A Case of Glutaric Aciduria Type I with Unique Abnormalities in the Cerebral CT Findings

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Glutaric aciduria type I (GA-I) is an inherited metabolic disorder of organic acids due to a defect of glutaryl-CoA dehydrogenase (GCDH) in the intermediate metabolic step of lysine and tryptophan (Goodman et al. 1975). GA-I is characterized by some progressive neurological symptoms such as choreoathetosis or dystonia differing from the many other organic acidemias. Since the first report by Goodman et al. in 1975, only ten cases have been reported in the literatures (Goodman et al. 1975; Gregersen et al. 1977; Kyllerman and Steen 1977; Brandt et al. 1978; Floret et al. 1979; Whelan et al. 1979; Leibel et al. 1980; Dunger and Snodgrass 1984). However, these reported cases were limited so far in North
America and Europe where the screening of organic acidurias using gas chromatography mass spectrometry (GC/MS) have been performed. In the present paper we described the first Japanese case of GA-I with unique abnormalities in the cerebral CT findings detected in the screening of organic acid disorders using GC/MS along with the clinical course.

**METHOD**

*Analysis of urinary organic acids*

The urine samples equivalent to 0.2 mg of creatinine, added with 30 μg of heptadecanoate as an internal standard, underwent oximation, solvent extraction and trimethylsilylation. The oximation was performed by adding 1 ml of 2.5% hydroxylamine-hydrochloride to the above samples and keeping at room temperature under pH 14.0 for 1 hr. The mixture was acidified with dropping of 6N HCl and extracted with one 9 ml aliquot of ethylacetate and two 9 ml aliquots of diethylether with vigorous shaking. The organic layer was dried over with 5 g of anhydrous sodium sulphate and evaporated to dryness under a nitrogen stream. The evaporated residue was trimethylsilylated with 50 μl of bis(silyl)trimethylfluoracetamide and 10 μl of trimethylchlorosilane at 60°C for 30 min. This sample was analyzed by injecting 2.0 μl and 0.5 μl onto gas chromatograph (GC) and gas chromatography mass spectrometer, respectively. The GC/MS instrument was a Shimadzu GC/MS 9020 model and the computer used was Shimadzu SCAP 11/23 based on DEC PDP 11/23 model. The data were processed by the computer program of "Mass Chromatographic Screening Program For Organic Acidurias Of Gifu University (MCSCR)" (Yamaguchi 1985). Conditions of analysis were as follows: the column used was 2 m x 2 mm I.D. glass column packed with 3% OV-17 on chromosorb W (mesh 80–100). The column temperature was initially retained at 90°C for 4 min and then raised from 90°C to 290°C by the rate of 6°C/min. The temperatures of the injection part, separator and ion source were fixed at 300°C, 300°C and 280°C, respectively. The mass spectra were recorded at 4 min intervals from m/z 50 to 550 under a computer control. The acceleration voltage, ionization voltage and trap current were 3.0 kV, 70 eV and 60 μA, respectively.

*Enzyme assay*

Synthesis of (1, 5-14C) glutaryl-CoA and enzyme assay were performed according to the methods of Christensen and Brandt (1978). The skin fibroblasts were cultured in the MEM containing 10% fetal calf serum. The fibroblast extracts equivalent to 314–838 μg of protein were subjected to the enzyme assay. Protein amounts were measured by Lowry method (Lowry et al. 1951). The enzyme activity of GCDH was assayed by measuring released 14CO2 which was trapped in a filter paper moistened with 100 μl of 10% KOH and quantitated by a liquid scintillation counter.

*Carnitine measurement*

Concentrations of carnitine in blood and urine were measured according to the method of McGarry and Foster (1976). D, L-Carnitine was orally administered at a dose of 30 mg/kg/day and pre- and post-dose carnitine concentrations were measured.

**Case Report and Results**

*Patient.* Case E.N. was a 7-month-old girl. Her family history and her perinatal period were uneventful. She was born at 37 weeks of gestation weighing 3,400 g. She was pointed out to have slightly poor head control in the
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medical examination at 4 months of her age, and became to suffer from ill temper, irritability and sleeplessness since 5 months of her age. These symptoms had been deteriorative. At 7 months, her urine samples were sent to Gifu University for the screening of organic acid disorders leading to abnormality suggesting GA-I.

Findings of physical examination on admission to our hospital at 7 months of age were as follows; Her body weight was 8,240 g, body length 66.8 cm, head circumference 42.8 cm and chest circumference 45.0 cm. She was incapable of head control and rolling over. The tonus of her trunk was hypotonic while the tonus of her extremities was rather hypertonic. She was able to recognize and vocalized well, but she seemed to be bad-tempered and irritable, and she also suffered from sleeplessness. Laboratory data were as follows; Complete blood cell counts were within normal limits except WBC of 11,300/mm. Serum electrolytes were within normal ranges. On blood gas analysis, pH was 7.29, pCO₂ 36.9, BE -7.8 and HCO₃ 17.8, indicating slight metabolic acidosis. Liver functions, blood sugar, blood ammonia, blood lactate and pyruvate were all within normal ranges. Total cholesterol in blood was normal (185 mg/100 ml) while triglyceride was slightly elevated (217 mg/100 ml). Amino acids in blood and urine were not contributory and EEG showed no abnormality. However, cerebral CT scanning showed remarkable abnormalities such as striking fluid collection in bilateral fronto-temporal regions and a slight enlargement of the lateral ventricles as indicated in Fig. 1a.

Organic acids. Urinary organic acids were analyzed by GC and GC/MS. Fig. 2. shows a gas chromatogram in which a large peak of glutarate (4.08 mg/mg creatinine, 0.10-0.15 in normal), unusual appearances of glutaconate and 3-
hydroxyglutarate and increases in adipate and 2-ketoglutarate were observed. Blood glutarate in small amount (27.3 μg/ml) was evidently found (not detected in normal subjects). Glutarate in cerebrospinal fluid was also increased to 106.4 μg/ml (0–3.9 in normal subjects). These results suggested that she suffered from GA-I.

**Enzyme assay.** GCDH enzyme activity in her skin fibroblasts was undetectable as shown in Table 1.

**Carnitine concentration.** D, L-Carnitine, 30 mg/kg/day was administered orally and pre- and post-dose carnitine concentrations in blood and urine were compared. As shown in Table 2, total carnitine concentrations in both blood and urine samples before loading were within normal ranges but the ratio of acylcarnitine to total carnitine was higher, and free carnitine level in blood was remark-

![Fig. 2. Gas chromatogram of urinary organic acids.](image)

**Table 1.** Enzyme activity of glutaryl CoA dehydrogenase in cultured fibroblasts (nmole CO₂/ hr/mg protein)

<table>
<thead>
<tr>
<th></th>
<th>Patient (E.N.)</th>
<th>Control 1</th>
<th>Control 2</th>
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<tr>
<td></td>
<td>0</td>
<td>2.8</td>
<td>3.2</td>
</tr>
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</table>
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Clinical course. She was almost exclusively taking breast feeding until 7 months of age. Though riboflavin, a coenzyme of GCDH, was given orally at a dose of 100 mg/day after the diagnosis, her clinical symptoms were unaffected and no decrease in urinary excretion of the glutarate was seen. Then, lioresal, an analogue of gamma-aminobutyrate (GABA), was orally administered at a dose of 5 mg/kg/day, by which her symptoms such as ill temper, irritability and sleeplessness were improved dramatically and opisthotonus was reduced. However, the amount of urinary excretion of the glutarate remained unchanged before and after the administration of lioresal. When valproic acid, 5 mg/kg/day, was orally given in addition to lioresal for 1 week, her symptoms such as ill temper, sleeplessness and opisthotonus were aggravated during the period. Withdrawal of this drug resulted in reduction of these symptoms. Her diet was restricted by a special formula (lysine, tryptophan free milk) to reduce daily intakes of lysine, tryptophan and protein to 50 mg/kg/day, 10 mg/kg/day, and 1.5–2.0 g/kg/day, respectively, which decreased the urinary excretion of the glutarate to 1/5–1/7 of the initial amounts. However, the clinical symptoms showed no tendency of improvement. Although D, L-carnitine administration (30 mg/kg/day, p.o.) increased total, acyl- and free carnitine levels in both blood and urine, her clinical symptoms seemed to be unchanged. She is now 2 years old and can lift her head in the prone position, but she is incapable of rolling over and sitting alone. In spite of severe motor retardation, recognition and vocalization were established. These findings gave us an impression that the mental retardation was relatively mild comparing with the motor retardation in GA-I. On the other hand, the abnormalities in the cerebral CT examination were not deteriorative until 2 years of her age (Fig. 1b) at least comparing with those on admission (Fig. 1a).

### Table 2. Carnitine levels at pre- and post-loading of D, L-carnitine (30 mg/kg/day, p.o.) (nmole/ml)

<table>
<thead>
<tr>
<th></th>
<th>Carnitine in blood</th>
<th>Carnitine in urine</th>
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<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Free</td>
</tr>
<tr>
<td>Pre-carnitine load</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 months of age</td>
<td>32.0</td>
<td>11.9</td>
</tr>
<tr>
<td>15 months of age</td>
<td>29.2</td>
<td>8.0</td>
</tr>
<tr>
<td>Post-carnitine load</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 months of age</td>
<td>59.4</td>
<td>40.2</td>
</tr>
<tr>
<td>16 months of age</td>
<td>77.8</td>
<td>44.7</td>
</tr>
<tr>
<td>Control</td>
<td>66.7±36.0</td>
<td>30.5±19.4</td>
</tr>
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</table>

Values and means±s.d.
DISCUSSION

GA-I is diagnosed chemically by the detection of a large amount of glutarate and other specific unusual components such as glutaconate and 3-hydroxyglutarate in urine (Goodman et al. 1975). We detected this patient as the first Japanese case of GA-I by the screening using GC/MS.

In the present case, unique abnormalities of the cerebral CT findings were observed. Abnormal CT results were reported in two among three cases which had the description of cerebral CT findings in the literatures (Goodman et al. 1975; Leibel et al. 1980; Dunger et al. 1984). While the mechanisms of these abnormalities in the cerebral CT findings have not been known, the effect of accumulation of glutarate in the central nervous system might be suspected. These abnormalities of CT may be one of the specific findings of GA-I.

Lioresal is an analogue of GABA which is considered to be reduced in the cerebral basal ganglia of the patients with GA-I (Brandt et al. 1979). In the present case, several neurological symptoms such as ill temper or sleeplessness reduced soon after the oral administrations of lioresal and this substance was thought to exert its effect through supplying GABA. On the other hand, valproic acid which inhibits the degradation of GABA (Godin et al. 1969) aggravated the above symptoms. These findings might be explained by an excess of GABA or the some other effects of valproic acid in the central nervous system.

The possibility exists that GA-I might be concealed among the children confirmed to have developmental retardation, cerebral palsy, regression or acute encephalopathy due to unknown causes. The number of patients with GA-I may possibly increase in future by the screening test using GC/MS. The relation of the neurological manifestations and biochemical changes in GA-I should be made clear by further investigations.

Acknowledgments

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References


