Original Article

Two novel mutations of ornithine transcarbamylase gene identified from three Chinese neonates with ornithine transcarbamylase deficiency

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Abstract: We aim to analyze the blood metabolic profiling and the gene mutation of ornithine transcarbamylase (OTC) in three neonates with ornithine transcarbamylase deficiency (OTCD). Three neonates with OTCD were included in this study. The profiling of amino acids and acylcarnitine was determined using MS-MS assay. The OTC exons were amplified using PCR amplification. DNA sequencing was performed, based on which mutation analysis of OTC genes was carried out. For the clinical symptoms, all the three neonates showed poor reaction and feeding. In addition, convulsion and neonatal infection were noticed. A remarkable decrease of citrulline concentration was revealed by MS-MS assay. In case 1, a 548A > G substitution was identified in exon 6, which resulted in replacement of cysteine by tyrosine in codon 183. In case 2, a 1016T > G substitution was identified in exon 10, leading to replacement of valine by glycine in codon 339. In case 3, a 995G > C mutation was noted in exon 9, resulting in missense mutation of tryptophane to serine in codon332. Three types of OTC gene mutations were identified in Chinese neonates with OTC deficiency, among which two novel mutations, including 1016T > G and 995G > C, are presented uniquely in our study.

Keywords: Ornithine transcarbamylase deficiency, mutation, ornithine transcarbamylase, MS-MS assay

Introduction

Ornithine transcarbamylase deficiency (OTC) refers to an X-linked genetic disorder of the urea cycle that resulted in elevated ammonia in blood due to decreased activity or complete elimination of ornithine transcarbamylase (OTC) [1, 2]. The presentation of OTCD in males is usually in the neonatal period with a rapidly progressive metabolic encephalopathy, which is characterized by poor feeling, irritability, lethargy, coma, and respiratory failure [2].

The human OTC gene, with a full length of 73 Kb containing 10 exons, is located on the short arm of the X chromosome within band Xp21.1 [3]. To date, mutations of OTC encoding genes have been considered as the main cause for the decrease or elimination of OTC activity [4]. To our knowledge, the phenotype of OTCD is extremely heterogeneous. Until now, more than 379 mutations of human OTC gene have been identified. As confirmed using molecular methods, only approximately 80% of patients with OTC deficiency are found to have mutations [5].

Studies have been carried out to investigate the mutation of OTC gene in the residents in Asian countries, including Japan and Korea [6-8]. However, rare studies have been carried out to analyze the gene mutation of OTC in Chinese neonates. In this study, a mutation analysis of OTC gene was performed in three infants with OTC deficiency treated in our hospital. We present two newly identified gene mutations in OTC gene, which could add more information to the analysis of OTC polymorphism.

Materials and methods

Patients

Case 1 was a male neonate born to healthy parents. Two days after delivery, the patient
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Table 1. Specific primers for the PCR amplification

<table>
<thead>
<tr>
<th>Primers (5'-3')</th>
<th>Exon</th>
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<tbody>
<tr>
<td>GAGTTTCAGGGGATAGAATCGTC</td>
<td>1</td>
</tr>
<tr>
<td>AGGAATCATGGTGATGCATAAAAC</td>
<td>2</td>
</tr>
<tr>
<td>CACCATAGTACATGGGTCTTTTCTT</td>
<td>3</td>
</tr>
<tr>
<td>CACGTATTTGGGGGCTAGTTTACTA</td>
<td>4</td>
</tr>
<tr>
<td>AAGAGGGGATTGAGGTTGAAAGGAC</td>
<td>5</td>
</tr>
<tr>
<td>GTTGAGATGATGGCCAATTCTTGT</td>
<td>6</td>
</tr>
<tr>
<td>CAAGCTGATTTTCAGAATCTGATGG</td>
<td>7, 8</td>
</tr>
<tr>
<td>GCCACATAATAGCTAAGGAGTGG</td>
<td>9</td>
</tr>
<tr>
<td>TGGGGAAATAATAAGCAAGTGAGAT</td>
<td>10</td>
</tr>
</tbody>
</table>

showed intermittent convulsion, combined with progressive dyspnea. The blood ammonia was 1000.0 µg/dl. The patient was diagnosed with OTCD using liver biopsy. The patient was lost in the follow up period. Case 2 was a male neonate, who was admitted to our department due to poor reaction and convulsion on day 7 after delivery. His blood ammonia rose rapidly to 1000.0 µg/dl using liver biopsy. He died on day 11. Case 3 was a male neonate, who was admitted to our department due to neonatal pneumonia and convulsion on day 9 after delivery. His blood ammonia rose rapidly to 1000.0 µg/dl as revealed by liver biopsy. He died on day 10. The protocols were approved by the Ethic Committee of General Military Hospital of Beijing PLA.

Acylcarnitine analysis

Dried blood samples were prepared by using 25 µl blood, and then were placed to a filter paper. The acylcarnitine analysis of dried blood spots of each patient was performed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) as previously described [9].

Urine metabolites analysis

For the collection of urine sample, 2 ml urine was collected and prepared according to the previous report with slight modification. ULTRASIQ GC-MS instrument (Thermo Fisher Scientific Inc., Waltham, MA, USA) was used for the urine metabolites analysis as previously described by Song et al. In brief, the samples were mixed with urease at 37°C for 15 min. Then daturic acid (200 ppm) was added to the mixture and set as internal standard.

DNA isolation

Peripheral blood was obtained from three cases and two mothers. DNA was isolated using the TIANamp Blood DNA extraction Kit according to the manufacture's instructions.

PCR amplification

Specific primers targeted the human OTC gene were synthesized by Sangon Biotech (Shanghai, China). In total, 9 pairs of primers were generated targeting the 10 exons in OTC gene (Table 1). PCR reaction was performed in a total volume of 20 µL containing 10 µL 2 × mix, 0.5 µL of each primer, and 1 µL (50~100 ng/µL) DNA template. The PCR conditions used in the amplification were pre-denaturation at 94°C for 3 min, followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 56-60°C for 30 seconds, and extension at 72°C for 1 minute. Finally, a final extension was performed at 72°C for 5 minutes. The PCR products were separated in a 2% agarose gel.

Sequencing

The PCR products were purified and linked to a pUC18 vector. Sequencing was performed using ABI3130 automatic sequencer (Applied Biosystems, CA, USA). The sequencing results were analyzed using Chromas version 2.23.

Results

Blood metabolic profiling

Table 2 summarized the metabolic profiling of the peripheral blood of the patients. The profiling pattern was similar for the amino acids analyzed. The citrulline concentration was near the lower limit of the normal range. In addition, significant increase was noted in the concentration of alanine, glutamine, glutamic acid, histidine, leucine/isoleucine, methionine, piperidine, proline, serine, and tryptophane compared with the normal ranges, respectively.
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Metabolite analysis of urea

The metabolite analysis of the urea indicated that the concentration of orotate and uracil showed remarkable increase compared with the normal range. This indicated the urea cycles were aberrant in these cases (Figure 1). For the rest of the metabolites in urea, no statistical difference was noted compared with the normal ranges (P > 0.05).

Structure of mutations and gene analysis

In case 1, a 548A > G substitution in exon 6 resulted in the replacement of cysteine by tyrosine in codon 183 (Figure 2A). In case 2, a 1016T > G substitution in the exon 10 resulted in the replacement of lyophobic amino acid (valine) by a hydrophilic amino acid (glycine) in codon 339 (Figure 2B). In addition, a heterozygous mutation was noted in the same position in his mother (Figure 2C). In case 3, a 995G > C was noted in exon 9, which resulted in missense mutation of tryptophan to serine in codon 332 (Figure 2D).

Discussion

Extensive studies have been carried out to investigate the gene mutations resulting in OTC deficiency. In our study, we report three missense mutations in Chinese neonates. Among these mutations, two novel mutations have never been reported previously.

According to the previous description, a total of 341 mutations and 29 nondisease-causing mutations and polymorphisms have been reported in 2006 [5]. The latest updated data indicated that a total of 379 mutations have been reported. The mutation of OTC gene was significantly heterogeneous between the races. In addition, the mutation profiling of the OTC was remarkably different in various geographical locations. To our knowledge, only three neonates and five late-onset children with OTCD have been reported in China mainland [10]. What’s more, gene mutation analysis was performed in only one neonate and three late-onset children, which revealed one nonsense mutation in the OTC gene at the exon 9 (C.958 C > T) causing an arginine to terminate the code at position 320 of the protein (R320X). In addition, two other mutations were also detected at intron 9 (C.1005 + 132 InsT) and intron 5 (C.542 + 134 G > G/A). The authors concluded that the mutation of C.958 C > T in OTC gene may occur during neonatal period, which may result in a very severe symptom, even sudden death several days after birth especially in boys.

OCT mutation has been considered to be extremely associated with OTC deficiency. To be exact, neonatal onset patients usually showed significantly reduced or even no residual OTC enzyme activity, while patients with residual OTC enzyme activity were tended to develop late-onset disease in childhood with repeated vomiting or neurological defects or seizures [11]. In Case 1, an A to G substitution was noted at the 548th base, which resulted in replacement of cysteine by tyrosine in codon 183. This substitution resulted in reduced stability of ornithine domain, which finally led to decreased activity of OTC in neonates as previously described. In Case 2, a T to G substitution was noted at the 1016nt position in exon 10, which resulted in a replacement of a lyophobic amino acid (valine) by a hydrophilic amino acid (glycine) in codon 339. In the previous report, a C to G substitution was noted at the 1015nt position in exon 10, leading to a replacement of a lyophobic amino acid (valine) by a hydrophilic amino acid (leucine) in codon 339 [12]. In Case 3, a homozygous mutation of G to C was noted in the 995nt position in exon 9, leading to missense mutation of tryptophan to serine in codon 332. Previously, a G to A substitution was noted in the 996nt base in exon 9, which
resulted in tryptophane into termination codon [13]. Thus, we proposed that two novel gene mutations, including 1016T > G and 995G > C, have never been reported previously. In this study, gene sequencing was also performed in 2 mothers, which revealed the mutated genes in Case 2 was inherited from his mother who was a carrier of heterozygous mutation with normal phenotype (Figure 2C).

In conclusion, we report three OTC gene mutations in Chinese neonates with OTC deficiency. Among these mutations, two novel mutations are presented uniquely in our study. Our study could provide helpful information for the gene mutation analysis of OTC, and contribute to the diagnosis of OTC deficiency in clinical practices, as well as the screening of OTC gene mutation carriers, especially to the neonatal screening of those with family histories.

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Disclosure of conflict of interest

None.

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