

Case Report

A novel mutation in ornithine transcarbamylase gene identified from a Chinese child with ornithine transcarbamylase deficiency

Bo Wu^{1,2*}, Yi-Zheng Wang^{1*}, Li-Rong Cao^{1*}, Rui-Ping Zhang¹, Yu-Lian Fang³, Yu-Qin Zhang², Chun-Quan Cai⁴

¹Graduate College of Tianjin Medical University, Tianjin 300070, China; Departments of ²Neurology, ³Institute of Pediatric, ⁴Neurosurgery, Tianjin Children's Hospital, Tianjin 300134, China. *Equal contributors.

Received May 2, 2017; Accepted March 11, 2018; Epub June 15, 2018; Published June 30, 2018

Abstract: Objective: The purpose of this study was to analyze a novel pathogenic variant for ornithine transcarbamylase deficiency (OTCD) and to deepen understanding of OTCD. Methods: One case of 14-month-old female OTCD patient was analyzed. Clinical data of the child patient were collected and venous blood 2 ml from the patient and her parents was extracted respectively. The ornithine transcarbamylase (*OTC*) exons were amplified using PCR amplification. Results: In the clinical symptoms, the patient showed intermittent drowsiness, vomiting, and cerebellar ataxia. According to brain magnetic resonance imaging, the lesion was severe. Blood chemistry showed mild hepatic lesion and increased blood ammonia. According to gas chromatography mass spectrometry assay, there was a rise in urine uracil and urine orotic acid. Genetic sequencing results showed a nonsense mutation was identified in exon 8 of the *OTC* gene in the proband and her mother, and the gene mutation has never been reported previously. Meanwhile, the phenotype of her mother was normal. Conclusion: Classical OTCD has the symptoms of hyperammonemia and the resulting in varying degrees of damage to the nervous system and the liver. Without clinical specificity, this disease is easily to be misdiagnosed. Methods like blood ammonia and urine metabolic disease screening, blood amino acid analysis and genetic testing help confirm the disease earlier. As for treatment, early intervention and chronic control of blood ammonia level to guard against hyperammonemia will lead to a better curative effect.

Keywords: Gas chromatography mass spectrometry assay, hyperammonemia, liver damage, mutation, ornithine transcarbamylase deficiency

Introduction

Ornithine transcarbamylase deficiency (OTCD) is the most common type of congenital urea cycle disorders [1, 2] and is an X-linked inherited disease [3, 4]. The morbidity rate of OTCD is 1 in 14000 [5]. The human *OTC* gene, with a full length of 73 kb containing 10 exons, is mapped to the short arm of chromosome Xp21.1 [6], and at least 435 mutation types have been identified [7]. Age of onset varies from newborn to adult, with high mortality in infancy. Patients with OTCD usually present with poor feeding, vomiting, and may progress to seizures, encephalopathy, and death. Due to the low incidence and various manifestations, OTCD was usually misdiagnosed as intracranial infection, digestive system diseases, hepatic encephalopathy, Reye' syndrome or poisoning. Early diagnosis and treatment is the key to reduce mor-

tality and disability. Here, we report a novel mutation in the *OTC* gene in a Chinese girl who presented with cerebellar ataxia.

Case report

We reported a 14-month-old Chinese girl admitted to Tianjin Children's Hospital in May 2014. An informed consent was obtained from her parents. The child was a product of a non-consanguineous marriage, who was born at term by spontaneous vaginal delivery following an uneventful pregnancy. She was the second child in the family with a healthy brother. There was no family heredity history. The proband was hospitalized for lethargy and vomiting for 4 days in the local hospital at the age of 11 months. Her initial laboratory investigations showed serum ammonia of 94 $\mu\text{mol/L}$ (normal value: 27~82 $\mu\text{mol/L}$), with high levels of ala-

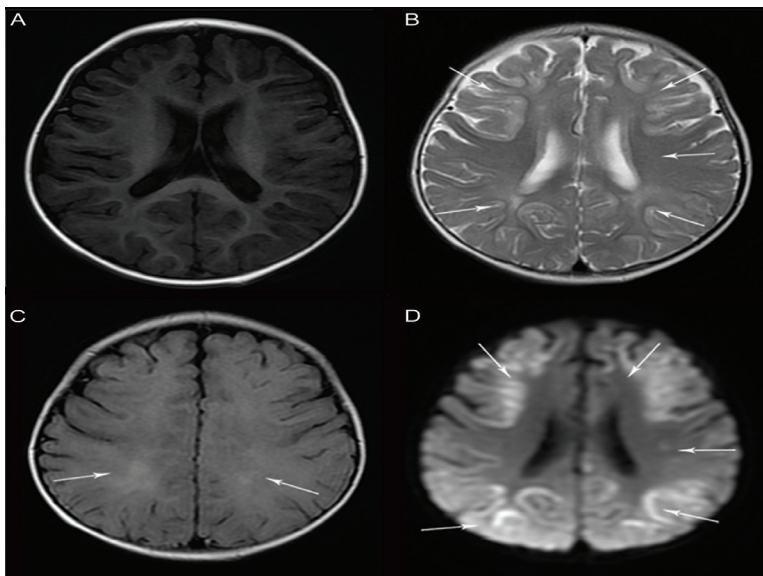


Figure 1. Brain MRI lesions in the child with ornithine transcarbamylase deficiency (arrow indicated the lesions of the case). A. T1WI shows enlarged ventricle, cistern and sulci; B. T2WI demonstrates enlarged ventricle, cistern and sulci and patchy hyper intense lesions in cortical areas of bilateral frontal, parietal lobes and left temporal lobe, as well as in bilateral parietal white matter; C. DWI sequence reveals patchy hyper intense lesions in bilateral parietal white matter; D. FLAIR sequence confirms patchy hyper-intense lesions in cortical areas of bilateral frontal and parietal lobes and left temporal lobe.

nine aminotransferase (ALT, 72 U/L) and aspartate aminotransferase (AST, 46 U/L). Moreover, electroencephalogram (EEG) showed theta activity of middle-high voltage, 5~6 c/s in the occipital area. In addition, a small amount of acetaminophen was detected in her cerebrospinal fluid (CSF). A recheck of serum ammonia was 62.0 $\mu\text{mol/L}$ on the third day in hospital. The patient discharged with improved spirit and was diagnosed as a drug response and upper respiratory tract infection. However, the proband presented psychomotor development regression in the next 3 months, and she could not sit or stand without support. She presented to our hospital with 3 days history of lethargy, complaint of vomiting and irritability. Physical examination revealed a poor spirit, confused child, with signs of developmental retardation. Her weight was 8.2 kg, head circumference was 44.5 cm. The heart and lung were normal. The liver was enlarged (below costal margin 4 cm). Muscle tension was remarkably decreased in all four extremities, and the muscle force grade of the limbs was IV. In addition, tendon reflex was enhanced (+++), and bilateral Babinski sign was negative. She could not sit without support and had volitional tremor.

Magnetic resonance imaging (MRI) showed enlarged ventricle, cistern and sulci in T1 weighted image (T1WI) and T2 weighted image (T2WI) (**Figure 1A, 1B**), patchy hyper intense lesions of the white matter in bilateral parietal lobes on T2WI and diffusion weighted imaging (DWI) sequence (**Figure 1B, 1C**). Meanwhile, on T2WI and fluid attenuated inversion recovery (FLAIR) sequence, patchy hyperintense lesions were seen in the bilateral parietal lobe and left temporal lobe cortex (**Figure 1B, 1D**). EEG showed delta activity of middle-high potential, 2~3 c/s in the occipital region. CSF was normal. Laboratory evaluation showed ALT of 117 U/L, AST of 71 U/L, blood ammonia of 94 $\mu\text{mol/L}$, and lactic acid of 6.22 mmol/L with partially compensated respiratory alkalosis

(pH 7.440, PaCO_2 3.875 kPa, and BE 3.1 mmol/L). The serum amino acids revealed mildly elevated aspartic acid, asparagine, glycine, methionine, lysine and tyrosine. Apart from a large elevation of orotate and uracil, pyrimidine metabolites and 4-Hydroxyphenylpyruvic acid also increased slightly in urine according to gas chromatography mass spectrometry (GC-MS) assay. The proband was clinically diagnosed as OTCD. She was on a low protein diet (1 g/kg.d). Lactulose, glucose and L-carnitine were also given. After three days of treatments, the review blood ammonia decreased to 80 $\mu\text{mol/L}$, and her clinical examination was normal. The child discharged from our department and maintained on oral L-carnitine and low protein diet. The symptoms of ataxia were relieved, but the psychomotor development disorder was not improved during follow-up. However, the patient was lost to follow-up 2 months later.

Gene mutation analysis

Genomic DNA extraction

Peripheral blood genomic DNA was extracted from the proband and her parents respectively. In brief, the white precipitate was extracted

Novel mutation in ornithine transcarbamylase gene

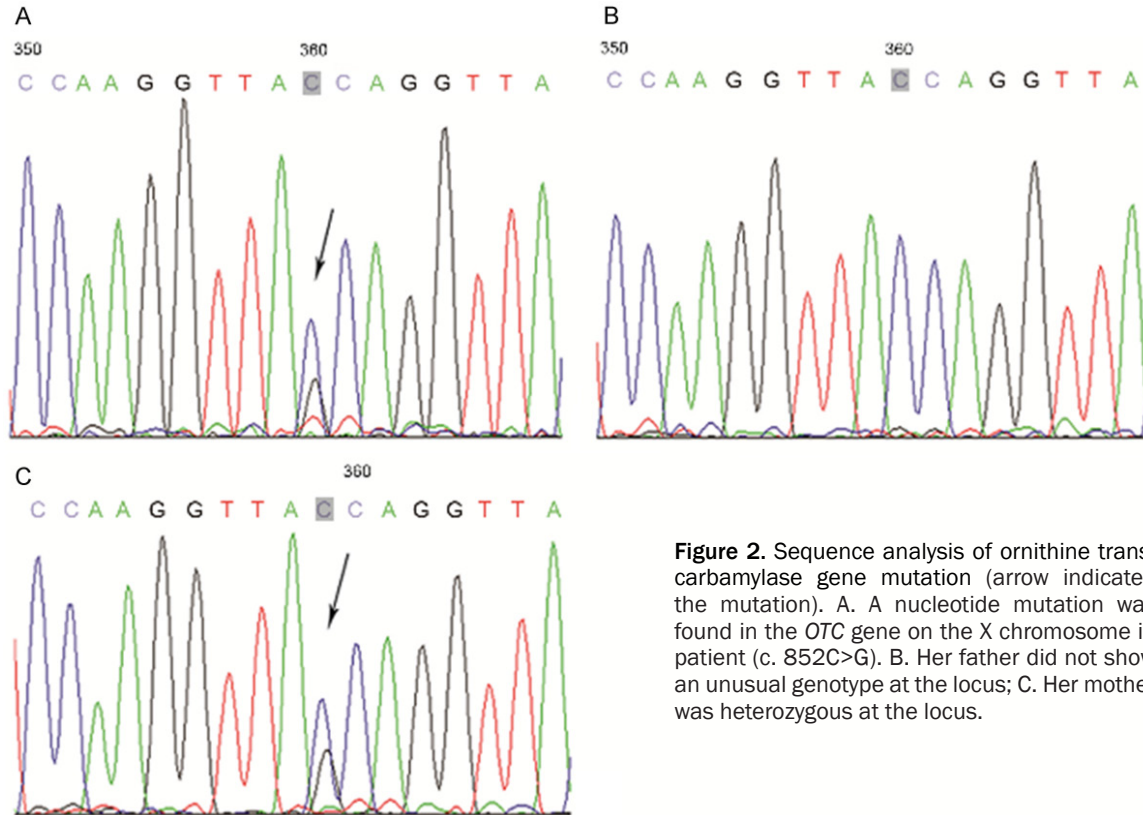


Figure 2. Sequence analysis of ornithine transcarbamylase gene mutation (arrow indicated the mutation). A. A nucleotide mutation was found in the *OTC* gene on the X chromosome in patient (c. 852C>G). B. Her father did not show an unusual genotype at the locus; C. Her mother was heterozygous at the locus.

using ethylene diamine tetra acetic acid (ED-TA) and tergitol-type NP-40. Then leucocytes were resuspended and lysed with 10% sodium dodecyl sulfate (SDS). After centrifugation, genomic DNA was extracted by gDNA mini kit (Biomiga, Jiaying, China) according to the manufacturer's instructions.

PCR amplification

Based on relevant articles [8, 9], primers produced by two companies (Sangon Biotech, Shanghai, China; Invitrogen, Shanghai, China) were used to amplify the 10 exons and adjacent introns of the *OTC* gene. The reaction mixture (final volume 50 μ L) contained 5 μ L 10 \times PCR buffer, 4 μ L dNTPs, 0.1 μ mol/L of each primer, 0.4 μ L EX *Taq* DNA polymerase (Takara, Dalian, China), and 6 μ L template DNA. The reaction conditions were as follows: pre-denaturation at 94 $^{\circ}$ C for 5 minutes; 35 cycles at 94 $^{\circ}$ C for 30 seconds, 54~60 $^{\circ}$ C for 35 seconds, and 72 $^{\circ}$ C for 30 seconds, 72 $^{\circ}$ C for 35 seconds; 72 $^{\circ}$ C for 7 minutes. The PCR products were identified by agarose gel electrophoresis and sequenced directly using ABI3730 automated sequencer (Invitrogen, Shanghai, China).

Sequencing analysis

Direct sequencing revealed a novel nonsense mutation at nucleotide 852 of exon 8 (c. 852C>G, p. Y284X) which resulted in a change of amino acid 284 from tyrosine to a stop codon. This mutation was also detected in her asymptomatic mother, but was not detected from the father. Sequencing results were shown in **Figure 2**.

Discussion

We performed a PubMed search using the keywords "Chinese" and "ornithine transcarbamylase deficiency" in English or Chinese literature and restricted the search to cases of patient who has relatively complete clinical information. A total of 80 cases of patients have been selected [10-19]. The clinical features and outcome of these patients were reviewed and analyzed (**Table 1**). Obviously, the clinical manifestations of OTCD are non-specific. Neonates with OTCD are often presented with poor suck, reduced intake and apnea, followed by lethargy progressing to somnolence and coma. Infant patients show vomiting, irritability, difficulty falling asleep, developmental delay. Child patients

Novel mutation in ornithine transcarbamylase gene

Table 1. Clinical data of 80 OTCD patients in mainland China

Authors	Cases	Age of onset	Sex	Type	Clinical presentation	Plasma amino acids		Urine orotate acid	Clinical outcome
						Ammonia	Citrulline		
Mak et al. [10], 2007	1	3 y	M	L	Encephalopathy, coma and gastroenteritis	Normal	Normal	↑	Recovery
Sun et al. [11], 2011	1	3 d	M	N	Vomiting, seizure	↑	↓	↑	Death
Mo et al. [12], 2011	3	20 d-2 y	3 M	1 N and 2 L	1 vomiting, 1 irritability and 1 somnolence	3 ↑	3 NA	3 ↑	3 Recovery
Han et al. [13], 2014	40	3 d-29 y	23 M and 17 F	9 N and 31 L	15 vomiting, 10 dyspnea, 9 seizure, 6 poor suck and 1 growth retardation	40 ↑	24 ↓ and 16 Normal	40 ↑	8 Death and 32 NA
Wang et al. [14], 2014	3	1 d-9 d	3 M	3 N	3 convulsion, 1 poor suck and 1 dyspnea	3 ↑	3 Normal	3 ↑	1 Death and 2 NA
Chen et al. [15], 2014	3	11 mo-1 y 8 mo	3 M	3 L	1 infectious diarrhea, 1 cerebral palsy, dysplasia and 1 upper respiratory infection	3 ↑	2 ↓ and 1 NA	2 ↑ and 1 Normal	2 Death and 1 Recovery
Gao et al. [16], 2015	1	2.5 y	F	L	Vomiting, irritability	↑	Normal	↑	Recovery
Tong et al. [17], 2015	1	9 y	M	L	Vomiting, irritability	↑	↓	↑	Death
Liu et al. [18], 2015	3	2 d-9 d	3 M	3 N	3 convulsion, 1 pneumonia and 1 poor reaction	3 ↑	3 ↓	3 ↑	2 Death and 1 NA
Shao et al. [19], 2017	24	1 d-15 y	17 M and 7 F	5 N and 19 L	13 vomiting, 6 seizure, 2 growth retardation, 3 irritability and 4 coma	24 ↑	9 ↓ and 15 Normal	NA	12 Death, 7 Recovery and 5 ND

y = years, d = days, mo = months, F = Female, M = Male, L = late onset OTCD (≤ 30 d), N = neonatal onset OTCD (> 30 d), NA = Not Available, ND = Neurocognitive Damage.

Novel mutation in ornithine transcarbamylase gene

manifest convulsions, sleep disorders, and neuropsychological complications. Adult patients manifest coma or mental behavior disorder. The age and severity of OTCD were associated with sex and gene mutation types. In hemizygous male patients, the phenotype is determined by the nature of mutation and other yet unknown factors (other genes/environment) [7, 20]. In contrast, besides the type of gene mutation, the clinical spectrum of female heterozygotes depends on the extent to which the abnormal gene is expressed [20]. The patients are subdivided into 2 groups based on age of onset and severity of clinical features, including neonatal onset OTCD (≤ 30 d) and late onset OTCD (LO-OTCD) (>30 d). Severe *OTC* gene mutations, which located in the active site or the protein interior, lead to hyperammonemic coma in the neonatal period or in early infancy, which is often fatal due to no residual enzyme activity, so it's also known as the early-onset; some affected patients may, however, exhibit a delayed onset of the disease, the so called "late onset OTCD" due to different *OTC* residual activity in the liver, which is resulted from the more milder mutation that located on the surface of the protein residues or away from the active site [21]. The patient with LO-OTCD can present from infancy to later childhood, adolescence or adulthood, but most of them are infancy. For some adolescence or adulthood, hyperammonemic encephalopathy can be triggered by high protein load [7]. The clinical presentation of patients with LO-OTCD shows a great individual difference in symptoms and course. Furthermore, the clinical manifestations of patients were distinctly different, even the patients have the same mutation in the same family. Notably, some *OTC* mutation carriers may have no onset throughout life.

The proband was sent to the hospital 2 times for treatment in 3 months, with liver damage and enlargement, as well as mild elevation of blood ammonia. Besides, her urine organic acids showed high levels of orotate and uracil. All these alerted us to the possibility of LO-OTCD. DNA sequencing revealed the presence of an 852C>G substitution on the *OTC* exon 8, which resulted in replacement of tyrosine by a stop codon in codon 284. Thus, it confirmed the diagnosis of LO-OTCD. Also, this mutation was detected in her asymptomatic mother. The mother rejected the test of blood biochemical and urine GC-MS. Therefore, her biochemical phenotype was unknown. To date, the same

gene mutation has never been reported previously. Additionally, studies have shown that the mutant *OTC* gene types were mostly missense mutation, and in half of late-onset patients, the mutant gene came from their parents [22, 23]. In this context, for the families which have positive patients, it was necessary to detect and manage other family members [24].

Detection of blood ammonia plays an important role in the diagnosis of new patients. Simultaneously, patients with hyperammonemia usually have severe symptoms and a poor prognosis. Moreover, hyperammonemic coma can cause permanent nerve injury or death. Wakiya et al. [25] observed that nervous system damage in some patients caused by hyperammonemia was difficult to alleviate even after liver transplantation. Besides, the long-term prognosis of patients with a low level of blood ammonia was significantly better than those with a high level of blood ammonia [26]. Acute liver failure (ALF) is another common indicator for the diagnosis. Gallagher et al. [27] observed that more than half of the 49 patients with symptomatic OTCD had liver damage, and the proportion with ALF was greatest in those with more severe OTCD. Notably, the extent of liver involvement is not always proportional to the level of high blood ammonia. And ALF may be the initial clinical presentation of some patients. Furthermore, Samuel et al. [28] reported 1 case of OTCD patient who had liver failure complicated with coagulopathy.

For the patients with typical OTCD, analysis of serum amino acids always shows a high level of glutamine and a lower level of citrulline. In addition, increased urinary orotic acid and uracil can be detected by GC-MS assay. The analysis of plasma amino acids and GC-MS in the intermittent period are always atypical. Accordingly, the sample analysis during the attack should be done. In recent years, genetic analysis has become an effective and feasible method for diagnosis of OTCD. Genetic analysis is readily available, which not only can be contributed to the diagnosis and prognosis for all probands, but also can be used for genealogy gene analysis to find asymptomatic carriers and to provide evidence for genetic counseling and prenatal diagnosis. As a previous gold standard for diagnosing OTCD, analysis of *OTC* enzyme activity in the liver requires a liver biopsy [29], which is rarely used now because of poor operability.

Novel mutation in ornithine transcarbamylase gene

For suspected OTCD patients with acute or chronic encephalopathy, unexplained hyperammonemia or liver damage, blood ammonia monitoring should be used as routine testing. Besides, GC-MS assay of urine should be carried out as soon as possible. Clinically diagnosed patients should be confirmed by genetic testing. To assess the condition of disease comprehensively, enzyme test, imaging examination and neuropsychological test should be performed. The treatment of OTCD includes acute stage and remission stage. During the acute stage, the treatment mainly includes the reduction of blood ammonia and supportive therapy. During the remission stage, the treatment should emphasize long-term strict management to patients focusing on avoiding inducements, including high protein diet, infection, fever, fatigue, drinking, drugs and other incentives. Living donor liver transplantation has achieved a certain effect in some countries. Also, liver cell transplantation and gene therapy are under study. The prognosis of patients with OTCD depends on several factors such as type of onset, the time of diagnosis and the management of long-term blood ammonia. Patients with neonatal-onset or with hyperammonemic coma have a poor prognosis. Frequent episodes of hyperammonemia can lead to learning disabilities and intellectual disability even if the diet therapy is given early.

In conclusion, early diagnosis and intervention can help to reduce the mortality rate and improve the prognosis as well as life quality. In this work, we have identified a novel mutation in the *OTC* gene in a patient with cerebellar ataxia and her asymptomatic mother. It could provide helpful information for the gene mutation analysis of *OTC*, and contribute to the diagnosis of OTCD in clinical practices, as well as provide meaningful exploration for identification of *OTC* in clinical core genetic pedigrees.

Acknowledgements

This work was supported by the Key Project of Tianjin Health Care Professionals [grant number 16KG166]; National Natural Science Foundation of China [grant number 81771589].

Disclosure of conflict of interest

None.

Address correspondence to: Chun-Quan Cai, Department of Neurosurgery, Tianjin Children's Hospital, No. 238 Longyan Road, Beichen District, Tianjin 300134, China. Tel: 86 15122656313; E-mail: tjps@126.com; Yu-Qin Zhang, Department of Neurology, Tianjin Children's Hospital, No. 238 Longyan Road, Beichen District, Tianjin 300134, China. Tel: 86 13920351807; E-mail: zhangyuqin0809@sina.com

References

- [1] Rüegger CM, Lindner M, Ballhausen D, Baumgartner MR, Beblo S, Das A, Gautschi M, Glahn EM, Grünert SC, Hennermann J, Hochuli M, Huemer M, Karall D, Kölker S, Lachmann RH, Lotz-Havla A, Möslinger D, Nuoffer JM, Plecko B, Rutsch F, Santer R, Spiekerkoetter U, Staufner C, Stricker T, Wijburg FA, Williams M, Burgard P, Häberle J. Cross-sectional observational study of 208 patients with non-classical urea cycle disorders. *J Inher Metab Dis* 2014; 37: 21-30.
- [2] Batshaw ML, Tuchman M, Summar M, Seminara J. A longitudinal study of urea cycle disorders. *Mol Genet Metab* 2014; 113: 127-130.
- [3] Kido J, Kawasaki T, Mitsubuchi H, Kamohara H, Ohba T, Matsumoto S, Endo F, Nakamura K. Hyperammonemia crisis following parturition in a female patient with ornithine transcarbamylase deficiency. *World J Hepatol* 2017; 9: 343-348.
- [4] Chantreuil J, Favrais G, Fakhri N, Tardieu M, Rouillet-Renoleau N, Perez T, Travers N, Barantin L, Morel B, Saliba E, Labarthe F. Intracranial pressure monitoring demonstrates that cerebral edema is not correlated to hyperammonemia in a child with ornithine transcarbamylase deficiency. *JIMD Rep* 2016; 27: 55-62.
- [5] Kang ES, Snodgrass PJ, Gerald PS. Ornithine transcarbamylase deficiency in the newborn infant. *J Pediatr* 1973; 82: 642-649.
- [6] Lindgren V, de Martinville B, Horwich AL, Rosenberg LE, Francke U. Human ornithine transcarbamylase locus mapped to band Xp21.1 near the Duchenne muscular dystrophy locus. *Science* 1984; 226: 698-700.
- [7] Cavicchi C, Donati M, Parini R, Rigoldi M, Bernardi M, Orfei F, Gentiloni Silveri N, Colasante A, Funghini S, Catarzi S, Pasquini E, la Marca G, Mooney S, Guerrini R, Morrone A. Sudden unexpected fatal encephalopathy in adults with *OTC* gene mutations-clues for early diagnosis and timely treatment. *Orphanet J Rare Dis* 2014; 9: 105.
- [8] 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.

Novel mutation in ornithine transcarbamylase gene

- [9] Summar ML, Dobbelaere D, Brusilow S, Lee B. Diagnosis, symptoms, frequency and mortality of 260 patients with urea cycle disorders from a 21-year, multicentre study of acute hyperammonaemic episodes. *Acta Paediatr* 2008; 97: 1420-1425.
- [10] Mak CM, Siu TS, Lam CW, Chan GC, Poon GW, Wong KY, Low LC, Tang NL, Li SK, Lau KY, Kwong NS, Tam S. Complete recovery from acute encephalopathy of late-onset ornithine transcarbamylase deficiency in a 3-year-old boy. *J Inherit Metab Dis* 2007; 30: 981.
- [11] Sun WH, Yang Y, Zhang YP, Li XY, Zhang M, Cao Y, Wang Y. Analysis of clinical features, biochemical analysis and gene mutations in one Chinese pedigree with neonatal-onset ornithine transcarbamylase deficiency. *Zhonghua Er Ke Za Zhi* 2011; 49: 356-360.
- [12] Mo WQ, Liu L, Chen YY, Chen J, Li XZ, Zhou ZH, Mao XJ, Zhang W. Clinical and gene mutation analyses of three patients with ornithine carbamoyltransferase deficiency. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi* 2011; 28: 328-331.
- [13] Han F, Han L, Ye J, Qiu H, Gao X, Wang Y, Gong Z, Gu X. Clinical characteristics and analysis of mass spectrometric data in patients with ornithine transcarbamylase deficiency. *Zhonghua Yi Xue Za Zhi* 2014; 94: 2684-2686.
- [14] Wang Y, Liu X, Wu H, Liu H, Wang C, He X. Analysis of clinical features, metabolic profiling and gene mutations of patients with ornithine transcarbamylase deficiency. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi* 2014; 31: 148-151.
- [15] Chen Z, Wen P, Wang G, Liu X, Chen S, Wan L, Cui D, Shang Y, Li C. Analysis of ornithine transcarbamylase gene mutation in three boys affected with late-onset ornithine transcarbamylase deficiency. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi* 2014; 31: 565-569.
- [16] Gao J, Gao F, Hong F, Yu H, Jiang P. Hyperammonemic encephalopathy in a child with ornithine transcarbamylase deficiency due to a novel combined. *Am J Emerg Med* 2015; 33: 474, e1-e3.
- [17] Tong WJ, Jin TQ, Sun JM. Report of a case with late-onset ornithine transcarbamylase deficiency with gas chromatography-mass spectrometry and DNA sequencing confirmation and literatures review. *Zhonghua Er Ke Za Zhi* 2015; 53: 366-369.
- [18] Liu J, Dong L, Wang Y, Zhang M. Two novel mutation of ornithine transcarbamylase gene identified from three Chinese neonates with ornithine transcarbamylase deficiency. *Int J Clin Exp Med* 2015; 8: 2656-2661.
- [19] Shao Y, Liang M, Lin Y, Mei H, Zhang W, Cai Y, Su X, Hu H, Li X, Li L. Clinical and mutation analysis of 24 Chinese patients with ornithine transcarbamylase deficiency. *Clin Genet* 2017; 92: 318-322.
- [20] Thurlow VR, Asafu-Adjaye M, Agalou S, Rahman Y. Fatal ammonia toxicity in an adult due to an undiagnosed urea cycle defect: under-recognition of ornithine transcarbamylase deficiency. *Ann Clin Biochem*. 2010; 47: 279-281.
- [21] Numata S, Koda Y, Ihara K, Sawada T, Okano Y, Matsuura T, Endo F, Yoo HW, Arranz JA, Rubio V, Wermuth B, Ah Mew N, Tuchman M, Pinner JR, Kirk EP, Yoshino M. Mutant alleles associated with late-onset ornithine transcarbamylase deficiency in male patients have recurrently arisen and have been retained in some populations. *J Hum Genet* 2010; 55: 18-22.
- [22] Brassier A, Gobin S, Arnoux JB, Valayannopoulos V, Habarou F, Kossorotoff M, Servais A, Barbier V, Dubois S, Touati G, Barouki R, Lesage F, Dupic L, Bonnefont JP, Ottolenghi C, De Lonlay P. Long-term outcomes in ornithine transcarbamylase deficiency: a series of 90 patients. *Orphanet J Rare Dis* 2015; 10: 58-71.
- [23] Choi JH, Lee BH, Kim JH, Kim GH, Kim YM, Cho J, Cheon CK, Ko JM, Lee JH, Yoo HW. Clinical outcomes and the mutation spectrum of the OTC gene in patients with ornithine transcarbamylase deficiency. *J Hum Genet* 2015; 60: 501-507.
- [24] Fujisawa D, Mitsubuchi H, Matsumoto S, Iwai M, Nakamura K, Hoshida R, Harada N, Yoshino M, Endo F. Early intervention for late-onset ornithine transcarbamylase deficiency. *Pediatr Int* 2015; 57: e1-e3.
- [25] Wakiya T, Sanada Y, Urahashi T, Ihara Y, Yamada N, Okada N, Egami S, Sakamoto K, Murayama K, Hakamada K, Yasuda Y, Mizuta K. Living donor liver transplantation from an asymptomatic mother who was a carrier for ornithine transcarbamylase deficiency. *Pediatr Transplant* 2012; 16: E196-E200.
- [26] Kido J, Nakamura K, Mitsubuchi H, Ohura T, Takayanagi M, Matsuo M, Yoshino M, Shigematsu Y, Yorifuji T, Kasahara M, Horikawa R, Endo F. Long-term outcome and intervention of urea cycle disorders in Japan. *J Inherit Metab Dis* 2012; 35: 777-785.
- [27] Gallagher RC, Lam C, Wong D, Cederbaum S, Sokol RJ. Significant hepatic involvement in patients with ornithine transcarbamylase deficiency. *J Pediatr* 2014; 164: 720-725.
- [28] Samuel N, Politansky AK, Hoffman R, Itzkovich S, Mandel H. Coagulopathy unmasking hepatic failure in a child with ornithine transcarbamylase deficiency. *Isr Med Assoc J* 2013; 15: 777-779.
- [29] Gascon-Bayarri J, Campdelacreu J, Estela J, Reñé R. Severe hyperammonemia in late-onset ornithine transcarbamylase deficiency triggered by steroid administration. *Case Rep Neurol Med* 2015; 2015: 453752.