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REVIEW ARTICLE

Genetics of Wilson's disease: a clinical perspective

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Abstract Hepatic Wilson's disease is often a difficult diagnosis to confirm. This review examines the current role of genetic tests for Wilson's disease and is aimed at clinicians caring for patients with this disease. We discuss how genetic testing is carried out for Wilson's disease, indications for these tests, and genetic counseling for the family. In contrast to the advances in diagnosis of Wilson's disease by testing for *ATP7B* mutations, genotype-phenotype correlations are not yet sufficiently established. The non-Wilsonian copper overload syndromes causing cirrhosis in children are another important area for study. The review also identifies further areas for research into the genetics of Wilson's disease in India.

Keywords *ATP7B* · Clinical utility · Genetic diagnosis · Wilson's disease

Wilson's disease, an inherited disorder of copper overload

Autosomal recessive disorders, like Wilson's disease, occur more frequently in children born to parents of consanguineous marriages. The chances of both parents sharing an abnormal allele of the gene causing an autosomal recessive disorder are higher in consanguineous marriages. Analysis of the family tree shows that in autosomal recessive disorders all affected persons are in one generation (horizontal pattern of transmission) [1]. Occasionally, we may encounter a family with a disorder of copper overload, wherein all 4 children are affected. Is this compatible with an autosomal recessive disorder like Wilson's disease? The explanation is that, in an autosomal recessive disorder running in a family, in each pregnancy there is a 25 % chance of the child being affected by the disease. It is good to remember that in Mendel's experiments to understand recessive traits, multiple trials of scientific experiments and a large sample size to make quantitative comparisons were needed [2]; Mendel studied 929 pea plants to analyse recessive traits in the second filial generation! The estimated worldwide prevalence of Wilson's disease is between 1 in 5,000 and 1 in 30,000 population [3, 4] and the carrier frequency is 1 in 90 [4, 5].

Prevalence of Wilson's disease in India

In India, population based data on prevalence of Wilson's disease is not available. Hospital based reports document copper related liver diseases to be a significant cause of cirrhosis with portal hypertension in the pediatric age group in India. In Vellore, between 2000 and 2007, Wilson's disease was the predominant cause of cirrhosis in 21 of 38 children (55 %) leading to portal hypertension in the pediatric population [6]. In Chandigarh, between 1991 and 2000, of 517 children with portal hypertension, 203 children had cirrhosis: the etiology of cirrhosis was Indian Childhood Cirrhosis in 35 (17 %) and Wilson's disease in 15 (7 %) [7].

When was the Wilson's disease gene identified?

In 1985, the gene abnormal in Wilson's disease was first localized to chromosome 13 using DNA linkage analysis studies which showed close linkage between Wilson's

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disease locus and the red cell enzyme, esterase D, gene [8]. In 1993 this gene, designated *ATP7B*, was identified by researchers in Toronto, Canada, and nearly simultaneously by a second research group using positional cloning techniques [9–12].

How does the genetic defect cause Wilson's disease?

The copper overload in Wilson's disease is due to defective action of the protein encoded by the *ATP7B* gene, which is highly expressed in liver, kidney and placenta [13]. The *ATP7B* gene product—the Wilson ATPase—is a transmembrane protein which functions as a copper transporting ATPase. Within the hepatocyte, the Wilson ATPase has at least two functions: it plays a role in the biliary excretion of copper (i. e: excretory function) and is part of the mechanism for incorporation of copper into the plasma protein ceruloplasmin (at trans-Golgi compartment) (i. e: synthetic function) [14]. Studies on cultured hepatoma cell lines show that copper concentrations in the culture medium influence the localization of Wilson ATPase within the hepatocyte. At low copper concentrations, Wilson ATPase is located at the trans-Golgi network; at higher copper concentrations, it moves to vesicles near the bile canaliculi [15]. Immunohistochemical studies support these findings [14, 16].

How is genetic testing for Wilson's disease done? Direct testing of *ATP7B* gene vs. tracking markers flanking the *ATP7B* gene (DNA linkage analysis) (Table 1)

Direct testing for mutations within the *ATP7B* gene is currently laborious and expensive, given the large size of the gene (80 kb, 21 exons). Direct gene testing for Wilson's disease is to look for homozygosity of one mutation or finding two different mutations, constituting compound heterozygosity, which still results in clinical Wilson's disease [17]. Most patients have compound heterozygosity.

One approach for direct gene testing is to sequence the entire *ATP7B* gene. Another cost-cutting approach is to do a preliminary step of mutation screening of different exons in the gene where mutations are especially frequent (for example: using conformation sensitive gel electrophoresis) followed by targeted sequencing [18]. A third approach is that once common mutations are identified within a population, these mutations can be looked for using polymerase chain reaction based restriction fragment length polymorphism assay (PCR-RFLP) [19, 20].

Genetic testing for pre-symptomatic Wilson's disease among siblings of an index patient with Wilson's disease can be done by direct gene testing (especially if the mutation has been identified in the index patient) [21] or DNA linkage analysis (as discussed below). Genetic testing is particularly effective for investigating first-degree relatives of an affected individual.

DNA linkage analysis is performed by tracking microsatellite markers (like D13S314, D13S316 and D13S133) flanking the *ATP7B* gene. This is a simpler technique than direct gene testing. The pattern of the different flanking markers is determined for both parents, siblings and the proband. Haplotype is constructed using these data, applying Mendelian pattern of inheritance of the markers in this family. If a sibling shares both sets of haplotypes with the proband, this indicates pre-symptomatic WD in this sibling [22]. In 0.5 % to 5 % of individuals, DNA linkage analysis can give erroneous results, caused by spontaneous recombination between the flanking markers and Wilson's disease loci [14]. To reduce such diagnostic errors during DNA linkage analysis, haplotypes are constructed for multiple flanking markers. Identifying markers within the *ATP7B* gene (intragenic single nucleotide polymorphism) can also be done to clarify any confusion related to potential recombination of the flanking markers [23]. The advantage of DNA linkage analysis is that actual identification of the mutation(s) present is not required.

If a sibling is to be screened for pre-symptomatic Wilson's disease and DNA from the proband is not available (for example, if the proband had died earlier), then DNA linkage analysis is not possible. Direct gene testing is the preferred method to assess the gene in the sibling, in this scenario (Table 1).

Table 1 Deciding which genetic test to do in an asymptomatic sibling of a proband (index patient) with Wilson's disease

DNA from proband available?	Preferred test			Preferred test
Yes	Direct gene analysis or Haplotype analysis[a]	*ATP7B* mutations detected on both chromosomes in proband ?	Yes	Direct gene analysis (to look for these mutations in sibling)
			No	Haplotype analysis or direct gene analysis
No	Direct gene analysis			

[a] Haplotype analysis needs DNA from both parents as well as from proband

Does Wilson's disease genetic testing have a role in day to day clinical practice?

While it is fairly easy to suspect the diagnosis of Wilson's disease, confirming the diagnosis by conventional tests is often a challenge, especially in patients suspected to have hepatic Wilson's disease. In one study, of 17 Wilson's disease patients with chronic liver disease, Kayser-Fleischer rings were present in 47 % of patients, low serum ceruloplasmin in 65 %, and increased urinary copper in 88 %; in contrast, in 20 patients with neurological Wilson's disease, Kayser-Fleischer rings were present in 90 % of patients, low serum ceruloplasmin level in 85 % and increased urinary copper in 78 % [24]. Specialized tests may be needed to confirm the diagnosis of hepatic Wilson's disease. Why is it important to make an accurate diagnosis of Wilson's disease? A diagnosis of Wilson's disease commits a patient to lifelong copper chelation treatment. On the other hand, a delay in making an early diagnosis often entails significant morbidity and mortality. We had reported deaths of presumed (undiagnosed) Wilson's disease in 8 children in 25 families, following which another sibling was diagnosed to have Wilson's disease [18].

The current conventional assay for serum ceruloplasmin is an immunological assay which detects ceruloplasmin protein irrespective of whether it is copper-containing or not. Ceruloplasmin is an enzyme whose activity is dependent upon its containing copper. An important development is the availability of accurate assays for enzymatic activity of serum ceruloplasmin to diagnose Wilson's disease [25–27]. Enzymatic assays are superior to immunological assays for serum ceruloplasmin. The original descriptions of Wilson's disease emphasized the very low serum ceruloplasmin because they employed this sort of enzymatic test. With the current immunological assay for ceruloplasmin many patients have only mild-to-moderate decreases in serum ceruloplasmin, and in some patients it is normal. Basal 24 h urinary copper excretion can be a highly informative test. This test largely depends on the copper in the plasma compartment which is not incorporated in ceruloplasmin and thus it reflects copper overload. Elaborate collection systems are not mandatory, but creatinine excretion can also be measured to check for completeness of the collection. "Spot" urine samples are not suitable for analysis. A major issue has been to determine the cut off for the upper limit of normal. The conventional cut off of 100 μg/24-hr is too insensitive and it fails to identify up to 20 % of patients. The cut off of 40 μg/24-hr (0.6 μmol/24-hr) is optimal [28].

Genetic tests for Wilson's disease are useful in two clinical situations: to clinch the diagnosis in patients with suspected Wilson's disease and to screen for Wilson's disease in asymptomatic siblings of an index patient with Wilson's disease. In 2001, an expert group suggested a consensus scoring system to diagnose Wilson's disease. In this scoring system, (Table 2), detection of *ATP7B* mutations on both chromosomes is the highest weighted parameter [29]. A retrospective study of this scoring system in 40 children with mild hepatic Wilson's disease and 58 controls showed good diagnostic accuracy (positive and negative predictive values of 93 % and 92 % respectively) when 40 μg/24-hr was taken as the upper limit of normal (UNL) for basal 24-hr urinary copper excretion [28]. However, the reality is that genetic testing for Wilson's disease, as well as specialized tests like liver copper estimation, is performed only in very few centers in India and worldwide. Thus, in most centers, clinical assessment, slit-lamp examination for Kayser-Fleischer rings, serum ceruloplasmin, basal 24-hr urinary copper excretion, brain imaging (in patients with suspected neuro-Wilson's) and staining for copper on liver biopsy comprise the basis for diagnosing Wilson's disease.

To conclude, detection of *ATP7B* mutations in an index patient establishes the diagnosis of Wilson's disease. However, >500 *ATP7B* mutations have been reported in Wilson's disease patients. In addition, apart from mutations in the exons (i. e: coding region) of *ATP7B* gene, mutations in the regulatory elements of *ATP7B* gene (promoter region, untranslated region) have also been reported in Wilson's disease [30]. Hence, it is difficult to be sure that we are not missing out on a mutation, when an *ATP7B* mutation is not found in an index patient who is suspected to have Wilson's disease. In contrast to the above mentioned limitations in utility of genetic testing in an index patient (that is, a proband) with suspected Wilson's disease in a family, genetic testing is the *test of choice* to evaluate for Wilson's disease in an asymptomatic sibling of a proband with Wilson's disease. If the asymptomatic sibling has non-diagnostic biochemical tests for Wilson's disease, then demonstration of wild type or simple heterozygous carrier state can prevent unnecessary treatment [21]. Antenatal genetic testing for Wilson's disease is possible [31, 32]. However, as Wilson's disease is eminently treatable, especially with early diagnosis and prompt treatment, antenatal testing for Wilson's disease is usually not performed [17].

Can genetic testing obviate the need for further invasive tests like liver biopsy?

An ideal genetic test for Wilson's disease would be cheap and widely available with a quick turn-around time. However such a test is not yet available and, if developed, is likely to be useful in all patients with suspected Wilson's disease whose initial evaluation is inconclusive. For the time being, liver biopsy will continue to be an important investigation in a patient with suspected Wilson's disease, if the diagnosis is unclear after initial evaluation [17].

After applying the diagnostic score for Wilson's disease [29], based on initial evaluation, a patient who is 'highly

Table 2 Scoring system to diagnose Wilson's disease (adapted from Ref [29])

Liver copper (μg/g dry weight) (in absence of cholestasis)	Score	Neuropsychiatric symptoms suggest WD and/or typical brain MR scan	Score
<50	−1	Absent	0
50–250	+1	Mild	+1
>250	+2	Severe	+2
Rhodanine stain (if liver copper dry weight not available)		Kayser-Fleischer rings	
Absent	0	Absent	0
Present	+1	Present	+1
Basal 24 hour urine copper		*ATP7B* mutations	
Normal	0	None	0
1-2 times ULN	+1	Mutation on one chromosome	+1
>2 times ULN	+2	Mutation on both chromosomes	+4
Normal, but >5 times ULN after Penicillamine challenge	+2		
Serum ceruloplasmin (mg/dL)		Coomb's negative hemolytic anemia	
>20	0	Absent	0
10–20	+1	Present	+1
<10	+2		

Score ≥4: WD highly likely, score 2 or 3: probable WD, further tests needed, score 0 or 1: WD unlikely
WD Wilson's disease

likely' to have hepatic Wilson's disease, may not need liver biopsy (Table 3). In patients with classic features of Wilson's disease (very low serum ceruloplasmin, Kayser-Fleischer rings, evidence of chronic liver disease) and coagulopathy a liver biopsy may not be necessary: it will provide information about the stage of liver disease, rather than information for the diagnosis of Wilson's disease. The penetrance of a disease-causing mutation refers to the proportion of individuals with the mutation who exhibit clinical symptoms. Penetrance of Wilson's disease is expected to be 100 % [33]; however, late onset Wilson's disease is well documented, raising the question whether *ATP7B* mutations are fully penetrant or whether the nature of the mutation influences the pattern of clinical disease [34–36].

What to advise regarding genetic counseling for the family?

In the absence of genetic tests for Wilson's disease (as is the case in most centers in India), how do we provide genetic counseling for the family? One question asked by parents with a child affected by Wilson's disease is whether they can plan for another child. It helps to explain the modes of inheritance of Wilson's disease—either both parents are simple heterozygotes or (rarely) one or both of them has Wilson's disease. If both parents are simple heterozygotes, there is a 25 % chance that another child born to the parents may have Wilson's disease, 50 % chance that the child will be a simple heterozygote and 25 % chance that the child will be entirely normal. If one parent has Wilson's disease and the other is a heterozygote carrier, then subsequent pregnancies are possible but should be monitored more closely. Another question is whether a person with Wilson's disease can marry. It is not practical to perform genetic tests for Wilson's disease in a prospective life partner. In an attempt to reduce the risk of Wilson's disease occurring in children, it appears reasonable to suggest that consanguineous marriage be avoided. It should be emphasized that Wilson's disease in a child, if detected early and promptly treated, will cause minimal morbidity, or none at all, so long as the patient responds well to treatment and is adherent to life-long treatment. All

Table 3 Probability of Wilson's disease after 3 initial tests, based on WD diagnostic score (See Table 2, also Ref. [29]): in a patient with isolated hepatic involvement (i.e.: no neurological manifestations)

Clinical scenario	Serum ceruloplasmin, urine copper, KF rings	WD score [29]	
		Total score	Probability of WD as per this score
10 year old boy, unexplained liver disease, LFT and imaging : consistent with cirrhosis, Child's A	2 or 3 test results abnormal	≥4	Highly likely
		2–3	Probable
	1 test result abnormal	2	
		1	Unlikely

WD Wilson's disease, *KF* Kayser-Fleischer, *LFT* liver function tests

siblings of an index patient with Wilson's disease should be screened for pre-symptomatic Wilson's disease. As most patients with Wilson's disease do not develop symptoms prior to 5 years of age [24], it is reasonable to screen for Wilson's disease before 5 years of age. There are uncommon reports of symptomatic Wilson's disease manifesting at 2 years of age [37] or earlier, thus supporting screening even from the age of 2 years [33]. If the child is entirely asymptomatic, then screening around the age of 3 years may be a practical plan.

Genotype phenotype correlation in Wilson's disease

It remains unclear as to why some Wilson's disease patients develop hepatic disease while others develop neurological disease. Cerebrospinal fluid copper concentration in Wilson's disease appears to reflect copper accumulation in the brain [38]. Standard classification of Wilson's disease phenotype is a pre-requisite for studies of genotype phenotype correlation [39]. Neuroradiological findings in Wilson's disease are now better characterized [40]. A Global Assessment Scale for Wilson's disease that grades the multi systemic manifestations of the disease has also been proposed [41]. Two approaches have been used to study genotype phenotype correlation in Wilson's disease. The most widely used approach has been to analyze Wilson's disease patients sharing a common genotype. Another approach is to study those families with more than one family member affected by symptomatic Wilson's disease. As affected siblings within a family will share identical *ATP7B* mutation, study of the Wilson's disease phenotype in these families could be informative. Study of phenotype in twins with symptomatic Wilson's disease has also been reported [42].

Genotype phenotype correlation in Wilson's disease patients sharing a common mutation

Wilson's disease phenotype has been analyzed in patients with H1069Q mutation (in Caucasians) or R778L mutation (in East Asians). A meta-analysis in Dutch patients with Wilson's disease reported the association of H1069Q mutation with late and neurological presentation [43]. However, other reports did not show this correlation [44]. Truncating mutations in *ATP7B* gene were associated with very low serum ceruloplasmin oxidase activity and early onset of Wilson disease [45]. Some other genes which could modify WD phenotype have been looked at. In Alzheimer's disease, temporal lobe epilepsy and cerebral trauma, the presence of the apolipoprotein E (ApoE) 4 is associated with an increased vulnerability of the brain to the effects of the disease, whereas the presence of the ApoE 3/3 appears to provide moderate neuroprotection. In an Austrian study of 121 symptomatic index patients with Wilson's disease, the ApoE genotypes were 3/3 (59 %), 3/4 (21 %), 3/2 (19 %), and 4/2 (1 %). Among 40 Wilson's disease patients who were H1069Q homozygotes, onset of symptoms was significantly delayed in patients with ApoE 3/3 (at 25 +/− 6 years) compared with patients with ApoE 3/4 (at 20 +/− 3 years). Thus, ApoE genotype appeared to play a role in delaying onset of symptoms, but did not modify phenotypic disease expression in this study [46]. The R778L mutation in the *ATP7B* gene is mostly found in Chinese, Japanese and Korean patients. In a study of Chinese Han children with Wilson's disease, ApoE 3/3 did not delay the onset of disease and there was no association between ApoE genotype and Wilson's disease clinical presentation, including those who were R778L homozygotes. The authors concluded that the onset of Wilson's disease was not related to ApoE 3/3, although the high frequency of ApoE 3/3 in Chinese Han children with Wilson's disease was not significantly different from that in controls [47]. Other genes which have been studied in Wilson's disease include *COMMD1* gene (earlier known as *MURR1* gene) which is the causative gene for Bedlingtoncanine copper toxicosis [48], antioxidant gene1 (ATOX1) [49] and *MTHFR* gene [50]. No influence of *COMMD1* on severity of Wilson's disease has been found, and evidence regarding the other two candidate modifiers is unconvincing.

Genotype phenotype correlation in affected siblings within a family

We reported the phenotype of 'presumed Wilson's disease' in siblings of patients with confirmed Wilson's disease: of eight such families, the affected family member within each family shared identical Wilson's disease phenotype in six families, while the phenotype was discordant among siblings in two families [18]. While symptomatic affected siblings within a family usually share identical Wilson's disease phenotype [51], uncommonly, the phenotype is discordant [52, 53]. In one report, in Family 1, two sons developed neurological Wilson's disease at 16 and 28 years of age, respectively, and while the daughter developed hepatic Wilson's disease at 38 years of age. In Family 2, the son showed neurological symptoms of Wilson's disease at 32 years of age while the 35-year-old daughter had hepatic Wilson's disease. The affected patients in both families were *ATP7B* compound heterozygotes [52]. In another report, two pairs of monozygotic twins discordant for Wilson's disease phenotype were studied. In the first set, the twins were *ATP7B* compound heterozygotes. The index case developed severe liver failure followed by depression, dysarthria, and tremor at the age of 36. Her sister remained presymptomatic at diagnosis at the age of 39. The second twins were *ATP7B* homozygotes for the mutation (H1069Q). The

Table 4 Lack of common *ATP7B* mutations in Wilson's disease patients in India

Centre	Number of WD patients studied	Commonest mutations of *ATP7B* gene
Chandigarh [61]	41	T33053 (6 %)
		C2975A (6 %)
		2977insA (6 %)
Kolkata [53]	62	C813A (19 %)
Vellore [18]	27	G3182A (16 %)
		C813A (12 %)

WD Wilson's disease

index case presented with dysarthria and tremor at the age of 26. Her sister remained pre-symptomatic at diagnosis at the age of 28 [42]. Such reports of discordant phenotypes among siblings sharing same *ATP7B* mutation may indicate that other as yet unidentified modifier genes determine Wilson's disease phenotype [53].

Apparent paradox: lack of common mutations of Wilson's disease, despite many parents being in consanguineous marriages in India

There are probably more consanguineous marriages in Southern India than in any other large non-isolated population in the world [54]. Uncle-niece and first-cousin marriages are favored for a variety of cultural reasons. Of 322 infants borne at two hospitals in Vellore in 1977, >39 % of the children were borne to consanguineous parents (16 % were uncle-niece marriages) [55]. Analysis of trends in consanguinity in National Family Health Survey (1992–93) in Southern Indian states of Andhra Pradesh, Karnataka, Kerala and Tamil Nadu showed that in Kerala, the frequency of consanguineous marriages was very low and uncle-niece marriage, a preferred type of marriage of the Dravidian marriage system, was conspicuously absent. In the other 3 states of Southern India, consanguinity and co-efficient of inbreeding were high [55]. It appears paradoxical that compared to most Western studies, predominant/common *ATP7B* mutations are uncommon in India, despite more frequent consanguineous marriages in India. H1069Q is the predominant *ATP7B* mutation seen in Northern European Caucasian populations (present in 18 % to 72 % of Wilson's disease patients) [56, 57], while R778L predominates in East Asians in Hong Kong Chinese (17 %) [58], Korean patients (65 %) [59]. In contrast, reports to date do not indicate a single common mutation among Wilson's disease patients in India [60], (Table 4). In the Vellore report, despite 44 % of parents of Wilson's disease patients studied being in consanguineous marriages, the two common mutations of *ATP7B* gene — G3182A and C813A — were found in only 4 (16 %) and 3 (12 %) of the patients respectively [18]. The gene pool in India is diverse. The Indian subcontinent is regarded as a natural genetic laboratory, owing to the co-existence and interaction of socio-culturally, linguistically, ethnically and genetically diversified endogamous populations in a geographical terrain [62]. In such a diverse gene pool in India, the small numbers of Wilson's disease patients studied may be one reason for this apparent paradox. Another explanation is that, in contrast to the West, many marriages in India still occur within castes [63]. It is possible that common mutations or exonic 'hot-spots' in the *ATP7B* gene may emerge, if one were to study Wilson's disease patients belonging to a particular ethnic group/caste/sub-caste in India.

Copper overload syndromes causing cirrhosis, other than Wilson's disease

A number of non-Wilsonian copper overload syndromes causing cirrhosis in children have been described, both in India (Indian childhood cirrhosis) [64, 65], atypical copper associated cirrhosis [66]) and in other parts of the world (endemic Tyrolean childhood cirrhosis) [67], idiopathic copper toxicosis [68] and (Indian childhood cirrhosis in a Saudi family) [69]. Two features appear to distinguish these disorders from Wilson's disease: the mode of inheritance and the organs affected by copper overload (Table 5). It is not known whether some patients with rare hepatic copper overload states actually have isolated or variant hepatic Wilson's disease, presenting with some very unusual features. However, it is clear that Bedlington canine copper toxicosis, once considered to be a model for Wilson's disease, is not canine Wilson's disease because the canine *ATP7B* gene is normal. The decline in incidence of Indian childhood cirrhosis has been attributed to the phasing out of the household use of brass utensils, with availability of cheaper alternatives like steel or aluminium utensils [64]. Copper may have leached into milk, when it was boiled in

Table 5 Two contrasting features of Wilson's disease and non-Wilsonian copper overload syndromes causing childhood cirrhosis

	Wilson's disease	Non-Wilsonian copper overload syndromes causing cirrhosis
Mode of inheritance	Monogenic (autosomal recessive) disorder	Complex (ecogenetic) disorder
Organs affected	Liver, brain (basal ganglia)	Liver

brass or copper containers. We looked for evidence of increased copper intake in 11 patients with atypical copper cirrhosis – 7 males, age: 16 (9–24) years, median (range). In one family, all 3 children, including 1 twin pair, were affected. Copper content was normal [2.32 (0.32–8.9) μg/L, median (range)] in drinking water samples obtained from homes of the study subjects. While a history of storing drinking water at home in brass vessels was elicited in 1 family, in no family was there a practice of cooking in brass vessels [70].

What are some of the research questions into genetics of Wilson's disease in India for the future?

Population based studies of the frequency of Wilson's disease, especially in different communities/ethic groups in India, are needed. Studies to identify predominant mutations of the *ATP7B* gene, if present, are needed among Wilson's disease patients within different communities/ethnic groups. If such predominant *ATP7B* mutations are identified, screening for these mutations would be the simple initial step to perform in patients from that community/ethnic group who are suspected to have Wilson's disease. We also need to analyze *ATP7B* gene and other candidate genes, as well as acquired factors, in non-Wilsonian copper overload states in India. Considering the disease burden in India, centres providing genetic testing for Wilson's disease as well as family genetic counseling services need to be set up across India. Improvements in technology can be expected to lead to simpler and better genetic testing in the future. We also need to develop appropriate strategies for genetic testing for Wilson's disease, tailored for Indian patients, considering the largely uncharted genetic spectrum of Wilson's disease across the diverse sections of society in India. Recently, the role of stem cell therapy to cure Wilson's disease has been explored. Bone marrow stem cell transplantation partially corrected the metabolic phenotype in a mouse model for Wilson's disease (toxic milk mouse) [71]. Induced pluripotent stem cells from a patient with Wilson's disease were differentiated into hepatocyte-like cells that displayed abnormal cytoplasmic localization of mutated *ATP7B* and defective copper transport; by correcting the genetic defect using a lentiviral vector, the functional defect could be reversed in vitro [72]. In the meantime, early accurate diagnosis of Wilson's disease and timely commencement of treatment are the best prospect for Indian patients with Wilson's disease. Considering Wilson's disease in any patient with unexplained hepatic or neurological disease, regardless of age, is an important first step.

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