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The genetics of Wilson disease

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Abstract

Wilson disease is an autosomal-recessive disorder of hepatocellular copper deposition caused by pathogenic variants in the copper-transporting gene, *ATP7B*. Early detection and treatment are critical to prevent lifelong neuropsychiatric, hepatic, and systemic disabilities. Due to the marked heterogeneity in age of onset and clinical presentation, the diagnosis of Wilson disease remains challenging to physicians today. Direct sequencing of the *ATP7B* gene is the most sensitive and widely used confirmatory testing method, and concurrent biochemical testing improves diagnostic accuracy. More than 600 pathogenic variants in *ATP7B* have been identified, with single-nucleotide missense and nonsense mutations being the most common, followed by insertions/deletions, and, rarely, splice site mutations. The prevalence of Wilson disease varies by geographic region, with higher frequency of certain mutations occurring in specific ethnic groups. Wilson disease has poor genotype–phenotype correlation, although a few possible modifiers have been proposed. Improving molecular genetic studies continue to advance our understanding of the pathogenesis, diagnosis, and screening for Wilson disease.

INTRODUCTION

In this chapter, we will discuss the inheritance, gene frequency, variants, genotype–phenotype correlation, and modifiers of the *ATP7B* gene, and the clinical molecular diagnosis and population screening for Wilson disease.

INHERITANCE

Wilson disease is a monogenic autosomal-recessive condition and carriers do not manifest any symptoms. Autosomal-recessive conditions are not usually present in consecutive generations, but may occur in populations with particularly high carrier frequency of Wilson disease (F. Wu et al., 2015). Our group and others have reported the presence of Wilson disease in two or more successive generations within the same family, reflecting a "pseudo-dominant" inheritance (Dziezyc et al., 2011, 2014; Bennett et al., 2013; H. Park et al., 2015). Therefore, the diagnosis of Wilson disease should not be excluded simply due to a

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misleading family history consistent with an autosomal-dominant inheritance pattern. Furthermore, recent studies have also identified Wilson disease due to atypical forms of inheritance, such as the presence of three concurrent mutations in a single patient or segmental uniparental disomy (Coffey et al., 2013). Uniparental disomy occurs when both homologs of a chromosome originate from a single parent. These findings have implications for clinical practice and genetic counseling, as clinicians may need to consider genotyping asymptomatic parents or obtaining full sequencing of *ATP7B* to confirm that pathogenic variants occur in *trans*.

ATP7B GENE ANDATPASE

Wilson disease is caused by homozygous or compound heterozygous mutations in the *ATP7B* gene (OMIM# 606882), which encodes a transmembrane copper-transporting P-type ATPase of the same name. Currently, *ATP7B* is the only identified gene known to cause Wilson disease (Bull et al., 1993; Petrukhin et al., 1993; Tanzi et al., 1993). Mutations in the *ATP7B* gene have been reported in almost all exons. Previous studies have reported individuals with both biochemical and clinical diagnosis of Wilson disease in the absence of two *ATP7B* mutations, raising the possibility of a second causative gene (Lovicu et al., 2006; Kenney and Cox, 2007; S. Park et al., 2007; Mak and Lam, 2008; Nicastro et al., 2010; Coffey et al., 2013). Nonetheless, *ATP7B* remains the only known gene responsible for Wilson disease.

Human dietary intake of copper is about 1.5–2.5 mg/day, which is absorbed in the stomach and duodenum, bound to circulating albumin, and transported to the liver for regulation and excretion (Culotta and Scott, 2016). The uptake of copper occurs on the basolateral side of hepatocytes via copper transporter 1 (CTR1), as illustrated in Figure 3.1. A specific copper chaperone, antioxidant protein 1 (ATOX1), delivers copper to the Wilson disease protein, ATP7B, by copper-dependent protein–protein interactions (Walker et al., 2004). Within hepatocytes, ATP7B performs two important functions in either the trans-Golgi network (TGN) or in cytoplasmic vesicles. In the TGN, ATP7B activates ceruloplasmin by packaging six copper molecules into apoceruloplasmin, which is then secreted into the plasma. In the cytoplasm, ATP7B sequesters excess copper into vesicles and excretes it via exocytosis across the apical canalicular membrane into bile (Bull et al., 1993; Tanzi et al., 1993; Yamaguchi et al., 1999; Cater et al., 2007). Due to the binary role of the ATP7B transporter in both the synthesis and excretion of copper, defects in its function lead to copper accumulation and the progressive features of Wilson disease (Fig. 3.1).

MOLECULAR STRUCTURE OF*ATP7B*

ATP7B is located on 13q14.3 and contains 20 introns and 21 exons, for a total genomic length of 80 kb (Bull et al., 1993; Petrukhin et al., 1993; Tanzi et al., 1993). The gene is synthesized in the endoplasmic reticulum, then relocated to the TGN within hepatocytes. *ATP7B* is most highly expressed in the liver, but is also found in the kidney, placenta, mammary glands, brain, and lung.

ATP7B (P-TYPE ATPASE) PROTEIN STRUCTURE AND FUNCTION

ATP7B belongs to class 1B (PIB) of the highly conserved P-type ATPase superfamily, which is responsible for the transport of copper and other heavy metals across cellular membranes (Gourdon et al., 2011). The protein contains 1465 amino acids, a phosphatase domain (A-domain), phosphorylation domain (P-domain, amino acid residues 971–1035), nucleotide-binding domain (N-domain, amino acid residues 1240–1291), and M-domain, which is comprised of eight transmembrane ion channels (Fig. 3.2) (Cater et al., 2004, 2007; Lenartowicz and Krzeptowski, 2010).

Unique amino acid motifs are present at the core structure of each domain, such as TGEA at the A-domain, DKTGT at the P-domain, and SEHPL in the N-domain. Specifically, the N-terminal metal-binding domain (MBD) is composed of six copper-binding sites, each with the conserved sequence motif GMXCXXC (Fatemi and Sarkar, 2002; Sazinsky et al., 2006). These MBDs play a central role in accepting copper from copper chaperone ATOX1 through protein–protein interactions. Previous studies have demonstrated unequal impact of MBDs on ATP7B activity, with MBD 5 and 6 having stronger effects on the catalytic activation of ATP7B than MBDs 1–4 (Lutsenko et al., 1997).

The active transport of copper across membranes is a complex process that begins with ATP7B binding copper at the N-terminal domain and transporting it across cellular membranes, using ATP as an energy source (Fig. 3.2). Next, free copper binds intracellularly to GG motifs in the MBDs, followed by transport on to the Cys-Pro-Cys (CPC) sequence motifs in MBD 6. Finally, dephosphorylation of acyl-phosphate at the A-domain discharges copper across the cellular membrane. Mutations causing copper accumulation may occur at any of these steps (Huster et al., 2006; Schushan et al., 2012).

Although the mechanism by which the histadine-containing SEHPL motif affects copper transport remains to be elucidated, it is clear that histidine-to-glutamate substitution at amino acid 1069 (p.H1069Q) in this motif is the most common cause of Wilson disease in northern Europeans. In the hepatocytes of patients homozygous for p.H1069Q, ATP7B was found in the endoplasmic reticulum instead of its usual TGN location, suggesting abnormal protein trafficking (Huster et al., 2003). Insect models with the p.H1069Q mutation in SF9 cells showed decreased ATP-mediated catalytic phosphorylation but no major protein misfolding, suggesting a role for p.H1069Q in the orientation of the ATP7B catalytic site for ATP binding prior to hydrolysis (Tsivkovskii et al., 2003).

VARIANTS IN THE *ATP7B* GENE

More than 600 pathogenic variants in *ATP7B* have been identified, with single-nucleotide missense and nonsense mutations being the most common, followed by insertions/deletions and splice site mutations (Human Gene Mutation Database, accessed 29 April 2016; Stenson et al. 2014). Other rare genetic mechanisms that have been reported in the literature include whole-exon deletions, promoter region mutations, three concurrent pathogenic variants, and monogenic disomy (Coffey et al., 2013; Bandmann et al., 2015). Mutation “hotspots” in *ATP7B* have also been reported to vary by geographic region (see regional gene frequency

section, below). The majority of pathogenic mutations are located in the M- and N-domains in presymptomatic patients or in those with hepatic symptoms (S. Park et al., 2007). The common mutations in *ATP7B* seen in various populations are listed in Figure 3.3.

The p.H1069Q mutation is one of the most common mutations, with a population allelic frequency of 10–40% (30–70% among Caucasians). Most patients are compound heterozygotes, carrying different mutations on each copy of the chromosome (Usta et al., 2014). The p.H1069Q mutation occurs when histidine of the conserved SEHPL motif in the N-domain of *ATP7B* is replaced by glutamic acid, resulting in N-domain protein misfolding, abnormal phosphorylation in the P-domain, and decreased ATP binding affinity (Rodriguez-Granillo et al., 2008). This mutation also leads to decreased heat stability and abnormal localization of the protein to the TGN (Ralle et al., 2010).

Other common mutations in *ATP7B* include p. E1064A, p.R778L, p.G943S, and p.M769V. Mutations in p.E1064A, also found in the SEHPL motif, completely disable ATP binding affinity but do not result in protein misfolding, transport abnormalities, or thermal instability. The p.R778L mutation affects transmembrane transport of copper (Dmitriev et al., 2011). The p.G943S and p.M769V mutations result in defective copper metabolism but preserved ceruloplasmin levels (Okada et al., 2010).

A substantial proportion of Wilson disease-associated missense mutations, including p.H1069Q and p.R778L, result in markedly decreased level of the protein caused by enhanced degradation (Payne et al., 1998; de Bie et al., 2007; van den Berghe et al., 2009). Other prevalent mutations, such as protein-truncating nonsense mutations (~13% of known point mutations) (Merle et al., 2010) and frameshift mutations (Vrabelova et al., 2005), are predicted to cause decay of mRNA (Mendell et al., 2004; Chang et al., 2007) or a severely truncated protein, resulting in absent or diminished levels of protein. It is therefore expected that most patients with Wilson disease have absent or significantly reduced levels of ATP7B.

REGIONAL GENE FREQUENCY

The prevalence of Wilson disease varies by geographic region, with higher prevalence of specific mutations reported in certain populations (Ferenci, 2006) (see Chapter 2 for more details). A list of the common regional variants of ATP7B mutations and geographic clustering of mutations are shown in Table 3.1 and Figure 3.4, respectively.

GENOTYPE–PHENOTYPE CORRELATION

Direct genotype–phenotype relationships in Wilson disease have been difficult to establish, despite several studies examining correlation (Panagiotakaki et al., 2004; Vrabelova et al., 2005; Nicastro et al., 2010; Cocoş et al., 2014; Usta et al., 2014). The numerous low-frequency and compound heterozygous nature of Wilson disease obfuscate the process of characterizing its numerous genetic variants and their clinical consequences. Descriptions of phenotypes are limited to age of onset and presenting symptoms, both of which may be affected by inaccurate diagnostic criteria, delayed diagnosis, and practitioner selection bias. Therefore, the marked variability in phenotype of Wilson disease is likely attributable to an amalgamation of genetic, metabolic, and environmental factors (Leggio et al., 2006).

The most consistent genotype–phenotype correlation in Wilson disease is that the most severe, early-onset disease with predominantly hepatic presentation is associated with mutations causing absent ATPase activity. Convincing studies have demonstrated fulminant hepatic disease in mouse models such as the toxic milk (tx) mouse and the Jackson tx mouse (tx^{j}), which harbor point mutations causing loss of ATP7B function, but not affecting ATP7B synthesis (Theophilos et al., 1996; Coronado et al., 2001; La Fontaine et al., 2001; Huster et al., 2006).

Genetic polymorphisms in *ATP7B*, other genes, and epigenetic factors have been shown to impact disease phenotype by affecting ATP7B protein structure and function. Of the over 600 mutations associated with Wilson disease, the majority are missense mutations that completely inactivate the copper-transporting function of ATP7B (Lutsenko, 2014). In general, individuals with protein-truncating mutations have earlier onset of disease by decreasing protein stability and quantity (Merle et al., 2010). However, other studies have demonstrated partial preservation of copper-transporting function, perhaps explaining the milder phenotypes associated with certain mutations (Rodriguez-Granillo et al., 2008; Dmitriev et al., 2011; Huster et al., 2012). Individuals with the R778L mutation have been shown to have an earlier onset of disease and predominantly hepatic presentation (Z. Y. Wu et al., 2003). In contrast, individuals with the H1069Q substitution have been shown to have mean onset of symptoms of 20–22 years old and predominantly neurologic presentations (Stapelbroek et al., 2004; Kalita et al., 2010). There is also some evidence that Kayser–Fleischer rings are more common in H1069Q homozygous patients in Hungary at time of diagnosis than in compound heterozygous individuals (Folhoffer et al., 2007).

Moreover, pathogenic variants may affect ATP7B targeting from the TGN to cytosolic vesicles. For instance, the p.Met875Val mutation results in a less stable protein and causes reversible ATP7B localization defects. Under a low-copper environment, the p.Gly875Arg variant is sequestered in the endoplasmic reticulum. However, addition of exogenous copper to the cellular growth medium stabilizes the protein, allowing it to complete its intended journey to the TGN and overcoming its disease-causing phenotype. Theoretically, patients with this specific variant may be more sensitive to dietary copper deficiency (Gupta et al., 2011).

The timing and location of copper buildup can also preferentially alter the hepatic transcriptome, based on homozygous $ATP7B^{-/-}$ mouse models. Proteomic analyses of mRNA profiles at each of these disease stages reflect unique patterns (Huster et al., 2006; Ralle et al., 2010). In the initial stage, mRNA for proteins responsible for cell cycle regulation, splicing, and cholesterol synthesis is present (Burkhead et al., 2011). This leads to early accumulation of copper bound to metallothioneins in the cytosol and free copper in the nuclei. In the progressive stage, mRNA changes throughout the cell are present, including the endoplasmic reticulum, mitochondria, and endocytic pathways causing copper to pathologically accumulate within hepatocytes. In the later stages, mRNA for lysosomal and endosomal proteins is upregulated. In these final stages, copper concentrations decrease in the cytosol and nuclei, and accumulate in the membranous cellular compartment, causing bile duct proliferation and hepatic neoplastic changes. Therefore, the location of copper

accumulation may convey more specific prognostic information about disease progression rather than total copper levels.

Other studies have compared homozygotes to compound heterozygotes of the same mutation to establish genotype–phenotype correlations. A study of 76 members of a large, consanguineous Lebanese family showed an association between c.2299insC and hepatic disease and between the p.Ala1003Thr mutation and neurologic disease (Usta et al., 2014).

Other candidate polymorphisms that are thought to modify the clinical phenotype of Wilson disease include MTHFR (Gromadzka et al., 2005), COMMD1 (Weiss, 2006), ATOX1 (Simon, 2008), XIAP (Weiss et al., 2010), PNPLA3 and hepatic steatosis (Stättermayer et al., 2012), and DMT1 (Przybyłkowski et al., 2014), although none of these genes has been demonstrated to have significant diagnostic or predictive value.

Significant phenotypic variation of Wilson disease exists between individuals with the same mutation, individuals within the same family, and even between monozygotic twins (Członkowska et al., 2009; Kegley et al., 2010). While some studies have documented high intra-familial concordance of clinical symptoms and biochemical results (Hofer et al., 2012; Chabik et al., 2014; Ferenci et al., 2015), others have reported a wide range in age of onset and presenting symptoms amongst siblings (Ala et al., 2007; Taly et al., 2007) and families carrying the same mutation (Takeshita et al., 2002). Indeed, disparate clinical presentations in monozygotic twins raise the suspicion for epigenetic modifiers in Wilson disease. See Chapter 4 for more details about the genetic and environmental modifiers of Wilson disease.

CLINICAL MOLECULAR DIAGNOSIS

The current gold standard for Wilson disease diagnosis is direct Sanger sequencing of *ATP7B* gene or molecular testing for familial mutations that were previously identified. Historically, most pathogenic variants in *ATP7B* were identified using a combination of polymerase chain reaction (PCR)/restriction fragment length polymorphism (RFLP), single-strand conformation polymorphism (SSCP), denaturing gradient gel electrophoresis (DGGE), temporal temperature gradient electrophoresis (TTGE), denaturing high-performance liquid chromatography (DHPLC), and Sanger sequencing (Loudianos et al., 1999; Shimizu et al., 1999; Margarit et al., 2005; Vrabelova et al., 2005; G. H. Kim et al., 2008). The critical demerits of this complex tiered approach are that the detection rate is not high enough to find most mutations and the turnaround time is often extended. Although regional clusters of specific mutations have been well described, a customized screening approach taking into account these regional variants may be complicated by ethnically diverse populations and inaccurate information provided with samples. Biochemical results are often imprecise, as elevations in urinary copper excretion tend to occur late in the disease process and fewer than 40% of presymptomatic patients excrete copper less than 100 μg/day (Sternlieb and Scheinberg, 1968; Nakayama et al., 2008). For these reasons, direct sequencing of the *ATP7B* gene has become the preferred standard and provided the greatest yield in clinical molecular diagnosis. Please refer to Chapter 14 for details about the diagnosis of Wilson disease.

Starting the diagnostic process with molecular testing may significantly reduce the need for invasive liver biopsy. Liver copper content alone was found to be insufficient to exclude Wilson disease, as levels may not be elevated in some affected patients. In several studies on patients with possible Wilson disease based on biochemical and clinical tests, disease-causing mutations in both alleles were identified in about 80% of them. Currently available screening tests may not definitely rule out the disease and no single test could permit *de novo* diagnosis. Of note, many patients may not possess the characteristic findings and may present when their clinical disease is relatively mild. Inappropriate treatment for false-positive cases has the potential of inducing copper deficiency, which can result in hematologic and neurologic sequelae (N. Kumar et al., 2003). These findings reinforce the need for reliable clinical diagnostic criteria and underscore the benefits of DNA testing prior to invasive procedures (Ferenci, 2005).

Multiplex PCR is used to amplify all 21 exons and splice sites of *ATP7B*, including promoter regions. Although the large deletions or duplications cannot be detected with this conventional Sanger sequencing method, the chance of these being present in Wilson disease appears low (Stenson et al., 2012). If clinical suspicion is still high with only one pathogenic variant present, then multiplex ligation-dependent probe amplification (MLPA) test should be considered. Microarray-based comparative genomic hybridization is another option to evaluate partial or full gene deletions or duplications with higher sensitivity. Cases with only one copy of mutation present should be carefully reviewed in the context of other biochemical and clinical findings. Molecular genetic testing using direct mutation analysis is very effective in identifying affected patients and presymptomatic siblings of probands (Manolaki et al., 2009).

Wilson disease is an autosomal-recessive disorder, which means that there is a 25% chance that a sibling of the index case also has Wilson disease. Once homozygous or compound heterozygous mutations in *ATP7B* have been established in the index patient, mutation detection becomes valuable in family screening. The same genotype in asymptomatic family members confirms diagnosis of the disease, thus allowing for early treatment before the onset of complications. In family members in whom clinical and biochemical features are uncertain, the demonstration of either heterozygous (carrier) or wild-type gene sequence prevents unnecessary treatment (Chang et al., 2007).

If the proband has secured a diagnosis of Wilson disease on the basis of clinical and biochemical evidence, but testing for *ATP7B* mutations is not available, family screening can be done by haplotype analysis of polymorphic markers flanking the disease gene (Thomas et al., 1995b; Gupta et al., 2005; Przybyłkowski et al., 2014). In this instance, the rare possibility of recombination events (typically 0.5–5% of cases) needs to be considered. The rate of recombination is dependent on which flanking markers are studied. Microsatellite or single-nucleotide polymorphisms in the *ATP7B* lateral wing are used for haplotyping, which is useful for screening relatives of patients with previously identified familial mutations. False-positive results may occur if haplotyping is used on patients with low-probability gene recombinations.

Genetic testing for *ATP7B* mutations can be valuable to confirm a diagnosis of Wilson disease, especially when presentation is unusual (Caprai et al., 2006). Attention has been drawn to this situation by the molecular confirmation of early-onset hepatic disease in a 3-year-old child (Wilson et al., 2000). Mutation analysis has also confirmed late-onset disease, including the case of two siblings in their 70s – the oldest reported patients so far at time of diagnosis (Nanji et al., 1997; Gupta et al., 2005; Perri et al., 2005; Weitzman et al., 2014).

ATP7B mutation analysis makes an important contribution to clinical practice. Unfortunately, systematic genetic testing for Wilson disease is still difficult and fairly expensive due to the plethora of different mutations, the occurrence of regulatory mutations in non-coding sequence, the large size of the gene, and the limitations of currently available methods. However, technical advances allowing high-throughput screening could be applied to the disease (Bost et al., 2012; Lepori et al., 2012). This new apparatus can sequence six million basepairs of DNA per hour with accuracy greater than 99%. Such advances might permit specialized laboratories to sequence the entire genomic Wilson disease gene from patients, including not only the translated exons, but also the important noncoding sequences that are not normally investigated, to detect all variants.

Interpretation of variants of uncertain significance has become a major challenge for accurate interpretation, genetic counseling, and prevention. Screening family members may help with the interpretation of variants of uncertain significance, but not all variants can be resolved with this approach. Functional analysis is often necessary; however, no clinical functional analysis is yet available. A computational approach to predict significance of mutations is often helpful but a further concrete model is required to demonstrate the efficacy in aiding the clinical decision.

POPULATION SCREENING

The purpose of newborn screening is to identify treatable congenital conditions that can affect a child's long-term health and development. Recent tandem mass spectrometry (MS/MS) applications have markedly expanded the ability to screen for >50 metabolic diseases from a single dried blood spot. In addition to the original Wilson–Jungner classic screening criteria (Wilson and Jungner, 1968), the American College of Medical Genetics convened the Newborn Screening Expert Group to develop a uniform screening panel in 2006. Of the primary tenants, Wilson disease is an ideal target for screening, given its relatively high prevalence and availability of effective treatment (Hahn et al., 2002; Roberts et al., 2008). Unfortunately, despite extensive discussion on the need for population screening, no cost-effective biomarkers or methods for early screening have been developed for Wilson disease yet. Several small pilot studies have been conducted using ceruloplasmin as a marker, with limited outcomes (Yamaguchi et al., 1999; Hahn et al., 2002; Owada et al., 2002; Schilsky and Shneider, 2002; Kroll et al., 2006). Ceruloplasmin alone is not sufficient to screen for Wilson disease in newborns, as a substantial number of newborns present with physiologically low ceruloplasmin. Ceruloplasmin assay around 3 years of age may be the most appropriate population-screening method, but mandatory health checkups at this age are not universally available in the USA and worldwide.

Many treatable congenital disorders are caused by mutations that result in absent or diminished levels of proteins; thus, protein biomarkers have enormous potential in the diagnosis/screening of congenital disorders. Liquid chromatography mass spectrometry with multiple reaction monitoring (LC-MRM-MS) has emerged as a robust technology that enables highly precise, specific, multiplex quantification of signature proteotypic peptides as stoichiometric surrogates of biomarker proteins.

Our lab is currently exploring the use of peptide immunoaffinity enrichment (Whiteaker et al. 2010, 2011) to quantify ATP7B in DBS. These promising proof-of-concept data open up the possibility of screening for Wilson disease in newborns. Further clinical validation on a large-scale pilot study will be required to determine the efficacy of the assay.

CONCLUSION

Wilson disease is an autosomal-recessive disease due to pathogenic mutations in *ATP7B*. *ATP7B* is the only identified gene known to cause Wilson disease, and encodes a transmembrane copper-transporting ATPase of the same name. While biochemical testing and clinical criteria may assist in the early diagnosis and treatment, the current gold standard for Wilson disease diagnosis is direct Sanger sequencing of ATP7B or molecular testing for known familial mutations. Genotype–phenotype correlations have been studied extensively but direct causations remain nebulous. Modifier genes may affect the penetrance and phenotypes but a large-scale study for clinical validation is warranted. The overall worldwide prevalence of Wilson disease is 1 in 30 000 individuals, with significant geographic variation. The most common mutation in Northern America and Europe is the missense mutation p.H1069Q and the most common mutation in East Asian populations is the missense p.R778L. Ceruloplasmin alone is insufficient to screen for Wilson disease in newborns. While peptide immunoaffinity assays show promise for newborn screening, further large-scale clinical studies are required to determine efficacy of these population-based screening methods for Wilson's disease.

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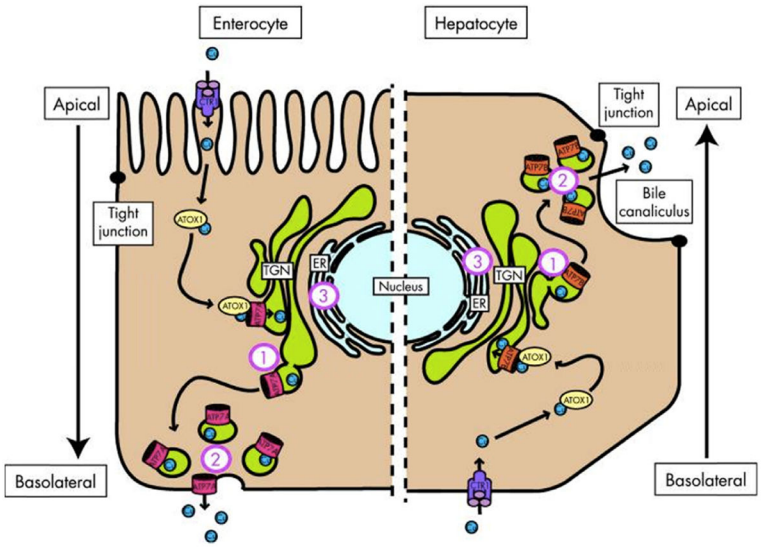

Fig. 3.1.

Schematic representation of copper-induced relocalization of ATP7A and ATP7B. The left side of the diagram represents an enterocyte and the right side represents a hepatocyte. On both sides, copper enters the cell through copper transporter 1 (CTR1) and is escorted by copper chaperone antioxidant protein 1 (ATOX1) to ATP7A or ATP7B in the trans-Golgi network (TGN). When copper levels rise above a certain threshold, ATP7A and ATP7B excrete copper into the plasma on the basolateral side of the enterocyte and into the bile on the apical side of the hepatocyte. Defects in localization of ATP7B may lead to copper accumulation at the (1) TGN due to unresponsiveness, (2) cell periphery, and (3) endoplasmic reticulum (ER) due to misfolding. (Reproduced from de Bie et al., 2007.)

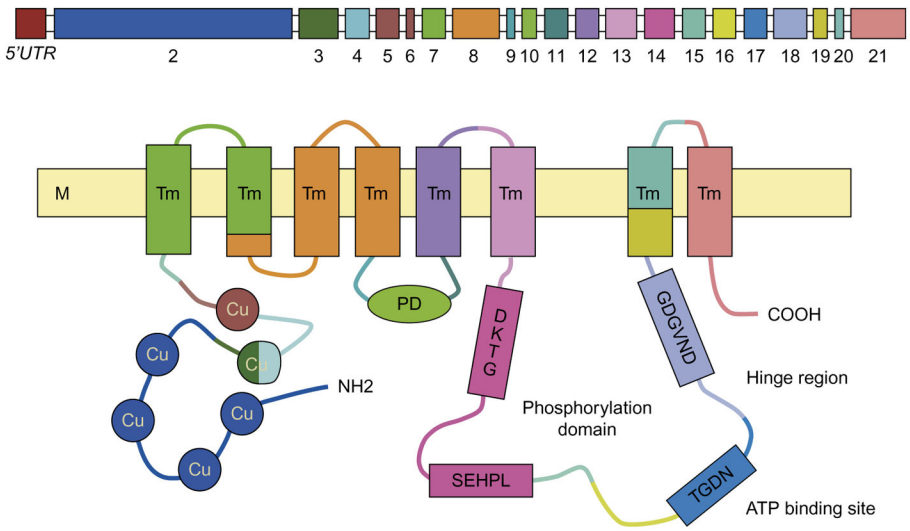

Fig. 3.2.

Schematic representation of *ATP7B* gene and corresponding human ATP7B protein. Top diagram shows 5'UTR promoter region and exons separated by introns. Bottom diagram shows the domain organization of human copper ATPase. Conserved amino acid motifs are present at the core structure of each functional domain, i.e., TGDN and GDGVND at the A-domain, DKTG at the P-domain, and SEHPL in the N-domain. M, phospholipidic bilayer of the membrane; Cu, the metal-binding domains of the trasmembrane cation channel; Tm, transmembrane domains; PD, phosphatase domain. (Reproduced from Fanni et al., 2005.)

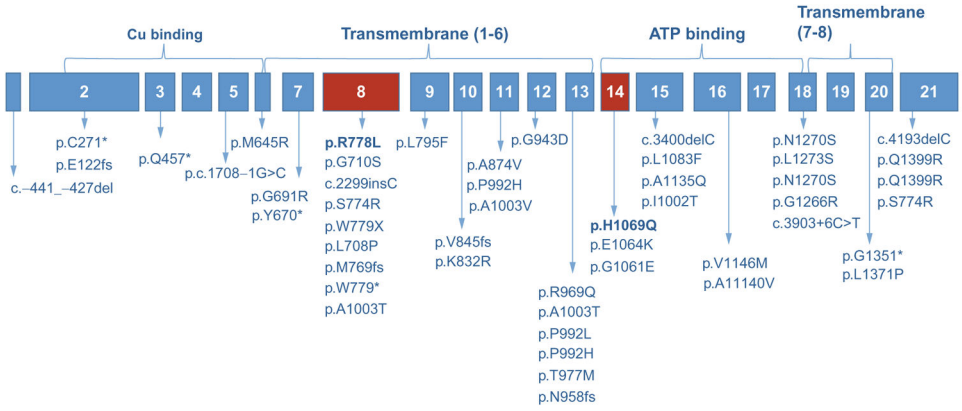

Fig. 3.3.

Schematic of the *ATP7B* gene with common mutation sites, including p.H1069Q (rs76151636), p.R778L (rs28942074), p.E1064K (rs376910645), c.3400delC, and p.Ala1135fs (rs137853281). Please refer to Table 3.1 for more details.

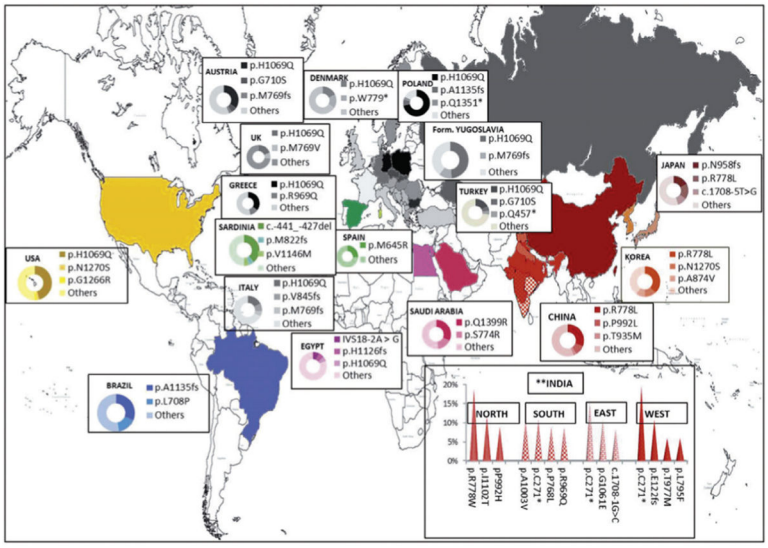

Fig. 3.4.

Prevalence of *ATP7B* mutation by geographic region; the darker the gradient, the higher the allelic frequency. (Reproduced from Gomes and Dedoussis, 2016, with permission from Taylor and Francis.)

Table 3.1

Regional distribution of common Wilson disease mutations by geographic location

		Prevalent mutations					
Region	**AF (%)**	**Protein**	**Nucleotide**	**RS**	**Exon**	**Type**	**Domain**
Europe							
Austria (Ferenci, 2006)	34.1	p.His1069Gln	c.3207C>A	rs76151636	14	Missense	ATP loop
	6.4	p.Gly710Ser	c.2128G>A		8	Missense	TM2
	3.6	p.Met769fs	c.2298_2299insC	rs137853287	8	Premature stop	TM4
Benelux (Ferenci, 2006)	53	p.His1069Gln	c.3207C>A	rs76151636	14	Missense	ATP loop
Bulgaria (Todorov et al., 2005)	58.8	p.His1069Gln	c.3207C>A	rs76151636	14	Missense	ATP loop
Canary Islands (García Villarreal et al., 2000)	64	p.Leu708Pro	c.2123 T>C		8	Missense	TM2
Czech Republic (Vrabelova et al., 2005)	57	p.His1069Gln	c.3207C>A	rs76151636	14	Missense	ATP loop
Denmark (Møller et al., 2011)	18	p.His1069Gln	c.3207C>A	rs76151636	14	Missense	ATP loop
	16	p.Trp779*	c.2336G>A	rs137853283	8	Nonsense	TM4
France (Bost et al., 2012)	15	p.His1069Gln	c.3207C>A	rs76151636	14	Missense	ATP loop
Germany (Ferenci, 2006)	47.9	p.His1069Gln	c.3207C>A	rs76151636	14	Missense	ATP loop
Germany (East, former) (Caca et al., 2000)	63	p.His1069Gln	c.3207C>A	rs76151636	14	Missense	ATP loop
Greece (Panagiotakaki et al., 2004;	35	p.His1069Gln	c.3207C>A	rs76151636	14	Missense	ATP loop
Dedoussis et al., 2005; Gomes and Dedoussis, 2016)							
	12	p.Arg969Gln	c.2906G>A	rs774028495	13	Missense	TM6
Hungary (Firneisz et al., 2002; Folhoffer et al., 2007)	42.9	p.His1069Gln	c.3207C>A	rs76151636	14	Missense	ATP loop
Iceland (Thomas et al., 1995a; Hofer et al., 2012)	100	p.Tyr670*	c.2007_2013del		7	Nonsense	TM1
Italy (Loudianos et al., 1999)	17.5	p.His1069Gln	c.3207C>A	rs76151636	14	Missense	ATP loop
	9	p.Val845fs	c.2530delA	rs755709270	10	Premature stop	Td
	6	p.Met769fs	c.2298_2299insC	rs137853287	8	Premature stop	TM4
Netherlands (Stapelbroek et al., 2004)	33	p.His1069Gln	c.3207C>A	rs76151636	14	Missense	ATP loop
Poland (Gromadzka et al., 2005)	72	p.His1069Gln	c.3207C>A	rs76151636	14	Missense	ATP loop
	7.3	p.Ala1135fs	c.3400delC	rs137853281	15	Premature stop	ATP loop
	3.7	p.Gln1351*	c.4051C>T		20	Nonsense	
Romania (Iacob et al., 2012)	38.1	p.His1069Gln	c.3207C>A	rs76151636	14	Missense	ATP loop
Russia (Ivanova-Smolenskaya et al., 1997)	49	p.His1069Gln	c.3207C>A	rs76151636	14	Missense	ATP loop

		Prevalent mutations					
Region	**AF (%)**	**Protein**	**Nucleotide**	**RS**	**Exon**	**Type**	**Domain**
Sardinia (Figus et al., 1995)	60.5		c.-441_-427del		5prime	Unknown	Promoter
	8.5	p.Met822fs	c.2463delC		10	Deletion	TM4/Td
	7.9	p.Val1146Met	c.3436G>A		16	Missense	ATP loop
Serbia	38.4	p.His1069Gln	c.3207C>A	rs76151636	14	Missense	ATP loop
(Tomić et al., 2013)							
	11.6	p.Met769fs	c.2304dupC		8	Missense	TM4
	9.3	p.Ala1003Thr	c.3007G>A	rs1801247	13	Missense	TM6/Ph
Spain (Margarit et al., 2005)	27	p.Met645Arg	c.1934 T>G	rs121907998	6	Missense	Cu6/TM1
Sweden (Shah et al., 1997)	38	p.His1069Gln	c.3207C>A	rs76151636	14	Missense	ATP loop
Turkey (Ferenci, 2006; Simsek Papur et al., 2013)	17.4	p.His1069Gln	c.3207C>A	rs76151636	14	Missense	ATP loop
	5.3	p.Gly710Ser	c.2128G>A	rs772595172	8	Missense	TM2
	4.53	p.Gln457*	c.1369C>T		3	Nonsense	Cu4/Cu5
UK (Coffey et al., 2013)	19	p.His1069Gln	c.3207C>A	rs76151636	14	Missense	ATP loop
	8	p.Met769Val	c.2305A>G		8	Missense	TM4
Yugoslavia (former) (Loudianos et al., 1999)	48.9	p.His1069Gln	c.3207C>A	rs76151636	14	Missense	ATP loop
	11.4	p.Met769fs	c.2298_2299insC	rs137853287	8	Premature stop	TM4
Asia							
China (Gu et al., 2003; Z.-Y. Wu et al., 2003; Wang et al., 2011; Wei et al., 2014)	31	p.Arg778Leu	c.2332C>T	rs28942074	8	Missense	TM4
	10	p.Pro992Leu	c.2975C>T	rs201038679	13	Missense	TM6/Ph
	9.6	p.Ile1148Thr	c.3443 T>C	rs60431989	16	Missense	ATP loop
	3.3	p.Thr935Met	c.2804C>T		12	Missense	TM5
	19	p.Arg778Leu	c.2332C>T	rs28942074	8	Missense	TM4
North India (S. Kumar et al., 2006; Gupta et al., 2007)							
	12	p.Ile1102Thr	c.3305 T>C	rs560952220	15	Missense	ATP loop
	9	p.Pro992His	c.2975C>A		13	Missense	TM6/Ph
South India (Santhosh et al., 2006; S. S. Kumar et al., 2012)	11	p.Ala1003Val	c.3008C>T		13	Missense	TM6/Ph
	11	p.Cys271*	c.813C>A	rs572147914	2	Nonsense	Cu3
	9	p.Pro768Leu	c.2303C>T		8	Missense	TM4
	9	p.Arg969Gln	c.2906G>A	rs121907996	13	Missense	TM6
East India (Gupta et al., 2005)	16	p.Cys271*	c.813C>A	rs572147914	2	Nonsense	Cu3

Handb Clin Neurol. Author manuscript; available in PMC 2017 October 19.

Region	AF (%)	Prevalent mutations					
		Protein	Nucleotide	RS	Exon	Type	Domain
	11	p.Gly1061Glu	c.3182G>A		14	Missense	ATP loop
	8.5		c.1708-1G>C	rs137853280	5	Splice	Cu6
West India (Aggarwal and Bhatt, 2013; Aggarwal et al., 2013)	20	p.Cys271*	c.813C>A	rs572147914	2	Nonsense	Cu3
	11	p.Glu122fs	c.365_366delins TTCGAAGC		2	Ins/Del	Cu1
	6	p.Thr977Met	c.2930C>T	rs72552255	13	Missense	TM6
	6	p.Leu795Phe	c.2383C>T		9	Missense	TM4/Td
Japan (Okada et al., 2000; Tatsumi et al., 2010)	17.95	p.Asn958fs	c.2871delC		13	Premature stop	TM5/TM6
	16.7	p.Arg778Leu	c.2332C>T	rs28942074	8	Missense	TM4
	10.5		c.1708-5 T>G		5	Splice	Cu6
Korea (E. K. Kim et al., 1998; Yoo, 2002; G.-H. Kim et al., 2008; Song et al., 2012)	37.9	p.Arg778Leu	c.2332C>T	rs28942074	8	Missense	TM4
	12.1	p.Asn1270Ser	c.3809A>G	rs121907990	18	Missense	ATP hinge
	9.4	p.Ala874Val	c.2621C>T	rs376355660	11	Missense	TM5
	8	p.Leu1083Phe	c.3247C>T		15	Missense	ATP loop
Lebanon (Usta et al., 2014)	44.7	p. Ala1003Thr	c.2299insC	rs137853287	8	Missense	TM4
Saudi Arabia (Al Jumah et al., 2004; Majumdar et al., 2004)	32	p.Gln1399Arg	c.4196A>G		21	Missense	After TM8
	16	p.Ser774Arg	c.2230 T>C	rs535217574	21	Missense	TM3
Taiwan (Lee et al., 2000; Wan et al., 2006)	29.6	p.Arg778Leu	c.2332C>T	rs28942074	8	Missense	TM4
	8.9	p.Pro992Leu	c.2975C>T	rs201038679	13	Missense	TM6
	4.8	p.Gly943Asp	c.2828G>A		12	Missense	TM5
Thailand (Panichareon et al., 2011)	10.52	p.Arg778Leu	c.2332C>T	rs28942074	8	Missense	TM4
	7.89	p.Leu1371Pro	c.4112 T>C		20	Missense	TM8
Iran (Zali et al., 2011)	19	p.His1069Gln	c.3207C>A	rs76151636	14	Missense	ATP loop
Africa							
Egypt (Abdelghaffar et al., 2008; Abdel Ghaffar et al., 2011)	42.2	IVS18+6 T>C	c.3903+6C>T	rs2282057	18	Splice	
	40.6	p.Ala11140Val	c.3419C>T		16	Missense	ATP loop
	26.5	p.Lys832Arg	c.2495A>G	rs1061472	10	Missense	TM4/Td
Americas							
USA (Kuppala et al., 2009)	40.3	p.His1069Gln	c.3207C>A	rs76151636	14	Missense	ATP loop
	1.9	p.Asn1270Ser	c.3809A>G	rs121907990	18	Missense	ATP hinge

Region	AF (%)	Prevalent mutations					
		Protein	Nucleotide	RS	Exon	Type	Domain
	1.9	p.Gly1266Arg	c.3796G>A	rs121907992	18	Missense	ATP hinge
Brazil (Deguti et al., 2004; Machado et al., 2008; Bem et al., 2013)	37.1	p.His1069Gln	c.3207C>A	rs76151636	14	Missense	ATP loop
	31.25	p.Ala1135fs	c.3400delC	rs137853281	15	Premature stop	ATP loop
	11.4	p.Ala1135GlnfsX13	c.3402delC	rs137853281	15	Premature stop	ATP loop
		p.Leu708Pro	c.2123 T>C		8	Missense	TM2
Costa Rica (Shah et al., 1997)	61	p.Asn1270Ser	c.3809A>G	rs121907990	18	Missense	ATP hinge
Venezuela (Paradisi et al., 2015)	26.9	p.Ala1135GlnfsX13	c.3402delC	rs137853281	15	Premature stop	ATP loop
	9.6	p.Gly691Arg	c.2071G>A		7	Missense	TM2

AF, RS,