Efficacy of sapropterin dihydrochloride (tetrahydrobiopterin, 6R-BH4) for reduction of phenylalanine concentration in patients with phenylketonuria: a phase III randomised placebo-controlled study

Harvey L Levy, Andrzej Milanowski, Anupam Chakrapani, Maureen Cleary, Philip Lee, Friedrich K Trefz, Chester B Whitley, François Feillet, Annette S Feigenbaum, Judith D Bebchuk, Heidi Christ-Schmidt, Alex Dorenbaum, for the Sapropterin Research Group*

Summary

Background Early and strict dietary management of phenylketonuria is the only option to prevent mental retardation. We aimed to test the efficacy of sapropterin, a synthetic form of tetrahydrobiopterin (BH4), for reduction of blood phenylalanine concentration.

Methods We enrolled 89 patients with phenylketonuria in a Phase III, multicentre, randomised, double-blind, placebo-controlled trial. We randomly assigned 42 patients to receive oral doses of sapropterin (10 mg/kg) and 47 patients to receive placebo, once daily for 6 weeks. The primary endpoint was mean change from baseline in concentration of phenylalanine in blood after 6 weeks. Analysis was on an intention-to-treat basis. The study is registered with ClinicalTrials.gov, number NCT00104247.

Findings 88 of 89 enrolled patients received at least one dose of study drug, and 87 attended the week 6 visit. Mean age was 20 (SD 9·7) years. At baseline, mean concentration of phenylalanine in blood was 843 (300) μmol/L in patients assigned to receive sapropterin, and 888 (323) μmol/L in controls. After 6 weeks of treatment, patients given sapropterin had a decrease in mean blood phenylalanine of 236 (257) μmol/L, compared with a 3 (240) μmol/L increase in the placebo group (p<0·0001). After 6 weeks, 18/41 (44%) patients (95% CI 28–60) in the sapropterin group and 4/47 (9%) controls (95% CI 2–20) had a reduction in blood phenylalanine concentration of 30% or greater from baseline. Blood phenylalanine concentrations fell by about 200 μmol/L after 1 week in the sapropterin group and this reduction persisted for the remaining 5 weeks of the study (p<0·0001). 11/47 (23%) patients in the sapropterin group and 8/41 (20%) in the placebo group experienced adverse events that might have been drug-related (p=0·80). Upper respiratory tract infections were the most common disorder.

Interpretation In some patients with phenylketonuria who are responsive to BH4, sapropterin treatment to reduce blood phenylalanine could be used as an adjunct to a restrictive low-phenylalanine diet, and might even replace the diet in some instances.

Introduction

One of the most compelling and repeated stories in medical genetics is the effective prevention of mental retardation from phenylketonuria by newborn screening and early dietary treatment to reduce the concentration of phenylalanine in blood.[1,2] Mutations in the gene that encodes phenylalanine hydroxylase (PAH) prevent hepatic conversion of phenylalanine to tyrosine, which leads to hyperphenylalaninaemia. The toxic agent in phenylketonuria is probably the increased concentration of phenylalanine.[3]

Although early dietary treatment for phenylketonuria is effective,[4,5] the diet has negative aspects such as its demanding schedule, unpleasant taste and odour, nutritional deficiencies, and a substantial psychosocial burden[3] and associated reduction in quality of life.[6] Most importantly, the life-long adherence that is essential to avoid neurological damage[7–9] is difficult in practice: by late adolescence and adulthood, at least 75% of patients with phenylketonuria are essentially non-compliant, with

a resulting increase in blood phenylalanine.[4] Non-compliance after successful treatment in early childhood can result in below-average IQ scores,[10] behavioural problems,[4] and severe emotional dysfunction, including attention deficit disorders,[11] depression, anxiety, and agoraphobia.[12,13]

One novel approach to treatment is to enhance the activity of residual PAH by treatment with pharmacological amounts of its cofactor, tetrahydrobiopterin (BH4), or its biologically active synthetic form 6R-BH4 (sapropterin dihydrochloride, known as sapropterin). Since 1999, studies have shown that a subset of patients with phenylketonuria respond to treatment with BH4[14–16] and that this effect is caused by increased oxidation of phenylalanine.[14] This responsiveness to BH4 is probably associated with mutations in the *PAH* gene that encode a variant form of the enzyme with some residual activity; however, the genotype–phenotype correlation is weak.[17] In some patients with phenylketonuria, 6R-BH4 treatment has been shown to increase the tolerance for phenyl-

Children's Hospital Boston and Harvard Medical School, Boston, MA, USA (H L Levy MD); **Instytutu Matki i Dziecka, Warsaw, Poland** (A Milanowski MD); **Birmingham Children's Hospital, Birmingham, UK** (A Chakrapani MD); **Great Ormond Street Hospital, London, UK** (M Cleary MD); **National Hospital for Neurology and Neurosurgery, London, UK** (P Lee MD); **Klinik für Kinder und Jugendmedizin Reutlingen, Reutlingen, Germany** (F K Trefz MD); **University of Minnesota, Minneapolis, MN, USA** (C B Whitley MD); **Hôpital d'Enfants—CHU Brabois, Vandoeuvre-lès-Nancy, France** (F Feillet MD); **Hospital for Sick Children, Toronto, ON, Canada** (A S Feigenbaum FRCPC); **Statistics Collaborative, Washington, DC, USA** (J D Bebchuk ScD, H Christ-Schmidt MSE); **and BioMarin Pharmaceutical, Novato, CA, USA** (A Dorenbaum MD)

Correspondence to:
Dr Harvey Levy, Division of Genetics, Children's Hospital Boston, 1 Autumn St, Boston, MA, USA
harvey.levy@childrens.harvard.edu

alanine sufficiently to allow a less restrictive diet[18] or even, in some cases, discontinuation of the diet.[19,20]

We aimed to assess the efficacy of sapropterin compared with placebo, for reduction of phenylalanine in blood in patients with phenylketonuria. Our secondary objective was to assess the safety of sapropterin compared with placebo.

Methods

Participants

We enrolled patients between March, 2005, and February, 2006, at 16 centres in North America and 14 centres in six countries in Europe. The protocol (PKU-003) was developed in collaboration with a Phenylketonuria Advisory Committee and approved at each centre by its institutional review board, research ethics board, or ethics committee. Each patient, or a parent or guardian for those aged younger than 18 years, gave written informed consent for participation in the study.

Figure 1 shows that we screened 89 patients with phenylketonuria who had relaxed or abandoned a strict low-phenylalanine diet and who had participated in a previous phase-I screening study (Protocol PKU-001).[21] Eligibility criteria included responsiveness in PKU-001, which was defined as a reduction of 30% or more in blood phenylalanine concentration after 8 days of treatment with sapropterin at a dose of 10 mg/kg per day. We started to screen patients 6 weeks before randomisation. Eligibility criteria for our study were blood phenylalanine of 600 μmol/L or greater (or ≥450 μmol/L after a protocol amendment) at the screening visit, age of 8 years or older, and willingness and ability to comply with study procedures and to adhere to their current diet. Women with childbearing potential had to have a negative urine pregnancy test to be eligible,

and sexually active men and women had to adopt acceptable birth control measures to prevent pregnancy.

Procedures

We took two baseline measurements of blood phenylalanine concentration at 1 and 2 weeks before randomisation. At week 0, we randomised all eligible patients in a 1:1 ratio. We used an interactive voice-response telephone system to maintain blinding. Patients were stratified by study centre and by blood phenylalanine at screening visit (<600 μmol/L and ≥600 μmol/L). Patients in the treatment group were allocated to receive 10 mg/kg sapropterin, and controls were given a placebo (which was similar in taste and appearance), orally once daily for 6 weeks.

We obtained randomisation lists from Statistics Collaborative (Washington, DC, USA); they were generated by a computer program that was verified for accuracy with strict quality-control procedures. Each randomisation list started with a block of two, followed by blocks of four, which ensured that the first two assignments in each stratum were to both the sapropterin and placebo groups, and that subsequent assignments in each stratum consisted of two sapropterin and two placebo assignments in random order.

Block size was not divulged to the study sponsor or to the study investigators until the study was completed. Investigators, patients, and sponsors were kept unaware of the treatment allocation until the database was locked.

Sapropterin and placebo tablets were dissolved in 120–240 mL of water, apple juice, or orange juice. Patients were instructed to continue their usual diet without modification. The concentration of blood phenylalanine was measured 2·5–5 h after breakfast at the screening assessment, each of the two baseline assessments, and treatment weeks 0, 1, 2, 4, and 6. We also measured the phenylalanine concentration in blood at the final visit of any patient who withdrew from the study early. Blood had been collected for assessment of *PAH* genotype during the screening study (PKU-001).

A central laboratory measured the concentration of phenylalanine in blood with the aminoacid analyser method. Centres and patients were forbidden to obtain such concentrations independently. Neither investigators nor patients were aware of postbaseline blood phenylalanine concentrations during the study. We also recorded medical histories, monitored adverse events and vital signs, and did physical examinations, thyroid function tests (thyroxine [T4] and thyroid-stimulating hormone [TSH]), and clinical laboratory tests (including chemistry, haematology, and urinalysis).

The primary endpoint, to measure efficacy of sapropterin compared with placebo, was the change in blood phenylalanine concentration from baseline to week 6. Secondary endpoints were changes in phenylalanine concentrations in blood at each of the 6 weeks of treatment, and the proportion of patients who had blood
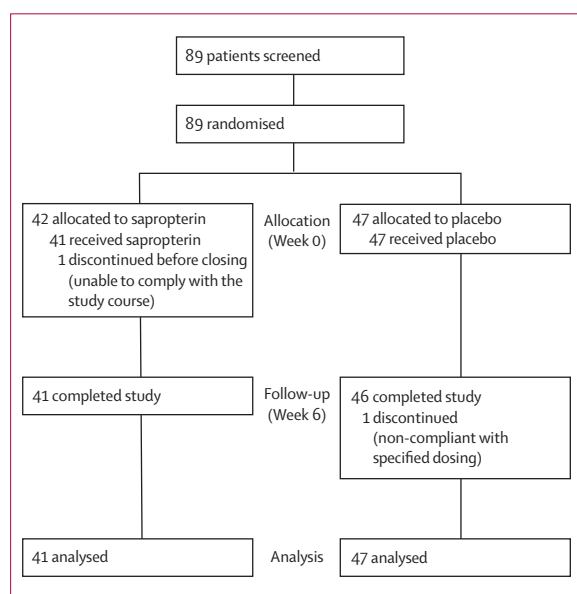

Figure 1: Trial profile

	Sapropterin treatment group (n=41)	Placebo group (n=47)	Total (n=88)
Sex			
Male	27 (66%)	24 (51%)	51 (58%)
Female	14 (34%)	23 (49%)	37 (42%)
Age (years)	21·5 (9·5)	19·5 (9·8)	20·4 (9·7)
Age range (IQR)	8–42 (15–29)	8–49 (13–23)	8–49 (14–25)
≥8 and ≤12 years	6 (15)	11 (23)	17 (19)
>12 years	35 (85)	36 (77)	71 (81)
Race			
White	39 (95%)	47 (100%)	86 (98%)
Non-white	2 (5%)	0	2 (2%)
Blood phenylalanine at screening			
Phenylalanine <600 µmol/L	7 (17%)	9 (19%)	16 (18%)
Phenylalanine ≥600 µmol/L	34 (83%)	38 (81%)	72 (82%)

Data are n (%) or mean (SD). For categorical variables, percentages include all patients with available data. For continuous variables, all patients with available data are included.
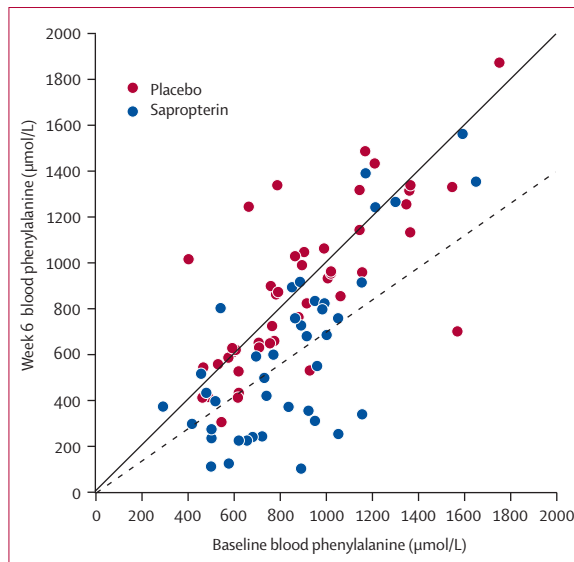
Table 1: **Baseline characteristics**

Figure 2: **Blood phenylalanine concentrations at baseline and week 6**
Black line shows no change from baseline; dotted line shows 30% reduction.

phenylalanine of less than 600 µmol/L at week 6. To assess safety, we also compared the frequencies of all adverse events and serious adverse events in the sapropterin and placebo groups. Adverse events were classified according to the Medical Dictionary for Regulatory Activities.[22]

Statistical analysis
We analysed all randomised patients who received at least one dose of study drug. Based on the results of a previous pilot study,[23] we assumed a difference between treatment groups in mean change at week 6 of 150 (SD 85) µmol/L, and a two-sided type I error rate of 0·05. Therefore, we calculated that a sample size of

80 patients (40 in each group) would provide over 95% power to detect a significant difference between treatment groups at week 6.

To assess change in blood phenylalanine concentration from baseline to week 6, we defined baseline blood phenylalanine as the average of two baseline assessments (1 and 2 weeks before randomisation) and the week 0 assessment. If the week 6 assessment of blood phenylalanine was missing, we used a last observation carried forward method to impute the data. We compared the mean change in blood phenylalanine concentration at week 6 with an analysis of covariance model, using change in blood phenylalanine from baseline to week 6 as the outcome variable, and treatment group as a categorical covariate.

We compared the mean change in blood phenylalanine concentration at week 6 by analysis of covariance, with change in blood phenylalanine from baseline to week 6 as the outcome variable, treatment group as a categorical covariate, and baseline blood phenylalanine concentration as a continuous covariate. We used Fisher's exact test to compare the proportion of patients whose blood phenylalanine concentration was less than 600 µmol/L at week 6. We also did a post-hoc analysis of the proportion of patients with a blood phenylalanine of less than 360 µmol/L at week 6. We analysed data with SAS statistical software (version 9.1.3). The study is registered with ClinicalTrials.gov, number NCT00104247.

Role of the funding source
The study protocol was drafted and developed by the study sponsor, BioMarin Pharmaceuticals, in collaboration with Merck Serono. The study sponsor collected and analysed the data. All authors had full access to all data and statistical analyses in this study and participated in writing the manuscript. The corresponding author had final responsibility for the decision to submit for publication.

Results
42 patients were randomly assigned to receive sapropterin, and 47 to placebo (figure 1). Sex was the only baseline demographic for which the treatment groups differed (table 1). Treatment compliance was high; 72/88 (82%) patients took all doses of the study drug correctly throughout the 6 weeks of the study. Numbers of missed doses or dosing errors were similar in the two treatment groups. Dietary compliance was also high; only 7/41 (17%) patients in the sapropterin group and 12/47 (26%) controls reported that their diet contained an increased or decreased intake of phenylalanine. None of these deviations from protocol were regarded as serious.

Concentrations of blood phenylalanine for each patient during the 6 weeks of the study are shown in figure 2. Figure 3 shows that mean blood phenylalanine concentration at baseline was 842·7 (SD 299·6) µmol/L for the

sapropterin group and 888·3 (323·1) µmol/L for controls. At 6 weeks, 18/41 (44%) patients given sapropterin (95% CI 28–60) and 4/47 (9%) controls (95% CI 2–20) had a blood phenylalanine reduction of 30% or more from baseline (p=0·0002). Blood phenylalanine concentration was reduced by 50% or more in 13/41 (32%) patients who received sapropterin (95% CI 18–48) and 1/47 (2%) controls (95% CI 0–11).

Figure 4 shows that mean blood phenylalanine in controls fluctuated only slightly from baseline over the 6 weeks of the study. By contrast, in the sapropterin group, the mean fell from 842·7 (299·6) µmol/L at baseline to 619·9 (354·7) µmol/L at week 1 and remained at this lower concentration for the duration of the study. At week 6, the mean change in blood phenylalanine from

baseline for the sapropterin group was −235·9 (257·0) µmol/L, compared with 2·9 (239·5) µmol/L for controls (p<0·0001). By week 6, the mean blood phenylalanine concentration was 606·9 (377·0) µmol/L in the sapropterin group. The estimated difference between treatment groups in mean change in concentration of blood phenylalanine from baseline at week 6 was −245 (52·5) µmol/L (p=0·0002).

Table 2 shows analyses of the efficacy of sapropterin at week 6. 7/41 (17%) patients assigned to sapropterin had blood phenylalanine of less than 600 µmol/L at screening; this proportion had increased to 22/41 (54%, 95% CI 38–69) after 6 weeks (p=0·004). In controls, this proportion was 9/47 (19%) at screening and 11/47 (23%, 95% CI 11–36) at week 6. 15/34 (44%) of patients assigned
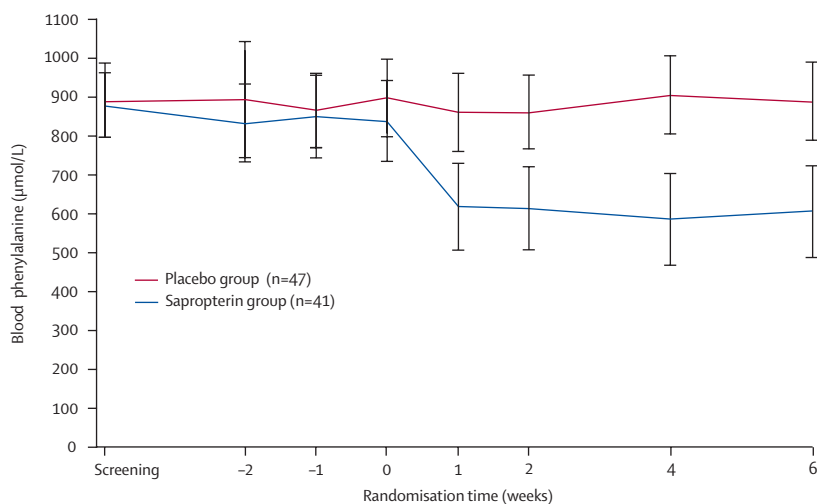

Figure 3: **Mean blood phenylalanine concentration over time**
Bars indicate 95% CI. Last observation carried forward method used to impute missing data. At week 2, n=40 for the sapropterin group.
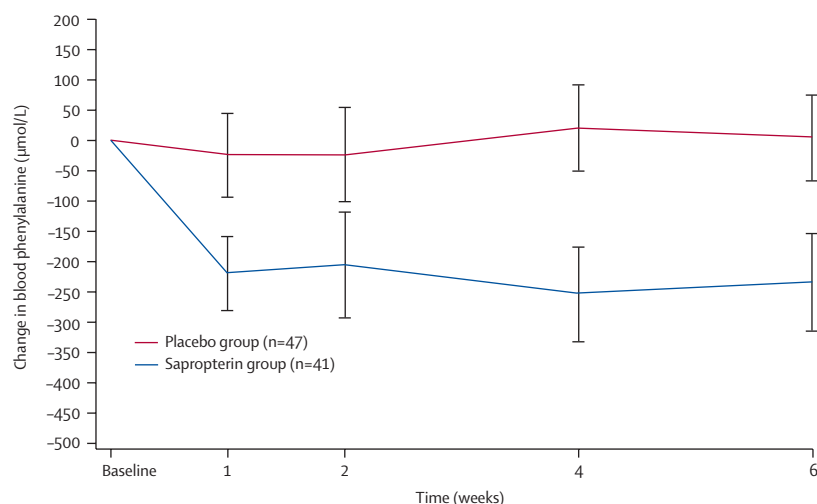

Figure 4: **Mean change in blood phenylalanine concentration over time**
Bars indicate 95% CI. Baseline data is average of duplicate measurements. At week 2, n=40 for the sapropterin group.

	Mean difference between treatment groups in phenylalanine level (µmol/L) at week 6	95% CI	p
Primary endpoint*	–245	–350 to –141	<0·0001
Secondary endpoint†	–230	–317 to –144	<0·0001

*ANCOVA model, with change from baseline as the response variable and both treatment group and baseline blood phenylalanine level as covariates. Missing values were imputed using the last-observation-carried-forward method †Longitudinal model with weekly blood phenylalanine measurements as the response variable and treatment group, visit, and baseline blood phenylalanine level as covariates.

Table 2: Analysis of efficacy of sapropterin at week 6

	Placebo group (n=47)		Sapropterin group (n=41)		Total (n=88)	
	Patients	Events	Patients	Events	Patients	Events
Any adverse events on or after first dose	34 (72%)	95	21 (51%)	53	55 (63%)	148
Adverse events that occurred in 5% or more of patients						
Upper respiratory tract infection	13 (28%)	13	7 (17%)	7	20 (23%)	20
Headache	7 (15%)	10	4 (10%)	5	11 (13%)	15
Vomiting	4 (9%)	4	2 (5%)	2	6 (7%)	6
Abdominal pain	4 (9%)	4	1 (2%)	1	5 (6%)	5
Diarrhoea	3 (6%)	3	2 (5%)	2	5 (6%)	5
Pyrexia	2 (4%)	2	2 (5%)	2	4 (5%)	4
Back pain	3 (6%)	3	1 (2%)	1	4 (5%)	4

Only adverse events with onset on or after first dose are summarised here. *A patient was counted at most once for a given adverse event. †Several events were counted if patients had the same adverse events with different onset dates or times.

Table 3: Adverse effects

to sapropterin and 4/38 (11%) of controls had blood phenylalanine of 600 µmol/L or more at screening, and of less than 600 µmol/L at week 6 (p=0·003). 13/41 (32%) in the sapropterin group and 1/47 (2%) controls had blood phenylalanine concentrations of less than 360 µmol/L at week 6 (p<0·001).

11/47 (23%) patients in the sapropterin group and 8/41 (20%) patients in the control group experienced adverse events that might have been drug-related (p=0·80). No patient withdrew from the study because of adverse events. Most adverse events were deemed to be unrelated to the study drugs. The most commonly reported adverse event was upper respiratory tract infection. Nervous system disorders (eg, headache) were more frequent in the placebo group than in the sapropterin group (table 3). No serious adverse events were recorded in either group, and no patient died during the study.

No patients in the sapropterin group and two controls had clinically significant changes in liver enzymes (alanine aminotransferase in one and aspartate transaminase in the other). One patient in the sapropterin group had a clinically significant low T4 at week 0 (before sapropterin exposure) and again at week 6. This patient had normal TSH concentration at week 0 and high TSH after 6 weeks.

Full *PAH* genotypes, obtained for the PKU-001 trial, were available for 17 of the 19 patients with a reduction of 30% or more in blood phenylalanine in response to sapropterin. In one patient, only a single mutation was identified and in the other a genotype was not available. Responsiveness to BH4 is thought to require at least one *PAH* mutation that allows for residual enzyme activity (non-null mutation).[17] 16/17 fully genotyped patients had at least one non-null mutation, as did the patient with only one identified mutation. However, responsiveness was not consistently linked with specific mutations; six mutations were associated with both responsiveness and non-responsiveness. Moreover, the two *PAH* mutations identified in a patient who had a 63% reduction in blood phenylalanine after 6 weeks of sapropterin treatment were *IVS10-3*C→T and *G272*X, neither of which would seem to allow for residual PAH activity.

Discussion

This 6-week study showed that sapropterin treatment can reduce the concentration of phenylalanine in blood in some patients with phenylketonuria. Mean blood phenylalanine fell in the sapropterin group at week 1 and remained lower than in patients in the placebo group for the duration of the study. Sapropterin treatment over a 6-week period was safe. Our results suggest that sapropterin treatment might be used as an adjunct to the restrictive low-phenylalanine diet in patients with phenylketonuria, and could even replace the diet in some instances.[14] These findings accord with those of earlier pilot studies that also described substantial reductions in blood phenylalanine in patients with phenylketonuria who were given oral doses of 5–20 mg/kg per day of 6R-BH4, and were able to normalise their diet.[19,20,24–26]

The proportion of patients assigned to receive sapropterin who had decreases in blood phenylalanine concentrations after 6 weeks of at least 30% was larger than that in controls (44% vs 9%). However, almost the same number of patients in each group, 12 given sapropterin and 10 given placebo, had an 11–29% decrease in blood phenylalanine concentration at week 6. Moreover, 7 (17%) patients given sapropterin and 21 (45%) controls had increased phenylalanine in blood. Some variations in blood phenylalanine could possibly have been caused by chance, or by undisclosed changes in dietary phenylalanine. Nevertheless, the much higher proportion of patients who had a blood phenylalanine decrease of at least 30% in the sapropterin group, compared with controls, suggests that the cause was a sapropterin response rather than a dietary difference. All patients with phenylketonuria who were enrolled in our study had prestudy reductions of at least 30% in blood phenylalanine while taking sapropterin. However, not all were necessarily truly responsive to BH4. Therefore, patients in our study for whom sapropterin treatment did not reduce blood phenylalanine might not have been BH4-responsive.

14/17 mutations we identified have been previously linked to a BH4-responsive phenotype;[27] two mutations, *G218V* and *Q226H*, have not been reported in responsive patients. BH4-responsiveness is thought to occur only when at least one *PAH* mutation allows for residual enzyme activity. However, one patient in our study, who had a 63% decrease in concentration of blood phenylalanine while taking sapropterin, had two *PAH* mutations, *IVS10-3*C→T and *G272*X, both of which would seem to be null. Because two intronic mutations have been linked with BH4-responsiveness,[27] the intronic mutation *IVS10-3*C→T could possibly be associated with residual PAH activity, and thus responsiveness. If so, the explanation could lie in alternative splicing, which would result in multiple transcripts, at least one of which might produce enzyme activity.[28]

Despite these genotype–phenotype associations, BH4-responsiveness cannot reliably be predicted from *PAH* genotype,[14,29] and must therefore be determined on the basis of blood phenylalanine in response to a loading dose of BH4. The most commonly used assessment method consists of administration of two 20 mg/kg doses of 6R-BH4, 24 h apart, followed by blood phenylalanine measurements at 0, 4, 8, 12, 24, and 48 h after the first dose.[30] A reduction in blood phenylalanine concentration of 30% or more at any of these timepoints is usually regarded as indicative of responsiveness, although a lesser decrease could also be therapeutically beneficial.

Several short-term dietary-intervention crossover studies in patients with early treated, well controlled phenylketonuria, and with IQs in the normal range, have shown that executive function and higher cognitive function deteriorate when the concentration of phenylalanine in blood exceeds 600 μmol/L and improve when phenylalanine is reduced to 600 μmol/L or below, even above 10 years of age.[31–37] In our study, more than half the patients in the sapropterin group had blood phenylalanine concentrations of 600 μmol/L or greater by week 6. Smith and colleagues showed a 4–10 point reduction in IQ, dependent on age, at 4–10 years of age.[38] Together, these findings emphasise the importance of maintaining low blood phenylalanine concentrations at all times, and support the use of sapropterin to lower blood phenylalanine in BH4-responsive patients with phenylketonuria. Furthermore, lifelong, reliable control of blood phenylalanine could also promote better overall central nervous system development and function, and alleviate the burden of a limited dietary regime.

Study organisation
Sapropterin Research Group: University of British Columbia, Children's and Women's Hospital, Vancouver, BC, Canada (L Clarke); Hospital for Sick Children, Toronto, Canada (A Feigenbaum); Hôpital Jeanne de Flandres, Lille (D Dobbelaere); Hôpital d'Enfants, Vandoeuvre-lès-Nancy, France (F Feillet); Hôpital Necker—Enfants Malades, Paris, France (V Abadie); Otto-Heubner-Centrum für Kinder-und-Jugendmedizin, Berlin, Germany (J Hennermann); Klinik für Kinder und Jugendmedizin, Reutlingen, Germany (F Treftz); The Children's University Hospital, Dublin, Ireland (E Treacy); Azienda Opedaliera San Paolo Clinica, Milan, Italy (M Giovannini); Azienda Ospedaliera Universitaria, Catania, Italy (C Meli); Instytutu Matki i Dziecka, Warsaw, Poland (A Milanowski); Birmingham Children's Hospital, Birmingham, UK (A Chakrapani); Great Ormond Street Hospital, London, UK (M Cleary); National Hospital for Neurology and Neurosurgery, London, UK (P Lee); Royal Manchester Children's Hospital, Manchester, UK (A Morris); Children's Memorial Hospital, Chicago, IL, USA (B Burton); University of California, Los Angeles, CA, USA (E Crombez); Washington University School of Medicine, St Louis, MO, USA (D Grange); Oregon Health and Sciences University, Portland, OR, USA (C Harding); Kaiser Permanente Medical Centre, Oakland, CA, USA (J Baker); Kaiser Point West Medical Offices, Sacramento, CA, USA (M Lipson); Kaiser Santa Teresa, San Jose, CA, USA (J Bergoffen); Children's Hospital and Harvard Medical School, Boston, MA, USA (H Levy); University of Utah, Salt Lake City, UT, USA (N Longo); Children's Hospital, Los Angeles, CA, USA (L Randolph); Yale University School of Medicine, New Haven, CT, USA (M Seashore); University of Texas Southwestern Medical Centre, Dallas, TX, USA (L Waber); Mount Sinai School of Medicine, New York, NY, USA (M Wasserstein); University of Wisconsin, Madison, WI, USA (J Wolff).
Data and Safety Monitoring Board: University of Washington, Seattle, WA, USA (S Emerson); Stanford University, Stanford, CA, USA (G M Enns); University of California at San Francisco, San Francisco, CA, USA (M Heyman); University of California, San Diego, San Diego, CA, USA (J Schneider); BioMarin Pharmaceuticals, Novato, CA, USA (non-voting member—A Dorenbaum).

References
1   Scriver CR, Clow CL. Phenylketonuria: epitome of human biochemical genetics (second of two parts). *N Engl J Med* 1980; **303:** 1394–400.
2   Scriver CR, Clow CL. Phenylketonuria: epitome of human biochemical genetics (first of two parts). *N Engl J Med* 1980; **303:** 1336–42.
3   Donlon J, Levy HL, Scriver CR. Hyperphenylalaninemia: phenylalanine hydroxylase deficiency. Chapter 77. In Scriver CR, Beaudet AL, Sly SW, et al, eds.The metabolic and molecular bases of inherited disease. New York: McGraw-Hill, 2004.
4   Koch R, Burton B, Hoganson G, et al. Phenylketonuria in adulthood: a collaborative study. *J Inherit Metab Dis* 2002; **25:** 333–46.
5   Walter JH, White FJ. Blood phenylalanine control in adolescents with phenylketonuria. *Int J Adolesc Med Health* 2004; **16:** 41–45.
6   Landolt MA, Nuoffer JM, Steinmann B, Superti-Furga A. Quality of life and psychologic adjustment in children and adolescents with early treated phenylketonuria can be normal. *J Pediatr* 2002; **140:** 516–21.
7   National Institutes of Health Consensus Development Panel. National Institutes of Health Consensus Development Conference Statement: phenylketonuria: screening and management, October 16–18, 2000. *Pediatrics* 2001; **108:** 972–82.

8   Medical Research Council Working Party on Phenylketonuria. Phenylketonuria due to phenylalanine hydroxylase deficiency: an unfolding story. *BMJ* 1993; **306:** 115–19.

9   Burgard P, Bremer HJ, Buhrdel P, et al. Rationale for the German recommendations for phenylalanine level control in phenylketonuria 1997. *Eur J Pediatr* 1999; **158:** 46–54.

10   Koch R, Azen C, Friedman EG, Williamson ML. Paired comparisons between early treated PKU children and their matched sibling controls on intelligence and school achievement test results at eight years of age. *J Inherit Metab Dis* 1984; **7:** 86–90.

11   Antshel KM, Waisbren SE. Timing is everything: executive functions in children exposed to elevated levels of phenylalanine. *Neuropsychology* 2003; **17:** 458–68.

12   Waisbren SE, Levy HL. Agoraphobia in phenylketonuria. *J Inherit Metab Dis* 1991; **14:** 755–64.

13   Levy HL, Waisbren SE. PKU in adolescents: rationale and psychosocial factors in diet continuation. *Acta Paediatr Suppl* 1994; **407:** 92–97.

14   Muntau AC, Roschinger W, Habich M, et al. Tetrahydrobiopterin as an alternative treatment for mild phenylketonuria. *N Engl J Med* 2002; **347:** 2122–32.

15   Fiori L, Fiege B, Riva E, Giovannini M. Incidence of BH4-responsiveness in phenylalanine-hydroxylase-deficient Italian patients. *Mol Genet Metab* 2005; **86** (Suppl 1)**:** S67–74.

16   Mitchell JJ, Wilcken B, Alexander I, et al. Tetrahydrobiopterin-responsive phenylketonuria: the New South Wales experience. *Mol Genet Metab* 2005; **86** (Suppl 1)**:** S81–85.

17   Blau N, Erlandsen H. The metabolic and molecular bases of tetrahydrobiopterin-responsive phenylalanine hydroxylase deficiency. *Mol Genet Metab* 2004; **82:** 101–11.

18   Hennermann JB, Buhrer C, Blau N, Vetter B, Monch E. Long-term treatment with tetrahydrobiopterin increases phenylalanine tolerance in children with severe phenotype of phenylketonuria. *Mol Genet Metab* 2005; **86** (suppl 1)**:** S86–90.

19   Lambruschini N, Perez-Duenas B, Vilaseca MA, et al. Clinical and nutritional evaluation of phenylketonuric patients on tetrahydrobiopterin monotherapy. *Mol Genet Metab* 2005; **86** (suppl 1)**:** S54–60.

20   Trefz FK, Scheible D, Frauendienst-Egger G, Korall H, Blau N. Long-term treatment of patients with mild and classical phenylketonuria by tetrahydrobiopterin. *Mol Genet Metab* 2005; **86** (suppl 1)**:** S75–80.

21   Burton BK, Grange DK, Milanowski A, et al. The response of patients with phenylketonuria and elevated serum phenylalanine to treatment with oral sapropterin dihydrochloride: a phase II, multicenter, open-label, screening study. *J Inherit Metab Dis* (in press).

22   IFPMA. Medical Dictionary for Regulatory Activities. Geneva, Switzerland: International Federation of Pharmaceutical Manufacturers and Associations, 2007. http://www.meddramsso.com/MSSOWeb/index.htm (accessed March 2, 2007).

23   Matalon R, Michals-Matalon K, et al. Response of patients with phenylketonuria in the US to tetrahydrobiopterin. *Mol Genet Metab* 2005; **86** (suppl 1)**:** S17–21.

24   Shintaku H, Kure S, Ohura T, et al. Long-term treatment and diagnosis of tetrahydrobiopterin-responsive hyperphenylalaninemia with a mutant phenylalanine hydroxylase gene. *Pediatr Res* 2004; **55:** 425–30.

25   Steinfeld R, Kohlschutter A, Ullrich K, Lukacs Z. Efficiency of long-term tetrahydrobiopterin monotherapy in phenylketonuria. *J Inherit Metab Dis* 2004; **27:** 449–53.

26   Cerone R, Schiaffino MC, Fantasia AR, Perfumo M, Birk Moller L, Blau N. Long-term follow-up of a patient with mild tetrahydrobiopterin-responsive phenylketonuria. *Mol Genet Metab* 2004; **81:** 137–39.

27   Blau N. BIOPKU: International Database of Patients and Mutations causing BH4-responsive HPA/PKU. http://www.bh4.org/BH4DatabasesBiopku.asp (accessed Oct 12, 2006).

28   Jonsson JJ, Aronovich EL, Braun SE, Whitley CB. Molecular diagnosis of mucopolysaccharidosis type II (Hunter syndrome) by automated sequencing and computer-assisted interpretation: toward mutation mapping of the iduronate-2-sulfatase gene. *Am J Hum Genet* 1995; **56:** 597–607.

29   Leuzzi V, Carducci C, Carducci C, et al. The spectrum of phenylalanine variations under tetrahydrobiopterin load in subjects affected by phenylalanine hydroxylase deficiency. *J Inherit Metab Dis* 2006; **29:** 38-46.

30   Fiege B, Bonafe L, Ballhausen D, et al. Extended tetrahydrobiopterin loading test in the diagnosis of cofactor-responsive phenylketonuria: a pilot study. *Mol Genet Metab* 2005; **86 (**suppl 1)**:** S91–95.

31   Griffiths P, Ward N, Harvie A, Cockburn F. Neuropsychological outcome of experimental manipulation of phenylalanine intake in treated phenylketonuria. *J Inherit Metab Dis* 1998; **21:** 29–38.

32   Griffiths P, Campbell R, Robinson P. Executive function in treated phenylketonuria as measured by the one-back and two-back versions of the continuous performance test. *J Inherit Metab Dis* 1998; **21:** 125–35.

33   Huijbregts SC, de Sonneville LM, Licht R, van Spronsen FJ, Sergeant JA. Short-term dietary interventions in children and adolescents with treated phenylketonuria: effects on neuropsychological outcome of a well-controlled population. *J Inherit Metab Dis* 2002; **25:** 419–30.

34   Krause W, Halminski M, McDonald L, et al. Biochemical and neuropsychological effects of elevated plasma phenylalanine in patients with treated phenylketonuria. A model for the study of phenylalanine and brain function in man. *J Clin Invest* 1985; **75:** 40–48.

35   Pietz J, Schmidt E, Matthis P, Kobialka B, Kutscha A, de Sonneville L. EEGs in phenylketonuria. I: Follow-up to adulthood; II: Short-term diet-related changes in EEGs and cognitive function. *Dev Med Child Neurol* 1993; **35:** 54–64.

36   Schmidt E, Rupp A, Burgard P, Pietz J, Weglage J, de Sonneville L. Sustained attention in adult phenylketonuria: the influence of the concurrent phenylalanine-blood-level. *J Clin Exp Neuropsychol* 1994; **16:** 681–88.

37   Schmidt H, Burgard P, Pietz J, Rupp A. Intelligence and professional career in young adults treated early for phenylketonuria. *Eur J Pediatr* 1996; **155** (suppl 1)**:** S97–100.

38   Waisbren SE, Leibowitz H, Fahrbach K, Noel K, Dorenbaum A, Levy H. Phenylketonuria blood levels and clinical outcomes in phenylketonuria: a systematic review and meta-analysis. 56th Annual Meeting of the American Society of Human Genetics (ASHG). New Orleans, LA, USA, 2006.