Recovery from Marked Altered Consciousness in a Patient with Adult-onset Type II Citrullinemia Diagnosed by DNA Analysis and Treated with a Living Related Partial Liver Transplantation

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Abstract

A 21-year-old woman was admitted with altered consciousness and hyperammonemia. She was diagnosed as having adult-onset type II citrullinemia (CTLN2) by DNA analysis. The patient had mutations of the SLC25A13 gene, which were compound heterozygotes of 851 del 4 and IVS11+1G>A. CTLN2 has a poor prognosis, in spite of various intensive medications, and we performed a living related partial liver transplantation (LRLT). Over a 2-year follow-up, the patient has been well. CTLN2 can be diagnosed by the DNA analysis and can be treated by LRLT. (Internal Medicine 41: 555-560, 2002)

Key words: citrulline, citrin, SLC25A13, coma, argininosuccinate synthetase, urea cycle

Introduction

Citrullinemia is an autosomal recessive disease caused by a deficiency of argininosuccinate synthetase (ASS), one of the five urea cycle enzymes. Saheki et al classified Japanese citrullinemia patients into three types based on enzyme abnormality and into two forms based on pathogenesis (1-6). The first is the classical form (CTLN1). Most CTLN1 are neonatal/infantile-onset citrullinemia (type I and type III). In CTLN1 the enzyme defect is found in all tissues and cells (1-4). Classical citrullinemia type I is characterized by abnormal kinetics caused by missense mutation, and type III is mainly caused by abnormal splicing of ASS gene. CTLN1 arises due to mutations in the ASS gene located on chromosome 9q34 (7-12). In contrast, the second form, adult-onset type II citrullinemia (CTLN2), is characterized by a liver-specific ASS deficiency with normal kinetic properties and there are no abnormalities in hepatic ASS mRNA or the ASS gene (1-3, 13-16). CTLN2 patients suffer from altered consciousness and coma, and most die with cerebral edema within a few years of onset (5). In 1999, Kobayashi et al reported that they identified the CTLN2 locus on chromosome 7q21.3, a novel SLC25A13 gene, by positional cloning. The SLC25A13 gene encodes a 3.4 kb transcript which is expressed most abundantly in the liver. The SLC25A13 protein, a putative calcium-binding mitochondrial carrier, is designated citrin (6). Before this mutation was identified, a liver biopsy was necessary to diagnose CTLN2. We report a case of a 21-year-old woman with CTLN2 who was diagnosed by DNA analysis without a liver biopsy. She had the mutations of the citrin gene, a compound heterozygote of 851 del 4 and IVS11+1G>A. (851 del 4 is a 4-bp deletion from nt 851 in exon 9 and IVS11+1G>A is a G>A substitution at 5' end of intron 11.) After liver transplantation, in fact, a selective marked decrease in ASS activity was found in her removed liver. In this case her condition suddenly grew worse and her consciousness was markedly altered. In the acute phase we employed conservative therapies and after her condition improved, a living related liver transplantation was performed.

Case Report

On July 5, 1999, a 21-year-old woman was admitted to our hospital because of sudden altered consciousness and convulsive seizures.

She had been well until two weeks before when she was admitted to another hospital for nausea, and vomiting, and behavioral aberrations such as crying like a child, raising her voice and struggling. They noted her short stature (150 cm, 41.5 kg) and delirium. Laboratory studies showed slight hyperammonemia...
nemia and abnormal electroencephalogram (EEG) which showed slow waves. A lumbar puncture revealed a cell count of 13/mm³ (all monocytes). A computed tomographic (CT) scan of the brain showed normal findings. Aciclovir was administered but she frequently developed periods of delirium lasting several hours. On July 4, as she was stable and showed no abnormal findings, she was discharged.

The day after discharge the patient had convulsive seizures and went into a deep coma. She was brought by ambulance to the emergency department of Nishiwaki City General Hospital. She was the product of full-term pregnancy but of low birth weight (2,400 gm). Development was normal. Her parents had no consanguinity. She had a past history of cyst-duodenostomy at age 8 for congenital biliary dilatation. At age 16 she had a hepatico-jejunostomy with Roux-en Y for cholangitis as well as a duodenostomy and papilloplasty for pancreatitis.

On July 5, her temperature was 36.2°C, pulse 68, respirations 30, blood pressure 128/66 mmHg. There were no murmurs and no rales on auscultation of the chest. She had no hepatomegaly. Skin and conjunctiva were not icteric. There were no spider nevi or palmar erythema. On neurologic examination, she was in a deep coma. Pupils were equal (3 mm) and round. Light reflex was complete and prompt. Muscle tonus of extremities was hypotonic. Deep tendon reflexes were absent. Pathologic reflexes and flapping tremor were not present.

Laboratory tests revealed hyperammonemia (481 μg/dl) and liver dysfunction (Table 1). Pancreatic secretory trypsin inhibitor (PSTI) was increased (Table 1). Laboratory tests for virus infection were negative. A lumbar puncture was performed (Table 2). There were no indications of encephalitis or meningitis. Plasma and urine amino acid analysis revealed high citrulline levels (Table 3). Threonine/serine ratio was increased in the plasma. The arginine level was increased in the urine. These findings suggested a diagnosis of adult-onset type II citrullinemia (CTLN2), and a DNA analysis was performed. She had mutations in the citrin gene (Fig. 1), indicating a compound heterozygote of 851del4 and IVS11+1G>A. She was diagnosed as having CTLN2.

Abdominal ultrasonography and CT revealed only mild fatty liver change without abnormal vessels or liver cirrhosis. On the first day, brain CT was normal. An EEG showed diffuse slow and nearly flat waves.

She was first treated with lactulose and branched-chain amino acids. Her altered consciousness and NH3 level improved slightly, but on day 5, anisocoria appeared and the light reflex was absent. Since she had very weak spontaneous respirations,
## Table 3. Amino Acid Analysis

<table>
<thead>
<tr>
<th>Plasma amino acid</th>
<th>Patient</th>
<th>Normal range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Threonine</td>
<td>112.6 nmol/ml</td>
<td>67–190</td>
</tr>
<tr>
<td>Serine</td>
<td>43.2 nmol/ml</td>
<td>72–160</td>
</tr>
<tr>
<td>Citrulline</td>
<td>210.1 nmol/ml</td>
<td>17–43</td>
</tr>
<tr>
<td>Arginine</td>
<td>129.8 nmol/ml</td>
<td>54–130</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Urine amino acid</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrulline</td>
<td>16,631.1 µmol/day</td>
<td>10–60</td>
</tr>
<tr>
<td>Arginine</td>
<td>1,444.7 µmol/day</td>
<td>10–60</td>
</tr>
</tbody>
</table>

She was placed on a ventilator. Brain CT showed remarkable edema (Fig. 2A). After D-mannitol was administered intravenously, she had a slight reaction to strong stimuli. But theNH₃ level was again elevated to 698 µg/dl. We suspected she had CTLN2, and treatment with intravenous arginine L-glutamate...

![Image of brain CT scan](image1)

**Figure 2.** On day 5, the patient was comatose with anisocoria. Brain CT (A) showed remarkable edema. After three months (on October 12, 1999, before liver transplantation), her brain was markedly atrophic on CT (B).

![Image of brain CT scan](image2)

**Figure 1.** The patient was a compound heterozygote with 851del4 and IVS11+1G>A. The 851del4 mutation was derived from her father, and IVS11+1G>A was from her mother.
Seven distinct mutations in the citrin gene were identified as CTLN2 (6, 21). Before the citrin gene was identified, a liver biopsy was necessary to measure the urea cycle enzyme activities in order to diagnose CTLN2. Now however, CTLN2 can be diagnosed from the DNA analysis.

It was reported that five patients with infantile cholestatic jaundice had mutations of the citrin gene (22, 23). One patient of the above-mentioned five cases developed the symptoms of CTLN2 at 16 years old and received a LRLT (23, 24). As far as we investigated, that case is the only reported case in which CTLN2 was diagnosed by DNA analysis without performing a liver biopsy. (But a liver biopsy had been performed in early infancy when he had developed transient hypoproteinemia and jaundice.)

The present patient had a mutation of the citrin gene, a compound heterozygote of 851 del 4 and IVS11+1G>A (Fig. 1). After surgery, the patient’s altered consciousness and general condition improved. The blood ammonia level was normalized (54 nmol/ml) and the plasma citrulline level, which was 35.3 nmol/ml, also became normal on November 27 (Table 4). She has been well for two years.

### Discussion

The clinical characteristics of adult-onset type II citrullinemia (CTLN2) are late onset (ages 11 to 72; 34±12.8, n=102) (17), and serious and recurring symptoms (18). Most CTLN2 patients suddenly suffer from altered consciousness associated with flapping tremor, disorientation, restlessness, drowsiness, and coma. The diagnosis of CTLN2 is made based on the clinical findings and biochemical investigations. PSTI gene is highly expressed in the liver of CTLN2 patients (19) and there is a significant increase in serum PSTI level (17). Some patients suffer from pancreatitis and two patients had a pancreaticojejunostomy (20). Serum PSTI level is a useful and convenient diagnostic marker (17). Though ASS activity in the liver is one of the most important factors for the diagnosis, ASS activity in a liver biopsy often cannot be measured because of the fulminant clinical course and the patient’s poor condition (18). Here also, we could not measure the ASS level in the acute phase for the same reasons.

Seven distinct mutations in the citrin gene were identified as CTLN2 (6, 21). Before the citrin gene was identified, a liver biopsy was necessary to measure the urea cycle enzyme activities in order to diagnose CTLN2. Now however, CTLN2 can be diagnosed from the DNA analysis.

Table 4. Amino Acid Profiles and Ammonia Level before and after Liver Transplantation

<table>
<thead>
<tr>
<th>Patient</th>
<th>Normal range</th>
</tr>
</thead>
<tbody>
<tr>
<td>before crisis</td>
<td>before LT</td>
</tr>
<tr>
<td>Amino acid</td>
<td></td>
</tr>
<tr>
<td>Citrulline</td>
<td>165</td>
</tr>
<tr>
<td>Arginine</td>
<td>77</td>
</tr>
<tr>
<td>Ammonia</td>
<td>339</td>
</tr>
</tbody>
</table>

LT: liver transplantation.

and oral L-carnitine was started. On the next day (day 6), the NH3 level markedly improved to 91 µg/dl. Oral sodium benzoate was started and oral protein intake was restricted. Her altered consciousness gradually improved. In August she was able to eat, in September she was speaking and by October she was writing and walking. But her citrulline level was high, her liver revealed severe fatty change, and her brain CT was markedly atrophic (Fig. 2B). Since the prognosis of CTLN2 is poor, on October 22, at Kyoto University Hospital, she received a living related partial liver transplantation (LRLT) from her father. DNA analysis of her father showed a mutation in citrin gene, in which he had the heterozygote of 851 del 4 without IVS11+1G>A (Fig. 1). After surgery, the patient’s altered consciousness and general condition improved. The blood ammonia level was normalized (54 nmol/ml) and the plasma citrulline level, which was 35.3 nmol/ml, also became normal on November 27 (Table 4). She has been well for two years.
In the present patient, amino acid analysis was performed at (32, 33).

There remain many unresolved problems about CTLN2, one before transplantation, DNA analysis of her father and pensates for the congenital citrin deficiency, so that until adult-
dered. We estimate that another pathway or substance com-

But her mother’s HBs antibody and HBc antibody were positive. We thought her father was a suitable donor. The patient’s removed liver showed marked enlargement with se-
vere fatty change. There were no cirrhotic changes. We measured the urea cycle enzyme activities in her removed liver and the father’s donor liver (Table 5). A selective marked decrease in ASS activity was found in her liver, her father’s was normal range (Table 5). (Her mother’s was not tested.) This data proved the usefulness of DNA analysis. Since the LRLT she has never had CTLN2 symptoms.

In the literature, CTLN2 patients have received a liver transplantation 4 to 144 months after the onset of symptoms (20, 25–31). In most of those patients, it was 4 to 6 months after symptom onset. In the present case, 4 months after the onset of symptoms, a LRLT was performed, but we recommend that a liver transplantation is performed as early as possible, because the CTLN2 prognosis is poor and all patients improve after a LRLT. On October 12, 1999, before LRLT, her brain CT revealed marked atrophy (Fig. 2B) and two years later brain atrophy was not changed. We considered cerebral edema on admission brought about atrophy, but CTLN2 may bring about atrophy and if so, a liver transplantation has to be performed as early as possible.

There remain many unresolved problems about CTLN2, one of which is the reason CTLN2 is a late-onset disease, or why the clinical symptoms do not appear until adulthood. Reportedly some cases of CTLN2 are triggered by alcoholic liver damage, and other cases by acacetaminophen or sodium valproate (32, 33).

In the present patient, amino acid analysis was performed at the other hospital when she was 17 years old, and her serum citrulline level had increased to 165 nmol/ml (Table 4). But CTLN2 symptoms had not occurred. This data indicates that before CTLN2 symptoms occur, the urea cycle becomes disor-
dered. We estimate that another pathway or substance comp-
ensates for the congenital citrin deficiency, so that until adult-
hood or until fatal liver damage happens, CTLN2 symptoms do not occur. CTLN2 symptoms may depend on the failure of compensation. If the failure of compensation is improved, CTLN2 symptoms such as consciousness disturbances and hyperammonemia may also improve. A few months before admission, the present patient began to eat too much. More protein than before might trigger the urea cycle’s disorder and CTLN2 symptoms.

When the mechanism of a late onset and reversibility is discussed, citrin is one of the most important proteins. Citrin as the aspartate/glutamate carrier (AGC) plays an important role in the urea cycle by providing aspartate for incorporation into argininosuccinate (34). The mutant citrin could not locate into the mitochondria membrane (6, 21). This impairment of the function of citrin would lead to a failure in supply of aspatate from mitochondria for formation of argininosuccinate. Citrin as AGC also plays an important role in the malate/aspartate shuttle (MAS) and MAS is essential to aerobic metabolism of glucose. The function of AGC and MAS might be compensated by other pathways. The disorder of other pathways and the impairment of the function of citrin would lead to the symp-
toms of CTLN2.

In conclusion, we reported a case of a 21-year-old woman with CTLN2 that was diagnosed using DNA analysis and was successfully treated with LRLT. At present, DNA analysis is useful to diagnose CTLN2 and LRLT is considered to be a useful and practical option that fundamentally improves this disorder.

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References


Table 5. Urea Cycle Enzyme Activities of the Removed Liver

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Patient</th>
<th>Donor</th>
<th>control (m±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbamoylphosphate synthetase (CPS)</td>
<td>0.035</td>
<td>0.034</td>
<td>0.036±0.013</td>
</tr>
<tr>
<td>Ornithine transcarbamylase (OTC)</td>
<td>1.57</td>
<td>0.72</td>
<td>0.88±0.35</td>
</tr>
<tr>
<td>Argininosuccinate synthetase (ASS)</td>
<td>0.0031</td>
<td>0.0165</td>
<td>0.033±0.012</td>
</tr>
<tr>
<td>Argininosuccinate lyase (ASL)</td>
<td>0.048</td>
<td>0.04</td>
<td>0.052±0.025</td>
</tr>
<tr>
<td>Arginase (ARG)</td>
<td>6.83</td>
<td>12.2</td>
<td>15.8±3.1</td>
</tr>
</tbody>
</table>


