Wilson’s disease (WD; hepatolenticular degeneration; OMIM, #277900) was first described in 1912 by Klökkern Wilson [1]. It is an autosomal recessive disorder of copper transport, characterized by the accumulation of intracellular copper in the liver and extrahepatic tissues. The majority of patients present between the ages of 5 and 35. The disease may present clinically as liver disease, as a progressive neurologic disorder, or as a psychiatric illness; diagnosis may therefore be difficult [2]. WD occurs worldwide with an incidence of between 1 in 35,000 and 1 in 100,000 live births [2].

In 1993, the gene responsible for WD was identified [3, 4]. The gene, named \( \text{ATP7B} \), exceeded 200 variations. The H1069Q mutation is the most common mutation in European and North American patients with WD, while the R778L mutation is found in most Asian patients. In the present study we identified 12 homozygotes and 22 heterozygotes for R778L. We summarize our results here along with those of previous studies. There were a total of 46 homozygotes and 66 heterozygotes for R778L. The phenotypes of R778L homozygotes and heterozygotes were grouped as hepatic presentation, neurologic presentation, or other presentation according to the most recent standards. No significant differences were found in three clinical features (mean age of onset, number of patients with hepatic presentation, and number of patients with neurologic presentation) between R778L homozygote and heterozygote groups, suggesting that the phenotype of WD is influenced by a series of factors, rather than only by the \( \text{ATP7B} \) gene.

**Key words:** genotype-phenotype, mutation, \( \text{ATP7B} \), Wilson’s disease.
has been classified to date as hepatic, neurologic, hepatic-neurologic or other [2]. To overcome this problem, recent standards for the diagnosis and phenotypic classification of WD were published after considerable discussion at the 8th International Meeting on Wilson’s Disease and Menkes Disease in Leipzig, Germany (April 16-18, 2001) [14]. Following the new criteria for the phenotype classification of WD, symptomatic patients are classified according to the major organ involved in their presenting symptoms: hepatic presentation (acute or chronic hepatic WD), neurologic presentation (associated or not associated with symptomatic liver disease; presence or absence of liver disease not investigated) or other [14].

In the present study, the genotype and phenotype (clinical features) relationship of R778L is discussed according to these new standards for the phenotype classification of WD.

Subjects, materials and methods

Subjects

The present study included a total of 58 unrelated patients with WD, who were examined for the R778L mutation. Forty patients were from mainland China, and 18 were from Japan. The diagnosis of WD was based on the clinical criteria of WD including Kayser-Fleischer rings, abnormal serum copper and ceruloplasmin levels, and a high level of copper in the urine [14]. Fifty unrelated healthy individuals were also examined as normal controls. Additionally, the homozygotes and heterozygotes for R778L who have been previously reported were searched for both in PubMed and in the Chinese journals website [http://www.sci.com.cn] [5, 9, 11, 13, 15-17].

Material and methods

Isolation of genomic DNA, polymerase chain reaction (PCR) amplification and direct sequencing

For detection of homozygotes and heterozygotes for R778L in our 58 WD patients, PCR and direct sequencing of exon 8 were performed as reported by Thomas et al. [5].

Statistical analysis

Phenotype classification was performed according to the above-mentioned new standards [14]. Data from symptomatic homozygotes and symptomatic heterozygotes for R778L were analyzed using a statistical package (SPSS, Version 10.0; SPSS Inc., Chicago, IL, USA). Statistical analysis was performed using the t test or chi-square test. The criterion for statistical significance was P<0.05.

Results

In the present study, 12 homozygotes and 22 heterozygotes for R778L were identified by PCR and direct sequencing; together with the patients who had been previously reported, we found a total of 46 homozygotes and 66 heterozygotes for R778L in Asian WD patients [9, 11, 13, 15-17].

Following the new standards, patients were classified as hepatic, neurologic or other presentation (Table 1). No significant difference of mean age of onset was observed between the homozygote and heterozygote groups (Table 1), however, in 75.6% of homozygotes the age of onset was between 10 and 20 years (Table 2). Neither the number of patients with hepatic symptoms nor the number of patients with neurologic symptoms was significantly greater among homozygotes than among heterozygotes for R778L (Table 1).

Discussion

Although R778L is a common mutation in Asian patients with WD, we found few homozygotes for R778L. For genotype and phenotype analysis, the homozygote and heterozygote for R778L are very useful, however the homozygote and heterozygote for R778L have been reported to date only sporadically. Wu et al. report that in 18 homozygotes R777L was associated with an earli-

Table 1. Genotype and phenotype relationships for Arg 778 Leu in patients with WD.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of patientsa</th>
<th>Age at onset, yearsb</th>
<th>Hepatic/neurologic/other symptoms/asymptom, No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homozygotes</td>
<td>43</td>
<td>15.19 ± 5.24</td>
<td>23/18/2/3</td>
</tr>
<tr>
<td>Heterozygotes</td>
<td>58</td>
<td>14.65 ± 7.76</td>
<td>26/31/1/8</td>
</tr>
</tbody>
</table>

a Includes patients in the present study and in references 5, 9, 11, 13, 15 and 16.
b Age data are given as mean ± SD.
er onset of WD than in 11 heterozygotes, and with hepatic presentation [11].

In the present study we summarized both our own and previously reported data, thereby examining a large number of homozygotes and heterozygotes, in order to analyze the genotype-phenotype relationship more accurately. We found that there were no significant differences in three clinical features (mean age of onset, number of patients with hepatic symptoms, and number of patients with neurologic symptoms) between homozygotes and heterozygotes for R778L.

Nevertheless, R778L homozygotes had an earlier age of onset than H1069Q homozygotes, in most of whom symptoms started at 20 years or older [5, 7, 18-20]. Although in Dutch, east German, Polish and Austrian homozygotes for H1069Q neurological presentation was predominant [7, 20-22], this finding has not been borne out in studies on other European and North American counterparts, in whom hepatic and neuropsychiatric presentations have been similarly represented [5, 18, 23]. Takeshita et al. investigated two Japanese families with WD in which siblings showed different clinical phenotypes and different ages of onset, in spite of their having the same mutation in each family [17], strongly suggesting that at least phenotypic variability in WD may be due to additional factors. Dietary copper intake, intestinal metallothionein inducibility and the capacity for countering copper stress at the cellular level via glutathione and the heat shock protein pathway may all be important factors modulating the phenotype response [5, 24]. Moreover, a recent study proposes that the copper-binding, antioxidant and membrane-stabilizing properties of the apolipoprotein E (ApoE) protein may be a possible mechanism by which the ApoE ε3/3 genotype may confer advantage by delaying clinical manifestations of WD [25].

In conclusion, the present study found no significant differences in three clinical features (mean age of onset, number of patients with hepatic presentation, or number of patients with hepatic presentation) between homozygotes and heterozygotes, suggesting that the phenotype of WD is influenced by a series of factors, including but not limited to the ATP7B gene.

Acknowledgements
This study was supported by Postdoctoral Fellowships for Foreign Researchers from the Japan Society for the Promotion of Science (JSPS).

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