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Case report

Identification of an inherited pathogenic *DNAJC12* variant in a patient with hyperphenylalalinemia

Yi Feng, Sichi Liu, Chengfang Tang, Xiang Jiang, Fang Tang, Bei Li, Xuefang Jia, Qianyu Chen, Jilian Liu, Yonglan Huang*

Department of Guangzhou Newborn Screening Center, Guangzhou Women and Children's Medical Center, Guangzhou Medical University, Guangzhou 510180, Guangdong Province, PR China

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ABSTRACT

Hyperphenylalaninemia (HPA), an abnormal condition of phenylalanine metabolism, was recently reported to be caused by *DNAJC12* mutations. As the heat shock co-chaperone, DNAJC12 prevents the aggregation of misfolded or aggregation-prone proteins and maintain the correct assembly and degradation. Here, we report a patient with unexplained HPA detected by newborn screening. Differential diagnoses of pterin profile and targeted next generation sequencing of excluded the most common causes of the defects of the enzyme phenylalanine hydroxylase or its cofactor tetrahydrobiopterin (BH4). Sanger sequencing revealed a novel homozygous deletion variant of c.262del in *DNAJC12*, which was predicted to produce the truncated protein (p.Q88SfsTer6) and was considered pathogenic to result in the symptoms of global developmental delays clinically. Treatment with the combination of BH4, the neurotransmitter precursors of dopamine and serotonin, and phenylalanine-restricted diet enabled the patient to improve his development and stabilize his phenylalanine level in a reasonable range. These findings expanded the spectrum of the *DNAJC12* mutations and provided new insights on patient management, further supporting the causal relationships of *DNAJC12* and HPA.

1. Introduction

Hyperphenylalaninemia (HPA) is an abnormal metabolic condition caused by the defects of the enzyme phenylalanine hydroxylase (PAH, EC1.14.16.1) or its cofactor tetrahydrobiopterin (BH4) with an incidence of 1:1000 approximately [1]. Patients with HPA can be detected by newborn screening for HPA or phenylketonuria. BH4 is catalyzed by multiple enzymes including GTP cyclohydrolase 1 (GCH1), 6-pyruvoyl-tetrahydropterin synthase (PTPS), sepiapterin reductase (SR), carbinolamine-4a-dehydratase (PCD) and dihydropteridine reductase (DHPR). Except for SR, whose diminished activity present without overt HPA, the defects in these enzymes can be detected by newborn screening [2]. Moreover, BH4 is also the coenzyme for tyrosine hydroxylase (TH, EC1.14.16.2) and tryptophan hydroxylase (TPH, EC1.14.16.4) besides PAH. The defects of BH4 assimilate the additional symptoms of dopamine- and serotonin-deficiencies. Hence, BH4-deficient HPA is usually treated with the combination of BH4 and the neurotransmitter precursors of dopamine and 5-hydroxytryptophan.

Notably, the gene *DNAJC12* (MIM: 606060, NM_021800.2) has been recently identified to implicate with HPA and a heterogeneous clinical spectrum. As a member of the heat shock co-chaperone family, DNAJC12 plays an inevitable important role in the proper folding of PAH, TH and TPH [3]. Thus, the defects of DNAJC12 will decrease the activity of these aromatic amino acid hydroxylases, mimicking BH4 deficiencies clinically. Herein, we report a novel *DNAJC12* variant in a patient detected by newborn screening and described clinical presentation, differential diagnoses, treatment and outcome in details. As far as we know, it is the first report of DNAJC12-defcient HPA in China.

2. Materials and methods

2.1. Ethical statement

This patient was from the newborn screening center of Guangzhou Women and Children's Medical Center. The study was approved by the ethical committee of Guangzhou Women and Children's Medical Center. And the written informed consent was obtained from the legal guardian of the patient. The clinical information and blood samples from the patient and his family members were collected and analyzed. The genomic DNA of this family was extracted from peripheral whole blood using nucleic acid extraction system (Zeesan Biotech, China). While the quantity and quality of DNA were evaluated using Nanodrop

* Corresponding author.
E-mail address: xxhuang321@163.com (Y. Huang).

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Table 1
PCR Primers of *DNAJC12* gene used in this study.

Exon no.	Primers
1 F	5′-ATGACATCTGGTGTATTGAC-3′
1 R	5′-TCAAGTAAAATCCCAACATAC-3′
2 F	5′-CAGGCTGGTCTTATTCC-3′
2 R	5′-TTACTATTCACTGGCTTTTG-3′
3 F	5′-TAAGAGCAGTTGGATAGTTTTG-3′
3 R	5′-CTTGGTTTTCTCCCTCCTA-3′
4 F	5′-ATTAGCACTACAGTAGTAGGTC-3′
4 R	5′-CATTATGTCAGTAAAGCCAAAG-3′
5 F	5′-TCCATATAAATTGTAATTCGTG-3′
5 R	5′ -ATGTTGGCAGCATAGGG-3′

F: forward primer; R: reverse primer.

2000 (Thermo Scientific, USA).

2.2. Molecular genetic analyses

The patient was firstly sequenced using the targeted next generation sequencing technology. There were totally 217 genes in this comprehensive panel including the genes known to be associated with HPA (*PTS*, *QDPR*, *GCH1*, *PCBD1* and *PAH*), covering approximately 110 major genetic diseases. The target sequencing was performed by the HiSeq X10 analyzer (Illumina, USA) with the average depth of coverage exceeding 200×. Data alignment and pathogenicity assessment were performed as described previously [4]. However, *DNAJC12* was not included in this panel. Hence, the Sanger sequencing technology was applied to confirm the genotype of *DNAJC12*. All the exons along with intron–exon boundaries of the *DNAJC12* gene were amplified by polymerase chain reaction with primers listed in the Table 1. Then the PCR products were sequenced by Sanger sequencing using the 3730 sequencer (Applied Biosystems, USA) and the results were analyzed using the software of sequence alignment DNAMAN (Lynnon Biosoft, USA).

3. Clinical report

The patient, the first child of his non-consanginous parents, was born at 41 weeks of gestation with a normal birth weight of 3380 g. Three days after his birth, dried-blood spot were made by collecting the heel blood for routine newborn screening. The analysis of inherited metabolic disorders *via* tandem mass-spectrometry showed an increased phenylalanine concentration of 185 μmol/L (cut off value: 120 μmol/L) and an elevated phenylalanine/tyrosine ratio of 5.3 (upper limit: 2.0). Hence, the infant was recalled to affirm the positive results of HPA. The repeated measurement of phenylalanine concentration was 247 μmol/L and the ratio of phenylalanine/tyrosine was 5.1, confirming the diagnosis of HPA in this newborn.

The results of differential diagnosis showed that the pterin profile (neopterin and biopterin) and DHPR activity were within the normal range. Moreover, neither any pathogenic mutation in *PAH* and *PTS* nor abnormal metabolite in the urine organic acids analysis was detected. And his liver function was normal, breastfeeding was therefore continued and the blood phenylalanine and tyrosine levels as well as his growth were regularly monitored. When the child was 8 months old, certain mild symptoms appeared, he could not roll over in either direction nor could he sit by himself. But the results of the targeted sequencing failed to discover any pathogenic variants, biochemically and genetically excluding the PAH or BH4 deficiencies-induced HPA. At the age of 11 months, he could not crawl and stand with help. The Gesell developmental scale indicated a low grade in development, and the maximum phenylalanine concentration in his follow-up period was about 284 μmol/L. To supply the need for the growth of the child, phenylalanine-restricted diet was supplemented with natural food including an appropriate amount of phenylalanine for a period of time. At the age of 13 months, he could not meet the developmental milestones of his age depicting the serious phenotype of global developmental delays. For example, neither could he crawl or stand alone nor say simple words like “mama” or “dada”. Moreover, he showed the tip-toe when standing with support and drooled during the past time, indicating the abnormality of his nervous system. The level of serum prolactin was 6.97 ng/mL (normal: 2.1–17.7 ng/mL) and brain magnetic resonance imaging (MRI) disclosed widening of the extracerebral space, suggesting a potential brain atrophy in this child.

Meanwhile, several studies have recently reported that the mutant *DNAJC12* gene resulted in HPA and diverse neurological phenotype. So we presumed that the patient herein with HPA and similar neurological

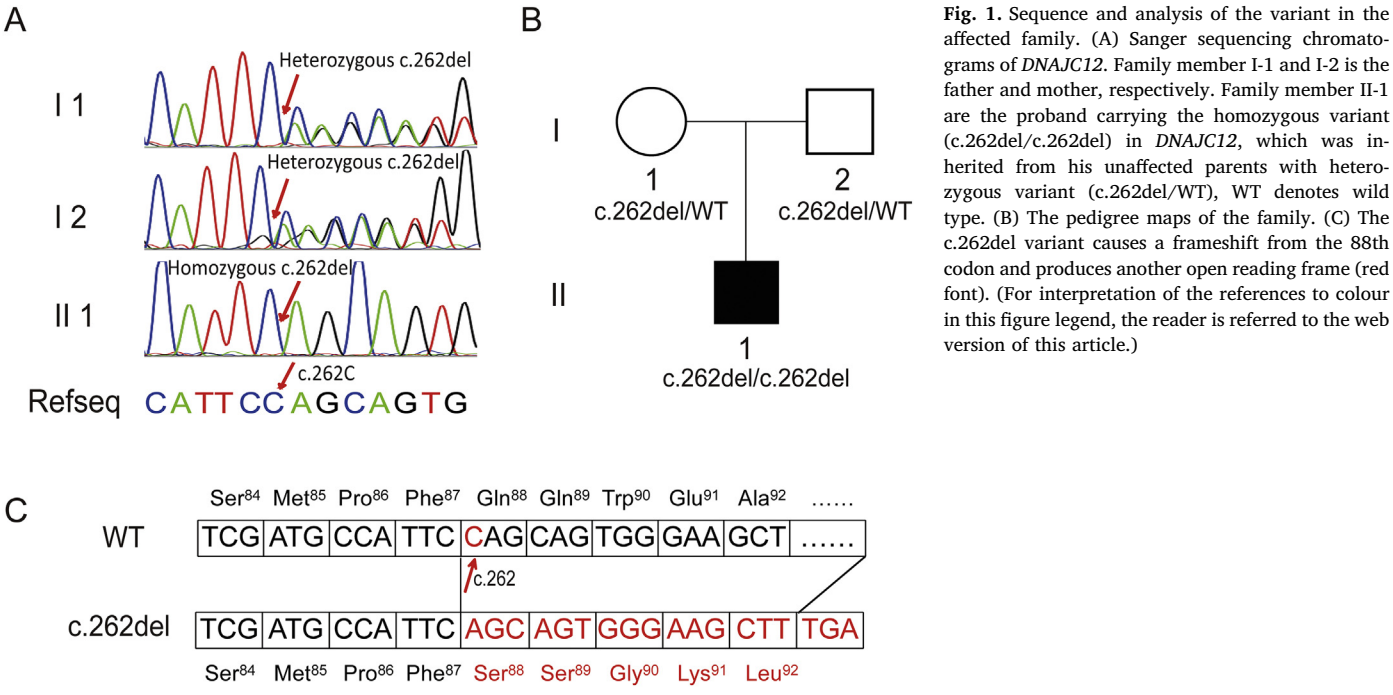

Fig. 1. Sequence and analysis of the variant in the affected family. (A) Sanger sequencing chromatograms of *DNAJC12*. Family member I-1 and I-2 is the father and mother, respectively. Family member II-1 are the proband carrying the homozygous variant (c.262del/c.262del) in *DNAJC12*, which was inherited from his unaffected parents with heterozygous variant (c.262del/WT), WT denotes wild type. (B) The pedigree maps of the family. (C) The c.262del variant causes a frameshift from the 88th codon and produces another open reading frame (red font). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

symptoms might possess DNAJC12 deficiency. As shown in Fig. 1A–B, the patient carried a biallelic deletion variant of c.262del in exon 3, which was inherited from his unaffected parents with heterozygous mutant alleles, coinciding with the inheritance of autosomal recessive in previous studies. The frameshift variant resulting in downstream premature termination of translation of messenger RNA was therefore predicted to produce the truncated protein (p.Q88SfsTer6) and influence the normal function of DNAJC12 (Fig. 1C). The *in silico* analysis of the population frequencies demonstrated that this variant was not found in Exome Variant Server database and was detected only once in Exome Aggregation Consortium (ExAC) database. Taken together, the variant was considered pathogenic to cause the clinical manifestations in the affected child. According to the recommended treatment of other studies, supplement of sapropterin dihydrochloride (an orally active synthetic form of BH4) was initiated in the dose of 3 mg/kg/day, as well as the neurotransmitter precursors of levodopa/benserazide (a dopamine precursor) and 5-hydroxytryptophan (a serotonin precursor). After two weeks, the follow-up phenylalanine concentration dropped to 170 μmol/L and he could crawl and stand with support. Nevertheless, the phenylalanine concentration rose again afterwards. Therefore, phenylalanine-free diet was added to the previous treatment. And the intake of the phenylalanine-restricted formula and natural food was adjusted based on frequent blood phenylalanine monitoring. At the time of writing, age of 18 months, the daily intake of phenylalanine was roughly 600 mg in the patient, but his phenylalanine level stabilized in 126 μmol/L, indicating a higher tolerance for phenylalanine. The Gesell developmental assessment suggested a definite improvement on adaptability, language, gross motor, fine motor and social-emotional with the developmental quotients of 73, 80, 76, 72, 86, respectively (higher than the score at his age of 11 months of 74, 63, 65, 59, and 65 in above items). Furthermore, there was a substantial improvement in motor development showing the capacity of walking independently in this child. Therefore, the patient was considered to be diagnosed with DNAJC12-defcient HPA.

4. Discussion

DNAJC12, a member of the heat shock co-chaperone HSP40/DnaJ protein family, was recently reported to participate in the catabolism of aromatic amino acids [5]. The pathogenic effect of DNAJC12 deficiency was first described in six individuals from four unrelated families [3]. Several reports were subsequently published. Most of these patients showed an elevated phenylalanine concentration and different degrees of neurological symptoms, such as dystonia, intellectual disability, non-progressive parkinsonism, extrapyramidal movement disorder, attention deficit and so on [3,6,7]. In this study, we identified a novel deletion variant c.262del (p.Q88SfsTer6) in *DNAJC12* in a Chinese family. Except HPA, the affected child herein showed global developmental delays, including the motor and language development, suggesting a future diagnosis of intellectual disability [8]. DNAJC12 functions as a molecular chaperone and interacts with its target proteins, comprising the aromatic amino acid hydroxylases, to maintain their correct assembly, folding and degradation [9]. Therefore, the defects of DNAJC12 would probably not assist the proper folding of these hydroxylases and hinder their activities and mimic the BH4-deficiencies clinically. As a frameshift mutation, the deletion variant c.262del was predicted to cause a truncation of the DNAJC12 protein, leading to loss of its biological function. Moreover, the homozygous variant of the affected child was from his heterozygous parents with normal phenotype. Hence, this variant was likely to be pathogenic inducing HPA and other neurological symptoms.

So far, accumulating evidence suggested that PAH deficiency caused by certain point missense mutations or small deletions was a kind of misfolding disease showing responsiveness to the cofactor BH4 [10]. BH4 was considered a pharmacological chaperone that could protect the aromatic amino acid hydroxylases through various mechanisms [11], namely: i) increase the activity of mutant enzymes by a “Michaelis-Menten” effect; ii) up-regulate the gene expression or mRNA stabilization; iii) protect the mutant proteins from degradation or inactivation [12]. Similarly, DNAJC12 deficiency, due to the defects of the molecular chaperone of DNAJC12, should be able to be treated with BH4 effectively serving as a pharmacological chaperone. Indeed, patients with DNAJC12-deficient HPA were effectively treated with the combination of BH4 and the neurotransmitter precursors of dopamine and serotonin [3]. The patient mentioned here was initially treated with the combination of BH4, levodopa/benserazide and 5-hydroxytryptophan. And then the treatment of phenylalanine-restricted diet was added because of the relatively high level of phenylalanine and the requirement of protein in the child. In particular, different from the relatively high dose exceeding 10 mg/kg/day in previous studies, the dose of BH4 herein was only 3 mg/kg/day, mainly because BH4 was costly and its treatment was empirical without sufficient experience. Although the dose of BH4 was not high, the symptoms of the patient with global developmental delays were greatly improved and the phenylalanine concentrations stabilized in a suitable range. Hence, the combination of phenylalanine-restricted diet, neurotransmitters precursors, and a relatively low dose of BH4 is worth considering for those with DNAJC12 deficiency.

Of note, all patients who have performed the cerebrospinal fluid (CSF) analysis in previous studies showed low levels of 5-hydroxyindoleacetic acid (5HIAA, the metabolite of serotonin) and homovanillic acid (HVA, the metabolite of dopamine), indicating the lack of these neurotransmitters in CSF [3]. But the invasive spinal tap limits the acceptance of the analysis of CSF. In the present study, despite the request of CSF investigation was rejected by the patient’s parents, we believe the concentrations of these neurotransmitters were less than the normal range.

However, it was reported that some of the DNAJC12-deficient patients with low CSF neurotransmitter metabolite concentrations showed the mild neurological symptoms [6], indicated the level of neurotransmitter metabolites in CSF was not proportional to the severity of the phenotype. Therefore, the optimal treatment should be decided based primarily on the clinical manifestation, and more experience in treating with DNAJC12 deficiency is demanded in the near future. What’s more, the result of BH4 loading test showed that all the patients responded to BH4 challenge, suggesting the universality of responsiveness to BH4 in DNAJC12 deficiency. However, we cannot preclude the influence of other genes that interact with DNAJC12, the epigenetic modifications or any other environmental factors. And further studies are needed to obtain a better understanding of *DNAJC12* mutant-induced HPA.

5. Conclusions

Collectively, we provided a detailed report on the diagnosis and treatment for DNAJC12 deficiency-mediated HPA with pathogenicity analysis of a novel variant and confirmed the role of DNAJC12 in catabolism of aromatic amino acids. Our finding not only expanded the variant spectrum of DNAJC12-defcient HPA but also provided new insights on its treatment and the phenotype-gene relationships, further supporting the importance of *DNAJC12* genotyping in the HPA patients in which deficiencies of PAH and BH4 are biochemically and genetically excluded. Moreover, to our knowledge, it is the first report in Chinese harboring *DNAJC12* variant.

Conflicts of interest

All authors declare that they have no potential conflict of interest.

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