**Abstract**

**Objective:** The objective of this study was to review the research on clinical genetics of Wilson’s disease (WD).

**Data Sources:** We searched documents from PubMed and Wanfang databases both in English and Chinese up to 2014 using the keywords WD in combination with genetic, ATP7B gene, gene mutation, genotype, phenotype.

**Study Selection:** Publications about the ATP7B gene and protein function associated with clinical features were selected.

**Results:** Wilson’s disease, also named hepatolenticular degeneration, is an autosomal recessive genetic disorder characterized by abnormal copper metabolism caused by mutations to the copper-transporting gene ATP7B. Decreased biliary copper excretion and reduced incorporation of copper into apoceruloplasmin caused by defunctionalization of ATP7B protein lead to accumulation of copper in many tissues and organs, including liver, brain, and cornea, finally resulting in liver disease and extrapyramidal symptoms. It is the most common genetic neurological disorder in the onset of adolescents, second to muscular dystrophy in China. Early diagnosis and medical therapy are of great significance for improving the prognosis of WD patients. However, diagnosis of this disease is usually difficult because of its complicated phenotypes. In the last 10 years, an increasing number of clinical studies have used molecular genetics techniques. Improved diagnosis and prediction of the progression of this disease at the molecular level will aid in the development of more individualized and effective interventions, which is a key to transition from molecular genetic research to the clinical study.

**Conclusions:** Clinical genetics studies are necessary to understand the mechanism underlying WD at the molecular level from the genotype to the phenotype. Clinical genetics research benefits newly emerging medical treatments including stem cell transplantation and gene therapy for WD patients.

**Key words:** ATP7B Gene; Clinic; Gene Mutation; Genetic; Hepatolenticular Degeneration; Phenotype; Wilson’s Disease

**Introduction**

Wilson’s disease (WD), also named hepatolenticular degeneration, is an autosomal recessive genetic disorder caused by defects of ATP7B gene. This disease occurs sporadically all over the world. It is found in individuals aged 3–80 years, but mainly in children and adolescents. Males have a slightly higher risk of developing WD than females, possibly because of differences in estrogen level and iron metabolism. Worldwide prevalence of WD is around 1:30,000, carrier rate is about 0.011, and the gene frequency is about 0.56. The prevalence of WD has been re-evaluated in a recent clinical study. In this study, 6,384 individuals were randomly selected and the full-length ATP7B gene (1008 individuals, 1 k data) and exons 8, 14, 18 (5376 individuals, 5 k data) were tested, respectively, by gene sequencing. The frequency of the 1 k data is 0.040–0.056 and for the 5 k data, the frequency is 0.0044–0.0057. The result indicates that theoretically the prevalence of WD should be higher than 1:30,000. The difference between the number of diagnosed cases and the theoretical value may be due to declined penetrance and diagnostic limitations. The clinical presentations of WD are highly varied, mainly consisting of hepatic and neurological symptoms. Hepatic symptoms include acute and chronic liver diseases, for example, fulminant hepatic failure (also named as abdominal Wilsonian disease) and liver cirrhosis. Neurological symptoms mainly include extrapyramidal symptoms and neuropsychiatric symptoms. The extrapyramidal symptoms are dystonia and tremor while the psychiatric symptoms of WD are often accompanied by cognitive and mood disorder. Hemolytic anemia and skeletal muscle disorder are also presented in a few patients.

Once diagnosed with WD, the patient should have a low-copper diet and receive anticopper treatment for the rest of their life. Western medicines for WD patients are d-penicillamine, sodium dimercaptosuccinate, dimercaptosuccinic acid, trientine, zinc preparation, tetrathiomolybdate, etc. Traditional Chinese medicine has also shown to be associated with significant positive
outcomes in the treatment of WD. WD patients treated with an anti-hepatolenticular degeneration decoction exhibited increased copper excretion.\textsuperscript{[7]} Effective treatments can greatly improve the quality-of-life for these patients. Unfortunately, diagnosis of this disease is usually difficult because of its complicated phenotypes and the lack of effective diagnostic tools. Over the last 10 years, an increasing number of clinical studies have used molecular genetics techniques as the clinical diagnosis index, leading to increased accuracy in diagnosis. This paper summarizes the current studies of genetics of WD.

**ATP7B Gene**

Not all the detected mutations are causative mutations in WD patients. ATP7B (OMIM\# 606882), located on 13q14.3, has a total genomic length of 80 kb and contains 21 exons encoding a copper-transporting P-type ATPase (Wilson ATPase) which consists of 1,465 amino acids.\textsuperscript{[8]} The gene is synthesized in the endoplasmic reticulum and then localized in the trans-Golgi network (TGN) of hepatocytes. Different levels of ATP7B expression are also detectable in the brain, kidney, lung, and placenta. There are over 500 mutations of the ATP7B gene,\textsuperscript{[9]} and most of them are extremely rare. Missense or nonsense mutations caused by single nucleotide variant are very common (60%), followed by insertions/deletions (26%) and splice-site mutations (9%). Compound heterozygote appears in the majority of WD patients. The mutations differ greatly in different geographic regions: H1069Q and R778L\textsuperscript{[10-12]} are relatively common mutant alleles in European and Asian populations, respectively, and the proportion of other reported mutations is mostly lower than 10%. The hotspots for WD gene mutations in European population are located in exons 8–18, while mutations in exons 2–5 that are associated with some severe phenotypes are found in Indian population.\textsuperscript{[10-12] The hotspots for WD gene mutations in Chinese population are in exons 2, 5, 8–13, 16, 18–19. Wang et al.\textsuperscript{[13]} performed a DNA sequencing of full-length ATP7B in 73 southern Chinese WD patients and detected 146 mutations. The R778L mutation was found in 32 patients (34/146, 23.39%) and 11148T mutation in 14 patients (14/146, 9.59%). Li et al.\textsuperscript{[14]} performed a mutational analysis of ATP7B gene in northern Chinese patients with WD and confirmed that R778L mutation is the most common mutation in Chinese population, followed closely by A874V and P992L mutations.

**ATP7B Protein Structure and Function**

Wilson’s disease gene-encoded ATP7B protein belongs to P-type ATPase superfamily. There are 11 classes in the P-type ATPase superfamily. P-type ATPase of class IB (PIB) are responsible for transporting Cu\textsuperscript{2+} and other heavy metal ions across biological membranes. Human PIB-type ATPases consist of ATP7A and ATP7B. The characteristic domains of PIB include actuator domain (A-domain), phosphorylation domain (P-domain), nucleotide-binding domain (N-domain), and the M-domain which are composed of eight transmembrane-spanning helices. The core structures of ATP7B protein are highly conserved, for example, TGEA in the A-domain, DKTGT motif in the P-domain and SEHPL in the N-domain. In addition, there are six CXXC motifs that can bind to Cu\textsuperscript{2+} in the heavy-metal binding domain.\textsuperscript{[15]} Recently, Gourdon et al.\textsuperscript{[16]} analyzed the LpCopA protein that is homologous to ATP7B by dividing the M-domain into six core helices M1–M6 and two PIB-specific helices MA and MB. The active transmembrane transport of Cu\textsuperscript{2+} is complex and involves a series of conserved domains: Intracellular free Cu\textsuperscript{2+} binding to the heavy-metal binding domain first approaches the double-glycine (GG) motif in the MB helix, then these ions are transported onto Cys-Pro-Cys motif in the M4 helix, and finally discharged across the membrane by phosphorylation in the A-domain.\textsuperscript{[16]}

Copper, an important microelement of the human body, participates in numerous physiological processes including mitochondrial respiratory chain, neurotransmitter synthesis, and iron metabolism. Excessively accumulated copper accelerates the formation of reactive oxygen species via the Haber–Weiss reaction, which induces oxidative DNA damage and mitochondria-mediated apoptosis. When the level of copper in the hepatocytes is normal or slightly low, Cu\textsuperscript{2+} is transported from the cytoplasm into the TGN and binds to blue copper proteins to form a six domain blue protein, that is, ceruloplasmin, which carries over 95% copper present in the plasm to the various organs. In the overloaded copper environment, Cu ions are secreted by hepatocytes into the bile capillary and then discharged through the bile out of the human body, maintaining intracellular copper homeostasis. Once the function and structure of ATP7B are impaired, the level of ceruloplasmin is decreased and copper overload occurs in the liver, resulting in various clinical presentations of WD.

**ATP7B Gene Mutations**

The majority of pathogenic mutations are located in M- and N-domains, especially in patients with presymptomatic or hepatic presentations.\textsuperscript{[17]} H1069Q mutation, in exon 14, is the most common type and has an allele frequency of 30–70% in WD patients.\textsuperscript{[18]} This mutation occurs when the conserved histidine of the SEHPL motif in the N-domain is replaced by glutamic acid. This leads to protein misfolding of N-domain, abnormal phosphorylation in the P-domain and decreased ATP binding affinity to half of the normal level.\textsuperscript{[19]} In addition, the thermal stability of H1069Q mutation is decreased and synthesized endoplasmic reticulum abnormally migrates towards and locates in the TGN. A meta-analysis has shown that most patients with homozygous or heterozygous H1069Q mutation present more frequently with neurologic disease at a later age than patients without the H1069Q mutation.\textsuperscript{[20]} However, a retrospective study on Bulgarian patients with WD has shown that the patients homozygous for H1069Q mutation present more frequently with hepatic signs. These studies indicate that genotype-phenotype correlations in WD are ethnic-specific.\textsuperscript{[21]}
E1064 mutation is also located in the SEHPL motif of the N-domain. This mutation can lead to complete loss of ATP binding affinity, presenting with severe symptoms. In a previous report,[23] a male WD patient homozygous for E1064K developed fulminant hepatic failure.[24] Interestingly, although E1064 is very close to its downstream H1069 site, its mutation does not alter the protein folding in the N-domain. Furthermore, the thermal stability and intracellular localization of the E1064A mutation are just slightly different as compared to wild-type.[25]

R778L mutation is located in exon 8, with an allele frequency of 14–19%.[18] The conserved arginine in the M2 transbilayer helix is replaced by leucine, which possibly influences the transmembrane transport of Cu²⁺. R778L and L770L are highly linked and originate from a single ancestor, and this East-Asian-specific mutation R778L/L770L is dated back at least 5,500 years.[24] In China, Liu et al.[23] concluded that R778L mutation was related to hepatic manifestations in WD. It is worth noting that the age of onset was very young in these two studies, being under 16 (55/57) and under 18 (72/75) years old, which may bias the study conclusions.

In patients with G943S and M769V mutations in the M-domain, copper metabolism is defected, but the level of ceruloplasmin is mostly normal. The common mutations in Sardinia population are in promoter regions −441/−427del, which possibly lead to the occurrence of WD by altering ATP7B expression. Frameshift mutations, nonsense mutations, insertions/deletions, and splice-site mutations often interrupt protein encoding, leading to earlier onset of WD, more severe metabolism disorder, and even occurrence of fulminant hepatic failure.[26] And more work should be done on studying the effect of mutations on protein function.

**Modifiers**

Modifiers are a group of genes that aggravate or relieve the phenotypes of other virulence genes. Apolipoprotein E (ApoE) gene, located in 19q13.2, is a modifier that has the strongest correlation with WD. The ApoE gene consists of three major alleles, designated as ε2, ε3, and ε4. ApoE ε3 and ε3/3 are the most common alleles and genotypes, and apoe plays an important role in lipid metabolism. ApoEε4 is considered to be related to lipid disorder and neurodegenerative diseases (for example, Alzheimer’s disease). There is evidence that the onset age of WD is late in patients with ε3/3, possibly because apoe3 has anti-oxidative and neuroprotective properties.[27] On the other hand, ApoE ε4 is related to the early onset for WD. For example, Litwin et al.[28] showed that ApoE ε4-positive women present WD symptoms 4 years earlier than women with wild-type ApoE ε3/ε3 genotype, particularly in women homozygous for H1069Q. In addition, it is presumed that estrogen exerts neuroprotective effects on nerve growth in individuals with ApoE ε3/ε3 genotype, but not ApoE ε4-positive genotype, in which the protection by estrogen is impaired, leading to decreased tolerance of the organism to toxic substance.

Prion-related protein has been shown to bind copper in vitro and is likely to participate in the regulation of copper homeostasis in the human body.[29] Prion protein gene codon 129 has polymorphism. If the 129M allele is replaced by 129V allele, the onset of WD symptoms will advance about 5 years, but the phenotype of the symptoms would not alter.[29]

Mthfr encodes a key enzyme, MTHFR, involved in folate/homocysteine pathway. Two common polymorphisms C677T and A1298C decreased the MTHFR activity, and thus increased homocysteine level, which influences copper homeostasis in vivo and intracellular Cu²⁺ toxicity. Individuals carrying double wild-type homozygotes 677CC/1298AA manifested WD symptoms 6 years later than those noncarriers.[30] Some genes encoding cytokines possibly influence the phenotypes of WD: Individuals carrying IL1B C−511T gene had increased blood copper level and ceruloplasmin level, those carrying ILIRN*2 gene had increased ceruloplasmin level and manifested WD symptoms about 3.5 years earlier.[31] Other potential modifiers include murr1, commdl, and atox1 genes. To our knowledge, there have been few studies reporting that these genes are associated with specific WD phenotypes.

**Genotype-phenotype Correlations in Wilson’s Disease**

Wilson’s disease has complex clinical phenotypes, including a wide range in age of onset, diverse clinical presentations, and greatly different metabolic disorders. On one hand, over 500 mutations of ATP7B gene have been identified. These mutations exert widely varying effects on the ATP7B protein structure (for example, loss of ATP7B integrity, misfolding, impaired interaction between proteins) and function (phosphorylation, abnormal copper-transportation, decreased ATP binding affinity, and abnormal intracellular transport).[32] Moreover, there are other possible modifying genes, given in addition to ATP7B, considering that different phenotypes exist in WD patients with same genotypes that in homozygotic twins.[33]

The majority of studies on genotype-phenotype correlations in WD have been inconclusive. Potential reasons for this include difficulties in determining the age of onset and the clinical phenotype. Another reason is that the prevalence of WD is relatively low in the Western countries. Rare genotypes in WD can be predicted only by the bioinformatics technique or heterogeneous protein detection, establishing genotype-phenotype correlations in WD is, therefore, difficult from a statistical perspective. In addition, the clinical presentations of WD cannot be simply attributed to specific genotypes; rather they are a manifestation of dynamic complex factors at the subcellular level. Nevertheless, establishment of genotype-phenotype correlations benefits clinical classification of WD, scientific clinical collaboration, and molecular mechanism research.
Direct gene sequencing can help identify the type of mutations and is currently the standard method of diagnosing WD. Since the exons of WD gene disperse over a 4.3-kb region, only mutational hotspots are generally sequenced, which is highly economic but ensures accurate results. Haplotyping acquires genetic information according to the molecular markers inside the target gene or in the lateral wing of the target gene. In general, microsatellite or single-nucleotide polymorphisms in the ATP7B lateral wing are used for haplotyping. There is no need to determine the type of mutations in haplotyping. Haplotyping is suitable for screening the relatives of patients with diagnosed WD. False positive results can occur if haplotyping is used for low probability gene recombination. Recently, novel sequencing technique enables sequencing of all exons even the whole genome. Therefore, gene diagnosis of WD in the future will be more efficient and comprehensive.

Wilson’s disease is conventionally diagnosed according to its clinical symptoms and biochemical indices. However, a few WD patients present with decreased ceruloplasmin level (usually 95%), nervous system problem and Kayser–Fleischer rings (+) in the cornea simultaneously. In most affected patients, WD manifests as liver dysfunction or decreased ceruloplasmin level with unknown reasons. At this time, diagnosis is made mainly based on gene detection. WD has an extremely high fatality rate without treatment, and screening family members of patients with diagnosed WD can avoid the unnecessary treatment of patients with heterozygote genotype. Antenatal diagnosis of WD has not been recommended as routine in obstetrical care. However, ATP7B gene has obvious heterogeneity. For example, mutations of untranslated region, including the promoter region, are also reported. Thus, a negative result of gene detection does not exclude a diagnosis of WD. Further studies in determining the relationships between high-frequency mutation type and region, race and or phenotype will greatly increase the accuracy and predictability of gene diagnosis of this disease.

Taken together, WD is not rare in China, and only a small number of patients can be effectively treated. Early diagnosis and medical therapy can markedly improve the prognosis of WD patients. Early detection and diagnosis, accurate disease progression prediction, and effective medical therapy are critically dependent on the transition from molecular genetics research to clinical studies. Modern genetics studies are necessary to clarify the mechanism underlying WD at the molecular level. Finally, clinical genetic tests also lead to newly emerging medical treatments including stem cell transplantation and gene therapy.

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