Hyperammonemonia in Pediatric Clinics: A review of ornithine transcarbamylase deficiency (OTCD) based on our case studies

Ichiro MATSUDA
Professor Emeritus, Kumamoto University

Abstract: Ornithine transcarbamylase, which is the enzyme to synthesize citrulline from carbamyl phosphate and ornithine, is located on the X chromosome. Male patients with OTCD present a wide clinical picture, as shown in the neonatal type (usually ending in death within one year) and the late-onset type (appearing between 2 and 56 years and characterized by long-term survival). OTC activity in the neonatal type is essentially undetectable, whereas those with the late-onset type have $8.1 \pm 6.3\%$ of the control level. Mutations of male patients with the neonatal type ($n=23$) include base insertion/deletion, exon skipping, and nonsense and missense mutations. Mutations may lead to unstable mRNA or truncated protein, or involve the active site or cord domain of the enzyme, leading to structural changes. Mutations associated with the late-onset type ($n=25$) are only of the missense type, with most occurring on the surface of the enzyme. We performed prenatal monitoring for OTCD in 21 cases, among which one male fetus with Arg129His and two male fetuses with Arg40His (both belonging to the late-onset type) were diagnosed on the basis of gene analysis of amniotic cells. Treatment was initiated immediately after birth, enabling them to enjoy a normal school life. Two fetuses diagnosed as having neonatal-type mutations were terminated. Gene analysis provides the most reliable information about the future consequences of OTCD, especially in male patients.

Key words: Hyperammonemia; Urea cycle disorder; Ornithine transcarbamylase deficiency (OTCD); Genotype-phenotype correlation

Introduction

Hyperammonemia in children is observed in association with a variety of diseases and conditions including liver diseases accompanied with liver cirrhosis (e.g., congenital biliary atresia) and hereditary diseases such as urea-cycle enzyme defects, defective transport of ornithine...
into mitochondria, and certain organic acido-
rias. In recent years, gene analysis of certain
hereditary diseases has revealed the relation-
ship between genotype and phenotype (the
pathologic condition), and the results of analy-
sis have come to be applied in clinical practice.

This paper describes ornithine transcarba-
mylase deficiency (OTCD), one of the most
frequent of such hereditary diseases in Japan
and the subject of our research since we
reported the first case of this disease in Japan
in 1971.1) Our group was the first to succeed
in elucidating the structure of the OTC gene
in 1988.2) In addition, identification of the
carbamyl phosphate synthetase and arginase
genes and elucidation of their mutant genes
were also initially carried out by our group.3–6)

OTC, a urea cycle enzyme localized in mito-
chondria of the liver, is involved in synthesizing
citrulline from carbamyl phosphate and orni-
thine. This enzyme is first synthesized as a pre-
cursor protein with a molecular mass of about
40,000 daltons in the cytosol, then transported
to the mitochondrial matrix, where it is pro-
cessed to the mature enzyme of about 36,000
daltons. It becomes active after being formed
into a trimer. The OTC gene maps to Xp21.1
and is 73 kb long with 10 exons.7) OTC defi-
ciency is an X-linked semidominant urea cycle
disorder, and has the highest incidence (1 out
of 50,000 people) among the various urea cycle
disorders. Clinically, the age of onset is wide-
ranging, involving infants to adults. The reason
for such variety remained unclear until gene
analysis provided relevant information.

Classification of Disease Types

About half of male patients (hemizygotes)
have a neonatal onset, with the disease occur-
rning within 1 month after birth; the other half
experience late-onset disease. Most female pa-
tients (heterozygotes) have late-onset disease.7)

1. Neonatal-onset type

OTCD in neonates is severe and manifests
with central nervous system (CNS) symptoms
such as vomiting, spasm, coma, and lethargy,
within 30 days after birth. In most cases, it ends
in patient death within the first several months
of life. Even if the patient survives, severe neu-
rological disorders will remain. OTC activity in
the liver is below the limit of detection.7)

2. Late-onset type

Late-onset disease occurs in patients of vari-
ous ages, ranging from those in infancy, puberty,
and adolescence, to middle age or even later.
One patient had been known to be asympto-
matic until the age of 65 years. Patients com-
monly lead a normal life until the onset of
disease. However, some patients have been
reported to have mild symptoms (vomiting and
mild neural confusion) particularly when they
have fever. Hepatic OTC activity in patients
with normal IQ and normal electrocardiographic
findings corresponds to 16.6 ± 5.5% of the nor-
mal level.7)

3. Female patients

Signs and symptoms vary widely among fe-
male patients (heterozygotes). Some are asymp-
tomatic, while others eventually die after onset.
Differences in the rate of inactivation of the X
chromosome owing to lyonization are involved
in how the disease manifests.7)

Relation between Disease Type
(Phenotype) and Mutant Gene (Fig. 1)

Mutation of the OTC gene is basically indi-
vidual, varying among those who are affected.8)

1. Gene mutation of neonatal-onset OTCD

About half the patients with neonatal-onset
disease have nonsense mutations (mutations
with a stop codon present in the sequence),
base insertion, and base deletion. In this case,
enzyme protein is always greatly decreased,
and the activity level is virtually nil. The other
half of patients have missense mutations (muta-
tions accompanied with substitution of amino
acids: e.g., D126G, R141Q, I172M, S192R, D196V, or L201R). All these mutations are missense mutations at sites important for enzyme activity, such as the site responsible for trimer formation and the active site of the enzyme.8) In any case, OTC activity as examined in gene expression studies was below the limit of detection.8,9)

2. Gene mutation of late-onset OTCD

In late-onset cases, all mutations are missense mutations, and most of them are located on the surface of the enzyme protein. The OTC activity corresponds to 10–15% of the normal level. Unlike neonatal-onset disease, identical gene mutations (R40H, R277W, R129H, M268T) are found in about 30% of the families of patients. The R40H mutation, in particular, has been found in 6 families. In these families, there was a male patient who was 65 years old at the first examination and had never experienced episodes of hyperammonemia, as well as male patients who developed the disease at the ages of 9, 15, 17, and 48 years. The 65-year-old patient was found to have a daily protein intake of less than 65 g. Interestingly, the disease manifested in all these patients in the 1980s, when the protein intake among Japanese adult men reached about 80 g/day.10)

3. Gene mutation of OTCD in females

In female patients, nonsense mutation, base insertion, base deletion, exon skipping, and missense mutation have been observed. However, mutations in female patients are basically of the same nature as those in male patients with neonatal-onset disease.8)

4. Patient gender and carrier diagnosis

It is known empirically that the percentage
of carriers among mothers of male patients is not the same as that among mothers of female patients. Whereas 92% of mothers of male patients are carriers, fresh mutation was common in female patients, with only 20% of mothers of female patients being carriers.11)

Prenatal Diagnosis of OTCD

The author has been involved in prenatal monitoring for OTCD in 18 patients from 13 families (Table 1). The male-female ratio was 11:7. Four of the 7 females were carriers, and 6 of the 11 males had a mutant gene. Three of these 6 had late-onset OTCD, showing an R40H or R129H mutant gene. In these cases, treatment was begun just after delivery without abortion. The patients are currently in elementary school and are showing healthy growth. Three of the 18 cases underwent abortion after prenatal diagnosis.12)

Table 1 Prenatal Diagnosis of OTCD

<table>
<thead>
<tr>
<th>Case</th>
<th>Family</th>
<th>Specimen</th>
<th>Sex</th>
<th>Site of mutation targeted for diagnosis</th>
<th>Mutation</th>
<th>Restriction enzyme</th>
<th>Result</th>
<th>Consequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>Villi</td>
<td>Male</td>
<td>Intron 1 (+4. A→C)</td>
<td>GAC→GTC</td>
<td>Rsal</td>
<td>Patient</td>
<td>Aborted</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>Villi</td>
<td>Male</td>
<td>Exon 2 CGT→CAT</td>
<td>Arg 40 His</td>
<td>Nla III, Mae II</td>
<td>Patient</td>
<td>Continued</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>Villi</td>
<td>Male</td>
<td>Exon 2 CGT→CAT</td>
<td>Arg 40 His</td>
<td>Nla III, Mae II</td>
<td>Patient</td>
<td>Continued</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>Villi</td>
<td>Male</td>
<td>Exon 4 GAC→GGC</td>
<td>Arg 126 Gly</td>
<td>Sdu I</td>
<td>Patient</td>
<td>Continued</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>Villi</td>
<td>Male</td>
<td>Exon 4 GAC→GGC</td>
<td>Arg 129 His</td>
<td>Msp I</td>
<td>Patient</td>
<td>Continued</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>Villi</td>
<td>Male</td>
<td>Exon 5 CGA→TGA</td>
<td>Arg 141 Ter</td>
<td>Taq I</td>
<td>Carrier</td>
<td>Continued</td>
</tr>
<tr>
<td>7</td>
<td>5</td>
<td>Amniotic fluid</td>
<td>Female</td>
<td>Exon 6 AGC→AGG</td>
<td>Ser 192 Arg</td>
<td>Pvu II</td>
<td>Patient</td>
<td>Aborted</td>
</tr>
<tr>
<td>8</td>
<td>6</td>
<td>Amniotic fluid</td>
<td>Female</td>
<td>Exon 8 GAG→AGA</td>
<td>Ser 332 Ter</td>
<td>Base sequence analysis</td>
<td>Carrier</td>
<td>Continued</td>
</tr>
<tr>
<td>9</td>
<td>7</td>
<td>Villi</td>
<td>Male</td>
<td>Exon 9 TGG→TGA</td>
<td>Msp I, RFLP</td>
<td>Normal</td>
<td>Continued</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>8</td>
<td>Villi</td>
<td>Male</td>
<td>Intron 8 (+1. G→A)</td>
<td>Msp I, RFLP</td>
<td>Normal</td>
<td>Continued</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>9</td>
<td>Villi</td>
<td>Male</td>
<td>Unknown</td>
<td>SSCP</td>
<td>Unavailable</td>
<td>Continued</td>
<td>(patient)</td>
</tr>
<tr>
<td>12</td>
<td>10</td>
<td>Amniotic fluid</td>
<td>Female</td>
<td>Exon 10 (G→A)</td>
<td>Normal</td>
<td>Normal</td>
<td>Continued</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>11</td>
<td>Amniotic fluid</td>
<td>Female</td>
<td>Exon 11 (G→A)</td>
<td>Normal</td>
<td>Normal</td>
<td>Continued</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>12**</td>
<td>Amniotic fluid</td>
<td>Unknown</td>
<td>Unknown</td>
<td>SSCP</td>
<td>Unavailable</td>
<td>Continued</td>
<td>(normal)</td>
</tr>
<tr>
<td>15</td>
<td>13</td>
<td>Villi</td>
<td>Male</td>
<td>SSCP</td>
<td>Normal</td>
<td>Normal</td>
<td>Continued</td>
<td></td>
</tr>
</tbody>
</table>

*Maternal blood contamination  **Bacterial contamination
SSCP: single-strand conformation polymorphism, RFLP: restriction fragment length polymorphism

Treatment and Prognosis

Administration of sodium benzoate or sodium phenylacetate is employed as pharmacotherapy, and a low protein diet (protein 1.0–1.5 g/kg/day plus essential amino acids at the required level) is used as nutritional therapy. However, since arginine is essential in this disease, in
addition to the usual essential amino acids, arginine (400 mg/kg/day) should be given to the patient in conjunction with other essential amino acids.

With regard to prognosis, most neonatal-onset cases end in death within several months after birth, as mentioned previously. In contrast, male patients with late-onset disease and female patients show various courses of illness ranging from a complete lack of symptoms to death after the initial onset, under the strong influences of both the gene mutation (remaining enzyme activity) and treatment. In general, patients with an initial blood ammonia level exceeding 1,000 μg/dl have a poor prognosis. Figure 2 shows the survival rate of Japanese patients.

In recent years, liver transplantation has been performed with the aim of radical treatment, achieving successful results in many cases. Although gene therapy is still in the experimental stage, AdexCAGhOTC, developed in Japan, has been demonstrated to be a considerably more efficient vector than AdexSRαhOTC, developed in the US. However, application to human subjects is not practical as long as basic immunological issues associated with adenovirus vectors remain unsolved.

**Conclusion**

In OTCD, gene mutations that severely affect the structure and function of OTC enzyme protein are associated with the complete absence of enzyme activity and neonatal onset, whereas gene mutations affecting the protein surface are associated with a 10–20% enzyme activity level and onset in late childhood or adulthood. In addition, a close association has been found between protein intake and disease onset. These findings represent a useful set of information for genetic counseling in relation to treatment of the patient and prenatal diagnosis of the disease.

**REFERENCES**


