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

Yoo-Mi Kim^{1*}, Han Hyuk Lim¹, Mi Hyeon Gang¹, Yong Wook Lee¹, Sook Za Kim¹, Gu-Hwan Kim², Han-Wook Yoo², Jung-Min Ko³, and Meayoung Chang¹

¹Department of Pediatrics, Chungnam National University Hospital, College of Medicine, Chungnam National University, Daejeon, Korea

²Medical Genetics Center, Asan Medical Center Children's Hospital, University of Ulsan College of Medicine, Seoul, Korea

³Department of Pediatrics, Seoul National University Children's Hospital, College of Medicine, Seoul National University, Seoul, Korea

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 Pakistani 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-

Received: 13 December 2019, Revised: 20 December 2019, Accepted: 20 December 2019, Published: 31 December 2019

*Corresponding author: Yoo-Mi Kim, M.D., Ph.D. <https://orcid.org/0000-0002-8440-5069>

Department of Pediatrics, Chungnam National University Hospital, College of Medicine, Chungnam National University, 282 Munhwa-ro, Jung-gu, Daejeon 35015, Korea.

Tel: +82-42-280-7244, Fax: +82-42-255-3158, E-mail: ym4805@gmail.com

Conflict of interest: The authors declare that they do not have any conflicts of interest.

© This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

© Copyright 2019 by the Korean Society of Medical Genetics and Genomics

www.e-kjgm.org

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%), Japanese (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 $\mu\text{mol/L}$ and no hyperammonemia occurred except for the first hyperammonemic event at diagnosis (ammonia was measured at 400 $\mu\text{mol/L}$). 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.

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
Ammonia, blood ($\mu\text{mol/L}$)	Newborn<100, after newborn<60	69	40	105	56	50	55	44
Homocitrulline, urine ($\mu\text{mol/g Cr}$)	ND	110	45	-	-	-	-	-
Orotic acid, urine ($\mu\text{mol/g Cr}$)	ND	242.9	ND	ND	-	-	-	-
Ornithine, urine ($\mu\text{mol/g Cr}$)	0-168	207	43	-	-	-	-	-
Ornithine, plasma ($\mu\text{mol/L}$)	Newborn: 38-207, 1-3 mo: 26-117, 3 mo-6 yr: 27-96	256	122	383	412	184	451	441
Glycine ($\mu\text{mol/L}$)	Newborn: 106-254, 1-3 mo: 105-222, 3 mo-6 yr: 125-318	269	188	206	226	243	151	135
Glutamine ($\mu\text{mol/L}$)	Newborn: 132-455, 1-3 mo: 332-1,084, 3 mo-6 yr: 475-746	413	543	592	409	506	600	567
Citrulline ($\mu\text{mol/L}$)	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 $43 \mu\text{mol/L}$. 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 $441 \mu\text{mol/L}$. 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
Ethnicity	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 $\mu\text{mol/L}$)	43	870	465	590	493
Serum ammonia (0-50 $\mu\text{mol/L}$)	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 $\mu\text{mol/L}$) [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.

Acknowledgements

We thank our patients and her family members for their participation in this study.

References

1. Häberle J, Boddaert N, Burlina A, Chakrapani A, Dixon M, Huemer M, et al. Suggested guidelines for the diagnosis and management of urea cycle disorders. *Orphanet J Rare Dis* 2012;7:32.
2. Shih VE, Efron ML, Moser HW. Hyperornithinemia, hyperammonemia, and homocitrullinuria. A new disorder of amino acid metabolism associated with myoclonic seizures and mental retardation. *Am J Dis Child* 1969;117:83-92.
3. Olivieri G, Pro S, Diodato D, Di Capua M, Longo D, Martinelli D, et al. Corticospinal tract damage in HHH syndrome: a metabolic cause of hereditary spastic paraplegia. *Orphanet J Rare Dis* 2019;14:208.
4. Panza E, Martinelli D, Magini P, Dionisi Vici C, Seri M. Hereditary spastic paraplegia is a common phenotypic finding in ARG1 deficiency, P5CS deficiency and HHH syndrome: three inborn errors of metabolism caused by alteration of an interconnected pathway of glutamate and urea cycle metabolism. *Front Neurol* 2019;10:131.
5. Martinelli D, Diodato D, Ponzi E, Monné M, Boenzi S, Bertini E, et al. The hyperornithinemia-hyperammonemia-homocitrullinuria syndrome. *Orphanet J Rare Dis* 2015;10:29.
6. Sokoro AA, Lepage J, Antonishyn N, McDonald R, Rockman-Greenberg C, Irvine J, et al. Diagnosis and high incidence of hyperornithinemia-hyperammonemia-homocitrullinemia (HHH) syndrome in Northern Saskatchewan. *J Inher Metab Dis* 2010;33 Suppl 3:S275-81.
7. Summar ML, Koelker S, Freedberg D, Le Mons C, Haberle J, Lee HS, et al.; European Registry and Network for Intoxication Type Metabolic Diseases (E-IMD); Members of the Urea Cycle Disorders Consortium (UCDC). The incidence of urea cycle disorders. *Mol Genet Metab* 2013;110:179-80.
8. Al-Hassnan ZN, Rashed MS, Al-Dirbashi OY, Patay Z, Rahbeeni Z, Abu-Amero KK. Hyperornithinemia-hyperammonemia-homocitrullinuria syndrome with stroke-like imaging presentation: clinical, biochemical and molecular analysis. *J Neurol Sci* 2008;264:187-94.
9. Boenzi S, Pastore A, Martinelli D, Goffredo BM, Boiani A, Rizzo C, et al. Creatine metabolism in urea cycle defects. *J Inher Metab Dis* 2012;35:647-53.
10. Joncquel-Chevalier Curt M, Voicu PM, Fontaine M, Dessein AF, Porchet N, Mention-Mulliez K, et al. Creatine biosynthesis and transport in health and disease. *Biochimie* 2015;119:146-65.
11. Zanatta Â, Rodrigues MD, Amaral AU, Souza DG, Quincozes-Santos A, Wajner M. Ornithine and homocitrulline impair mitochondrial function, decrease antioxidant defenses and induce cell death in menadione-stressed rat cortical astrocytes: potential mechanisms of neurological dysfunction in HHH syndrome. *Neurochem Res* 2016;41:2190-8.
12. Zanatta A, Viegas CM, Tonin AM, Busanello EN, Grings M, Moura AP, et al. Disturbance of redox homeostasis by ornithine and homocitrulline in rat cerebellum: a possible mechanism of cerebellar dysfunction in HHH syndrome. *Life Sci* 2013;93:161-8.