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Phenylalanine and tyrosine metabolism in DNAJC12 deficiency: A comparison between inherited hyperphenylalaninemias and healthy subjects

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ABSTRACT

DNAJC12 deficiency is a new cause of inherited hyperphenylalaninemia (HPA), besides phenylalanine hydroxylase (PAH) deficiency and tetrahydrobiopterin (BH4) deficiencies.

Differently from other inherited HPAs, no quantitative data on peripheral phenylalanine (Phe) and tyrosine (Tyr) metabolism are currently available in DNAJC12 deficiency.

Phe and Tyr metabolism in a patient with DNAJC12 after a simple Phe oral loading test (100 mg/kg) and a combined Phe (100 mg/kg) + BH4 (20 mg/kg) loading test is presented and compared to patients with disorders of BH4 metabolism, PAH deficiency, and healthy controls.

Phe and Tyr metabolism in DNAJC12 deficiency is similar to non-PKU HPA. Differently from BH4 deficiency, BH4 administration in DNAJC12 deficiency does not firmly enhance the rate of Phe hydroxylation. A central effect of BH4 treatment in DNAJC12 deficiency cannot be excluded.

1. Introduction

Inherited co-chaperone DNAJC12 deficiency was recently recognized as a new cause of primary hyperphenylalaninemia (HPA), besides phenylalanine hydroxylase (PAH) deficiency and disorders of tetrahydrobiopterin (BH4) metabolism [1,2]. DNAJC12 deficiency has peculiar clinical and biochemical features, including movement disorders and reduced neurotransmitters metabolites in the cerebrospinal fluid similar to BH4 deficiency, but normal BH4 metabolism, like PAH deficiency. After positive neonatal screening for HPA, DNAJC12 can be biochemically distinguished from BH4 deficiencies, as showing normal urinary pterin pattern and dihydropteridine reductase (DHPR) activity, whereas molecular testing is required for its distinction from PAH deficiency [3].

The response to standardized oral loading tests with phenylalanine (Phe), followed or not by BH4 administration, was described in various forms of BH4 deficiency and PAH deficiency. Under these experimental conditions, disorders of synthesis and regeneration of BH4 and different classes of PAH deficiency show characteristic metabolic patterns, as peripheral course of phenylalanine (Phe) and tyrosine (Tyr) concentration after loading allows an *in vivo* quantitative evaluation of individual Phe hydroxylation rate [4–9]. Such a standardized dynamic metabolic characterization is not currently available in DNAJC12 deficiency. Here we provide a comparative analysis of peripheral blood Phe and Tyr metabolism after a simple Phe loading test and a combined Phe + BH4 loading test in primary HPAs, including DNAJC12, PAH and BH4 deficiency. For a comprehensive comparison, moreover, Phe hydroxylation in healthy subjects was also evaluated by the same procedures.

2. Patients and methods

The metabolic outcomes after a simple Phe loading test (100 mg/kg) and a combined Phe (100 mg/kg) and BH4 (20 mg/kg, 3 h after Phe loading) loading test were evaluated in 16 patients with different forms of primary HPA (DNAJC12 deficiency, 6-pyruvoyl tetrahydropterin synthase (PTPS) deficiency, dihydropteridine reductase (DHPR) deficiency, and phenylalanine hydroxylase deficiency). Patients' characteristics are shown in Table 1. A simple Phe loading test was also performed in two healthy subjects. All the Phe loadings were performed at normal or near-normal blood Phe concentrations and a normocaloric non-protein diet was administered during the tests to avoid additional Phe and Tyr intake, as previously described [8]. Duration of the tests

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Table 1
Clinical, biochemical, and molecular characteristics patients with primary hyperphenylalaninemia (HPA) undergoing a simple phenylalanine (Phe, 100 mg/kg) and a combined Phe (100 mg/kg) plus tetrahydrobiopterin (BH4, 20 mg/kg) loading tests.

Patient	Disease	Genotype	Age at onset	Symptoms at onset	Blood Phe at onset (μmol/l)	Urine pterin pattern (mmol/mol crea)[a]	CSF HVA (nmol/l)**	CSF 5-HIAA (nmol/l)**	Treatment	Outcome
1	DNAJC12 deficiency	V27Wfs[a]14/ V27Wfs[a]14	32 years	Parkinsonism	449	N=0.4; B=0.9	37	7	BH4, l-dopa, 5-OH-trp	Anxiety, insomnia
2	PTPS deficiency	T76 M/D136V	Neonatal	Microcephaly, hypotonia	480	N=10.7; B: 0.04	31	6	BH4, l-dopa, 5-OH-trp, selegiline, entacapone	Mild dyskinesia
3	DHPR deficiency	G23D/Y150C	Neonatal	Hypotonia	600	N=1.7; B=1.1[b]	34	6.4	BH4, l-dopa, 5-OH-trp, selegiline, entacapone	Anxiety
4	DHPR deficiency	L14P/L14P	Neonatal	Hypotonia	520	N=1.9; B=1.4[b]	40	8.2	Low-Phe diet, l-dopa, 5-OH-trp, selegiline, entacapone, pramipexole	Minimal dyskinesia
5	Classic PKU	IVS10-11G > A/ R158Q	Neonatal	None	1000	N=10.9; B=5.9	NA	NA	Low-Phe diet	No symptoms
6	Classic PKU	IVS4+1G > C/ R252W	Neonatal	None	840	N=6.6; B=4.4	NA	NA	Low-Phe diet	No symptoms
7	Classic PKU	R158Q/ P211Hfs[a]130	Neonatal	None	1080	N=7.9; B=5.4	NA	NA	Low-Phe diet	No symptoms
8	Mild PKU	L48S/S67P	Neonatal	None	300	N=2.1; B=1.2	NA	NA	Low-Phe diet	No symptoms
9	Mild PKU	Y343C/L48S	Neonatal	None	520	N=3.9; B=2.5	NA	NA	Low-Phe diet	No symptoms
10	Mild PKU	IVS4+5G > T/ V388 M	Neonatal	None	340	N=2.0; B=1.2	NA	NA	Low-Phe diet	No symptoms
11	Mild PKU	I174V/R261Q	Neonatal	None	370	N=3.0; B=4.7	NA	NA	Low-Phe diet	No symptoms
12	Non-PKU HPA	R158Q/ A403V	Neonatal	None	252	N=1.6; B=1.0	NA	NA	None	No symptoms
13	Non-PKU HPA	R261Q/ E390G	Neonatal	None	330	N=3.3; B=2.2	NA	NA	None	No symptoms
14	Non-PKU HPA	R408Q/A300S	Neonatal	None	246	N=1.8; B=0.6	NA	NA	None	No symptoms
15	Non-PKU HPA	I174V/E178G	Neonatal	None	228	N=3.2; B=1.7	NA	NA	None	No symptoms
16	Non-PKU HPA	IVS10-11G > A/ A300S	Neonatal	None	237	N=4.0; B=2.4	NA	NA	None	No symptoms

PTPS (6-pyruvoyl tetrahydropterin synthase); DHPR (dihydropteridine reductase); PKU (phenylketonuria); CSF (cerebrospinal fluid); HVA (homovanillic acid); 5-HIAA (5-hydroxyindoleacetic acid); N (neopterin); B (Biopterin); l-dopa (levodopa); 5-OH-Trp (5-hydroxytryptophan).
[a] Normal range urinary pterin: N: 0.3–4 mmol/mol crea; B: 0.5–3 mmol/mol crea. **Normal range CSF metabolites: HVA: 115-455 nmol/l; 5-HIAA: 51-204 nmol/l.
[b] DHPR activity on dried blood spot: <0.01 nM reduced ferricytochrome C/minute/5 mm disk.

lasted 24 h after Phe administration.

The DNACJ12 patient was diagnosed at 32 years with early-onset tremulous non-progressive Parkinsonism. Cognitive assessment revealed mild intellectual disability (intelligent quotient 71). Basal blood Phe concentration was 449 μmol/l (normal value 37-115 μmol/l). Cerebrospinal fluid pterin pattern was normal (neopterin: 19 nM, normal value 10-31 nM; 7,8 dihydrobiopterin: 2 nM, normal value < 18 nM) whereas concentrations of homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) were low (HVA: 37 nM, normal value 115-455 nM; 5-HIAA: 7 nM, normal value 51-204 nM). Treatment with l-dopa was effective in controlling tremors but drug-induced dyskinesias corrected by fractionation of l-dopa dose (300 mg/day in 3 administrations). A short prolactin (PRL) profile revealed morning pre-treatment mild hyperprolactinemia (45.6 ng/ml, normal range 4.8–23.3 ng/ml) followed by PRL normalization 3 h after l-dopa administration (15.6 ng/ml) and PRL increase 6 h later (33.8 ng/ml), consistent with intermittent dopaminergic stimulation [10]. Informed consent was obtained from all subjects. The study was conducted according to the World Medical Association Declaration of Helsinki regarding ethical conduct of research involving human subjects.

3. Results

The differential outcomes of the simple Phe and combined Phe + BH4 loading tests in healthy controls and inherited HPAs are depicted in Fig. 1. The healthy response to an oral Phe load is characterized by a rapid enhancement of Phe hydroxylation (not preventing transient HPA peaking 30 min after Phe administration), reflected by immediate Tyr rise after Phe loading. In patients with disorders of synthesis and regeneration of BH4 (PTPS and DHPR deficiency, respectively), cofactor administration differentially enhanced Phe hydroxylation. In patients with PTPS deficiency, BH4 administration rapidly boosted Phe hydroxylation, allowing full correction of HPA within 4 h and concomitant Tyr rise. In patients with DHPR deficiency, the BH4-related enhancement of Phe hydroxylation allowed near-complete Phe clearance 9 h after loading.

In patients with PAH deficiency (classic PKU, mild PKU, and non-PKU HPA), BH4 administration did not alter Phe and Tyr metabolism. Twenty four hours AFTER loadings, classic PKU showed sustained HPA and no Tyr increase, non-PKU HPA showed ~80% reduction of blood Phe and concomitant Tyr rise, and mild PKU showed intermediate results.

In the patient with DNAJC12 deficiency, the outcome of the simple Phe loading showed progressive blood Phe decrease until near-normal concentration reached 24 h after Phe administration. A similar metabolic behavior was observed after the combined Phe + BH4 loading test, showing slightly increased Phe clearance 3-9 h after BH4 administration but overlapping Phe concentration 24 h after Phe loading, with virtually unchanged Tyr metabolism.

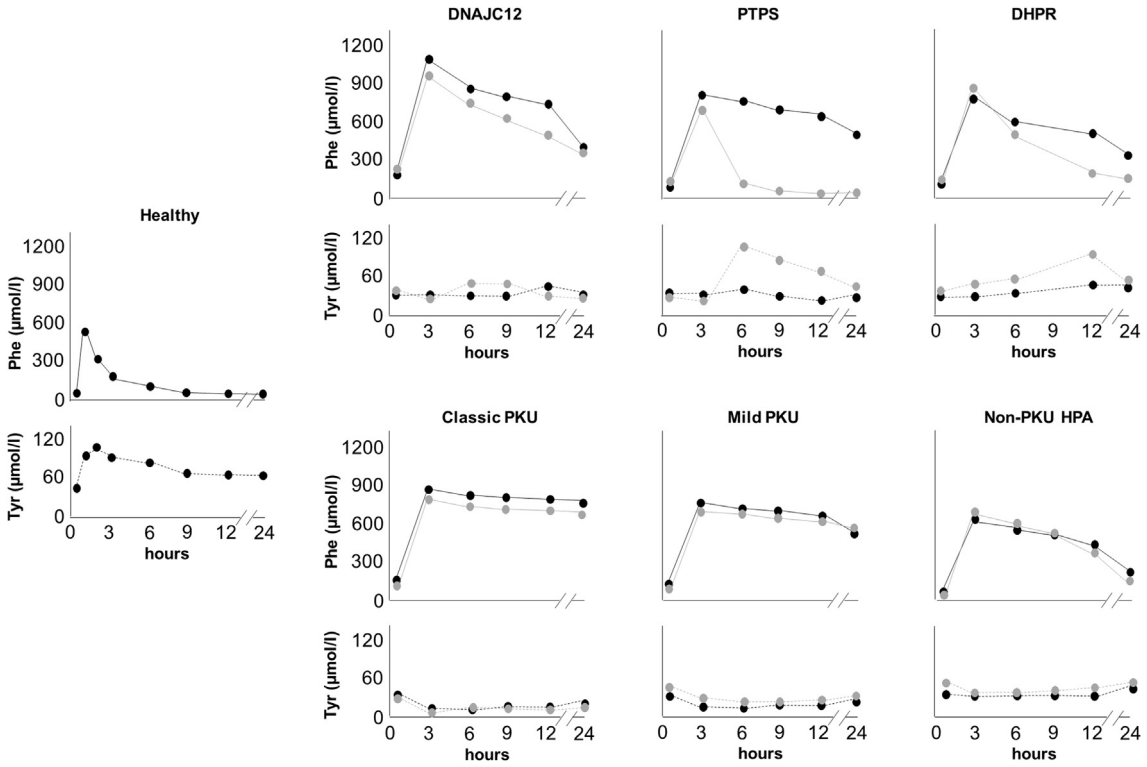

Fig. 1. Mean phenylalanine (Phe, continuous lines) and tyrosine (Tyr, dotted lines) concentrations after a simple Phe oral loading test (100 mg/kg, black lines) or a combined Phe plus tetrahydrobiopterin (BH4) oral loading test (Phe 100 mg/kg + BH4 20 mg/kg, gray lines) in different forms of inherited hyperphenylalaninemia (HPA) and in healthy controls.

4. Discussion

DNAJC12 is a newly identified cause of inherited HPA, besides PAH deficiency and BH4 deficiencies. DNAJC12 is a member of the J-proteins family, also known as heat shock proteins 40 (HSP40s), co-chaperoning with the HSP70s and controlling folding, degradation, and translocation of their protein clients [11,12]. In particular, DNAJC12 interacts with the human hydroxylases of aromatic amino acids, namely PAH, Tyr hydroxylase, and tryptophan hydroxylase, having a role in the processing of misfolded PAH by the ubiquitin-dependent proteasome [13]. An impairment of proper folding of aromatic amino acid hydroxylases has been hypothesized as a main pathogenetic mechanism in DNAJC12 deficiency, consistent its peculiar biochemical picture characterized at peripheral level by HPA and by dopamine and serotonin deficiency at central level, in the absence of BH4 deficiency. Recently, DNAJC12 deficient cell clones were generated, ensuring the addition of new insights on the pathophysiology of the disease [14]. To date, 29 DNAJC12-deficient patients were reported with variable clinical characteristics (Table 2). Their compound phenotype is highly heterogeneous, including different degree of HPA, juvenile Parkinsonism, dystonia, autism, intellectual disability, attention deficit hyperactivity disorder, psychiatric symptoms, or no symptoms [1–3,15–19]. Treatment was variable, with patients receiving both BH4 and neurotransmitter replacement therapy with or without Phe-restricted diet [1,18], only BH4 [16,19], only neurotransmitters [2], or no drugs [17]. No experience is available with the use of the l-dopa sparing therapies commonly employed in BH4 deficiencies [20–22].

Differently from other inherited HPAs, a peripheral metabolic characterization of DNACJ12 deficiency through standardized oral loading tests was not available. Such an experimental approach was historically designed to distinguish different forms of HPA (i.e. PAH deficiency and BH4 deficiency) and successively suggested as the

Table 2
Main clinical features of 29 patients with DNAJC12 deficiency previously described.

Reference	Number of patients	Age at diagnosis (years)	Parkinsonism	Dystonic features	Autism	Intellectual disability/ Developmental delay	Attention disorder	Psychiatric symptoms	Asymptomatic
			Number of patients						
Anikster et al. (2017) [1]	6	2–20	1	6	1	5	1	0	0
Straniero et al. (2017) [2]	3	13–51	3	0	0	3	0	2	0
Leal et al. (2017) [19]	11	2–38	0	0	0	1	0	0	10
Van Spronsen et al. (2018) [17]	5	2–13	0	3	0	0	1	0	1
De Sain-van der Velden et al. (2018) [16]	1	15	1	0	0	1	0	0	0
Veenma et al. (2018) [15]	2	6–17	0	0	0	2	0	0	0
Feng et al. (2019) [18]	1	1	0	0	0	1	0	0	0
Total	**29**	**1–51**	**5 (17%)**	**9 (31%)**	**1 (3%)**	**13 (45%)<**	**2 (7%)**	**2 (7%)**	**11 (38%)**

ideal approach to investigate BH4-responsiveness in PKU, as potentially more reliable than the commonly recommended simple BH4 loading test [5,23–25]. Actually, standardized loading conditions (normal basal Phe level, defined doses of Phe and BH4, scheduled sampling collection, and non-protein diet during the test) allow quantitative evaluations in single patients or metabolic comparisons within small cohorts of patients with rare disorders, as in the present study. Moreover, this approach can be useful for the reliable assessment of new treatments [8,9,26]. Here, we described Phe and Tyr metabolism in a patient with DNAJC12 deficiency after both a simple Phe and a combined Phe + BH4 loading test and compared the metabolic outcomes with other inherited HPAs and healthy controls. The outcome of the loading tests in DNAJC12 deficiency was similar to non-PKU HPA, being unsuitable for the biochemical differential diagnosis of these disorders. This finding is consistent with the normal availability of endogenous BH4 characterizing both conditions. Actually, in presence of normal DHPR activity, BH4 functions catalytically in the Phe hydroxylating system so that the administration of exogenous cofactor is not required for the correct electron transferring to molecular oxygen and hydroxylation [7]. The essential role of normal DHPR activity in the Phe hydroxylating system is clearly demonstrated by the outcome of the loading tests in patients with PTPS deficiency. In this condition, in which synthesis of BH4 is impaired but recycling through DHPR is normal, the administration of exogenous BH4 (even at very low dose) ensure a catalytic activity of the cofactor, with virtually physiological Phe hydroxylation [5,6,27]. On the contrary, in patients with DHPR deficiency, BH4 is not recycled and can function only stoichiometrically (i.e the amount of tyrosine formed cannot exceed the amount of BH4 present), as evidenced by the higher cofactor dose to partially restore Phe hydroxylation [4,7]. The stoichiometric activity of BH4 also applies to an *in vitro* model of Phe hydroxylation, lacking in DHPR activity [28]. Obviously, in this model, only the adjunct of BH4 to the system ensures Phe hydroxylation either by wild type PAH or mutant PAH with residual activity, as BH4 is consumed during the coupled conversion of Phe into Tyr and BH4 to carbinolamine. Although obtained in a small cohort, our quantitative data on peripheral Phe and Tyr metabolism integrate the current knowledge on the Phe hydroxylation system *in vivo*, also allowing a functional comparison among different inherited HPAs. In particular, our experimental data in DNAJC12 deficiency suggest an incomplete peripheral effect of the cofactor. A potential central effect of BH4 on neurotransmitter synthesis in DNAJC12 was not evaluated but cannot be excluded. Further evaluations in larger cohorts of DNAJC12 patients are needed to investigate this issue.

In conclusion, the metabolic evaluation of DNAJC12 showed high natural Phe tolerance and hydroxylation. Differently from BH4 deficiency, the peripheral effect of exogenous BH4 in DNAJC12 deficiency is not striking and the dynamic metabolic characterization virtually overlaps non-PKU HPA.

Declaration of competing interest

None.

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