Molecular Genetics Diagnosis of Wilson Disease: the First Reported Case of ATP7B Gene Mutation at Codon 778 in Southwest Iran

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ABSTRACT

Wilson disease is a metabolic disorder with an autosomal recessive genetic pattern and occurs in 1-4 of every 100000 individuals. Inactivation of the ATP7B gene leads to accumulation of the toxic copper to liver and brain causing hepatic and neurological complication. Therefore, most patients suffer from chronic hepatic inflammation and central nervous system disorder. Nowadays, up to 500 mutations were found in the ATP7B gene that weaken or fully disrupt the function of the gene product. Recurrent mutations were found in different population. We found a homozygous pathogenic missense mutation at codon 778 (R778W) in an individual from southwest Iran. This mutation has been reported in previous studies in the continents America and Europe. The present study is the first report from Wilson disease that has been diagnosed in southwest Iran. This mutation has been shown in previous studies in patients from continents America and Europe.

Keywords: Wilson Disease, ATP7B Cu-binding P type ATPase, Iran

Introduction

Organic copper is modified and restored in the liver in a safe way. However, inorganic copper mostly bypasses the liver and enters to the blood directly. Because of toxic nature of copper, its penetration through the blood/brain barrier may cause neurological difficulties (1). Approximately, 95% of copper in blood is associated with Ceruloplasmin, a major copper-carrying protein in blood, and the rest of copper exists free in blood (2).
Wilson’s disease (MIM# 277900) is a copper metabolic disorder that inherits with an autosomal recessive genetic pattern. Wilson’s disease (WD) occurs in 1 per 30,000 people (3), but its prevalence in Iran may be higher, because of consanguinity. Wilson disease was first described by Kiennear Wilson in 1912 (4), but Bull et al. described in 1993 that the ATP7B gene is associated with Wilson disease (5).

Genetically, the WD is caused by mutation in the ATP7B gene localizing to the q14.3 band of chromosome 13 (5) and encodes a copper-transporting P-type ATPase (6).

The ATP7B protein is expressed mostly in the liver (5). Immunohistochemical studies have shown the trafficking of the ATPase from the Golgi network to cytoplasmic vesicles in response to an increase in copper concentration (7).

Lacking or decreased of ATP7B protein directs to reduction of hepatocellular copper secretion into bile, which results in copper accumulation and injury leading to chronic liver dysfunction as psychiatric and neurological complications (8, 9).

The age of diagnosis is reported from very young patients from 3 years to eighth decade of life. However, the majority of patients are diagnosed between 5 and 35 years (10, 11). Younger WD patients show clinical feature and histological findings that mimic to autoimmune hepatitis, which makes a definite diagnosis difficult (12).

Numerous studies show recurrent and novel mutations in the mentioned gene, which emphasizes the necessity of mutation survey. According to the Human Gene Mutation Database (www.HGMD.org), there are more than 500 pathogenic nucleotide changes in the ATP7B gene. However, a large portion of the detected mutations is missense or nonsense.

Apparently, the frequency of mutations within the ATP7B gene differs between ethnic groups. Nevertheless, to date, there are few data about molecular diagnosis of Wilson disease in Iran, and no reported cases from southwest Iran. We present here a case of Wilson disease from Khuzestan Province.

**Case presentation**

A 16 years young man, as the first child of a double cousin marriage, was referred to our canter with the asymptomatic hepatomegaly and difficulty to movement (tremor, involuntary movement), experience of seizure, migraine headaches, and persistently elevated serum aminotransferase activity (AST, ALT). No other biochemical tests and clinical signs were available. He was also suspected to be affected with Wilson’s disease. After informed consent, whole blood of the patient and his parents were subjected for genomic DNA extraction by routine salting out method.

Genomic DNA was extracted using a standard protocol and 21 coding exons of the ATP7B gene were amplified with the following PCR condition: 100 ng genomic DNA, 200 µM dNTP’s, 1.5 mM MgCl₂, 2.5 units SuperTaq polymerase (Genfanavaran, Iran), and 25 pmol each primer. Primers for amplification of exons and flanking introns were designed by the software ‘Primer3out’. Primer sequences are available by request. Amplification was carried out in 25 µl volume and 35 cycles: 95°C for 30 sec., 58-65°C for 45 sec., and 72°C for 60 sec. Direct sequencing of the coding exons and the flanking intron sequences was performed using the Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) on an ABI Prism 3700 automated genetic analyser (Applied Biosystems).

Direct sequencing analysis of the proband demonstrated a homozygous missense mutation at codon 778 (CCG>TGG), which causes an amino acid exchange of Arginine to Tryptophan (Fig. 1).
The human \textit{ATP7B} gene (on chromosome 13q14.3) consists of 80,000 base pairs including 21 coding exons (upper image). The spliced form of the gene processed into the 7500 bases mRNA that translated into the 1411 amino acid making a 159 kDa ATPase that expresses in liver, brain, kidney and placenta tissue with several functionally important domains (lower image): six copper binding sites (Cu 1-6); 7 transmembrane Domains; Transducing domain (Td) for the passage of copper through the membrane.

The parents were heterozygous for the mentioned nucleotide substitution. The pathogenic nature of this mutation has been confirmed in previous studies by expression analysis (Table 1).

\textbf{Table 1-} The missense mutation at codon 778 (C>T) of the \textit{ATP7B} gene in individuals with Wilson’s disease has been reported in different ethnic groups in the continents: America, Europe, and East Asia.

<table>
<thead>
<tr>
<th>Position</th>
<th>Amino acid change</th>
<th>Kind of mutation</th>
<th>Exon</th>
<th>Affected domain</th>
<th>Ethnic group *</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.2332C&gt;T</td>
<td>p.Arg778Trp</td>
<td>missense</td>
<td>8</td>
<td>TM4**</td>
<td>American, British, Sardinian, Spanish (Grand Canaria), Japanese, Bulgarian, Italian, Sardinian, Bulgarian, Persian (Southwest Iran)</td>
</tr>
</tbody>
</table>

* http://www.wilsondisease.med.ualberta.ca/database

**TM4: transmembrane
Discussion

Excessive deposition of copper in the liver, brain, and cornea is the major cause of Wilson disease, an autosomal recessive disorder, which is caused by mutation in the \textit{ATP7B} gene (13). In the liver, ATP7B inactivation leads to copper accumulation in the cytosol and nuclei (14), down regulation of lipid metabolism and increased cell proliferation (14).

Symptomatic or successful treated patients show success by a chelating agent therapy such trientine or penicillamine (15). The major effect of penicillamine in WD is to promote the urinary excretion of copper. Penicillamine may also act by inducing metallothionein in individuals with WD. Penicillamine also interferes with collagen cross-linking and has some immunosuppressant actions (15). Trientine was introduced in 1969 as an alternative to penicillamine. Few data exist about the pharmacokinetics of trientine. It is poorly absorbed from the gastrointestinal tract, and what is absorbed is metabolized and inactivated. About 1% of the administered trientine and about 8% of the biotransformed trientine metabolite, acetyltrien, ultimately appears in the urine (16). Generally, it is possible to have a normal lifespan, if the disease is diagnosed in early stage (3).

Nevertheless, prevention is quit the best way to avoid the WD. A suitable preventing way is the use of molecular diagnostics as a powerful tool in respect of its accuracy and time saving. But, this requires the knowledge about kind and frequency of mutations in the \textit{ATP7B} gene.

We report here the first molecular diagnosis of Wilson’s disease in southwest Iran (Khuzestan Province). The disease is caused by a missense mutation at codon 778 of the \textit{ATP7B} gene, which is reported, previously (17). This mutation affects negatively the structure of domain 4 leading to a dysfunction of phosphatase activity of the ATP7B protein (17). The mentioned pathogenic mutation has been detected in other countries in Europe and East Asia with different prevalence. Very recently, the mutation H1069Q was identified as the most common mutation in the \textit{ATP7B} gene in WD patients from north and central Iran (18), which probably shows diverse distribution of mutation frequency of the \textit{ATP7B} gene in Iran. However, further studies with large-scale samples are strongly needed to find the real mutation frequency in southwest Iran, an area that is characterized by multi-ethnic population and high consanguineous marriages.

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References