



# Key European guidelines for the diagnosis and management of patients with phenylketonuria

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We developed European guidelines to optimise phenylketonuria (PKU) care. To develop the guidelines, we did a literature search, critical appraisal, and evidence grading according to the Scottish Intercollegiate Guidelines Network method. We used the Delphi method when little or no evidence was available. From the 70 recommendations formulated, in this Review we describe ten that we deem as having the highest priority. Diet is the cornerstone of treatment, although some patients can benefit from tetrahydrobiopterin (BH4). Untreated blood phenylalanine concentrations determine management of people with PKU. No intervention is required if the blood phenylalanine concentration is less than 360  $\mu\text{mol/L}$ . Treatment is recommended up to the age of 12 years if the phenylalanine blood concentration is between 360  $\mu\text{mol/L}$  and 600  $\mu\text{mol/L}$ , and lifelong treatment is recommended if the concentration is more than 600  $\mu\text{mol/L}$ . For women trying to conceive and during pregnancy (maternal PKU), untreated phenylalanine blood concentrations of more than 360  $\mu\text{mol/L}$  need to be reduced. Treatment target concentrations are as follows: 120–360  $\mu\text{mol/L}$  for individuals aged 0–12 years and for maternal PKU, and 120–600  $\mu\text{mol/L}$  for non-pregnant individuals older than 12 years. Minimum requirements for the management and follow-up of patients with PKU are scheduled according to age, adherence to treatment, and clinical status. Nutritional, clinical, and biochemical follow-up is necessary for all patients, regardless of therapy.

## Introduction

Phenylketonuria (PKU) is a rare autosomal recessive inborn error of phenylalanine metabolism with an estimated frequency in Europe of 1 in 10 000 newborn babies (panels 1 and 2).<sup>1</sup> Deficiency of the hepatic-based enzyme phenylalanine hydroxylase (PAH) results in a complete or partial inability to convert phenylalanine (from the diet or derived from catabolism of proteins in the body) to tyrosine (figure 1),<sup>2</sup> which can lead to high blood phenylalanine concentrations that cross the blood-brain barrier causing detrimental effects on brain development and function. Available treatments aim to decrease the blood phenylalanine concentration, which is considered the surrogate marker for brain phenylalanine concentrations. Nevertheless, the precise pathophysiology of PKU remains unclear (figure 2).<sup>3</sup> Clinically, untreated PKU is characterised by irreversible intellectual disability, microcephaly, seizures, aberrant behaviour, psychiatric symptoms, motor disturbances, and eczematous rash. When diagnosed by newborn screening and treated immediately, patients essentially show normal development, although neuropsychological deficits and behavioural and social issues can occur.<sup>2,5</sup> Dietary treatment consists of a low-phenylalanine diet, restrictive in natural protein combined with phenylalanine-free L-aminoacid supplements and low protein foods. Some patients with PKU respond to tetrahydrobiopterin (BH4), a naturally occurring essential cofactor for PAH that acts as a pharmaceutical chaperone (prescribed as sapropterin dihydrochloride), decreases blood phenylalanine concentrations and increases dietary phenylalanine tolerance.<sup>2</sup> In some (mainly non-European) countries, casein glycomacropeptide or large neutral aminoacids are used for treatment, but both

## Panel 1: Information on genetics and pathophysiology of phenylketonuria (PKU)

### Genetics of PKU<sup>2</sup>

PKU is caused by mutations in the gene encoding phenylalanine hydroxylase (PAH). The PAH gene is located on chromosome 12 (region q22–24.1) consisting of 13 exons and 12 introns, in total covering 100 kb of genetic data. More than 950 mutations in the gene encoding PAH (BIOPKU database) are known to be associated with PAH deficiency. Most of the mutations are missense, usually resulting in protein misfolding or impairment of catalytic functions. The prevalence of PKU shows considerable geographic variation; in Europe it occurs in one in 10 000 newborn babies, with a higher rate in some countries, such as Ireland and Turkey, but a very low rate in Finland.

### Phenylalanine hydroxylase<sup>2</sup>

Phenylalanine is hydroxylated by PAH, necessitating tetrahydrobiopterin (BH4) as a cofactor, with iron and oxygen. The structure of PAH is a tetramer. During the hydroxylation of phenylalanine, BH4 is oxidised to a 4a-hydroxy intermediate, which is subsequently regenerated to BH4 by the enzymes carbinolamie-4a-dehydratase and dihydropteridine reductase. BH4 is synthesised from guanosine triphosphate (GTP) by three additional enzymes: GTP cyclohydrolase I, 6-pyruvoyl-tetrahydropterin synthase, and sepiapterin reductase. Variants in the genes encoding the enzymes involved in BH4 metabolism result in BH4 deficiency, mostly treated with BH4 and neurotransmitter precursors. Some patients with PAH deficiency also respond to BH4. This response is referred to as BH4 responsiveness.

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## Panel 2: Definitions of genetics and pathophysiology of phenylketonuria (PKU)

### Executive functioning

Executive functioning describes cognitive processes that regulate behaviour; examples are inhibitory control, working memory, and cognitive flexibility.

### High-risk pregnancy

A pregnancy that threatens the health or life of the mother or her fetus.

### Hyperphenylalaninaemia

Any blood phenylalanine concentration more than 120  $\mu\text{mol/L}$ .

### Late treated or untreated adults

Late diagnosis refers to children diagnosed between ages 3 months and 7 years ( $\geq 3$  months to  $< 7$  years). Untreated refers to patients who are untreated at age 7 years or older.

### Maternal PKU syndrome

The teratogenic effects of elevated maternal phenylalanine levels during pregnancy to the fetus.

### Phenylalanine tolerance

The amount of phenylalanine (mg/kg per day or mg per day) that maintains plasma phenylalanine concentrations within the target range. This amount might also be described as natural protein tolerance expressed as g per day, taking a phenylalanine content in natural protein as 50 mg of phenylalanine per gram of natural protein.

### Protein requirements

The lowest level of dietary protein intake that will balance the loss of nitrogen from the body, and thus maintain the body protein mass in a person at energy balance with modest levels of physical activity.

### Protein substitutes (phenylalanine-free L-aminoacid supplements and low phenylalanine glycomacropeptide protein)

Protein replacements or substitutes are essential in treatment of PKU to prevent protein deficiency and optimise metabolic control. Protein substitutes are mainly sourced from phenylalanine-free L-aminoacid supplements and less commonly from low phenylalanine glycomacropeptide.

### BH4

Cofactor of phenylalanine hydroxylase. BH4 also acts as a chaperone protein in some patients who are deficient in phenylalanine hydroxylase. BH4 is a cofactor of tyrosine and tryptophan hydroxylase and has a role in the conversion of L-arginine to nitric oxide by nitric oxide synthases.

### BH4 deficiencies

Defects in BH4 metabolism (either synthesis or turnover) result in a deficiency of BH4. These include three known defects for synthesis and two for regeneration (see also phenylalanine hydroxylase in panel 1).

### Potential BH4 responsiveness

More than 30% reduction in blood phenylalanine in a BH4 loading test or two BH4-responsive mutations. BH4 responsiveness should be proven in a treatment trial adjusting the BH4 dosing, natural protein intake, and phenylalanine-free L-aminoacid supplement.

### BH4 responsiveness

In this Review we defined BH4 responsiveness as an increase of 100% or more in natural protein or improved biochemical control ( $> 75\%$  of phenylalanine levels in target range), or both, on a dose of BH4 that ranges between 1–20 mg/kg bodyweight (with a maximum dose of 1000 or 1400 mg per day in some countries).

safety and efficacy of these treatment strategies need further study.<sup>2</sup>

PKU management differs across Europe, with various local and national guidelines. A need exists for more standardised management tailored for optimal outcomes.<sup>6,7</sup> The development of these European guidelines was driven both by health professionals and the patient organisation European Society for Phenylketonuria and Allied Disorders Treated as Phenylketonuria (ESPKU).<sup>7–9</sup> Generally, guidelines should result in measurable improvements in patient care,<sup>10,11</sup> consistent, high-quality treatment without inequity, and awareness of rare diseases.<sup>12</sup> Here we report on the development of European PKU guidelines by 19 European PKU experts, and we highlight ten clinical recommendations (out of a total of 70) that we deem as having the highest priority for implementation. The key recommendations were based on the best available evidence for management of PKU that would achieve optimal clinical and neuropsychological outcomes without overtreatment and unnecessary costs.

## Guideline development

The scientific advisory committee of the ESPKU was tasked with organising a group of European PKU experts. 19 health professionals were invited on the basis of their experience and expertise rather than nationality; one of them declined and one resigned for personal reasons. The 17 professionals included the following: paediatric and adult metabolic physicians, a paediatric neurologist, a biochemist, metabolic dietitians, neuropsychologists, and psychologists. They were divided into five working groups on (1) nutritional treatment and biochemical or nutritional follow-up (Working Group A); (2) neurocognitive outcomes including imaging (Working Group B); (3) psychosocial outcome, and adherence (Working Group C); (4) adult and maternal PKU, and late diagnosis of PKU and untreated PKU (Working Group D); and (5) diagnosis of PKU including treatment initiation and drugs in PKU (Working Group E). The working groups were supported by a project assistant and guidelines leader. We consulted an obstetrician for maternal PKU. We developed the guidelines between October, 2012, and December, 2015.

We developed the guidelines with the Appraisal of Guidelines, Research, and Evaluation in Europe method. We did the literature search, critical appraisal, and evidence grading according to the Scottish Intercollegiate Guidelines Network (SIGN) method (version 2011) using eight levels of evidence being transferred into grades of recommendations (from A [highest] to D [lowest], with an extra recommendation for so-called good practice points that are not based on any evidence).

All working groups defined key questions, after which they searched the published work for relevant papers with the help of the project assistant. All working groups and plenary sessions, to discuss as adequately as possible the statements without enough evidence,

were facilitated by the guidelines leader or project assistant, or both. We sent a concept of the full guidelines worldwide to 14 external consultants (physicians and dietitians or nutritionists) and the board of the ESPKU. Further data about the process (including the questions for each of the key recommendations reached) are shown in the appendix.

## Guidelines

### Overview

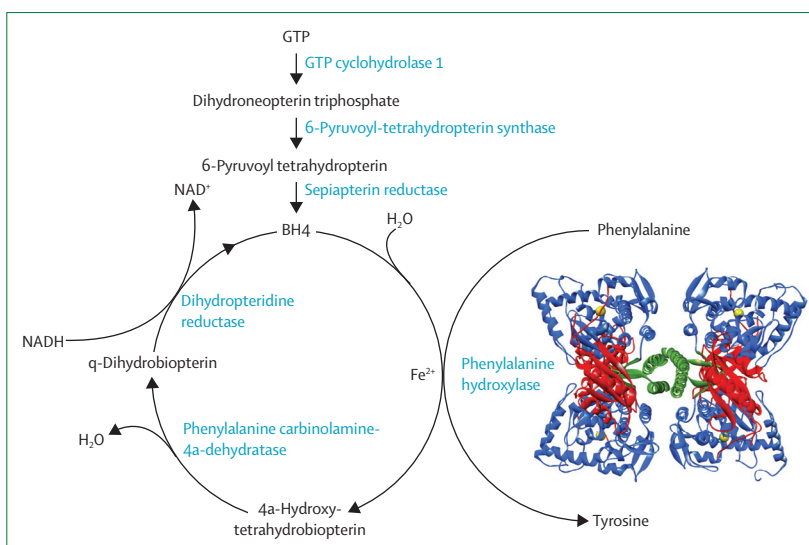
The main topics of these guidelines are (1) diagnosis, treatment initiation, and treatment duration; (2) treatment targets; (3) practical issues in dietary and BH4 treatment; (4) follow-up; and (5) special conditions (maternal PKU; late PKU diagnosis and untreated PKU). In the final section, we discuss the future goals of PKU management. Panel 3 lists the ten key statements arising from these main topics. Some aspects of this Review are lacking a discussion in depth in part related to the length of the paper but also in part related to a lack of evidence. Where this is the case it is stated.

### Diagnosis and treatment initiation and duration

Most of the statements on diagnosis and classification are experience-based rather than evidence-based.<sup>2</sup> PAH deficiency is usually classified as mild, moderate, or severe (also referred to as classic) PKU. This classification is commonly based on the highest untreated blood phenylalanine concentration following a clinical diagnosis or at newborn screening. However, blood samples for newborn screening might be collected before infants have achieved their peak phenylalanine concentration without treatment. Since this sample collection time limits the use of the traditional classification for severity, a new classification is proposed on the basis of whether patients with PAH deficiency—to maintain blood phenylalanine concentrations in the recommended range—do not need treatment, or require dietary intervention, or BH4, or both.

Newborn screening for PKU meets all accepted screening criteria including cost–benefit ratio.<sup>13</sup> Therefore, newborn screening for PKU should be done in every European country. This screening not only needs a robust infrastructure in which blood is taken from all newborn babies within a few days of birth, but also a well equipped laboratory that can manage and assess bloodspots efficiently. Low-income countries might consider using newborn screening laboratory facilities of another country as a possible option.

In any child referred for hyperphenylalaninaemia, BH4 deficiencies and liver disease should be excluded, as these require other treatment strategies. Before starting treatment or doing a BH4 challenge, samples for pterins in blood and urine and dihydropteridine reductase activity in dried blood spots are taken. Additionally, a 24 h BH4 loading test might be useful, especially if time until receipt



**Figure 1: Phenylalanine hydroxylating system<sup>2</sup>**

BH4=tetrahydrobiopterin. GTP=guanosine triphosphate.

of laboratory results of pterins and dihydropteridine reductase activity is an issue. BH4 deficiencies should be considered in any child with neurological problems of unknown origin with even only slightly increased blood phenylalanine concentrations.

In patients with PKU, every 4 week delay in starting treatment results in a decline of approximately four intelligence quotient (IQ) points,<sup>14</sup> which underscores the knowledge that neurological damage starts early after birth. Therefore, the organisation of newborn screening and referral to PKU centres is strongly advocated so that treatment is started no later than 10 days of age.

A clear consensus exists that patients with untreated blood phenylalanine concentrations of less than 360  $\mu\text{mol/L}$  do not require treatment and that treatment is required in those with phenylalanine concentrations of more than 600  $\mu\text{mol/L}$ . The necessity of treatment for untreated phenylalanine concentrations between 360  $\mu\text{mol/L}$  and 600  $\mu\text{mol/L}$  is less clear;<sup>15,16</sup> only Weglage and colleagues<sup>17</sup> have adequately studied patients with untreated phenylalanine concentrations within this range and suggested it was safe. However, in that study only seven of the 31 patients had an untreated phenylalanine concentration of more than 500  $\mu\text{mol/L}$ , and on such evidence we consider it inappropriate to recommend that untreated phenylalanine concentrations of 360–600  $\mu\text{mol/L}$  are safe.<sup>17</sup> Therefore, we advise treatment for any child with untreated phenylalanine concentrations of more than 360  $\mu\text{mol/L}$ . This advice is not based on conclusive evidence and further data are still required for a definitive recommendation.

With regards to the duration of treatment, we advise treatment until age 12 years in individuals with untreated phenylalanine concentrations between 360  $\mu\text{mol/L}$  and

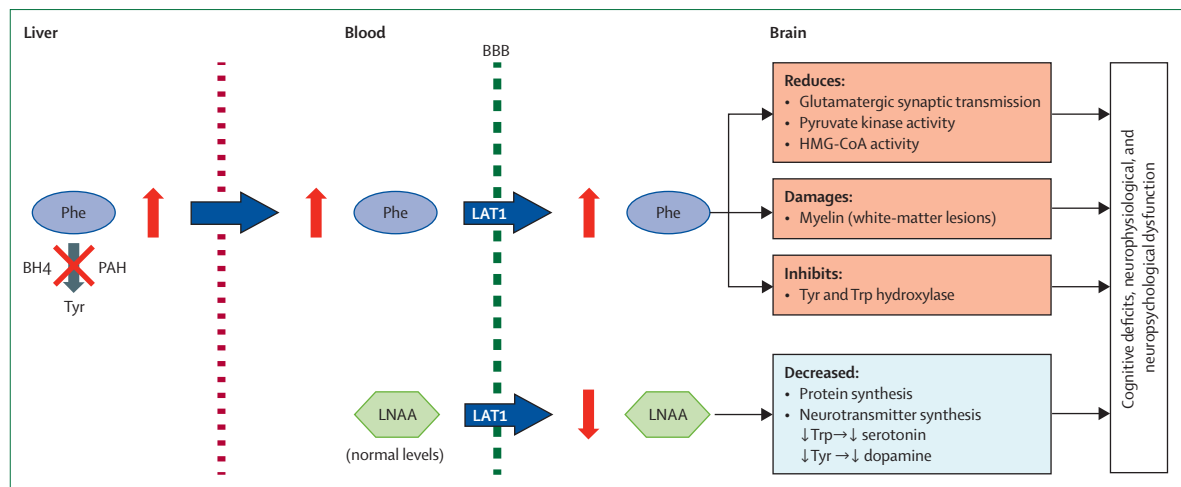
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Correspondence to: Prof Franjan J van Spronsen, Division of Metabolic Diseases, Beatrix Children's Hospital, University Medical Center Groningen, 9700 RB, Groningen, Netherlands f.j.van.spronsen@umcg.nl For more on the BIOPKU database see <http://www.biopku.org/home/pah.asp>

For more on the **Appraisal of Guidelines, Research, and Evaluation in Europe** method see [http://www.agreertrust.org/wp-content/uploads/2013/10/AGREE-II-Users-Manual-and-23-item-Instrument\\_2009\\_UPDATE\\_2013.pdf](http://www.agreertrust.org/wp-content/uploads/2013/10/AGREE-II-Users-Manual-and-23-item-Instrument_2009_UPDATE_2013.pdf)

For more on **SIGN** see <http://www.sign.ac.uk>

See Online for appendix



**Figure 2: Pathophysiology of phenylketonuria**

Summary of potential mechanisms of neurocognitive impairment by high phenylalanine concentrations. This figure shows the detrimental effects of high phenylalanine in the brain, as well as the effects of very low concentrations of LNAA in the brain. These low concentrations are the result of the effect of high blood phenylalanine concentrations on the transport of LNAA from blood to brain, even when the blood concentrations of LNAA are within the normal range. Reproduced with permission from *Pediatrics*, 126, 333–41, Copyright © 2010 by the AAP.<sup>4</sup> Phe=phenylalanine. BBB=blood-brain barrier. LNAA=large neutral aminoacids. LAT1=L-type aminoacid carrier. BH4=tetrahydrobiopterin. HMG-CoA=3-hydroxy-3-methylglutaryl-coenzyme A. Tyr=tyrosine. Trp=tryptophan.

600  $\mu\text{mol/L}$ , providing blood phenylalanine remains less than 600  $\mu\text{mol/L}$  without treatment afterwards. We suggest this duration because of scarce and inconsistent evidence and the necessity for good metabolic control to retain cognitive function, especially during the first 12 years of life.<sup>18,19</sup> In untreated women with phenylalanine concentrations of more than 360  $\mu\text{mol/L}$ , treatment is also required preconception and during pregnancy with the aim of reducing phenylalanine concentrations to less than 360  $\mu\text{mol/L}$ .

In patients with untreated phenylalanine concentrations of more than 600  $\mu\text{mol/L}$ , it is clearly unsafe to discontinue treatment before reaching adulthood (age 18 years),<sup>20–23</sup> but the need to continue treatment during adulthood has been challenged. Evidence from the results of studies summarised in a systematic review show that in adults treated early (with treatment continued or discontinued) for PKU (usually >600  $\mu\text{mol/L}$ ), significant suboptimal outcomes occur compared with healthy individuals, including deficits in executive function, attention problems, decreased verbal memory, expressive naming and verbal fluency, mood, and social and emotional difficulties.<sup>4</sup> Whether these suboptimal outcomes are because of suboptimal treatment during adulthood, adolescence, or even before is unclear. Adults treated early (treatment was both continued and discontinued) for PKU usually show a clear association between concurrent blood phenylalanine concentrations and certain aspects of brain function, brain metabolism, and differences in myelination, as summarised by van Spronsen and colleagues.<sup>24</sup> Adult patients with PKU, who are no longer on a phenylalanine restricted diet, can experience neurological complications<sup>25</sup> and vision loss<sup>26,27</sup> that can improve or even reverse after reinstatement of treatment.<sup>25–27</sup>

In adults continuing treatment for PKU, results of health-related quality of life surveys suggest that this quality of life is normal, with an improvement of health-related quality of life in some patients who had returned to a low phenylalanine diet having discontinued PKU treatment.<sup>28,29</sup> However, non-PKU generic health-related quality of life questionnaires are insensitive to the subtle negative effects of PKU, which has led to the development of a validated PKU-specific questionnaire (PKU-QOL).<sup>30,31</sup>

On the basis of these data, we advise treatment for life for patients with untreated phenylalanine concentrations of more than 600  $\mu\text{mol/L}$ , even though it is acknowledged that dietary management is associated with a substantial patient burden.

### Treatment targets

Blood phenylalanine is the primary marker for guiding available treatments.<sup>18,19,32</sup> Table 1 lists the studies that were included through use of the SIGN method to achieve the highest level of evidence available for the statements that describe age-related target ranges. For children up to the age of 12 years, the results of meta-analyses by Albrecht and colleagues<sup>32</sup> and Waisbren and colleagues<sup>18</sup> suggested safe upper target blood phenylalanine concentrations of 320  $\mu\text{mol/L}$ <sup>32</sup> and 420  $\mu\text{mol/L}$ ,<sup>18</sup> and Fennesbeck and colleagues<sup>19</sup> indicated that a mean concentration of 400  $\mu\text{mol/L}$  was associated with an increased risk of an IQ less than 85. Some evidence also exists that shows more favourable outcomes when the mean blood phenylalanine concentrations are less than 240  $\mu\text{mol/L}$  rather than 360  $\mu\text{mol/L}$  (mean age <12 years).<sup>41</sup> Jahja and colleagues<sup>41</sup> reported a better neuropsychological task performance by patients with PKU when blood phenylalanine concentrations were less than 240  $\mu\text{mol/L}$  than

**Panel 3: Key recommendations for patients with phenylketonuria (PKU)**

The grades range from ✓ (no possibility to assess the level of evidence because of a lack of any published work on this issue) to as high as B. The key recommendations were either based on evidence (if level of evidence was A or B using the SIGN method) or by consensus (using the Delphi method) if the level of evidence was C or D, or the so-called good practice points that are not based on any evidence if the level was ✓.

**Statement 1**

*Grade of recommendation:* ✓

To maintain blood phenylalanine concentrations in the recommended range, patients with phenylalanine hydroxylase (PAH) deficiency can be classified as either not requiring treatment, or requiring diet or tetrahydrobiopterin (BH4), or both.

**Statement 2**

*Grade of recommendation\*:* C

In the differential diagnosis of hyperphenylalaninaemia, of any degree, BH4 deficiencies should be excluded by measurement of pterins in blood or urine and dihydropteridine reductase activity in dried blood spot.

**Statement 3**

*Grade of recommendation:* D/C

Patients with untreated blood phenylalanine concentrations less than 360 µmol/L do not require treatment. Patients with untreated blood phenylalanine levels more than 360 µmol/L should be treated. Patients with untreated phenylalanine levels between 360 µmol/L and 600 µmol/L should be treated until the age of 12 years. Patients with untreated phenylalanine levels more than 600 µmol/L should be treated for life.

**Statement 4**

*Grade of recommendation:* C

All adults with PKU should have life-long, systematic follow-up in specialised metabolic centres, because of specific risks that might occur during adulthood.

**Statement 5**

*Grade of recommendation:* B

In treated patients with PKU up to the age of 12 years, target phenylalanine concentrations should be 120–360 µmol/L.

**Statement 6**

*Grade of recommendation:* D

In treated patients with PKU aged 12 years or older, the target phenylalanine concentrations should be 120–600 µmol/L.

**Statement 7**

*Grade of recommendation:* B

In pregnant patients treated for PKU the target phenylalanine concentrations should be 120–360 µmol/L.

**Statement 8**

*Grade of recommendation:* B

Women with untreated blood phenylalanine concentrations less than 360 µmol/L do not require treatment to lower blood phenylalanine before or during pregnancy.

**Statement 9**

*Grade of recommendation:* C

An annual nutritional review is required for any patient who is on a prescribed low phenylalanine diet or is self-restricting high protein foods. Such review must include a clinical examination including the anthropometric parameters (weight, height, BMI). We also recommended that plasma aminoacids, plasma homocysteine or methylmalonic acid, haemoglobin, mean corpuscular volume, and ferritin are measured. All other micronutrients (vitamins and minerals including calcium, zinc, selenium) or hormones (parathyroid hormone) can be considered if clinically indicated.

**Statement 10**

*Grade of recommendation:* ✓

In patients younger than 12 years, when more than 50% of the phenylalanine concentrations are out of target range over a period of 6 months, consider: (1) increased frequency of blood phenylalanine monitoring and outpatient visits and re-education, (2) psychology consultation or social worker intervention, and (3) hospital admission. When around 100% of blood phenylalanine concentrations are out of target range over a period of 6 months and there are other signs of failure of adherence, such as lack of cooperation, clinic non-attendance, or unresolved issues outside PKU consider consultation with social services and child safeguarding measures.

\*Level of evidence is chosen as C because of the high number of data notwithstanding that most included papers are of descriptive nature.

patients with concentrations between 240 µmol/L and 360 µmol/L (mean age <12 years); however, despite being statistically significant, differences of inhibitory control and cognitive flexibility, and motor control were not clinically significant.

Therefore, in view of the overall available evidence, we advise 360 µmol/L as the upper target phenylalanine blood concentration for the first 12 years of life. Data about adolescents and adults are scarce, necessitating

further data collection within long-term international collaborative studies. Using the data shown in table 1, we advise an upper target phenylalanine blood concentration of 600 µmol/L for all individuals older than 12 years. This statement is different when compared to the upper target phenylalanine concentration of 360 µmol/L for adults with PKU, as advised by the American College of Genetics and Genomics.<sup>45</sup> The upper target of 360 µmol/L is based on

a meta-analysis with three starting conditions: measures of cognitive outcomes other than IQ are either used non-uniformly across studies or are too difficult to combine; IQ variation is not an important component of variation of bias; and the effect of historical (lifetime) and momentary phenylalanine concentrations after 6 years of age is seen as one factor only.<sup>19</sup> We acknowledge the importance of this meta-analysis,<sup>19</sup> and think that the bias due to variation in IQ measures and variation of individual IQ over time should not be underestimated. Additionally, the effect of the metabolic control on adult

IQ as a measure of neurocognitive outcome should not be overestimated, and in adulthood, the target phenylalanine concentration should be decided by the effect of phenylalanine concentrations in adult age.

As the absolute phenylalanine concentration is the most crucial factor in directing treatment, phenylalanine measurements should be robust. The accuracy of aminoacid analysers, high-performance liquid chromatography, and tandem mass spectrometry is well established, but phenylalanine concentrations from dried blood spots are 8–26% lower than venous blood

	Studied population	Measurement of Phe	Measured outcome	Conclusion
MRI studies*†: Weglage et al (2001), <sup>27</sup> Bick et al (1993), <sup>33</sup> Kono et al (2005), <sup>34</sup> Cleary et al (1994), <sup>35</sup> and Manara et al (2009) <sup>36</sup>	Children, adolescents, and adults with PKU; range of n=2–77	Concurrent Phe, lifetime Phe, or Phe during childhood	White matter alteration by magnetic resonance imaging	No white matter changes when blood Phe concentrations were <300–600 µmol/L
Waisbren et al (2007) <sup>18*‡</sup>	Meta-analysis of 40 studies reported in 40 papers; 3361 patients with PKU aged 0–31 years	(1) Phe concentrations during critical period (birth to 6 or 12 years); and (2) lifetime Phe	IQ	(1) Phe range 423–750 µmol/L: each increase of 100 µmol/L results in 1.3–3.1 point reduction of IQ points; and (2) Phe range 394–666 µmol/L: each increase of 100 µmol/L results in 1.9–4.1 point reduction of IQ points
Fonnesbeck et al (2013) <sup>19*‡</sup>	Meta-analysis of 17 studies reported in 21 papers; 432 patients with PKU aged 2–32 years	(1) Phe concentrations during critical period of age <6 years; (2) lifetime Phe for age ≥6 years; and (3) IQ measurement concurrent with Phe in both periods	Probability of lower IQ (<85); general population probability was approximately 15%	(1) Probability of IQ <85 during the critical period (<6 years) is 19% with Phe concentrations of 400 µmol/L and 30% with Phe concentration of 600 µmol/L; (2) probability of historical Phe concentrations during the non-critical period (≥6 years) to result in IQ <85 is 14% with Phe concentration of 400 µmol/L and 20% with Phe concentration of 600 µmol/L; and (3) concurrently measured Phe was weakly correlated with probability of low IQ
Weglage et al (2013) <sup>23*</sup>	28 adult patients with PKU younger than 32 years with a relaxed diet in young adulthood, 16 adult patients with PKU older than 32 years with a relaxed diet from age 10 years, and 46 controls	Lifetime and concurrent Phe	IQ and EF	Stable outcomes in 5-year interval for both groups. Older group had lower IQ and poorer EF than younger group. IQ and EF were inversely associated with lifetime Phe
Diamond et al (1997) <sup>37‡</sup>	37 patients with PKU (0.5–7 years) and 86 controls	Concurrent Phe (defined as mean Phe during a 6-week period before testing) <360 µmol/L and 360–600 µmol/L	EF	Those with concurrent Phe of 360–600 µmol/L did less well in EF tasks (working memory and inhibitory abilities) than concurrent Phe <360 µmol/L and controls
Leuzzi et al (2004) <sup>38‡</sup>	14 patients with PKU (mean age 10.8 years, range 8–13 years) and 14 age-sex-IQ-SES-matched controls (mean age 10.9 years, range 8–13 years)	Lifetime Phe ≤400 µmol/L (n=5) and >400 µmol/L (n=9)	EF	Patients with lifetime Phe >400 µmol/L did worse than those with lifetime Phe ≤400 µmol/L in all tests of EF
Huijbregts et al (2002) <sup>39‡</sup>	67 patients with PKU (7–14 years) and 73 age-matched controls	Concurrently measured Phe ≤360 µmol/L (n=29) and >360 µmol/L (n=38)	Neuropsychological speed tests, including tests of EF	Those with concurrent Phe >360 µmol/L did significantly worse in several tests regarding EF than healthy controls. Patients with concurrent Phe concentrations ≤360 µmol/L did not differ from controls and were significantly better than patients with concurrent Phe concentrations >360 µmol/L
Schmidt et al (1996) <sup>40‡</sup> (included in Albrecht et al [2009]) <sup>21*‡</sup>	93 patients with PKU (61 with good metabolic control and 32 with poor metabolic control) and 60 controls; mean age of both groups was 8.7 years	Mean concurrent Phe of cluster with good metabolic control was 240 µmol/L (n=30) and with poor metabolic control was 620 µmol/L (n=31)	Neuropsychological speed tests, including tests of EF	Sustained attention and calculation speed of long-term well-controlled children (from birth to age 9 years) were vulnerable to high concurrent Phe concentrations, whereas children with low concurrent concentrations could not counterbalance the effects of long-term (from birth to age 9 years) poor dietary control
Schmidt et al (1996) <sup>40†</sup>	15 patients with PKU (17–24 years) and 19 controls (18–24 years)	Phe measured at three timepoints concurrently with assessed outcomes; strict diet with aminoacid supplement vs no diet	Neuropsychological speed tests, including tests of EF; tested three times with intervals of 4–5 weeks	Better neuropsychological task performance for patient group on diet (mean Phe of 630 µmol/L [range 280–966]) vs patient group off diet (mean Phe of 1320 µmol/L [720–1800] and 1410 µmol/L [1040–2200])

(Table 1 continues on next page)

	Studied population	Measurement of Phe	Measured outcome	Conclusion
(Continued from previous page)				
Jahja et al (2014) <sup>†‡</sup>	64 patients with PKU (mean age 10.8 years; range 6–15 years) and 73 controls (mean age 10.9 years)	Lifetime Phe of $\leq 240$ $\mu\text{mol/L}$ (n=10); 240–360 $\mu\text{mol/L}$ (n=33); or $\geq 360$ $\mu\text{mol/L}$ (n=21) concurrent Phe	Neuropsychological speed tests, including tests of EF	Patients with PKU aged 6–15 years with mean lifetime Phe concentrations $< 240$ $\mu\text{mol/L}$ did better than those with mean lifetime Phe concentrations between 240–360 $\mu\text{mol/L}$ , and those with lifetime Phe concentrations $> 360$ $\mu\text{mol/L}$ on performed speed EF tasks
ten Hoedt et al (2011) <sup>††</sup>	Nine patients with PKU (19–34 years), double-blind randomly assigned crossover trial; treatment arm duration of 4 weeks	Mean Phe during treatment with placebo capsules and Phe-containing capsules	IQ, mood, and neuropsychological speed tests, including tests of EF	Improved mood and neuropsychological task performance with lower Phe concentrations (Phe 709 $\mu\text{mol/L}$ [SD 322] for placebo vs 1259 $\mu\text{mol/L}$ [332] for experimental condition)
Hoeksma et al (2009) <sup>†‡</sup>	16 patients with PKU (16–47 years)	Phe measured concurrently with outcome	Cerebral protein synthesis rate by brain positron emission tomography studies	Recommended Phe concentrations $< 600$ – $800$ $\mu\text{mol/L}$ (because plasma Phe concentrations $> 600$ – $800$ $\mu\text{mol/L}$ decreased cerebral protein synthesis rates)
Sanayama et al (2011) <sup>††</sup>	40 patients with PKU (15–50 years) and 30 age-BMI-biological data-matched controls	Phe measured concurrently with outcome	Oxidative stress markers, antioxidant concentrations, and enzyme activities in erythrocytes	Recommended Phe concentrations $< 700$ – $800$ $\mu\text{mol/L}$ because the oxidative stress changed greatly at 700–800 $\mu\text{mol/L}$
Phe=phenylalanine. PKU=phenylketonuria. EF=executive function. IQ=intelligence quotient. SES=socioeconomic status. Evidence included for target Phe concentrations during †childhood ( $< 12$ years), †adolescence (12–18 years), and †adulthood ( $\geq 18$ years).				
<b>Table 1: Evidence for target phenylalanine concentrations during childhood, adolescence, and adulthood</b>				

samples.<sup>46–48</sup> Data from studies investigating neuro-cognitive outcomes are largely based on plasma phenylalanine concentrations, whereas routine practice is dried blood spots measurements. The guideline statements here on phenylalanine targets present the upper target phenylalanine concentrations, whereas the published work discusses means or medians. Consequently, the guideline statements are stricter than study findings. However, this difference might also compensate for other factors, such as dried blood spots instead of plasma phenylalanine concentrations, and not testing blood phenylalanine after an overnight fast, which is usually the highest point in 24 h.<sup>49,50</sup> We advise that blood phenylalanine samples are taken at the same time of day and under the same nutritional conditions in each individual patient.

Other markers, such as the phenylalanine-to-tyrosine ratio or the variation in the phenylalanine concentration, could be of additional value,<sup>41,51–55</sup> but any advantage offered in addition to blood phenylalanine measurements still remains to be determined, particularly as tyrosine concentrations vary considerably throughout 24 h.<sup>49,56</sup>

### Practical issues in dietary and BH4 treatment

Dietary phenylalanine restriction is the mainstay of treatment. This treatment consists of three parts: natural protein restriction using individual phenylalanine tolerance,<sup>57</sup> phenylalanine-free L-aminoacid supplements (usually with added vitamins and minerals) to meet protein and non-protein requirements, and low protein food to meet energy requirements. Although we have

longstanding experience with dietary treatment, it is only since the 2008 introduction of sapropterin dihydrochloride that more of our treatment is evidence-based rather than experience-based, but important evidence gaps still exist in several areas. Patients with the most severe PAH deficiency usually tolerate a daily intake of less than 350 mg phenylalanine, and therefore the quality of aminoacid supplements is important. Total protein intake should supply the age-related safe levels of protein intake (FAO/WHO/UNU 2007) with an additional 40% of L-aminoacids, given as natural protein or supplements according to FAO/WHO/UNU, using ideal bodyweight to calculate the dose ( $[(\text{ideal bodyweight [kg]} \times 0.8) - \text{intake of natural protein (g)}] \times 1.4 = \text{dose [g]}$ ). This calculation encompasses an additional 20% of L-aminoacids to compensate for the so-called digestible indispensable aminoacid score and a further 20% of L-aminoacids to optimise the effect of L-aminoacids on blood phenylalanine control. If inadequate doses of L-aminoacids are given, these aminoacids become rate-limiting for protein synthesis, and protein metabolism becomes catabolism. In net protein catabolism, phenylalanine will not only remain unused for protein synthesis, but will also be released, and the concentration will increase. Therefore, giving L-aminoacids decreases the blood phenylalanine concentration.

Aminoacid supplements can cause gastrointestinal upset (abdominal pain, diarrhoea, and constipation) in young children.<sup>58</sup> In adult patients, life-long and higher intake of aminoacid supplements has been linked to proteinuria and decreased glomerular filtration rate.<sup>59</sup> Aminoacid supplements should be evenly administered,

For more on the FAO/WHO/UNU 2007 report see [http://apps.who.int/iris/bitstream/10665/43411/1/WHO\\_TRS\\_935\\_eng.pdf?ua=1](http://apps.who.int/iris/bitstream/10665/43411/1/WHO_TRS_935_eng.pdf?ua=1)

at least three times throughout the day, to minimise fluctuations in blood phenylalanine variability.<sup>50</sup>

Low protein foods should contain 50 mg of phenylalanine or less per 100 g (equivalent to 1 g of protein per 100 g) of dry product. Fruit and vegetables (excluding potatoes) containing less than 75 mg of phenylalanine per 100 g of food product should not adversely affect blood phenylalanine control and can be included without restriction in the diet.<sup>60</sup>

The optimal amount of tyrosine provided in a low phenylalanine diet is unknown, but additional supplementation in excess of amounts provided by aminoacid supplements is not associated with a benefit.<sup>61</sup> At present, no statement can be given regarding daily practice with reference to supplementation of large neutral aminoacids treatment (figure 2) or the use of glycomacropeptide because of insufficient evidence.

As illness and fever cause increased catabolism and higher blood phenylalanine concentrations, aminoacid supplements and energy requirements should be met during infection. Antipyretics or analgesics, such as paracetamol and ibuprofen, should be considered to control temperature and improve appetite.

Aspartame (E951) is an intense sweetener composed of 50% phenylalanine,<sup>62,63</sup> and needs to be avoided in patients with PKU. In neotame, a similar artificial sweetener to aspartame, phenylalanine content is comparable but bioavailability is largely reduced.<sup>64</sup> Some medications contain aspartame (such as antibiotics), and short treatment courses might need to be given if no alternatives are readily available.

Patients with PKU, especially with a higher residual PAH activity, might respond to BH4 administration with a significant increase in phenylalanine tolerance, or decrease in blood phenylalanine concentrations, or both.<sup>65-67</sup> All patients warrant testing for BH4 responsiveness either by genotyping or BH4 loading.<sup>68</sup> According to the group's consensus, in any patient without two known null mutations for PKU or two known BH4 responsive mutations, a 48 h BH4 loading test should be done by analysing the blood phenylalanine concentrations before and after a single daily dose (20 mg/kg per day) on two consecutive days.<sup>68</sup> In the neonatal period, to avoid delays in dietary treatment, a 24 h instead of 48 h BH4 loading test is advised before starting the diet. Treatment with BH4 should only be prescribed in cases of proven long-term BH4 responsiveness, defined as an increase in the amount of natural protein of 100% or more or improved biochemical control (phenylalanine >75% in target range) and proven by a trial (of up to 6 months) of treatment with BH4. The treatment trial starts at 10–20 mg of BH4 per kg bodyweight and natural protein intake is increased (with the advice of a dietician), the BH4 dose is adjusted, and the aminoacid supplements are decreased accordingly, while blood phenylalanine concentrations are still maintained in the target range. BH4 treatment should be stopped if blood phenylalanine concentrations are

consistently above the upper target range, with a lack of response to an increase in BH4 dose. If nutritional status deteriorates (eg, development of obesity or nutritional deficiencies), discontinuation of BH4 treatment should be discussed. BH4 treatment can be given during pregnancy, but only if women are known to be BH4 responders and dietary treatment alone is unsuccessful.

### Follow-up and adherence

Treatment and follow-up of patients should be done in specialised metabolic centres, with at least an experienced metabolic physician and dietician for both children and adults, and a specialised metabolic laboratory. Establishing adult metabolic teams and centres to care for and monitor older patients with PKU is important. Ideally, these teams will be led by an adult metabolic disease physician who can coordinate care and liaise with different specialties as necessary. Access to a neuropsychologist, or psychologist, and a social worker is strongly advised, but this advice is on the basis of clinical rather than evidence-based practice.

Follow-up includes home blood sampling and outpatient visits, the frequency of both being largely dependent on age (table 2). Various life events, such as change of school, starting work, and living independently, as well as adherence issues (eg, during adolescence) might necessitate a higher frequency of blood phenylalanine testing or visits. The transition process not only includes the transition of patients from paediatric to adult care, but also the transition of the responsibility for treatment from parents to the patient. The pathway to greater autonomy generally starts at around 12 years with progression to adult services from the age of 16 years.

Outpatient visits include taking a medical and dietary history, collecting anthropometric data, such as body mass index, undertaking a physical and neurological examination, especially observing for clinical signs of phenylalanine toxicity and nutrient (including phenylalanine) deficiency,<sup>69,70</sup> and discussing issues of treatment and outcome (such as neurological and psychiatric issues, behaviour, and mood). Table 2 lists the additional investigations necessary, and these focus on potential nutritional deficiencies (especially iron and vitamin B<sub>12</sub>),<sup>71-74</sup> neurocognitive measures, and bone mineral density. Clinical symptoms of nutrient deficiency are rare and are mostly described because of vitamin B<sub>12</sub> deficiency (if micronutrient supplementation, which is usually added to aminoacid supplements, is stopped or partially taken, alongside a meat-free diet).<sup>73,74</sup> The incidence of osteopenia and osteoporosis in PKU is somewhat higher than in the general population, but only one study indicated that increased risk of fractures exists.<sup>3,75,76</sup> Follow-up is advised with dual-energy x-ray absorptiometry scanning once during late adolescence. If normal, a repeat scan is unnecessary. If abnormal, repetition with or without a change of treatment should follow after 1 year. Additionally, micronutrient deficiencies (selenium, zinc, coenzyme Q10, and



L-carnitine) have been linked to oxidative stress in PKU,<sup>77</sup> and oxidative stress is associated with poor metabolic control.<sup>44</sup> However, because of the lack of clinical symptoms associated with oxidative stress, and the absence of increased oxidative stress with adequate metabolic control, we do not propose routine biochemical monitoring of oxidative stress.

Routine neurocognitive assessments should be done at ages 12 years and 18 years in all patients, since blood phenylalanine treatment targets and life-phase changes occur at these ages (including change of school, living situation, starting work, and becoming an adult). We also advise measurement of quality of life and discussion of psychosocial functioning during clinic visits with PKU-specific health-related quality of life instruments.<sup>30,31</sup>

To achieve long-term adherence, changing the attitude and motivation of the patient might be more effective than improving knowledge.<sup>78</sup> Regular professional health

support might be needed throughout life to encourage normal, healthy feeding behaviours, with a positive acceptance of a low phenylalanine diet.

As health-care providers have a legal obligation to protect and care for each child in their care, the following considerations are proposed when a child (<12 years of age) is in poor metabolic control. When more than 50% of the phenylalanine concentrations are out of the target range during a period of 6 months (approximately 13 measurements per year), health-care providers should consider increased frequency of blood phenylalanine monitoring and outpatient visits, and re-education, plus psychology consultation or social worker interventions, and hospital admission. When around 100% of measured blood phenylalanine concentrations are out of the target range during a period of 6 months and other signs of failure of adherence; safety issues; or lack of cooperation, clinic non-

	Childhood (<12 years)	Adolescence (12–18 years)	Adulthood (≥18 years), excluding maternal PKU	Maternal PKU
Outpatient visit	Every 2 months at age 0–1 years and twice per year at age 1–12 years given good clinical and metabolic control; extra clinic visit as indicated	Twice per year given good clinical and metabolic control; extra clinic visit as indicated	Once per year given good clinical and metabolic control; extra clinic visit as indicated	Once during each trimester given good clinical and metabolic control; extra clinic visit as indicated
Clinical nutritional assessment	Dietary assessment (3 day food record or 24 h recall), anthropometric parameters (weight, height, BMI), and clinical features of micronutrient and Phe deficiency (especially anorexia, listlessness, alopecia, and perineal rash) at every outpatient visit	Dietary assessment (3 day food record or 24 h recall), anthropometric parameters (weight, height, BMI), and clinical features of micronutrient and Phe deficiency at every outpatient visit	Dietary assessment (3 day food record or 24 h recall), anthropometric parameters (weight, height, BMI), and clinical features of micronutrient and Phe deficiency every 12–24 months	Dietary assessment (3 day food record or 24 h recall) and weight at every outpatient visit
Metabolic control assessment	Weekly Phe at age 0–1 year and fortnightly Phe at age 1–12 years; increased frequency as indicated. Plasma aminoacids annually	Monthly Phe. Increased frequency as indicated; plasma aminoacids annually	Monthly Phe. Increased frequency as indicated; plasma aminoacids annually	Pre-conceptionally: weekly Pregnancy: twice weekly; increased frequency as indicated Pre-conceptionally: plasma aminoacids
Biochemical nutritional assessment	Annual measurement of plasma homocysteine or methylmalonic acid, or both, haemoglobin, mean corpuscular volume, and ferritin; all other micronutrients (vitamins and minerals including calcium, zinc, and selenium) or hormones (parathyroid hormone) if clinically indicated	Annual measurement of plasma homocysteine or methylmalonic acid, or both, haemoglobin, mean corpuscular volume, and ferritin; all other micronutrients (vitamins and minerals including calcium, zinc, and selenium) or hormones (parathyroid hormone) if clinically indicated	Annual measurement of plasma homocysteine or methylmalonic acid, or both, haemoglobin, mean corpuscular volume, and ferritin; all other micronutrients (vitamins and minerals including calcium, zinc, and selenium) or hormones (parathyroid hormone) if clinically indicated	Folic acid, vitamin B12, plasma homocysteine or methylmalonic acid, or both, ferritin, full blood count assessed pre-conceptionally and at the start of pregnancy; assess when indicated during pregnancy
Bone density	BMD measurement only indicated when there are specific clinical reasons or when patients are known to be at particular risk of metabolic bone disease	First BMD measurement should be done during late adolescence; when BMD is abnormal, the measurement (with or without change of treatment) should be repeated after 1 year; if the results are still low but stable, yearly BMD is unnecessary. When BMD is normal, no repeat measurement is necessary; further study need only be considered when clinical reasons exist to do so	BMD measurement only indicated when specific clinical reasons exist or when patients are known to be at particular risk of metabolic bone disease	Not indicated
Neurocognitive examination	Neurocognitive testing only when indicated	Testing at age 12 years. Proposed domains of testing for Phe toxic effects are IQ, perception or visuospatial functioning, EF (divided into inhibitory control, working memory, and cognitive flexibility), and motor control. Extra neurocognitive tests as indicated	Testing at age 18 years. Proposed domains of testing for Phe toxic effects are IQ, perception or visuospatial functioning, EF (divided into inhibitory control, working memory, and cognitive flexibility), and motor control. Extra neurocognitive tests as indicated	Not indicated

(Table 2 continues on next page)

	Childhood (<12 years)	Adolescence (12–18 years)	Adulthood (≥18 years), excluding maternal PKU	Maternal PKU
(Continued from previous page)				
Adaptive issues function examination (eg, clinically relevant behavioural problems)	Annual clinical assessment or discussion	Annual clinical assessment or discussion; screening at age 12 years	Annual clinical assessment or discussion; screening at age 18 years	Not indicated
Neurological examination	If neurodegeneration occurs	If neurodegeneration occurs	Annual clinical examination	Not indicated
Investigations of psychosocial functioning and wellbeing, and health-related quality of life	Annual clinical assessment or discussion; once during childhood: PKU-specific quality of life questionnaire	Annual clinical assessment or discussion; once during adolescence: PKU-specific quality of life questionnaire	Annual clinical assessment or discussion; once during adulthood PKU-specific quality of life questionnaire	Additional support might be required for women who are unable to conceive
Psychiatric examination	At onset of symptoms of psychiatric disturbances	At onset of symptoms of psychiatric disturbances	At onset of symptoms of psychiatric disturbances	Not indicated
Neuroimaging examination (MRI)	When there is an unexpected clinical course and/or unexpected neurological deficits	When there is an unexpected clinical course and/or unexpected neurological deficits	When there is an unexpected clinical course and/or unexpected neurological deficits	Not indicated
Investigations specific to age group	..	..	..	Ultrasound at 18–22 weeks of pregnancy with screening for organ development (especially if a lack of optimal metabolic control exists); echocardiogram in all infants who are conceived by women with either high blood Phe concentrations or poor maternal blood Phe control during pregnancy

PKU=phenylketonuria. Phe=phenylalanine. BMD=bone mineral density. IQ=intelligence quotient. EF=executive function.

**Table 2: Minimum requirements for the clinical management and follow-up of patients with PKU**

attendance, or unresolved issues outside PKU, are also apparent, health-care providers should consider consultation with social services and child safeguarding measures. These proposals are based on the Delphi consensus method and are suggested in order to improve the outcome of patients from families who are resistant to routine advice and commonly fail to engage successfully with health professionals.

**Special conditions**

Within treatment for PKU, some specific conditions require a very specific treatment strategy. These groups include maternal PKU and late diagnosed or untreated PKU. Treatment for women with PKU also aims to prevent fetal effects of high maternal phenylalanine concentrations, called maternal PKU syndrome.<sup>79</sup> Blood phenylalanine concentrations should ideally be kept between 120 µmol/L and 360 µmol/L from preconception onwards.<sup>80–84</sup> Maintaining these concentrations necessitates continuous education throughout preadolescence, adolescence, and adulthood.<sup>85</sup> Contraceptive strategies should only be discontinued after stable phenylalanine concentrations within the target range have been achieved for at least 2 weeks, thus preventing sustained phenylalanine

concentrations above or below the target range.<sup>86</sup> phenylalanine intake must immediately be increased by 50–100 mg per day when blood phenylalanine is 120 µmol/L or less at anytime during pregnancy. However, no treatment is necessary if untreated blood phenylalanine concentrations are less than 360 µmol/L.<sup>87–89</sup> Stable weight gain during pregnancy improves outcome,<sup>90,91</sup> and energy intake should be monitored to ensure it is adequate but not excessive.

Referral to a fertility centre should be considered for patients with well controlled phenylalanine concentrations who do not conceive within 6 months. Women with an unplanned pregnancy should be seen within 24 h to initiate immediate dietary treatment. Treatment will require intensive intervention—eg, consider the use of the support of a home “Resource Mother”<sup>45,92</sup> (available in the USA) or even hospital admission.<sup>93</sup> Treatment might be difficult because of nausea, which can necessitate dietary adjustments such as small and frequent low protein meals and snacks,<sup>94</sup> tube feeding, safe antiemetic therapy, and acid-reducing medications.

Blood phenylalanine concentration should be checked at least once weekly before pregnancy and at least twice

weekly during pregnancy. This check requires a rapid laboratory turnaround time. High blood phenylalanine concentrations above target range (120–360  $\mu\text{mol/L}$ ) imply a high-risk pregnancy, necessitating the need for a fetal ultrasound at 18–22 weeks of pregnancy with screening for organ development (depending on the country, as for some countries this scan is an additional scan).

Aminoacid supplements usually supply sufficient tyrosine (intake should be at least 6 g per day). We advise 400  $\mu\text{g}$  per day of folic acid preconceptually and during the first 12 weeks of pregnancy, which corresponds with general antenatal recommendations.

We encourage breastfeeding and do not consider there to be contraindications for breastfeeding in maternal PKU, but clinical experience would suggest that the provision of breast feeding to an infant with PKU whose mother also has PKU requires careful monitoring with regard to the phenylalanine concentrations both in the mother and the infant.

Patients with PKU who are diagnosed late or patients with untreated PKU, especially because of newborn screening failures or lack of newborn screening or treatment (eg, immigrants), also need special consideration.<sup>95–97</sup> Patients with PKU who are diagnosed late can improve their IQ with treatment,<sup>97–99</sup> and improvements in seizures and behaviour can occur in patients with untreated PKU.<sup>99</sup> Therefore, regardless of age, we advise the identification of these patients. Treatment for patients with a late diagnosis of PKU should always be considered, whereas in patients with untreated PKU, introduction of treatment is on an individual basis. If treatment is started, a treatment trial of 6 months should be given before the outcomes are assessed (including motor function [less tremors or spasticity] and behaviour [less restless and irritable, more alert or responsive, and less aggressive with decreased frequency and severity of self-injury behaviours]), but further data are required to give clear recommendations for the care of late diagnosed or untreated PKU.

## Conclusion and future directions

To set a standard of care for all patients with PKU in Europe, we have developed this first set of European guidelines for the diagnosis and treatment on the basis of the highest quality of available evidence. We have identified knowledge gaps that require further research to direct better care for the future.

Optimisation of all current treatment strategies is essential, with a focus on adult and elderly patients and a definition of their difficulties and any explanatory pathophysiological mechanisms. Such future treatment strategies might target the blood phenylalanine concentrations through the use of phenylalanine ammonia-lyase modified with polyethylene glycol, new chaperones, and gene treatment.<sup>100</sup> Other treatments might directly affect cerebral metabolism, such as the

## Search strategy and selection criteria

We searched PubMed (MEDLINE), Embase, the NHS Economic Evaluations Database (NEED), the Cochrane Library, and reference lists for relevant publications in English. We used the search terms listed in the appendix. All literature that was reviewed was published up to Dec 31, 2015, and was without any exclusion of publications before a specified year. In total, 975 publications were reviewed. The quality of the studies was assessed by two group members independently, or by group discussion, or both, before we developed the conclusions into recommendations. Recommendations with no or low level of evidence were discussed with all participants during five face-to-face plenary sessions until consensus was reached, in line with the Delphi method. The key recommendations were either based on evidence (if level of evidence was A or B [SIGN method]) or by consensus (Delphi method) if the level of evidence was C or D, or are so-called good practice points that are not based on any evidence.

use of large neutral aminoacids. Both glycomacropeptide and large neutral aminoacids are already being used in some countries but require further proof of safety and efficacy.<sup>101,102</sup> We also need to improve the quality of care delivered by some PKU centres, particularly in low-income and middle-income countries, to achieve acceptable care for all patients with PKU in Europe.<sup>8</sup>

To facilitate attainment of these objectives, the ESPKU and the development of European Reference Networks for PKU are important. Especially for rare diseases, an international consortia to investigate outcomes in relation to treatment strategies would be very useful. The European Reference Network for metabolic diseases, including PKU, is an important international consortium that can facilitate studying outcomes associated with varying treatments options and target phenylalanine concentrations in different age ranges.

The availability of a device for home monitoring might further optimise outcomes,<sup>103</sup> as a shorter turnaround time to attain blood phenylalanine concentrations could help patients improve phenylalanine control.

## Contributors

FJvS led the project, chaired the plenary discussions, and was the lead writer of the manuscript. AMJvW co-chaired the plenary discussions and was the second lead writer of the manuscript. All other authors contributed in the plenary discussions, and co-wrote and approved the manuscript. FJvS contributed to groups B and E (initiation of treatment [a topic of group E] and methods of measuring phenylalanine [a subtopic of group B]). AMJvW was the project assistant and assisted all working groups in the literature search, selection, and grading of evidence, and processed the data. KA, FF, AM, and JHW were members of working group A (nutritional treatment and nutritional and biochemical follow-up). AB, JC, SCH, and VL were members of working group B (neurocognitive outcomes including imaging). AMB, SK, and MvR were members of working group C (psychosocial outcomes and adherence). MG, FM, and FKT were members of working group D (adult and maternal phenylketonuria [PKU], late diagnosed, and untreated PKU). AB-Q, NB, and ACM were members of working group

E (diagnosis of PKU and drugs in PKU). KA worked on the large neutral aminoacids main topic. AB-Q worked on the emerging therapies main topic. AB and VL worked on the magnetic resonance imaging main topic. NB worked on the diagnosis, differential diagnosis, genotyping, and BH4 loading test main topics. AMB worked on the psychosocial functioning (including quality of life) and mental health main topics. JC worked on the target phenylalanine levels, biochemical markers, and neurocognitive functioning main topics. FF worked on the bone mineral density, oxidative stress, and biochemical follow-up main topics. MG and FKT worked on the maternal PKU and late diagnosed and untreated PKU main topics. SCH worked on the target phenylalanine levels, biochemical markers, and neurocognitive functioning main topics. SK worked on the frequency of visits and phenylalanine level measurement main topics. AM worked on the dietary treatment main topic and contributed to group D on dietary treatment in maternal PKU and untreated or late-treated adults. FM worked on the adult PKU main topic. ACM worked on the BH4 treatment main topic. MvR worked on the adherence main topic and contributed to group D on untreated or late-treated adults. JHW worked on the parenteral nutrition main topic.

#### Declaration of interests

KA has been a member of the European Nutrition Expert Panel (Merck Serono International). AB-Q has received honoraria as a speaker for Nutricia International, Vitaflo International, Merck Serono, and Recordati, and is a member of the European Nutrition Expert Panel (supported by Merck Serono International), the Sapropterin Advisory Board (supported by Merck Serono International and BioMarin), and KAMPER Advisory Board (supported by Merck Serono International, BioMarin). NB has been a member of the Merck Serono and BioMarin and Censa Pharmaceuticals scientific advisory boards for phenylketonuria (PKU), and has received grants and honoraria from Merck Serono and BioMarin. AMB, AB, FF, MG, and ACM have been members of scientific advisory boards for PKU (supported by Merck Serono, BioMarin, and Nutricia International). AMB has received grants from Nutricia International and honoraria from Merck Serono, BioMarin, and Nutricia International. JC and MG have received honoraria as consultants and speakers from Merck Serono and Nutricia International/Danone. FF has received honoraria from BioMarin, Merck Serono, and Nutricia International/Danone. SCH has participated in strategic advisory boards and received consultant and speaker honoraria from Merck Serono, Biomarin, and Nutricia International. SK has received honoraria from Merck Serono and BioMarin. VL has received honoraria as a consultant from Nutricia International. AM has received research funding and honoraria from Nutricia International, Vitaflo International, and Merck Serono, chairs the European Nutrition Expert Panel (supported by Merck Serono International), has been a member of the Sapropterin advisory board (supported by Merck Serono International), is a member of the Advisory Boards Element (Danone-Nutricia), and is a member of the scientific advisory board of Arla Foods International. FM has received consultant and speaker honoraria from Merck Serono, Nutricia International, Vitaflo International, and Arla Foods International, and research grants from Merck Serono. ACM has received research funding from Nutricia International, Vitaflo International, and Merck Serono, and has received honoraria as a speaker from Merck Serono and Arla Foods International. MvR was a member of the European Nutritionist Expert Panel in PKU (supported by Merck Serono International; until 2015), is a member of the ELEMENT (Leading Education in Metabolic Error Nutritional Therapy) steering committee for Nutricia International, and has received grants and fees for educational and research activities from Nutricia International and Orphan Europe. FJvS is a member of scientific advisory boards for PKU and aminoacid defects that are supported by Merck Serono (past), BioMarin, Arla Foods International, SoBi, and Nutricia International, has received research grants from Nutricia International, SoBi, Alexion, and Merck Serono, and honoraria as a consultant and speaker from Merck Serono and Nutricia International/Danone, and honoraria as a speaker from Vitaflo International. FKT has received grants from Vitaflo and honoraria as a speaker from Merck Serono. AMJvW and JHW declare no competing interests.

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