Phenotypic variability in male patients carrying the mutant ornithine transcarbamylase (OTC) allele, Arg40His, ranging from a child with an unfavourable prognosis to an asymptomatic older adult

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Abstract
In five different Japanese families, we identified six male hemizygotes (aged 6, 9, 15, 17, 56, and 65 years) and a putative candidate (aged 48 years), carrying a mutant allele of the ornithine transcarbamylase (OTC) gene, a G to A substitution at nucleotide 119 in exon 2 generating histidine in place of arginine. OTC activity in the necropsied liver tissue was reduced to ~12% of the control and that of COS 1 transfected with Arg40His OTC cDNA was 10.2 ± 1.8% of the control transfected with wild type OTC cDNA. Clinical features ranged from death during a hyperammonaemic attack (a 9 year old) to a 65 year old asymptomatic man. We consider that the amount of protein ingested by these subjects may be one predisposing factor leading to the manifestation of this disease.

Key words: urea cycle disorders; OTC deficiency; phenotypic variability.

Ornithine transcarbamylase (OTC, EC 2133) is the second enzyme of urea genesis in the liver. The structural organisation of the entire human OTC gene has been reported by Horwich et al. and Hata et al. OTC deficiency (McKusick, 311250) is a common, X linked urea cycle disorder, the incidence of the disease being approximately 1 in 80 000. Female heterozygotes have manifestations ranging from being asymptomatic to mild or severe symptoms. Even in male hemizygotes there is a range of phenotypes from neonatal onset with a high mortality rate to a late onset with a better prognosis. This variability is related to allelic heterogeneity producing different activities of residual enzyme in the liver. Since mutations of the OTC gene are heterogeneous in individual patients, and cover all exons and some introns, less attention has been directed to clinical manifestations in patients carrying the identical OTC mutant allele. We describe here the clinical spectrum of six hemizygotes and a possible candidate with mutant allele Arg40His. These subjects were from five different families and were identified by gene analysis of 51 families with OTC deficiency.

Case reports
The proband of family A, a 9 year old boy, was admitted to hospital because of repeated vomiting, which occurred one day after a school trip. Seizures, unconsciousness, coma, and decerebrated posture occurred following admission to hospital. Therapy included peritoneal dialysis, oral administration of sodium benzoate and L-carnitidine, and intravenous administration of arginine. Blood ammonium levels decreased, but on the fifth day in hospital he died of multisystem organ failure. Gene tracking was undertaken in family members, including prenatal monitoring of a fourth pregnancy. Chorionic villi were obtained in the tenth week of gestation.

The proband of family B, a 15 year old boy, had a first attack of refractory seizure and frequent vomiting and subsequently anuria and hemiplegia on the left side occurred. There was no apparent cause of the attack. Treatment was not effective and he died on the ninth day in hospital.

The proband of family C, a 17 year old boy, was admitted as he had been found unconscious and in a coma for no apparent reason. Treatment including exchange blood transfusion and intravenous administration of arginine was without effect and he died on the seventh day in hospital.

Clinical and biochemical features of our patients are summarised in table 1. All subjects had had a normal school and daily life and had eaten similarly to other boys on a daily basis before the onset of symptoms. Case reports of families D and E have been reported elsewhere.

Material and methods
GENE ANALYSIS
Genomic DNA, isolated from liver specimens, chorionic villi, or blood samples as described previously, were amplified in vitro by PCR using Taq DNA polymerase and 10 pairs of synthetic oligonucleotide primers of sense and antisense standards, which covered each exon and adjacent introns. The conditions for
amplification have been described elsewhere. The amplified DNA was sequenced using T7 sequence kits (United States Biochemical Corporation, Cleveland, OH) or a Pharmacia ALF DNA sequencer.

**Expression study of Arg40His mutant OTC cDNA in COS 1 cells**

Transient expression of the Arg40His mutant cDNA in COS 1 cells was performed as described elsewhere. Briefly, full length normal and mutant cDNAs were subcloned separately into expression vector pcAGGS. These cDNAs were transfected in cultured COS 1 cells using lipofectin. To normalise for any variability in transfection efficiency, plasmid pCH110, which contained the E. coli Lac Z sequence, was co-transfected as an internal standard. Cells were harvested three days later and were used for enzyme assay after three cycles of freezing and thawing. The transfected cDNA was confirmed with reverse transcription (RT) PCR followed by sequencing. Enzyme protein was determined in western blots.

The study was approved by the Institutional Review Board of Kumamoto University Hospital.

**Results**

**Mutation analysis**

A G to A substitution at nucleotide 119 of the OTC gene in exon 2, generating histidine (CAT) in the place of arginine (CGT) at codon 40, was found in all five probands of families A to E and no other mutation was found in other exons or exon/intron boundaries. The mutation destroys the restriction site for endonuclease MaeIII; therefore amplification of exon 2 by PCR (250 bp), followed by MaeIII digestion, was used for gene tracking of the families. A wild type allele produces 103 bp, 102 bp, 24 bp, and 21 bp fragments, while a mutant allele produces 205 bp, 24 bp, and 21 bp fragments by MaeIII digestion. As shown in fig 1, the mother of family A carried an undigested 205 bp fragment of a mutant OTC allele and two digested fragments (103 bp and 102 bp bands are seen as a single band in fig 1) of the wild type allele indicating carrier status (24 bp and 21 bp bands are not seen in the prepared plate). In the father and brothers, there were two digested fragments thereby indicating a normal state. The maternal grandfather and the fetus carried an undigested 205 bp fragment. Thus, they are hemizygotes for the mutant allele; the grandfather has so far had no hyperammonaemic attacks. Gene tracking of families B to E using the same method showed that the mothers of these families and a sister of family B had mutant and wild type alleles, and were therefore carriers (data not shown).

**Expression study of mutant cDNA, Arg40His**

Western blots of the extract of COS 1 cells transfected by the mutant cDNA showed a decreased signal but of a size similar to that in the control transfected with the wild type cDNA (data not shown). The calculated activ-

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**Table 1 Clinical and laboratory features of male patients carrying the mutant allele Arg40His**

<table>
<thead>
<tr>
<th>Family</th>
<th>Onset (y)</th>
<th>Maximum blood ammonium (mmol/l)</th>
<th>Blood urea (mmol/l)</th>
<th>Serum citrulline (mmol/ml)</th>
<th>Serum glutamine plus glutamic acid (mmol/ml)</th>
<th>OTC in necropsied liver (%)</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2</td>
<td>65*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Died</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>17</td>
<td>1585</td>
<td>3.6</td>
<td>19.3</td>
<td>1487</td>
<td>3.4</td>
<td>Living</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>15</td>
<td>2322</td>
<td>2.4</td>
<td>26.3</td>
<td>2513</td>
<td>2.4</td>
<td>Died</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>103</td>
<td>321</td>
<td>2.1</td>
<td>15.0</td>
<td>1155</td>
<td>1.3</td>
<td>Died</td>
<td>11</td>
</tr>
<tr>
<td>E</td>
<td>17</td>
<td>2322</td>
<td>2.4</td>
<td>13.7</td>
<td>2231</td>
<td>5.4</td>
<td>Died</td>
<td>11</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>-50</td>
<td>3.0-6.5</td>
<td>28-52</td>
<td>574-949</td>
<td>100</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*An asymptomatic grandfather of A-1.
†A brother of A-1, who was diagnosed by prenatal monitoring.
‡A brother of D-1, who died with similar symptoms; gene analysis had not been performed.
All were physically and mentally normal before onset.

**Fig 1 Gene tracking of family A.** A PCR product of exon 2 (250 bp) of the wild type allele produces 103 bp, 102 bp, 24 bp, and 21 bp fragments by MaeIII digestion, while that of the mutant allele produces 205 bp, 24 bp, and 21 bp fragments (103 bp and 102 bp bands are seen as a single band, and 24 bp and 21 bp bands are not seen in the prepared plate), since the mutation destroys a restriction site for MaeIII. An undigested fragment (205 bp) is seen in proband II.2, a 65 year old asymptomatic man (I.3), and a fetus (III.4), indicating hemizygosity for the mutant allele, Arg40His. M: marker, P: PCR product, N: normal control.
Phenotypic variability in male patients carrying the mutant OTC allele Arg40His

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>20%</td>
<td>15%</td>
<td>10%</td>
<td>5%</td>
<td>0%</td>
</tr>
</tbody>
</table>

**Discussion**

All five families in the present study reside in the Fukuoka Prefecture of Japan and may possibly have the same ancestors. An analysis of the RFLP haplotype of the mutant gene was only feasible in families A and B, since the materials used for this purpose were no longer available for families C to E. The RFLP haplotype was the same in families A and B (6.2 kbp/5.1 kbp by MspI and 4.0 kbp by TaqI). The possibility that the five mutations occurred independently could not be excluded, since the mutation is located at a CpG hot spot. Arg40 was conserved in OTCs of human and rat, but not in other species studied. The observation of the expression of a mutant allele in COS 1 cells (10.2 ± 1.8% of control) corresponded to hepatic OTC activity in patients which ranged from 1.3 to 12% of the control. Such a wide range of enzyme activity appears to depend on the condition of the liver obtained at necropsy. Male patients with OTC deficiency were classified into two types, neonatal and late onset types. The residual enzyme activities of the liver and the serum citrulline levels (products of OTC) were significantly higher in the latter than in the former group. The data obtained in the present study are comparable to those of the late onset group shown in table 1.

Of interest in the present study are the ages at onset ranging from 6 years (D-3) to 56 years (D-1) and the findings in an asymptomatic man aged 65 years who was a hemizygote (A-2). The same mutation in a 3 year old male patient was reported by Tuchman et al. Valproate ingestion is a risk factor in the development of hyperammonaemic attacks in OTC deficiency. However, this was not the case in our patients. Male patients with onset after 5 years of age had a higher mortality rate than did those with onset between 1 and 5 years, as in the present study, even with appropriate treatment. This may relate to factors including increased metabolism during adolescence or a diminished capacity to recover from multisystem organ failure in older adults. Non-protein energy intake, particularly during illness, could be another factor. In addition, the amount of protein ingested may play an important role in the development of hyperammonaemia.

We reported another five male hemizygotes from three different families with the late onset type of OTC deficiency, all of whom carried Arg277Trp alleles. Among them, two were asymptomatic subjects on a relatively low protein diet and one 8 year old boy experienced a hyperammonaemic attack when he ate six boiled eggs after returning from an athletics meeting (unpublished observation). Accordingly, we checked the mean protein ingestion of Japanese adults, as officially recorded by the Japanese Ministry of Health and Welfare, since 1945. Before 1945 it was probably similar to or less than that in 1945—1950. As shown in fig 2, the mean daily protein ingestion has increased gradually year by year, and attacks of hyperammonaemia occurred during the last 15 years in all patients in the present study, regardless of age, when the protein intake was close to the value of 80 g/day in adults. Although the exact amount of protein ingestion (per kg of body weight) of each subject was uncertain, the three elderly hemizygotes (A-2, D-1, D-2) may have ingested lesser amounts of dietary protein during childhood, as compared to the younger patients. The age at onset of OTC deficiency seems to depend on the amount of dietary protein ingested in younger subjects. At present, the 65 year old man (A-2) ingests ~65 g/day of protein.

The parents of family A decided not to terminate the pregnancy of a fetus with a mutant allele, because the grandfather was asymptomatic. We are now following the baby (1 year of age) prospectively with dietary control; the serum amino acid profile is practically normal, except for citrulline levels, which are significantly lower than controls (table 1). Urinary orotic acid is within the normal range. Thus, prenatal diagnosis and early appropriate treatment, including protein restriction, can lead to good results, especially in the case of late onset OTC deficiency with mutations such as Arg40His or Arg277Trp.

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![Figure 2](https://example.com/figure2.png)  
**Figure 2** Life span and age of onset of each hemizygote (A-1, A-2, B, C, D-1, D-2, D-3, E in table 1) with Arg40HIs and average protein ingestion by the Japanese.

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