# The first European guidelines on phenylketonuria

Evers, Roeland A F; van Wegberg, Annemiek M J; Anjema, Karen; Lubout, Charlotte M A; van Dam, Esther; van Vliet, Danique; Blau, Nenad; van Spronsen, Francjan J

*Published in:*
Journal of Inherited Metabolic Disease

*DOI:*
10.1002/jimd.12173



*Publication date:*
2020

Received: 4 July 2019 | Revised: 28 August 2019 | Accepted: 9 September 2019

DOI: 10.1002/jimd.12173



ORIGINAL ARTICLE

JIMD SSIEM WILEY

# The first European guidelines on phenylketonuria: Usefulness and implications for $BH_4$ responsiveness testing

**Roeland A. F. Evers BSc[1] | Annemiek M. J. van Wegberg MSc[1] | Karen Anjema MD[1] | Charlotte M. A. Lubout MD[1] | Esther van Dam MSc[1] | Danique van Vliet MD[1] | Nenad Blau PhD[2] | Francjan J. van Spronsen MD, PhD[1]**

[1]Beatrix Children's Hospital, Division of Metabolic Diseases, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

[2]University Children's Hospital Zürich, Zürich, Switzerland

**Correspondence**
Francjan J. van Spronsen, University Medical Center Groningen, Hanzeplein 1, 9700 RB Groningen, The Netherlands.
Email: f.j.van.spronsen@umcg.nl

**Communicating Editor:** Wilcken Bridget

**Funding information**
Tyrosinemia Foundation; Stichting PKU research; Stofwisselkracht; NPKUA; Metakids; ESPKU; Vitaflo; SoBi; Nutricia; Codexis; Alexion; Rare Disease Foundation; Merck Serono; Nutricia, honoraria; Biomarin

## Abstract

**Objective:** This study aimed to investigate and improve the usefulness of the 48-hour $BH_4$ loading test and to assess genotype for $BH_4$ responsiveness prediction, using the new definition of $BH_4$ responsiveness from the European guidelines, as well as an amended definition.

**Method:** Applying the definition of the European guidelines (≥100% increase in natural protein tolerance) and an amended definition (≥100% increase in natural protein tolerance or tolerating a safe natural protein intake) to a previous dataset, we first assessed the positive predictive value (PPV) of the 48-hour $BH_4$ loading test using a cutoff value of 30%. Then, we tried to improve this PPV by using different cutoff values and separate time points. Last, using the BIOPKU database, we compared predicted $BH_4$ responsiveness (according to genotype) and genotypic phenotype values (GPVs) in $BH_4$-responsive and $BH_4$-unresponsive patients.

**Results:** The PPV of the 48-hour loading test was 50.0% using the definition of the European guidelines, and 69.4% when applying the amended definition of $BH_4$ responsiveness. Higher cutoff values led to a higher PPV, but resulted in an increase in false-negative tests. Parameters for genotype overlapped between $BH_4$-responsive and $BH_4$-unresponsive patients, although $BH_4$ responsiveness was not observed in patients with a GPV below 2.4.

**Conclusion:** The 48-hour $BH_4$ loading test is not as useful as previously considered and cannot be improved easily, whereas genotype seems mainly helpful in excluding $BH_4$ responsiveness. Overall, the definition of $BH_4$ responsiveness and $BH_4$ responsiveness testing require further attention.

**KEYWORDS**
definition, European guidelines, loading test, phenylketonuria, responsiveness, tetrahydrobiopterin

## 1 | INTRODUCTION

The cornerstone of treatment in phenylketonuria (PKU; MIM 261600) is restricting phenylalanine (Phe) intake by a natural protein-restricted diet combined with intake of Phe-free amino acid supplements. Additionally, some patients benefit from pharmacological treatment with tetrahydrobiopterin ($BH_4$), which can increase residual phenylalanine hydroxylase

activity leading to better metabolic control and/or an increase in natural protein tolerance. However, different definitions of $BH_4$ responsiveness exist.[1-3]

Recently, the first European guidelines on PKU were published.[4,5] In these guidelines, $BH_4$ responsiveness is defined as "establishing an increase in natural protein tolerance of ≥100% with blood Phe concentrations remaining consistently within the target range" or by improved metabolic control, which is defined as ">75% of blood Phe levels remaining within target range without any decrease in natural protein intake associated with $BH_4$ treatment." Since these criteria are stricter than previously used in the Netherlands,[1] some patients in our population might no longer be considered $BH_4$ responsive when applying this definition. We noticed that this would even be the case for some patients who could actually tolerate a safe natural protein intake as a result of $BH_4$, meaning these patients could meet their protein requirements (according to WHO guidelines) using only natural protein sources, therefore not requiring additional amino acid supplements. Since these patients clearly benefit from $BH_4$ treatment, we felt that the definition of $BH_4$ responsiveness from the European guidelines may need to be amended to include patients who can tolerate a safe natural protein intake due to $BH_4$.

The European PKU guidelines also give recommendations on the method of $BH_4$ responsiveness testing. With the exception of patients with a genotype consisting of two null mutations, in whom $BH_4$ responsiveness does not need to be further considered, it is recommended that $BH_4$ responsiveness testing is performed by a 48-hour $BH_4$ loading test. If Phe concentrations decrease with at least 30% during this test, a treatment trial should be performed to evaluate whether the patient is indeed $BH_4$ responsive. Although the 48-hour $BH_4$ loading test with a cutoff value of 30% is often cited as a reliable way to predict $BH_4$ responsiveness,[1,6] its predictive value has not been assessed using the expert-based definition of $BH_4$ responsiveness that is stated by the European guidelines. Specifically, the study by Anjema et al. is cited as conformation of the utility of the 48-hour $BH_4$ loading test, but this study defined $BH_4$ responsiveness as an increase in natural protein intake of ≥50% or ≥4 g/day, which is a much lower threshold.[1] Therefore, the present study aimed to investigate and improve the usefulness of the 48-hour $BH_4$ loading test, and to assess the predictive value of genotype, first using the new definition of $BH_4$ responsiveness from the European guidelines, and second using an amended definition that also includes patients who can tolerate a safe natural protein intake due to $BH_4$.

## 2 | METHODS

### 2.1 | Patients and protocol

We used data that were collected for a previous study on $BH_4$ responsiveness testing.[1] Detailed information on the data collection, subjects and protocol were described by Anjema et al.[1] Here, the most relevant methodological aspects are summarized.

Data were collected retrospectively from 183 pediatric and adult patients who performed the 48-hour $BH_4$ loading test between November 2009 and December 2010. None of these tests took place in the neonatal period. For the 48-hour $BH_4$ loading test, baseline Phe concentrations were required to be over 400 μmol/L. Patients who had Phe concentrations below 400 μmol/L were, therefore, supplemented with Phe. Patients received 20 mg/kg $BH_4$ for 2 days (at $t = 0$ and 24 hours), while blood samples were collected at $t = 0$, 8, 16, 24, and 48 hours. Patients who showed a reduction in blood Phe concentrations ≥30% compared to $t = 0$ at any moment during the loading test were invited for a $BH_4$ treatment trial. Three-day dietary records were taken before and after the treatment trial to assess natural protein intake. During this treatment trial, $BH_4$ was introduced at 20 mg/kg/day (with a maximum of 1400 mg/day), dietary Phe was increased to reach the maximal Phe tolerance, and $BH_4$ dose was finally decreased if possible. In the original protocol, true $BH_4$ responsiveness was defined according to previously used guidelines in the Netherlands as "a reduction in blood Phe concentrations of 30% or more compared to mean blood Phe concentrations prior to the 48-hour $BH_4$ loading test with the same diet, and/or an increase in dietary Phe tolerance of ≥ 50% or ≥ 4 grams of natural protein without increasing the Phe concentrations above the upper target." Data on genotype was collected if available. In total, 175 PKU patients correctly performed the 48-hour $BH_4$ loading test, and 65 patients performed the treatment trial (Table 1). Two patients from the original cohort were excluded, since it was found out that these patients had a DNAJC12 deficiency.[7]

### 2.2 | Genotype

Using the BIOPKU database (http://www.biopku.org, accessed on 29 January 2019), we assessed two ways for using genotype to predict $BH_4$ responsiveness. First, we used the BIOPKU database to collect the percentage of $BH_4$-responsive patients (including "slow" responders) with a corresponding genotype, when information on $BH_4$ responsiveness was available for ≥5 cases. Second, we used the BIOPKU database to assign genotypic phenotype values (GPVs) to the genotypes of the patients in this cohort. GPVs are as a numerical representation of predicted PAH activity

**TABLE 1** Demographic and clinical details of the study cohort

| 48-hour $BH_4$ loading test result | No potential $BH_4$ responsiveness[a] | Potential $BH_4$ responsiveness[a] (n = 78) | | |
|---|---|---|---|---|
| Treatment trial result | (n = 97) | $BH_4$ responsive[b] (n = 31) | $BH_4$ unresponsive[b] (n = 34) | Other[c] (n = 13) |
| Gender (% female) | 51.5 | 64.5 | 47.1 | 46.2 |
| Age (years) | 14.7 (10.3-23.0) | 12.0 (6.6-17.1) | 12.5 (7.9-22.2) | 14.1 (10.4-19.4) |
| Baseline Phe (μmol/L)[d] | 663 (530-915) | 458 (362-549)*** | 509 (408-623)** | 485 (386-590)* |
| Phe supplementation (%) | 29.9 | 71.0*** | 58.8* | 92.3*** |
| Phe supplementation (mg/day)[e] | 200 (125-300) | 335 (202-500) | 350 (150-1000) | 400 (210-1000)* |
| Natural protein intake prior to treatment trial (g/kg bw) | | 0.34 (0.23-0.43)[f] | 0.52 (0.29-0.70) | |

*Note:* Data are presented as median (IQR) or as percentage of patients.
[a]Based on a decrease in Phe levels of 30% as cutoff value.
[b]Based on an increase in natural protein tolerance of 100% as cutoff value.
[c]No treatment trial despite a positive 48-hour $BH_4$ loading test.
[d]At $t = 0$ during the 48-hour $BH_4$ loading test.
[e]Amount of Phe supplementation in patients who were supplemented with Phe.
[f]$P < .05$ compared to $BH_4$-unresponsive patients (Mann-Whitney U test).
*$P < .05$; **$P < .01$; ***$P < .001$ compared to patients with no potential $BH_4$ responsiveness (Mann-Whitney $U$ test, corrected for multiple comparisons according to Bonferroni).

in PKU patients, ranging from 0 (lowest PAH activity) to 10 (highest PAH activity).[8] Since $BH_4$ responsiveness is associated with higher levels of residual PAH activity, it was hypothesized that GPVs could be helpful in predicting $BH_4$ responsiveness. Patients who had a 48-hour $BH_4$ loading test that was considered positive (eg, ≥30% reduction in Phe levels) but did not perform a treatment trial were not included in these analyses.

## 2.3 | Analyses

No analyses were copied from the original study. $BH_4$-responsiveness was assessed based on natural protein intake. The positive predictive value (PPV) of the 48-hour $BH_4$ loading test was calculated as the number of $BH_4$-responsive patients (based on the results of the treatment trial, using different definitions) divided by the number of potentially $BH_4$-responsive patients (based on the results of the 48-hour $BH_4$ loading test, using different cutoff values). Similarly, the negative predictive value (NPV) was calculated as the number of $BH_4$-unresponsive patients divided by the number of not potentially $BH_4$-responsive patients. Descriptive statistics were used to present most of the data. Normality of data was checked visually using histograms and QQ-plots, and tested using the Shapiro-Wilk test. Other statistical tests are mentioned where used. A two-tailed $P$-value <.05 was considered statistically significant. Statistical analyses were performed using IBM SPSS Statistics version 23 and GraphPad Prism version 7 for Windows.

# 3 | RESULTS

## 3.1 | $BH_4$ responsiveness as defined by the European guidelines: The 48-hour $BH_4$ loading test and genotype

Of the 65 patients who performed a treatment trial, three had two putative null mutations (here defined as a GPV of 0 in the BIOPKU database) and were therefore excluded from the analyses in this part, as they would not have been involved in any $BH_4$ responsiveness testing following the recommendations of the European guidelines. All three patients were not considered to be $BH_4$ responsive in the original protocol. The PPV of the 48-hour $BH_4$ loading test was 50.0% when using the recommended cutoff value of 30% and the definition of $BH_4$ responsiveness recommended in the European guidelines.

Increasing the cutoff value to 35% led to a PPV of 57.4%, but beyond that, higher cutoff values were associated with a reduction in NPVs (Table 2). The PPV of separate time points varied between 51.7% (at $t = 8$) and 59.1% (at $t = 24$) using 30% as a cutoff value, with higher cutoff values again resulting in lower NPVs (Supplemental material 1). Furthermore, again looking at separate time points, 51.6%, 29.0%, 16.1%, and 6.5% of $BH_4$-responsive patients showed no Phe decrease ≥30% at $t = 8$, $t = 16$, $t = 24$, and $t = 48$, respectively.

In total, 58 patients had a genotype for which the percentage of $BH_4$-responsive patients in the BIOPKU database with a corresponding genotype (≥5 cases) was available

(Figure 1A). This BIOPKU responsiveness percentage was compared between patients considered $BH_4$ responsive and $BH_4$ unresponsive in this study. Significant differences were found between $BH_4$-responsive patients vs a total group of $BH_4$-unresponsive patients (including patients with no potential responsiveness after the loading test), as well as between patients considered $BH_4$ unresponsive after the loading test vs patients considered $BH_4$ unresponsive after the treatment trial. However, within the group of patients who performed the treatment trial, there was no difference between $BH_4$-responsive and $BH_4$-unresponsive patients. In 106 patients, it was possible to assign GPVs to their genotype (Figure 1B), showing a similar pattern. The lowest GPV in a $BH_4$-responsive patient was 2.4.

### 3.2 | $BH_4$ responsiveness using an amended definition: the 48-hour $BH_4$ loading test and genotype

For a second set of analyses, we defined $BH_4$ responsiveness as "an increase in natural protein tolerance ≥ 100% or tolerating a safe natural protein intake," in which a safe natural protein intake was determined based on gender and age according to recommendations.[9] Using this definition, the number of $BH_4$-responsive patients in our cohort increased from 31 to 43, which is a significant increase of 38.7% ($P < .001$, McNemar test). Equally, again excluding the three patients with two null mutations, the PPV of the 48-hour $BH_4$ loading test (using the 30% cutoff value) increased from 50.0% to 69.4%.

Higher cutoff values resulted in higher PPVs, but were also associated with a decrease in the NPV (Supplemental material 2). The PPVs of having at Phe decrease ≥30% at separate time points were between 71.7% (at $t = 48$) and 82.8% (at $t = 8$) (Supplemental material 3), with higher cutoff values again generally resulting in lower NPVs. Of $BH_4$-responsive patients, 44.2%, 23.8%, 19.0%, and 11.6% showed no decrease in Phe ≥30% at $t = 8$, $t = 16$, $t = 24$, and $t = 48$, respectively.

Parameters of genotype showed similar patterns as with the definition in European guidelines, although significant differences were now found between $BH_4$-responsive and $BH_4$-unresponsive patients who performed the treatment trial (Supplemental material 4). With the amended definition, the lowest GPV in a $BH_4$-responsive patient again was 2.4.

**TABLE 2** Predictive values using different cutoff values for a decrease in Phe levels during the 48-hour $BH_4$ loading test

| Phe decrease[a] | PPV[b] | Phe decrease[a] | NPV[c] |
|---|---|---|---|
| ≥30% | 50.0% | | |
| ≥35% | 57.4% | 30-35% | 100.0% |
| ≥40% | 58.7% | 30-40% | 75.0% |
| ≥45% | 57.5% | 30-45% | 63.6% |
| ≥50% | 56.8% | 30-50% | 60.0% |

[a]Maximum Phe decrease compared to the baseline value.
[b]Positive predictive value (PPV) using the corresponding cutoff value.
[c]Negative predictive value (NPV) for a decrease in Phe in the corresponding range.

## 4 | DISCUSSION

Following the publication of the first European guidelines for PKU, this study assessed the usefulness of the 48-hour $BH_4$ loading test and genotype for $BH_4$ responsiveness prediction. Our results indicate that the 48-hour $BH_4$ loading test is not useful for predicting $BH_4$ responsiveness as defined by the European PKU guidelines, whereas genotype seems mainly helpful in excluding $BH_4$ responsiveness. Additionally, we introduced an amended definition of $BH_4$ responsiveness, which in our opinion better defines which patients benefit from $BH_4$ treatment. This definition also leads to an increase in $BH_4$-responsive patients and results in a somewhat more effective 48-hour $BH_4$ loading test.

Before discussing our findings in more detail, some limitations of this study need to be addressed. Since this study used retrospectively collected patient care data, patients with a negative 48-hour $BH_4$ loading test did not perform a treatment trial, and the number of false negative tests is therefore unknown. Furthermore, we determined $BH_4$ responsiveness based on natural protein intake, and therefore did not assess using ">75% of blood Phe levels remaining within target range" as a definition. We anticipate however that this definition will be less regularly used, mostly since increasing the natural protein tolerance is the main goal of $BH_4$ treatment, as shown by longer-term follow-up studies of $BH_4$-treated patients.[10-12] Besides these points, it is important to note that this protocol of the 48-hour $BH_4$ loading test involved Phe supplementation in case of too low Phe concentrations, and did not take measurements of Phe levels at $t = 32$ and $t = 40$.

Since the introduction of $BH_4$ as a new treatment option for PKU, different definitions of $BH_4$ responsiveness have been proposed ([1-4]). The definition of $BH_4$ responsiveness recently proposed in the European guidelines is stricter than the definition previously used in the Netherlands, as shown by a decrease in the number of $BH_4$-responsive patients in this study compared with the original publication (33 vs 58 $BH_4$-responsive patients). As mentioned in the introduction, we noticed that some patients who did not meet the criteria of $BH_4$ responsiveness set by the European guidelines could tolerate a safe natural protein intake due to $BH_4$.

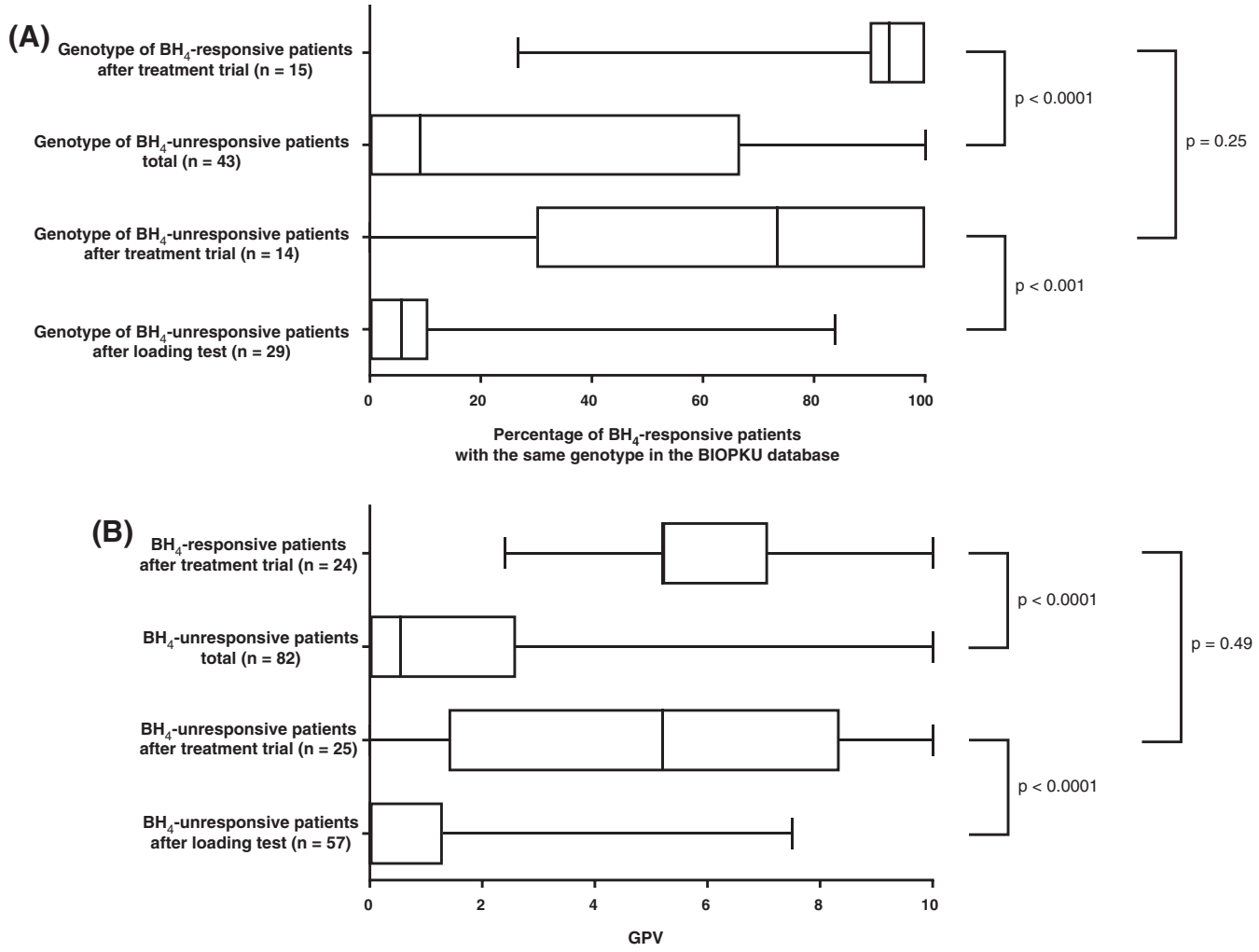


**FIGURE 1** Association between genotype and $BH_4$ responsiveness. A, Boxplots (median, 25th-75th percentile, min, max) of the percentage of $BH_4$-responsive patients with a similar genotype in the BIOPKU database in $BH_4$-unresponsive and $BH_4$-responsive patients according to the definitions of the European PKU guidelines. Differences are tested with the Mann-Whitney U test. B, Boxplots (median, 25th-75th percentile, min, max) of the genotypic phenotype value (GPV) in $BH_4$-unresponsive and $BH_4$-responsive patients according to the definitions of the European PKU guidelines. Differences are tested with the Mann-Whitney U test

In our cohort, this was the case for 12 patients. These patients could increase their tolerance with a mean of 22 g of natural protein (range: 13 to 35 g) during the $BH_4$ treatment trial, enabling them to adopt a more normal diet. Moreover, these patients typically did not need amino acid supplementation anymore. Considering these major advantages of dietary liberalization to this extent, the consensus-based definition in the European guidelines could, in our opinion, be improved by defining $BH_4$ responsiveness as "an increase in natural protein tolerance $\geq$ 100% *or tolerating a safe natural protein intake*."

Clearly, this amended definition is arbitrary as well. The criterion of increasing the natural protein tolerance by at least 100% may still be too strict, and seems especially difficult for patients with a relatively high baseline natural protein tolerance. This is also indicated by a significant difference in baseline natural protein intake between $BH_4$-responsive and $BH_4$-unresponsive patients (according to the definition of the European PKU guidelines) who performed a treatment trial (Table 1). Ultimately, to be able to give a more definitive definition of $BH_4$ responsiveness that is evidence based rather than consensus-based, long-term follow up studies comparing outcomes in $BH_4$ treated to only dietary-treated PKU patients are necessary. Such studies should identify the exact advantages of better metabolic control and/or increased natural protein intake as a result of $BH_4$ treatment in PKU. Since these data are not yet available, developing a (better) consensus-based definition of $BH_4$ responsiveness may be the best alternative. However, different definitions of $BH_4$ responsiveness may require different $BH_4$ responsiveness testing methods, as shown by the results in this study.

Our results show a low PPV of the 48-hour $BH_4$ loading test when applying the definition of $BH_4$ responsiveness from the European guidelines. Using the amended definition resulted in a higher PPV, albeit still much lower than the PPV of 87% that was reported in the original publication. Given the widespread use of the loading test, this is a worrying result, since it impairs the cost-effectiveness of $BH_4$ testing and may also lead to an increase in disappointed patients with a false-positive $BH_4$ loading test. Using the amended definition, our data show that a larger reduction in Phe during the loading test is associated with a higher chance of being $BH_4$ responsive. However, considering the increase in false negatives with larger cutoff values, using a cutoff value higher than 30% would not be recommended according to the results in this cohort. Equally, the PPV of a decrease in Phe at separate time points may be somewhat higher, but is also associated with an increase in false-negative tests. Overall, the 48-hour $BH_4$ loading test cannot be improved easily, and its shortcoming should be kept in mind, especially when using the definition for $BH_4$ responsiveness stated in the European PKU guidelines.

To improve testing for potential $BH_4$ responsiveness, the simple 48-hour $BH_4$ loading test may need to be developed further. In this study, we unsuccessfully tried to improve the test by selecting higher cutoff values and by using separate measurements, but we were unable to investigate the predictive value of Phe measurements at $t = 32$ and $t = 40$, and Phe-to-tyrosine ratios. These latter approaches may thus deserve further attention. Additionally, extending the $BH_4$ loading test to 7 days[13] or even longer may be helpful in differentiating between daily Phe variation and a $BH_4$-induced decrease in Phe. On this topic, a comparison between the outcomes of the $BH_4$ testing regime in Europe (using a 48-hour $BH_4$ loading test) and the United States (using a 28-days $BH_4$ loading test) would be interesting. Recently, a group of experts proposed a $BH_4$ testing algorithm which combines the 48-hour $BH_4$ loading tests with testing periods up to 4 weeks.[14] Apart from the fact that Muntau et al. did not recommend a specific definition of long-term $BH_4$ responsiveness, the value of the recommended testing protocol remains to be established. Alternatively, it could be argued that completely different testing methods are simply superior. Potential examples of this include comparing simple Phe loading with Phe + $BH_4$ loading,[15] using a [$^{13}$C]Phe breath test,[16] or assessing genotype.[8]

With regard to genotype, results of this study showed that all patients with a GPV below 2.4 showed no (potential) $BH_4$ responsiveness, which is in line with the report of Gerbade et al. that mentions a cutoff value of 2 as a minimum for (potential) $BH_4$ responsiveness.[8] Nevertheless, in line with that study, we found considerable overlap between $BH_4$-responsive and $BH_4$-unresponsive patients, with some unresponsive patients having a GPV as high as 10 (using the definition of the European guidelines) or 8.9 (using the amended definition). It should be noted that many $BH_4$-unresponsive patients only performed a loading test and no treatment trial, and some of them might therefore be false negatives. Nevertheless, this is an important finding that requires further examination. Overall, GPVs seem to be primarily helpful to exclude $BH_4$ responsiveness in patients in case of low-residual PAH activity. Predicting $BH_4$ responsiveness using data on $BH_4$ testing in the BIOPKU database from patients with the same genotype shows similar results. Importantly, the BIOPKU database defines $BH_4$ responsiveness as a decrease in Phe levels of at least 30% during a short-term $BH_4$ loading test only, regardless of the specific protocol used. While genotype may be strongly related to the results of 48-hour $BH_4$ loading test,[8] our results show that the 48-hour $BH_4$ loading test is not a good predictor for $BH_4$ responsiveness. Therefore, although genotype is helpful in excluding $BH_4$ responsiveness, it is not an overall good predictor of $BH_4$ responsiveness. These results show the shortcomings of both genotype and the 48-hour $BH_4$ loading to predict $BH_4$ responsiveness, thereby underlining the importance of a treatment trial.

To conclude, the 48-hour $BH_4$ loading test with a cutoff value of 30% does not effectively predict long-term $BH_4$ responsiveness as defined by the European PKU guidelines, whereas genotype seems mainly helpful to exclude $BH_4$ responsiveness. We recommend amending the definition of $BH_4$ responsiveness from the European PKU guidelines to include patients who can tolerate a safe natural protein intake as a result of treatment with $BH_4$. Methods to predict $BH_4$ responsiveness require further attention, although a sound definition of $BH_4$ responsiveness may be even more important to optimize $BH_4$ responsiveness testing as well as treatment.

## ACKNOWLEDGEMENTS

We thank everyone who was involved with the original publication, including patients, physicians, dieticians, researchers, and laboratory technicians. The authors received no funding for this research.

## CONFLICTS OF INTEREST

R.A.F.E. has received financial support from Biomarin for attending symposia. A.M.J.v.W. has received a research grant from Nutricia, honoraria from Biomarin as speaker, and travel grants from Nutricia, and Vitaflo K.A. has received research funding from Merck Serono. C.M.A.L. has received a speaker fee from the Recordati Rare Disease Foundation. E.v.D. has received advisory board fees from Merck Serono and Biomarin. D.v.V. has

received speaker's honoraria from Biomarin. N.B. has no conflicts of interest to declare. F.J.v.S. is a member of scientific advisory boards for defects in amino acid metabolism of APR, Arla Food International, BioMarin, Eurocept Int, Lucana, Moderna TX, Nutricia, Rivium, and SoBi, has received research grants from Alexion, Biomarin, Codexis, Nutricia, SoBi, and Vitaflo, has received grants from patient organizations ESPKU, Metakids, NPKUA, Stofwisselkracht, Stichting PKU research and Tyrosinemia Foundation, and has received honoraria as consultant and speaker from APR, Biomarin, MendeliKABS, and Nutricia.

## AUTHOR CONTRIBUTIONS

R.A.F.E. designed the study, analyzed the data, interpreted the results, and was the lead writer of the manuscript. A.M.J.v.W. designed the study, interpreted the results, and was the second lead writer of the manuscript. K.A. collected the data, interpreted the results, and cowrote the manuscript. C.M.A.L., E.v.D., D.v.V., and N.B. interpreted the results and cowrote the manuscript. F.J.v.S. interpreted the results, cowrote the manuscript, and supervised the project. All authors read and approved the final version of the manuscript.

## DATA ACCESSIBILITY

Original data will be made available on reasonable request.

## ETHICAL APPROVAL

For the original data collection, the $BH_4$ testing protocol was considered standard patient care by the Medical Ethical Committee of the University Medical Center Groningen. Informed consent was not required for this study.

## REFERENCES


1. Anjema K, van Rijn M, Hofstede FC, et al. Tetrahydrobiopterin responsiveness in phenylketonuria: Prediction with the 48-hour loading test and genotype. *Orphanet J Rare Dis*. 2013; 8:103.
2. Singh RH, Quirk ME. Using change in plasma phenylalanine concentrations and ability to liberalize diet to classify responsiveness to tetrahydrobiopterin therapy in patients with phenylketonuria. *Mol Genet Metab*. 2011;104:485-491.
3. Vockley J, Andersson HC, Antshel KM, et al. Phenylalanine hydroxylase deficiency: Diagnosis and management guideline. *Genet Med*. 2014;16:188-200.
4. van Spronsen FJ, van Wegberg AM, Ahring K, et al. Key European guidelines for the diagnosis and management of patients with phenylketonuria. *Lancet Diabetes Endocrinol*. 2017b;5; 743-756.
5. van Wegberg AMJ, MacDonald A, Ahring K, et al. The complete European guidelines on phenylketonuria: Diagnosis and treatment. *Orphanet J Rare Dis*. 2017;12:162.
6. Cerone R, Andria G, Giovannini M, Leuzzi V, Riva E, Burlina A. Testing for tetrahydrobiopterin responsiveness in patients with hyperphenylalaninemia due to phenylalanine hydroxylase deficiency. *Adv Ther*. 2013;30:212-228.
7. van Spronsen FJ, Himmelreich N, Rüfenacht V, et al. Heterogeneous clinical spectrum of DNAJC12-deficient hyperphenylalaninemia: From attention deficit to severe dystonia and intellectual disability. *J Med Genet*. 2017a;55:249-253.
8. Garbade SF, Shen N, Himmelreich N, et al. Allelic phenotype values: A model for genotype-based phenotype prediction in phenylketonuria. *Genet Med*. 2018;21:580–590.
9. WHO/FAO/UNU. *Protein Amino Acid Requirements in Human Nutrition*. Geneva, Switzerland: WHO/FAO/UNU; 2007.
10. Evers RAF, Wegberg v, Annemiek MJ, et al. Anthropomorphic measurements and nutritional biomarkers after 5 years of BH4 treatment in phenylketonuria patients. *Mol Genet Metab*. 2018; 124:238-242.
11. Keil S, Anjema K, van Spronsen FJ, et al. Long-term follow-up and outcome of phenylketonuria patients on sapropterin: A retrospective study. *Pediatrics*. 2013;131:1881.
12. Scala I, Concolino D, Della Casa R, et al. Long-term follow-up of patients with phenylketonuria treated with tetrahydrobiopterin: A seven years experience. *Orphanet J Rare Dis*. 2015;10:14.
13. Nielsen JB, Nielsen KE, Güttler F. Tetrahydrobiopterin responsiveness after extended loading test of 12 Danish PKU patients with the Y414C mutation. *J Inherit Metab Dis*. 2010;33:9-16.
14. Muntau AC, Adams DJ, Bélanger-Quintana A, et al. International best practice for the evaluation of responsiveness to sapropterin dihydrochloride in patients with phenylketonuria. *Mol Genet Metab*. 2019;127:1-11.
15. Porta F, Spada M, Ponzone A. Early screening for tetrahydrobiopterin responsiveness in phenylketonuria. *Pediatrics*. 2017;140:e20161591.
16. Muntau AC, Röschinger W, Habich M, et al. Tetrahydrobiopterin as an alternative treatment for mild phenylketonuria. *N Engl J Med*. 2002;347:2122-2132.


## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

**How to cite this article:** Evers RAF, van Wegberg AMJ, Anjema K, et al. The first European guidelines on phenylketonuria: Usefulness and implications for $BH_4$ responsiveness testing. *J Inherit Metab Dis*. 2020;43:244–250. https://doi.org/10.1002/jimd.12173