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RESEARCH & REVIEW

Novel *ATP7B* mutations in Vietnamese patients with Wilson disease

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ABSTRACT

Wilson disease (WD) is an autosomal recessive disorder of copper metabolism. It is caused by defects of ATP7B encoding a copper transporting P-type ATPase. This gene is located on chromosome 13q14.3. Mutation of ATP7B disrupts copper homeostasis, resulting in copper accumulation in liver, brain, kidneys, and corneas as well as copper poisoning at these sites. Until today, more than 300 disease-specific mutations have been identified. To detect mutations of the entire ATP7B gene in Vietnamese patients with WD, sequencing analysis was applied. We detected three mutations in ATP7B that have not previously been reported. Two are missense mutations and one is a nonsense mutation. Furthermore, segregation analysis showed no mutation transmission patterns within each family of WD patients. These newly discovered mutations of ATP7B in Vietnamese patients are important for WD research and, therefore, should be included in public genetics epidemiology and population genetics databases.

1. Introduction

Copper is an essential component of many enzymes such as: lysyl oxidase, superoxide dismutase, dopamine-β-hydroxylase and cytochrome C oxidase. These copper-dependent enzymes are needed for diverse processes of oxidase metabolism including respiration, free-radical detoxification, neurotransmitter synthesis, maturation of connective tissue and iron uptake (1-2). However, copper is only required in trace amounts. The accumulation of copper can damage plasma membranes, peroxisomes, mitochondria, microtubules, enzymes and even DNA (3). The effect of copper imbalance can be described best in two human genetic disorders: Wilson's Disease (WD) and Menkes's Disease. WD is an inherited autosomal recessive disease first described in 1912 by Samuel Alexander Kinnier Wilson. Prevalence of the disease is approximately 1 in 30,000. In 1993, Bull et al. identified the genetic defect of WD within the copper-transporting P type ATP7B gene located on chromosome 13 (4). According to Wilson's Disease Mutation Database (http:// www.wilsondisease.med.ualberta.ca/database.asp), more than 300 disease-causing mutations have been detected and this number is still growing because mutations in the ATP7B gene vary in different populations. A recent study identified a mutation hotspot in exon 2, 8, and 10 within a sample of Northern Vietnamese population (the data have been submitted for publication). In this study we have identified three new mutations in ATP7B with WD by using DNA sequencing. All of them are heterogeneous mutations.

2. Materials and Methods

2.1 Subjects and DNA extraction

Three Vietnamese patients from unrelated families were selected. All patients had been diagnosed with WD and treated at the National Hospital of Pediatrics in Hanoi, Vietnam. WD diagnosis was based on multiple clinical symptoms, including liver failure and/or neurological issues and/or the presence of the Kayser-Fleischer ring in their eyes, and biochemical markers, such as low serum ceruloplasmin (CP < 20 mg/dL) and high level of urinary copper (Cu > 100 mg/24h) (5). Genomic DNA was extracted from peripheral blood collected in EDTA-containing tubes using Wizard Genomic DNA Purification Kit (Promega, Madison, Wisconsin, USA) following manufacturer's protocols.

2.2 Polymerase Chain Reaction (PCR)

The entire exons of ATP7B gene were successfully amplified by using 24 primer pairs (four primer pairs for exon 2, one primer pair each for others) (IDT, Coralville, Iowa, USA). PCR was performed using GoTaq Green Master Mix (Promega, Madison, Wisconsin, USA) with 100 ng of genomic DNA in a mix containing 1U of Taq Polymerase, 10 pmol of each primer and distilled water. The thermocycle program included an initial denaturation step at 95 ºC for 5 min, followed by 35 cycles at 95 ºC for 30 s, 55 ºC for 45 s, and 72 ºC for 30 s, with a final extension at 72 ºC for 3 min. The size and quantity of PCR products were verified by electrophoresis in 1.5% agarose gel.

2.3 DNA sequencing

PCR products were directly sequenced using BigDye Terminal Kit (Amersham, UK) and Avant 3100 Genetic Analyzer automated sequencer (Applied Biosystems Inc., Foster City, California, USA), and data was analyzed using CLC Genomics Workbench (CLC Bio, Qiagen, Venlo, Limburg, Germany). All products were sequenced in both forward and reverse strands.

3. Results

All three patients demonstrated similar symptoms and laboratory analyses revealed low ceruloplasmin levels, low serum copper concentrations and increased urinary copper excretion (Table 1). Genomic DNA was isolated from the peripheral blood leukocytes of the subjects. DNA sequencing analysis was applied to identify mutations and polymorphisms of all 21 exons of ATP7B gene in 3 Vietnamese patients with WD. One nonsense mutation and two missense mutations in Table 2 were found to be novel: c.3233C>G; c.2943T>G; c.2705T>C (Figure 1). This procedure used Polyphen, an automatic tool predicting possible impacts of amino acid substitutions on the structure and function of human protein in analyzing the novel missense mutations. The mutations were correctly identified as novel mutations in the WD database (http:// www.wilsondisease.med.ualberta.ca/database.asp).

Clinical manifestation of these 3 patients showed association of liver, brain, and cornea damages with low levels of serum ceruloplasmin (Table 1). Segregation analysis within their families (including parents and one brother) was performed for these 3 patients whose mutations in the ATP7B gene had been detected, but no mutations were identified in their family members.

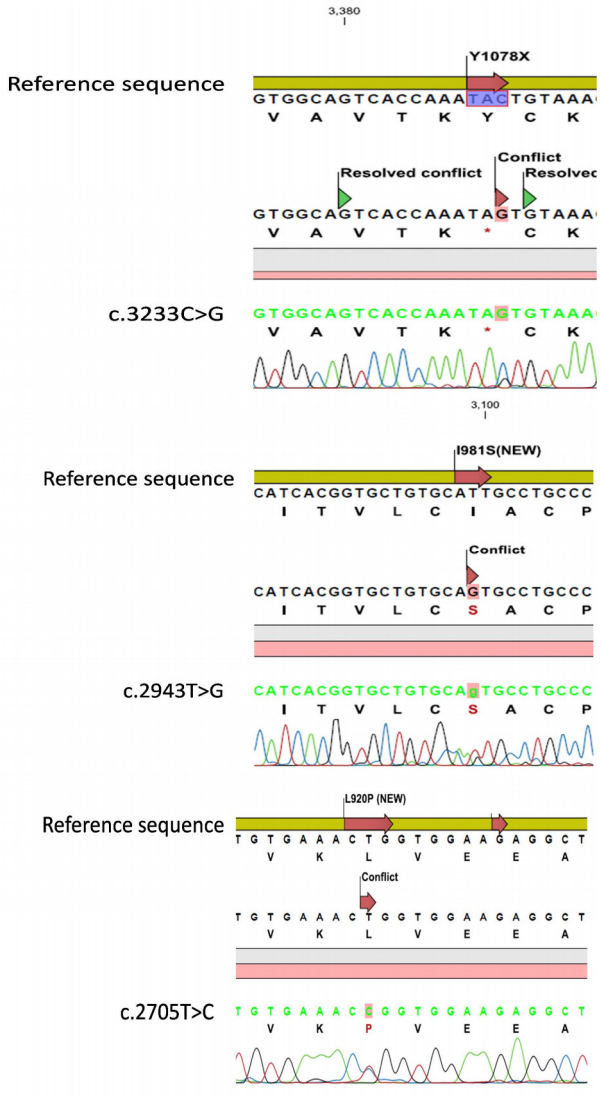

Figure 1. *Sequencing results of three new mutations on ATP7B gene of Wilson's disease patients*

4. Discussion

Wilson disease is an infrequent disorder worldwide in almost all populations. Frequencies of 1:30,000 to 1:40,000 are usually quoted (6), except for Crete and Sardinia with 6:100 and 1:7,000 live births respectively (7-8). At present, diagnosis of WD is extremely difficult especially for clinically asymptomatic individuals or those with atypical clinical manifestations. Moreover, WD patients have different clinical features (9). Particularly, WD is one of the rare diseases that are difficult to be treated; however, early diagnosis of WD may increase the probability of recovery (9).

Mutation	Patient	Affected organ			Laboratory finding	
		Liver	Brain	KF-ring	Serum CP (mg/dL)	Urine Cu (µg/day)
c.2705T>C	WD 52	-	+		6.0	325
c.2943T>G	WD 27	+	-	+	<10	220
c.3233C>G	WD 23	+	-	+	<10	180

Notes: Genbank Accession NM_000053.
The first nucleotide of ATG translation codon is considered nt + 1.
Serum CP was measured by Immnoturbidmetric test. Normal values for serum CP is 20-60 mg/dL (5).
Normal values for urine Cu is 10-40 µg/day (5).

Table 1: *ATP7B genotype, clinical and laboratory findings*

Nucleotide change	Exon/intron	Codon change	Mutation	Type
c.2705T>C	11	L902P	novel	missense
c.2943T>G	13	I981S	novel	missense
c.3233C>G	14	Y1078X	novel	nonesense

Notes: Genbank Accession NM_000053.
The first nucleotide of ATG translation codon is considered nt +1.

Table 2: *Mutations distributed throughout the entire coding region of ATP7B*

Currently, more than 300 WD-induced mutations have been reported in various populations but a few of them show relatively high frequencies in certain geographic areas. The most frequent mutation in Europe is H1069Q with a frequency of more than 65% (10). In Sardinia, c.-441_- 427del accounts for 61.5% of mutated chromosomes (8). The mutation M645R is present in 55% of patients in Spain (11), and R778L mutation is very common in Hong Kong patients (12). In contrast, deletion c.3402delC at exon 15 has been reported worldwide at a low frequency (usually below 2%) in several populations but at a high frequency (34.8%) among Brazilians (13). Our study also shows

that the most frequent mutation in Vietnam is S105X with 21.05% (data submitted for publication).

In recent years, assisted by the development of molecular analysis, the diagnostic rate for WD has been improved. However, clinical laboratory molecular diagnosis is expensive because there are many types of ATP7B genetic mutations. Due to its central role in WD etiology, it is essential to widely share new ATP7B mutations with the scientific and medical communities for improving WD research and diagnosis.

5. Conclusion

In our study, three new mutations (c.3233C>G; c.2943T>G; c.2862A>G) of ATP7B were detected in 3 Vietnamese WD patients by exon sequencing. Our findings enrich the database of Vietnamese WD genetics mutations as well as the WND database.

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