Molecular diagnosis for presymptomatic patients with Wilson disease
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Abstract
Wilson disease is a genetic disorder of copper metabolism characterized by hepatic and/or neurological manifestations. This disease is caused by mutations in the gene of copper transporting ATPase (ATP7B). Early diagnosis is very important to improve the prognosis of this disease. However, biochemical studies are not sufficiently effective for the definitive diagnosis of young patients. This study presents the molecular diagnosis of presymptomatic patients with Wilson disease. Three patients, two infants and one young child without any symptoms, and one carrier, were diagnosed by ATP7B gene analysis. We conclude that the molecular diagnosis of Wilson disease is very useful for the identification of young patients and familial analysis.

Keywords: Wilson disease, ATP7B, gene analysis, presymptomatic diagnosis, familial analysis

Introduction
Wilson disease is an autosomal recessive disorder based on an inborn error of copper metabolism. Copper is accumulated primarily in the liver, brain, cornea, kidney, and other organs. The clinical features of this disease are liver cirrhosis, extra pyramidal signs and a Kayser-Fleischer ring. Copper accumulation is believed to result from the loss of the ability to excrete copper via bile due to a dysfunction of intracellular copper transport in the liver. The Wilson disease gene (ATP7B) encodes a putative copper-transporting P-type ATPase. More than 200 disease-specific mutations have been reported¹).

Wilson disease is a treatable disorder. Treatment involves the removal of excess copper by chelating agents (D-penicillamine or trienthine 2HCl), and/or blocking intestinal copper absorption by oral administration of zinc salt. Early diagnosis is very important for the improvement of prognosis. In this study, the authors report the presymptomatic diagnosis of Wilson disease by ATP7B gene analysis.

[Patients]
1) Case 1 (Table 1)
Seven-year-old boy. He presented no any symptom. Liver dysfunction was identified by biochemical findings before inguinal hernia operation. His serum ceruloplasmin level was 2.0 mg/dl and urinary copper excretion (usually<40μg/day) was elevated. From these data, Wilson disease was suspected.

2) Case 2 (Table 1)
An eight-month-old boy was found to have hypoceruloplasminemia by a pilot study of mass screening for Wilson disease³). The serum AST, ALT levels and urinary copper excretion were normal. Differential diagnosis included Wilson disease, aceruloplasminemia and others.

3) Case 3 (Fig. 1)
Fifteen-year-old boy. The proband was his 18-year-old...
Table 1  Biochemical and molecular data from cases 1 & 2

<table>
<thead>
<tr>
<th>Case</th>
<th>AST/ALT (U/L)</th>
<th>S-Cp (mg/dl)</th>
<th>U-Cu (µg/day)</th>
<th>ATP7B gene mutations (exon)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>122/137</td>
<td>2.0</td>
<td>125.5</td>
<td>R778L(8) / 2659delG (11)</td>
</tr>
<tr>
<td>2</td>
<td>40/31</td>
<td>0.95</td>
<td>17.0</td>
<td>2299insC (8) / 2299insC (8)</td>
</tr>
</tbody>
</table>

S-Cp: serum ceruloplasmin levels  U-Cu: urinary copper excretion

Results

1) Case 1 (Table 1)

He had R778L mutation in exon 8 and 2659delG mutation in exon 11. These results revealed that he is a presymptomatic patient with Wilson disease.

2) Case 2 (Table 1)

ATP7B gene analysis showed that he had 2299insC mutation (exon 8) in both alleles, indicating that he is a presymptomatic patient with Wilson disease.

3) Case 3 (Fig. 1)

ATP7B gene analysis for his sister (proband) disclosed two mutations, 2871delC mutation in exon 13 and N1270S mutation in exon 18. ATP7B gene analysis for case 3 was then carried out and only 2871delC mutation was detected. Thus, he was diagnosed as a carrier of Wilson disease.

4) Case 4 (Fig. 2)

One-year-old girl. Her elder sister (5 years old) was diagnosed with Wilson disease because of liver dysfunction, hypoceruloplasminemia and a mild elevation of urinary copper excretion. The serum ceruloplasmin level of case 4 was decreased, but was definitely higher than her sister. Urinary copper excretion was not measured.

Materials and Methods

[ATP7B gene analysis]

ATP7B gene was analyzed in the cases described above and their siblings. Genomic DNA was isolated from peripheral blood leukocytes of patients and their family. All exons of the ATP7B gene were amplified by genomic polymerase chain reaction (PCR), and then analyzed by direct sequencing according to the method described previously. Gene analysis was performed under written informed consent.

Fig. 1 The family pedigree of case 3. The arrow shows case 3, and the arrow with p shows the proband. S-Cp: serum ceruloplasmin levels. U-Cu: urinary copper excretion.

Fig. 2 The familial pedigree of case 4. The arrow shows case 4, and the arrow with p shows the proband. S-Cp: serum ceruloplasmin levels. U-Cu: urinary copper excretion. ND: not performed.
Wilson disease can be diagnosed based on low serum ceruloplasmin levels and urinary excess copper excretion. Early diagnosis in the presymptomatic period is important to improve the prognosis of Wilson disease patients. As the urinary copper excretion of young (under 4-5 years old) patients may not increase, hepatic copper contents must be measured for definitive diagnosis. However, liver biopsy is invasive, and we do not know how copper accumulates in the liver of infantile patients, such as case 2 and case 4. When ATP7B gene mutations are detected in both alleles, we can diagnose Wilson disease. Thus, molecular analysis for diagnosis should be carried out for young patients with this disease.

Wilson disease is an autosomal recessive disorder with a wide-ranging onset age. Familial analysis is important to identify presymptomatic patients and carriers. When ATP7B gene mutations of probands are found, a familial study using molecular analysis will be rapid and reliable. Molecular diagnosis should be performed for family members, especially for siblings, if Wilson disease patients are identified.

In conclusion, the molecular diagnosis of Wilson disease is very useful for the definitive diagnosis of young patients and familial analysis.

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References