Milder Form of Urea Cycle Defect Revisited: Report and Review of Hyperornithinaemia-Hyperammonaemia-Homocitrullinuria (HHH) Syndrome Diagnosed in a Teenage Girl Presenting with Recurrent Encephalopathy

Dear Editor,

Hyperornithinaemia-hyperammonaemia-homocitrullinuria (HHH) syndrome (OMIM #238970) is a rare autosomal recessive disorder associated with mutations of the *SLC25A15* gene which encodes the mitochondrial ornithine transporter 1 (ORNT1).[1] ORNT1 is responsible for the transport of cytosolic ornithine into the mitochondria in exchange for citrulline in the urea cycle and ornithine degradation pathway. A defect in this transporter results in accumulation of ornithine in the cytosol (resulting in hyperornithinaemia), disruption of the urea cycle (resulting in hyperammmonaemia) and increased secretion of homocitrulline in urine (a product of transcarbamoylation of lysine). This biochemical reaction cascade is affected by protein load in the diet.[2]

It is challenging to diagnose HHH syndrome as it is not detected on newborn metabolic screening.[3] Hyperornithinaemia develops beyond infancy and hyperammonaemia tends to be milder compared to other urea cycle disorders. However, it is a preventable cause of intellectual disability, and prompt diagnosis with appropriate management can improve long-term outcome and quality of life for patients. HHH syndrome was first described in 1969 by Shih and colleagues[4] and it constitutes only 1% to 3% of all urea cycle disorders.[5] HHH syndrome has the highest prevalence among French-Canadians, followed by Italians and Japanese.[6] HHH syndrome has not been reported locally and only been reported once previously in an individual of Indian descent.[7] We describe an additional case of a girl of Indian descent, who presented with recurrent episodes of altered mental state in association with febrile illnesses and was subsequently diagnosed with HHH syndrome.

Clinical Report

An 11-year-old Indian girl presented to our hospital with altered mental state in association with acute upper respiratory tract illness. She was feeding less due to poor appetite and had vomiting prior to admission. She had no fever. She took oral dexamethasone, clarithromycin and dextromethorphan for 2 days before admission. She was found to be confused and unable to recognise her family or surroundings. Physical examination revealed a well-thrived child. She had spontaneous eye opening and was able to move her limbs voluntarily. However, she was disoriented to time, place and person. She was afebrile. She was mildly tachypnoeic but the respiratory examination did not reveal any abnormality. Neurological evaluation revealed symmetrical hyperreflexia over bilateral knee and ankle joints with bilateral abnormal Babinski sign. She did not have ankle clonus and her strength was full. Her sensory examination was normal and she did not exhibit neck stiffness or any signs of cerebellar involvement (nystagmus, dysmetria, dysdiadokinesia, ataxia or pendular reflexes). She had no hepatosplenomegaly.

She is the second child of non-consanguineous parents of Indian descent. She had a maternal uncle who passed away in infancy of unknown cause. She had a personal preference for a protein restricted vegetarian diet because she felt unwell after consuming meat. Besides asthma, she had a previous admission at 5 years of age where she had experienced a brief period of altered mental state. It occurred during an episode of acute asthma exacerbation and had resolved spontaneously. She did not require intravenous hydration during that admission. No further workup was done at that point of time in view of the spontaneous remission of symptoms. She was developmentally appropriate and was coping in mainstream school, albeit with poor performance in Mathematics.

Brain magnetic resonance imaging (MRI), electroencephalogram and cerebrospinal fluid (CSF) biochemical analysis were normal. Blood investigations were significant for mild respiratory alkalosis (pH 7.466, pCO_2 34.4 mmHg, pO_2 68 mmHg, BE 1, HCO_3 24.8 mmol/L) and mildly elevated alanine transaminase at 44 U/L, (normal range 9 to 25 U/L). Her coagulation profile was normal. Ammonia levels revealed mild hyperammonaemia (84 μmol/L, reference range 9 to 33 μmol/L). In view of her encephalopathy, hyperammonaemia, respiratory alkalosis and a history of protein aversion, a possible diagnosis of a urea cycle disorder was suspected. Plasma amino acid analysis showed elevations of glutamine (881 μmol/L; normal range 400 to 750 μmol/L) and ornithine (197 μmol/L; normal range 35 to 155 μmol/L) with citrulline and arginine levels within their respective normal reference ranges. No argininosuccinic acid was detected. Orotic acid was present and abnormally raised in the urine [orotic acid to creatinine

ratio 14.6 µmol/mmol (normal range 0.0-3.5 µmol/mmol)]. Urine amino acid analysis showed a small but distinguishable peak corresponding to the retention time of homocitrulline. This finding in combination with hyperornithinaemia and hyperammonaemia suggested a possible diagnosis of HHH syndrome. Due to the presence of mild orotic aciduria in the biochemical workup, as well as family history of male neonatal death, the differential diagnosis considered for our patient was that of a heterozygous female presenting with partial ornithine transcarbamylase (OTC) deficiency, a more well known metabolic cause of recurrent encephalopathy in females. Sanger sequencing of *OTC* gene was negative. Sanger sequencing of *SLC25A15* gene revealed that she was compound heterozygous for 2 variants (*c.88T>G; p.F30V* and *c.113A>C; p.Q38P*). One of the variants *c.113A>C*; *p.Q38P* is known to be a pathogenic mutation, while the second is a novel variant *c.88T>G; p.F30V*. This variant was absent from dbSNP141, 1000 Genomes, Exome Aggregation Consortium and Exome Variant Server. The novel variant alters a highly conserved amino acid residue and was predicted by in silico prediction algorithms to be pathogenic. Parents were offered genetic testing of *SLC25A15* gene to phase the variants, as well as to determine their carrier status, but they declined due to financial constraints.

She was given intravenous dextrose drip after admission with improvements in her ammonia level to 44 µmol/L. After she was diagnosed with HHH syndrome, she was continued on a protein restricted diet (0.9 g protein/kg/day) and started on oral sodium benzoate which led to normal ammonia

Table 1. Comparison of the Clinical Presentation of Previously Reported Cases of HHH with Our Patient

	Debray et al[*]	**Miyamoto et al**[†]	**Tunali et al**[‡]	**Tezcan et al**[§]	**Lee et al**[‖]	**Our Patient**
Ethnic group	French Canadian	Japanese	Turkish	Indian	Chinese	Indian
Number of patients	16	2	1	1	1[¶]	1
Mutation	Homozygous *p.F188del*[**]	Homozygous *p.R179X*	Homozygous *p.A15V*	Compound heterozygous *p.G220R* and *p.R275del*	Compound heterozygous *p.R179X* and *p.T272I*	Compound heterozygous *p.F30V* and *p.Q38P*
Age of presentation (years)	2.7[#]	52[††] and 10[‡‡]	6	35	<1 (1 month)	11
Initial clinical presentation						
Neurological symptoms	9/16	2/2	Yes	Yes	No	Yes
Encephalopathy	3/16	2/2	Yes	Yes	No	Yes
Liver dysfunction	6/16	Unknown	Unknown	Unknown	Yes	Mildly elevated transaminases
Hyperammonaemia	12/16	2/2	Yes	Yes	Yes	Yes
Ammonia level at presentation (µmol/L)	146	90[††] and 132[‡‡]	300	114	132	84

[*]Debray FG, Lambert M, Lemieux B, Soucy JF, Drouin R, Fenyves D, et al. Phenotypic variability among patients with hyperornithinaemia-hyperammonaemia-homocitrullinuria syndrome homozygous for the *delF188* mutation in *SLC25A15*. J Med Genet 2008;45:759-64.
[†]Miyamoto T, Kanazawa N, Kato S, Kawakami M, Inoue Y, Kuhara T, et al. Diagnosis of Japanese patients with HHH syndrome by molecular genetic analysis: a common mutation, *R179X*. J Hum Genet 2001;46:260-2.
[‡]Ersoy Tunali N, Marobbio CM, Tiryakioglu NO, Punzi G, Saygili SK, Onal H, et al. A novel mutation in the *SLC25A15* gene in a Turkish patient with HHH syndrome: functional analysis of the mutant protein. Mol Genet Metab 2014;112:25-9.
[§]Tezcan K, Louie KT, Qu Y, Velasquez J, Zaldivar F, Rioseco-Camacho N, et al. Adult-onset presentation of a hyperornithinemia-hyperammonemia-homocitrullinuria patient without prior history of neurological complications. JIMD Rep 2012;3:97-102.
[‖]Lee HH, Poon KH, Lai CK, Au KM, Siu TS, Lai JP, et al. Hyperornithinaemia-hyperammonaemia-homocitrullinuria syndrome: a treatable genetic liver disease warranting urgent diagnosis. Hong Kong Med J 2014;20:63-6.
[¶]Patient 2 in the study was excluded as he was diagnosed by screening due to family history.
[#]Median.
[**]All are homozygous *p.F188del* except 1 patient with *p.F188del* with a second unidentified mutation.
[††]Patient 1.
[‡‡]Patient 2.

levels as well as initial improvement in her cognition and academic performance. She was educated on emergency feeding regimen including being on protein-free diet and high caloric carbohydrate drinks during sick days. She is now 12 years old and occasionally misses her medications and medical appointments. We continue to engage her and her family to improve compliance.

Discussion

This is the first case reported in the local population and the second case reported in an individual of Indian ethnicity, the former being a 35-year-old college graduate male from India with a history of protein restriction in his diet who had no neurological or neurocognitive deficits prior to presentation.[7] Both individuals had a history of self-restriction of protein in the diet and thus were generally asymptomatic. This likely contributed to the late clinical presentation and diagnosis. They both had episodic decompensation causing neurological symptoms and their conditions are stable with diet control and medications. Our patient had homocitrullinuria, while this was absent in the previously published case.

Compared to the reported clinical features of patients with HHH syndrome,[6] she shared some of the commonest clinical features of pyramidal signs, lethargy and abnormal behaviour. Most patients, like ours, were diagnosed between 1 to 12 years of age. In Table 1, we compared our patient with those reported previously in literature and include individuals of French-Canadian, [8] Japanese,[9] Chinese[10] and Turkish[2] descent. Although, there is a common founder mutation in the French-Canadian patients (*p.F188del*), there are no obvious differences in the phenotype among patients of different ethnic groups. In addition, there is a lack of phenotype-genotype correlation among patients with HHH syndrome, where individuals with the same genetic mutation may have different age of presentation and varying clinical outcomes.[2] Affected individuals have unremarkable antenatal and birth histories and varied clinical presentations ranging from prolonged hyperbilirubinaemia,[10] mild neurocognitive deficits to seizures, gait disturbances, stroke-like episodes and life threatening presentations of encephalopathy and liver failure.[11] The diagnosis can be easily missed because the encephalopathy and mild hyperammonaemia can be treated merely by intravenous dextrose drip which is typically given for patients with vomiting and poor feeding.

Conclusion

In conclusion, we report the first local case of HHH syndrome. Although our patient is only the second case of Indian origin, she shared many clinical features with previously reported patients from other ethnic groups. HHH syndrome should be considered in any female with mild hyperammonaemia, raised urine orotic acid and negative gene analysis for *OTC* gene. Early diagnosis and appropriate management can prevent life threatening complications and improve overall quality of life.

Acknowledgement

The authors would like to thank the patient and her family reported herein for their participation in this research. SSJ is supported by the Nurturing Clinican Scientist Scheme, SingHealth Duke-NUS Academic Clinical Programme.

REFERENCES

1. Bachmann C. Inherited hyperammonemias. In: Blau N DM, Blaskovic ME, Gibson KM, editor. Physician's guide to the laboratory diagnosis of metabolic diseases. Berlin: Springer; 2003. p. 261-76.
2. Ersoy Tunali N, Marobbio CM, Tiryakioglu NO, Punzi G, Saygili SK, Onal H, et al. A novel mutation in the SLC25A15 gene in a Turkish patient with HHH syndrome: functional analysis of the mutant protein. Mol Genet Metab 2014;112:25-9.
3. 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 Inherit Metab Dis 2010;33 Suppl 3:S275-81.
4. 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.
5. Batshaw ML, Tuchman M, Summar M, Seminara J. A longitudinal study of urea cycle disorders. Mol Genet Metab 2014;113:127-30.
6. Martinelli D, Diodato D, Ponzi E, Monne M, Boenzi S, Bertini E, et al. The hyperornithinemia-hyperammonemia-homocitrullinuria syndrome. Orphanet J Rare Dis 2015;10:29.
7. Tezcan K, Louie KT, Qu Y, Velasquez J, Zaldivar F, Rioseco-Camacho N, et al. Adult-onset presentation of a hyperornithinemia-hyperammonemia-homocitrullinuria patient without prior history of neurological

complications. JIMD Rep 2012;3:97-102.

8. Debray FG, Lambert M, Lemieux B, Soucy JF, Drouin R, Fenyves D, et al. Phenotypic variability among patients with hyperornithinaemia-hyperammonaemia-homocitrullinuria syndrome homozygous for the delF188 mutation in SLC25A15. J Med Genet 2008;45:759-64.
9. Miyamoto T, Kanazawa N, Kato S, Kawakami M, Inoue Y, Kuhara T, et al. Diagnosis of Japanese patients with HHH syndrome by molecular genetic analysis: a common mutation, R179X. J Hum Genet 2001;46:260-2.
10. Lee HH, Poon KH, Lai CK, Au KM, Siu TS, Lai JP, et al. Hyperornithinaemia-hyperammonaemia-homocitrullinuria syndrome: a treatable genetic liver disease warranting urgent diagnosis. Hong Kong Med J 2014;20:63-6.
11. Mhanni AA, Chan A, Collison M, Seifert B, Lehotay DC, Sokoro A, et al. Hyperornithinemia-hyperammonemia-homocitrullinuria syndrome (HHH) presenting with acute fulminant hepatic failure. J Pediatr Gastroenterol Nutr 2008;46:312-5.

Syeda Kashfi Qadri*, [1]*MD*, Teck Wah Ting*, [1]*MBBS*, James SC Lim, [2]*PhD*, Saumya Shekhar Jamuar, [1]*MBBS*

[1]Department of Paediatrics, KK Women's and Children's Hospital; Paediatrics Academic Clinical Programme, SingHealth Duke-NUS Medical School, Singapore
[2]Department of Laboratory Medicine, KK Women's and Children's Hospital, Singapore

*Joint first authors.

Address for Correspondence: Dr Saumya Shekhar Jamuar, Department of Paediatrics, KK Women's and Children's Hospital, 100 Bukit Timah Road, Singapore 229899.
Email: Saumya.s.jamuar@kkh.com.sg