



## Case Report

## DNAJC12 deficiency: Mild hyperphenylalaninemia and neurological impairment in two siblings

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## ABSTRACT

**Background:** DNAJC12 co-chaperone protein deficiency has been recently described as a stand-alone metabolic disorder explaining many cases of mild hyperphenylalaninemia (HPA) that are not caused by variants in the *PAH* gene, which encodes for the hepatic enzyme phenylalanine hydroxylase (PAH), or in *GCH1*, *PTS*, *QDPR*, *PCBD1* and *DHPR*, involved in tetrahydrobiopterin (BH4) biosynthesis and activity.

**Results:** We describe two sisters born to consanguineous parents. The youngest sister (Patient 1), initially asymptomatic, tested positive at NewBorn Screening (NBS) for mild HPA. After variants in the *PAH* and BH4 related-genes were excluded, we performed *DNAJC12* genetic analysis and found a previously described homozygous deletion [NM\_021800.3: c.58\_59del p.(Gly20Metfs\*2)]. The older sister (Patient 2), homozygous for the same variant and exhibiting mild HPA, was diagnosed subsequently and presented with ataxia and repeated falls, upper limb dyskinesia, intentional tremor, and mild intellectual disability. Patient 1 was started on treatment with low Phenylalanine (Phe) diet, BH4, 1-3,4-dihydroxyphenylalanine/carbidopa (L-DOPA) and 5-OH-Tryptophan, soon after diagnosis, and despite poor adherence to the dietary regimen, only manifested language impairment at last follow-up (age 5 years and 4 months). Patient 2, who started the same treatment at school age, experienced a minimal progression of neurological symptoms, with some improvement in her motor skills.

**Conclusions:** These two new patients with *DNAJC12*-associated HPA, in addition to previous reports, point to *DNAJC12* deficiency as a new metabolic syndrome that must be considered in patients with unexplained HPA.

## 1. Introduction

Hyperphenylalaninemia (HPA) is a neurometabolic condition defined by blood phenylalanine (Phe) concentrations over 120  $\mu\text{mol/L}$ . Approximately 98% of patients who test positive for HPA carry a biallelic loss-of-function variant in the *PAH* gene, which encodes for the hepatic enzyme phenylalanine hydroxylase (PAH) [4]. This enzyme is involved in the conversion of Phe to tyrosine (Tyr), with tetrahydrobiopterin (BH4) cofactor being essential for this reaction.

Variants in the *PAH* gene can cause different HPA phenotypes. Currently, the main classification of *PAH*-related HPAs includes Classic Phenylketonuria (PKU), Moderate PKU, Mild PKU and mild HPA (M. J. [10]).

When untreated, this condition leads to progressive and irreversible neurological symptoms including intellectual disability, motor impairment and behavioural disorders (M. J. [10]).

The remaining 2% of patients with HPA exhibit BH4 deficiencies (M. J. [10]).

Genetic alterations in five genes (*GCH1*, *PTS*, *QDPR*, *PCBD1* and *DHPR*), all encoding for proteins involved in BH4 synthesis, functioning and metabolism, have been associated with BH4 deficiencies.

Recently, biallelic variants in the *DNAJC12* gene (OMIM: 606060, locus 10q21.3) have been described in patients with HPA not caused by *PAH* gene alterations or BH4 deficiency [1]. The *DNAJC12* gene encodes for a protein family named DnaJ, which is cofactor of molecular chaperones in the reactions needed to enable cellular proteins to assume and

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maintain the correct conformation required for their function. Molecular chaperones, also known as Heat Shock 70 kDa Proteins (Hsp70s) as they can be upregulated by heat, exert their activity by producing a stable binding based on ATP hydrolysis in their ATPase domain. Such reaction produces a conformational modification in Hsp70s, allowing them to bind to the client protein in specific sites, resulting in correct protein folding, transport, activity control and degradation.

Since Hsp70s' ATPase activity alone is insufficient, a cofactor is required. DnaJ proteins are a family of cofactor proteins encoded by approximately 41 genes, united by the same J-domain, through which they can interact with the Hsp70s' ATPase domain and increase its activity over the minimal threshold of effectiveness [2].

After the first 6 patients were described by [1], 45 patients with *DNAJC12* biallelic variants were subsequently reported in association not only with HPA, progressive neurodevelopmental delay and dystonia [6,7,12], but also to milder neurological phenotypes (including autistic features and hyperactivity) [9,13] and early onset Parkinsonism [8] outlining a broader phenotypical spectrum.

All the variants reported in the literature are biallelic, with predominance of point mutations. HPA was detected in almost all cases described, but only in 22/51 (43%) cases was diagnosed at NewBorn Screening (NBS). In most cases where lumbar puncture (LP) was performed, Homovanillic Acid (HA) and 5-Hydroxyindoleacetic Acid (5-HIAA) were below the reference range [5]. 13/51 (25%) patients were treated with Phe diet restriction. Most of the reported patients received supplemental therapy with oral Sapropterin, while only 13/51 (25%) received oral l-3,4-dihydroxyphenylalanine/carbidopa (L-DOPA) and 9/51 (18%) 5-OH-tryptophan. A clinical response was observed in all patients treated with at least two concomitant interventions (including dietary treatment), and clinical improvement was observed regardless of the time of treatment initiation [5].

Here we present two previously unreported siblings with HPA caused by *DNAJC12* homozygous deletion, one diagnosed after NBS without clinical manifestation, and the other diagnosed at a later stage with global developmental delay and movement disorder. In both patients, targeted treatment was effective in either preventing symptom onset or improving clinical symptoms.

### 1.1. Subject and methods

We describe two sisters born to consanguineous parents evaluated at the Metabolic and Neuromuscular Unit, Meyer Children's Hospital IRCCS, Florence, Italy. Patient 1 was evaluated in her fifth day after delivery, due to positive NBS for HPA. Patient 2, the older sister, was evaluated following Patient 1 diagnosis. Subsequent evaluations, laboratory tests, imaging and neurophysiological examinations were carried out at the Meyer Children's Hospital IRCCS, Florence, Italy.

## 2. Results

### 2.1. Patient 1

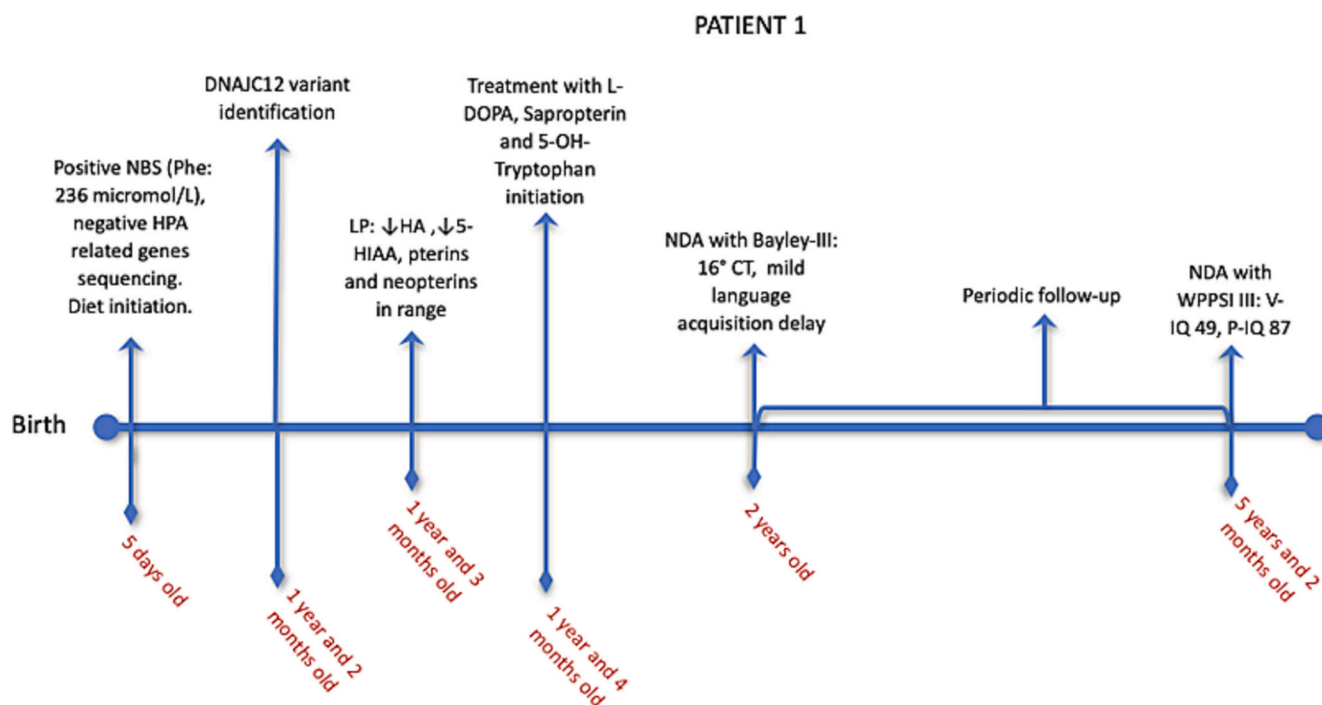
Patient 1, female, was born at term in Italy to consanguineous parents (first cousins) of Pakistani origin. Pregnancy and delivery were uneventful. She came to our attention at 5 days of life as NBS detected HPA (plasma level of Phe 236 micromol/L, reference range < 120 micromol/L), along with a Phe/Tyr ratio of 3. Our initial laboratory assessment confirmed HPA and detected normal dihydropteridine reductase (DHPR) on dried blood spot (DBS) and normal levels of urinary pterins. Since blood Phe levels were above 360 micromol/L, we initiated a specific diet with controlled intake of Phe, as per our centre's protocol. Targeted multiplex ligation-dependent probe amplification (MLPA) and sequencing of the *PAH* gene showed no pathogenic variants. Extended next-generation sequencing (NGS) to the genes associated with HPA and related to neurotransmitter defects (including *DBH*, *DDC*, *GCH1*, *MAOA*, *PAH*, *PCBD1*, *PTS*, *QDPR*, *SLC18A2*, *SLC6A3*, *SPR*, and *TH*

genes) was unrevealing. Considering the laboratory findings, the clinical condition of her older sister (Patient 2, see below) and the recently described *DNAJC12* genetic alterations in patients with similar conditions, we performed *DNAJC12* gene sequencing analysis and found a homozygous deletion NM\_021880.3: c.58\_59del p.(Gly20Metfs\*2). The same deletion was detected in both *DNAJC12* alleles of Patient 2, while both parents were heterozygous carriers. We subsequently performed cerebrospinal fluid (CSF) neurotransmitters assay and detected low levels of HA (275 nmol/L, reference range 364–870) and 5-HIAA (19 nmol/L reference range 155–359). CSF pterins and neopterin levels were normal. Consequently, we started therapy with L-DOPA and 5-OH-Tryptophan at the initial dosage of 2.5 mg/Kg/die (later increased up to 6 mg/Kg/die), and Sapropterin at the dosage of 10 mg/Kg/die [1,3]. Phe intake restriction was continued, although with limited compliance to the dietary regimen. We evaluated the response to treatment with periodic clinical and laboratory assessments, including yearly LP to measure CSF HA and 5-HIAA levels and adjust the dosage of supplemental therapy with L-DOPA and 5-OH-Tryptophan accordingly. At the age of 2 years and 6 months, neurodevelopmental assessment with Bayley-III scale revealed mild delay in language acquisition (though likely related to poor socio-cultural status and bilingualism). A further assessment at 5 years and 2 months with the Wechsler Preschool and Primary Scale of Intelligence (WPPSI III) highlighted a relatively more severe impairment in language (Verbal Intelligent Quotient, IQ = 49) when compared to non-verbal skills (Performance IQ = 87). The neurological examination has never shown any motor symptoms nor movement disorders. During follow up, a poor compliance to Phe restriction diet was constantly observed and confirmed by blood Phe levels above 360 micromol/L, despite continuous therapy with Sapropterin and periodic counselling by our specialised dietitians (Fig. 1A).

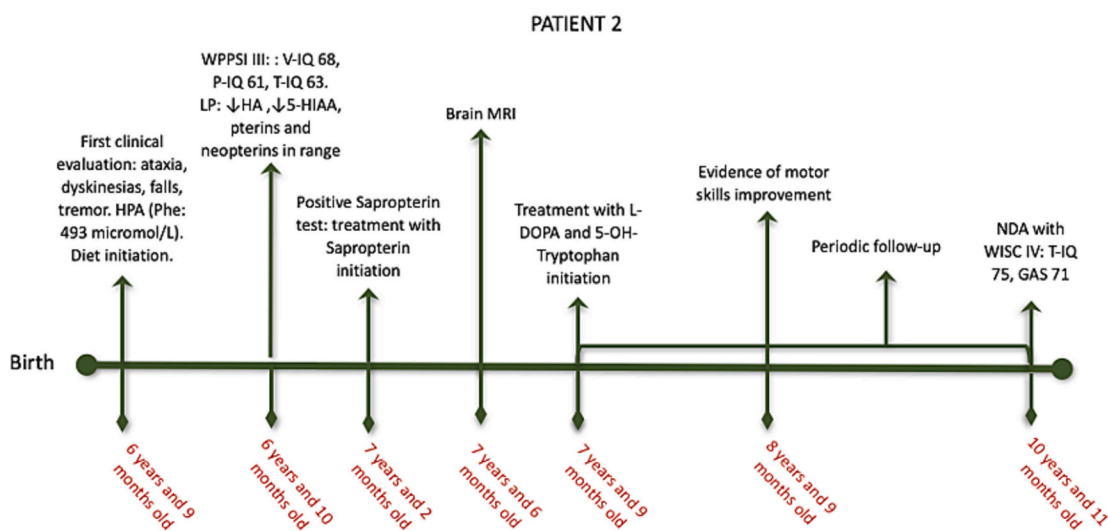
### 2.2. Patient 2

Patient 2 was referred to our service after the HPA diagnosis of her younger sister (Patient 1), at the age of 6 years 9 months. She was born in Pakistan at term by an uncomplicated pregnancy and normal delivery. NBS was not performed in her native country. Her parents reported global developmental delay: she could sit at 12 months, produce her first words at 12 months, walk by 24 months. At the first clinical evaluation, she presented with ataxic gait, dyskinetic movements mostly affecting the upper limbs, and gross motor impairment. Routine blood tests were normal whereas Phe plasma level was increased at 493 micromol/L (reference range <120 μmol/L). A low Phe diet was started, with excellent compliance. Neurodevelopmental assessment with WPPSI-III showed mildly impaired cognitive abilities with Verbal IQ score of 68, Performance IQ score of 61 and Total IQ score of 63. Electroencephalogram (EEG) was normal.

As soon as genetic analysis confirmed the same homozygous *DNAJC12* deletion as in Patient 1, we performed LP and found reduced levels of HA (133 micromol/L reference range 364–870) and 5-HIAA (5 nmol/L, reference range 155–359). A Sapropterin-loading test resulted positive, i.e., administration of 10 mg/Kg of Sapropterin at T0 (baseline) led to decreased Phe plasma levels >30% from baseline in a series of Phe plasma level assays after 4, 8, 12, 24 and 48 hours. Thereafter oral Sapropterin treatment at the dosage of 10 mg/Kg/die was initiated. At the age of 7 years 5 months brain MRI resulted normal, supporting the hypothesis of a neurotransmitters-related movement disorder. We started treatment with oral L-DOPA and Tryptophan at the dosage of 2.5 mg/Kg/die (later increased up to 6 mg/Kg/die). Patient 2 underwent yearly clinical assessment and LP for CSF neurotransmitters measurement, to monitor treatment response. A significant improvement of ataxia, dyskinesias and gross motor function was apparent one year after initiation of oral L-DOPA and tryptophan. The latest cognitive assessment at the age of 10 years and 11 months using the Wechsler Intelligence Scale for Children, IV edition (WISC IV), showed a Total IQ score of 75, and a General Ability Score of 71 (Fig. 1B).



**Fig. 1A.** Timeline illustrating disease course of Patient 1 (NBS= NewBorn Screening; HPA=Hyperphenylalaninemia; LP=Lumbar Puncture; HA=Homovanillic Acid; 5-HIAA=5-Hydroxyindoleacetic Acid; L-DOPA=1-3, 4-dihydroxyphenylalanine/carbidopa; NDA=Neurodevelopmental Assessment; WPPSI III=Wechsler Preschool and Primary Scale of Intelligence, III edition).



**Fig. 1B.** Timeline illustrating disease course of Patient 2 (HPA=Hyperphenylalaninemia; WPPSI III=Wechsler Preschool and Primary Scale of Intelligence; LP=Lumbar Puncture; HA=Homovanillic Acid; 5-HIAA=5-Hydroxyindoleacetic Acid; L-DOPA=1-3, 4-dihydroxyphenylalanine/carbidopa; NDA=Neurodevelopmental Assessment; WISC IV=Wechsler Intelligence Scale for Children, IV edition).

### 3. Discussion

These two new patients with *DNAJC12*-associated HPA and neurological impairment, in addition to previous reports, point to *DNAJC12* deficiency as a new metabolic syndrome that must be considered in patients with unexplained HPA.

The *DNAJC12* variant found in our patients [homozygous deletion NM\_021800.3: c.58\_59del p.(Gly20Metfs\*2)] causes the deletion of two nucleotides and frameshift, introducing a premature stop codon in the mRNA. The same variant was described by Veenma et al. in two siblings born to consanguineous parents of Afghani origin, of whom the younger tested positive for HPA at NBS and was later found to carry the *DNAJC12*

deletion. The second sibling, with NBS reported as negative, was subsequently diagnosed with the same condition [11]. Although it is not possible to exclude a founder effect due to the geographical proximity of the countries of origin of the two pairs of siblings, there are no other reports for this variant in the Human Gene Mutation Database (HGMD), at the moment of writing.

In our report, NBS in Patient 1 was critical to diagnose this condition at an early and asymptomatic stage and to start treatment with Sapropterin, L-Dopa and 5-OH-Tryptophan, and low Phe diet immediately, after detecting the *DNAJC12* gene deletion and before manifest clinical onset. During her follow-up, poor compliance to dietary treatment reflected in the finding of isolated elevated plasma Phe levels regardless

Sapropterin therapy. This is likely explained by the role of BH4 as cofactor in the reaction catalyzed by the phenylalanine hydroxylase (PAH) enzyme. DNAJC12, on the other hand, is involved in the biosynthesis and proper functioning of the enzyme itself, among others. Therefore it seems likely that supplementation with BH4 alone would not be sufficient to ensure proper functioning of the enzyme and to keep plasma Phe levels within neurotoxicity limits. In our case, the finding of elevated Phe values due to poor compliance to dietary treatment did not, however, result in neurological symptoms referable to Phe neurotoxicity or neurotransmitter deficit.

Although Patient 2 was diagnosed at a later stage, when neurological symptoms were already manifest, she still benefited from the same treatment, with improved motor skills and cognitive profile.

#### 4. Conclusions

Based on our experience and according to the recent findings on DnaJ proteins, we recommend early *DNAJC12* gene sequencing analysis for children with unexplained HPA, in order to start targeted treatment before symptoms onset. This diagnosis should also be considered when evaluating patients born in low-income countries, who did not undergo NBS, and presenting with unexplained neurological symptoms such as developmental delay, movement disorder, motor and cognitive impairment.

In our opinion, patients previously diagnosed with HPA by NBS, or laboratory analysis, who did not undergo genetic assay, should be tested for *DNAJC12* variants, as targeted treatment may improve the neurologic manifestations even if started late.

Finally, our report emphasizes that an appropriate dietary regimen combined with targeted supplementation therapy based on genetic evaluation can be effective in both preventing the onset of symptoms and improving the clinical outcome of already symptomatic patients.

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#### Author contributions

EF contributed to review of the literature, study design, and writing of the draft; AB contributed to review of the literature, study design, and writing of the draft; EP contributed to data collection, data analysis, data interpretation and critical revision of the manuscript; GMS contributed to data collection, data analysis, data interpretation and critical revision of the manuscript; SB contributed to data interpretation and critical revision of the manuscript. All authors read and approved the final manuscript.

#### Ethics approval

Approval was granted by the Tuscan Regional ethics committee for clinical trials - Pediatric Section, % Azienda Ospedaliero Universitaria Meyer, Viale Gaetano Pieraccini 24, 50193 Florence, Italy.

#### Consent to participate

Not applicable.

#### Consent to publication

Informed consent was obtained from all the individual participants included in the study.

#### Declaration of Competing Interest

The authors have no relevant financial or non-financial interests to disclose.

#### Data availability

Data will be made available on request.

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