determined to be appropriate by clinician)			BMI between 18.5 and 24.9	N/A
<i>Biomarkers (PKU specific)</i>							
Phe (μmol/L)	120–360 [9,14,88,89]			120–360 [9,14,89]	120–360 [14]		120–360
Tyr	Within normal limits						
Phe:Tyr	<6 [104]					No data in adults	
Nutritional status	Intake records consistent with		Intake records consistent with		Intake records consistent with		
Diet record					recommendations for age and size, except protein and Phe. Depending on medical foods used, may need special attention to tyrosine, essential fatty acids, vitamins, and minerals		
<b>calculations</b>	recommendations for age and size, except protein and Phe [88,116,127,130]		recommendations for age and size, except protein and Phe [88,115,116,129]. Depending on medical food used, may need special attention to tyrosine, essential fatty acids [144], vitamins, and minerals				
<i>Biochemical</i>							
<b>Albumin</b>	Normal concentrations as expected for age and health status						
<b>Prealbumin</b>							
<b>RBC essential fatty acids</b>							
<b>Zinc</b>							
<b>Copper</b>							
<b>Iron status</b>							
Bone health	Normal concentrations as expected for age and health status					Some reports of ↓ bone mineralization, especially with less than optimal management [138]; no recommendations for routine clinical monitoring	
<b>Vitamin D</b>							
<b>DXA</b>	No recommendations for routine clinical monitoring		Normal bone mineralization with appropriate management [22,138]; no recommendations for routine clinical monitoring				
<i>Clinical Assessment</i>							
<b>Hair and skin findings</b>	Normal with appropriate management						

Abbreviations: BMI, body mass index; CDC, Centers for Disease Control and Prevention; WHO, World Health Organization.

maintains blood Phe levels within treatment range should be evaluated for an accompanying increase in dietary Phe tolerance.

**3.3.4.2. Best practices for making dietary modifications in sapropterin-responsive individuals.** Protocols for dietary modification with sapropterin treatment vary. In Europe and Canada, BH<sub>4</sub> loading tests have been used widely to determine BH<sub>4</sub>-responsive PKU and BH<sub>4</sub> deficiency. Because these protocols generally test response over a short period of time, generally 48 h or less, patients who exhibit a higher Phe tolerance and are more likely to have a significant and rapid response may be represented in greater proportion. These individuals are more likely to tolerate a complete discontinuation of dietary restriction, and this is predominantly reported in publications using these protocols [159,160].

Protocols that extend for longer periods of time, frequently used in the United States, are more likely to identify individuals who respond later and with a less significant decrease in blood Phe. These protocols should include a dietary Phe challenge to adequately measure any increased Phe tolerance. A Phe challenge is especially informative in those who consistently maintain lower Phe levels on dietary treatment because they may not show a further decrease in Phe levels while on sapropterin therapy, but may demonstrate increased Phe tolerance. In such cases, a Phe challenge may be started before the usual trial period is completed [162].

Although Phe tolerance on sapropterin is usually greater in patients with milder PKU, Phe tolerance also may increase in patients with more severe forms of PKU [163]. The goals of protocols used to determine Phe tolerance are to ascertain how much natural protein can be increased in the diet, if and how much medical food may be decreased, and whether the use of foods modified to be low in protein is still needed. Increases in dietary Phe intake from natural protein are reported to range from less than two-fold to more than three- or four-fold over baseline intake [153,157,160,164–167].

The best approach for determining increased dietary Phe tolerance is to add incrementally a natural protein (e.g., powdered milk) that can be easily calculated, measured, and adjusted without significantly affecting the patient's usual diet. This can then be converted to dietary protein if the Phe levels remain in the target range. Regular blood Phe monitoring and evaluation of dietary intake records are important throughout the Phe challenge and subsequent dietary modification. Illness, missed sapropterin dosage, or changes in diet, exercise, or lifestyle may complicate the evaluation. After determining increased Phe tolerance, dietary modifications can be made to include reevaluation of requirements for medical food and foods modified to be low in protein. Reeducation of the patient and/or family on implementation of the new dietary regimen is essential [151,166]. Regular monitoring of blood Phe levels and dietary intake is required to assess compliance and nutritional adequacy. Recommended indicators of nutritional status also should be monitored, especially in children. A reassessment of Phe tolerance may be needed subsequent to major changes in growth, body mass, or lifestyle. Recommendations for determining Phe tolerance and making subsequent diet modifications have been published [168].

There are few studies describing the long-term effect of sapropterin therapy along with modified dietary management [153,164,165]. Those that are available report success in maintaining treatment-range blood Phe levels with increased natural protein intake and decreased medical food [164]. Diet liberalization has the potential to augment improved adherence to treatment with associated flexibility in food choices and quantity in addition to enhancing the nutritional quality of the diet.

In summary, it may be appropriate to liberalize the diet of individuals who have responded to a sapropterin trial with decreased blood Phe concentrations. The length of time it takes for a response and the magnitude of the decrease in blood Phe do not predict an ultimate increase in dietary Phe tolerance. And finally, Phe tolerance can be a criterion for response to sapropterin, as it identifies the range of liberalization possible from a slight increase in Phe intake, to two to four times baseline intake, to achieving a normal diet [162,163,169].

### 3.3.5. Use of LNAA and GMP as dietary interventions (see Table 5)

**3.3.5.1. LNAA.** The oral ingestion of a mixture of LNAA, excluding Phe, with the purpose of lowering brain Phe concentrations was first studied in rats in 1976 [170]. LNAA share a common transporter into the brain, and when blood Phe levels are elevated, Phe preferentially crosses the blood–brain barrier by competitively inhibiting the transport of the other LNAA. As a consequence, brain Phe is increased (although the magnitude of the increase is variable among patients studied) and other LNAA concentrations are decreased [171], thereby impairing protein synthesis, increasing myelin turnover, and disrupting amine neurotransmitter systems [172,173]. Studies in individuals with PKU, however, have shown a poor correlation between blood and brain Phe levels with the use of LNAA, particularly when blood Phe levels are <1200 μmol/L [174,175]. It is also known that LNAA competitively inhibit uptake of Phe in the gut [176]. Current LNAA product composition has improved based on this functional effect, and it more accurately mimics the natural affinity to amino acid carriers in the gut and brain. Although LNAA may have some effect on decreasing blood Phe concentrations [177], this effect is not consistent or predictable [75].

Some literature suggests that the use of LNAA may be beneficial in adolescents and adults with elevated blood Phe who have relaxed or do not follow conventional dietary treatment recommendations [174,178–180] or who were late diagnosed and previously untreated [181].

LNAA may be used in conjunction with conventional dietary treatment, with a calculated amount of natural protein but without medical foods [182], or with an unrestricted diet [179,181]. LNAA typically provide 20–30% of the total protein requirement with 70–80% of protein supplied by natural sources [182,183]. Total protein has been limited to the DRI of 0.8 g/kg or 1 g/kg [183]. However, LNAA supplementation combined with unrestricted natural protein intake has been suggested to increase cerebral essential amino acid concentrations [182].

LNAA are contraindicated in pregnancy and in young children. Maternal blood Phe levels may not be sufficiently reduced during pregnancy to prevent MPKUS. There are no data on safety and efficacy of LNAA in young children. Precaution should be taken when using LNAA in individuals who are on psychotropic medications as the LNAA affect neurotransmitters in the brain. LNAA should be gradually increased to the prescribed dose as psychotropic medications are decreased in a process that may take up to 3 months (Kathryn Moseley, personal communication).

Most currently available LNAA products do not contain minerals and vitamins, and their use requires frequent monitoring of relevant biomarkers. As with any individual with PKU, nutritional status should be assessed and monitored according to standard guidelines for PKU. Protein intake should be monitored to ensure essential amino acid sufficiency.

**3.3.5.2. GMP.** GMP is a protein derived from whey and used in the dietary management of PKU. It is a natural protein source that contains minimal Phe, but also has insufficient amounts of histidine, leucine, tryptophan, and tyrosine that must be supplemented in the medical food for use in PKU [184]. In addition, threonine and isoleucine content is two to three times higher than in other natural protein sources. Even though a limited number of studies have been conducted utilizing GMP, increased palatability [184,185] and compliance and improved nutritional status have been documented [74]. Growth and Phe levels in brain and plasma of mice with PKU treated with GMP products were normal [186]. Insulin concentrations and ghrelin levels in short-term human studies were elevated, suggesting that the product may enhance satiety [187]. GMP has not been studied in children less than age 11 years. All published studies are short term, include a small number of subjects, and do not follow study participants over a long period of time.

Products that include GMP as the protein source serve as an alternative to Phe-free amino acid medical foods currently available, but they

also must be used in conjunction with a Phe-restricted diet. In addition, commercially available GMP supplies a small amount of Phe ranging from 2.5 to 5.0 mg Phe per gram of protein (<http://www.arlafoodsingredients.com>). Thus, the specific dietary therapy design will have to take into account the amount of Phe supplied through the GMP product.

### 3.3.6. Influence of individual patient variation on management

Patient variation may be categorically defined by phenotype (Phe tolerance and blood Phe levels) and genotype. With the advent of tandem mass spectrometry newborn screening and early hospital discharge, pretreatment Phe levels are not always informative. Thus, other variables such as individual Phe tolerance, genotype, results of neurological and IQ testing, and the specific clinical course of the disease are important factors to consider in the management of individuals with PKU.

Phe tolerance has been described as the amount of dietary Phe an individual can tolerate while maintaining blood Phe concentrations within the accepted treatment range [188]. A comprehensive document on diagnosis and classification of PKU in children along with a way to distinguish between different forms and various degrees of severity of PAH deficiency has been proposed [189]. Although Phe tolerance is usually established by age 5 years, more recent reports suggest that an individual's Phe tolerance can be predicted as early as age 2 years. Tolerance at ages 2, 5, and 10 years appears to be correlated [190]. However, the importance of reassessing Phe tolerance in adults as body mass changes has been documented [150].

Several studies have specifically examined the relationship between genotype and biochemical phenotype, and the corresponding dietary Phe tolerance and genotype-phenotype correlations have been examined in different populations [191–195]. For some ethnic populations, informative mutations have been collected and further classified into the four categories of classical, moderate, mild PKU, or mild HPA using blood Phe concentrations [196,197]. In addition, several reports classify PKU based on individuals' reported dietary Phe intake [189,196,197] (see also Table 2). Blau and colleagues summarized the available knowledge on diagnosis and classification of PKU in 2011 following a workshop dedicated to PKU held in Lisbon, Portugal, in March 2011 [188].

Classifying the severity of PKU using blood Phe concentrations prior to diet initiation has been reported (Table 2) [198]; however, an accurate maximal pretreatment Phe level is almost never obtained in current practice when newborns are identified via newborn screening.

### 3.3.7. Treatment considerations for persons with PKU who were either never treated or had inadequate treatment

Included in this category are individuals with PKU who have never been treated; who discontinued or were taken off a Phe-restricted diet as a school-age child; who were treated in the first 2 years of life, but the level of treatment was ineffective in achieving blood Phe levels within target ranges; or who are adults who cannot adequately follow dietary Phe restrictions and/or medical food, but would consider other treatment modalities. In these situations, treatment options include Phe-restricted diets with medical foods and the use of LNAA, GMP as the medical food protein source, sapropterin, or any combination of these treatments. Few well-controlled prospective studies with sufficient patient numbers are available to make broad recommendations.

Adults with classical PKU who were born prior to newborn screening generally have severe intellectual disability, behavioral problems, and neurological deficits [181,199–201]. The ability to study this group of individuals is limited by ethical concerns, and recommendations for treatment are not readily available. Anecdotal and small study group results suggest that severe behavioral manifestations may be reduced with nutritional therapy [181,200].

Children who were diagnosed late, defined as not detected through screening and put on a low-Phe diet after ages 2–3 months [202], or were inadequately treated, have been shown to have partial reversal

of IQ loss and catch up in development after initiating nutritional therapy [202]. Children who have previously not had optimal care should be offered standard dietary therapy and be considered for treatment with sapropterin or LNAA as deemed clinically appropriate.

Adults who are unable to follow standard dietary therapy present a special dilemma regarding whether to treat. Evidence suggests that they may benefit from any level of treatment that reduces plasma Phe concentrations and improves behavioral problems, executive function, and neurological dysfunction. Therefore, outreach efforts to encourage patients to reinstate their involvement in managing their Phe concentrations are warranted. With the availability of newer medical food products, including LNAA, and the possibility of an effective pharmacological option, return to therapy may be less challenging for those who have discontinued treatment. The goal of treatment should be any benefit to the patient that can be achieved with a realistic level of compliance.

Until more rigorous studies are performed, a prudent approach is to follow the statement that was generated in the NIH 2000 PKU Consensus Development Conference. Individuals who go "off diet" and suffer declines in central nervous system (CNS) function may be helped by returning to a Phe-restricted diet. Older individuals who are not on treatment should receive appropriate care. Individuals who cannot tolerate or refuse to accept the use of medical food should be offered sapropterin or LNAA, as they may be of benefit.

### 3.3.8. Breakout session input

Breakout session participants addressed the following: issues pertaining to evaluating adolescents and adults for individualized therapy goals; important elements of the diet for PKU that are in need of research; forging partnerships between dietitians and other health care providers; and international perspectives that might inform U.S. practice.

To set goals for optimal blood Phe control in adolescents and adults with PKU, clinicians need uniform and consistent approaches regarding frequency of blood evaluations, timing of blood tests, and frequency of clinic visits. Evaluations should include patient goals, a physical examination, medical and diet history, historical blood Phe concentrations, blood Phe control, cognitive and adaptive functioning, and genotype.

The most important elements of the diet of an individual with PKU that impact optimal outcome should be targeted for future research including agreement on optimal macro- and micronutrient profiles; identification of nutritional deficits; formulation of medical foods; examination of the nutritional value of natural proteins versus free amino acids in medical foods; the effects of chronic, lifelong, synthetic diets, including the impact on body weight and the development of eating disorders; the impact on parent-child and child-food relationships due to dietary restrictions; and depression and mood disorders related to nutrient deficiencies versus the pathophysiology of PKU. Other research questions include the reliability of blood spot samples relative to plasma samples for measuring Phe concentrations and whether brain or blood Phe concentrations are more important.

In considering how the metabolic dietetic community can partner with other disciplines to promote optimal outcomes, participants focused on the use of tools that could measure and improve adherence to treatment and educate families and patients. Other important collaborations include programs designed for each patient and family with input from clinics, schools, local support groups, and communities.

### 3.3.9. Summary and key points (see also Table 5)

The Diet Control and Management Working Group supported the premise that the primary treatment for PKU is the Phe-restricted diet and should be continued throughout the lifetime of individuals with PKU. Currently, blood Phe concentrations between 120 and 360  $\mu\text{mol/L}$  (2–6 mg/dL) are the treatment goal. Appropriate growth and development are expected in children, while avoiding underweight or obesity



and maintaining normal body weight are expected in adolescents and adults.

There are a number of factors that can influence management of individuals with PKU and predict response to treatment. Individualized treatment is necessary to maximize nutritional status, cognitive outcomes, behavior, mood, and quality of life. Hence, regular monitoring of blood Phe and Tyr and nutritional biomarkers to evaluate treatment adequacy and adherence is necessary. Blood Phe concentrations along with assessment of corresponding actual Phe intake is critical to determine Phe tolerance. Phe tolerance and genotype can inform clinical expectations and the design of effective treatment strategies. Additional treatment options should be individually assessed, particularly for "off-diet" individuals or others who do not adhere to treatment.

Full access across the lifespan to medical foods and foods modified to be low in protein provides the tools to succeed in managing PKU effectively on a daily basis. However, availability is inconsistent due to a patchwork of state laws and state programs that impact access. Even an optimally designed and well-managed dietary treatment for PKU may not replicate the quality of a normal diet in the general population. Treatment strategies that increase the allowance of natural protein food sources leading to increased consumption of a variety of foods should be encouraged. The range of nutrients needed for optimal body growth and development as well as a palatable diet contributing to improved quality of life will result from approaches that increase the ability to consume a more typical diet.

Barriers to management include lack of access to care (especially for adults with PKU) and inadequate coverage and reimbursement for provider services, medical foods, genetic testing, and other tools critical for effective, individualized PKU management.

### 3.4. Pharmacologic interventions

#### 3.4.1. Scope of work

The Pharmacologic Interventions Working Group was charged with investigating the role of BH<sub>4</sub> in individuals with PKU. Key areas of investigation included:

- BH<sub>4</sub> deficiency, mechanisms of action of BH<sub>4</sub> in PKU, and related biochemical defects
- Pharmacodynamics and pharmacokinetics of BH<sub>4</sub> in PKU
- Considerations for pharmacologic treatment with synthetic BH<sub>4</sub> (sapropterin) with regard to Phe and Tyr levels, clinical phenotype, and PAH genotype, and in whom sapropterin treatment might not be beneficial
- Best methods and predictors of determining responsiveness to BH<sub>4</sub>
- Considerations for pharmacologic intervention for newborns and infants with PKU and for those who are previously untreated, discontinued dietary therapy early, or refuse dietary therapy.

#### 3.4.2. Introduction

The BH<sub>4</sub> co-factor is essential for a number of enzyme activities in humans [203], including PAH, and is essential for the metabolism of catecholamines, serotonin, and nitric oxide in the CNS [203].

Clinically significant reductions in blood Phe levels were seen in some patients with PKU following oral administration of BH<sub>4</sub>, raising the prospect of oral pharmacotherapy for PKU [204,205]. BH<sub>4</sub> can cross the blood-brain barrier, its transport is dose dependent, and it stimulates CNS biogenic amine synthesis. BH<sub>4</sub> may act as a pharmacological chaperone to stabilize mutant proteins [206–209], promoting normal metabolism of Phe by increasing hydroxylation via kinetic mechanism [210] and lowering its concentration in the blood in patients who are BH<sub>4</sub> responsive. The frequency of BH<sub>4</sub> responsiveness reported in the literature varies depending on the severity of PKU and duration of the BH<sub>4</sub> challenge and ranges from 10% in individuals with classical PKU to >80% in those with mild PKU [155,205,211,212]. Although genotype cannot predict BH<sub>4</sub> responsiveness with 100%

accuracy, residual PAH activity (due to specific mutations) is strongly associated with the BH<sub>4</sub>-responsive phenotype [209]. Furthermore, the presence of two inactive alleles (severe classical PKU) effectively excludes the possibility of BH<sub>4</sub> responsiveness [213–216].

#### 3.4.3. BH<sub>4</sub> deficiency, mechanisms of action of BH<sub>4</sub> in PKU, and related biochemical defects

BH<sub>4</sub> deficiencies, a heterogeneous group of autosomal recessive neurological diseases, may present phenotypically with or without HPA [217]. BH<sub>4</sub> deficiencies affect either all organs, including the CNS, or only the peripheral hepatic PAH system, and they present with typical signs and symptoms of catecholamine and serotonin deficiencies. Abnormal signs may be observed in the neonatal period (i.e., poor sucking, decreased spontaneous movements, hypotonia), but symptoms are usually noted at about age 4 months. BH<sub>4</sub> deficiency presenting with HPA can be caused by mutations in genes encoding the enzymes involved in its (BH<sub>4</sub>'s) biosynthesis [218] or regeneration [219,220]. Patients presenting with HPA are usually detected through the newborn screening programs for PKU, whereas those presenting without HPA (e.g., Segawa disease or dopa-responsive dystonia and sepiapterin reductase deficiency [221]) are recognized clinically or by analysis of neurotransmitter metabolites and pterins in cerebral spinal fluid [222].

Dihydropteridine reductase (DHPR) deficiency is the most severe form of BH<sub>4</sub> deficiency, possibly due to accumulation of potentially neurotoxic dihydrobiopterin [223]. BH<sub>4</sub> also is connected to folate pathways, as patients with DHPR deficiency present with depletion of 5-methyltetrahydrofolic acid in the CNS, and substitution with folinic acid is mandatory [224].

#### 3.4.4. Pharmacodynamics and pharmacokinetics of BH<sub>4</sub> in PKU

The safety and efficacy of BH<sub>4</sub> were demonstrated in clinical trials leading to FDA and European Medicines Agency approval as an orphan drug to treat BH<sub>4</sub>-responsive PAH deficiency, but the molecular mode of action was not well understood [225]. The therapeutic efficacy of BH<sub>4</sub> depends on a number of factors, including quality, stability, dosage, mode of administration, and its pharmacokinetic properties [226]. To achieve the successful use of BH<sub>4</sub> in individuals with PKU and related disorders, it is critical to understand both its pharmacokinetics (the time course of the drug's absorption, distribution, metabolism, and excretion) and pharmacodynamics (the concentration of the drug at the site of action and the resulting effects) [227]. Some information about the pharmacokinetic and pharmacodynamic properties of BH<sub>4</sub> is available [228–230].

As with healthy subjects, pharmacokinetic parameters for individuals with HPA or PKU are characterized by a rapid absorption and distribution phase, followed by a prolonged elimination phase [226]. Pharmacologic response following oral administration of BH<sub>4</sub> appears to be delayed, as demonstrated by a reduction in blood Phe concentrations 8–24 h after each dose [231]. The findings from a population pharmacokinetic analysis in a subgroup of subjects from a phase 3 trial on safety and efficacy of sapropterin supported body-weight-based, once-daily dosing of sapropterin 5–20 mg/kg/day [232]. Greater reductions in plasma Phe occurred with doses of 10 or 20 mg/kg/day compared with 5 mg/kg/day [230].

There is a substantial lack of knowledge regarding pharmacodynamic properties of BH<sub>4</sub> and the effect of genotype on response to treatment with the natural co-factor. In several mouse models for compound heterozygous BH<sub>4</sub>-responsive PAH, blood Phe elimination, blood Phe:Tyr ratio, and kinetics of *in vivo* Phe oxidation were used as endpoints to discriminate the different mouse strains. When the pharmacodynamic properties of BH<sub>4</sub> were compared in wild-type, *Pah<sup>enu1/1</sup>* (mild HPA), and *Pah<sup>enu1/2</sup>* (mild HPA/classical PKU) mice, crucial differences were observed in effect size, effect kinetics, and dose response [233].

Overall, results of these studies suggest great variability of pharmacokinetic parameters among subjects, possibly due to the first-pass effect and/or factors affecting gastrointestinal absorption. Therefore,

investigation of the variability in BH<sub>4</sub> responsiveness among individuals with HPA and PKU is warranted. Although sapropterin is marketed for once-daily administration, more studies are needed to examine pharmacokinetic parameters with multiple daily dosing regimens [234].

#### 3.4.5. Considerations for pharmacologic treatment with sapropterin with regard to Phe and Tyr levels, clinical phenotype, and PAH genotype, and in whom sapropterin treatment might not be beneficial

It is important to understand the clinical phenotype of patients when considering initiation of treatment, including the use of sapropterin. Table 2 provides a comparison of definitions for classical PKU, moderate PKU, mild HPA gray zone, and mild HPA not requiring treatment. See also Section 3.5 for a discussion of the challenges in assigning current pretreatment blood Phe levels to a specific category of PKU severity.

**3.4.5.1. Initiating standard treatment.** The levels of blood Phe that trigger the initiation of treatment vary around the world and range from 200 to 600  $\mu\text{mol/L}$  [235,236]. In the United States, it is common practice to initiate therapy when blood Phe is  $>360 \mu\text{mol/L}$ . However, some individuals with non-PKU HPA who have plasma Phe concentrations consistently below 600  $\mu\text{mol/L}$  may not be at higher risk of developing intellectual, neurological, and neuropsychological impairment than are individuals without PAH deficiency. Although some specialists debate the advisability of nontreatment, others believe that dietary treatment is unnecessary for individuals in this class. In a study by Weglage et al., 31 individuals with HPA who were never treated and whose plasma Phe concentrations did not exceed 600  $\mu\text{mol/L}$  had normal IQ and education and career outcomes and the authors suggest that dietary treatment is unlikely to be of benefit in these individuals [237]. The use of Phe:Tyr ratio as one factor that may help in the decision of whether to treat mild HPA has been proposed [12], but its use requires caution as there are large diurnal variations in Tyr [12]. Further evidence for and against treatment of mild HPA is provided in several reviews [12,13].

**3.4.5.2. Initiating pharmacological treatment.** A minimum blood Phe level of 400  $\mu\text{mol/L}$  at the time of BH<sub>4</sub> testing has been suggested as a requirement to demonstrate responsiveness [188]. Lowering blood Phe levels by diet in four previously documented BH<sub>4</sub> nonresponders and retesting did not result in a response at the lower blood Phe level [238]. However, studies of PAH enzyme kinetics have shown that some mutations (e.g., R261Q) have maximum response to BH<sub>4</sub> at high levels of Phe, while other mutations (e.g., P314S) respond at lower levels of Phe [208]. This type of metabolic and genetic variation may explain some of the inconsistencies of BH<sub>4</sub> responsiveness reported in the literature.

Patients with a higher Phe:Tyr ratio were less likely to respond to BH<sub>4</sub>, even though baseline Tyr in BH<sub>4</sub> nonresponders and responders was similar [238]. Phe:Tyr ratio may then be a marker of disease severity because those individuals with higher ratios are less responsive to BH<sub>4</sub>, and hence are more likely to have more severe PKU. The value of using PAH genotype to identify BH<sub>4</sub> responsiveness was evaluated in 250 German [239] and in 588 Turkish patients [215]. Although genotype generally predicted nonresponsiveness, there was no clear genotype-phenotype correlation. Predicting responsiveness for gene mutations that affected regulatory domains was difficult, and 10 patients with identical genotypes had inconsistent responses to BH<sub>4</sub> loading. Genotype-phenotype correlations are discussed in greater detail in Section 3.5.3.

#### 3.4.6. Best methods of determining the responsiveness to BH<sub>4</sub>

**3.4.6.1. Measuring response to BH<sub>4</sub>.** Criteria have been proposed to measure the response to BH<sub>4</sub>, including reductions in blood Phe levels, an increase in dietary Phe tolerance, and improved psychological outcomes.

A 30% reduction in blood Phe levels is the most commonly used biochemical criterion to define BH<sub>4</sub> responsiveness [240], although reductions of 20% and 40% have been reported in the literature. Some investigators have attempted to further refine calculation of BH<sub>4</sub> responsiveness, but in general, there is poor agreement about definitions of responders using different models [241,242]. Complicating the interpretation of BH<sub>4</sub> responsiveness is the variability in day-to-day Phe levels and Phe:Tyr ratios [238].

As discussed in Sections 3.3.4.1 and 3.3.4.2, Phe tolerance, rather than a decrease in blood Phe levels, has been proposed as a measure of response to BH<sub>4</sub> because a decrease in blood Phe levels alone fails to capture the possible benefit of the ability to increase natural protein intake [163,164,240]. Several studies have shown a significant increase in dietary Phe tolerance with sapropterin treatment [153,165], and liberalization of natural protein intake may increase nutritional status and growth [243]. Although there is no universally accepted definition of what constitutes an increase in Phe tolerance, 300–400 mg/day dietary Phe above baseline has been suggested [153,163].

Improved psychological functioning may occur as a result of lower Phe levels, but it also could result from benefits of BH<sub>4</sub> via non-PAH-related effects, such as changes in neurotransmitter levels. However, neuropsychological testing is not standardized and is often difficult to perform outside of a research setting. Currently, there are limited data regarding the use of psychological outcomes as an indication of BH<sub>4</sub> responsiveness. One short-term study did not demonstrate an effect [244], although Table 3 includes a proposal for neuropsychological testing strategies across the lifespan.

**3.4.6.2. BH<sub>4</sub> testing protocols.** There is currently no standard protocol to test for BH<sub>4</sub> responsiveness. BH<sub>4</sub> dosing, minimum blood Phe levels prior to testing, timing of blood Phe sampling, prescribed dietary changes during the testing period, and intercurrent illness protocols vary among loading test protocols used in clinical practice [151,152]. Additional factors such as gastrointestinal absorption [245] and basal blood Phe levels [208] may influence the outcome of the test. BH<sub>4</sub> loading protocols are either of short or long duration, with advantages and disadvantages to each.

Short tests involve a single dose given in 24 h or two doses in 48 h. Multiple blood Phe levels are obtained at baseline and over a 24- to 48-hour period, the frequency of which may require hospital admission. Many responders show a response in 24 h; however, the last 24 h of the 48-hour load may be the most important in determining response [246]. Advantages compared with longer tests include fewer dietary inconsistencies and fewer false positives, while the disadvantages are frequent blood tests and missed late responders.

BH<sub>4</sub>-responsiveness testing in North America favors longer protocols with BH<sub>4</sub> administration for 1 to 4 weeks. A possible advantage of longer tests is the detection of both fast and slow responders [247]; however, some centers only obtain blood Phe levels once per week, leading to potential error in interpretation of response secondary to normal Phe variability [248]. In addition, there is the potential for bias in individuals with a great desire to be on medication who may unwittingly modify their diet to reduce Phe levels that may be erroneously attributed to BH<sub>4</sub>. Reports suggest that an initial BH<sub>4</sub>-positive response should be followed by a longer “effectiveness” trial to ensure that the initial response was true and to optimize Phe tolerance and BH<sub>4</sub> dosing [156]. This concept was reinforced during a workshop at the 3rd European PKU Group Symposium (Lisbon, March 25–26, 2011) where it was agreed that a short-term screening challenge with BH<sub>4</sub> should be followed by a long-term efficiency test [188].

One protocol that may provide more objective data about BH<sub>4</sub> responsiveness is the Sapropterin Therapy Actual Response Test (START) [216]. The START protocol combines a double-blind, placebo-controlled design and two 7-day testing periods to capture “slow responders.” Clinical benefit is defined as  $>20\%$  reduction in blood Phe [216], and Phe measurements are obtained after periods of protein

catabolism following overnight fasting [102]. This approach provides an accurate and rapid BH<sub>4</sub> response test, while minimizing the cost of testing.

The Phe breath test is the only noninvasive method to measure PAH activity in vivo [249], and it has been used as a research procedure to determine BH<sub>4</sub> responsiveness. Cumulative <sup>13</sup>CO<sub>2</sub> formation in the first 60 min after ingestion of the labeled Phe is a measure of immediate Phe uptake and oxidation in the liver [250]. Thus, the rate of <sup>13</sup>CO<sub>2</sub> production measures residual enzyme activity, and changes in PAH activity after BH<sub>4</sub> administration are an indicator for BH<sub>4</sub> responsiveness [251]. In patients on dietary restriction and with low blood Phe concentrations, a single Phe challenge should be included in the standard breath test [211]. This method of testing for BH<sub>4</sub> responsiveness may avoid some of the confounding factors associated with more traditional testing protocols, but its availability is a limiting factor.

**3.4.6.3. Specific predictors of responsiveness to BH<sub>4</sub>.** The ability to predict BH<sub>4</sub> response in PKU patients could reduce costs and help guide clinical decisions in managing PKU. Unfortunately, the results of pharmacogenetic studies to identify BH<sub>4</sub>-responsive genotypes have been inconsistent [214,239,252–254]. Variables within and between these studies may have contributed to inconsistent results and include differences in BH<sub>4</sub> test doses and test durations, variable definitions of response, differences in plasma Phe testing times relative to protein catabolism and dietary protein intake, and inconsistent dietary Phe and energy intake [214,239,252–257].

BH<sub>4</sub> responsiveness has been identified within all PKU phenotypes (mild, moderate, and classical) [167,214,239,252,254] and among all ages [165]. Importantly, age does not appear to play a role in the distribution, clearance, and half-life of BH<sub>4</sub> [232]. Using the START protocol, the presence of residual mutant PAH enzyme activity correlated with BH<sub>4</sub> responsiveness [216]. Among the 46 genotypes in this study, 25 contained at least one allele with a known residual PAH enzyme activity of greater than 25% of wild-type PAH activity. Of these 25 genotypes, 22 (88%) were found to be BH<sub>4</sub> responsive. Nine genotypes that were non-responsive to BH<sub>4</sub> contained alleles for which known activity was <10% for each allele, and the combined additive activity of both alleles was <11.85%.

**3.4.7. Considerations for pharmacologic intervention in infancy and for individuals with PKU who are previously untreated, discontinued dietary therapy early, or refused dietary therapy**

**3.4.7.1. Pharmacologic interventions in infancy.** Infants and children with disorders of BH<sub>4</sub> synthesis have been treated with BH<sub>4</sub> for over 20 years without adverse effects [258]. BH<sub>4</sub> loading tests are commonly performed in Europe subsequent to an abnormal newborn screen for PKU. Some BH<sub>4</sub>-responsive infants may have a defect in BH<sub>4</sub> synthesis, whereas others may have PKU due to PAH mutations that are associated with BH<sub>4</sub> responsiveness. Infants loaded with BH<sub>4</sub> achieved Phe levels within the treatment range more quickly than those treated with diet alone [228], so BH<sub>4</sub> loading may be beneficial in reducing the time that Phe levels remain significantly elevated in the newborn period. Some infants found to have BH<sub>4</sub>-responsive PKU by this method have been successfully continued on a BH<sub>4</sub> supplement since birth with good developmental outcomes and excellent control of Phe levels [228]. In the United States, clinical trials that formed the basis of FDA approval for sapropterin were conducted on individuals with PKU who were age 4 years or older. Although no age restriction was placed on the approval for Kuvan®, some physicians and parents have been reluctant to use the drug in younger children or in infants. Additional clinical studies are underway for younger children treated with sapropterin, specifically addressing neuropsychological outcomes (ClinicalTrials.gov Identifier: NCT01376908). Case reports suggest that sapropterin along with diet in infants and young children achieve better

control of Phe levels than with diet therapy alone [259]. Hypothetically, there may be potential benefits to an increased amount of natural protein in the diets of infants and young children being treated with sapropterin who can liberalize their diet; however, this has never been tested.

**3.4.7.2. Individuals with PKU previously untreated, discontinued dietary therapy early, or refused dietary therapy (see also Section 3.3.7).** Lowering blood Phe levels in late-diagnosed, developmentally disabled adult patients with PKU using dietary therapy has been shown to improve cognitive function and behavior [202,260]. Thus, it may be reasonable to consider sapropterin treatment in this population as a means to maintain lower Phe levels in those individuals determined to be BH<sub>4</sub> responsive. A limited number of anecdotal reports suggest that late-diagnosed adults with PKU may benefit from sapropterin treatment. Behavior and psychiatric problems improved with low-dose BH<sub>4</sub> therapy in one patient with mild HPA [261]. A pilot study of sapropterin in 10 previously untreated adults showed significant improvement in anxiety, nervousness, unexplained sadness, and negative behaviors, as well as increased blood Tyr levels and decreased Phe:Tyr ratio despite no significant change in blood Phe levels [262]. Thus, there may be beneficial effects of sapropterin in the CNS even without a decrease in blood Phe levels, although further research is warranted.

**3.4.8. Breakout session input**

Breakout session participants felt that testing for BH<sub>4</sub> responsiveness should be available to all individuals with PKU with the recognition that currently there are no uniform policies to guide testing for any given individual. There was almost unanimous agreement that blood Phe levels should be the measure of responsiveness to BH<sub>4</sub> testing, with a smaller majority of participants indicating that Phe tolerance should also be a measure. Standardization of BH<sub>4</sub> testing was deemed to be important, but due to regional differences in costs, requirements regarding inpatient treatment, and duration of testing, participants felt it would be difficult to implement. Participants noted that whereas FDA required only one biomarker for approval of sapropterin, ongoing clinical trials are examining additional endpoints such as improved behavior and executive function, among others.

**3.4.9. Summary and key points**

BH<sub>4</sub> is essential for metabolism of catecholamines, serotonin, and nitric oxide in the CNS. Inborn errors of BH<sub>4</sub> metabolism present with deficiencies of biogenic amines, and some may present with or without elevated blood Phe. BH<sub>4</sub> can cross the blood–brain barrier, and its transport is dose dependent. In some individuals with PKU, BH<sub>4</sub> acts as a pharmacological chaperone, stimulates residual PAH activity, and reduces blood Phe levels. There is no standard test for BH<sub>4</sub> responsiveness. Short duration protocols will identify the majority of responders but will miss the slow responders, whereas longer duration protocols might be prone to variability. Double-blind, placebo-controlled protocols may provide the most objective and comprehensive measure of responders, but they may be difficult to implement in all centers.

BH<sub>4</sub> responsiveness is variable and associated with milder phenotypes and specific genotypes. The decreased Phe levels and increased Phe tolerance in responsive individuals may increase diversity of the diet and decrease out-of-pocket medical food expenses. Although clinical diagnostic tests can assist in predicting genotype–phenotype correlations, not all genotypes have been described and a seemingly infinite number of combinations of alleles likely exist.

Significant gaps regarding the use of sapropterin in those with PKU remain. Individuals with PKU who are not followed in specialty clinics, which include the majority of patients with PKU over age 19 years [263], do not have access to BH<sub>4</sub>-responsiveness testing. Furthermore, although sapropterin is useful for some with PKU, for others, responsiveness is either minimal or nonexistent. There is a great need for

new drugs that are safe, efficacious, and impact a larger proportion of individuals with PKU.

### 3.5. Molecular testing, new technologies, and epidemiologic considerations

#### 3.5.1. Scope of work

The Molecular Testing, New Technologies, and Epidemiologic Considerations Working Group was charged with determining whether the information in the 2000 NIH Consensus Development Statement should be updated regarding newborn screening and molecular testing for PKU. Key areas of investigation included:

- Established genotype–phenotype correlations
- Ethnic or epidemiologic considerations for specific genotype differences
- Psychosocial and ethical implications associated with BH<sub>4</sub> response
- Emerging technologies that will impact treatment and management for PKU in the future.

#### 3.5.2. Introduction

Maximal pretreatment blood Phe concentrations have historically been used to categorize the severity of the PKU phenotype (Table 2). With the advent of tandem mass spectrometry a decade ago, screen-positive newborns are now recognized earlier and often have their initial clinic visit during the first week of life. Thus, confirmatory testing is performed and a Phe-restricted diet initiated prior to blood Phe reaching peak concentrations. Other techniques previously used to help determine the degree of PAH deficiency included Phe or standardized protein loading tests, which have safety concerns, and multiple quantitative amino acid assessments, which are not useful when dietary therapy needs to be initiated immediately after a diagnosis of PKU is made. Therefore, the traditional methods that were used to characterize the PKU phenotype are no longer as helpful as they were in the past.

Mutational analysis of the *PAH* gene provides an indication of the residual PAH activity in PAH-deficient patients in many cases [264,265]. However, genotype-based prediction of disease phenotype using estimated residual enzyme activity has limitations. Furthermore, the residual enzyme activity of many PKU mutations is not known. Correlating phenotype with a complete genotype, that is, both *PAH* mutations, and cataloging this correlation is more effective for future clinical use. Reliable genotyping is widely available through academic and private DNA diagnostic laboratories (GeneTests; Genetic Testing Registry), and the costs to sequence are now reasonable and decreasing. Both causative mutations can be identified in 95% of affected individuals [215,266].

As new molecular technologies emerge and evolve, such as whole exome and whole genome sequencing and identification of modifier genes, the potential to identify the degree of severity of PKU and tailor treatment to the individual based on non-*PAH* genetic changes will improve.

#### 3.5.3. Genotype–phenotype correlations

Genotype–phenotype correlations are being established for PAH deficiency. The Phenylalanine Hydroxylase Locus Knowledgebase (<http://www.pahdb.mcgill.ca/>) includes data on about 600 complete PAH genotypes and their associated disease phenotype, although this database has not been updated since 2009. The BIOPKU database ([www.biopku.org/home/biopku.asp](http://www.biopku.org/home/biopku.asp)) includes PAH genotypes from over 6100 individuals; disease phenotype and response or refractivity to sapropterin therapy in almost 3000 individuals; and a direct link to another locus-specific PAH database (PAHvdb; [www.biopku.org/home/pah.asp](http://www.biopku.org/home/pah.asp)) with 843 gene variations and corresponding 3D protein structures. New data are continually added to both databases, providing a useful resource for researchers and for clinical management of those with PKU.

As *PAH* gene mutations have been characterized, the association of these mutations with disease phenotypes has emerged [191,196,264].

Mutations associated with classical and with mild disease are now well characterized [206]. Mutations that retain substantial residual PAH enzyme activity are most often associated with mild phenotypes, a notable exception being c.1066-3C>T, a splice acceptor mutation. Mutations associated with inconsistent disease phenotypes include R261Q in the homozygous state or in combination with R158Q, the L48S mutation in the homozygous state or in combination with R158Q, and the Y414C mutation in combination with R408W. Although the relative disease severity associated with these mutation combinations is inconsistent, their response to BH<sub>4</sub> is consistent except for the 20% of L48S homozygotes that may not respond [188]. BH<sub>4</sub> response testing may be warranted in patients with the mutation combinations listed above that are associated with inconsistent phenotype.

Initial genotype-based predictions of the disease phenotype were made by predicting the *in vivo* residual enzyme activity of the patient. The predicted residual activity was calculated by averaging the relative *in vitro* residual enzyme activity associated with each of the mutant alleles of the patient's genotype. However, the *in vitro* residual enzyme activity of each mutant PAH allele is not known, and there can be an interplay between mutant PAH monomers that results in less than average activity. Therefore, an approach where the phenotype associated with each genotype, that is, each combination of *PAH* mutations was cataloged, emerged as a more effective way to predict disease phenotype in newly diagnosed individuals with PKU [209].

**3.5.3.1. The *PAH* genotype and BH<sub>4</sub> responsiveness.** The observation that a subset of PAH-deficient individuals benefits from pharmacological intervention with sapropterin changed management for many of these individuals [204]. Mild phenotype and the presence of a so-called responsive mutation(s) in the *PAH* gene [206] suggest that these individuals may benefit from sapropterin therapy. Those with classical PKU have been successfully treated with sapropterin; however, most are not responsive to BH<sub>4</sub> treatment [155,206,267]. Several reports have identified genotypes unresponsive to BH<sub>4</sub>, for example, homozygosity for p.R408W. Mutations associated with severe classical disease and refractivity to sapropterin are documented in the BIOPKU database and have been reviewed [188,206].

Mutations frequently associated with a positive response to BH<sub>4</sub> include p.E380G, p.V190A, p.V245A, p.Y414C, and p.A104D. Importantly, only knowledge of both mutations is informative with regard to BH<sub>4</sub> responsiveness and phenotype. Some residual PAH activity (>25% of normal) seems to be important for BH<sub>4</sub> responsiveness, although individuals with less residual activity have been shown to respond to BH<sub>4</sub> (BIOPKU database). There are mutations that have been inconsistently associated with BH<sub>4</sub> response, notable among these is p.L48S described above in Section 3.5.3, which provide evidence supporting the hypothesis that other loci influence the way individuals will respond to BH<sub>4</sub>.

Providers in metabolic centers vary in their rationale for ordering *PAH* mutation analysis. In a survey of metabolic centers reported by Blau et al. [188], 40% of centers indicated that all of their patients receive *PAH* genotyping, while 53% indicated that some patients receive genotyping. Among the reasons given for genetic testing were to elucidate the PKU phenotype based on genotype–phenotype correlations (53%), to inform genetic counseling (72%), and to provide information for individuals with PKU who want to try sapropterin (20%). The reasons given by centers who do not perform mutation analysis include that the genotype is not necessary to understand the phenotype (54%), too little information is available regarding genotype–phenotype correlations (40%), and lack of insurance coverage for mutation analysis (36%). In summary, providers are mixed in their use of genetic testing for individuals with PKU and their rationale for doing so.

#### 3.5.4. Ethnic and epidemiologic considerations for specific *PAH* genotypic changes

There are large variations in incidence and severity of PKU within differing subpopulations. The frequency and severity of *PAH* mutations

within subpopulations influence disease phenotypes within these groups. In more homogeneous populations, disease presentation is associated with frequently observed mutations, whereas in heterogeneous populations, such as those collectively observed in North America, there is broad distribution of a variety of mutations and phenotypes. Among individuals with PKU in the United States, homozygosity is observed at a rate of approximately 12%, and approximately half of these involve the common classical p.R408W mutation [268].

There are *PAH* mutations whose prevalence is observed at greater frequency among particular ethnic groups, and the prevalence of these mutations will influence disease phenotypes in the particular ethnic group. The central European populations (East Germany, Poland, Czech Republic, Lithuania, others) display a high prevalence of severe classical mutations [269,270]. For example, in Lithuania, 73% of mutant *PAH* alleles are p.R408W, and Hardy–Weinberg equilibrium suggests that 53% of PKU patients will be homozygous for this allele. Severe phenotypes also are associated with PKU in Ireland and the Scandinavian countries. Milder mutations are observed among Italian populations with a relatively high frequency of the p.L48S mutation, which retains approximately 39% residual *in vitro* enzyme activity [271].

### 3.5.5. Psychosocial and ethical implications associated with BH<sub>4</sub>-responsiveness testing and treatment decisions

Psychosocial and ethical concerns associated with BH<sub>4</sub> therapy exist and are related to the clinical decision to test for responsiveness and the subsequent treatment decisions based on the test findings. Understanding these implications is important because they guide other decisions about further needed research, the development of educational materials, and how best to develop policies about testing and treatment decisions.

**3.5.5.1. Ethical issues in BH<sub>4</sub>-responsiveness testing.** Genotype tests for *PAH* mutations can be used to (1) phenotypically categorize patients with regard to the severity of their disease; (2) classify patients by potential for BH<sub>4</sub> responsiveness; and (3) inform parental reproductive decisions. Although the cost of genotype testing is decreasing, it is still expensive and is often not covered by health care payers. Clinicians and families may emphasize the importance of the “informational value” of genetic testing differently, and it is, therefore, important that clinicians and parents understand the benefits and limitations of *PAH* mutational analysis.

Phenotype categorization based on analysis of Phe levels and dietary Phe intake may also predict BH<sub>4</sub> responsiveness, but it is less predictive than a specific BH<sub>4</sub> challenge test. Currently, there are different protocols for responsiveness challenges (see Section 3.4.6), each differing by effort of clinicians and families, complexities of interpretation, and costs.

Until a consensus is developed for a standardized approach to BH<sub>4</sub>-responsiveness testing, opinions will differ about the best way to approach this issue. Even if clear and directive recommendations about testing are developed, it will be important to develop educational materials that are balanced and understandable so that professionals and families can make informed decisions.

Divergent opinions between clinicians and families about treatment approaches are common. These differences may stem from patients and providers weighing the benefits and risks of treatment decisions differently. It is important to maintain a therapeutic alliance with the individual with PKU and/or family members, and strive for mutually acceptable goals of Phe control. While sapropterin may offer advantages for those who respond, some individuals and their families may prefer dietary treatment approaches. Any decision made about a specific treatment should be based on understanding the benefits versus risks to each individual with PKU and his/her family.

Other individuals or their families may request sapropterin treatment even though the clinician may not be convinced that it will be beneficial. In some cases, although there may not be objective evidence

of benefit through changes in Phe levels, some individuals with PKU may observe subjective cognitive benefits that justify treatment. In the last 15 years, there has been a gradual shift to placing greater emphasis on patient-oriented outcomes in clinical decision-making. However, such decisions are further complicated by the high cost of sapropterin, which may influence some providers because of their social obligations to manage costs.

**3.5.5.2. Cost/access issues.** Currently, some health care payers do not cover the genetic analysis of the *PAH* gene, sapropterin treatment, or even the medical foods that are essential for traditional clinical management approaches. Even though the cost of sapropterin per patient is high, the overall cost of this treatment across the entire population would not be great due to the rarity of PKU. Newer technologies, such as cell-based therapies and gene transfer, also may offer promise for PKU, and the expected cost of these technologies may be even higher than for currently available pharmaceuticals.

### 3.5.6. Emerging technologies that will impact treatment and management for PKU in the future

Among the technological advances in the study and treatment of PKU in the past decade are the development and approval of the first medication for PKU (sapropterin), the elucidation of genotype–phenotype correlations for *PAH* mutations, and the improvement in medical food formulations. The future holds the potential for paradigm-changing technologies such as cell-based therapies, gene therapy, and home Phe monitoring. Advances in diagnosis and treatment, as well as better understanding of modifying factors in determining disease severity and response to treatment, are likely to alter significantly the landscape in this field.

Many questions about the use and efficacy of sapropterin remain unanswered and are under investigation [155,156,165]. Sapropterin has been approved as an adjunct therapy to decrease blood Phe levels in a subset of patients with PKU, but its labeling in the United States does not include increasing dietary Phe tolerance as an approved therapeutic indication. In reality, these two parameters are inseparable, and future clinical studies should address the issues in tandem. The non-Phe-related effects of sapropterin are also under investigation. Specifically, a clinical trial has been conducted ([ClinicalTrials.gov](http://ClinicalTrials.gov) Identifier: NCT01274026) to determine whether the medication has any effect on neuropsychiatric status in patients with PKU even if there is no lowering of blood Phe when treated with sapropterin. The results of this study are being tabulated and prepared for publication.

Polyethylene glycol-conjugated phenylalanine ammonia lyase (PEG-PAL) represents a new class of medications to treat PKU now under investigation. Currently in phase 3 clinical trials, the medication is classified as an enzyme substitution therapy (see Section 4.1). The major side effect has been an early immunoglobulin M-mediated immune response to the polyethylene glycol component of the medication that appears to resolve with time and/or dose reduction. In contrast to the other therapies discussed thus far, PEG-PAL has the potential to normalize Phe in the majority of patients while on a normal diet, regardless of genotype, although formal studies to demonstrate this have not been completed as yet. It is important to note that the normal transformation of Phe to Tyr catalyzed by *PAH* does not occur in individuals treated with PEG-PAL, and thus they remain at risk for Tyr deficiency and Phe:Tyr imbalance. Delivery of an enzyme substitute to the bloodstream through an orally administered enzyme replacement agent has been proposed, but has been unsuccessful in limited attempts [272,273].

Additional therapeutic options for PKU exist on the more distant horizon. Gene therapy for genetic diseases has been a research target for many years, and hepatocyte transplantation also offers an alternative mechanism to treat PKU by providing liver cells with active *PAH* (see Section 4.1). Translation to human trials and routine clinical therapy remains a long-term goal.

Regardless of the form of therapy used, its effect on blood Phe levels must be monitored to avoid unexpected changes in metabolic status. Currently, this requires sending a blood sample to a diagnostic laboratory and then waiting for the sample to be processed and a result returned. While the time from sample collection to result has decreased over time, and convenience has improved by allowing home collection of blood on filter paper spots with analysis by tandem mass spectrometry, the lag is still considerable and makes management of therapy based on real-time Phe levels impossible. Development of a home Phe meter, analogous to a home glucose meter, may ultimately resolve this issue [274].

Technical advances in human genome sequencing, bioinformatic analysis, and functional analysis of mutant *PAH* alleles are all likely to have an impact on the treatment of PKU. Decreased cost and improved availability of *PAH* gene sequencing make precise genotyping of all patients with PKU increasingly possible, and some would argue, necessary. Complete data on broad populations will be necessary to achieve better understanding of genotype–phenotype correlations for *PAH* mutations [275]. The availability of relatively inexpensive whole exome or whole genome sequencing will allow identification of modifier genes that provide better prediction of clinical outcome. Similarly, the elucidation of the X-ray crystal structure of *PAH* and improved protein folding assays provide a platform to better understand the effects of individual mutations on protein structure and function as well as potential drugs to overcome these defects. Integration of a wide range of disparate data to make clinical predictions will be the purview of the bioinformatics community working in concert with knowledgeable metabolic physicians [276].

Finally, rapid advances in information technology have led to the development of a wealth of new options to improve communication and access to educational materials for individuals with PKU, their families, and clinicians and researchers. Moving forward, it will be important to develop an evidence base through the use of registries collecting data about patient-oriented outcomes related to diet, treatments, and emerging screening and diagnostic approaches.

In summary, there is great potential for new treatments for PKU to be developed in the next two decades. The recognition of the need for treatment for life and the inadequacy of current therapies will be the drivers to developing new therapies and guaranteeing access to care for all individuals with PKU.

### 3.5.7. Breakout session input

Breakout session participants addressed five specific questions. The first question related to availability and access to *PAH* genotype testing. GeneTests ([www.genetests.org](http://www.genetests.org)) was noted as a resource for locating laboratories that perform genetic testing for PKU; another resource is the Genetic Testing Registry (<http://www.ncbi.nlm.nih.gov/gtr/>). Challenges for patients and providers center around the time-intensive processes required for authorization and reimbursement for testing and subsequent genetic counseling. It was noted that because *PAH* is not a patented gene, there are no restrictions on the ability to test for it. The second question concerned the use of genotyping to predict BH<sub>4</sub> responsiveness; it was noted that some clinics will conduct BH<sub>4</sub> testing even when the genotype predicts that the individual is likely nonresponsive. Participants concluded that there is not enough evidence at this time to prohibit BH<sub>4</sub>-responsiveness testing in patients with unfavorable genotypes. The third question addressed whether information from newborn screening is useful to classify individuals with PKU, and if so, what Phe and Phe:Tyr ratio cutoffs should be used. Participants concluded that even though Phe levels and Phe:Tyr ratios are a valuable newborn screening tool for PKU, Phe and Tyr levels obtained in the newborn period are impacted by timing and amount of protein in the diet. Thus, confirmatory testing and classification of severity of PKU require further evaluation to include quantitative measurements and genotyping (Table 2). In addressing the fourth question about whether carrier testing for *PAH* mutations in prospective parents would be practical or

helpful for predicting the risk of having a child with PKU, participants concluded that although it is technically feasible, issues of cost, consent, and lack of clarity regarding the goals of prenatal testing complicate such testing. However, it was noted that although it is possible to perform prenatal testing in families who already have a child with PKU, it is not known how common this practice is.

### 3.5.8. Summary and key points

The value of obtaining the *PAH* genotype in individuals with PKU has been established to inform genetic counseling and predict, at least partially, the biochemical phenotype as well as responsiveness to BH<sub>4</sub>. Genotyping specific populations and ethnic groups has provided a rich database of sequence information that is publicly available and can be a valuable resource in counseling patients. The use of genotype information in the newborn period may also yield valuable insights about the severity of the condition before Phe tolerance can be ascertained in infants who may be diagnosed before maximal Phe levels are reached. Although emerging and established genotype–phenotype correlations have the potential to improve our understanding of PKU, genotype correlation with the intellectual phenotype may be more difficult to elucidate. Moreover, the complex psychosocial and health care system issues that impact patients and families influence the decisions they make regarding therapeutic choices, including the use of sapropterin. Moving forward, it is likely that new treatment modalities will be developed that can improve quality of life for a greater proportion of those with PKU, and also have the potential to have an even greater impact on the health care delivery system and health care payers. In this environment, it is imperative that treatment guidelines and the decision processes for determining access to treatments be tied to a solid evidence base with rigorous standards for robust and consistent data collection.

## 4. Other reports

### 4.1. New treatments on the horizon for PKU (Cary O. Harding, M.D.)

Novel therapeutic approaches to PKU are needed, including cell and gene therapy and enzyme substitution therapy, which do not rely solely upon dietary Phe restriction [277]. Although PKU is caused by deficiency of liver *PAH* activity, the proximate pathophysiology of the disease is due to effects of elevated Phe upon the brain [278]. Therefore, any treatment that lowers brain Phe will ameliorate the symptoms of PKU. Liver transplantation to provide a fully functional Phe hydroxylation system is curative [279] but certainly not common practice. Alternatively, therapeutic liver repopulation with *PAH*-expressing hepatocytes is an alternative approach that is less invasive than whole-organ transplant [280]. Successful therapeutic liver repopulation requires both a stimulus for hepatocyte regeneration and a selective growth advantage for donor hepatocytes [281]; unfortunately, *PAH*-positive hepatocytes have no selective growth advantage over *PAH*-negative cells, and transplantation of *PAH*-positive hepatocytes into *PAH*-deficient *Pah<sup>enu2</sup>* mice without such a growth advantage yields no therapeutic benefit [282]. However, if a selective growth advantage for donor cells can be achieved, liver repopulation as little as 10% with *PAH*-positive hepatocytes yields complete correction of HPA in murine models [282,283]. A clinical trial of hepatocyte transplantation with a novel method for inducing a growth advantage for donor cells is currently underway at the University of Pittsburgh for adults with PKU (<http://www.clinicaltrials.gov/>).

Gene therapy for PKU also has been intensely investigated primarily in murine PKU models [277]. The most common gene therapy approach is the delivery of a correct copy of the *PAH* gene, usually to the liver, using either a recombinant viral vector or by a DNA-mediated method. Correction of HPA in *Pah<sup>enu2</sup>* mice was first achieved using a recombinant adenovirus vector [284], but the success of this treatment was limited by an inflammatory immune response against the viral particle. More recently, recombinant adeno-associated virus (AAV) vectors,

particularly AAV serotype 8 vectors, have been used because of their low immunogenicity and facile ability to infect the liver [285]. A single intravascular injection of a recombinant AAV8 vector expressing murine PAH resulted in restoration of liver PAH activity and correction of blood Phe to therapeutic levels in *Pah<sup>enu2</sup>* mice [286–288]. The AAV8 vector can even be successfully delivered by intramuscular injection [289]. However, the recombinant AAV genome does not integrate into the host liver genome, and time-related hepatocyte turnover is associated with loss of vector genomes and reemergence of HPA, typically by 1-year post-AAV8 vector administration [289]. Methods to allow vector reinjection, currently limited by the immune response following initial infection, or to allow increased frequency of safe viral genome integration are under development. Liver-directed gene therapy with PAH-expressing nonviral DNA minicircles [290] or constitution of a complete Phe hydroxylation system in skeletal muscle [291] are also under investigation as potential methods of achieving permanent correction of HPA.

One of the most promising experimental therapies for PKU is enzyme substitution therapy using recombinant phenylalanine ammonia lyase (PAL), an enzyme employed by many plants, fungi, and bacteria to metabolize Phe. Enzyme replacement therapy using native human PAH protein also has been explored [292], but this approach is limited by difficulties in producing recombinant tetrameric PAH protein, the requirement for BH<sub>4</sub> co-factor, and the need to deliver the protein to the liver [293]. Alternatively, PAL is active as a monomer and has no exogenous co-factor requirement. Subcutaneous injection of recombinant PAL protein from the yeast *Rhodospiridium toruloides* into *Pah<sup>enu2</sup>* mice yielded reduction of blood Phe to the normal range [294]. Coating the protein with PEG led to suppressed immune responses against the recombinant protein and sustained treatment efficacy out to 1 year with weekly PEG-PAL injections [295]. PAL protein from the cyanobacterium *Anabaena variabilis* ultimately proved to be a better clinical candidate because of improved stability, more favorable kinetics, and proteolytic resistance [296]. Ultimately, PEGylated recombinant *A. variabilis* PAL (rAvPAL-PEG) was chosen by BioMarin Pharmaceutical Inc., Novato, California, for further clinical development. A successful phase 1 trial was initiated in 2008 with evidence of treatment efficacy in the highest-dose cohort; phase 2 trials have recently been completed [297]; and pivotal phase 3 trials to include a blinded, placebo-controlled evaluation of rAvPAL-PEG have been initiated in adults with PKU (<http://www.clinicaltrials.gov/>). Enzyme substitution therapy with rAvPAL-PEG is a very promising novel treatment approach for adults with PKU who are unable to maintain adherence to the PKU diet.

#### 4.2. Perspectives from industry and advocacy organizations

This interactive session was moderated by Dianne Frazier, Ph.D., M.P.H., R.D. The panelists were Suyash Prasad, M.D.; Steven Yannicelli, Ph.D., R.D.; David Paoella; Christine Brown, M.S.; and Amy Oliver. The session sought to obtain the insight of individuals who represent industry (Drs. Prasad and Yannicelli and Mr. Paoella) and advocacy organizations (Ms. Brown and Ms. Oliver) regarding treatments to achieve optimal outcomes for PKU. The representatives described their organizations; the products and/or services they provide for the PKU community; their organizational goals for the development of future treatments; the barriers or challenges to developing treatments and/or accessing care for individuals with PKU; and the ways in which industry, clinicians, researchers, advocacy groups, professional organizations, and individuals with PKU can partner to advance treatments for PKU.

In addition to providing products used in the management of PKU, industry provides educational tools and supports families as they navigate the health care system. Industry is also focusing on making the diet for PKU appear as normal as possible. A broader approach to the functionality of medical food products is being sought. It is a challenge to make products that fit the needs of individuals at each life stage, are palatable, are integrated with new drug treatments, and are

covered by insurance. However, one industry representative cautioned that there is the risk of decline in insurance coverage for innovative products that resemble normal foods, creating a disincentive for their development. These challenges will require partnerships between industry and regulatory groups to innovate beyond food science technology and develop therapeutic approaches and new diagnostic and monitoring tools for all of the disorders detectable through newborn screening.

The advocacy groups work to empower those with PKU and other IEM by providing platforms for communication and a variety of educational resources. Of concern to these organizations is that the science to detect and diagnose rare IEM is ahead of the implementation of a mechanism to ensure equitable access to treatment for all who need it. One of the main barriers to access to treatment in the United States is the inconsistent coverage of medical foods by health care payers. Advocacy work requires support from all those involved in the care of individuals with PKU. It is important that policymakers, legislators, regulatory agencies, and health care payers are thoroughly educated by patients, clinicians, and research scientists about the vital need for continued treatment and monitoring of individuals with PKU and other IEM.

Both industry and advocacy representatives voiced concerns related to the very difficult day-to-day dietary management of PKU. One advocacy representative would like to see researchers, clinicians, and others work together to ease the burden of these difficult diets on patients and families so that all children have an equal opportunity to achieve their full potential as adults.

Personalized care, now and in the future, will require a range of therapies and a greater societal focus on the field of rare diseases. However, clinical effectiveness data will be needed to understand how treatment selections can optimize management of PKU, and clinical trial data are needed to inform regulation and insurance coverage decisions. The relative rarity of PKU makes clinical trials difficult to perform, and mechanisms to locate and enroll patients in clinical trials are needed. An industry representative suggested that an important area of research that could confirm efficacy of treatments and enable the targeting of products is the development of valid biomarkers. This would allow for a better understanding of the pathophysiology of PKU at the level of the CNS. Further, development of a successful home blood Phe meter would empower patients and families and help move research forward.

#### 4.3. Perspectives from the international community

This interactive session was moderated by Uta Lichter-Konecki, M.D., Ph.D. The panelists were metabolic clinicians and researchers from several countries: Nenad Blau, Ph.D. (Germany and Switzerland); Anita MacDonald, Ph.D., R.D. (United Kingdom); John Mitchell, M.D. (Canada); and Susan Thompson, R.D. (Australia). This session focused attention on the management of PKU from an international perspective. The session's purpose was to identify differences in the management of PKU in these countries compared with the United States; identify barriers and challenges to providing dietary interventions and/or medications; describe the status of treatment guidelines; and articulate lessons learned that would help support partnerships between industry, clinicians, researchers, advocacy groups, professional organizations, and individuals with PKU to advance treatments for PKU. The panel discussion and audience question-and-answer session revealed common themes that are described below.

##### 4.3.1. The status of management guidelines for PKU

Although Europe comprises many different countries, each with its own national guidelines and associations, a process is currently underway to develop consensus and guidelines for Europe as a whole. In Germany, PKU management guidelines are being reviewed. In the United Kingdom, recommendations are used that were developed in 1993 and reinforced by the National Society for Phenylketonuria

([www.nspku.org](http://www.nspku.org)) in 2004 and again in 2010; however, they are not uniformly followed across the country. Australia has national guidelines for newborn screening, but not national treatment guidelines, and clinics vary in how they manage patients. A national protocol for BH<sub>4</sub>-responsiveness testing and the introduction of sapropterin is being developed in Australia in preparation for the availability of the drug. Canada does not have uniform guidelines on who should be treated, and management differs across centers and as compared with other countries. The time needed to develop a consensus in Canada, such as in the United States, is a lengthy process despite the small number of experts in Canada. A participant from the United States suggested that because insufficient knowledge exists about optimal treatment within the broad range of low and high blood Phe levels, patients also are not treated uniformly in the United States. Large collaborative studies will be needed to organize data in a consistent manner to inform treatment decisions. Several participants suggested that development of international guidelines that included clinical judgment due to variability among patients would be beneficial.

There was general acknowledgment among the panelists and participants that blood Phe cutoffs for defining a category of PKU severity is not uniform among countries, and an international conversation about this issue should be undertaken.

#### 4.3.2. PKU clinics

In the United Kingdom, PKU care is fairly well centralized into specific clinics due to the small size of the country. In Australia, although there are centralized metabolic clinics, there is a risk of resources being diverted from the management of PKU to the acute management needs of other metabolic diseases being diagnosed as a result of expanded newborn screening. Staffing issues must be addressed in light of how this impacts PKU management. In Canada, there are relatively few metabolic centers, and some patients travel long distances to be seen. However, care is centralized at these centers, and the follow-up is cohesive. In addition, wait lists for patients to be seen by specialty providers such as psychologists and psychiatrists can be long. Critical needs include improving patient self-monitoring, greater access to dietitians, and sufficient numbers of providers to facilitate the transition from pediatric to adult care.

#### 4.3.3. Dietary issues

With respect to dietitians, it was suggested that dietitians in the United States have more responsibilities and play a different role than those in some European countries. In the United Kingdom, however, there are dietitian-led PKU clinics. Standards of education for dietitians are not uniform across Europe, which explains the different roles and levels of responsibility that might be seen in Europe and the United States.

Dietary management challenges were described, and one panel participant noted that the diet for PKU is relentless and intrusive on a day-to-day basis. Some families do not cope well, and less than optimal results are seen. In Australia, families and patients do not have the cooking and food skills that they had in the past, and there is a limited range of medical food products compared with the United States, which exacerbates the problem.

In a discussion about the differences between the United States and other countries, it was noted that in the United Kingdom, approximately 50% of adults are on diet for life, whereas only 30% adults in the United States are even followed in a clinic [263], let alone treated with diet for life. The uniform provision of medical foods is one of the strengths of the United Kingdom health care system.

With respect to coverage of medical foods, the panel participants described the differing landscapes that characterize coverage in their countries. Although Canada has universal health care coverage, there is limited funding, and it does not necessarily apply to orphan diseases such as PKU. Medical foods and formulas are covered for all ages in Quebec, but not necessarily in other provinces, leading to disparities

across Canada. In the United Kingdom, all special medical foods are paid for by the state, although individuals over age 16 years are expected to pay a small prescription charge for each item dispensed. Australia has a federal and state system, and medical foods are subsidized with the cost to patients around \$5 a month. Additional funding for low protein foods is available, but the range of products is limited.

#### 4.3.4. Provision of sapropterin and general research challenges

In the United Kingdom, funding for sapropterin is not usually provided by the National Health Service, even though it is registered in the country. Equally, in Australia, most patients with PKU do not have access to sapropterin, even though it is designated as an orphan drug in the country. Canada does not have an orphan disease policy to direct how these medications should be handled. In Quebec, the government has not recognized the cost versus the benefits of sapropterin and has declined to cover this therapy, despite recognizing its clinical benefits. However, pregnant patients in Quebec are covered for sapropterin, and there is some leeway to test responsiveness in women considering pregnancy. In the United Kingdom, pregnant women with PKU have to fail dietary management to potentially receive sapropterin, an untenable situation.

Currently, there is general inertia around PKU within the United Kingdom. New therapies are not available to use routinely; little money is being directed to research; and there are very few postdoctoral students working in PKU. Basic research is needed to bring more focus to this field.

#### 4.4. Transitions to adult health care (Sandra Sirrs, M.D.)

Transition is defined as “the purposeful, planned process that addresses the medical, psychosocial, educational, and vocational needs of youth and young adults with disabilities as they move from child-centered to adult-oriented health care systems” [298]. For youth with complex health care needs, transition involves care within a “medical home,” which provides coordinated services for the youth and family within the community [299]. Recent studies suggest that a minority of young people with complex health needs receive counseling in the areas of health insurance, transition planning, and anticipated changes in their health with age [300], suggesting that more work needs to be done to enhance the transition of youth to the adult health care system.

Barriers to transition are articulated by patients, families, and health care providers. Women with PKU noted that the challenge of informing new health care providers about their past medical history presented a major barrier [68]. Patients and families reported feeling anxious about leaving their longstanding relationship with a pediatric care provider and both meeting a new person and receiving care in a new setting [301]. In a recent publication, a group of patients with PKU provided concrete suggestions to ease this anxiety, including a combined visit with both their pediatric and adult health care providers followed by an opportunity to return once to the pediatric clinic after the transfer of care was complete [302]. Pediatricians noted that there was a shortage of adult physicians with either the necessary expertise in rare disorders or the willingness to care for patients with complex health care needs [303]. A needs assessment identified a lack of suitable opportunities for medical residents to obtain experience in the care of patients with chronic health conditions [304], and strategies to address this may increase the willingness of adult physicians in the future to care for these patients.

One of the biggest obstacles in the transition of youth to adult health care is the time it takes to plan and execute the transition process. For some common conditions such as diabetes mellitus and renal transplantation, effective transition care has been shown to improve health outcomes [305] and result in cost savings [306], thereby justifying the costs of providing support for physicians and patients in the transition process. In the field of rare diseases such as PKU, one of the greatest obstacles to finding sustainable funding to support the transition process is



the lack of data on the effects of such planning on clinical outcomes in adult patients. This PKU consensus conference may help identify clinical targets, monitoring protocols, and research priorities that can help provide data over time to justify funding to support transition processes for youth and young adults with PKU.

In summary, transition processes are not optimal for youth with complex health care needs due to limitations in resources to support adequate transition planning and post-transition care. Future work to improve the transition of youth with PKU into the adult health care system will require research targeted at the effects of therapeutic interventions on clinical outcomes that can then be used to project the resources needed to support this transition process.

#### 4.5. Sound clinical trials: a regulatory perspective from the FDA (Anne R. Pariser, M.D.)

##### 4.5.1. Clinical trials in rare diseases

Drug development is a complex process, and rare diseases, defined as diseases affecting fewer than 200,000 individuals in the United States [307], present many challenges for the pharmaceutical industry. Rare disease patient populations are small; thus, there is limited opportunity for the study of candidate therapeutics in clinical trials. Among other challenges, rare diseases are usually poorly understood, knowledge of disease natural history is incomplete, there is little or no regulatory precedent for drug development, and elements important to clinical trial design, such as disease-specific endpoints and assessment measures, are often lacking. While careful planning of drug development programs is essential for all medications, it is especially important for rare disease drugs given these known clinical research challenges.

Statutory standards for the approval of new drugs in the United States require that substantial evidence of product quality, effectiveness, safety, and a favorable benefit-to-risk profile be demonstrated for a drug in the treatment of a specified patient population. Approval standards are the same for drugs intended to treat either rare or common diseases, although there is flexibility in determining the type and quantity of data needed to meet these standards [308,309]. Substantial evidence of effectiveness is generally obtained through the conduct of adequate and well-controlled trials. An adequate and well-controlled trial is defined as a trial that is generally recognized by the scientific community as having been designed, conducted, and analyzed well enough such that the results obtained could be “fairly and responsibly” concluded as having shown that the drug will have the effects it claims under the “conditions of use prescribed, recommended, or suggested” in the product labeling [310].

The main purpose of conducting clinical investigations is to distinguish the drug-mediated effects from other effects, such as spontaneous change in the course of the disease, bias, or other influences (e.g., placebo effect). An adequate and well-controlled trial should be prospectively designed to meet this goal by including a clear statement of purpose, methods for minimizing bias, and methods of assessing response that are well defined and reliable [311]. Randomized, concurrently controlled trials are the gold standard, although other designs may be appropriate in special circumstances. To ensure appropriate use of a drug postapproval, indications and instructions for use (e.g., intended population, dose, and duration) will need to be determined, typically in the premarket period. All of this information is generally not obtained from a single trial, and most drugs will undergo evaluation in sequentially conducted trials within a comprehensive drug development program. Well-conducted clinical development programs also can identify additional areas of study that may be further evaluated in the postmarketing period (e.g., long-term safety assessments in a larger patient population).

The sapropterin clinical development program is an example of the application of established scientific principles that were flexibly applied in a rare disease patient population. The AHRQ report notes that the sapropterin program provided most of the evidence for an intervention

in PKU obtained from adequate and well-controlled trials. Key considerations that informed the design and conduct of the sapropterin clinical development program included some a priori knowledge of the mechanism of action of the drug, disease pathophysiology, natural history of the disease, and description of patient subgroups that, when taken together, informed the design and conduct of a series of adequate and well-controlled trials. These trials were able to provide substantial evidence of effectiveness, safety, and favorable benefit-risk to support drug approval. However, the mechanism of action of sapropterin in PKU has not been fully elucidated, and at the time, it was thought to act as a pharmacologic chaperone that stabilizes and augments mutant PAH [229,312]. During the premarket clinical trials, it was predicted that only a subset of PKU patients would respond to sapropterin. (Subsequent work has led to a greater understanding of the mechanism of action and sapropterin-responsive genotypes [216,313]). The biochemical pathway of the disease was well understood, with availability of a short-term biomarker (Phe) able to be used as an endpoint in clinical trials to assess the pharmacodynamic effect of sapropterin administration. Use of Phe as a surrogate endpoint also was supported by existing consensus recommendations [1] and long-term clinical outcomes data [14]. The major clinical outcome assessment (COA) of interest in PKU – long-term neurocognitive outcomes – is not practical for most drug development programs since assessments would need to be performed over many years to decades. Finally, the short-term effectiveness of sapropterin (reduction in Phe) was demonstrated in four clinical trials, which were mainly inclusive of two different patient populations: poorly controlled patients, age  $\geq 8$  years, and children, ages 4–12 years, who were well controlled on diet [314,315].

A key feature of the clinical development program was the use of an enrichment design, in which sapropterin responsiveness was first assessed in an open-label, single-arm, short-term exposure trial (or phase), and responders were then offered entry into the randomized, double-blind, placebo-controlled trial. Otherwise, much larger clinical trials would have been necessary to achieve a statistically significant change in mean Phe in a nonenriched patient population.

Limitations of the premarket program were recognized, and steps were taken to address some of them in the postmarketing period [316]. A long-term observational postmarketing registry was established as a condition of approval, the PKUDOS (Phenylketonuria [PKU] Demographic, Outcomes, and Safety) Registry. Because sapropterin's efficacy and safety in pregnancy were not evaluated in the premarket period, a PKU maternal outcomes survey subregistry within PKUDOS known as PKUMOMS, intended to follow maternal-fetal outcomes in women in the United States with PKU who become pregnant, also was established. Additional assessments in children, including long-term assessment of effects on neurocognitive outcomes and growth in children age  $\leq 8$  years at study entry, and a 6-month study of the safety, efficacy, and pharmacokinetics of sapropterin in children age  $< 4$  years were required. As a condition for approval, the FDA also required that PAH gene mutation analysis be performed in banked specimens for the purpose of determining whether patients with specific PAH mutations are likely to be responders.

##### 4.5.2. Directions for the future

Two fundamental principles of human experimentation are: (1) a clinical trial is inherently ethically unjustifiable if it is not scientifically reliable and valid; and (2) the needs of patients are best served through scientific rigor and good-quality trial designs that are able to achieve their stated outcomes. Descriptions of disease natural history, drug mechanism of action, expected effects of the drug upon intended targets, and the ability to measure these effects reliably are of considerable importance in designing rational clinical development programs. With careful planning in premarket phases and additional evaluation factored into the postmarket period, drug development programs can be directed toward addressing knowledge gaps and the needs of patients in an ongoing manner. Important among these gaps is the need for

well-defined and reliable COA tools for all PKU patient subgroups. Intermediate COAs (measured between short-term pharmacodynamic effects such as blood Phe and long-term neurocognitive outcomes) that are well substantiated, such as measures of executive function, are especially needed for patients who are outside the “critical period,” defined historically as during the first 6 years of life [14].

The AHRQ report and experience from recent clinical trials have focused attention on the need for a more complete public health approach to PKU that should include comprehensive prospective data collections and longitudinal outcome assessments, and will require involvement of the entire PKU community. This approach could help generate the necessary data to allow individuals across the entire PKU disease spectrum to have access to additional therapeutic options and to make informed choices regarding the management and intervention of their disease.

### 5. Development of a research agenda: needs, priorities, and next steps (Melissa A. Parisi, M.D., Ph.D.)

Following the conference presentations and discussion sessions, the entire audience joined in an open dialog facilitated by Drs. Parisi and McPheeters to develop a research agenda that would identify needs, priorities, and next steps. The topics of discussion were distilled into seven main areas: outcome measures and endpoints for PKU, basic research on the pathophysiology of PKU, response to therapy, issues of treatment access and social supports, clinical trial design, genotyping issues, and resources and technology development. In addition, soon after the PKU State-of-the-Science Conference, the AHRQ EPC led by Dr. McPheeters, which published the Evidence-based review of adjuvant therapies for PKU, also convened a group of stakeholders to develop a Future Research Needs document that was released in September 2012 [317]. Many of the themes identified at the conference were represented in this AHRQ report.

#### 5.1. Outcome measures and endpoints for PKU

A recurring theme of the conference was the notable lack of robust, reliable, and clinically valid outcome measures or endpoints across all domains of function that can be used to monitor disease status and the effects of treatment for individuals with PKU throughout the lifespan. Although the standard outcome measure has been the blood Phe level, this serves as a proxy measure of disease control at that moment in time and does not capture the fluctuations inherent in blood Phe levels that reflect nutritional status, diurnal variation, acute illness, and/or a host of other variables. Some experts have suggested that the more important measure is the degree of fluctuation in blood Phe levels as a function of time. For example, greater variability of blood Phe concentrations may predict more severe effects on fetal outcomes for women with PKU who are pregnant [58]. The ability to measure Phe levels in “real-time” relative to meal consumption, activity level, and other parameters would be of great benefit in this regard and reflects the need for more personalized methods of measuring blood Phe levels, such as using home Phe meters (see Section 5.7). The use of blood Tyr levels or Phe:Tyr ratios as measures of disease control has been suggested, although these biochemical markers have their own limitations.

The core question of the effects of elevated Phe (and possibly by extension, reduced or altered neurotransmitter levels) on cognitive function and neuropsychological outcomes are not addressed by the biomarkers currently measured. This reflects the inherent limitations of using serum markers to approximate CNS phenomena. There is a real need for valid surrogate endpoints to represent executive function, psychiatric co-morbidity, neurological impairment such as tremor, and other domains of function as identified by the Long-Term Outcomes and Management across the Lifespan Working Group (Section 3.1) to determine if they correlate with blood markers such as Phe levels, and if they can be reliably identified via screening tools. Successful and

clinically useful outcome measures will also need to be cost effective and require minimal labor and expertise to measure, monitor, and interpret.

The validation of appropriate and sensitive short-term and long-term clinical outcome assessments for identifying effects of interventions with regard to nutrition, cognition, behavior, and other symptoms (or other patient-reported outcomes) for individuals with PKU is a major identified need. A discussion of surrogate endpoints as a proxy for clinical outcome measures for use in clinical trials of new interventions or medications has been described from the FDA perspective (see Section 4.5), and the FDA guidance for the development of patient-reported outcome measures exists (<http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM193282.pdf>).

With the high prevalence of personal digital devices and computers, Internet access, and social media, opportunities to administer some measures electronically are a real possibility. Some standardized psychological measures or diet records could be collected in “real time” using these technologies and could be facilitated or piloted by professional and/or advocacy organizations. There is often a “disconnect” between the types of research questions and goals of research performed by academic investigators, the needs of the pharmaceutical industry and FDA regarding drug approval, and the desired measures articulated by advocacy groups and patients. Greater collaboration among clinicians, researchers, industry representatives, advocacy organizations, and individuals with PKU could facilitate more relevant and effective endpoint development.

Related to this research theme is the need to develop treatment strategies for PKU that would optimize outcomes such as growth, nutrition status, cognition, and behavior. With regard to diet, the role of macro- and micronutrients, the formulation of new medical foods, the timing of dietary component intake, and other parameters remain unanswered, particularly considering long-term impacts. Development of drugs such as PEG-PAL and other interventions, such as hepatocyte transplantation and gene therapy, show great promise but need to be developed with an eye toward clinically relevant outcome assessments that will improve quality of life for those with PKU and have a favorable benefit-to-risk ratio, as articulated by FDA. Therapies that have significant morbidity and mortality must be carefully considered in such a scheme; as noted, liver transplantation can cure PKU, but at a high cost to the individual and society.

#### 5.2. Basic research on the pathophysiology of PKU

Another domain in which there are significant research gaps is the basic science understanding of PKU, in particular, the effects of elevated blood Phe levels on the brain levels and neurocognitive development and function. In spite of over 70 years of research in PKU, it is still unknown whether the neurological effects of PKU are related solely to elevated Phe levels or whether other neurometabolic consequences of elevated blood Phe may play a larger role in disease pathology. There is still a limited understanding of the basic mechanisms of neurotoxicity of Phe itself, and how elevations in this simple amino acid result in negative consequences on cognition and behavior during development and throughout life. New tools and technologies that offer a better window into brain function are sorely needed, and those that can identify biomarkers that might be valuable for monitoring PKU and response to therapy would be particularly valuable. The appropriate neurological outcomes that demonstrate the effects of elevated Phe on the brain remain to be developed, as noted by several of the working groups. One group speculated that insights from studying disorders of BH<sub>4</sub> metabolism could inform studies in PKU. Other discussions centered on the need for accurate tools to diagnose psychiatric disorders in individuals with PKU, including attention deficit hyperactivity disorder, depression, and anxiety, and whether sapropterin could alleviate these symptoms. Moreover, it was recognized that an improved

understanding of the indications for, treatment response to, and tolerability of psychotropic medications that are frequently used to target symptoms such as inattention, depression, and anxiety is needed.

Certainly, one area in which research could shed light on these basic science questions is in studies that evaluate the metabolic, neurochemical, and neurotransmitter derangements that occur in animal models of PKU. Mice with a targeted mutation in the *PAH* gene that reproduces the human phenotype [318,319] could facilitate such studies, by allowing detailed evaluations of CNS structural and biochemical defects using newer imaging modalities and of cognitive function using established behavioral paradigms. These strategies can also be applied to humans. High-resolution MRI with proton magnetic resonance spectroscopy for aberrant Phe signatures and diffusion tensor imaging for white-matter integrity afford an opportunity to evaluate neurotransmitter function and structural consequences of perturbations in Phe metabolism in individuals with PKU [320,321].

Although the effects of high Phe levels on the brain are of great importance, research is also needed on the consequences of high Phe, and/or potential deficiencies in nutrient intake on a Phe-restricted diet, on other organ systems. Osteopenia has been documented with relatively high frequency in those with PKU. However, the mechanism and risk factors that underlie poor bone mineral density in PKU remain poorly understood [322]. One of the most significant complications of PKU for adult women is the risk of MPKUS given the known teratogenic effects of elevated Phe on the developing heart, brain, and other organs of the fetus. The Pregnancy and PKU Working Group identified a number of areas where more research was needed, including the question of whether optimal maternal blood Phe levels during pregnancy should be the current recommendation of 120–360  $\mu\text{mol/L}$ , or whether they should be maintained closer to the normal physiological range of 60–120  $\mu\text{mol/L}$  to afford even greater protection to the developing fetus. In raising this question, it was recognized that the lowest “safe” limit of blood Phe for maternal PKU has not been established, as there is a real risk of endogenous protein catabolism in the mother if her Phe levels are too low. The fetal milieu remains an unexplored domain, and it is unknown whether long-term developmental outcomes are better for children born to mothers who had blood Phe in good control before conception versus those who achieved it during the first 8 weeks of gestation. Finally, the frequency of monitoring blood Phe levels in lactating mothers and the safety of sapropterin use during breastfeeding remain open questions.

### 5.3. Response to therapy

In spite of exciting progress in the realm of new therapeutics for PKU with the development and approval of sapropterin, there are still a number of unanswered questions about its utility and application. Conference participants differed in their opinions regarding which individuals should have  $\text{BH}_4$ -responsiveness testing and which algorithms were the best for testing responsiveness. Many members of the Pharmacological Interventions Working Group believed that all individuals with PKU deserved a  $\text{BH}_4$ -responsiveness trial, in spite of the observation that certain genotypes have never been associated with response to the drug. Although a number of protocols for  $\text{BH}_4$  responsiveness have been developed, there was no consensus on a single algorithm that was reliable, simple, and objective enough to identify both early and late responders. Studies are needed on the use of sapropterin in special populations, including children, pregnant women, and late or untreated adults with neurocognitive deficits. These studies will be important to determine how sapropterin may be safely and effectively prescribed to provide maximal benefit.

For other emerging therapies, similar questions remain. For example, well-designed, controlled trials are needed to determine if LNAA are efficacious, particularly in children and individuals with PKU who are taking psychotropic medications. Additional studies are needed to determine the effects of LNAA on nutritional status and growth, to

delineate the impact of LNAA competition at the gut and blood–brain barrier in humans, and to develop validated surrogate markers to measure changes in brain function while being treated with LNAA. For GMP, studies are needed on the long-term efficacy and impact on nutritional status for those with PKU.

An intriguing question was raised during the discussion about whether individuals with PKU can be overtreated. Is it possible that a person with PKU could have a blood Phe concentration that is too low, and experience a metabolic crisis resulting in muscle breakdown and catabolism? Particularly vulnerable periods might be during infancy, adolescence, or pregnancy, when active growth may demand larger amounts of protein consumption. Finally, an unresolved issue in need of additional research is the best treatment strategy for mild HPA, particularly for those in the “gray zone” with Phe levels of 360–600  $\mu\text{mol/L}$  at baseline.

### 5.4. Issues of treatment access and social supports

One of the topics that garnered the most intense discussion at the conference was the importance of access to treatments and social supports for individuals with PKU that facilitate the best clinical outcomes. Although studies show that social support, a positive attitude toward treatment, and ease and ability to manage the disease predict adherence to medical recommendations for those with PKU [55], evidence is needed to demonstrate that social support services have a long-term, sustained impact. A parallel activity that could inform studies designed to demonstrate the efficacy of treatments is the development of standardized, uniform medical practice guidelines for PKU treatment that include measures with known ceiling and floor effects so that they can be compared across clinics and practitioners. To this end, the American College of Medical Genetics and Genomics (ACMG) and Genetic Metabolic Dietitians International (GMDI) have created practice guidelines that are based on many of the activities described in this proceedings [323,324] (see Section 5.8).

A number of discussants made the compelling observation that resources devoted to the development of new drugs and treatment strategies for PKU were wasted if individuals with PKU could not access these treatments or obtain health care coverage to pay for them. Although it seems intuitively obvious that individuals with better access to treatments will have outcomes that are superior, there is a relative paucity of data to support this assertion. Barriers to obtaining appropriate care would appear to be an obvious problem that needs to be addressed. Several participants shared anecdotal evidence about state-by-state differences in legislative requirements to provide coverage for PKU treatments, ranging from states with no insurance mandate to states with mandates that cover all newborn screening disorders. A small number of articles describe the inconsistencies of access to treatment in the United States due to consumer protection laws that stipulate different coverage requirements. Other obstacles in coverage result from the Healthcare Common Procedure Coding System established by the Centers for Medicare & Medicaid Services that do not reflect the clinical purpose of the medical foods required for treatment [325,326]. Furthermore, there is no national or uniform policy that addresses coverage for medical foods for individuals with PKU [4]. Although PKU is the paradigm for newborn screening, some families still have a difficult time obtaining the dietary interventions that have been shown to reduce long-term intellectual disability, loss of productivity, and severely impaired quality of life [263]. Ironically, it is sometimes the more expensive treatment, such as sapropterin, that is covered by health care payers because it is an FDA-approved medication, rather than a dietary intervention. Nevertheless, more data are needed to demonstrate the value of specific treatments in PKU to make the case for comprehensive and uniform coverage. To this end, partnerships with the National PKU Alliance and other advocacy groups could lead to surveys of members regarding treatments used and insurance reimbursement issues. Another possibility that was raised was

implementation research, such as comparative effectiveness studies between countries to evaluate outcomes in which different treatment strategies have been employed, or even mandated. One example could be a comparison between outcomes in the United Kingdom, where sapropterin is not readily available, and in the United States, where a known proportion of patients with PKU utilize this treatment.

Research is needed to address aspects of the health care delivery system that negatively affect individuals with PKU, such as health care disparities and lack of support for the transition to adulthood. In the realm of health care disparities, research is needed that focuses on ways to increase access to medical care and increase life expectancy, particularly among underrepresented minorities. In addition, there are limited numbers of adult health care providers with adequate expertise to provide medical care for youth and adults with PKU. For example, little is known about the skills and knowledge that adolescents with PKU need as they transition into adulthood, the factors that facilitate and influence successful transition, and the indicators of a successful system for transition. A fundamental question is whether adolescents and young adults with PKU who have a strong identity also attain better metabolic control during this period of transition to adult health care. While no studies about identity achievement have been undertaken with regard to PKU, studies that included college students suggest that establishing an identity is an important milestone in the transition to adulthood [327].

Conference participants also discussed the strategies that can be used to overcome barriers and improve adherence to treatment during all phases of life, including adolescence, pregnancy (including the pre-conception and postpartum periods), adulthood, and late adulthood. To accomplish this goal, reliable and sensitive measures of treatment adherence need to be developed and measured. Moreover, management approaches vary, nutrient intake is not always quantified, and studies of the growth of individuals with PKU are inconsistent [328,329], making it difficult to tease out clinical management versus patient actions as factors in nonadherence. Rigorous and reliable investigations of PKU and growth (including obesity and body composition) need to be included in discussions about adherence measures. With regard to the postpartum period and lactation in the mother with PKU, a life stage about which very little is known, approaches to optimize maternal and newborn health need to be addressed. At all stages of life, the domains most sensitive to treatment and relevant to positive long-term outcomes need to be agreed upon and measured.

### 5.5. Clinical trial design

One of the recurring issues in PKU treatment and management is how to determine which individuals with PKU should be eligible for new and emerging treatments and, by extension, how best to study their responsiveness to these treatments. Determining the guiding principles for designing clinical trials for pharmacologic agents to be used in PKU is not trivial, as with any rare metabolic disorder in which the patient population is small and dispersed. Trial design strategies that may be beneficial include enrichment design (as applied for sapropterin, in which those who demonstrated early response were included in the main study), crossover study design (in which each subject serves as his or her control at some point during the study), and N-of-1 studies for ultra-rare conditions. No matter how rare the condition or how complicated the study design, the general principles must include rigor in all aspects of the study, with randomization and blinding wherever feasible.

As discussed in Section 4.5, the strategies for clinical trial design that will most likely lead to FDA approval need to be considered early in the process, and after regular and frequent discussions with personnel at regulatory agencies. In developing therapeutics for rare diseases, drug manufacturers must be held to the same high standards required of any drug under development. There must be substantial evidence of product quality, effectiveness, safety, and a favorable benefit-to-risk

profile in the treatment of a specified patient population. Considerations for testing combinations of therapies in individuals with PKU add to the challenge, particularly when the standard of care is a dietary intervention that is individualized, difficult to administer, and without uniformly agreed-upon measures of adherence. Finally, as the cost of obtaining genetic and genomic information has plummeted and as the ability of such information to potentially predict response to therapy has improved, the issue of whom to genotype is becoming more relevant. Genotyping was made a condition of approval for sapropterin in the United States, and this is a trend that is likely to continue for drugs that have a specific genetic and metabolic target. As genomic sequencing becomes more widespread, genotyping of at least the gene target of interest is likely to be required in many clinical trials that measure efficacy; furthermore, genotype information will be incorporated increasingly in algorithms to determine responsiveness to treatment.

### 5.6. Genotyping issues

In addition to issues of genotyping for the purposes of study design, there are also fundamental issues related to genotyping for management in PKU. Although the 2000 NIH Consensus Development Statement recommended that all individuals with PKU be genotyped, *PAH* gene mutational analysis is not performed for many individuals. This recommendation was explored in great detail by the participants at the 2012 conference; while there was not uniform consensus on its utility for management purposes, the majority felt that there was value in collecting genotype information. More research is needed to improve the understanding of how genotypes influence phenotypes through the support of comprehensive genotype-phenotype cataloging of *PAH* mutations. Furthermore, given the challenges of predicting severity from Phe measurements in the newborn period, prospective multicenter studies that would genotype all infants with positive newborn screens for PKU, establish strict criteria for phenotyping, and determine the appropriate pretreatment Phe level for initiation of dietary therapy are needed. To have broad uptake of genetic testing, individuals with PKU, their families, and the health care community need to be educated about the value of genotyping in predicting the degree of severity of PKU and response to therapy, both dietary and drug-related.

In this era of modern genomics, discussion of the genotype with regard to single gene testing is too limiting. Given that PKU is a continuum of severity, and that some variability exists with regard to prediction of phenotype based on genotype, it is highly likely that modifier genes or genomic variants play a role in disease expression for those with PKU. Also, there are likely to be a number of epigenetic and/or environmental modifiers of PKU that, if known, would lay the framework for an enriched understanding of the nuances of disease course and treatment response. Several NIH-supported centers are piloting genomic testing, either whole exome or whole genome sequencing, in newborns and/or newborn screening programs to determine the utility of these comprehensive approaches to understanding newborn screening disorders in infants with known conditions such as PKU or in otherwise healthy newborns (<http://www.genome.gov/27554919>). These strategies may inform future efforts to more fully characterize IEM from a metabolic, genetic, and epigenetic perspective.

### 5.7. Resources and technology development (see also Table 4)

There was a great deal of discussion about resources and technological developments that would facilitate progress in PKU research. One suggestion was the creation of a national registry of individuals with PKU that could serve as a source of subjects for natural history studies and future clinical trials as additional therapies or approaches to management became available. Clearly, there is benefit to collecting longitudinal data on individuals with rare diseases to assess changes with age that are part of the evolution of the condition versus related

to a particular intervention or complication. Although a registry exists for those taking Kuvan® (sapropterin) for the benefit of postmarketing surveillance, there is no single patient registry that allows for tracking individuals with PKU over time outside of a clinical trial.

Resources that have been developed for other rare diseases have potential to be utilized by the PKU community as well. For example, the NICHD-funded Newborn Screening Translational Research Network (NBSTRN; <https://www.nbstrn.org/>) has a number of services and resources for investigators, including a virtual repository of dried blood spots that can be accessed by investigators for research projects, a longitudinal pediatric data resource that can be used to collect clinical information over time for individuals with IEM who are participating in research studies, and a consultation service that can help investigators with grant applications, even addressing the ethical and legal issues related to newborn screening and state rules and regulations that govern newborn screening research. Common Data Elements (CDEs) have been developed for many IEM, including PKU, by the NBSTRN and other groups such as ORDR (<https://grdr.ncats.nih.gov/>), and an NIH-wide effort to catalog CDE initiatives is available (<http://www.nlm.nih.gov/cde/>). The ORDR-funded Rare Disease Clinical Research Network is a group of consortia developed to address pressing research needs that will speed therapy development for a host of rare diseases; although none of the existing consortia support PKU directly, this entire network is being recomputed in 2013–2014, so there may be opportunities for IEM such as PKU to become a part of this ambitious and productive program. Table 4 lists additional resources applicable to the PKU community.

Under the category of technology development, there was near unanimous agreement on the need for improved treatments for PKU, with enthusiasm for PEG-PAL and other related medications that have the potential to be effective for a larger segment of the PKU community than sapropterin. Home monitoring using a blood Phe meter, analogous to a glucose meter for diabetes management, is a technology that has not yet been realized. However, the development and availability of such a device remains an important goal of PKU management and treatment. Many in the PKU community would welcome the opportunity to play a more active role in self-management via the use of such a device that could provide immediate feedback for both the individual with PKU and their health care providers based on a simple finger prick. Such a technology remains a lofty but achievable goal for PKU disease management.

#### 5.8. Development of clinical practice guidelines

One of the major goals of the conference was to provide the knowledgebase for professional organizations to craft clinical practice guidelines for PKU. ACMG and GMDI have developed harmonized medical and nutrition guidelines being published as companion documents [323,324]. The international PKU community has also embraced many of these themes as guidelines under development in several European countries and Australia. Canadians will adapt the ACMG guidelines to meet their needs. These coordinated efforts highlight the important role that professional organizations play in the development of guidelines that will ultimately improve care for individuals with these disorders.

## 6. Conclusions

In conclusion, although PKU is arguably the IEM about which we know the most, there are still many unanswered questions regarding diagnosis, treatment, and long-term outcomes. As technology has evolved, those questions have become even more complex. The needs of an aging population of adults with PKU have emerged that reflect long-term outcomes that were never imagined when newborn screening for PKU was initiated 50 years ago. Given the societal commitment to newborn screening, we have an obligation to provide the best

possible diagnosis, care, and management for individuals who are identified with IEM via newborn screening; ideally, our ability to diagnose should not outstrip our ability to treat. PKU demonstrates the great challenges that exist in effectively treating IEM, and if we can address these challenges, we will have a framework in place that will make it easier to treat other IEM. We must use all of the tools at our disposal and create others that will evolve to facilitate development of new diagnostic, management, and therapeutic strategies for these conditions.

The value of a comprehensive team for improving outcomes in IEM cannot be overstated. The team includes those engaged in clinical care and research and constitutes the medical and nutrition providers, the investigators who conduct basic and clinical research and explore new treatment paradigms, and the industry partners who develop new drugs. Another critical component of the team is the federal and state agencies that fund research, collect epidemiological data on prevalence and outcomes, provide infrastructure and public health care systems that conduct newborn screening, and establish laws that guide regulation of drug products. Advocacy organizations serve as the collective “voice” for individuals with an IEM and their families, providing education to a wide variety of stakeholders, encouraging research participation, and advocating to promote coverage of treatments. At the center of this “team” is the person with PKU and his/her family. The perspectives of those who live with a condition or who care for an affected individual are essential for understanding their most important needs, integrating practice guidelines into daily life, determining what will work, and ultimately, participating in the establishment of research priorities. Above all, the autonomy of the individual with PKU and his/her family must be respected.

As evidenced by existing literature and ongoing research in PKU and other IEM, considerable strides have been made in understanding the molecular basis of these disorders and in the application of therapeutic strategies to prevent their adverse sequelae. However, the recognition that evaluative outcomes research is needed to provide robust evidence for effective and appropriate care for IEM has resulted in at least two international initiatives. Potter et al. [330] describe a practice-based evidence framework to inform the design and delivery of health services for patients with IEM in Canada, while Camp et al. [11] describe the need for research on the nutritional interventions used to manage IEM, the current U.S. research infrastructure, and the need for novel approaches for IEM product development.

Because PKU is the paradigm for many other IEM, establishing a framework for approaching its evaluation and management holds hope for these other rare disorders. Partnerships among the metabolic community, federal and state agencies, pharmaceutical and medical food industry, advocacy groups, and individuals are essential. If we do not get it right for PKU, how can we possibly get it right for all the less common metabolic disorders about which even less is understood?

## Conflict of interest

The following authors declared a financial relationship with these companies and organizations:

*BioMarin Pharmaceutical Inc.:* Bilder, Blau, Bodamer, Brown, Burton, Cunningham, Grange, Harding, Huntington, Lichter-Konecki, Mitchell, Mofidi, Moseley, Prasad, Pridjian, Rohr, Singh, Sirrs, Stremer, Utz, Vockley, Waisbren, White, Whitley

*Merck Serrano:* Blau, Bodamer, Burton, Cunningham, MacDonald, Mitchell, White

*Nutricia NA:* Bodamer, Brown, Frazier, Lichter-Konecki, MacDonald, Rohr, Yannicelli

*Cambrooke Foods:* Brown, MacDonald, Moseley

*VitaFlo:* Brown, MacDonald, Mitchell

*Abbott:* Acosta, Brown, Frazier, Rohr

*Mead Johnson:* Brown

*National PKU Alliance:* Brown, Harding, Levy, Waisbren

*SIMD/GMDI/ACMG:* Berry, Bodamer, Whitley.

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*BioMarin*: Bilder, Frazier, Mitchell, Utz

*Merck Serrano*: Berlina

*Nutricia NA*: Berlina, Stremer

*Cambrooke Foods*: Stremer

*VitaFlo*: MacDonald, Stremer

*National PKU Alliance*: Cunningham, Franklin, Harding, Huntington, Vockley, Lichter-Konecki, Moseley, White

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### Dedication

This paper is dedicated to Dr. Richard Koch, a pioneer in the management of PKU and research on PKU and maternal PKU syndrome. He participated early in the information gathering phase of the conference, but sadly, he passed away before the conference took place. The PKU community lost a champion. Through his tireless clinical, research, and teaching efforts, the world gained an immense understanding of PKU.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.ymgme.2014.02.013>.

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