

Brief Communication

## Long-term follow-up of a patient with mild tetrahydrobiopterin-responsive phenylketonuria

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### Abstract

We report on the long-term follow-up of the first Italian patient with the tetrahydrobiopterin (BH<sub>4</sub>)-responsive type of phenylalanine hydroxylase deficiency (R243X/Y414C genotype). The patient was diagnosed by the newborn screening for phenylketonuria (PKU) and with a positive BH<sub>4</sub> loading test. Introduction of BH<sub>4</sub> (initially 10 and later 20 mg/kg/day) in addition to reduced low-phenylalanine diet resulted in therapeutic plasma phenylalanine concentrations (<340 μmol/L). Very good compliance and no side effects in this patient demonstrate the great potential of BH<sub>4</sub> in the treatment of some patients with mild PKU.

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### Introduction

Since the first report of a BH<sub>4</sub>-responsive type of phenylalanine hydroxylase (PAH) deficiency [1], an increasing number of new cases has been reported (for review see Spaapen and Rubio-Gonzalbo [2]). It has been further documented that almost 70% of all patients with mild hyperphenylalaninemia (HPA) and mild PKU respond to BH<sub>4</sub> by lowering plasma phenylalanine levels 8 h after administration [3]. Although a number of patients with mild HPA and PKU are now on BH<sub>4</sub> treatment worldwide, reports on long-term follow-up are scarce. Here, we report the first Italian patient with BH<sub>4</sub>-responsive PAH deficiency (mild PKU) and discuss the therapeutic efficacy of BH<sub>4</sub> supplementation in this patient during the last two years.

### Case report and methods

A girl, born December 2000, after normal pregnancy and delivery was found in the newborn screening pro-

gram with blood phenylalanine levels of 696 μmol/L and at 13 days of age with 1005 μmol/L. BH<sub>4</sub> loading (20 mg/kg body weight) resulted in a decrease of blood phenylalanine to 725 and 528 μmol/L, 4 and 8 h post loading, respectively. Pterin analysis in urine and dihydropteridine reductase activity in red blood cells were both normal (for methodology see Blau et al. [4]). The BH<sub>4</sub> used was from Schircks Laboratories (Jona, Switzerland).

Mutation analysis was performed using denaturing gradient gel electrophoresis (DGGE) and direct sequencing of exons 7 and 12.

### Results and discussion

Fig. 1 shows plasma phenylalanine concentrations in relation to different treatment protocols. From 14 days of age, the patient was put on a low-phenylalanine diet (160 mg/day). Because of rather poor compliance phenylalanine concentrations ranged between 350 and 520 μmol/L. While still on low-phenylalanine diet, supplementation with 10 mg/kg body weight of BH<sub>4</sub> per day, divided into two doses, was started. Under this treatment phenylalanine levels persisted between 380 and 470 μmol/L. Subsequently, the BH<sub>4</sub> dose was increased to 20 mg/kg

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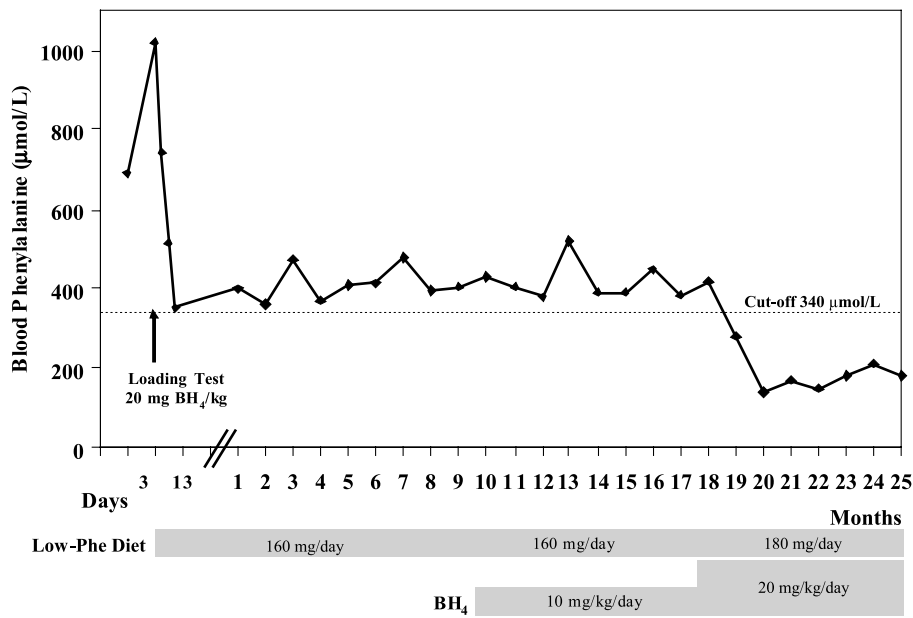


Fig. 1. Plasma phenylalanine concentrations in a patient with BH<sub>4</sub>-responsive PKU under different therapy protocols.

body weight per day, resulting in phenylalanine concentrations mostly below 300 µmol/L. At the same time, the low-phenylalanine diet was adjusted to 180 mg/day. In contrast to some patients who benefit from the BH<sub>4</sub> alone, our patient need a combination of BH<sub>4</sub> and a low-phenylalanine diet. To achieve optimal BH<sub>4</sub> dosage, each single patient need to be titrated. In some patients BH<sub>4</sub> dosage can be reduced from the initial 10 mg/kg and in some need to be increased. The preliminary pharmacokinetic data show the elimination half-life time for BH<sub>4</sub> between 3.3 and 5.1 h [5]. Thus, for the optimal activation of phenylalanine hydroxylase, BH<sub>4</sub> should be given in at least two doses.

DNA analysis of the *PAH* gene revealed two mutations: R243X (727C>T) and Y414C (1241A>G). The Y414C mutation is the most frequent one found in patients with BH<sub>4</sub>-responsive HPA/PKU. According to the database of BH<sub>4</sub>-responsive HPA/PKU (BIOPKU; www.bh4.org) this mutation found in 19 out of 77 alleles is potentially associated with BH<sub>4</sub>-responsiveness. Y414C has been reported as inconsistently associated with BH<sub>4</sub>-responsiveness [6]; however, this was probably due to the use of a different BH<sub>4</sub> product in the past [7]. When expressed recombinantly in the eukaryotic cell system the Y414C mutant protein expresses 28% of the wild-type activity.

Between 1984 and 2002 53 HPA patients were investigated at the G. Gaslini Institute. In all of them BH<sub>4</sub> deficiency was excluded, but four out of the 53 patients showed a significant decrease of phenylalanine levels 8 h after BH<sub>4</sub> loading (20 mg/kg). However, one should consider that some older patient, tested before October 1999, used an old formulation of BH<sub>4</sub> containing about

33% of inactive 6S-BH<sub>4</sub>. These patients may have respond to the new fully active BH<sub>4</sub> product (6R-BH<sub>4</sub>) and thus, the actual number of BH<sub>4</sub>-responsive patients may be higher. In three of these four patients without protein restriction or BH<sub>4</sub> supplementation phenylalanine concentrations never exceeded 300 µmol/L; the patients are developing normally. In one patient, the genotype is A403V/W187X, while in the other two the DNA genotyping is still in progress.

Our data confirm the therapeutic potential of BH<sub>4</sub> in some patients with mild BH<sub>4</sub>-responsive PKU; however, further long-term studies are necessary to evaluate the optimal dosage of BH<sub>4</sub> in this group of patients.

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