Screening for mutations in ATP7B gene using conformation-sensitive gel electrophoresis in a family with Wilson’s disease

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This study was supported by the Department of Science and Technology, Government of India, under project no. SP/SO/BS4/99

Source of support: Departmental sources

Summary

Background: Wilson’s disease (WD) is an autosomal recessive disorder leading to copper overload, mainly in the liver and brain, due to mutations in the ATP7B gene. About 10% of heterozygous carriers of ATP7B gene mutations have decreased serum ceruloplasmin, posing diagnostic difficulties.

Case Report: We report a four-member family wherein the 11-year-old daughter was diagnosed as having WD based on standard biochemical tests and the presence of Kayser Fleischer rings. On screening the entire family for WD, both parents and her eight-year-old brother had no clinical evidence of WD. However, serum ceruloplasmin was markedly decreased in the brother and was borderline low in the father, raising the possibility that the brother also had WD. We used conformation-sensitive gel electrophoresis (CSGE) to screen for mutations in the ATP7B gene in this family.

Using CSGE we found that the patient’s father and brother had an aberrant pattern in exon 8 of the ATP7B gene, the mother an aberrant pattern in exon 13, while the daughter (the index patient) had aberrant patterns in exons 8 and 13 of the ATP7B gene. DNA sequencing revealed that the index patient was a compound heterozygote with 2292-2312del21bp (a novel mutation) and Arg969Gln mutations, while the father and brother were heterozygous for the 2292-2312del21bp mutation and the mother for the Arg969Gln mutation.

Conclusions: This case report illustrates the utility of CSGE in analyzing mutations in the ATP7B gene to resolve diagnostic dilemmas arising in heterozygous carriers with low serum ceruloplasmin.

key words: ATP7B gene • conformation-sensitive gel electrophoresis • heterozygous carrier • Wilson’s disease
**BACKGROUND**

The likelihood of Wilson disease (WD) in the siblings and parents or children of an index patient is 25% and 0.5% respectively [1]. More than 250 mutations of the \(ATP7B\) gene have been reported to date. Conventional tests to diagnose WD include low serum ceruloplasmin, elevated urine copper, and the presence of Kayser Fleischer rings in the cornea. However, about 10% of heterozygous carriers of \(ATP7B\) mutations can pose a diagnostic dilemma, with low serum ceruloplasmin but without clinical symptoms or signs of the disease. These individuals, who represent approximately 1 in 2000 persons in the general population, may present a difficult diagnostic problem if they develop chronic hepatitis or cirrhosis due to some other cause, thereby developing the clinical, biochemical, and histological features of WD [2]. Genetic testing is the definitive test to exclude WD in a sibling of an index WD patient [3]. This is the first report demonstrating the utility of direct screening of the \(ATP7B\) gene using conformation-sensitive gel electrophoresis (CSGE) to exclude WD in a sibling and to provide genetic counseling to the family members.

**CASE REPORT**

**Family history**

The index patient, an 11-year-old girl from southern India with chronic liver disease and renal tubular acidosis, was diagnosed to have WD on the basis of decreased serum ceruloplasmin (13 U/l, normal value: 62–140 U/l), Kayser Fleischer rings in the cornea, and elevated urine copper (1326 μg/24 hrs, normal value: <150 μg/24 hrs). Her asymptomatic eight-year-old brother was screened for WD. His physical examination and liver function tests were normal and he did not have Kayser Fleischer rings on slit lamp examination of the cornea. However, his serum ceruloplasmin was markedly decreased (5 U/l), while urinary copper was normal (81 μg/24 hrs). They were born to parents of a second-degree consanguineous marriage. Both parents were asymptomatic and had normal liver function tests. Serum ceruloplasmin was borderline low (59 U/l) in the father and normal (185 U/l) in the mother (Table 1).

**Table 1. Copper chemistry in the family of an index WD patient.**

<table>
<thead>
<tr>
<th>Status</th>
<th>Serum ceruloplasmin (U/l)</th>
<th>Serum copper (μg/dl)</th>
<th>Urinary copper (μg/24 hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Father</td>
<td>59</td>
<td>68</td>
<td>Not available</td>
</tr>
<tr>
<td>Mother</td>
<td>185</td>
<td>106</td>
<td>Not available</td>
</tr>
<tr>
<td>1st child (WD patient)</td>
<td>13</td>
<td>28</td>
<td>1326</td>
</tr>
<tr>
<td>2nd child (brother)</td>
<td>5</td>
<td>90</td>
<td>81</td>
</tr>
</tbody>
</table>

Normal ranges: serum ceruloplasmin 62–140 U/l, serum copper 70–170 μg/dl, and urinary copper <150 μg/24 hr.

**Genetic studies**

We performed genetic studies on the entire family, after obtaining informed consent, to resolve whether the low se-
rum ceruloplasmin in the index patient’s brother was due to WD or to a heterozygote carrier status. DNA was isolated from peripheral blood lymphocytes by the standard phenol chloroform method [4]. Exonic and flanking intronic regions of $ATP7B$ gene were amplified by polymerase chain reaction (PCR) using primers described elsewhere [5]. Mutation screening was performed using CSGE [6] and the PCR products displaying abnormal CSGE profiles relative to those in a normal individual were sequenced using a Big Dye terminator kit on an ABI310 Genetic Analyzer. The aberrant band produced in exon 8 of the $ATP7B$ gene was excised from the 10% polyacrylamide gel and DNA sequencing was performed to confirm the deletion.

Using CSGE we found that the patient’s father and brother had an aberrant pattern in exon 8 (Figure 1A) of the $ATP7B$ gene, while the mother had an aberrant pattern in exon 13 (Figure 1B) and the daughter (the index patient) had aberrant patterns in exons 8 and 13 of the $ATP7B$ gene. DNA sequencing revealed that the index WD patient was a compound heterozygote (a novel 2292-2312del21bp mutation and an Arg969Gln mutation), while the father and brother were heterozygous for the 2292-2312del21bp mutation (Figure 1C,D) and the mother for the Arg969Gln mutation (Figure 1E).

**DISCUSSION**

To date, 51 short (<21-base-pair) deletions have been identified in the $ATP7B$ gene, and three of these were found in exon 8 [7]. The novel 2292-2312del21bp mutation in exon 8 reported in the current study causes a deletion of codon 764 to 771 which induces the loss of seven amino-acid residues (NTPPMLF), leading to a shortening of transmembrane domain 4 (Tm4) of the WD protein. Arg969Gln, a missense mutation described in the Mediterranean population, disrupts the channel and transmembrane 6 domain (Tm6) of the WD protein [8]. The disease-causing compound heterozygous mutation (2292-2312del21bp and Arg969Gln) was present only in the index patient, while the family members were heterozygous carriers for WD (Figure 1). Thus the parents and the brother of the index patient are not at risk of developing WD.

DNA Linkage analysis using microsatellite markers [9] and direct gene analysis using single-stranded conformation polymorphism [10] are other methods applied to detect haplotypes and mutations in WD.

**CONCLUSIONS**

CSGE is a simple and cheap mutation screening tool which, to our knowledge, has so far not been applied to screen for mutations in WD. This report highlights the utility of CSGE to screen for mutations in the $ATP7B$ gene and its use in genetic counseling by differentiating WD from a heterozygote carrier.

**Acknowledgement**

We thank the family members for cooperating in this study.

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