



DNACJ12 deficiency in patients with unexplained hyperphenylalaninemia: two new patients and a novel variant

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Received: 1 February 2021 / Accepted: 10 May 2021 / Published online: 20 May 2021
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Abstract

In addition to tetrahydrobiopterin deficiencies and phenylalanine hydroxylase deficiency (phenylketonuria) due to *PAH* variants, the deficiency of the co-chaperone protein DNAJC12 was identified in 2017 as a novel cause of inherited hyperphenylalaninemia, revealing the genetic etiology in previously unresolved cases. In this study, we aimed to investigate DNAJC12 deficiency in non-tetrahydrobiopterin-deficient persistent hyperphenylalaninemia cases without biallelic *PAH* variants in a single pediatric metabolic center. It was determined retrospectively that 471 patients with non-tetrahydrobiopterin deficiency-hyperphenylalaninemia had undergone *PAH* gene sequencing and 451 patients had biallelic variants in *PAH*. *DNAJC12* sequencing was performed in the remaining 20 patients, identifying a previously reported homozygous splice-site variant (c.158-2A > T) in one patient with axial hypotonia and developmental delay, and a novel, homozygous c.404del (p.Arg135Lysfs*21) frameshift variant in an asymptomatic patient. In segregation analysis, the asymptomatic patient's both parents were also found to be homozygous for this variant and hyperphenylalaninemic. The parents may have had academic difficulties but intellectual disability could not be confirmed due to lack of cooperation. The symptomatic patient significantly benefited from treatment with sapropterin dihydrochloride and neurotransmitter precursors. DNAJC12 deficiency might be responsible for approximately 10% or more of cases with unexplained hyperphenylalaninemia. The phenotypic spectrum is broad, ranging from early infantile hypotonia to incidental diagnosis in adulthood. Similar to tetrahydrobiopterin deficiencies, early diagnosis and treatment with sapropterin dihydrochloride and neurotransmitter precursors can be beneficial, supporting the analysis of *DNACJ12* gene in patients with unexplained hyperphenylalaninemia.

Keywords DNAJC12 · Hyperphenylalaninemia · Neurotransmitter deficiencies · Phenylalanine hydroxylase · Phenylketonuria · Tetrahydrobiopterin

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Introduction

Phenylketonuria (OMIM #261600), caused by deficiency of phenylalanine hydroxylase (PAH) enzyme, is one of the most common inborn errors of metabolism. However, it is not the only primary cause of hyperphenylalaninemia (HPA), as disorders of tetrahydrobiopterin (BH₄) metabolism leading to BH₄ deficiency, which impair the activity of not only PAH, but also the enzymes tyrosine hydroxylase (TH) and tryptophan hydroxylase (TPH) involved in neurotransmitter synthesis, account for approximately 1–2 percent of patients with persistent HPA. Early identification of BH₄ deficiencies in the differential diagnosis of HPA is crucial and standard-of care because early treatment with neurotransmitter precursors is known to drastically improve outcomes (Blau et al. 2011). However, there had long been a cohort of patients with persistent HPA without BH₄ deficiency or any detectable pathogenic *PAH* variants.

In 2017, Anikster et al. were the first to describe a novel type of HPA in six individuals from four families who had HPA and monoamine neurotransmitter deficiencies. Variant analyses of the patients for *PAH* or and all genes implicated in BH₄ metabolism were normal, although PAH enzyme activities were low. Biallelic variants in *DNAJC12* gene (RefSeq NM_021800.3) were detected in all of these patients. *DNAJC12* gene encodes a co-chaperone protein with the same name, which is responsible for the intracellular stabilization, homeostasis and folding process of the BH₄-dependent enzymes PAH, TH and TPH (Anikster et al. 2017; Blau et al. 2018; Bouchereau et al. 2018; Jung-Kc et al. 2019). In this study, we aimed to screen for *DNAJC12* deficiency (OMIM #617384) in our patients with unexplained persistent HPA.

Materials and methods

Patient selection

The study was performed at the pediatric metabolic clinic of a referral university hospital. Patients with persistent hyperphenylalaninemia (blood Phe > 120 μmol/L) who had undergone sequencing of the coding exons and exon–intron junctions of *PAH* gene were screened through hospital records retrospectively. Hyperphenylalaninemic patients with confirmed disorders of BH₄ metabolism, either via abnormal pterin or dihydropteridine reductase (DHPR) levels, or via genetic analysis were excluded. Information regarding the genotypes, maximum Phe levels and the metabolic phenotype was recorded. Patients with biallelic pathogenic or likely pathogenic variants in *PAH* were accepted to have PAH deficiency (Richards et al. 2015). Patients with no

variants or with a single monoallelic variant detected in *PAH* gene sequencing (RefSeq NM_001354304.2) were selected for Sanger sequencing of *DNAJC12* gene (NM_021800.3). Those with biallelic pathogenic or likely pathogenic *DNAJC12* variants underwent further clinical evaluation and family screening as recommended previously (Blau et al. 2018), to the extent detailed below (see Case description in Results).

DNAJC12 sequencing

Genomic DNA was isolated from peripheral blood samples (10 mL) using a standard salting-out method. All *DNAJC12* exonic sequences, including exon–intron junctions were amplified (Table 1). PCR reactions were performed in a total reaction volume of 25 μL (10X PCR buffer, 1.5 mM MgCl₂, 200 μM of each dNTPs, 30 pmol of each primer, and 0.5 U Taq DNA polymerase), using 50 ng of genomic DNA. The PCR products were analyzed on 2% agarose gels stained with ethidium bromide. The PCR products were purified with the MinElute 96 UF PCR purification kit (Qiagen Inc., Valencia, CA, USA) and pathogenic variant analysis was performed by direct sequencing of the purified PCR products using the BigDye Terminator Cycle Sequencing kit (version 3.1) and an ABI 3130 automated DNA sequencer (Applied Biosystems, CA, USA). The results were evaluated based on a reference sequence (GenBank NM_021800.3) of the *DNAJC12* gene using Sequencing Analysis Software v5.2 Patch 2 (Applied Biosystems, CA, USA).

Results

Selection of *DNAJC12* deficiency candidates

471 patients with non-BH₄ deficiency-HPA had undergone sequencing of the coding exons and exon–intron junctions of *PAH* gene. 451 patients (247 female, 54.8%) from 427 families had biallelic variants in *PAH*. 145 (32%) of these

Table 1 *DNAJC12* primer sequences

DNAJC12-1F:	5'-TGGTGTATTGACTGTGGCCA-3'
DNAJC12-1R:	5'-ACTTTCTCTGAGCCAAAAGTGC-3'
DNAJC12-2F:	5'-CTTGCTGTGTTACCCAGGCAG-3'
DNAJC12-2R:	5'-GTTAACACTCAATAGCTGGTG-3'
DNAJC12-3F:	5'-CACATCTGTGTCATCAGCAC-3'
DNAJC12-3R:	5'-GTAGTCACAGGCTCTTCTGG-3'
DNAJC12-4F:	5'-CCCTCCATTCATTCATCCATCTG-3'
DNAJC12-4R:	5'-AGACACTGAGAATTGTCTCAAGG-3'
DNAJC12-5F:	5'-ACATTGTACCTGCTGCAGTGA-3'
DNAJC12-5R:	5'-TCAGCAATTCACAGACATGACA-3'

451 patients had classical PKU, 126 (28%) had mild PKU, and 180 (40%) had mild HPA (Hillert et al. 2020). They had 238 distinct genotypes, the most common of which was homozygosity of c.1066-11G>A (IVS10-11G>A) (6.9% of patients), associated with classical PKU. 162 (36%) patients had a single homozygous variant and 289 (64%) were compound heterozygous for two variants. 102 distinct alleles were recorded (71 missense, 17 splice-site, 6 nonsense, 5 frameshift, 2 small deletion, 1 promoter site variant). The most prevalent allele was c.1066-11G>A (133 or 14.7% of all 902 alleles), followed by c.898G>T (p.Ala300Ser, 68 alleles, 7.5%) and c.782G>A (p.Arg261Gln, 61 alleles, 6.8%). Five variants (c.227A>T, c.826del, c.842+6T>C, c.1100del, and c.1255C>A) were not reported in Phenylalanine Hydroxylase Locus Specific Database (PAHvdb) (Blau, Yue and Perez 2021), ClinVar (Landrum et al. 2018) or Human Gene Mutation Database (Stenson et al. 2003), and were identified as novel variants. Maximum Phe levels, PAH deficiency phenotypes and PAH genotypes of these 451 patients are presented in Online Resource 1.

PAH deficiency genotype could not be confirmed in 20 patients (4.2% of all HPA patients), 12 of whom had a single heterozygous monoallelic PAH variant, and eight of whom had no detectable variants. These 20 patients (11 female) from 20 families were selected for DNAJC12 sequencing. Their maximum Phe levels ranged between 204 and 1728 $\mu\text{mol/L}$ (Online Resource 2). Two of them (10% of selected patients, 0.4% of all non-BH4 deficiency-HPA) had homozygous DNAJC12 variants. Family screening of one of these patients revealed both parents to be hyperphenylalaninemic and DNAJC12-deficient as well. Thus, four patients in total were diagnosed with DNAJC12 deficiency.

Case descriptions

Case 1

A 26-day-old male infant was referred to our hospital for suspicion of hyperphenylalaninemia in newborn screening. He was born from a primigravid mother via spontaneous vaginal delivery with a birth weight of 3000 gr and head circumference of 34.5 cm. Parents were not consanguineous, but they were from the same small town, suggesting possible inbreeding. According to the parents, his prenatal and family history was unremarkable. Physical examination of the patient was normal. Blood phenylalanine level was 318 $\mu\text{mol/L}$. He was followed up without treatment with the diagnosis of mild hyperphenylalanemia. Neopterin, biopterin levels in dried blood spots (DBS) and urine, and DHPR activity on DBS were

within the normal range. No pathogenic variants were identified in PAH gene. In follow-up, Phe levels ranged from 224 to 539 $\mu\text{mol/L}$. Upon elevation of Phe levels above 360 $\mu\text{mol/L}$, BH₄ loading test was requested, but not performed because of parental refusal. Phe-restricted diet was started, which maintained Phe levels between 300 and 360 $\mu\text{mol/L}$. DNAJC12 gene sequencing analysis of the patient revealed a novel, homozygous c.404del (p.Arg135Lysfs*21) frameshift variant. As per the guidelines of the American College of Medical Genetics and Genomics (Richards et al. 2015), this variant is interpreted as likely pathogenic, based on the information that it is a null variant in a gene where loss of function is a known mechanism of disease (Anikster et al. 2017), and that it is absent in the chromosomes of 60,146 control individuals in the Genome Aggregation Database (Karczewski et al. 2020). Serum prolactin level was normal. Lumbar puncture for determination of neurotransmitter metabolites and initiation of L-dopa and 5-hydroxytryptophan (5HT) could not be performed due to parental refusal. In his latest follow-up visit at the age of six, the patient's growth, development, physical examination and Denver-II Developmental Screening Test results were normal.

Segregation analysis of the novel DNAJC12 c.404del variant surprisingly revealed that both parents were also homozygous for this variant. Their Phe levels were elevated at 409 and 527 $\mu\text{mol/L}$ in the mother and father, respectively. Although their physical and neurological examination was normal, they had not attended formal education beyond five years of school. We also wanted to perform neuropsychometric evaluation, but they did not comply.

Case 2

A 23-day-old female infant cared for by child protective services was evaluated in our hospital due to elevated blood phenylalanine in newborn screening. Prenatal and family history could not be obtained. Her first physical examination was normal. Blood phenylalanine level was 410 $\mu\text{mol/L}$. Urine neopterin and biopterin levels, and DHPR enzyme activity on DBS were within normal range. A 96-h BH₄-loading test showed 49% reduction in Phe, and sapropterin dihydrochloride (20 mg/kg/day) was started. At the age of 2 months, she was transferred to the care of a foster family, who would later become her adoptive parents. Axial hypotonia and developmental delay was firstly noticed by the adoptive family at the age of 3 months. She started holding her head at the age of 5 months, and sitting without support at 9 months. Delay in gross motor skills was also demonstrated by Denver-II Developmental Screening Test periodically performed at the ages of 9, 13, and 18 months. Personal-social, language, and fine motor skills in Denver-II test were normal. She said her first word at the age of 14 months. She started getting up on her

feet and walking with support at the age of 20 months. Cognitive, language and motor composite scores in Bayley Scales of Infant and Toddler Development at the age of 22 months were 65, 59 and 67, respectively, showing developmental delay in these areas. *PAH* gene sequencing was normal. *DNAJC12* gene sequencing analysis revealed a homozygous splice-site variant (c.158-2A>T), which had been reported in the literature (Anikster et al. 2017). Identities of the DNA samples of the biological parents were not available. Lumbar puncture was performed for quantification of biogenic amines. Levels of cerebrospinal fluid (CSF) homovanillic acid (HVA), L-dopa and 5-hydroxyindolacetic acid (5-HIAA) were 332 nmol/L (normal: 364–870), 11 nmol/L (0–15) and 9 nmol/L (155–359), respectively, suggesting significant insufficiency in serotonin synthesis. 5-methyltetrahydrofolate and pterin levels were within the normal range in CSF. Serum prolactin level was normal. 5HT and L-dopa replacement, combined with peripheral dopa decarboxylase inhibitor benserazide were started. She is now 41 months old and receiving special education. Treatment with L-dopa (6.4 mg/kg/day) with benserazide, 5HT (4.8 mg/kg/day), sapropterin dihydrochloride (20 mg/kg/day) are being continued.

Discussion

A member of the heat shock protein 40 (HSP40) family, *DNAJC12* is a chaperone protein, responsible from the regulation, intracellular stabilization and folding of PAH, TH and TPH. It also inhibits the aggregation of alpha-synuclein protein, which is involved in neurodegenerative diseases (Bouchereau et al. 2018; Jung-Kc et al. 2019). *DNAJC12* deficiency shows autosomal recessive inheritance, and the responsible gene is located in chromosome 10q21.3. 39 patients have so far been reported with biallelic *DNAJC12* variants, aged between 2 and 40 years. Mild attention disorder and hyperactivity, speech delay, infantile encephalopathy, axial hypotonia, hypertonia, dystonia, movement disorders, parkinsonism, oculogyric crisis, psychosis, depression, autism, global developmental delay and neurodegenerative disorders have been reported in these patients (Blau et al. 2018; de Sain-van der Velden et al. 2018; Feng et al. 2019; Gallego et al. 2020; Li et al. 2020; Straniero et al. 2017; van Spronsen et al. 2017; Veenma et al. 2018).

Asymptomatic patients have also been reported. Similar to our Case 1, Gallego et al. from Spain reported that 16 out of 20 patients with *DNAJC12* deficiency showed no neurological symptoms. It was not specified whether these patients were formally investigated for intellectual disability. They reported that only four out of 20 patients had shown clinical symptoms, including psychomotor delay and seizures, autistic symptoms or hyperactivity. They detected four variants (c.524G>A, c.502+1G>C, c.309G>T and c.298-2A>C) in

DNAJC12 gene in their patients, all different from those in our patients (Gallego et al. 2020). In addition, one of the five patients reported by van Spronsen et al. was asymptomatic and had a homozygous variant, c.214C>T (van Spronsen et al. 2017). These 17 asymptomatic patients were aged between 2–38 years (Gallego et al. 2020; van Spronsen et al. 2017). It cannot be reliably determined whether the parents of Case 1 are symptomatic or not, as they have not consented to formal neuropsychometric testing. It could not be specified whether the limited duration of formal education was due to poor academic performance, or to unrelated personal or social issues.

Similar to Case 2, all reported patients were found to be BH₄-responsive, HVA and 5-HIAA levels in CSF were found to be low in all patients in whom the tests were performed, similar to Case 2. Lumbar puncture was not performed in Case 1 because the family did not consent to a lumbar puncture. Serum prolactin levels were elevated in some patients, and normal in others, similar to our two patients (Blau et al. 2018).

Experience with treatment of *DNAJC12* deficiency is limited and there are no consensus treatment guidelines; but neurotransmitter precursors, Phe-restricted diet and sapropterin dihydrochloride seem to be beneficial in most of the reported symptomatic patients (Anikster et al. 2017; de Sain-van der Velden et al. 2018; Feng et al. 2019; Li et al. 2020). Asymptomatic patients are reported to be observed without medical treatment or only with sapropterin dihydrochloride (Gallego et al. 2020; van Spronsen et al. 2017). In our patients, Case 1 is not receiving any medical treatment because he is not showing any symptoms, and neurotransmitter deficiencies could not be confirmed or ruled out. Medical treatment was started in Case 2 due to developmental delay and documented neurotransmitter deficiency. (Blau et al. 2018; Feng et al. 2019; Straniero et al. 2017; Veenma et al. 2018).

It is difficult to establish a genotype–phenotype relationship due to the limited number of cases. Case 1 was asymptomatic despite having a homozygous frameshift variant. Symptomatology could not be documented in his parents due to insufficient cooperation on their part. Currently we do not know if intellectual disabilities develop over time in otherwise asymptomatic cases in *DNAJC12* deficiency, and whether it may be related to neurotransmitter deficiency, the long term effect of untreated HPA, or perhaps to alpha-synuclein aggregation. Case 2 had developmental delay and axial hypotonia, while one of the two siblings with the same homozygous splice-site variant from an Arab family with the same genotype had similar symptoms in early childhood, the other had later-onset cognitive deficits and hypertonia. They showed a favorable response to sapropterin dihydrochloride (Anikster et al. 2017). Since we do not know the parental ethnicities of Case 2, it is not possible to speculate whether she also has Arabic heritage, suggesting a founder effect, or if the c.158-2A>T variant represents a mutational hot-spot.

In a study from an HPA reference center in Spain, Gallego et al. reported that they found biallelic variants in *PAH* in 95% of cases and detected biallelic *DNAJC12* variants in 20 patients out of 50 (40%) who had unexplained hyperphenylalaninemia (Gallego et al. 2020). In our cohort, two (10%) out of 20 patients with non-BH₄-deficiency HPA without biallelic *PAH* variants were found to have biallelic *DNAJC12* variants. However, this may be an underestimation, since *PAH* gene analysis in our cohort has only been performed by Sanger sequencing of the coding exons and exon–intron junctions, which achieves a variant detection rate of 95–99% in *PAH* gene. Variants in deep intronic regions, or regulatory regions such as enhancer sequences are not covered in this analysis. Our data are also limited by the fact that complex rearrangements, large deletions or duplications that are not recognized by standard PCR-based methods can be found in a small proportion (2%) of *PAH* alleles; they may be detected by other methods, including but not limited to next generation sequencing (NGS), multiplex ligation-dependent probe amplification (MLPA) and quantitative real-time PCR (qPCR) (Birk Møller et al. 2007; Hillert et al. 2020; Razipour et al. 2017). Thus, it can be estimated that in our total cohort of 471 patients, utilization of these genetic analysis methods could have diagnosed an additional 9–10 patients ($471 \times 2\% = 9.47$) with *PAH* deficiency, possibly bringing down the number of unexplained HPA patients from 20 to approximately 10–11. In other words, if *PAH* variants are assessed not only with Sanger sequencing, but also with additional methods, *DNAJC12* deficiency could potentially be responsible for 18–20% of unexplained HPA in our retrospective cohort. It is also possible that additional patients with *DNAJC12* deficiency could have been detected, if we had been able to sequence deep intronic and regulatory regions of *DNAJC12* gene, and to perform deletion / duplication analyses.

Patients having developmental delay, movement disorders, parkinsonism, autism, or those without symptoms with high blood phenylalanine levels, in whom BH₄ deficiencies and *PAH* variants are ruled out, should be evaluated for *DNAJC12* variants. Symptomatic patients should be evaluated for neurotransmitter deficiencies in CSF and neurotransmitter replacement should be commenced if necessary, in addition to BH₄ therapy (Blau et al. 2018).

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11011-021-00753-0>.

Authors' contributions Kısmet Çıkrı: Conceptualization, methodology, investigation, writing—original draft; Yılmaz Yıldız: Conceptualization, methodology, investigation, writing, review and editing; Didem Yücel Yılmaz: Genetic analysis, writing, editing; Emine Pektaş: Investigation, review; Ayşegül Tokatlı: Methodology, review and editing, supervision; R. Köksal Özgül: Genetic analysis, editing; H. Serap Sivri: Methodology, review and editing, supervision; Ali Dursun: Methodology, review and editing, supervision. All authors approved the final manuscript as submitted.

Funding No funding was received for conducting this study.

Data availability Study data are available from the corresponding author upon reasonable request.

Declarations

Ethics approval Approval was obtained from the ethics committee of Hacettepe University (Date: August 25, 2020; Approval no: 2020/16–21). The procedures used in this study adhere to the tenets of the Declaration of Helsinki.

Informed consent to participate and for publication Informed consent was obtained from parents of children enrolled in the study.

Conflict of interest The authors have no conflicts of interest to declare that are relevant to the content of this article.

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