Neurosci. Bull. June, 2017, 33(3):323–330
DOI 10.1007/s12264-017-0107-4

www.neurosci.cn
www.springer.com/12264

REVIEW

Wilson's Disease in China

Juan-Juan Xie[1] · Zhi-Ying Wu[1]

Received: 13 August 2016 / Accepted: 10 January 2017 / Published online: 6 March 2017

Abstract Wilson's disease (WD) is an autosomal recessive disorder of copper metabolism. Its incidence is higher in China than in western countries. *ATP7B* is the causative gene and encodes a P-type ATPase, which participates in the synthesis of holoceruloplasmin and copper excretion. Disease-causing variants of *ATP7B* disrupt the normal structure or function of the enzyme and cause copper deposition in multiple organs, leading to diverse clinical manifestations. Given the variety of presentations, misdiagnosis is not rare. Genetic diagnosis plays an important role and has gradually become a routine test in China. The first Chinese spectrum of disease-causing mutations of *ATP7B* has been established. As a remediable hereditary disorder, most WD patients have a good prognosis with an early diagnosis and chelation treatment. However, clinical trials are relatively few in China, and most treatments are based on the experience of experts and evidences from other countries. It is necessary to study and develop appropriate regimens specific for Chinese WD patients.

Keywords Wilson's Disease · Copper · Epidemiology · Pathogenesis · Management

Introduction

Wilson's disease (WD), also termed hepatolenticular degeneration, is an autosomal recessive disorder attributed to disease-causing mutations of the culprit gene *ATP7B*, which plays a key role in copper metabolism. In 1932, Cheng first reported two cases of WD in China [1], and research on WD in China has soared since the 1950s. To estimate the incidence of WD in China, Hu *et al*. conducted the first official epidemiological investigation [2]. WD is likely to be misdiagnosed due to the heterogeneity of the clinical manifestations, and many investigators have focused on finding the relationship between genotype and phenotype. Molecular diagnosis is a robust means of establishing an accurate determination. From 2004 to 2015, we identified 58 novel mutations and established the first Chinese spectrum of disease-causing mutations of *ATP7B* [3]. As for management, D-penicillamine (PCA) is recommended for the majority of WD patients, but not for those with severe spasticity, malformation, or dysarthria, and those who are allergic to PCA [4]. Dimercaptosuccinic acid (DMSA) was first used to treat WD in China [5], and is recommended as an alternative for patients with severe neurological manifestation [4]. In addition, zinc monotherapy is suitable for pre-symptomatic individuals and patients after liver transplantation [6, 7]. Here, we retrospectively review some of the research in Chinese WD patients.

Clinical Features

Symptoms and Signs

WD was first reported in China in 1932 by Cheng, who published detailed clinical histories of two 24-year-old patients who manifested a pure extrapyramidal motor disturbance characterized by poverty of movement, disturbed associated movements, and involuntary movements including tremor, as well as athetotic and choreiform

✉ Zhi-Ying Wu
zhiyingwu@zju.edu.cn

[1] Department of Neurology and Research Center of Neurology in the Second Affiliated Hospital, and the Collaborative Innovation Center for Brain Science, Zhejiang University School of Medicine, Hangzhou 310009, China

movements [1]. Zhang summarized the research on WD in the 1950s and concluded that the main clinical manifestations were due to neurological damage, especially in the extrapyramidal system [8]. Research on WD in China has soared since then and the number of reports was higher than that in western countries during the same period, according to Zhang [8]. It is worth noting that five cases had pathological results attached.

With the development of diagnostic technology, more WD patients are identifiable before the occurrence of evident neurological abnormality. The age at onset ranges from 5 to 35 years [9]. More recently, molecular genetic testing has confirmed an 8-month-old Chinese boy who presented with elevated liver enzymes and a low serum ceruloplasmin (CP) level [10]. Pediatric WD patients are often picked up incidentally with isolated elevation of serum alanine transaminase [11]. In a retrospective review of the medical records of WD patients in Shengjing Hospital of China Medical University during 1993-2011, 69.9% (93/133) had hepatic manifestations at the time of diagnosis, and 20.3% (27/133) had neurological manifestations [12]. The liver dysfunctions include acute or chronic hepatitis, cirrhosis, hepatic encephalopathy, and fulminant hepatitis. Patients without a neurological presentation are likely to be misdiagnosed. The statistics show that only 33.1% (44/133) are diagnosed at the initial medical consultation. Neurological manifestations, such as involuntary movements and rigidity, are common in WD patients. Tremor and oropharyngeal dysfunction like slurring and dysphonia are the most common and obvious symptoms, and appear in the early stage [13]. Oropharyngeal dysfunctions are prominent in WD patients, such as keeping the mouth open with different degrees of salivation as a result of dysphagia, and spastic laughter due to rigidity of the mimetic muscles [13]. Other symptoms, including dystonia, dysgraphia, gait abnormality, ataxia, autonomic dysfunction, memory deterioration, and impaired concentration and cognition, as well as hostility, are not rare in Chinese WD patients. Psychiatric symptoms are often nonspecific, such as depression, mania, personality changes, and disturbed intelligence. When the symptoms are controlled by antipsychotic drugs, the correct diagnosis is likely to be delayed until extrapyramidal symptoms occur. In addition, it has been reported that patients show neurological or psychiatric deterioration after surgery or trauma, indicating that WD patients should avoid trauma and unnecessary surgery [14]. Clinical manifestations resulting from copper damage to other tissues and organs are relatively infrequent (Table 1).

Kayser-Fleischer (K-F) rings are the most common ocular signs. In a nonrandomized retrospective case series [15], the positive rate of K-F rings was 100% (11/11) in patients with neurological disorder and 23.1% (12/52) in those with hepatic disease. However, the absence of K-F rings in patients with overt neurological symptoms cannot be used to exclude the possibility of WD. As K-F rings are caused by the deposition of copper, they also exist in patients with chronic cholestasis [9], since biliary excretion is the predominant means of decreasing the copper concentration. Li *et al.* found a significantly older age at symptom onset for patients positive for K-F rings [16], which might be the reason why they had a longer diagnostic delay if the physician of their first visit knew little about WD. In addition, patients with K-F rings have a higher level of 24-h urinary copper but a lower level of serum alanine aminotransferase (ALT) [16].

Table 1 Clinical features in other tissues and organs.

Tissue, organ, or system	Clinical features
Kidney	Edema of both lower limbs, microscopic or macroscopic hematuria, proteinuria, renal tubular acidosis
Blood	Hemolytic anemia, thrombocytopenia, leukopenia
Bone and muscle	Osteoporosis, osteomalacia, joint deformity, amyotrophia, muscle and joint ache, pathological fracture
Skin	Hyperpigmentation
Endocrine system	Male feminization, paramenia, habitual abortion, infertility, sexual retardation

Laboratory Examinations

The normal range of serum CP is 200–500 mg/L. As ATP7B plays a key role in the incorporation of copper into apo-ceruloplasmin, dysfunctional ATP7B often results in a decreased CP level. However, CP can be >200 mg/L when the estrogen level is elevated in pregnant women or individuals given estrogen supplementation, or in patients with rheumatoid arthritis. CP levels that give false-positive can be seen in babies <2 years old, in ~20% WD carriers [17], and in patients with chronic hepatitis, severe hepatitis, or nephrotic syndrome. The 24-h excretion of urinary copper in patients is >100 μg due to the high levels of serum copper, and in pediatric patients a concentration >40 μg should be considered a high level. Disease-causing mutations of *ATP7B* identified on both homologous chromosomes are deemed to be the gold standard for the diagnosis of WD.

Imaging Examination

Brain magnetic resonance imaging (MRI) in WD patients mainly shows hypointensity on T1 and hyperintensity on

T2 on both sides in the putamen, caudate nucleus, thalamus, midbrain, pons, and cerebellum, while in rare cases hyperintensity on T1 or hypointensity on both T1 and T2 have also been found [18–21]. Various degrees of sulcal widening, ventriculomegaly and encephalomalacia in the frontal cortex have also been found. The MRI presentation is not in accordance with the severity of disease, but lesions can shrink during management, making it an applicable method to monitor the efficacy of treatment. In addition, marked hypointensity on susceptibility-weighted imaging has been found in the head of the caudate nucleus, globus pallidus, putamen, thalamus, substantia nigra, and red nucleus on both sides [22].

Pathology

The first systematic report on pathological changes in the central nervous system of patients with WD was published in 1957 [23]. According to that report, the remarkable changes in the brain are atrophy of the putamen with brown sediment and the transformation of astrocytes ("Alzheimer's cells"). Alzheimer's cells are part of a continuously evolving process, from gradual hypertrophy and proliferation to retrogression. The nucleus of such a giant glial cell can reach ~6 times the size of a normal glial nucleus. The whole course of transformation is divided into four successive stages according to the morphology as follows: (1) hypertrophy; (2) emergence of the nucleolus and deformation of the nucleus; (3) prominent deformation of the nucleus and the appearance of multiple nuclei; and (4) disruption of the nuclear membrane, dissolution of the nucleus, disappearance of the nuclear membrane, and fragmentation of neurites. In addition to Alzheimer's giant glial cells, Opalski's cells are also characteristic and generally considered to be homologous to Alzheimer's cells as they are both derived from astrocytes. Diffusely scattered giant glial cells are found in brain without a tendency to form fibers because of retrogression. The occurrence of Alzheimer's cells is not totally secondary to neuronal damage, because the changes in astrocytes do not closely accompany those in neurons. Liver damage manifests as postnecrotic cirrhosis [23], and black-brown copper particles can be observed by microscopy after rubeanic acid staining.

Epidemiology

The prevalence rate of WD is estimated to be 1/10,000 to 1/30,000 worldwide [24–26]. It is believed that the prevalence is higher in China than in western countries. Hu *et al.* [2, 27] conducted two successive investigations in three counties of Anhui Province, surveying a total of 153,370 individuals, and detected 9 WD patients, inferring a prevalence rate of 5.87/100,000. Diagnosis of all the above cases was based on clinical features, biochemical parameters and the presence of K-F rings. Considering the existence of asymptomatic patients without K-F rings or other common biochemical abnormalities, as well as a screening level confined by the technology and the uneven expertise of doctors, the actual prevalence is likely to have been underestimated. By sequencing *ATP7B* in 1000 control participants in the United Kingdom, the frequency of individuals predicted to carry two mutant pathogenic *ATP7B* alleles was calculated to be 1/7026 [25]. In addition, in an investigation involving 500 healthy Korean participants [28], the extrapolated prevalence was ~1/3000 and the carrier frequency was 1/27. Furthermore, the estimated incidence of WD in Hong Kong Chinese was 1/5400, based on haplotype analysis of 660 participants [29]. Molecular epidemiological investigations of *ATP7B* in different populations noted above confirm the higher prevalence rate in Asians than in Caucasians and that the previous WD incidence was underestimated.

Genetics

Molecular diagnosis has been increasingly widely applied. Direct sequence analysis is a relatively accurate method to identify mutations in *ATP7B* at present. The most common type is point mutations; but other types have also been identified in WD patients, such as small deletions or insertions, gross deletions and mutations in splicing sites. A previous study showed that WD in China seems to result from a few relatively common mutations and a large number of rare mutations [30]. Reports of novel *ATP7B* mutations are frequent. Li *et al.* identified 14 novel mutations in 62 WD cases [16]. Dong *et al.* have summarized a long-term investigation of *ATP7B* variants in 632 unrelated Chinese WD patients from 2004 to 2015, and 173 variants were detected, 58 of which were novel [3]. The distribution and allele frequency of common disease-causing variants are listed in Table 2. The top three – p.R778L, p.P992L, and p.T935M – have been identified in 78% of patients, which has enabled the development of a rapid and cost-effective genetic test, the multiplex allele-specific PCR to screen for WD [3]. Ninety percent of patients are genetically diagnosed as having WD with two or more disease-causing variants [3]. It is worth noting that 6 unrelated WD patients have been found to carry three disease-causing variants [3]. This suggests that two disease-causing variants might come from a single chromosome, so it is necessary to test the genotype of the parents of a potential patient to determine the exact location when the clinical

Table 2 Distribution and allele frequency of 14 common disease-causing mutations.

Exon	Mutation	Allele frequency (%)
2	p.V176Sfs×28	1.7
3	p.Q511X	2.6
5	c.1708-1g>c*	1.6
8	p.R778L; p.R778Q	31.9; 2.1
11	p.A874V	3.8
12	p.T935M; p.G943D;p.R919G	7.7; 2.4; 2.1
13	p.P992L	15.5
15	p.V1106I	1.1
16	p.I1148T	3.5
17	p.V1216M	2.1
18	p.N1270S	2.2

* Adjacent to exon 5.

features do not support a diagnosis of WD. If both variants are confirmed to be from a parent, the individual should be diagnosed as a heterozygous carrier.

At present, many investigators focus on exploring the relationship between genotype and phenotype in an attempt to explain the complex and diverse clinical manifestations. One study has shown that patients with a homozygous p.R778L mutation display obvious liver damage at an earlier age with a lower CP concentration than a heterozygous group; that is, p.R778L has a strong effect on ATP7B function [31, 32]. Cellular function experiments have confirmed that p.R778L is a more serious type of mutation than some others, such as p.T935M and p.R919G [33, 34]. However, many reports deny this conclusion. Some have suggested that p.R778L postpones the age at onset and is not related to the first symptoms and disturbance of copper metabolism when comparing WD patients with and without p.R778L [35]. In addition, Lu *et al.* found that the frequencies of p.R778L, p.P992L and p.A874V did not significantly differ in pediatric patients with distinct clinical manifestations [36]. To sum up, a consensus has not been reached. This may be due to the different populations used in different research centers, the distinct standards used in grouping participants, and the complex and different variant combinations, since compound heterozygous mutations occur often in WD patients while homozygous mutations are rare.

COMMD1 (copper metabolism domain containing 1) is a protein that accounts for canine copper metabolism disorder [37]. It is considered to be one of the factors that modulate the function of ATP7B, the expression of which shows a negative correlation with the amount of COMMD1 [38]. One study indicated that variants in *COMMD1* influence human copper metabolism and the clinical features of WD [39]. However, by directly sequencing the entire coding region and adjacent splice sites of *COMMD1* in 120 unrelated healthy Chinese and 218 unrelated Chinese patients with WD, no disease-causing variants were detected, indicating no correlation between *COMMD1* mutation and WD in the Chinese population [40].

Pathogenesis

Excessive copper accumulation is the accepted cause of WD. The maintenance of copper homeostasis requires exquisite manipulation by a regulatory system in which ATP7B plays a critical role. It is generally situated in the trans-Golgi network and transfers copper to be incorporated into newly-synthesized apoceruloplasmin [41]. With an increasing copper concentration in the cellular milieu, ATP7B is relocated from the trans-Golgi network to the peripheral compartment and copper is excreted into the bile [41].

Previous investigations have shown that pathogenic mutations of *ATP7B* disturb its normal structure and function. Structurally, variants may result in the loss of ATP7B integrity, misfolding, or impaired interaction between proteins. Functionally, variants may have decreased ATP-binding affinity, and abnormal phosphorylation or copper transport [42]. Wu *et al.* found that p.R778L and p.T935M exert no influence on the quantity of ATP7B, while p.Q914X increases its expression level [43]. Zhu *et al.* confirmed that p.E332X, p.Q511X, p.Q547X, p.Q819X, and p.R778L have a diffuse and homogenous distribution pattern *in vitro*, whereas p.R919G, p.T935M, and p.P992L are normally located in the Golgi apparatus when copper is not added. However, after exposure to $CuCl_2$, p.T935M mutants move from the Golgi apparatus to a diffuse vesicular distribution, similar to wild-type ATP7B, while p.R919G and p.R992L mutants remain mainly in the Golgi apparatus, and p.R778L mutants exhibit a diffuse and homogenous distribution in the cytoplasm. In short, p.T935M has mild effects on the function of ATP7B. p.R919G and p.P992L influence its trafficking, while p.R778L disturbs both its localization and trafficking. These results may explain why the clinical manifestations in WD patients with p.R778L are often more severe than in those with p.T935M [33, 34].Yang *et al.* found no influence of -183C>T on the activity of the promoter [44]. Diao *et al.* found that c.3244-2A>C causes a 13-nucleotide sequence to be skipped [45]. Wan *et al.* elucidated the consequences of the mutations p.S986F, p.I1348N, p.G1355D, p.M1392K, p.A1445P, and c.2810delT in the coding region of *ATP7B* and two nucleotide substitutions, -133A>C and -215A>T, in the promoter region [46]. The p.I1348N, p.G1355D, p.M1392K, and c.2810delT mutations completely inhibit

copper-transporting activity, while p.S986F and p.A1445P decrease enzyme activity by ~50%. The -133A>C, -215A>T, or both mutations reduce promoter activity to 51%, 25% and 13%, respectively. The c.2810delT mutation completely destroys the function of ATP7B but increases the expression levels of alternatively-spliced variants of exon 12 (ATP7B-d12). Alternative splicing of exon 12 generates ATP7B cDNA without exon 12, and since further experiments showed that deletion of exon 12 does not alter the function of ATP7B, Wan *et al.* concluded that enough ATP7B activity is maintained by the increased expression of ATP7B-d12 influenced by c.2810delT [46]. Cheng *et al.* detected two patients in a family carrying a heterozygous p.R778W mutation and a homozygous p.P992L mutation. One parent carried a heterozygous p.R778W mutation and the other a p.P992L heterozygous mutation, so the author concluded that loss of heterozygosity is also involved in the pathogenesis of WD [47].

The final outcome of the disease-causing mutations of *ATP7B* is that superfluous copper accumulates in hepatocytes, overflows into the blood, and then is deposited in the brain, kidney and cornea [48], initiating oxidative damage and mitochondrial dysfunction [49, 50], leading to hepatocyte apoptosis and neuronal degeneration. With the availability of $Atp7b^{-/-}$ mice, a valid animal model of WD, Dong *et al.* confirmed the markedly increased copper content and abnormal ultrastructural findings in the liver, which suggested that $Atp7b^{-/-}$ mice are an excellent model in which to study pathogenesis in the liver in WD [51]. Unexpectedly, there was no evident increase in the whole brain and the cerebral cortex, basal ganglia, and cerebellum of $Atp7b^{-/-}$ mice. Furthermore, the copper concentration in mitochondria in the basal ganglia also displayed no significant difference, even though mitochondria were confirmed to be the first responder under the condition of copper imbalance. However, ultrastructural abnormalities were apparent in neurons in the basal ganglia of $Atp7b^{-/-}$ mice. These discrepancies hint that other morbidity factors might enhance the toxicity of copper in neurons in WD patients [51].

Management

Early and lifelong treatment is the principle of WD management. Evaluation of efficacy and side-effects is also essential. Since copper is derived from food, a low-copper diet must be adopted once the diagnosis is established. The core component of drug therapy is the chelation of excessive copper.

D-penicillamine, the first choice for WD, chelates copper inside the body, facilitating its excretion, and effectively reducing its level in the patient. However, the final effect varies in different subtypes, which makes it necessary to individualize the regimen according to age, type of clinical manifestation, course of disease, and amount of 24-h urinary copper. Adverse effects of PCA are not rare, and include neurological deterioration. Both hydroxyl radicals and free copper are increased in the striatum during PCA administration in toxic milk mice, which might contribute to the neurological deterioration [52]. So it should be used cautiously or even be forbidden in patients with severe neurological symptoms, especially those with rigidity. The most serious side-effect is anaphylaxis, manifesting as fever and rash in the first several days. When this happens, PCA should be terminated immediately. Nonetheless, PCA is still the main drug used in China because of its effectiveness, abundance, and low price.

DMSA is a broad-spectrum metal antidote and an appropriate alternative oral drug for maintenance treatment. It was first used to treat WD in China [5]. DMSA, Na-DMS, and sodium dimercaptosulphonate (DMPS) are recommended for patients with neurological and psychiatric symptoms, mild-moderate liver symptoms, and for those who are not tolerant of PCA. The adverse effects of these drugs include an allergic reaction and a tendency to bleed. The administration of DMSA, Na-DMS, and DMPS has Chinese characteristics, as these drugs are not mentioned in the practice guidelines published by the American Association for the Study of the Liver Diseases [17, 53] and the European Association for the Study of the Liver[9]. PCA along with intravenous DMPS could possibly avoid the continued adverse effects of the drug or a poor curative effect [54]. Theoretically, excreting copper rapidly worsens the neurological symptoms. But Yang *et al*. reported that by the end of strengthened copper-removal therapy comprising intravenous infusion of DMPS and oral intake of Gandou tablets (a kind of traditional Chinese medicine) and zinc gluconate, the total effectiveness was 84.85% [55].

Hu *et al.* conducted a clinical study of several antidotes against heavy metal poisoning in WD and compared the urinary copper, clinical effectiveness and side-effects before and after treatment in each group [56]. The urinary copper and clinical effectiveness values suggested that PCA is the best among the oral drugs, and DMSA takes second place. DMPS is the best among injection drugs.

Zinc has been confirmed as a valid adjuvant agent in WD patients to reduce the absorption of copper. With zinc therapy, the serum CP values gradually increase and the 24-h urinary copper values gradually diminish in pre-symptomatic siblings of WD patients, indicating that the disease progression is blocked and even reversed in potentially symptomatic WD patients [6]. Zinc is an ideal drug for the pre-symptomatic state and for the maintenance of neuropsychiatric patients.

Traditional Chinese Medicine, as an adjuvant therapy, promotes the excretion of copper in the bile, feces, and urine [57]. Representative drugs are Gandou tablets and Gandou Decoction, which are mainly comprised of Radix et Rhizoma Rhei, Rhizoma coptidis, and Radix Scutellariae [57]. The effect of Gandou Decoction on the excretion of intracellular copper and the accumulation of intracellular zinc has been assessed [58]. Xue *et al.* found that DMPS combined with Gaudou Decoction improves liver function and reverses the progress of hepatic fibrosis by indirectly enhancing the degradative activity of matrix metalloproteinase-1 in the extracellular matrix [59]. A systematic review of 9 randomized controlled trials revealed that traditional Chinese medicine as a monotherapy or an adjuvant therapy generally appears to be effective, safe and well-tolerated, but the evidence presented is insufficient to warrant a clinical recommendation due to the generally low methodological quality of the included studies [60]. So, additional well-designed, randomized, placebo-controlled clinical trials are still needed [60].

Surgical treatment mainly consists of splenectomy and liver transplantation. The former is aimed at WD patients with severe hypersplenism [61]. Since the leukocyte count would fall further in hypersplenism patients taking PCA, copper-removal treatment can continue only after splenectomy [50]. Fulminant hepatic failure that fails to respond to medical therapy is the best indication to undertake orthotopic liver transplantation (OLT). Taking its risks and the uncertain long-term effects into account, OLT should be prudently implemented only in patients who are unresponsive to standard copper-revomal therapy [50]. The shortage of liver donors is an important restriction. Auxiliary partial orthotopic liver transplantation (APOLT) is considered an attractive alternative. Cheng performed APOLT in $Atp7b^{-/-}$ mice with donor livers from $Atp7b^{+/+}$ mice and reported that the postoperative survival in the APOLT group was higher than that in the OLT group. In addition, early APOLT restored normal copper metabolism and prevented disease progression [62]. Neurological complications are quite common due to the surgery itself, together with the inevitable application of immunosuppressive agents. A clinical follow-up study for 3-9 years on 8 postoperative patients with WD has shown that it is necessary to continue chelation therapy after liver transplantation in patients with neurological deterioration [7].

In addition, symptomatic treatment and rehabilitation training are also indispensable. The visible amelioration of symptoms improves the quality of life, and increases confidence in and compliance with the therapy. This encourages and helps patients to take part in moderate activity with an optimistic attitude to restore social function.

Summary and Expectation

The incidence of WD is higher in China than in western countries, and the first Chinese spectrum of disease-causing mutations of *ATP7B* has been established. Genetic diagnosis plays an important role in diagnosing WD and is gradually becoming a routine test in China. However, clinical trials are relatively few in China, and most treatments are based on the experience of experts and evidence from other countries. Individualized therapeutic schedules have been put into practice, oriented by studies on the relationship between genotype and phenotype, but this is far from being enough. Therefore, it is necessary to study and develop appropriate regimens aimed at Chinese WD patients.

Acknowledgements This review was supported by the National Natural Science Foundation of China (81125009).

References

1. Cheng YL. Hepatolenticular degeneration (pseudosclerosis, progressive lenticular degeneration and torsion spasm) review of literature and report of two cases. Chin Med J 1932, 46: 347–364.
2. Hu WB, Han YZ, Xue BC, Cheng N, Sun DY, Ye DQ, *et al.* Epidemiological investigation of Wilson disease in Hanshan county, Anhui Province. Chin Med J 2010, 91: 894–897.
3. Dong Y, Ni W, Chen WJ, Wan B, Zhao GX, Shi ZQ, *et al.* Spectrum and classification of ATP7B variants in a large cohort of Chinese patients with Wilson's disease guides genetic diagnosis. Theranostics 2016, 6: 638–649.
4. Liang XL, Yang RM, Wu ZY, Wang N, Li XH, Wang X. Diagnosis and treatment guidelines of Wilson disease. Chin J Neurol 2008, 41: 566–569.
5. Zhang YD, Yang RM. Therapeutic assessment of dimercaptosuccinic acid capsule in the treatment of hepatolenticular degeneration. New Drugs Clin Rem 1990, 9: 73–76 (in Chinese with English abstract).
6. Wu ZY, Lin MT, Murong SX, Wang N. Molecular diagnosis and prophylactic therapy for presymptomatic Chinese patients with Wilson disease. Arch Neurol 2003, 60: 737–741.
7. Ni W, Dong QY, Zhang Y, Wu ZY. Zinc monotherapy and a low-copper diet are beneficial in patients with Wilson disease after liver transplantation. CNS Neurosci Ther 2013, 19: 905–907.
8. Zhang YC. Research of hepatolenticular degeneration in the past 10 years. Zhonghua Shen Jing Jing Shen Ke Za Zhi 1959, 5: 326–327 (in Chinese).
9. Ferenci P, Czlonkowska A, Stremmel W, Houwen R, Rosenberg W, Schilsky M, *et al.* EASL clinical practice guidelines: Wilson's disease. J Hepatol 2012, 56: 671–685.
10. Abuduxikuer K, Li LT, Qiu YL, Wang NL, Wang JS. Wilson disease with hepatic presentation in an eight-month-old boy. World J Gastroenterol 2015, 21: 8981–8984.
11. Hui J, Yuen YP, Chow CM, Chong J, Chiang G, Cheung CK, *et al.* Isolated persistent elevation of alanine transaminase for early diagnosis of pre-symptomatic Wilson's disease in Chinese children. World J Pediatr 2013, 9: 361–364.
12. Lin LJ, Wang DX, Ding NN, Lin Y, Jin Y, Zheng CQ. Comprehensive analysis on clinical features of Wilson's disease: an

experience over 28 years with 133 cases. Neurol Res 2014, 36: 157–163.
13. Wu ZY, Zhang Y, Sun YM. Metabolic Disorder of Neurogenetic Disease. Shanghai: Science and Technology Press of Shanghai, 2014: 799–830.
14. Wu ZY, Murong SX, Chen SR. Wilson disease starting with psychiatric disorder. Chin J Birth Health Heredity 1997, 5: 97–98.
15. Huo LJ, Liao RR, Chen XM. Ophthalmic manifestations of Wilson's disease. Chin J Ophthalmol 2008, 44: 128–130.
16. Li XH, Lu Y, Ling Y, Fu QC, Xu J, Zang GQ, *et al.* Clinical and molecular characterization of Wilson's disease in China: identification of 14 novel mutations. BMC Med Genet 2011, 12: 6.
17. Roberts EA, Schilsky ML. Diagnosis and treatment of Wilson disease: an update. Hepatology 2008, 47: 2089–2111.
18. Huang L, Li XH, Liang XL. Correlation between the findings by MRI in brain of Wilson disease and clinical manifestations. J Apoplexy Nerv Dis 2011, 28: 216–219.
19. Tang YB, Lu Q, Huang JJ, Yuan NP. MRI diagnosis of brain with hepatolenticular degeneration. J Clin Radiol 2008, 27: 1454–1456.
20. Yu XE, Yang RM. 132 cases brain MR imaging of hepatolenticular degeneration. J Apoplexy Nerv Dis 2007, 24: 30–34.
21. Wang ZB, Liu B, Liu HQ, Li DL, Wang XD. MRI features of brain with hepatolenticular degeneration in adults. Chin J CT MRI 2014, 12: 37–39.
22. Yang JJ, Li XH, Yang RM, Yu XE, Yu CL, Qian YF, *et al.* Susceptibility-Weighted Imaging manifestations in the brain of Wilson's disease patients. PLoS One 2015, 10: e0125100.
23. Zhang YC, Qin ZJ, Zou HW. Observation of 25 patients with hepatolenticular degeneration. Zhonghua Shen Jing Jing Shen Ke Za Zhi 1957, 1: 45–59 (in Chinese).
24. Reilly M, Daly L, Hutchinson M. An epidemiological study of Wilson's disease in the Republic of Ireland. J Neurol Neurosurg Psychiatry 1993, 56: 298–300.
25. Coffey AJ, Durkie M, Hague S, McLay K, Emmerson J, Lo C, *et al.* A genetic study of Wilson's disease in the United Kingdom. Brain 2013, 136: 1476–1487.
26. Gialluisi A, Incollu S, Pippucci T, Lepori MB, Zappu A, Loudianos G, *et al.* The homozygosity index (HI) approach reveals high allele frequency for Wilson disease in the Sardinian population. Eur J Hum Genet 2013, 21: 1308–1311.
27. Hu WB, Han YZ, Yang RM, Cheng N, Xue BC, Sun DY, *et al.* Epidemiological investigation on hepatolenticular degeneration of Han population in Jinzhai county and Lixin county, Anhui province: report of a family pedigree. Anhui Med J 2012, 33: 734–737 (in Chinese with English abstract).
28. Park HD, Ki CS, Lee SY, Kim JW. Carrier frequency of the R778L, A874V, and N1270S mutations in the ATP7B gene in a Korean population. Clin Genet 2009, 75: 405–407.
29. Mak CM, Lam CW, Tam S, Lai CL, Chan LY, Fan ST, *et al.* Mutational analysis of 65 Wilson disease patients in Hong Kong Chinese: identification of 17 novel mutations and its genetic heterogeneity. J Hum Genet 2008, 53: 55–63.
30. Wu ZY, Wang N, Lin MT, Fang L, Murong SX. Detection and analysis of mutations in Chinese patients with Wilson disease. Chin J Neurol 2001, 34: 152–156 (in Chinese with English abstract).
31. Wu ZY, Wang N, Lin MT, Fang L, Murong SX. Genotype-phenotype correlation of patients with Wilson disease in Chinese population. Chin Med J 2003, 83: 309–311.
32. Liu XQ, Zhang YF, Liu TT, Hsiao KJ, Zhang JM, Gu XF, *et al.* Correlation of ATP7B genotype with phenotype in Chinese patients with Wilson disease. World J Gastroenterol 2004, 10: 590–593.
33. Zhu M, Dong Y, Ni W, Wu ZY. Defective roles of ATP7B missense mutations in cellular copper tolerance and copper excretion. Mol Cell Neurosci 2015, 67: 31–36.
34. Zhu M, Ni W, Dong Y, Wu ZY. EGFP tags affect cellular localization of ATP7B mutants. CNS Neurosci Ther 2013, 19: 346–351.
35. Huang F, Liang XL, Wang Y, Xu PY, Yang CS, Yan ZW. Study on correlation between gene Arg778Leu mutation and clinical manifestation in Wilson' s disease. Chin J Neurol 2000, 33: 290–291 (in Chinese with English abstract).
36. Lu Y, Wang JS, Yu H, Wang XH. Correlation of clinical features of Wilson's disease with ATP7B gene mutation in Children. Chin J Evid Based Pediatr 2013, 8: 346–351 (in Chinese with English abstract).
37. van De Sluis B, Rothuizen J, Pearson PL, van Oost BA, Wijmenga C. Identification of a new copper metabolism gene by positional cloning in a purebred dog population. Hum Mol Genet 2002, 11: 165–173.
38. Materia S, Cater MA, Klomp LW, Mercer JF, La Fontaine S. Clusterin and COMMD1 independently regulate degradation of the mammalian copper ATPases ATP7A and ATP7B. J Biol Chem 2012, 287: 2485–2499.
39. Stuehler B, Reichert J, Stremmel W, Schaefer M. Analysis of the human homologue of the canine copper toxicosis gene MURR1 in Wilson disease patients. J Mol Med (Berl) 2004, 82: 629–634.
40. Wu ZY, Zhao GX, Chen WJ, Wang N, Wan B, Lin MT, *et al.* Mutation analysis of 218 Chinese patients with Wilson disease revealed no correlation between the canine copper toxicosis gene MURR1 and Wilson disease. J Mol Med 2006, 84: 438–442.
41. Hung IH, Suzuki M, Yamaguchi Y, Yuan DS, Klausner RD, Gitlin JD. Biochemical characterization of the Wilson disease protein and functional expression in the yeast Saccharomyces cerevisiae. J Biol Chem 1997, 272: 21461–21466.
42. Chen C, Shen B, Xiao JJ, Wu R, Duff Canning SJ, Wang XP. Currently clinical views on genetics of Wilson's disease. Chin Med J (Engl) 2015, 128: 1826–1830.
43. Wu ZY, Wang N, Murong SX. Construction of site-directed mutant variants of ATP7B in vitro and their expression. Chin J Neurol 2003, 36: 339–342 (in Chinese with English abstract).
44. Yang CS, Liang XL, Li JY, Yan ZW, Huang F. Effect of the mutation of promoter region in Wilson disease ATP7B gene on the expression of reporter gene. Chin J Med Genet 2005, 22: 566–568 (in Chinese with English abstract).
45. Diao SP, Hong MF, Huang YQ, Wei ZS, Su QX, Peng ZX, *et al.* Identification and characterization of a novel splice-site mutation in the Wilson disease gene. J Neurol Sci 2014, 345: 154–158.
46. Wan L, Tsai CH, Hsu CM, Huang CC, Yang CC, Liao CC, *et al.* Mutation analysis and characterization of alternative splice variants of the Wilson disease gene ATP7B. Hepatology 2010, 52: 1662–1670.
47. Cheng N, Wang X, Yu X, Zhou Z, Gao M, Rao R, *et al.* Clinical and genetic study of Wilson's disease in affected twins and siblings. Chin J Med Genet 2013, 30: 261–265 (in Chinese with English abstract).
48. Huster D. Wilson disease. Best Pract Res Clin Gastroenterol 2010, 24: 531-539.
49. Koopman WJ, Nijtmans LG, Dieteren CE, Roestenberg P, Valsecchi F, Smeitink JA, *et al.* Mammalian mitochondrial complex I: biogenesis, regulation, and reactive oxygen species generation. Antioxid Redox Signal 2010, 12: 1431–1470.
50. Zischka H, Lichtmannegger J, Schmitt S, Jägemann N, Schulz S, Wartini D, *et al.* Liver mitochondrial membrane crosslinking and destruction in a rat model of Wilson disease. J Clin Invest 2011, 121: 1508–1518.

51. Dong Y, Shi SS, Chen S, Ni W, Zhu M, Wu ZY. The discrepancy between the absence of copper deposition and the presence of neuronal damage in the brain of Atp7b(-/-) mice. Metallomics 2015, 7: 283–288.
52. Zhang JW, Liu JX, Hou HM, Chen DB, Feng L, Wu C, *et al.* Effects of tetrathiomolybdate and penicillamine on brain hydroxyl radical and free copper levels: a microdialysis study in vivo. Biochem Biophys Res Commun 2015, 458: 82–85.
53. Roberts EA, Schilsky ML. A practice guideline on Wilson disease. Hepatology 2003, 37: 1475–1492.
54. Xu SQ, Li XF, Zhu HY, Liu Y, Fang F, Chen L. Clinical efficacy and safety of chelation treatment with typical penicillamine in cross combination with DMPS repeatedly for Wilson's disease. J Huazhong Univ Sci Technolog Med Sci 2013, 33: 743–747.
55. Yang RM, Cheng N. Observation on short-term effect and follow-up of integrative medicine in treating 198 patients with hepatolenticular degeneration. Chin J Integr Trad West Med 2002, 22: 657–659 (in Chinese with English abstract).
56. Hu JY, Yang RM, Han YZ, Hong MF, Wang X, Li K, *et al.* The clinical study of six antidotes against heavy metal poisoning in Wilson' s disease. Anhui Med J 2004, 25: 361–365 (in Chinese with English abstract).
57. Li WJ, Wang JF, Wang XP. Wilson's disease: Update on integrated chinese and western medicine. Chin J Integr Med 2013, 19: 233–240 (in Chinese with English abstract).
58. Tang QQ, Yang RM, Han YZ, Ren MS, Hong MF, Wang XP. Effect of gandou decoction on copper metabolism of skin fibroblast of hepatolenticular degeneration model. Chin J Integr Trad West Med 2000, 20: 37–39 (in Chinese with English abstract).
59. Xue BC, Yang RM, Hu JY, Han YZ, Wang JH. Effect of Gandou Decoction IV combined with short-term decoppering therapy with sodium dimercapto-sulphonate on serum indexes of hepatic fibrosis in patients with Wilson' s disease. Chin J Integr Trad West Med 2007, 27: 785–788 (in Chinese with English abstract).
60. Wang Y, Xie CL, Fu DL, Lu L, Lin Y, Dong QQ, *et al.* Clinical efficacy and safety of Chinese herbal medicine for Wilson's disease: a systematic review of 9 randomized controlled trials. Complement Ther Med 2012, 20: 143–154.
61. Huang F, Yu ZG, Liang XL. Therapeusis for Wilson's disease accomplicated with splenosis-16 cases with splenectomization analysis. Chin J Nervous Mental Dis 2000, 26: 6–8 (in Chinese with English abstract).
62. Cheng Q, He SQ, Gao D, Lei B, Long X, Liang HF, *et al.* Early application of auxiliary partial orthotopic liver transplantation in murine model of Wilson disease. Transplantation 2015, 99: 2317–2324.