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## Minireview

## Fluctuations in phenylalanine concentrations in phenylketonuria: A review of possible relationships with outcomes

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## ABSTRACT

Fluctuations in blood phenylalanine concentrations may be an important determinant of intellectual outcome in patients with early and continuously treated phenylketonuria (PKU). This review evaluates the studies on phenylalanine fluctuations, factors affecting fluctuations, and if stabilizing phenylalanine concentrations affects outcomes, particularly neurocognitive outcome. Electronic literature searches of Embase and PubMed were performed for English-language publications, and the bibliographies of identified publications were also searched. In patients with PKU, phenylalanine concentrations are highest in the morning. Factors that can affect phenylalanine fluctuations include age, diet, timing and dosing of protein substitute and energy intake, dietary adherence, phenylalanine hydroxylase genotype, changes in dietary phenylalanine intake and protein metabolism, illness, and growth rate. Even distribution of phenylalanine-free protein substitute intake throughout 24 h may reduce blood phenylalanine fluctuations. Patients responsive to and treated with 6R-tetrahydrobiopterin seem to have less fluctuation in their blood phenylalanine concentrations than controls. An increase in blood phenylalanine concentration may result in increased brain and cerebrospinal fluid phenylalanine concentrations within hours. Although some evidence suggests that stabilization of blood phenylalanine concentrations may have benefits in patients with PKU, more studies are needed to distinguish the effects of blood phenylalanine fluctuations from those of poor metabolic control.

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**Abbreviations:** BH<sub>4</sub>, 6R-tetrahydrobiopterin; ECT, early and continuously treated; HPA, hyperphenylalaninemia; IDC, index of dietary control; IQ, intelligence quotient; LNAA, large neutral amino acid; PAH, phenylalanine hydroxylase; PKU, phenylketonuria; SD, standard deviation; SEE, standard error of the estimate; Tyr, tyrosine; WISC, Wechsler Intelligence Scale for Children.

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## 1. Introduction

Treatment for phenylketonuria (PKU) includes diet restriction of phenylalanine intake and/or administration of 6R-tetrahydrobiopterin (BH<sub>4</sub>) to BH<sub>4</sub>-responsive patients. Treatment can prevent neurological impairment and mental retardation [1]. There is a consensus that, for an optimal outcome, treatment should start as early as possible and that strict blood phenylalanine level control is of primary importance, particularly during the first years of life [1]. BH<sub>4</sub> stimulates phenylalanine hydroxylase (PAH) activity in about 20% of patients with PKU, and in those patients it serves as a useful adjunct to the phenylalanine-restricted diet because it increases phenylalanine tolerance and allows significant dietary relaxation [2]. Sapropterin dihydrochloride (Kuvan<sup>®</sup>, Merck Serono SA Geneva, Switzerland<sup>2</sup> and BioMarin, Novato, CA, USA), a pharmaceutical formulation of BH<sub>4</sub>, is an approved drug for the treatment of PKU. It has been shown to lower blood phenylalanine concentrations significantly in those patients with PKU who respond to sapropterin therapy [3–6].

A wealth of data has shown that high blood and brain phenylalanine concentrations in patients with PKU are associated with deleterious effects on neurocognitive outcomes including executive function [7,8]. Some studies have suggested that the fluctuations in phenylalanine concentrations (phenylalanine fluctuation) may be of particular significance [9–11]. Although there is currently no standard definition of *phenylalanine fluctuation*, studies reporting phenylalanine fluctuations have used the following methods for its assessment: standard deviation (SD), standard error of the estimate (SEE) of the regression of phenylalanine concentration, and mean (SD) of the index of dietary control (IDC) as measured by the mean of the 6-month median phenylalanine values [12]. Clearly, the mean of IDC requires assessment over at least 6 months, whereas SD and SEE can be assessed over shorter time frames. The mechanism by which fluctuations in phenylalanine concentrations affects outcomes is not known.

The aim of this paper is to review the literature on phenylalanine fluctuations, factors affecting fluctuations, and whether stabilizing blood and brain phenylalanine concentrations has a positive effect on outcomes, particularly on general intelligence and neurocognitive outcome.

## 2. Selection criteria for publications

Electronic literature searches of Embase and PubMed were performed for English-language publications on 24 May 2012 using the following terms: *phenylalanine*, *fluctuat\** or *variation\**, *PKU* or *phenylketonuria*, *intellectual outcome* or *IQ*, *Kuvan* or *sapropterin* or *phenoptin* or *6R-BH4* or *6RBH4* or *6R BH4* or *BH4*. The search was supplemented by additional relevant secondary references and published materials known to the authors.

The electronic literature search identified 112 papers from the search criteria used. From this search, 39 papers were identified as

relevant to the subject matter of the review. Only one meta-analysis [13] and five papers reported studies assessing the impact of phenylalanine fluctuations on outcomes in patients with PKU [9–11,14,15], and one in offspring from mothers with PKU [16].

## 3. Fluctuations in phenylalanine concentrations

### 3.1. Fluctuation in healthy subjects

In healthy individuals, blood phenylalanine concentrations fluctuate by no more than 50% over 24 h [17,18]. In healthy infants and children up to the age of 18 years, reference blood phenylalanine concentrations are between 21 and 137 μmol/L, and in adults, 35 to 85 μmol/L [19–21]. Fasting blood concentrations of amino acids, including phenylalanine, are reasonably constant. Values vary throughout the day according to dietary intake, with less fluctuation with more frequent meals [17,18]. In children without PKU on a normal diet, blood phenylalanine concentrations were higher in the evening than in the morning [22].

### 3.2. Diurnal fluctuation in PKU

Evidence suggests that physiological fluctuations in phenylalanine concentrations in patients with PKU within a day are different to those seen in healthy individuals on a normal diet.

Inverse diurnal variation with phenylalanine concentrations that were highest in the morning was reported as early as 1969 in two children with PKU [22] and subsequently confirmed in 1985, in a pregnant 22-year-old woman with PKU [23]. Larger studies have since confirmed this inverse diurnal variation. In a study of 16 patients with classic PKU aged 1 to 18 years, the highest phenylalanine concentration occurred in the morning between 6 and 9 a.m. and the lowest between 6 p.m. and midnight in 63% of patients [24]. Diurnal variations were also demonstrated in seven patients with PKU aged less than 1 year [25]. This suggests that protein catabolism predominates over protein anabolism during fasting periods. In line with this, prolonged fasting results in a small rise in phenylalanine concentrations [26].

However, it appears that the timing of protein substitutes also affects diurnal variation. In a study of 19 patients (15 girls, 4 boys) aged 1 to 16 years, considerable fluctuation was seen between early morning and late afternoon plasma phenylalanine concentrations, which was related to intake of protein substitute [27]. In the above-mentioned study of 16 patients with classic PKU aged 1 to 18 years [24], a significant correlation was seen between the timing of protein substitute consumption and percentage change in plasma phenylalanine concentrations: the greater the quantity of protein substitute consumed by 4 p.m., the larger the decrease in daytime phenylalanine concentration ( $r = -0.7030$ ,  $p < 0.005$ ). The less the protein substitute consumed after 4 p.m., the larger the plasma phenylalanine concentration between 4 p.m. and 6 a.m. the following morning ( $r = -0.7337$ ;  $p < 0.005$ ). Similarly, in a randomized controlled study of 16 patients with well-controlled PKU, median differences in blood phenylalanine concentrations within a day were 40 μmol/L when protein substitutes were given every 4 h,

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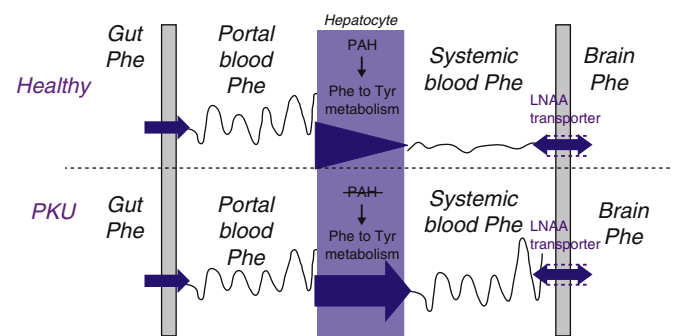
including administration at midnight and 4 a.m., whereas with three equal protein substitute doses given over 10 h, such differences were 140  $\mu\text{mol/L}$  [28].

The distribution of phenylalanine intake is not a crucial factor in the determination of the diurnal variation [26]. Blood phenylalanine concentrations rose slightly (+16%) in the 90 min following breakfast with an individually tailored, tolerance-based intake in a study of nine patients with PKU aged 1 to 20 years, whereas blood tyrosine concentrations rose 4- to 5-fold. In the study of 16 patients with classic PKU aged 1 to 18 years [24], blood phenylalanine concentrations generally did not rise in response to phenylalanine consumption. Regarding the size of fluctuations in phenylalanine concentrations, the median variation in plasma phenylalanine concentrations was 155  $\mu\text{mol/L}$  per day, with a minimum of 80  $\mu\text{mol/L}$  and a maximum of 280  $\mu\text{mol/L}$ , among 16 patients with classic PKU aged 1 to 18 years [24]. In the study of seven patients with PKU aged less than 1 year, diurnal variations did not differ significantly when infants were fed natural protein together with phenylalanine-free formula or followed a feeding scheme in which these were alternated [25]. The effect of equally and unequally divided distribution of daily phenylalanine intake was studied in seven patients with PKU aged 1 to 17 years [29]. In three different tests, patients were fed breakfast and lunch consisting of varying percentages of daily phenylalanine allowance and total daily protein intake (including the protein substitute). The maximum rise in blood phenylalanine concentration above baseline was 26%, and it was suggested that unequal distributions of intake of the daily phenylalanine allowance are justified, provided that the patient is adjusted to the diet adequately and that the daily allowance was not exceeded, although long-term effects were not studied [29].

In summary, a number of studies have shown a diurnal variation in blood phenylalanine concentrations, with highest concentrations in the morning in patients with PKU. Some studies suggest that even distribution of protein substitute intake throughout 24 h stabilizes phenylalanine concentrations.

### 3.3. Day-to-day variation in PKU

In a study of six adult patients with PKU who had been continuously treated since diagnosis, day-to-day variations in blood phenylalanine concentrations were between 55 and 257  $\mu\text{mol/L}$  and individual SDs varied from 6 to 99  $\mu\text{mol/L}$  [30]. Within 4 days after various phenylalanine challenges, variation in SDs increased to between 23 and 208  $\mu\text{mol/L}$ . Thus, in adults with well-controlled PKU, day-to-day blood phenylalanine concentrations may vary by up to 400% [30].



**Fig. 1.** Fluctuations in blood phenylalanine (Phe) concentrations in healthy subjects and patients with phenylketonuria (PKU) [31,32]. In healthy subjects and patients with PKU, phenylalanine fluctuations are similar in portal blood. In systemic blood, however, phenylalanine fluctuations are greater in patients with PKU than in healthy subjects due to reduced phenylalanine hydroxylase (PAH) activity in the liver. LNAAs, large neutral amino acid; Tyr, tyrosine.

Fig. 1 [31,32] depicts portal and systemic blood phenylalanine concentrations in healthy subjects and patients with PKU, and shows how systemic blood phenylalanine fluctuates to a greater extent in patients with PKU than in healthy subjects.

### 3.4. Long-term fluctuation in PKU

In patients with PKU, day-to-day phenylalanine fluctuations over months and years may have effects on cognition that are cumulative with detrimental effects on intelligence quotient (IQ). Two studies have reported increases in phenylalanine concentrations and fluctuations with age [14,33].

A cross-sectional, longitudinal study of 105 patients with hyperphenylalaninemia (HPA) summarized plasma phenylalanine concentrations throughout patients' lives using the IDC. Patients with good dietary control had more stable blood phenylalanine concentrations than those with poor dietary control [14]. Both the mean IDC and SD IDC increased with age, particularly after 12 to 13 years.

In a retrospective study of 37 patients with PKU on sapropterin therapy (22 mild-to-moderate PKU, 17 classic PKU) and a mean age 12.6 years, mean (SD) phenylalanine concentration under dietary therapy was 403.8 (254.3)  $\mu\text{mol/L}$ , with a mean (SD) within-subject variance of 417.5 (26.0)  $\mu\text{mol/L}$  [33]. Phenylalanine concentrations were significantly positively associated with age and increased by 14.5  $\mu\text{mol/L}$  per year ( $p < 0.0001$ ) after adjustment for repeated measures. Phenylalanine fluctuations also seemed to increase with age (Table 1) [33]. In contrast, other studies investigating dietary treatment of PKU suggest that phenylalanine fluctuations within a day may decrease with age [24,29].

Mean (SD) lifetime blood phenylalanine concentrations of 182 (72)  $\mu\text{mol/L}$  and mean (SD) phenylalanine concentrations of 312 (132)  $\mu\text{mol/L}$  were reported in a retrospective study of 46 patients (23 male, 23 female; 26 classic PKU, 15 moderate PKU, 4 mild PKU, 1 unclassified) with early and continuously treated (ECT)-PKU, and a mean (SD) age of 7.5 (3.32) years [9].

In summary, blood phenylalanine concentrations may increase and fluctuations may or may not decrease with age. The increase in phenylalanine concentrations with age may be due, at least in part, to relaxation of diet [13,34] and poor dietary adherence.

### 3.5. Factors that affect day-to-day fluctuations

Factors such as diet, intake of protein substitute, and age affect day-to-day phenylalanine fluctuations. Other factors may also include PAH genotype and changes in protein metabolism [35]. Phenylalanine fluctuations, expressed as SEE, were related to the predicted (from genotype information) in vitro PAH enzyme activity and the severity of the disease in a study of 64 patients with HPA [11]. Patients with predicted activity greater than 20% had lower SEE values than patients with lower residual enzyme activity.

**Table 1**

Variances and standard deviations in blood phenylalanine concentrations as a function of age in a study of 37 patients with phenylketonuria. Reprinted from B.K. Burton et al. [33], Copyright (2010), with permission from Elsevier.

Comparison	Age group (years)	Estimate of variance ( $\mu\text{mol/L}$ )	Standard deviation ( $\mu\text{mol/L}$ )
Between subjects	<3	89.0	9.4
Between subjects	3–10	380.2	19.5
Between subjects	>10	462.2	21.5
Within subjects	<3	223.8	15.0
Within subjects	3–10	528.4	23.0
Within subjects	>10 <sup>a</sup>	575.10	24.0

<sup>a</sup> Range 10–32 years.

Illness, such as vomiting, diarrhea, or other infections, may decrease intake of protein substitute and energy, induce protein catabolism, and increase blood phenylalanine concentrations [14,35,36], and thus potentially also increase day-to-day blood phenylalanine fluctuations. Variations in growth rate may also affect fluctuations in blood phenylalanine concentrations; if natural protein or total energy intake is inadequate to support growth, blood phenylalanine levels may increase due to protein catabolism [36,37].

In sapropterin-responsive patients with PKU, sapropterin therapy appears to reduce fluctuations in blood phenylalanine concentrations. In a retrospective study of 37 patients with PKU treated with sapropterin for 12 to 31 months, sapropterin therapy significantly reduced phenylalanine fluctuations [33]. The SD of blood phenylalanine concentration (of the mean of averaged individual measurements) after sapropterin treatment was 228.8  $\mu\text{mol/L}$  compared with 254.3  $\mu\text{mol/L}$  before treatment. The mean (SD) within-subject variance, estimated via linear mixed modeling, was also lower after (290.5 [16.3]  $\mu\text{mol/L}$ ) than before sapropterin therapy (417.5 [26.0],  $p = 0.0017$ ).

Blood phenylalanine variability over 8 years (2002–2010) has been compared in patients with HPA detected at birth who responded to  $\text{BH}_4$  therapy ( $n = 9$ ) and in patients unresponsive to  $\text{BH}_4$  ( $n = 25$ ) [38]. Although median phenylalanine concentrations were the same in both groups, fluctuations in blood phenylalanine level, as assessed by differences in confidence intervals of the mean and median phenylalanine concentrations, were significantly lower in the  $\text{BH}_4$ -responsive group treated with  $\text{BH}_4$  ( $p = 0.025$ ) than in the  $\text{BH}_4$ -nonresponsive group. However, patients who respond to  $\text{BH}_4$  therapy tend to have milder disease than  $\text{BH}_4$ -nonresponsive patients and may also have lower levels of phenylalanine fluctuation.

To summarize, although prospective, controlled studies are lacking, phenylalanine fluctuations in patients with PKU may be influenced by diet, adherence to diet, illness, growth rate, and genotype. Treatment with sapropterin or  $\text{BH}_4$  appears to reduce phenylalanine fluctuations, but this effect may be influenced by additional factors such as treatment adherence.

### 3.6. Impact of fluctuations on the brain

Phenylalanine concentrations can be easily measured in the blood, but it is brain concentrations rather than blood concentrations that are considered to affect neurocognitive development [39]. Brain phenylalanine concentration is a direct result of blood phenylalanine concentration and phenylalanine transport across the blood–brain barrier [40,41]. Such transport occurs via the neutral amino acid transporter (LAT1) and increases with phenylalanine blood concentration, but it may also be affected by blood concentrations of other amino acids [40].

In patients with PKU, the brain phenylalanine concentration ranges from 16% to 44% of the blood concentration [42–45]. Following phenylalanine challenge, increases in brain phenylalanine concentrations are less steep than in the blood but occur over a longer period of time [41,42]. The variance in blood phenylalanine concentrations (4.6%) appeared to be similar to the variance in brain values, and significant ( $p < 0.01$ ) individuality of blood–brain phenylalanine ratios was seen [45]. In healthy controls, however, the blood and brain phenylalanine concentrations are about equal [43]. Some patients with PKU have elevated blood phenylalanine concentrations but good clinical status and IQ within the normal range, which has been attributed to low brain phenylalanine concentrations [43,46]. It has been suggested that interindividual variations in the kinetics of phenylalanine uptake and metabolism lead to different brain phenylalanine concentrations at comparable blood levels [46–49], but not all studies support that hypothesis [44].

In summary, in patients with PKU, the increase in phenylalanine concentrations in the brain is smaller than in the blood. Peaks in blood concentrations occur later in the brain, are less steep, but last longer.

### 3.7. Fluctuation in PKU, neurocognitive outcome, and measures of brain activity

The main influence of phenylalanine on the developing brain, and hence on intelligence in patients with PKU, appears to be during the first 10 years of life [50,51]. High blood phenylalanine concentrations in children with PKU aged 7 to 14 years have been associated with slower speeds of information processing than in healthy controls [8]. In addition, lowering brain phenylalanine concentrations via the ingestion of large neutral amino acids has been shown to prevent the slowing of electroencephalogram activity seen with high brain phenylalanine concentrations [41].

It may be difficult to separate the effects of more severe PKU and/or poor disease control from the effects of phenylalanine fluctuations on neurocognitive outcomes. The relationship between IQ and blood phenylalanine levels is clear [52]. However, only a few studies have specifically addressed phenylalanine fluctuation and intellectual outcome. One study of 76 patients with early diagnosed PKU noted that intelligence correlated negatively with both current IDC ( $r = -0.468$ ,  $p < 0.0001$ ) and phenylalanine fluctuations ( $r = -0.347$ ,  $p < 0.004$ ) [14], where phenylalanine fluctuations were assessed by the SEE of the regression of phenylalanine concentration over age; however, the relationship between these two variables was not determined. In another study of 64 patients with HPA [11], IQ on the revised Wechsler Intelligence Scale for Children (WISC) correlated significantly with phenylalanine fluctuations expressed as SEE ( $r = -0.37$ ,  $p < 0.05$ ). However, phenylalanine concentrations and phenylalanine fluctuations could not be separated with regard to their influence on IQ. The effect of maternal blood phenylalanine concentrations on outcomes in 105 offspring born to 67 mothers with PKU [16] supports an effect of phenylalanine fluctuations on IQ in exposed infants.

Two studies have failed to determine this relationship between phenylalanine fluctuations and cognitive impairment. In a retrospective study of 46 children with ECT-PKU, the correlation between the SD of blood phenylalanine concentrations and the most recent full-scale IQ was not significant ( $-0.36$ ,  $p = 0.058$ ) [9]. In another study of 55 patients, fluctuations in phenylalanine concentrations (assessed as SD) did not significantly affect IQ as measured by the WISC-III, WISC-IV, and Wechsler Adult Intelligence Scale-III [15].

One study of 18 children with classic PKU assessed standardized measures of executive functions, and noted executive functions correlated best with increased individual variation in blood phenylalanine concentrations ( $r = -0.65$ ,  $p < 0.02$ ) [10]. However, individual variation was significantly correlated with increased current blood phenylalanine concentrations ( $r = 0.53$ ,  $p = 0.027$ ). Studies that tease out the individual effects of phenylalanine concentrations versus fluctuations are needed to help better understand the importance of the two parameters.

In addition to decreasing the blood phenylalanine concentration, stabilization of blood phenylalanine concentrations may be a goal of treatment in PKU. However, although the evidence suggests that greater fluctuation in phenylalanine concentrations in patients with PKU has detrimental effects on IQ, direct evidence from prospective, randomized controlled trials is lacking.

In summary, several publications showed correlations between phenylalanine fluctuations and intellectual outcome, cognition, or executive functioning. Two studies, however, found no association between phenylalanine fluctuations and IQ. In addition, it is difficult to differentiate the effects of blood phenylalanine fluctuations and phenylalanine concentrations. Prospective, randomized studies of the effect of fluctuations in blood phenylalanine concentrations on outcomes in PKU are needed.

### 3.8. Review limitations

Relatively few papers have been published on blood phenylalanine concentration fluctuations in PKU, and due to the rarity of the disease,



most involve small numbers of patients. Data in adults are particularly limited. Increased individual fluctuations in phenylalanine concentrations have been shown to be correlated with increased current phenylalanine concentrations ( $r = 0.53$ ,  $p = 0.027$ ), and it is difficult to differentiate between the effects of these two variables [10]. However, individual fluctuations and lifetime phenylalanine concentrations were not correlated in that particular study.

In the papers reviewed, phenylalanine fluctuations were determined by a number of different methods: SD, SEE of the regression of phenylalanine concentration, and mean (SD) of the IDC. Further data are needed to decide on which method of fluctuation assessment is the one that correlates best with patient outcomes.

Further studies on the influence of genotype on fluctuations in phenylalanine concentration would be useful as this has not been investigated in the studies performed to date. Further studies are also needed to investigate whether short-term fluctuations in phenylalanine concentrations have an impact on IQ or whether long-term fluctuations are more important.

#### 4. Conclusions

Evidence that fluctuations in blood phenylalanine concentrations are associated with less optimal outcome is limited. In contrast, there is substantial evidence that high blood phenylalanine concentrations have detrimental effects on neurocognitive outcomes.

In patients with PKU, blood phenylalanine concentrations vary hourly, day by day, weekly, and over months and years. Such fluctuations may be due to various factors, including dietary phenylalanine intake, intake of protein substitutes, changes in growth rate, and illness. In healthy subjects, day-to-day fluctuation in blood phenylalanine concentrations does not normally exceed 50%, but in patients with PKU, day-to-day fluctuation can be much higher. Increases in phenylalanine intake may result in increased blood phenylalanine concentrations and these are followed by increases in brain phenylalanine concentrations, which are longer lasting than those observed in the blood. Some studies have shown that greater fluctuations in blood phenylalanine concentration in patients with PKU are associated with lower neurocognitive outcome.

Treatment of PKU includes diet restriction for all patients and/or administration of BH<sub>4</sub> or sapropterin to BH<sub>4</sub>-responsive patients. Diet therapy is effective in controlling blood phenylalanine concentrations but it is socially restrictive. Some evidence suggests that intake of protein substitute at regularly spaced intervals over 24 h can reduce fluctuations in blood phenylalanine concentrations, and presumably brain phenylalanine concentrations.

Similarly, sapropterin produces significant and sustained reductions in blood phenylalanine concentrations in patients with PKU responsive to BH<sub>4</sub> and appears to reduce fluctuations in responders. Similar to reduction of mean phenylalanine concentration, reduction of fluctuations in blood phenylalanine may also be desirable for improving outcomes in patients with PKU. Further studies are needed to try to separate the effects of raised phenylalanine concentrations and increased fluctuations of phenylalanine concentrations.

#### Conflict of interest

MC has participated in a strategic advisory board for Merck Serono SA Geneva, Switzerland<sup>2</sup>.

FT has served as a member on Merck Serono SA Geneva, Switzerland<sup>2</sup> advisory boards or similar committees, has current or recent participation in a clinical trial sponsored by Merck Serono SA Geneva, Switzerland<sup>2</sup> has assisted in the design of and/or participated in clinical studies using products manufactured by Merck Serono SA Geneva, Switzerland<sup>2</sup> and has received consulting fees or other remuneration including speaker fees from Merck Serono SA Geneva, Switzerland<sup>2</sup>.

ACM has participated in strategic advisory boards and has received honoraria as a consultant and as a speaker from Merck Serono SA Geneva, Switzerland<sup>2</sup>.

FF has served as a member on Merck Serono SA Geneva, Switzerland<sup>2</sup> advisory boards or similar committees, has current or recent participation in a clinical trial sponsored by Merck Serono SA Geneva, Switzerland<sup>2</sup> has assisted in the design of and/or participated in clinical studies using products manufactured by Merck Serono SA Geneva, Switzerland<sup>2</sup> and has received consulting fees or other remuneration including speaker fees from Merck Serono SA Geneva, Switzerland<sup>2</sup>.

FjvS has participated in strategic advisory boards and received grants and fees for presentations from Merck Serono SA Geneva, Switzerland<sup>2</sup> and Nutricia.

AB has served as a member on Merck Serono SA Geneva, Switzerland<sup>2</sup> advisory boards or similar committees, has current or recent participation in a clinical trial sponsored by Merck Serono SA Geneva, Switzerland<sup>2</sup> has assisted in the design of and/or participated in clinical studies using products manufactured by Merck Serono SA Geneva, Switzerland<sup>2</sup> and has received consulting fees or other remuneration including speaker fees from Merck Serono SA Geneva, Switzerland<sup>2</sup>.

AB-Q has participated in strategic advisory boards and received grants and fees for presentations from Merck Serono SA Geneva, Switzerland<sup>2</sup> and Nutricia.

MG has participated in strategic advisory boards for Merck Serono SA Geneva, Switzerland<sup>2</sup> and Nutricia, and has received honoraria as a speaker from Merck Serono SA Geneva, Switzerland<sup>2</sup> and Nutricia.

CG and EB were employees of Merck Serono SA Geneva, Switzerland<sup>2</sup> at the time of the study. Currently, CG is an employee of Abbott Product Operations, Allschwil, Switzerland.

NB has received research grants from Merck Serono SA Geneva, Switzerland<sup>2</sup> and BioMarin Pharmaceutical Inc., Novato, CA, USA.

AM has received compensation from Merck Serono SA Geneva, Switzerland<sup>2</sup> as a member of the European Nutritionist Expert Panel in Phenylketonuria and as a member of the Scientific Advisory Board on Phenylketonuria. She has received honoraria for consulting or lecturing from SHS International, Nutricia, and Merck Serono SA Geneva, Switzerland<sup>2</sup>. She has received research grant funding from VitaFlo International, Nutricia, and SHS International.

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