A novel mutation in ornithine transcarbamylase gene causing mild intermittent hyperammonemia

Sarar Mohamed, MD, FRCPCH, *Muddathir H. Hamad,* MD, ABP, *Altaf A. Kondkar,* MSc, PhD, *Khaled K. Abu-Amero,* MSc, PhD.

ABSTRACT

نستعرض في هذا المقال حالة مريض سعودي يبلغ من العمر 3 سنوات وقد أتى إلى المستشفى وهو يعاني من قيئ متكرر وتغذية ضعيفة، بالإضافة الى تغير مزاجه مصحوباً بفرط أمونيا الدم الخفيفة المتقطعة، وزيادة في حمض الأورتيك البولي. وقد أظهر فحص الجينات بتسلسل سانغر عن وجود طفرة جينية جديدة في جين اورنيثين ترانس كاربوميليز. وقد كانت هذه الطفرة الجينية هي المسؤولة عن ظهور نقص اورنيثين ترانس كاربوميليز.

We report a 3-year-old Saudi boy with recurrent episodes of vomiting, poor feeding, and altered mental status accompanied by an intermittent mild hyperammonemia, and a large elevation of urinary orotic acid. Sanger sequencing of the ornithine transcarbamylase (OTC) gene revealed a novel hemizygous deletion at the fourth nucleotide of intron 4 (c.386+4delT) in the proband and his asymptomatic mother. This novel mutation in the OTC gene is responsible for the late-onset phenotype of OTC deficiency.

Saudi Med J 2015; Vol. 36 (10): 1229-1232
doi: 10.15537/smj.2015.10.12127

From the Department of Pediatrics (Mohamed, Hamad), and the Ophthalmic Genetics Laboratory (Kondkar, Abu-Amero), Department of Ophthalmology, College of Medicine, King Saud University, Riyadh, Kingdom of Saudi Arabia

Received 23rd April 2015. Accepted 1st August 2015.

Address correspondence and reprint request to: Dr. Sarar Mohamed, Department of Pediatrics (39), College of Medicine, King Saud University, PO Box 2925, Riyadh 11461, Kingdom of Saudi Arabia. E-mail: sararmohamed@hotmail.com

Disclosure. Authors have no conflict of interests, and the work was not supported or funded by any drug company. This study was supported by the College of Medicine Research Center, Deanship of Scientific Research, King Saud University, Riyadh, Kingdom of Saudi Arabia.

Hyperammonemia is a life-threatening condition caused by inherited and acquired diseases. Urea cycle disorders (UCD) are the leading inborn errors that present with hyperammonemia.[1] All the 6 urea cycle enzyme defects are inherited as autosomal recessive except ornithine transcarbamylase (OTC) deficiency (OTCD; MIM#311250), which is an X-linked disease.[2] Ornithine transcarbamylase is a mitochondrial enzyme that catalyzes the second step of the urea cycle leading to the synthesis of citrulline from ornithine and carbamoyl phosphate.[1] The prevalence of OTC deficiency is estimated to range from 1 in 40,000 to 1 in 80,000.[3,4] The phenotype of OTC deficiency is extremely heterogeneous. Patients with OTC deficiency usually present with poor feeding, vomiting, and respiratory alkalosis, and may progress to seizures, encephalopathy, and death.[1] Late-onset disease presents beyond the neonatal period with life-threatening hyperammonemia and occasionally with intermittent episodes of metabolic decompensation.[5] These patients with late-onset disease usually have more residual enzyme activity compared to classic OTC phenotype. Aside from the clinical phenotype, the diagnosis of OTC deficiency is based on the presence of hyperammonemia, high glutamine, and low arginine in serum amino acid and demonstration of orotic aciduria.[2] Previously, enzyme study using liver biopsy tissues was the gold standard for the diagnosis of this condition.[1] Currently, the diagnosis is confirmed by detection of the pathogenic mutation(s) in the OTC gene. The human OTC gene was mapped to the short arm of chromosome Xp21.1 and encodes a 354 amino acids protein.[2] There exists mutational heterogeneity in the OTC gene. More than 341 mutations have been identified in the OTC gene that are distributed throughout the gene and most of which are private mutations.[5] A large number of reported variants are missense mutations (68%), followed by nonsense, insertions, and deletions in the coding region (18%). The remaining variants include splice site variants affecting splicing of OTC mRNA.[5,6] In this study, we report a

novel mutation in the OTC gene in a Saudi boy who presented with mild intermittent hyperammonemia.

Case Report. We report a 3-year-old Saudi boy attending King Saud University Medical City (KSUMC), Riyadh, Saudi Arabia in March 2014. An informed consent was obtained from the family of the patient. The proband is a product of a non-consanguineous marriage, was born at term by spontaneous vaginal delivery following an uneventful pregnancy. His development was appropriate for his age. He was the third child in the family with 2 healthy sisters (**Figure 1**). There is no family history of inherited diseases or early neonatal death, and the mother was healthy and remained asymptomatic. Our patient was apparently well until the age of one year when he was admitted to the hospital with pneumonia. He presented again at the age of 2 years with 3 days history of vomiting, poor feeding, and altered mental status. His physical examination showed a lethargic, confused child with signs of moderate dehydration. His liver and spleen were not palpable. Central nervous system examination revealed normal cranial nerves, and normal upper, and lower limbs. His initial laboratory investigations showed serum ammonia of 135 µmol/L (normal <50 µmol/L) with a normal serum lactate and negative urine ketones (**Table 1**). Complete blood count, liver function tests, and renal function tests were all normal. His cerebrospinal fluid analysis was negative for viral and bacterial infections. His brain CT was unremarkable. Intravenous dextrose 10% was started, and he was kept on protein-free diet. He improved clinically, and the ammonia level returned to normal. He was discharged and booked for follow-up in the outpatient clinic, but unfortunately, missed his appointment. One year later, he presented to our hospital with vomiting, decreased activity, and feeding associated with sleepiness. Clinical assessment revealed signs of moderate dehydration with a normal chest, cardiovascular system, and abdominal examination. Apart from lethargy, central nervous system examination showed normal power, tone, and reflexes. He was commenced on intravenous infusion of ½ normal saline containing 5% dextrose. His initial ammonia level was 178 µmol/L and increased to 269 µmol/L in 6 hours. Intravenous fluids were changed to ½ normal saline containing 10% dextrose at 150 ml/kg/d (10 mg/kg/minute of glucose). Arginine, Sodium benzoate, and Sodium phenylbutyrate infusion were also given. His urine organic acids showed large elevation of orotate, uracil, 3-hydroxybutyrate, and acetoacetate.

In addition, glutarate and 3-hydroxyglutarate were also detected in a small amount. Serum amino acids showed glutamine of 630 µmol/l (normal <700), arginine of 105 µmol/l (normal 100-150), and citrulline of 80 µmol/l (normal <200). Also, acylcarnitine profile was normal. Our patient responded nicely to ammonia scavenger drugs. His ammonia levels returned to normal within 48 hours, and his clinical examination was normal. He was discharged home and maintained on oral Arginine, Sodium benzoate, Sodium phenylbutyrate, and low protein formula. He continued follow-up in the outpatient clinic for one year without metabolic decompensation or hyperammonemia. His most recent neurological and developmental assessment were normal.

Molecular genetic testing. Sample collection and DNA preparation. DNA from our patient and his family members was obtained from peripheral blood (7 mL) collected in EDTA tubes. The extraction was performed using the Illustra Blood GenomicPrep Mini Spin Kit (GE Healthcare, Buckinghamshire, UK) and stored at -20°C in aliquots until further use. Quantification of extracted DNA was performed using a NanoDrop ND-2000c spectrophotometer (Thermo Scientific, Wilmington, DE, USA).

Sequencing. All 10 exons of the OTC gene and their flanking intron regions were amplified using the polymerase chain reaction (PCR), and the nucleotide sequences were determined using Sanger sequencing.[6] Sequencing was performed using BigDye terminator v3.1 cycle sequencing kit (Applied Biosystems Inc., Foster City, CA, USA). Fragments were electrophoresed on the ABI 3130xl Genetic Analyzer (Applied Biosystems) according to the manufacturer's protocol. All the sequenced fragments were then analyzed using SeqScape software v2.6 (Applied Biosystems).

Genetic findings. Direct sequencing of the region encompassing exon 4 revealed a hemizygous deletion at the fourth nucleotide of intron 4 (c.386+4delT), which is a novel variant. This variant is in proximity to the Invariant splicing site. For this reason, we used computer-based programs, Splice Site Finder, to study the effects of this variant on splicing.[7] This database predicted that this variant is likely to affect normal splicing and may represent destruction of the highly conserved GT dinucleotide sequence at the splice donor site. This mutation was also detected in the mother, but none from the 2 sisters.

Discussion. Ornithine transcarbamylase deficiency is the most common urea cycle enzymopathy that

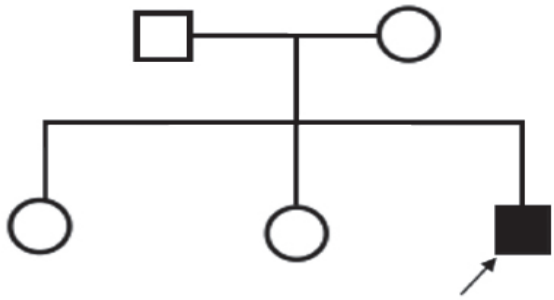

Figure 1 - Family pedigree. ■ - Proband (affected), □ - healthy male, ○ - healthy female

Table 1 - Characteristic of the patient with ornithine transcarbamylase (OTC).

Variables	Results
Gender	Male
Age at initial presentation	2 years
Consanguinity	Non-consanguineous
Urine pH	7.47
Ketonuria	Absent
Initial ammonia	135 μmol/L
Highest ammonia	269 μmol/L
Urine organic acids	Large elevation of orotate, uracil, 3-hydroxybutyrate, and acetoacetate, low levels of glutarate and 3-hydroxyglutarate
Serum amino acids	Normal
Acylcarnitine profile	Normal
OTC gene mutation	Hemizygous novel variant c.386+4delT

presents in the neonatal period, or after, as a late-onset disease. Recent data show that the late-onset disease is more prevalent than the classic neonatal phenotype.[8] Our patient presented with mild elevation of ammonia, at 2 occasions, associated with unexplained poor feeding, lethargy, and altered consciousness. These non-specific symptoms are frequently seen in sick infants and young children and usually attributed to infections. In order to save the life of such children, pediatricians should think of other diagnoses, such as acute metabolic disorders, especially urea cycle defect. The probability of these disorders is high if the patient is too sick, rapidly deteriorated or had subtle signs unexplained by sepsis such as respiratory alkalosis. For these reasons, ammonia should be measured in any child with unexplained acute illness. The recurrent non-specific symptoms of our patient together with the high ammonia alerted us to the possibility of late-onset UCD. However, the serum amino acids did not show the typical pattern of high glutamine and low arginine usually seen with OTC deficiency and certain UCD. Also, increased urinary orotic acid was suggestive of OTC deficiency, but this can also be seen in other distal UCD, lysinuric protein intolerance (LPI) and hyperammonemia, hyperornithinemia homocitrulinuria (HHH) syndrome. The presence of normal serum and urine amino acids virtually excluded LPI and HHH syndrome. Molecular defects in OTC deficiency have been increasingly identified since the mapping of human OTC gene. For this reason, we opted to sequence the OTC gene, which revealed the presence of a novel hemizygous mutation (c.386+4delT); thus, it confirmed the diagnosis of late-onset OTC deficiency in our patient. Although the splice site variant (c.386+4delT) identified in this patient has not been previously reported, but some other splice site variants had been previously reported.[6] These include c.386+1G>C, c.386+1G>T, and c.386+1G>A involving the highly conserved GT dinucleotide sequence at the splice donor site in patients with OTC deficiency.[6] This strongly indicates that the c.386+4delT is the mutation responsible for the late-onset OTC deficiency phenotype seen in our patient. Splice site mutations occur in number of other genes. Such mutations can have a variety of consequences including exon skipping and intron retention.[6,7,9] Most of them affect the splice sites or their surrounding consensus sequences.

Females who are heterozygous for OTC gene mutations may exhibit skewed X-inactivation, and for this reason, may become symptomatic under metabolic stress as in the post-partum period and during severe infections.[2] We detected the OTC novel mutation in both the proband and his mother, who remained asymptomatic and never had complaints related to UCD. Also, her ammonia and serum amino acid were found to be normal following the result of the OTC gene mutation. We plan to monitor her closely and intervene on any suspicion of hyperammonemia.

Management of patients with OTC deficiency entails measures to reduce acute hyperammonemia including high calories, low protein intake, intravenous ammonia scavenger drugs, and renal dialysis if needed.[2] Maintenance therapy relies on low protein formula and oral ammonia detoxifying medications. Liver transplantation is indicated in patients with severe disease and frequent metabolic decompensation.[2] The long-term complications of OTC disease are developmental delay, intellectual disability, growth retardation, and attention deficit hyperactivity disorder.[10]

A recent review[8] found that the mortality rate of UCD including OTC was 24% in neonatal-onset cases and 11% in late-onset.[8] Identification of OTC deficiency is highly critical for patient management, and can help to reduce mortality rate. Although gene mutations are found in ~80% of patients with OTC deficiency, a combination of clinical, biochemical, and molecular analysis can facilitate definitive diagnosis of OTC deficiency and related disorders.[2,6]

In conclusion, we have identified a novel splice site mutation in the OTC gene in a patient with mild intermittent hyperammonemia and his asymptomatic mother.

References

1. Häberle J. Clinical and biochemical aspects of primary and secondary hyperammonemic disorders. ***Arch Biochem Biophys*** 2013; 536: 101-108.
2. 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.
3. Testai FD, Gorelick PB. Inherited metabolic disorders and stroke part 2: homocystinuria, organic acidurias, and urea cycle disorders. ***Arch Neurol*** 2010; 67: 148-153.
4. Summar ML, Koelker S, Freedenberg D, Le Mons C, Haberle J, Lee HS, et al. The incidence of urea cycle disorders. ***Mol Genet Metab*** 2013; 110: 179-180.
5. Yamaguchi S, Brailey LL, Morizono H, Bale AE, Tuchman M. Mutations and polymorphisms in the human ornithine transcarbamylase (OTC) gene. ***Hum Mutat*** 2006; 27: 626-632.
6. Shchelochkov OA, Li F-Y, Geraghty MT, Gallagher RC, Van Hove JL, Lichter-Konecki U, et al. High-frequency detection of deletions and variable rearrangements at the ornithine transcarbamylase (OTC) locus by oligonucleotide array CGH. ***Mol Genet Metab*** 2009; 96: 97-105.
7. Houdayer C. In silico prediction of splice-affecting nucleotide variants. ***Methods Mol Biol Clifton NJ*** 2011; 760: 269-281.
8. Batshaw ML, Tuchman M, Summar M, Seminara J, Members of the Urea Cycle Disorders Consortium. A longitudinal study of urea cycle disorders. ***Mol Genet Metab*** 2014; 113: 127-130.
9. Ogino W, Takeshima Y, Nishiyama A, Okizuka Y, Yagi M, Tsuneishi S, et al. Mutation analysis of the ornithine transcarbamylase (OTC) gene in five Japanese OTC deficiency patients revealed two known and three novel mutations including a deep intronic mutation. ***Kobe J Med Sci*** 2007; 53: 229-240.
10. Diaz GA, Krivitzky LS, Mokhtarani M, Rhead W, Bartley J, Feigenbaum A, et al. Ammonia control and neurocognitive outcome among urea cycle disorder patients treated with glycerol phenylbutyrate. ***Hepatol Baltim Md*** 2013; 57: 2171-2179.

Related Articles

Al-Sannaa NA, Cheriyan GM. Carnitine-acylcarnitine translocase deficiency. Clinical course of three Saudi children with a severe phenotype. ***Saudi Med J*** 2010; 31: 931-934.

Hertecant JL, Al-Gazali LI, Karuvantevida NS, Ali BR. A novel mutation in ARG1 gene is responsible for arginase deficiency in an Asian family. ***Saudi Med J*** 2009; 30: 1601-1603.