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# Gene Mutation Analysis and Prenatal Diagnosis of the Ornithine Transcarbamylase (OTC) Gene in Two Families with Ornithine Transcarbamylase Deficiency

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**Background:** The aim of this study was to perform gene detection in 2 clinical cases of highly suspected ornithine transcarbamylase deficiency (OTCD) pediatric patients by first-generation sequencing technology in order to confirm the pathogenic genetic factors of their families and allow the families to undergo genetic counselling and prenatal diagnosis.





**Material/Methods:** The peripheral DNA samples of 2 children with highly suspected OTCD (the probands) and their parents were collected. DNA fragments corresponding to exons 1–10 of the OTC gene from the samples were amplified using polymerase chain reaction (PCR), and then subjected to Sanger sequencing to confirm the pathogenic mutation sites.

**Results:** The probands were both confirmed to have OTCD. The proband in Family 1 was a male carrying a c.867+1G>C mutation at a splice site within the OTC gene. The gene detection results of amniotic fluid cells at 16 weeks of pregnancy showed that the fetus was a male who also carried the c.867+1G>C mutation. The proband in Family 2 was a male carrying a c.782T>C(p. I261T) mutation in the OTC gene. The gene detection results of amniotic fluid cells at 18 weeks showed that the fetus was a male without pathogenic mutations in the OTC gene. The gene detection results of peripheral blood from the fetus after birth were consistent with those obtained from amniotic fluid cells.

**Conclusions:** Pediatric children who are clinically suspected of OTCD can receive a definitive diagnosis through OTC gene detection.

**MeSH Keywords:** **Mutation • Ornithine Carbamoyltransferase Deficiency Disease • Prenatal Diagnosis**

**Full-text PDF:** <https://www.medscimonit.com/abstract/index/idArt/911295>

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## Background

Ornithine transcarbamylase deficiency (OTCD, OMIM: 311250) is a hereditary metabolic disease with the main symptom of hyperammonemia caused by OTCD. This disease is the most common form of the urea cycle disorders, and the total number of affected people accounts for 50% of urea cycle disorder prevalence [1–3]. The pathogenic gene for OTCD is ornithine transcarbamylase (OTC). OTCD belongs to the X-linked recessive genetic disorders; therefore, almost all hemizygous males develop this disease. Approximately 20% of female carriers present some neurocognitive disorders due to unfavorable random X-inactivation; however, the symptoms are substantially milder than those of male patients [4].

The OTC gene is located on chromosome Xp21.1. The full-length gene is 73 kb and contains 10 exons and 9 introns. The encoded OTC protein contains 322 amino acids [5,6]. This enzyme is mainly expressed in the liver with only a small portion expressed in the intestinal mucosa [7]. The reduction of functional OTC causes a disorder of the urea cycle, thus resulting in reduced citrulline and arginine and increased alanine and glutamine in the plasma, significantly increased orotic acid and uracil in the urine, and significantly increased ammonia in the blood [8]. The severity of clinical symptoms of OTCD is associated with the level of enzyme deficiency. When mutations occur within the protein or at active sites, the catalytic functions of OTC are almost completely lost. Patients with these mutations usually experience disease onset during the neonatal stage and have severe forms of OTCD. The major presentations are vomiting, apastia, lethargy, convulsion, muscle hypotonia, and coma [9,10]. When mutations occur on the protein surface or distant from active sites, the affinity between the enzyme and the substrate is reduced or the stability of the enzyme decreases; patients with these mutations have late disease onset and milder forms of OTCD. The major presentations are intermittent recurrent lethargy, irritability, restlessness, and ataxia. Feeding of high-protein food, infection, and trauma can all induce the onset of OTCD. Mental retardation, microcephaly, and hepatomegaly are common [11]. Because OTCD symptoms are nonspecific, it can easily be mistaken for general digestive system infection or other neurological diseases. In addition, treatment and prognosis between OTCD and these other diseases are largely different; therefore, early diagnosis is particularly important to guide treatment and prognosis.

Currently, the diagnosis of OTCD is mainly based on clinical symptoms, blood ammonia levels, blood tandem mass spectrometry (MS-MS), urine gas chromatography–mass spectrometry (GC-MS), and imaging examination results. The definitive diagnosis depends on detection of OTC activity or genetic testing. This study used first-generation sequencing technology to perform gene detection in 2 clinical cases of highly suspected OTCD

pediatric patients in order to confirm the pathogenic genetic factors of their families and allow the families to undergo genetic counselling and prenatal diagnosis.

## Material and Methods

### Study participants

**Family 1:** The parents of the proband were both healthy and were not consanguineous. The first child was a girl who was born after full-term normal delivery and has thus far appeared healthy. The second child was a baby boy who was born after full-term normal delivery. The baby boy was admitted into the neonatal department because of poor feeding, poor spirit, coma, and lethargy on the 2<sup>nd</sup> day after birth. The maternal grandmother of the proband in this family had given birth to 3 boys and 2 girls. Two boys died within 1 month after birth (Figure 1).

**Family 2:** The parents of the proband were both healthy and were not consanguineous. There was no similar patient in this family (Figure 1). The proband was a boy and the first-born. He was admitted to the hospital due to poor mental response, feeding difficulty, vomiting, and convulsions on the 7<sup>th</sup> day after birth.

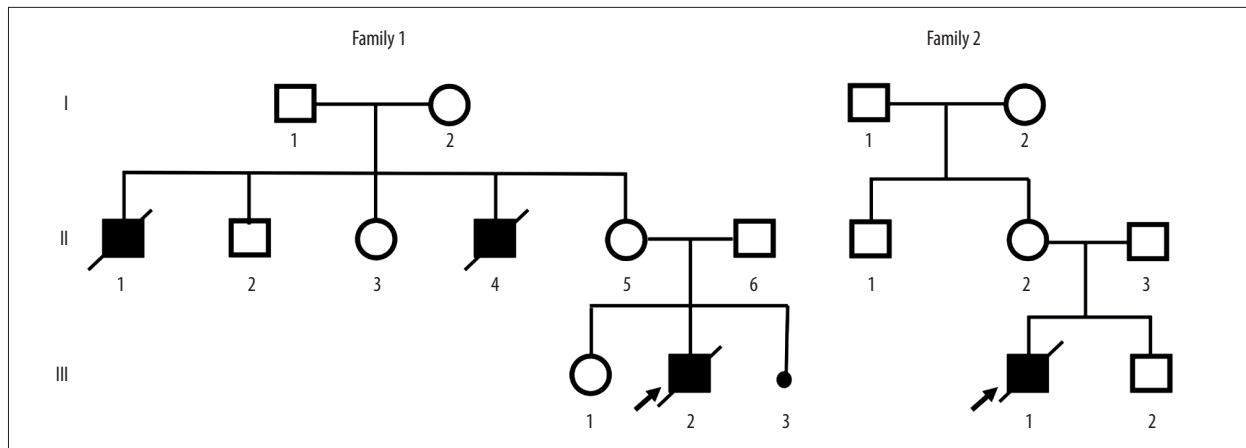
This study was approved by the Ethics Committee of the Sixth Affiliated Hospital of Sun Yat-sen University. The patients' parents provided informed consent for both gene detection studies and prenatal diagnosis.

### Specimen collection and genomic DNA extraction

Venous blood samples (2 mL) were collected from the probands and their parents in the 2 case Families, additionally, samples were collected from the maternal grandmother and the maternal uncle in the case of Family 2. We also collected 10 mL amniotic fluid from the mother in Family 1 at 16 weeks and the mother in Family 2 at 18 weeks when they became pregnant again. Genomic DNA was extracted using the QIAamp DNA Blood Midi Kit (Qiagen, Duesseldorf, Germany). Quality control assessment of DNA samples was performed using the NanoDrop 2000 ultra-microvolume nucleic acid and protein spectrophotometer (Thermo, Waltham, MA, USA). The purity of DNA was required to be between 1.8 and 2.0.

### Primer design

The OTC gene sequences were retrieved from the National Center for Biotechnology Information (NCBI) database. Primers were designed using the Primer Premier 5.0 (La Jolla, CA, USA) and synthesized by Thermo Fisher Scientific (Waltham, MA, USA). The primer sequences are shown in Table 1.



**Figure 1.** The pedigree maps of 2 OTCD families. □ indicates males with the normal phenotype, ○ indicates females with the normal phenotype, ■ indicates males who died of this disease, ♀ indicates the probands of these families, and ↓ the early termination of the affected fetus.

**Table 1.** Amplification primers of base sequences for 10 exons in OTC gene.

Exon	Primer sequence (5'-3')	Amplification length (bp)	Annealing temperature (°C)
1	F: TACCTTTGCTCCCTCACTGC	446	58.0
	R: CAAACGAAAGATGTCAAGAACG		
2	F: ATGCCTTATCAACAGTAAAACAATG	502	58.0
	R: CAGACAAATGATTCTTAGGTTTCG		
3	F: CACTTATTGGGGGTAGTTATTACT	374	56.5
	R: TCTGCTTGACGCTTTTATGAG		
4	F: TGAATAAGAGGGTGGAGAAATGG	509	60.0
	R: TTCAACCGATGGAAAGTGGC		
5	F: CACTGGATAAGTCGTGGGAGG	570	60.0
	R: GGAATAGGGTTGAGTGCAGAGC		
6	F: TGTCTGGATGTTAATGGTGCTC	577	58.0
	R: GCTGGTAACGTAACCTAAATTCTG		
7/8	F: AAGAAGTCGGAGATAGTGAAAGC	648	57.0
	R: CTAGGGGATACAGTCAGTGAGTC		
9	F: CCATCACTCTGCTCCTTTGTC	561	58.0
	R: ATAATGGTTGGCAAAGTGGT		
10	F: TTAAGTGAACCCTGCAAAGCTG	314	59.5
	R: GTTTCACAATGGCAAAGCATATC		

### Polymerase chain reaction (PCR) amplification and sequencing

The polymerase chain reaction (PCR) components were as follows: 5.0 µL 10× Buffer, 4.0 µL dNTP, 0.3 µL Taq DNA polymerase, 2 µL upstream primer (10 µM), 2 µL downstream primer (10 µM), and 3 µL DNA template (50 ng/µL); deionized water was added to obtain a final volume of 50 µL. The PCR conditions were as follows: pre-denaturation at 94°C for 5 min;

35 cycles of denaturation at 94°C for 30 sec, annealing at 58°C for 30 sec and extension at 72°C 30 sec; and a final extension at 72°C for 7 min. The system was then kept at 4°C.

After the PCR amplification was completed, 8-µL products were subjected to 2% agarose gel electrophoresis. The results were observed using an ultraviolet imaging apparatus to confirm whether the product had a single target band. Qualified PCR

**Table 2.** Gene mutation analysis and prenatal diagnosis in the two OTCD families.

Family	Member	Gene	Exon	Nucleotide mutation	Protein mutation	Heterozygote/ homozygote
1	Proband	OTC	IVS8	c.867+1G>C	Splice site	Hemizygote
	Mother			c.867+1G>C	Splice site	Heterozygote
	Foetus			c.867+1G>C	Splice site	Hemizygote
2	Proband	OTC	8	c.782T>C	p. I 261 T	Hemizygote
	Mother			c.782T>C	p. I 261 T	Heterozygote
	Foetus			No mutation	–	–
	Maternal grandmother			c.782T>C	p. I 261 T	Heterozygote
	Maternal Uncle			No mutation	–	–

products were sent to Thermo Fisher Scientific (Waltham, MA, USA) for Sanger sequencing.

### Result analyses

The sequencing results were viewed using Chromas software. The sequencing results for the target sequences were compared with the OTC gene sequence from the NCBI database (NM\_000531.5) to identify whether any gene mutations were present.

## Results

### Analyses of clinical information

The major laboratory test result for the proband in Family 1 showed the blood ammonia level was higher than 600 µmol/L. The MS-MS results showed that the concentration of citrulline was lower than the reference value (3.66 µmol/L; reference value: 5.00–30.00 µmol/L) and the concentrations of alanine, glutamic acid, and tryptophan were higher than the reference values. The urine GC-MS results showed that urine orotic acid was significantly higher than the reference value (41.60 µmol/L; reference value: 0.00–1.50 µmol/L), which was accompanied by increased concentrations (relative to the reference values) of lactic acid, oxalic acid, pyruvic acid, 3-hydroxybutyric acid, adipic acid, pimelic acid, suberic acid, and sebacic acid. Combined with the results regarding blood and urine metabolism, OTCD was highly suggested; this patient died after an ineffective rescue.

The major laboratory test results of the proband in Family 2 showed that the blood ammonia level was higher than 700 µmol/L. The blood MS-MS results showed the concentrations of citrulline (1.77 µmol/L; reference value: 4.00–40.00 µmol/L) and proline

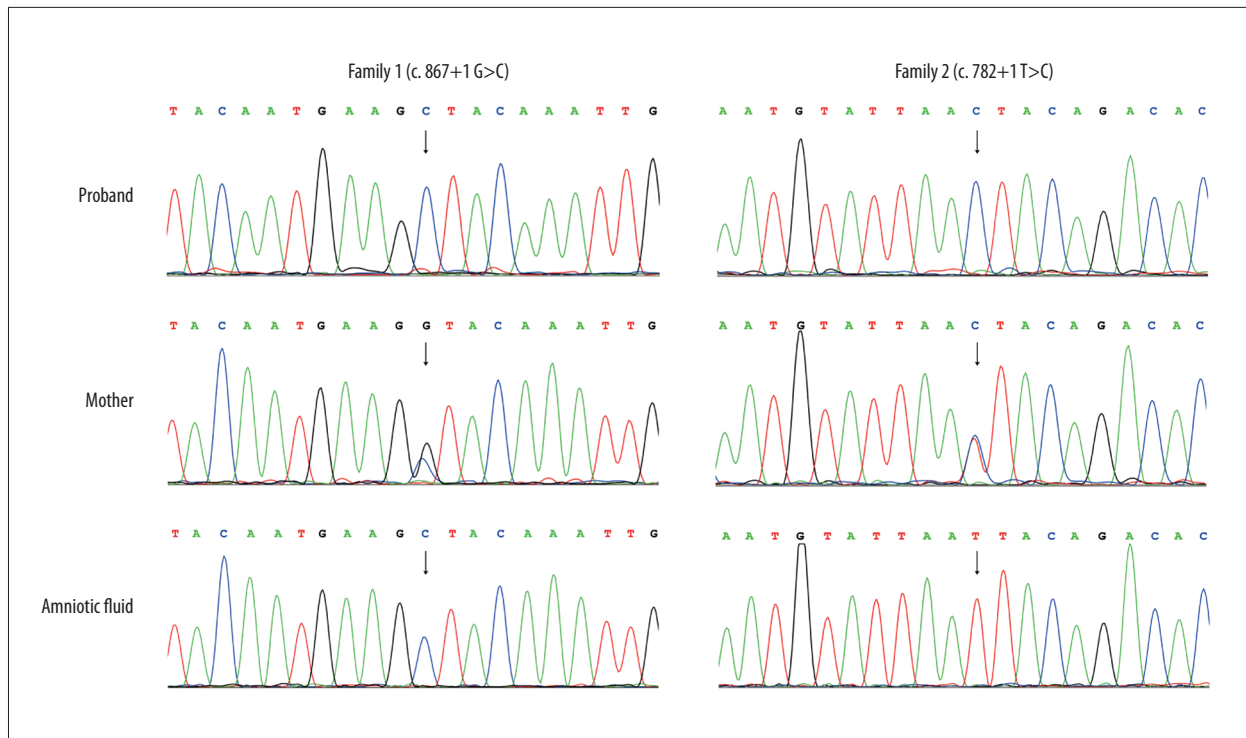
were lower than the reference values and the concentrations of alanine, glutamine, lysine, and methionine were higher than the reference values. The urine GC-MS results showed that urine levels of orotic acid (analytic value 33.11; baseline value 0.033) and uracil (analytic value 2.15; baseline value 0.041) were significantly higher than the baseline values. In addition, the urine concentrations of lactic acid, succinic acid, and glycerol acid were also increased with respect to the baseline values. Combined with the blood and urine metabolism results, OTCD was highly suggested; this patient died after an ineffective rescue. The proband's mother became pregnant again, and the prenatal diagnosis results showed that this fetus was not an OTCD patient. None of MS-MS or GC-MS analyses conducted after the fetus was born showed abnormal metabolic products.

### Sequencing results and prenatal diagnosis results for these 2 families

Sequencing results confirmed that the pathogenic gene for these 2 families was the OTC gene. The probands were both confirmed to have OTCD. The sequencing results are shown in Table 2.

The proband of Family 1 was a male who carried an OTC gene mutation of c.867+1G>C at a splice site. This mutation was inherited from his mother. Genetic analyses of the amniotic fluid at 16 weeks showed that this fetus was a male carrying the c.867+1G>C hemizygous mutation. Combined with the genotype of the proband, this fetus was speculated to have OTCD; therefore, the parents decided to terminate the pregnancy. The results of genetic analyses of the abortive tissues were consistent with those from amniotic fluid (Figure 2).

The proband from Family 2 was a male who carried the c.782T>C mutation in the OTC gene. This mutation was inherited from



**Figure 2.** Sanger sequencing results of probands, mothers, and amniotic fluid in the 2 OTCD families. ↓ indicates the positive mutation sites.

his mother. Genetic analyses of the amniotic fluid at 18 weeks showed that this fetus was a male who did not carry any known pathogenic mutations in the OTC gene. After the fetus was delivered normally, the gene detection results from the peripheral blood were consistent with those from the amniotic fluid (Figure 2). The gene detection results from the maternal grandmother of the proband suggested that she was a carrier of the c.782T>C mutation; however, the exon sequences of the OTC gene from the proband's maternal uncle did not show any known pathogenic mutations.

## Discussion

The clinical manifestation of OTCD includes the toxic symptom of hyperammonemia. The initial presentations are usually brain injury, liver injury, and other digestive system symptoms that involve many organs and systems. Acute hyperammonemia induces vomiting, convulsions, poor responses, coma, and lethargy; however, chronic hyperammonemia induces psychomotor retardation, growth retardation, and abnormal liver function. Currently, the diagnosis of OTCD is mainly based on biochemical detection, blood gas analysis, blood MS-MS detection, urine GC-MS detection, and imaging examinations [12]. The typical OTCD laboratory examinations can reveal increased blood ammonia levels and abnormal liver function; blood MS-MS detection can determine reductions in citrulline concentration and

increases in glutamine and alanine; urine GC-MS can detect significant increases in the concentrations of uracil and orotic acid; and cranial imaging examination can display changes such as brain edema and brain atrophy. The laboratory test results for the pediatric patients in the 2 families described in this study all met the typical OTCD features. In addition, the urine GC-MS detection results for these 2 children showed that in addition to the increased concentrations of uracil and orotic acid, the concentration of lactic acid was also significantly increased. This lactic acid level might have been caused by the formation of glutamine due to excessive ammonia and  $\alpha$ -ketoglutaric acid or glutamic acid, which would in turn block the tricarboxylic acid cycle and cause pleomorphic changes to the liver mitochondria [13] and further induce hyperlactacidemia. The 2 children in this study did not receive cranial imaging examinations because they both had disease onset within several days after birth and had rapidly developing disease courses.

Ever since Horwich et al. [5] successfully cloned and sequenced the OTC gene, more than 400 types of mutations and 29 polymorphism *loci* have been discovered in China and other countries. Approximately 84% of mutations are single base substitution mutations, 12% are small-fragment deletions or insertion mutations, and 4% are large-fragment deletion mutations. Approximately 42% of mutations are associated with disease onset during the neonatal stage, 21% with late disease onset, and 37% with disease onset in females [7,14–19].



The mutation sites in the OTC gene are associated with the age at disease onset and the severity of OTCD. Mutations on the protein surface or distant from the active sites cause partial loss of the enzyme activity, late age at disease onset, and milder symptoms, whereas the mutations inside the protein or on active sites cause loss of most enzyme activity or even complete loss of enzyme activity, which is typically associated with disease onset in the neonatal stage and severe clinical symptoms. The 2 children in this study both had disease onset during the neonatal stage and rapid disease progression; both patients died after ineffective rescue. Therefore, it was speculated that the c.867+1G>C and c.782T>C mutation sites in the OTC that caused disease onset in these 2 patients both had important effects on OTC activity. Of the 2 mutations, the c.867+1G>C mutation has already been reported as a pathogenic mutation. It has been shown that OTCD pediatric patients harboring this mutation usually have severe disease onset during the neonatal stage [20]. The c.782T>C mutation is a newly discovered mutation. The results of this study suggest that this mutation is a pathogenic mutation of OTCD; however, functional validation experiments on this site have not yet been performed.

A mutation of the 785 base in exon 8 of the OTC gene from C to T switches threonine at the 262 amino acid residue to isoleucine. This mutation only affects the binding between OTC and its substrate ornithine; therefore, pediatric patients with this mutation mostly have late onset and milder symptoms [21]. However, the pathogenic T to C mutation of the proband in Family 2 in this study was at the 782 base in exon 8 of the OTC gene, thus switching the isoleucine at the 261 amino acid residue to threonine. This patient was admitted to the hospital due to the presence of a poor mental response, poor feeding, vomiting, and convulsions on the 7<sup>th</sup> day after birth. The patient died on the 12<sup>th</sup> day. The results suggested that the c.782T>C mutation could cause disease onset during the neonatal stage, and the induced symptoms were more severe.

## References:

1. Caldovic L, Abdikarim I, Narain S et al: Genotype-phenotype correlations in ornithine transcarbamylase deficiency: A mutation update. *J Genet Genomics*, 2015; 42: 181-94
2. Prasun P, Altinok D, Misra VK: Ornithine transcarbamylase deficiency presenting with acute reversible cortical blindness. *J Child Neurol*, 2015; 30: 782-85
3. Lee JH, Kim GH, Yoo HW, Cheon CK: OTC gene in ornithine transcarbamylase deficiency: Clinical course and mutational spectrum in seven Korean patients. *Pediatr Neurol*, 2014; 51: 354-59
4. Goldstein ED, Cannistraro R, Atwal PS, Meschia JF: Undiagnosed partial ornithine transcarbamylase deficiency presenting postoperatively as agitated delirium. *Neurohospitalist*, 2018; 8: 82-85
5. Horwich AL, Fenton WA, Williams KR et al: Structure and expression of a complementary DNA for the nuclear coded precursor of human mitochondrial ornithine transcarbamylase. *Science*, 1984; 224: 1068-74
6. Balasubramaniam S, Rudduck C, Bennetts B et al: Contiguous gene deletion syndrome in a female with ornithine transcarbamylase deficiency. *Mol Genet Metab*, 2010; 99: 34-41
7. Yamaguchi S, Brailey LL, Morizono H et al: Mutations and polymorphisms in the human ornithine transcarbamylase (OTC) gene. *Hum Mutat*, 2006; 27: 626-32
8. Nassogne MC, Heron B, Touati G et al: Urea cycle defects: Management and outcome. *J Inher Metab Dis*, 2005; 28: 407-14
9. Kaneko M, Ogasawara K, Go H et al: Continuous hemodialysis therapy for an extremely low-birthweight infant with hyperammonemia. *Pediatr Int*, 2013; 55: 656-58
10. Mukhtar A, Dabbous H, El SR et al: A novel mutation of the ornithine transcarbamylase gene leading to fatal hyperammonemia in a liver transplant recipient. *Am J Transplant*, 2013; 13: 1084-87

When these 2 families were pregnant again after having OTCD children, they both chose prenatal diagnosis. The prenatal diagnosis results of the mother in Family 1 at 16 weeks suggested that the fetus carried the c.867+1G>C hemizygous mutation. Combined with the gene detection results and clinical symptoms of the proband, this fetus was determined to be a potential OTCD patient. The parents decided to terminate the pregnancy after being made aware of these results. The gene detection results from the abortive tissues were consistent with those from the amniotic fluid cells. The prenatal diagnosis results from Family 2 suggested that the OTC gene of the fetus did not have any known pathogenic mutations. Considering that this fetus did not carry pathogenic mutations and combined with the gene detection results and clinical symptoms of the proband, the possibility of OTCD in this fetus was excluded. The detection results of peripheral blood from the fetus after birth were consistent with those of the amniotic fluid. Therefore, it is very necessary to perform prenatal diagnosis in OTCD families when they again become pregnant to avoid the birth of affected children.

## Conclusions

Clinical gene detection for OCTD patients can not only obtain definite diagnoses for suspected patients but can also reveal heterozygous carriers in the family and aid in prenatal diagnosis. There are hundreds of OTC gene mutation types. The direct DNA sequencing method can confirm the mutation site; therefore, it is a reliable gene diagnostic method for detection of mutations in this gene.

## Conflicts of interest

None.

11. Kim GH, Choi JH, Lee HH et al: Identification of novel mutations in the human ornithine transcarbamylase (OTC) gene of Korean patients with OTC deficiency and transient expression of the mutant proteins *in vitro*. *Hum Mutat*, 2006; 27: 1159
12. Yang YL, Sun F, Qian N et al: [Clinical and laboratory screening studies on urea cycle defects]. *Zhonghua Er Ke Za Zhi*, 2005; 43: 331–34 [in Chinese]
13. Ivanovski I, Jesic M, Ivanovski A et al: Metabolically based liver damage pathophysiology in patients with urea cycle disorders – a new hypothesis. *World J Gastroenterol*, 2017; 23: 7930–38
14. Jang YJ, LaBella AL, Feeney TP et al: Disease-causing mutations in the promoter and enhancer of the ornithine transcarbamylase gene. *Hum Mutat*, 2018; 39: 527–36
15. Lopez-Corella E, Ibarra-Gonzalez I, Fernandez-Lainez C et al: Kernicterus in a boy with ornithine transcarbamylase deficiency: A case report. *Neuropathology*, 2017; 37: 586–90
16. Choi DE, Lee KW, Shin YT, Na KR: Hyperammonemia in a patient with late-onset ornithine carbamoyltransferase deficiency. *J Korean Med Sci*, 2012; 27: 556–69
17. Kim HJ, Park SJ, Park KI et al: Acute treatment of hyperammonemia by continuous renal replacement therapy in a newborn patient with ornithine transcarbamylase deficiency. *Korean J Pediatr*, 2011; 54: 425–28
18. Caballero F, Ris J, Puig M et al: Brain-Dead donors with ornithine transcarbamylase deficiency: A big learning opportunity in clinical evaluation. *Am J Transplant*, 2017; 17: 2229
19. Kim GH, Choi JH, Lee HH et al: Identification of novel mutations in the human ornithine transcarbamylase (OTC) gene of Korean patients with OTC deficiency and transient expression of the mutant proteins *in vitro*. *Hum Mutat*, 2006; 27: 1159
20. Shao Y, Jiang M, Lin Y et al: Clinical and mutation analysis of 24 Chinese patients with ornithine transcarbamylase deficiency. *Clin Genet*, 2017; 92: 318–22
21. Giorgi M, Morrone A, Donati MA et al: Lymphocyte mRNA analysis of the ornithine transcarbamylase gene in Italian OTCD male patients and manifesting carriers: Identification of novel mutations. *Hum Mutat*, 2000; 15: 380–81