Hyperornithinemia–hyperammonemia–homocitrullinuria 증후군을 유발하는 *SLC25A15* 유전자의 새로운 변이

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A Novel *SLC25A15* Mutation Causing Hyperornithinemia–Hyperammonemia–Homocitrullinuria Syndrome

Hyperornithinemia–hyperammonemia–homocitrullinuria syndrome (HHH syndrome) is a neurometabolic disorder with highly variable clinical severity ranging from mild learning disability to severe encephalopathy. Diagnosis of HHH syndrome can easily be delayed or misdiagnosed due to insidious symptoms and incomplete biochemical findings, in that case, genetic testing should be considered to confirm the diagnosis. HHH syndrome is caused by biallelic mutations of *SLC25A15*, which is involved in the urea cycle and the ornithine transport into mitochondria. Here we report a boy with spastic paraplegia and asymptomatic younger sister who have compound heterozygous mutations of c.535C>T (p.R179*) and c.116C>A (p.T39K) in the *SLC25A15* gene. We identified that p.T39K mutation is a novel pathogenic mutation causing HHH syndrome and that p.R179*, which is prevalent in Japanese and Middle Eastern heritage, is also found in the Korean population.

Key Words: HHH syndrome, Urea cycle disorders, *SLC25A15*, Genetics

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Introduction

Hyperornithinemia–hyperammonemia–homocitrullinuria syndrome (HHH syndrome, MIM #238970) is an autosomal recessive neurometabolic disorder due to ornithine degradation defect¹. HHH syndrome accounts for only 1–3.8% of all urea cycle disorders (UCDs), and the incidence based on UCDC (Urea Cycle Disorders Consortium) and newborn screening is less than 1: 2,000,000^{2,3}. The condition represents highly variable clinical severity ranging from mild learning disability to severe encephalopathy with hepatic failure. Patients may show recurrent vomiting, lethargy, liver dysfunction, coagulopathy, protein intolerance and in severe cases, progression to coma and death^{1,4}. Neurological manifestations encompass a variable combination of the following signs: cognitive impairment, behavioral disorders, spastic paraplegia, pyramidal and extrapyramidal signs, stroke-like episodes, hypotonia, seizures, and ataxia^{5–8}. Symptoms may appear at any time between birth and adulthood, and clinical severity does not correlate with the age of onset, genotype or ammonium/ornithine plasma

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levels^{9,10}). Since the syndrome was first reported in 1969, more than 100 affected individuals have been reported worldwide^{10,11}. *SLC25A15* encodes mitochondrial ornithine transporter 1 (ORNT1), which is involved in the urea cycle and the ornithine degradation pathway. Therefore, mutations in the *SLC25A15* (solute carrier family 25, member 15) gene result in cytoplasmic ornithine accumulation and impaired ammonia detoxication and raised carbamoyl phosphate. There is a typical metabolic profile comprising the name-giving triad with urine homocitrulline as specific marker allowing for diagnosis on the basis of just biochemical analysis¹²). Homocitrullinuria is presumably formed from the reaction of mitochondrial carbamyl phosphate with lysine, which can become a substrate for the OTC reaction when ornithine is deficient.

There have been several patients with HHH syndrome in Korea but their genotypes have not been reported. Hwang et al. reported two cases of HHH syndrome in Siblings in Korea¹³). Similar to our case, the first of his siblings was diagnosed at the age of 5 years of age who had difficulty standing at 13 months of age. His younger brother was also diagnosed at 13 month of age, who had recurrent vomiting but there was no developmental delay.

Here we reported a boy whose diagnosis was delayed because of mild clinical manifestation during infancy. Furthermore, we identified a novel *SLC25A15* mutation associated with HHH syndrome.

Case report

1. Clinical presentation

A 5 year old Korean boy presented with spastic paraplegia. He was born after an uneventful pregnancy at 39 weeks of gestation and had a birthweight of 3,300 grams. Newborn screening (NBS) within a few days of birth revealed a negative result. He showed normal development until he started to drag along his right leg at 15 months of age. At the age of 5 years old, the patient's weight and height were 19.1 kg (25-50th percentile) and 112 cm (50-75th percentile), respectively. On examination, manual muscle tests of both legs graded 4/5 and muscle tone was increased in right leg. The deep tendon reflex of right ankle was increased but the others were normal. The cranial nerves and sensation were normal. After several years of rehabilitation therapy, his gait became normal, but still had some difficulty climbing stairs. He had no history of hepatopathy or neurological problems aside from paraplegia. He had been voluntarily avoiding a high-protein diet such as fish or meat, and vomited

occasionally after meals. Brain magnetic resonance imaging (MRI) which had been previously performed 3 times at different hospitals were normal. Genetic tests of hereditary spastic paraplegia including DYT5 and SPG3A were performed in a previous hospital and the results were negative. There was no known family history of metabolic disorders. His parents were healthy, nonconsanguineous, and of Korean descent (Fig. 1). He had a healthy 13-month-old sister, who had normal development including growth. Brain MRI and metabolic tests were performed on his 13-month-old sister. Her brain MRI was normal and NBS results within a few days of birth of her were also negative.

2. Metabolic defect workup

Laboratory findings showed hyperammonemia with a level of 86 $\mu\text{mol/L}$ (normal range: less than 50 $\mu\text{mol/L}$), slightly elevated liver enzymes with aspartate transaminase (AST) of 53 U/L (normal range: 15-40 U/L) and alanine transaminase (ALT) of 84 U/L (normal range: 5-45 U/L). Serum lactate was within normal limits. Plasma amino acid analysis showed elevated serum ornithine concentration of 480.2 $\mu\text{mol/L}$ (normal range: 27-96 $\mu\text{mol/L}$). Urinary homocitrulline and Urine orotic acid were 79.7 mmol/mol creatinine (normal range: less than 11 mmol/mol creatinine) and 117 mmol/mol creatinine (normal range: 0.05-6 mmol/mol), respectively which were also highly increased. The metabolic tests on his 13-month-old sister also showed hyperornithinemia of 823.6 $\mu\text{mol/L}$ (normal range: 27-96 $\mu\text{mol/L}$), hyperammonemia of 170 $\mu\text{mol/L}$ (normal range: less than 50 $\mu\text{mol/L}$), and homocitrullinuria of 94.5 mmol/mol creatinine (normal range: less than 4 mmol/mol creatinine).

3. Genetic testing

Whole blood was obtained from the family members: the father, mother, patient and younger sister. The institutional review board of Kyungpook National University Hospital approved the protocol, and informed consent forms were obtained for genetic analysis and for utilization of the results for diagnosis and research purposes from the participants or from their legal guardian (IRB no. KNUH 2016-06-011). Sanger sequencing of *SLC25A15* was performed by using dye terminator chemistry (Big-Dye) on an automated DNA sequencer (ABI3130, Applied Biosystems, Foster City, CA). Two sets of primers were used to amplify a target sequence from DNA of affected and unaffected individuals (primer sequences available upon request). Sanger sequencing of *SLC25A15* revealed that he and his sister have

compound heterozygous mutations of c.535C>T (p.R179*) and c.116C>A (p.T39K) (Fig. 1). p.R179*, a known pathogenic mutation, was inherited from their father. p.T39K (chr13:41373253), a novel mutation, was inherited from mother. p.T39K were verified by the in silico analysis database, PROVEAN (<http://provean.jcvi.org/index.php/>), SIFT (<http://sift.jcvi.org/>), and PolyPhen-2 (<http://genetics.bwh.harvard.edu/pph2/>). Prediction results were deleterious (-5.56) in PROVEAN, damaging (0.001) in SIFT, and possibly damaging (0.911) in PolyPhen-2, indicating that the amino-acid substitutions has high probability of pathogenicity.

Discussion

Diagnosis of HHH syndrome can easily be delayed or misdiagnosed. More than one fourth of cases are identified in adulthood although symptoms frequently begin during neonatal period or infancy¹⁰. The most common causes of delayed diagnosis are insidious symptoms and incomplete biochemical findings. Needless to say, it is important to consider detailed metabolic tests on patients with undiagnosed neurologic symptoms. However, diagnosis could still be delayed in patients presenting with incomplete metabolic triad of hyperammonemia, hyperornithinemia, and urinary excretion of homocitrulline. Individuals taking low protein diet can have little or no homocitrulline in the urine, although homocitrullinuria is a hallmark for detecting HHH syndrome. For laboratories that do not measure homocitrulline directly, an increase in urinary methionine may indicate homocitrullinuria because the peaks of methionine overlap with that of homocitrulline¹⁴. N-Bromo-

succinimide (NBS) with tandem mass spectrometry (MS/MS) measures plasma ornithine as a marker for HHH syndrome. A negative result of NBS does not exclude HHH syndrome because affected newborns may not present hyperornithinemia in the first few days after birth¹⁵. Once hyperornithinemia is detected in MS/MS, ornithine aminotransferase (OAT) deficiency should be included in differential diagnosis. However, OAT deficiency never presents with hyperammonemia and homocitrullinuria; presents mostly with ophthalmologic findings with gyrate atrophy of the choroid and retina^{16,17}.

When clinical or biochemical findings are imprecise, genetic testing should be used to confirm the diagnosis. *SLC25A15* maps on chromosome 13q14.11, spans about 23 kb and contains 7 exons, and encodes a 301 amino acid protein composed of six α -helices that traverse the inner mitochondrial membrane with the C- and N-termini exposed to the cytosolic side of the membrane¹⁸. Thirty five mutations have been identified up until now and the two most common mutations are F188del and p.R179*¹⁰. F188del accounts for about 30% of patients with HHH syndrome and it is common in French-Canadian descent because of a founder effect⁸. 179R* accounts for 15% of HHH patients and appears to be prevalent in patients of Japanese and Middle Eastern origin^{19,20}.

Although there is no genotype-phenotype correlation in HHH syndrome, genetic testing enables early treatment and prevents progression of the condition. Genetic testing of asymptomatic family members is extremely important because it is possible that two affected siblings may have completely different clinical outcomes like our patient and his younger sister. This is the first report describing mutations of Korean patients with HHH syndrome. Here we report siblings having compound

Fig. 1. Pedigree and sequencing chromatograms of each family members.

heterozygous mutations of c.535C>T (p.R179*) and c.116C>A (p.T39K) in the *SLC25A15* gene. We identified that p.T39K mutation is a novel pathogenic mutation causing HHH syndrome and that p.R179*, which is prevalent in Japanese and Middle Eastern heritage, is also found in Korean population.

요약

Hyperornithinemia-hyperammonemia-homocitrullinuria 증후군(HHH 증후군)은 경한 학습장애에서 심각한 뇌증에 이르기까지 다양한 신경학적 증상을 보일 수 있는 신경대사질환이다. HHH 증후군은 임상 증상이나 대사 검사 결과가 뚜렷하지 않아 진단이 늦어지는 경우가 흔한데, 이러한 경우 유전자 검사로 확진하는 것이 중요하다. 저자들은 강직성 하반신 마비를 가진 소아와 무증상의 여동생에서 HHH 증후군을 진단하고 유전자 검사를 통해 *SLC25A15* 유전자의 c.535C>T (p.R179*)와 c.116C>A (p.T39K) 변이를 확인하였다. p.R179*는 일본과 중동의 HHH 환자들에서 흔히 발견되는 변이로 한 국민에서도 발견됨을 확인하였고, p.T39K는 HHH 증후군을 유발하는 새로운 변이임을 최초로 보고하는 바이다.

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