

Case Report

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).

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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 live. 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 center 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 μ M dNTP's, 1.5 mM MgCl₂, 2.5 units SuperTaq polymerase (Genfanavar, 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 μ l 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).

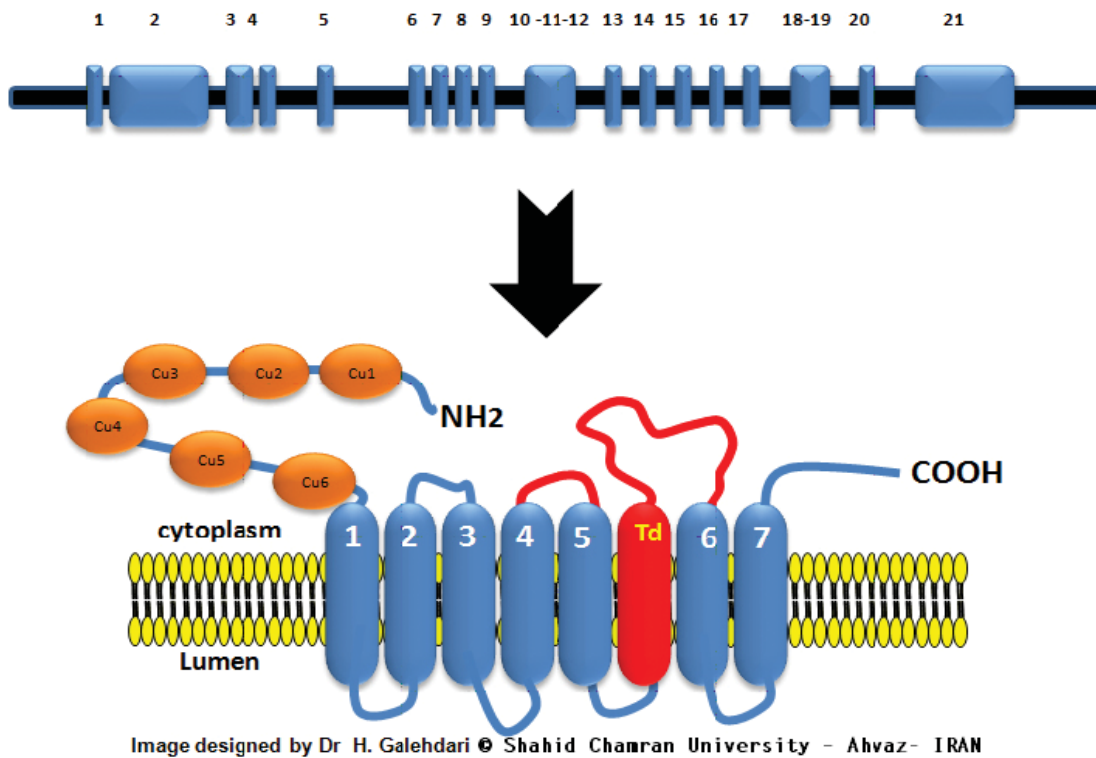


Fig. 1- The human *ATP7B* gene (on chromosome 13q14.3) consists of 80.000 base pairs including 21 coding exons (upper image). The spliced form of the gene processed into the 7500 bases mRNA that translated into the 1411 amino acid making a 159 kDa ATPase that expresses in liver, brain, kidney and placenta 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).

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

Position	Amino acid change	Kind of mutation	Exon	Affected domain	Ethnic group *
c.2332C>T	p.Arg778Trp	missense	8	TM4**	American British Sardinian Spanish (Grand Canaria) Japanese Japanese Bulgarian Italian Sardinian Bulgarian Persian (Southwest Iran)

* <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 *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 successfully treated patients show success by a chelating agent therapy such as 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 quite 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 *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 *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 *ATP7B* gene in WD patients from north and central Iran (18), which probably shows diverse distribution of mutation frequency of the *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.

Acknowledgments

The authors declare that there is no conflict of interests.

References

1. Brewer GJ. The risks of copper toxicity contributing to cognitive decline in the aging population and to Alzheimer's disease. *J Am C Nut* 2009;28(3):238–42.
2. Gaw A, Murphy MJ, Cowan RA. "Copper," in *Clinical Biochemistry: An Illustrated Color Text*. Philadelphia:Churchill Livingstone;2008.
3. Ala A, Walker AP, Ashkan K, Dooley JS, Schilsky ML. Wilson's disease. *Lancet* 2007;369(9559): 397–408.
4. Wilson SAK. Progressive lenticular degeneration: a familial nervous disease associated with cirrhosis of the liver. *Brain* 1912;34:295–508.
5. Bull PC, Thomas GR, Rommens JM, Forbes JR, Cox DW. The Wilson disease gene is a putative copper transporting P-type ATPase similar to the Menkes gene. *Nat Genet* 1993;5:327–337.
6. Nagano K, Nakamura K, Urakami K, Umeyama K, Uchiyama H, Koiwai K, *et al.* Intracellular distribution of the Wilson's disease gene product (ATPase7B) after in vitro and in vivo exogenous expression in hepatocytes from the LEC rat, an animal model of Wilson's disease. *Hepatology* 1998; 27(3):799–807.
7. Hung IH, Suzuki M, Yamaguchi Y, Yuan DS, Klausner RD, Gitlin JD. Biochemical characterization of the Wilson disease protein and functional expression

in the yeast *Saccharomyces cerevisiae*. *J Biol Chem* 1997;272(34):21461-6.

8. Merle U, Schaefer M, Ferenci P, Stremmel W. Clinical presentation, diagnosis and long-term outcome of Wilson's disease: a cohort study. *Gut*. 2007;56(1):115-20.

9. Shiono Y, Wakusawa S, Hayashi H, Takikawa T, Yano M, Okada T, *et al*. Iron accumulation in the liver of male patients with Wilson disease. *Am J Gastroenterol* 2001;96(11):3147-51.

10. Kalach N, Seidman EG, Morin C, Rasquin-Weber A, O'Regan S, Laberge JM, *et al*. Acute liver failure from Wilson's disease in a five year-old child. *Can J Gastroenterol* 1993;7:610-2.

11. Wilson DC, Phillips MJ, Cox DW, Roberts EA. Severe hepatic Wilson's disease in preschool-aged children. *J Pediatr* 2000;137(5):719-22.

12. Milkiewicz P, Saksena S, Hubscher SG, Elias E. Wilson's disease with superimposed autoimmune features: report of two cases and review. *J Gastroenterol Hepatol* 2000;15(5):570-4.

13. Yamaguchi Y, Heiny ME, Gitlin JD. Isolation and characterization of a human liver cDNA as a candidate gene for Wilson disease. *Biochem Biophys Res Commun* 1993;197(1):271-7.

14. Huster D. High copper selectively alters lipid metabolism and cell cycle machinery in the mouse model of Wilson disease. *J Biol Chem* 2007;282(11):8343-55.

15. Scheinberg IH, Sternlieb I, Schilsky M, Stockert RJ. Penicillamine may detoxify copper in Wilson's disease. *Lancet*. 1987;2(8550):95.

16. Kodama H, Murata Y, Iitsuka T, Abe T. Metabolism of administered triethylene tetramine dihydrochloride in humans. *Life Sci* 1997;61(9):899-907.

17. Walshe JM, Dixon AK. Dangers of non-compliance in Wilson's disease. *Lancet*. 1986;1(8485):845-7.

18. Zali N, Mohebbi SR, Esteghamat S, Chiani M, Haghghi MM, Hosseini-Asl SM, *et al*. Prevalence of ATP7B gene mutations in Iranian patients with Wilson disease. *Hepatitis monthly*. 2011;11(11):890-4.