Molecular Diagnosis and Prophylactic Therapy for Presymptomatic Chinese Patients With Wilson Disease

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**Background:** The potential for therapy for Wilson disease (WD) emphasizes the importance of presymptomatic diagnosis in families with WD (WD families).

**Objectives:** To investigate the feasibility of presymptomatic DNA diagnosis and evaluate the efficacy of zinc sulfate therapy in WD families.

**Methods:** Seventy-eight clinically unaffected siblings were studied from 51 unrelated WD families that were ascertained by affected individuals. The diagnosis in presymptomatic patients was established by a combination of direct mutational analysis and haplotype analysis with 3 short tandem repeat markers. The presymptomatic patients were treated with 50 mg of elemental zinc sulfate twice a day from the time of molecular diagnosis and followed up for 3 to 5 years.

**Results:** Of the 78 siblings, 17 were diagnosed as presymptomatic patients. Kayser-Fleischer rings were absent in 7 and faint in 4 of the 17 presymptomatic patients. The serum ceruloplasmin values gradually increased and 24-hour urinary copper values gradually diminished during zinc therapy, which indicate effective control of copper metabolism. None of the siblings developed clinical symptoms of WD or adverse effects from zinc therapy.

**Conclusion:** We conclude that presymptomatic DNA diagnosis and zinc therapy are effective treatment of patients with WD.

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**Wilson Disease** (WD), an autosomal recessive disorder of copper metabolism, causes toxic accumulation of copper in the liver, brain, cornea, and kidney. The disease phenotype includes progressive liver cirrhosis, neurologic impairment, and Kayser-Fleischer (K-F) rings and/or renal malfunction. Wilson disease can be treated successfully, but treatment must be lifelong. Without effective anticooper treatment, WD is usually crippling and frequently fatal. However, prophylactic therapy in affected but presymptomatic patients can prevent the onset of symptoms. Thus, prompt and accurate presymptomatic diagnosis is critically important.

Clinical and biochemical screening, including liver biopsy for hepatic copper analysis, remains the standard for diagnosis. However, these standard tests may give false-positive results or cause the diagnosis of WD to be missed. A missed diagnosis can result in lost opportunities for prophylactic therapy, whereas false-positive diagnosis may result in inappropriate lifelong administration of potentially toxic drugs in heterozygote carriers and those with non-wilsonian liver disease. Furthermore, the standard criteria cannot be applied for carrier detection or presymptomatic diagnosis. Molecular diagnosis has the potential to overcome all of these limitations when the siblings of an index case are screened.

Therapy with penicillamine in presymptomatic patients was reported many years ago. However, penicillamine is an exceedingly toxic drug and may lead to adverse effects primarily involving the immune system or connective tissue. Also, it can cause the onset of neurologic disease when used in the initial therapy for presymptomatic patients. Zinc has been developed as an effective and nontoxic therapy in WD that blocks the absorption of copper and increases copper excretion in the stool. In this study, we used molecular diagnosis to identify 17 presymptomatic patients from 78 clinically unaffected siblings of 51 families with WD (hereafter referred to as WD families). Of the 17 presymptomatic patients, 14 were prescribed zinc sulfate as prophylactic therapy from the time of diagnosis and remained well 3 to 5 years later. The other 3 refused the prophylactic therapy and manifested symptoms later.

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Seventy-eight clinically unaffected siblings were studied from 51 unrelated WD families that were ascertained by the presence of affected individual family members. Genotypes of 51 propositi and 102 parents have been described elsewhere.7 Of 78 siblings, 53 were from 32 families with 2 known mutations and 25 were from 19 families with 1 known mutation. Informed consent for each subject was obtained according to the policy of Fujian Medical University, Fuzhou, People’s Republic of China. Genomic DNA was isolated from peripheral-blood lymphocytes by use of standard methods.

**DIRECT MUTATIONAL ANALYSIS**

According to the genotypes of propositi,7 genotypes of 78 siblings were detected by single-stranded conformational polymorphism (SSCP) analysis of specific coding regions generated by polymerase chain reaction amplification. For example, the genotype of the propositus from family 4 was Thr935Met (exon 12)/Arg778Leu (exon 8), so genotypes of siblings from this family were identified by SSCP analyses of exon 12 and exon 8. Procedures of polymerase chain reaction amplification and SSCP analysis with nondenaturing polyacrylamide gel electrophoresis have been described elsewhere.7

**HAPLOTYPE ANALYSIS**

The siblings from 19 families with 1 known mutation were investigated by haplotype analysis. Three short tandem repeat (STR) markers, D13S133, D13S301, and D13S314, were analyzed in the confirmed propositi together with the parents and the siblings. Procedures of polymerase chain reaction amplification and measurement of these STR polymorphisms with non-denaturing polyacrylamide gel electrophoresis have been described elsewhere.6

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**RESULTS**

Polymerase chain reaction–SSCP analysis was performed to identify the genotypes of 78 siblings from 51 WD families, with the propositus and parents used as positive control subjects. If the propositus was a homozygote, he or she could be used as a homozygote control and parents as heterozygote controls. If the propositus was a compound heterozygote, then the propositus and parents were all used as heterozygote control subjects. The exhibiting bands in nondenaturing polyacrylamide gel (SSCP bands) of siblings were compared with those of the propositus and parents, so the results could be obtained definitely. Among
53 siblings from 32 families with 2 known mutations, 11 siblings who inherited mutations on both alleles, like their propositi, were diagnosed as presymptomatic patients; 17 as heterozygote carriers; and 25 as normal individuals. Among the other 25 siblings from 19 families with 1 known mutation, 16 siblings from 11 families were shown to have different SSCP bands from their propositi, so they were excluded from the presymptomatic diagnosis. They may have been heterozygote carriers or unaffected individuals. The other 9 siblings from 8 families were shown to have the same SSCP abnormal bands as their propositi. They may have been presymptomatic patients or heterozygote carriers. Haplotypes of alleles at 3 STR loci on the same chromosome were constructed for these 25 siblings and their propositi and parents to perform linkage analysis. Among 9 siblings from 8 families, 6 siblings from 6 families who inherited the same haplotypes as their propositi were diagnosed as presymptomatic patients, the other 3, as heterozygote carriers. Among 16 siblings from 11 families, 9 were diagnosed as heterozygote carriers and 7, as unaffected individuals. In conclusion, we identified 17 presymptomatic patients, 29 heterozygote carriers, and 32 unaffected individuals from 78 siblings. The genotypes of these presymptomatic patients are shown in Table 1. The diagnosis of presymptomatic patient and heterozygote carrier in family 53 with SSCP analysis of exon 12 and haplotype analysis by means of 3 STR markers is shown in the Figure.

PROPHYLACTIC THERAPY AND FOLLOW-UP STUDIES OF PRESYMPTOMATIC PATIENTS

At the time of molecular diagnosis, routine clinical studies were carried out and showed normal results among all presymptomatic patients. All of them were asked to follow a low-copper diet and undergo prophylactic treatment with zinc. Fourteen of the 17 presymptomatic patients accepted, but the other 3 refused the therapy. During the subsequent 3 to 5 years, none of the 14 presymptomatic patients who accepted the therapy developed clinical symptoms of WD or adverse effects from zinc therapy. Among the other 3 patients, 1 presented with neurologic symptoms at the age of 26 years, and 2 presented with liver symptoms at 12 and 18 years of age, respectively. The clinical information about these patients, in terms of age, sex, the presence of K-F rings, serum ceruloplasmin levels, and 24-hour urinary copper levels at the time of diagnosis, are given in Table 1. In addition, yearly serum ceruloplasmin values and 24-hour urinary copper values in the presymptomatic patients who accepted the prophylactic therapy are given in Table 2.

The normal ranges of serum ceruloplasmin and urinary copper excretion in our laboratory are 20 to 40 mg/dL and 20 to 50 µg/24 h, respectively. As shown in Table 2 and Table 3, 13 of 14 presymptomatic patients had lower serum ceruloplasmin values (<20 mg/dL). The average serum ceruloplasmin values gradually increased from a baseline mean of 13.94 to 17.60 mg/L, and the mean value after more than 4 to 5 years of zinc therapy was significantly higher than the baseline value by t test (P = .045). The 24-hour urinary copper values of these presymptomatic patients were all above the normal value at the time of diagnosis (baseline) and gradually diminished during zinc therapy. It can be clearly seen in Table 3 that the average 24-hour urinary copper values decreased substantially from a baseline mean of 132.7 to 105.0 µg/24 h, and mean values after more than 2 to 3, more than 3 to 4, and more than 4 to 5 years of zinc therapy were significantly lower than the baseline value by t test (P = .01, .03, and .047, respectively).

The potential for treatment of WD emphasizes the importance of diagnosis. Because it is inherited in a recessive
mode, the siblings of affected patients are at a 25% risk of having the disease. The disease is fully penetrant and can present without warning at a variety of ages and in different forms, so once a sibling is diagnosed, it is imperative to treat that person prophylactically. Normally, the diagnosis of WD is based on clinical and biochemical criteria. Although the presence of K-F rings and a low level of ceruloplasmin are sufficient to diagnose WD, they are increasingly recognized to have low sensitivity. Twenty percent of carriers have low ceruloplasmin levels, which will result in a false-positive diagnosis. The K-F rings are indicative of pathological copper accumulation load in the central nervous system and are a late manifestation of WD; thus, they are often absent in younger patients, especially asymptomatic siblings in WD families need to be confirmed or ruled out by genetic analysis.

Wilson disease is caused by mutations in the ATP7B gene encoding a putative copper-transporting P-type adenosine triphosphatase. According to the genotypes of propositi that had been confirmed by sequencing previously, the genotypes of siblings can be identified by SSCP analysis and do not need to be confirmed by sequencing, which has simplified the approach. With the cloning of the ATP7B gene, several STR markers from chromosome 13 were found and used for gene diagnosis within families. The accuracy and informativeness of the haplotype analysis depend on the polymorphism information content of each locus and on the relative positions of marker loci around the candidate gene. D13S301 is within the WD locus, while D13S133 and D13S314 are positioned in a 150-kilobase region on either side of the WD locus. The polymorphism information content of these markers is 0.88, 0.88, and 0.89, respectively, in the Chinese population, and the polymorphism information content of D13S133 and D13S314 is 0.83 and 0.76, respectively, in the non-Chinese population. Therefore, these markers are sufficiently close to the WD locus and sufficiently polymorphic to be useful in Chinese or non-Chinese populations. The methods based on these 3 STRs are simple to perform, provide reproducible results, and are highly informative.

In this study, the diagnoses of siblings were established by a combination of direct mutational analysis and haplotype analysis with STR markers. Among 78 siblings from 51 families, 17 siblings were diagnosed as presymptomatic patients, 29 as heterozygote carriers, and 32 as unaffected individuals. At the time of presymptomatic diagnosis, a clinical follow-up study including genetic and psychological counseling and legal safeguarding was begun. Detailed laboratory investigation showed that K-F rings were absent in 7 of 17 presymptomatic pa-

![Table 3. Statistical Analysis of Serum Ceruloplasmin and 24-Hour Urinary Copper Values*](reprinted Arch Neurol. 2003;60(5):647-651)

<table>
<thead>
<tr>
<th>Years of Follow-up</th>
<th>No. of Patients</th>
<th>Serum Ceruloplasmin, mg/dL</th>
<th>24-Hour Urinary Copper, µg/24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Baseline</td>
<td>&gt;0-1 y</td>
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<tr>
<td></td>
<td></td>
<td>14</td>
<td>13.94 ± 4.54</td>
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<tr>
<td></td>
<td></td>
<td>&gt;0-1 y</td>
<td>13.64 ± 4.53†</td>
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<tr>
<td></td>
<td></td>
<td>&gt;1-2 y</td>
<td>14.41 ± 4.31†</td>
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<tr>
<td></td>
<td></td>
<td>&gt;2-3 y</td>
<td>14.83 ± 4.44‡</td>
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<tr>
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<td>&gt;3-4 y</td>
<td>15.70 ± 3.09†</td>
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<tr>
<td></td>
<td></td>
<td>&gt;4-5 y</td>
<td>17.60 ± 0.97§</td>
</tr>
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Abbreviations: CP, ceruloplasmin; ellipses, not applicable; UC, urinary copper.

*Data are given as mean ± SD.
†Not significantly different from baseline value by t test.
‡P = .05.
§P = .03.
||P = .045.
¶P = .047.
The molecular diagnosis system is the most accurate, and fast diagnostic method that is well suited for routine use in clinical laboratories engaged in determining the status of the siblings in WD families.

Drs Wu and Wang contributed equally to this work.

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