Novel mutations found in the ATP7B gene in Chinese patients with Wilson's disease

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1 | BACKGROUND

Wilson's disease (WD, OMIM#277900) is an autosomal recessive genetic disease caused by mutations in ATP7B (HGNC: 870, VERSION NG_008806.1). ATP7B is located on 13q14.3 and contains 20 introns and 21 exons, for a total genomic length of 80 kb (Tanzi et al., 1993). ATP7B encodes copper-transporting P-type ATPase, which is a group of transmembrane copper transport proteins (Petrukhin et al., 1993). This protein is composed of 1,465 amino acids that contain a phosphatase domain (A-domain), a phosphorylation domain (P-domain), a nucleotide-binding domain (N-domain), and eight transmembrane ion channels (M-domain) (Cater, Fontaine, & Mercer, 2007).

Mutation of the ATP7B gene is closely linked to the impairment of copper excretion, leading to abnormal deposition of copper in the target organs (Dong & Wu, 2012). Variants in the ATP7B gene have been reported in almost all exons. More than 700 variants in ATP7B have been identified, of which single-nucleotide missense and nonsense mutations is the most common, followed by insertions/deletions and splice site mutations. Most patients are compound heterozygotes, carrying different mutations on each copy of the chromosome. Due to the diverse clinical manifestations of WD, it can sometimes be difficult to diagnose.

We conducted a molecular analysis of 14 probands and 12 family members and identified six novel variants in the ATP7B gene.

Abstract

Background: Wilson's disease (WD) is an autosomal recessive genetic disease caused by mutations in ATP7B and characterized by copper metabolism disorders.

Methods: Direct sequencing of the ATP7B gene is the most sensitive and widely used confirmatory testing method. Fourteen probands with WD and 12 family members participated in this study. The ATP7B gene was analyzed by direct sequencing.

Results: Twenty-nine different variants (27 substitutions, 1 duplication, 1 deletion) were found. Of the 23 reported variants, nine nondisease variants, 11 disease variants, one silent variant, and two variants with uncertain functions were identified. The six novel variants included c.1875T>A, c.2306T>C, c.3028A>G, c.3243G>A, c.3437_3438 delTG, and c.3903+5G>A.

Conclusion: These findings will assist in the diagnosis of WD. The novel variants have enriched the WD database.

Keywords

ATP7B, mutation, Wilson's disease
PATIENTS AND METHODS

Fourteen probands (three males and 11 females, age from 4 to 4 years old), who presented with hepatic symptoms and decreased ceruloplasmin (<200 mg/L, normal 200–400 mg/L), were diagnosed with WD from 2012 to 2015 in the YouAn Hospital of Capital Medical University. All probands had at least four points according to the WD scoring system (European Association for the Study of the Liver, 2012). Additionally, 11 parents and one sibling of the 14 probands were recruited for the study. They were of the Han ethnicity from North China. Written informed consent was obtained from the participants or their guardians before the genetic investigation was conducted. The Ethics Committee of the Beijing YouAn Hospital of Capital Medical University approved the present work. This study protocol conformed to the ethical guidelines of the Declaration of Helsinki.

The ATP7B gene was analyzed by direct sequencing using genomic DNA extracted from leukocytes in peripheral blood (QIAGEN, Germany). The Primers used for PCR assay were showed in Table 1. The amplified products were detected by agarose gel electrophoresis and sequenced using an ABI3730 DNA Analyzer (Applied Biosystems, USA). The pathogenicity of the genetic variants was ascertained using the WD allelic variant database (http://www.wilsondisease.med.ualberta.ca/database.asp).

RESULTS

By direct sequence analysis of the entire ATP7B gene coding and promoter regions, we identified 29 different variants.
| Variant name (nucleotide) | Nucleotide sequence | Variant type | Amino acid change | Result of change | Area of protein | Reported status | Classification | No. of alleles | Allele frequency (%) |
|--------------------------|---------------------|--------------|-------------------|-----------------|---------------|----------------|----------------|--------------|---------------------|
| 5′ c.-128C>A             | Substitution        | Unknown      | 5UTR              | NDV             |               |               |                | 4            | 14.3               |
| 5′ c.-75A>C              | Substitution        | Unknown      | 5UTR              | NDV             |               |               |                | 5            | 17.9               |
| Exon2 c.588C>A          | GAC-GAA            | Substitution | p.Asp196Glu       | Missense Cu2    | DV            | Pathogenic     |                | 1            | 3.6                |
| Exon2 c.1216T>G         | TCT-GCT            | Substitution | p.Ser406Ala       | Missense Cu4    | NDV           | Pathogenic     |                | 4            | 14.3               |
| Exon3 c.1366G>C         | GTG-CTG            | Substitution | p.Val456Leu       | Missense bet Cu4/Cu5 | NDV          | Uncertain      |                | 6            | 21.4               |
| Exon5 c.1708-5T>G       | Substitution        | Splice       | Cu6               | DV              |               |               |                | 1            | 3.6                |
| Exon6 c.1875T>A         | ATT-ATA             | Substitution | p.Ile625Ile       | Synonymous Cu6  | Novel         |                |                | 1            | 3.6                |
| Exon8 c.2304dupC        | CCCCATG           | Duplication  | p.Met769Hisfs*26  | Termination TM4 | DV            | Pathogenic     |                | 2            | 7.1                |
| Exon8 c.2306T>C         | ATG-CTG             | Substitution | p.Met769Thr       | Missense TM4    | Novel         | Uncertain      |                | 1            | 3.6                |
| Exon8 c.2310G>G         | CTC-CTG             | Substitution | p.Leu70Leu        | Synonymous TM4  | Sil           | Likely Benign  |                | 7            | 25                 |
| Exon8 c.2333G>T         | CGG-CTG             | Substitution | p.Arg778Leu       | Missense TM4    | DV            | Likely Pathogenic | 6          | 21.4               |
| Exon10 c.2495A>G        | AAG-AGG             | Substitution | p.Lys832Arg       | Missense TM4/Td | NDV           | Uncertain      |                | 6            | 21.4               |
| Exon11 c.2621C>T        | GCG-GTG             | Substitution | p.Ala874Val       | Missense bet Td/TM5 | DV        | Pathogenic     |                | 2            | 7.1                |
| Exon12 c.2827G>A        | GGT-AGT             | Substitution | p.Gly943Ser       | Missense TM5    | DV            | Pathogenic     |                | 1            | 3.6                |
| Exon12 c.2855A>G        | AAA-AGA             | Substitution | p.Lys952Arg       | Missense bet M5/TM6 | NDV      |                |                | 12           | 42.9               |
| Exon13 c.2975C>T        | CCC-CTC             | Substitution | p.Pro992Leu       | Missense bet TM6/Ph | DV     | Likely Pathogenic | 1          | 3.6                |
| Exon13 c.3028A>G        | AAG-GAG,            | Substitution | p.Lys1010Glu      | Missense bet TM6/Ph | Novel   | Pathogenic     |                | 1            | 3.6                |
| Exon13 c.3053C>T        | GCG-GTG             | Substitution | p.Ala1018Val      | Missense bet TM6/Ph | DV | Pathogenic     |                | 1            | 3.6                |
| Exon14 c.3243G>A        | GAG-GAA             | Substitution | p.Gln1081Gln      | Synonymous ATP loop | Novel |               |                | 1            | 3.6                |
| Exon15 c.3316G>A        | GTC-ATC             | Substitution | p.Val1106Ile      | Missense ATP loop | DV or NDV  | Pathogenic     |                | 2            | 7.1                |
| Exon16 c.3419C>T        | GCC-GTC             | Substitution | p.Val1140Ala      | Missense ATP loop | NDV       |                |                | 12           | 42.9               |
| Exon16 c.3437_3438delTG | TGC                 | Deletion     | p.Val1146Ala fs*6 | Frameshift ATP loop | Novel | Pathogenic     |                | 1            | 3.6                |
| Exon16 c.3443T>C        | ATT-ACT             | Substitution | p.Ile1148Thr      | Missense ATP loop | DV or NDV  | Pathogenic     |                | 1            | 3.6                |
| Exon17 c.3646G>A        | GTG-ATG             | Substitution | p.Val1216Met      | Missense ATP bind | DV | Pathogenic     |                | 1            | 3.6                |
| Exon18 c.3809A>G        | AAT-AGT             | Substitution | p.Asn1270Ser      | Missense ATP hinge | DV | Pathogenic     |                | 1            | 3.6                |
| Exon18 c.3889G>A        | GTC-ATC             | Substitution | p.Val1297Ile      | Missense bet ATP hinge/TM7 | NDV |                |                | 1            | 3.6                |
| Exon18 c.3903+5A>G      | gaatgag-gacgcgtg    | Substitution | Splice            | bet ATP hinge/TM7 | Novel |               |                | 1            | 3.6                |
| Exon18 c.3903+6T>C      | gaatgag-gacgcgtg    | Substitution | Splice            | bet ATP hinge/TM7 | NDV |                |                | 11           | 39.3               |
| Exon20 c.4114C>T        | CAG-TAG             | Substitution | p.Gln1372Ter      | Nonsense TM8    | DV            | Pathogenic     |                | 1            | 3.6                |

Note: Reported status: variants according to the WD allelic variant database. Classification: variants into “Benign”, “Likely benign”, “Uncertain significance”, “Likely pathogenic”, and “Pathogenic” based on ACMG/AMP 2015 guideline. DV: disease variants, NDV: nondisease variants, UTR: untranslated regions, Cu: copper binding domain, TM: transmembrane domain, Ph: phosphorylation loop, bet: between; WD: Wilson's disease.
(27 substitutions, one duplication, one deletion). Of these 29 variants, six were novel variants and 23 reported variants previously (Table 2). The variants occurred most frequently in exons 8, 13, 16, and 18. No variants were found in exon 1, 4, 7, 9, and 19. Among the 23 reported mutations, we found nine nondisease-variants (NDV), 11 disease-variants (DV), one silent-variant, and two uncertain function variants (DV or NDV) according the WD allelic variant database (http://www.wilsondisease.med.ualberta.ca/database.asp). The variants were classified into benign, likely benign, uncertain significance, likely pathogenic and pathogenic based on ACMG/AMP 2015 guideline (Richards et al., 2015) (http://wintervar.wglab.org/). The most frequent variants were c.2855A>G, c.3419C>T, and c.3903+6T>C, which were NDV. For 11 DVs, the most frequent was c.2333G>T, followed by c.2304dupC, c.2621C>T, c.588C>A, c.1708-5T>G, c.2827G>A, c.2975C>T, c.3053C>T, c.3646G>A, c.3809A>G, and c.4114C>T. 1 silent-variant is c.2310C>G. 2 controversial variants (DV or NDV) are c.3316G>A and c.3443T>C.

The six novel variants included two synonymous mutations (c.1875T>A and c.3243G>A) and four possible disease variants (DVs) (c.2306T>C, c.3028A>G, c.3437_3438delTG, and c.3903+5G>A) (Figure 1). The disease variants and novel variants from the 14 probands with WD showed in Table 3.

4 | DISCUSSION

Mutation hotspots in ATP7B vary by geographic region, with a higher prevalence of specific variants reported in certain populations. The predominant variants in the Chinese population include c.2333G>T (p.Arg778Leu), c.2975C>T (p.Pro992Leu), c.3443T>C (p.Ile1148Thr), and c.2804C>T (p.Thr935Met) (Gu et al., 2003; Wang et al., 2011; Wei et al., 2014). In our study, the most frequently observed DVs were c.2333G>T, c.2304dupC, c.2621C>T, c.588C>A, c.1708-5T>G, c.2827G>A, c.2975C>T, c.3053C>T, c.3646G>A, c.3809A>G, and c.4114C>T. The one silent variant was c.3316G>A and c.3443T>C.

FIGURE 1 Chromatograms of six novel ATP7B variants. The lower nucleotide symbols in each frame represents the variant, while the upper one represents the normal sequence. The red arrow shows the variation point. (a) c.1875T>A, (b) c.2306T>C, (c) c.3028A>G, (d) c.3243G>A, (e) c.3437_3438delTG, (f) c.3903+5G>A and reported c.3903+6T>C.
c.2310C>G. The two uncertain variants (DVs or NDVs) were c.3316G>A and c.3443T>C.

In our study, we found six novel variants, of which two were synonymous mutations (c.1875T>A and c.3243G>A) and four were possible DVs (c.2306T>C, c.3028A>G, c.3437_3438 delTG, and c.3903+5G>A).

The c.2306T>C (ATG‐ACT, p.Met769Thr) mutation was newly found. At the same amino acid position, two mutations (c.2305A>G, ATG‐GTG, p.Met769Val and c.2306T>G, ATG‐AGG, p.Met769Arg) have been reported as DVs. The novel c.2306T>C heterozygous mutation was found in a child proband and his father. This mutation affects Cu transport by creating a conservative amino acid change in Tm4.

The c.3028A>G (AAG‐GAG, p.Lys1010Glu) mutation is regarded as a new DV. At the same amino acid position, three DVs have been verified previously (Santhosh et al., 2008). It is found a compound heterozygote patient carrying c.3028A>G and the known pathogenic variant c.3053C>T. We found a novel variant in exon 16, c.3437_3438 delTG (p.Val1146Ala fs+6). In a previous study, it was found that the c.3436G>A (p.Val1146Met) missense mutation at amino acid position 1146 is a DV (Antonietta et al., 2008). Generally, frameshift and missense mutations are associated with more severe phenotypes of WD. Furthermore, the compound heterozygote proband with the novel variant (c.3437_3438 delTG) is likely to be a DV. A novel variant (c.3903+5G>A) was found in exon 18. Similarly, the c.3903+6T>C splice variant is a nondisease variant (NDV) (Gu et al., 2003) and the novel c.3903+5G>A splice variant was speculated to be a NDV. However, in our study, a patient carried the novel variant (c.3903+5G>A), a DV (c.2621C>T), and three NDVs (c.2855A>, c.3419C>T, and c.3903+6C>T). The pathological significance of the novel variant (c.3903+5G>A) requires more study in future.

Summary, genetic testing is a valuable tool to detect WD. The results add data to the spectrum of known mutations in the ATP7B gene in Chinese Han population.

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**CONFLICT OF INTEREST**

The authors declared that they have no conflict of interest.

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**REFERENCES**

Antonietta, Z., Olympia, M., Lepori, M. B., Valentina, D., Stefania, D., Simona, I., … Georgios, L. (2008). High incidence and allelic homogeneity of Wilson disease in 2 isolated populations: A
prerequisite for efficient disease prevention programs. *Journal of Pediatric Gastroenterology and Nutrition*, 47, 334–338. [https://doi.org/10.1097/MPG.0b013e31817094f6](https://doi.org/10.1097/MPG.0b013e31817094f6)

Cater, M. A., La Fontaine, S., & Mercer, J. F. (2007). Copper binding to the N-terminal metal-binding sites or the CPC motif is not essential for copper-induced trafficking of the human Wilson protein (*ATP7B*). *Biochemical Journal*, 401(1), 143–153. [https://doi.org/10.1042/BJ20061055](https://doi.org/10.1042/BJ20061055)

Dong, Q. Y., & Wu, Z. Y. (2012). Advance in the pathogenesis and treatment of Wilson disease. *Translational Neurodegeneration*, 1, 23–31. [https://doi.org/10.1186/2047-9158-1-23](https://doi.org/10.1186/2047-9158-1-23)

European Association for the Study of the Liver. (2012). EASL clinical practice guidelines: Wilson’s disease. *Journal of Hepatology*, 56, 671–685. [https://doi.org/10.1016/j.jhep.2011.11.007](https://doi.org/10.1016/j.jhep.2011.11.007)

Gu, Y. H., Kodama, H., Du, S. L., Gu, Q. J., Sun, H. J., & Ushijima, H. (2003). Mutation spectrum and polymorphisms in *ATP7B* identified on direct sequencing of all exons in Chinese Han and Hui ethnic patients with Wilson’s disease. *Clinical Genetics*, 64, 479–484. [https://doi.org/10.1046/j.1399-0004.2003.00179.x](https://doi.org/10.1046/j.1399-0004.2003.00179.x)

Petrukhin, K., Fischer, S. G., Pirastu, M., Tanzi, R. E., Chernov, I., Devoto, M., ... Gilliam, T. C. (1993). Mapping, cloning and genetic characterization of the region containing the Wilson disease gene. *Nature Genetics*, 5(4), 338–343. [https://doi.org/10.1038/ng1293-338](https://doi.org/10.1038/ng1293-338)

Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J., ... Rehm, H. L. (2015). Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genetics in Medicine*, 17(5), 405–424. [https://doi.org/10.1038/gim.2015.30](https://doi.org/10.1038/gim.2015.30)

Santhosh, S., Shaji, R. V., Eappen, C. E., Jayanthi, V., Malathi, S., Finny, P., ... Chandy, G. M. (2008). Genotype phenotype correlation in Wilson’s disease within families—a report on four south Indian families. *World Journal of Gastroenterology*, 14, 4672–4676. [https://doi.org/10.3748/wjg.14.4672](https://doi.org/10.3748/wjg.14.4672)

Tanzi, R. E., Petrukhin, K., Chernov, I., Pellequer, J. L., Wasco, W., Ross, B., ... Gilliam, T. C. (1993). The Wilson disease gene is a copper transporting ATPase with homology to the Menkes disease gene. *Nature Genetics*, 5(4), 344–350. [https://doi.org/10.1038/ng1293-344](https://doi.org/10.1038/ng1293-344)

Wang, L.-H., Huang, Y.-Q., Shang, X., Su, Q.-X., Xiong, F. u., Yu, Q.-Y., ... Xu, X.-M. (2011). Mutation analysis of 73 southern Chinese Wilson’s disease patients: Identification of 10 novel mutations and its clinical correlation. *Journal of Human Genetics*, 56, 660–665. [https://doi.org/10.1038/jhg.2011.76](https://doi.org/10.1038/jhg.2011.76)

Wei, Z., Huang, Y., Liu, A., Diao, S., Yu, Q., Peng, Z., & Hong, M. (2014). Mutational characterization of *ATP7B* gene in 103 Wilson’s disease patients from Southern China: Identification of three novel mutations. *NeuroReport*, 25, 1075–1080. [https://doi.org/10.1097/WNR.0000000000000216](https://doi.org/10.1097/WNR.0000000000000216)

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