Case Report

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A neonate with hyperornithinemia-hyperammonemia-homocitrullinuria syndrome from a consanguineous Pakistani family

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Hyperornithinemia-hyperammonemia-homocitrullinuria (HHH) syndrome is a rare autosomal recessive urea cycle disorder. HHH is caused by a deficiency of the mitochondrial ornithine transporter protein, which is encoded by the solute carrier family 25, member 15 (*SLC25A15*) gene. Recently, government supported Korean newborn screening has been expanded to include a tandem mass spectrometry (MS/MS) measurement of ornithine level. We report a case of a neonate with HHH syndrome showing a normal MS/MS measurement of ornithine level. A female newborn was admitted to neonatal intensive unit due to familial history of HHH syndrome. Her parents were consanguineous Parkistani couple. The subject's older sister was diagnosed with HHH syndrome at age of 30 months based on altered mental status and liver dysfunction. Even though the subject displayed normal ammonia and ornithine levels based on MS/MS analysis, a molecular test confirmed the diagnosis of HHH syndrome. At 1 month of age, amino acid analysis of blood and urine showed high levels of ornithine and homocitrulline. After 11 months of follow up, she showed normal growth and development, whereas affected sister showed progressive cognitive impairment despite no further hyperammonemia after protein restriction and standard therapy. Our report is in agreement with a previous Canadian study, which showed that neonatal samples from HHH syndrome patients demonstrate normal ornithine levels despite having known mutations. Considering the delayed rise of ornithine in affected patients, genetic testing, and repetitive metabolic testing is needed to prevent patient loss in high risk patients.

Key words: Urea cycle disorders, inborn, Neonatal screening, HHH syndrome, Ornithine.

Introduction

Hyperornithinemia-hyperammonemia-homocitrullinuria (HHH) syndrome (MIM #238970) is a rare autosomal recessive urea cycle disorder (UCD) [1]. This disorder caused by a deficiency of mitochondrial ornithine transporter encoding by *SLC25A15* gene (MIM*603861) located on chromosome 13q14 [1]. Shih and colleagues published the first report of an HHH syndrome patient in 1969 [2]. This report described a 3 year-old boy with cognitive impairment and myoclonic seizures along with inter-

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mittent hyperammonemia, high plasma ornithine levels and homocitrullinuria [2]. As of now, more than 100 patients with HHH syndrome have been reported. The incidence of all UCDs is 1:35,000 live births. HHH syndrome is estimated to have an incidence of 1:6,000,000 live births [3,4]. Among 111 HHH syndrome patients, the distribution included many Canadians (Quebec, 25 patients, 23%), Japanense (14 patients, 13%), and Italian (18 patients, 17%) [3]. Since October 2018, government supported Korean newborn screening (NBS) has been expanded to include mass spectrometry (MS/MS) based measurements of ornithine level. However, normal NBS test results cannot guarantee the lack of a metabolic disorder, since some patients take time to accumulate the substrate detected in NBS. Herein, we report the case of a neonate with HHH syndrome showing a normal MS/MS test result.

Case

A female newborn was born at 38 weeks with a weight of 3.21 kg. She was placed in neonatal intensive care due to a familial history of HHH syndrome. She was the 3rd baby from consanguineous Pakistani parents (Fig. 1A). Her older sister presented with lethargy and liver dysfunction at age of 30 months and was finally diagnosed with HHH syndrome 3 months before our subject's birth. Older brother and parents were already known as a carrier through family genetic testing. This affected sister showed developmental regression and autistic behavior despite of ammonia scavenger and 3 months on protein restricted diet. The affected older sister showed normal walking and neurologic examination including normal deep tendon reflex. Her plasma ornithine level was within 521 to 687 μmol/L and no hyperammonemia occurred except for the first hyperammonemic event at diagnosis (ammonia was measured at 400 μmol/L). Her full-scale intelligence quotient (FSIQ) was measured to 44 at 43 months of age. We performed a metabolic analysis at birth and again 4 days after *ad lib* feeding. Measurements performed at birth showed elevated levels not only for ornithine in plasma and urine, but also homocitrulline and orotic acids in urine. However, follow-up measurements performed at 4 days after full feeding revealed that these abnormal findings returned to normal

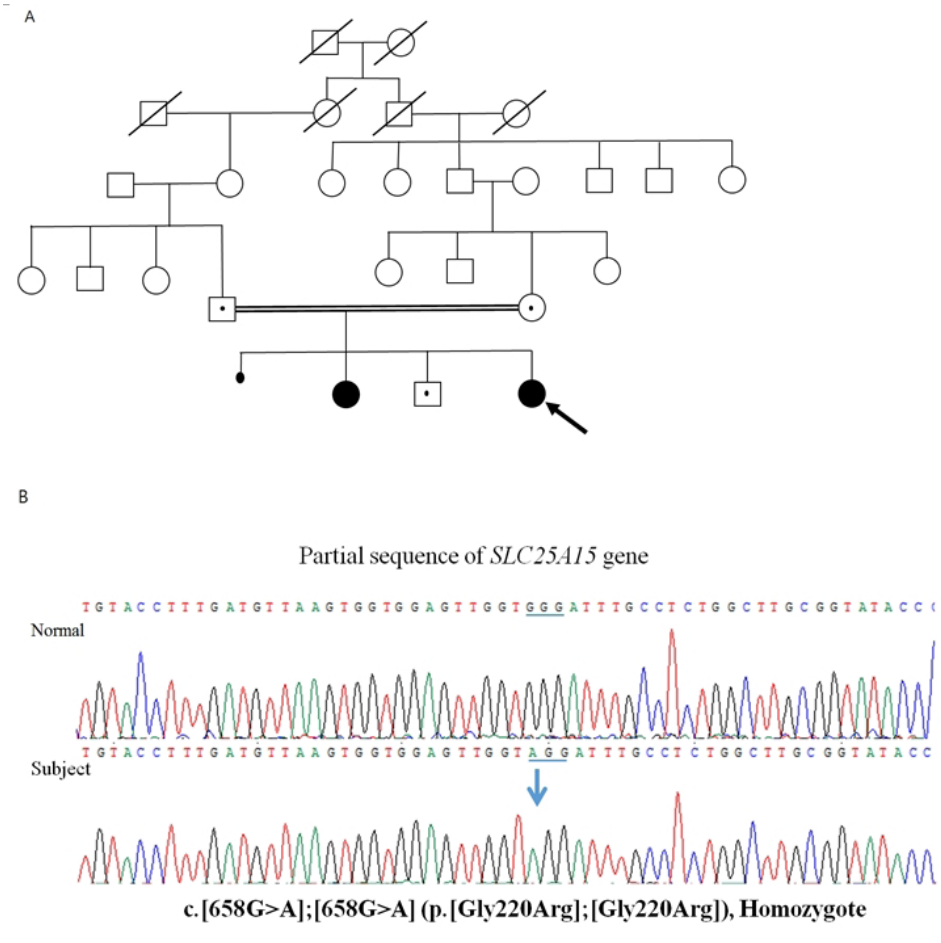

Fig. 1. Pedigree and Sanger sequencing of *SLC25A15* in the hyperornithinemia-hyperammonemia-homocitrullinuria (HHH) syndrome patient. (A) Pedigree showing consanguineous parents and affected patients with HHH syndrome. (B) Partial Sanger sequencing of *SLC25A15* showing homozygous mutations for G220R. Each mutation was inherited from her father and mother.

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Table 1. The laboratory findings in patient with hyperornithinemia-hyperammonemia-homocitrullinuria syndrome from birth to 11 months of age

	Reference range	Age						
		At birth	4 Days	3 Weeks	2 Months	5 Months	8 Months	11 Months
Ammoina, blood (μmol/L)	Newborn<100, after newborn<60	69	40	105	56	50	55	44
Homocitrulline, urine (μmol/g Cr)	ND)	110	45	-	-	-	-	-
Orotic acid, urine (μmol/g Cr)	ND	242.9	ND	ND	-	-	-	-
Ornithine, urine (μmol/g Cr)	0-168	207	43	-	-	-	-	-
Ornithinie, plasma (μmol/L)	Newborn: 38-207, 1-3 mo: 26-117, 3 mo-6 yr: 27-96	256	122	383	412	184	451	441
Glycine (μmol/L)	Newborn: 106-254, 1-3 mo: 105-222, 3 mo-6 yr; 125-318	269	188	206	226	243	151	135
Glutamine (μmol/L)	Newborn: 132-455, 1-3 mo: 332-1,084, 3 mo-6 yr; 475-746	413	543	592	409	506	600	567
Citrulline (μmol/L)	Newborn: 3-36, 1-3 mo: 6-36, 3 mo-6 yr: 8-47	7	6	18	6	15	22	19

Cr, creatine; ND, not detected.

(Table 1). The plasma ornithine was decreased to 43 μmol/L. NBS by MS/MS was performed at 4 days of age and again at 7 days of age and the results were also normal. The ammonia level was consistently within normal range. Although the normal metabolic results indicated a lack of HHH syndrome, we started the subject on a protein restricted formula (protein 2.5 g/kg/day). Two weeks after discharge, genotyping of this newborn revealed that the subject, as well as her sister, were *SLC25A15* c.658G>A (G220R) homozygotes (Fig. 1B). This mutation was not identified in control population but reported in HHH syndrome patients. It was predicted as damaging by in-silico analysis (Sorting Intolerant From Tolerant [SIFT] and Polyphen-2) and considered as pathogenic for disorder according to American College of Medical Genetics classification. Moreover, at two weeks after discharge, the amino acid analysis of blood and urine showed high levels of ornithine and homocitrulline (Table 1). The subject was started on the medications citrulline (100 mg/kg/day) and sodium phenylbutyrate (250 mg/kg/day). We gave the patient's emergency card to their parents and educated them on how to prevent an acute phase of hyperammonemia.

For 11 months of follow up, the subject showed normal growth and development (Table 2) [5]. The latest ornithine level was 441 μmol/L. Until now, her development and growth are normal for her age based on height, weight, and head circumference. Her height, weight and head circumference were 76.3 cm (75th percentile), 9.6 kg (75-90th percentile), and 43 cm (25th percentile), respectively. However, her affected sister showed memory loss and reduced communication, although she has been on protein restriction (0.8 g/kg/day) and medication (phenyl butyrate and citrulline). Brain magnetic resonance image and gene panel using the TruSight One panel of 4,813 genes were performed in the affected sister to determine if her behavior could be attributed to autism, but no explanation for the regression of her neurological status was found except for HHH syndrome. The level of creatine of both sisters was checked and the result was normal. The result of patient and affected older sister was 0.8 mg/dL and 0.9 mg/dL (reference range, 0.3 to 1.2 mg/dL), respectively. Treatment with a creatine dose of 500 mg per day was prescribed, however, it is too early to assess the effectiveness of creatine.

Discussion

HHH syndrome patients with a founder mutation (p.F118del) and normal ornithine levels during the neonatal period were identified by a FrenchCanadian group [6]. Despite the small

Table 2. Clinical characteristics of patients having homozygous G220R mutations of *SLC25A15*

Characteristic	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Sex	Female	Female	Female	Female	Female
Ethinicity	Pakistan	Pakistan	Saudi Arabia	Saudi Arabia	Saudi Arabia
Onset (yr)	Family screening	1.9	1	Family screening	Family screening
Diagnosis (yr)	0.06	2.5	1.5	13	7
Late evaluation (yr)	0.9	4	4	13	7
Recurrent vomitus	No	Yes	Yes	No	No
Lethargy	No	Yes	No	No	No
Coma	No	No	No	No	No
Liver dysfunction	No	Yes	Yes	No	No
Coagulopathy	No	Yes	Yes	No	No
Intellectual disability	No	Moderate (FSIQ 42)	Language delay	Mild	Mild
MR, brain	Not done	No abnormal finding	Multiple stroke lesions	Normal	Normal
Seizures, myoclonic	No	No	No	No	No
Pyramidal signs	No	No	Yes	Yes	Yes
Ornithine at diagnosis (25-175 μmol/L)	43	870	465	590	493
Serum ammonia (0-50 μmol/L)	40	400	532	77	54
Urine homocitrulline (<10 mM/M Cr)	45	143	108	13.3	21
Reference	This study	This study	[5]	[5]	[5]

FSIQ, full-scale intelligence quotient; MR, magnetic resonance; Cr, creatine.

number of HHH syndrome mutants in this study, they showed that no wild, carrier, and mutants had normal ornithine levels during the newborn period (cut off <120 μmol/L) [6]. Our neonatal patient showed decreasing ornithine levels despite the 4 days of normal formula feeding. At birth, the ornithine level was quite high, it may be related with stressful status during delivery. Interestingly, the ornithine level was decreased to normal level for a few days. It may be the reason that her residual enzyme activity might be acceptable to decrease the ornithine level for first several days, but the ornithine level was finally increasing due to exceeding the amount of intake. This patient had G220R, which was previously reported in a 4-year-old Saudi girl presenting with recurrent Reye-like episodes and multiple supratentorial stroke-like lesions [5]. The Saudi girl had two affected siblings. The siblings aged 13 and 7 years, had mild symptoms with learning difficulties and protein intolerance, respectively, and showed mild elevation of homocitrulline level in urine (Table 2) [5]. There were no progressive neurological symptoms described in the three G220R homozygotic Saudi siblings.

Even though half of patients with HHH syndrome (46%) presented symptoms until infantile period, the diagnosis tended to be delayed (mean period between symptoms and diagnosis, 6.3±10.1 years) [3]. HHH syndrome is characterized by acute and chronic symptoms from hyperammonemia and liver failure to mental regression and signs of motor dysfunction [3,6]. In our study, the affected older sister showed progressive neurologic deterioration despite receiving standard therapy including citrulline supplementation, ammonia scavenger, and protein restriction. Progressive neurologic symptoms including hereditary spastic paraplegia and variable cognitive impairment are prevalent complications in HHH syndrome as well as in argininemia [7,8]. However, the pathogenesis underlying progressive neurological manifestations of HHH syndrome remains to be elucidated. It might be related to other mechanisms apart from hyperammonemia [8]. Secondary creatine deficiency in brain has been suggested to play a role in neurologic manifestation, since ornithine excess inhibits creatinine biosynthesis which is important role in energy storage and transmission [3,9,10]. Another study showed that excess ornithine and homocitrulline lead to astrocyte injury in rat by impairing mitochondrial function, and decreasing antioxidant defenses and cell viability [11,12]. The *SLC25A15* gene and the ornithine metabolic pathway are expressed in the liver, as well as other tissues, including those in the brain and kidney, etc. Liver transplantation cannot be a used to cure HHH syndrome considering the chronic neurological manifestations. Further investigation is needed to develop

treatments for preventing progressive neurological symptoms in HHH syndrome.

In conclusion, we report a neonatal HHH syndrome case with a normal MS/MS test result. Molecular testing in HHH syndrome may be more useful for diagnosis and management in familial cases compared to biochemical findings considering phenotypic heterogeneity despite of same mutations. Chronic neurologic progression should be monitored in HHH syndrome patients in addition to hyperammonemic events.

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