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An Asymptomatic Patient of Phenylketonuria: A Case Report of 2 Siblings

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Abstract

A 6-year-old girl presented to our hospital with a genetic result indicating a homozygous pathogenic variant (c.G898T) in the phenylalanine hydroxylase (PAH) gene and a heterozygote variant (c.94dupT) in the HECT domain and ankyrin repeat containing E3 ubiquitin protein ligase 1 (HACE1) gene. The study was performed due to her brother's earlier diagnosis of phenylketonuria (PKU) through a genetic analysis (homozygote for PAH). Her 4-year-old brother was also admitted to our hospital with symptoms of hypotonicity, which started at birth and deteriorated when he was 6 months old. He developed a prolonged fever from the age of 8 months until the age of 3 years. All infectious and rheumatologic workups were normal. He was screened for PKU twice at birth, both showing negative results. The plasma phenylalanine (Phe) level was checked several times in the first 2 years of his life, and all of them were in the borderline range (2 - 4 mg/dL). He was tested again at the age of 2 years for the plasma Phe level twice, both showing positive results (14 and 8 mg/dL, respectively). Both positive results for the plasma Phe level led to a genetic study, indicating that this case is homozygote for both variants, c.G898T in the PAH gene and c.94dupT in the HACE1 gene. Then, a Phe-restricted diet was given. At the age of 3 years, a Kuvan test was performed on the patient, indicating a non-BH4-responsive PKU (classic type of PKU). However, to reduce diet restriction, he was treated with Kuvan and responded to the treatment. The symptoms (such as hypotonia and developmental retardation) improved after treatment with Kuvan, probably due to HACE1 gene dysfunction.

Keywords: Phenylketonuria, Child, Disease

1. Introduction

Phenylketonuria (PKU) is an inherited autosomal recessive disorder occurring due to the increased level of phenylalanine (Phe) in the blood with various mild to severe phenotypes. The pathogenic variant of the phenylalanine hydroxylase (PAH) gene is the primary cause of PKU (1-3). Although it occurs in 1 in 25,000 live births worldwide, in Iran, it appears in 1 in 8000 births (4, 5). An estimated 1 - 2% of cases of hyperphenylalaninemia are secondary to a deficiency in tetrahydrobiopterin (BH4), which is a required cofactor for PAH and other amino acid-metabolizing enzymes (6, 7).

Despite the rarity of this autosomal recessive disease, it can cause neurological complications in children if untreated. Therefore, a lifelong protein-controlled diet is commonly needed in PKU patients to prevent severe complications. However, a small group of patients can use a diet alleviated by sapropterin dihydrochloride (Kuvan) as a synthetic form of the cofactor BH4. It can be administered with a Phe-restricted diet for children aged > 1 month (1, 8) because they can tolerate it, and no significant complication has been noted after its use (9, 10).

Moreover, spastic paraplegia and psychomotor retardation with or without seizures (SPPRS) is a complex autosomal recessive neurodevelopmental disorder due to the HECT domain and ankyrin repeat containing E3 ubiquitin protein ligase 1 (HACE1) gene mutations (11-13). HACE1 is involved in the subcellular localization and proteasomal degradation of target proteins. This disorder commonly has an infantile onset and presents hypotonia at birth or by age 3 - 4 months. It can negatively affect normal development, intellectual ability, and early motor milestones. Also, it induces slowly progressive bilateral lower limb spasticity, which by their 20s, not only limits all patients to a wheelchair and bed, but some patients may also miss their walking ability (12, 14).

Although it seems that both mentioned autosomal recessive disorders (PKU and SPPRS) affect different genes and need other treatments, we aimed to present a boy

with homozygote features for both genes and his sister. In this case presentation, surprisingly, SPPRS symptoms relatively improved after treatment with Kuvan, probably due to HACE1 gene dysfunction.

2. Case Presentation

2.1. Case 1

A 6-year-old girl presented to our hospital with her brother, who was earlier diagnosed as a PKU patient through a genetic analysis (homozygote for PAH). The 6-year-old girl was born at 39 weeks of gestation by cesarean section (due to breech presentation) to consanguineous parents with a birth weight of 3300 gr (67th percentile). The patient had no symptoms specific to PKU. The only signs were mild impatience and a slight lack of concentration, which started last year when she started kindergarten. Physical examinations were normal except for some reticular skin lesions, which had an additive incidence pattern according to her mother's inspections. He had normal neurological examinations.

2.2. Case 2

The 4-year-old brother of the described patient has also presented to our hospital with a genetic study indicating that this case is homozygote for both variants, c.G898T in the PAH gene and c.94dupT in the HACE1 gene. He was born at 39 weeks of gestation by cesarean section (due to breech presentation) to consanguineous parents with a birth weight of 3670 gr (92nd percentile). The only remarkable family history is one of the maternal uncles, who had been diagnosed with Cerebral palcy (CP) and deceased at 31 due to a seizure. According to the patient's mother, the described uncle had similar symptoms to her son.

He, the 4-year-old patient, had been screened for PKU twice at birth, showing negative results. He tested for the plasma Phe level several times in the first 2 years of his life, and all results were within the borderline range (2 - 4 mg/dL). His first symptom was hypotonicity, which started at birth and deteriorated when he was 6 months old. Other neurological parameters were normal.

He developed a prolonged fever from the age of 8 months until the age of 3 years. All infectious and rheumatologic workups were normal. He was tested again at the age of 2 years for the plasma Phe level, in which a positive result was reported (14 mg/dL). The repeated test results also showed a plasma Phe level of 8 mg/dL. Both positive results for the plasma Phe level led to a genetic study described above.

A peripheral blood sample containing K3-EDTA anticoagulant was taken from the patients in the study. A genetic analysis was performed on the presented male case when he was 2 years old. The genetic research aimed to conduct a mono whole sequencing test, followed by mutation confirmation by direct Sanger sequencing. Primary/diagnostic sequence variants and secondary/incidental sequence variants were reported. Due to the gained results, his consanguineous parents and his sister were also investigated for PAH gene mutations by Sanger sequencing.

The 6-year-old girl's genetic study indicated a homozygous pathogenic variant (c.G898T) in the PAH gene and a heterozygote variant (c.94dupT) in the HACE1 gene.

The 4-year-old brother of the described patient had a genetic study indicating that this case is homozygote for both variants, c.G898T in the PAH gene and c.94dupT in the HACE1 gene.

The results are shown in Tables 1 and 2.

After the genetic study, a Phe-restricted diet was given to the male patient. At the age of 3 years, the Kuvan test was performed on him, showing a non-BH4-responsive PKU (classic type of PKU). However, to reduce diet restriction, he was treated with Kuvan and responded to the treatment. As mentioned, the symptoms (such as hypotonia and developmental retardation) fortunately improved after treatment with Kuvan, probably due to HACE1 gene dysfunction.

Both patients had normal head circumference, anthropometric indices, and developmental progression. Also, magnetic resonance imaging and electroencephalographic findings were normal.

3. Discussion

We reported a family with mutations in 2 genes. Although PAH is the common cause of PKU, one of the affected cases in this family is a heterozygote for HACE1. Thus, the variant in PAH is causal in this family. Pathogenic variants of HACE1 lead to spastic paraplegia and psychomotor retardation with or without seizures.

PKU patients manifest neurological symptoms, including seizures, tremors, and mental retardation (15). The male sibling developed symptoms such as hypotonia and developmental retardation, which started at birth and deteriorated from 6 months, while his plasma Phe level was normal in the first 2 years of his life. Kuvan-responsive PKU patients can benefit from a Kuvan treatment; it can improve clinical features and a better low Phe diet tolerance. It can also cause a less restricted diet in these patients (16). Although he is suffering from the classic type of PKU (non-responsive to Kuvan), the treatment with Kuvan has improved his symptoms, which might be due to HACE1 gene dysfunction. This phenomenon requires more investigation and further studies on the Kuvan treatment's efficacy

Table 1. Primary/Diagnostic Sequence Variants

Gene	Cytogenetic Location	DNA Change	Protein Change	dbSNP rsID	Associated Disease	OMIM	Inheritance	Zygosity	Classification (ClinVar)
HACE1	6q16.3	NM_00132108: exon2:c.94dupT	Y32fs	-	Spastic paraplegia and psychomotor retardation with or without seizures	610876	AR	Patient: Hem; Father: Het; Mother: Het; Sister: Het	NR

Abbreviations: HACE1, HECT domain and ankyrin repeat containing E3 ubiquitin protein ligase 1; AR, autosomal recessive.

Table 2. Incidental/Secondary Sequence Variants

Gene	Cytogenetic Location	DNA Change	Protein Change	dbSNP rsID	Associated Disease	OMIM	Inheritance	Zygosity	Classification (ClinVar)
PAH	12q23.2	NM_000277:Exon8: c.G898T	A300S	Rs5030853	1: [hyperphenylalaninemia,Non-PKU mild]; 2: Phenylketonuria	612349	AR	Patient: Hem; Father: Het; Mother: Het; Sister: Hem	R-pathogenic

Abbreviations: HACE1, HECT domain and ankyrin repeat containing E3 ubiquitin protein ligase 1; AR, autosomal recessive.

in managing HACE1-positive patients and/or classic types of PKU.

The female sibling is also homozygote for PAH, manifesting mild and nonspecific symptoms as described above. A low Phe diet is the base treatment in these patients to lower the levels of plasma Phe, and lack of this diet causes severe symptoms such as mental retardation and developmental delay (17). She has not been on any low Phe diet, but she had normal developmental stages, and there are no neurologic signs but mild impatience and a slight lack of concentration. The absence of neurologic symptoms can be due to a transportation error in the blood-brain barrier that reduces the Phe concentration in cerebrospinal fluid (CSF). Therefore, the brain is not exposed to toxic amounts of Phe. Further workups and a long-term follow-up seem to be essential for her to evaluate the progression of the symptoms.

With this case presentation, we tend to recommend more investigation and further studies on Kuvan treatment for neurologic symptoms in HACE1-positive patients and/or classic types of PKU and the justification of symptoms in patients with both HACE1 and PAH dysfunction.

Footnotes

Authors' Contribution: Study concept and design: AHR, SHK, and SD; Acquisition of data: AMGH, TH, RB, and SD; Analysis and interpretation of data: AHR, SHK, AMGH, TH, RB, and SD; Drafting of the manuscript: AHR, SHK, AMGH, TH, RB, and SD; Critical revision of the manuscript for important intellectual content: AHR, SHK, AMGH, TH, RB, and SD; Statistical analysis: SD; Administrative, technical, and material support: AHR, SHK, AMGH, TH, RB, and SD; Study supervision: AHR, SHK, AMGH, TH, RB, and SD.

Conflict of Interests: There was no conflict of interest to be declared.

Ethical Approval: This study was approved by the Ethics Committee of Guilan University of Medical Sciences (code: IR.GUMS.REC.1401.275; Link: ethics.research.ac.ir/EthicsProposalView.php?id=278928).

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