Pediatric Neurology 145 (2023) 135–147

Contents lists available at ScienceDirect

Pediatric Neurology

journal homepage: www.elsevier.com/locate/pnu

Research Paper

A Systematic Review and Meta-Analysis of the R778L Mutation in *ATP7B* With Wilson Disease in China

Ziru Xue, MM [a], Hongyu Chen, MM [b], Lan Yu, PhD [b, *], Peifang Jiang, PhD [a, b, *]

[a] *Department of Neurology at The Children's Hospital, Zhejiang University School of Medicine, National Clinical Research Center for Child Health, Hangzhou, Zhejiang, China*
[b] *The Children's Hospital, Zhejiang University School of Medicine, National Clinical Research Center for Child Health, National Regional Medical Center for Children, Hangzhou, Zhejiang, China*

ARTICLE INFO

Article history:
Received 19 July 2022
Accepted 30 April 2023
Available online 8 May 2023

Keywords:
Wilson disease
Genetics
Mutation
R778L

ABSTRACT

Background: Wilson disease (WD) is a hereditary disorder of copper metabolism, caused by mutations in the *ATP7B* gene. There are more than 1000 pathogenic variants identified in *ATP7B*. R778L is the most common *ATP7B* mutation in China.
Methods: To estimate whether R778L is associated with the onset age of WD and other clinical variables. Genotyping results of *ATP7B* gene were collected in our 22 patients with WD. We then conducted a systematic review and meta-analysis in databases, using the keywords Wilson disease and R778L mutation.
Results: After the screening, a total of 23 studies were included, including 3007 patients with WD. Patients with R778L mutation presented at an earlier age (standardized mean difference [SMD] = −0.18 [95% confidence interval, −0.28 to 0.08], $P = 0.0004$) and had lower ceruloplasmin concentration (SMD = −0.21 [95% confidence interval, −0.40 to −0.02], $P = 0.03$) than the patients without the R778L mutation. However, sex (odds ratio [OR] = 1.07 [95% confidence interval, 0.89 to 1.29], $P = 0.32$) and first presentation were not associated with R778L mutation in WD (hepatic: OR = 1.37 [95% confidence interval, 0.87 to 2.16, $P = 0.17$; neurological: OR = 0.79 [95% confidence interval, 0.48 to 1.30, $P = 0.35$; mix: OR = 1.04 [95% confidence interval, 0.42 to 2.53, $P = 0.87$; asymptomatic/others: OR = 1.98 [95% confidence interval, 0.49 to 7.96, $P = 0.34$).
Conclusions: Our results indicated that the R778L mutation is associated with an earlier presentation and lower ceruloplasmin concentration in China.

Introduction

Wilson disease (WD) is an inherited chromosomal recessive disorder of copper metabolism. The recent genetic prevalence studies suggest the incidence of WD is approximately 1:20,000, which is more common than previously estimated.[1,2] Furthermore, it is believed that the prevalence is higher in Asian countries than in Western countries, especially in East Asia, with an estimated prevalence of 29.5 per 100,000.[3-5] The onset of WD may occur at any age, but the majority of patients with WD present between ages five and 35 years.[6] Symptoms of WD vary widely; hepatic and neurological manifestations are the most common symptoms, and ophthalmologic and psychiatric symptoms are also frequently reported. The excess copper leads to impaired copper homeostasis and overloaded copper in the liver, brain, and other organs, resulting in toxic effects.[6] WD can be successfully treated with appropriate pharmacologic agents early after diagnosis but requires lifelong adherence.

It is known that the biallelic mutations in *ATP7B* result in WD. *ATP7B* is mapped to chromosome 13q14.3 in 1993,[7] consisting of 20 introns and 21 exons. This gene encodes copper-transporting ATPase (Cu-ATPase), a transmembrane protein that translocates copper with the energy of ATP hydrolysis. Cu-ATPase has several

Funding: This work was supported by NSFC Grant No. 81501295 (L.Y.) and Grant Nos. 81671287 and 82171438 (P.J.).

Authors' contributions: Z.X., P.J., and L.Y. conceptualized and designed the analyses, drafted the initial manuscript, and reviewed and revised the manuscript; H.C. and Z.X. designed the data collection instruments, collected data, and conducted the initial analyses; L.Y. coordinated and supervised data collection, and critically reviewed the manuscript for important intellectual content. Z.X., P.J., and L.Y. contributed to study concept and design, critical review, and manuscript revision. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

* Communications should be addressed to: Drs. Jiang and Yu; The Children's Hospital; Zhejiang University School of Medicine; National Clinical Research Center for Child Health; 3333 Binsheng Road; Hangzhou 310052, China.
E-mail addresses: yulan20@zju.edu.cn (L. Yu), jiangpeifang@zju.edu.cn (P. Jiang).

https://doi.org/10.1016/j.pediatrneurol.2023.04.026
0887-8994/

functional domains: the copper-binding domain regulates Cu-ATPase activity, the transmembrane domain forms the Cu channel pore, the ATP-binding domain has a distinct nucleotide-coordination environment (consists of two portions: the P-domain and the signature motifs for the P-type ATPases and the N-domain), and the A-domain is essential for the enzymatic function of P-type ATPases.[8] There are more than 1098 pathogenic variants in the *ATP7B* gene identified in patients with WD according to the Human Gene Mutation Database.[9] The H1069Q in exon 14 is the most common *ATP7B* mutation in Central, Eastern, and Northern Europe populations. The R778L in exon 8, the most common mutation in the Chinese population, was found to have an allele frequency of 28.4% to 49.2% in patients with WD.[10] The founder effect was indicated in this prevalent pathogenic variant based on the haplotype studies.[11,12] This mutation results in the changes of arginine to leucine, remarkably decreasing the level of copper-transporting ATPase by enhanced degradation.[13]

Some studies showed a correlation between R778L mutation in the *ATP7B* gene and several clinical indices, including the age of onset, clinical manifestation, and serum ceruloplasmin (CP) concentration,[14-18] but the other studies failed to reproduce the correlation.[19-21] The limited number of patients in each study may decrease the power to analyze the genotype-phenotype correlations, resulting in discrepancies. Therefore, aiming to better decipher the correlation between the mutation and clinical indices, we utilized a meta-analysis, including a total number of 23 studies, to analyze the mutation-outcome correlation.

Materials and Methods

Patients and screening for the R778L mutation

This retrospective study was conducted from April 2004 to September 2020. The study was approved by the ethics committee of the Children's Hospital, Zhejiang University School of Medicine (2022-IRB-169), and written informed consent was received from the patients or patients' legal guardians.

Twenty-two unrelated patients with WD were included for *ATP7B* gene analysis. The sex, age of onset, phenotypes of the first presentation, laboratory confirmation of the clinical diagnosis, the *ATP7B* mutations, and the province data for each patient were recorded when available. Among them, 14 patients had clinical genetic testing with confirmed *ATP7B* variants. Peripheral blood from the other eight patients was obtained. Genomic DNA was extracted, and Sanger sequencing was performed to screen all the exons and splice junctions of the *ATP7B* gene. A total of 29 pairs of primers were designed to amplify the target regions of the *ATP7B* gene (Supplementary Table 1). Pathogenic variants in *ATP7B* were identified using the American College of Medical Genetics and Genomics (ACMG) guidelines.[22] Patients were categorized as hepatic when they showed signs and symptoms of acute or chronic liver disease without any extrapyramidal symptoms (nausea, vomiting, darkening of the color of urine, lightening of the color of stool, jaundice, or ascites). Patients were categorized as neurological when they showed the typical extrapyramidal symptoms (problems with speech, swallowing, physical coordination, stiff muscles, or tremors).

Search strategy

We registered the protocol in the International Prospective Register of Systematic Reviews (PROSPERO CRD42021275791), which is available in full on the NIHR HTA program web site (https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42021275791).[23] The protocol was followed with no change, and the review followed the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines.[24] A protocol-driven systematic search for articles reporting on the association between WD and R778L mutation in *ATP7B* was conducted using PubMed, EMBASE, Cochrane Library, Web of Science, and CNKI databases. Each database was searched from the inception data until October 5, 2021. A comprehensive search strategy was including a combination of database-specific controlled vocabulary terms and free-text terms relating to WD (e.g., 'Wilson* disease,' 'WD,' 'Kinnier-Wilson,' 'Westphal-Strumpell Syndrome*,' 'copper,' 'copper storage,' 'storage disease*,' 'degeneration*,' 'hepatolenticular,' 'hepatocerebral,' 'pseudoscleroses,' 'pseudosclerosis,' 'cerebral,' 'hepatic form,' 'neuropathic,' 'hepatic neurologic,' 'hepato-neurologic,' 'lenticular') and R778L mutation (e.g., 'c.2333G>T,' 'p. Arg778Leu,' 'R778L'). The search was restricted to English and Chinese languages with any study type. The search strategy for each database is described in Supplementary Table 2.

Inclusion criteria and study selection

We identified and selected records retrieved through a search of the electronic databases and indexing services. All articles were uploaded to Endnote X9 for further screening. In the first stage of screening, reviewing titles and abstracts were screened by two independent reviewers (Z.X. and H.C.) for the relevance of both WD and R778L mutation. In the second stage, full texts of the articles were retrieved to be assessed for eligibility. To be eligible, we included the study that provided both the detailed genotyping data and the corresponding clinical data (sex or age or phenotypes, or serum CP concentration). In addition, the study was included describing at least two patients with R778L mutation, as well as at least two non-R778L patients for comparison. Besides, we exclude review papers, meta-analyses, organizational guidelines, editorial letters, and expert opinions to avoid duplication. Conference abstracts to be assessed were also excluded as they had not gone through rigorous peer review. Possible discordance at any stage was resolved by a third reviewer (L.Y.) to reach a consensus.

Clinical variables

The detailed clinical variables of this study were as follows: (1) the age at onset of WD; (2) the sex of the patients; (3) the phenotypes at the first presentation of WD: hepatic, neurological, mixed phenotypes, and other or no phenotypes; and (4) the serum CP value at first diagnosis.

Data extraction

Two authors (Z.X. and H.C.) independently extracted data from the eligible following study characteristics: (1) publication details (study author(s); year published, and the reference type); (2) patients' general details (the patient's country and province or the hospital at diagnosis); and (3) clinical variables. We took age, sex, phenotypes of the first presentation, and serum CP concentration as independent variables. For continuous variables (age and CP value), mean and S.D. were extracted from the study or after calculation. For categorical variables (sex and phenotypes), the number of participants or events was extracted.

Quality assessment

Considering that all the included studies in this meta-analysis were observational, the Newcastle-Ottawa Scale,[25] which was developed to assess the quality of nonrandomized studies, was

used for quality assessment by two reviewers (Z.X. and H.C.), respectively. Any discordance in the evidence of quality during the assessment was discussed with the third reviewer (L.Y.) to reach a consensus. The quality assessment criteria consisted of eight components: (1) adequate definition of cases; (2) representativeness of the cases; (3) selection of controls; (4) definition of controls; (5) comparability of cohorts based on the design or analysis; (6) ascertainment of exposure; (7) same method of ascertainment for cases and control; and (8) nonresponse rate. The total quality assessment score of each study was 9, which was divided into three levels: good quality (scoring 7 to 9), medium quality (scoring 4 to 6), and poor quality (scoring 0 to 3). Studies with medium and good quality were included for further analysis.

Statistical analysis

All analyses for the R778L mutation reported in WD were performed using the meta package in R version 5.0-1. The R package contains the algorithm, and the results reported in the analysis could be found on GitHub (https://github.com/xueziru0/Meta-analysis_-Wilson-disease.git). To perform a meta-analysis, we have to find a suitable effect size that can be summarized across all studies and as a metric quantifying the relationship between two entities. Continuous variables (age and CP value) were assessed based on the standardized mean difference (SMD) with 95% confidence intervals (CIs), whereas categorical variables (sex and phenotype) were analyzed using odds ratio (OR) with 95% CIs. SMD was used to express the size of the intervention effect in the study, often interpreted using the convention by Cohen.[26] SMD $\approx$0.20 suggested a small effect, SMD $\approx$0.50 suggested a moderate effect, and SMD $\approx$0.80 suggested a large effect. An effect size could be small according to the criteria by Cohen, but it could still be extremely important. In categorical data, ORs were the quantification of odds to compare the relative odds of mutation. OR $<$ 1 suggested lower odds between the variable and mutation. OR $=$ 1 suggested no association between the variable and mutation. OR $>$ 1 suggested higher odds between the variable and mutation.

By the Cochrane Handbook for Systematic Reviews of Interventions,[27] potential heterogeneity was assessed by visualization of the forest plot and two statistical methods (Q-test and Higgins I^2 statistic).[28,29] Q-test was commonly used to quantify between-study heterogeneity; however, it heavily depended on the type of data. Therefore, another way to quantify between-study heterogeneity, the I^2 statistic, which is directly based on Q-test, was applied. Finally, data were pooled and analyzed using common-effects models if I^2 $<$ 50%, whereas random-effects models have otherwise been employed.

Additional analysis

When heterogeneity was observed, factors leading to heterogeneity would be discussed. The heterogeneity would be further evaluated via three additional analyses.

Subgroup analysis

When data were available, we would perform subgroup analysis to investigate whether the pooled effect size was associated with the following factors.

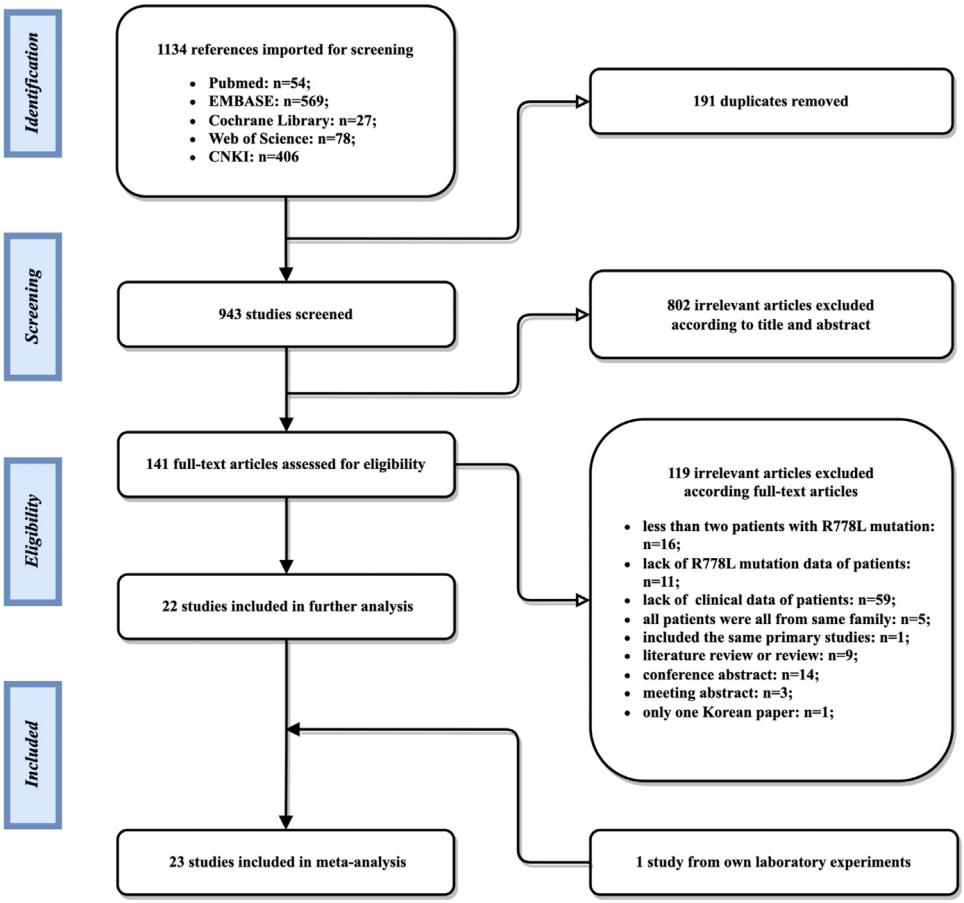

FIGURE 1. Flow chart depicting the article selection process. The color version of this figure is available in the online edition.

TABLE 1
Characteristics of the Included Studies About Non-R778L Mutation

Reference	Reference Type	Province/Hospital	Participants			Onset Age (years)			Symptom Onset					Serum CP (mg/L)		
			♂	♀	*n*	Mean	S.D.	*n*	A/O	H	N	H + N	*n*	Mean	S.D.	*n*
Sheng Ye, 2003[34]	Thesis	Zhejiang Province	NA	NA	NA	9.33	1.87	15	NA	NA	NA	NA	NA	NA	NA	NA
Yan-Hong Gu et al., 2005[35]	Journal article	Shandong, Jiangsu, Anhui, Liaoning, Hebei, and Sichuan provinces	NA	NA	NA	10.56	3.08	16	0	7	9	0	16	NA	NA	NA
Ai-Hong Qi, 2006[36]	Thesis	Hubei and Henan provinces	10	10	20	14.7	8.9	20	1	8	11	0	20	94.15	70.97	20
Jing-Fan Yang et al., 2006[57]	Journal article	The First Affiliated Hospital of Sun Yat-sen University	3	0	3	7.5	2.5	3	1	0	1	1	3	NA	NA	NA
Zhong-Sheng Zhang et al., 2008[38]	Journal article	Guizhou province	9	3	12	22.83	3.2	12	NA	3	6	NA	12	50.4	4.5	12
Ming-Ran Sun, 2010[39]	Thesis	The First Hospital of Jilin University	3	1	4	29.25	10.28	4	0	2	0	2	4	NA	NA	NA
Nan Cheng, 2010[40]	Thesis	The Hospital of Anhui University of Chinese Medicine	48	25	73	14.7	6.54	73	NA	NA	36	NA	73	54.46	25.15	73
Ye-Qing Huang, 2011[41]	Thesis	The First Affiliated Hospital/ School of Clinical Medicine of Guangdong Pharmaceutical University	28	15	43	16.84	9.56	43	NA	25	NA	NA	43	67	24	43
Ying-Qian Li, 2011[42]	Thesis	The First Affiliated Hospital of Medicine of Shanxi University	2	5	7	NA	NA	NA	NA	1	5	NA	7	NA	NA	NA
Shao-Juan Gu et al., 2013[43]	Journal article	The Third Xiangya Hospital of Central South University	3	9	12	20	11.19	12	0	5	7	0	12	75.2	31.08	12
Ming-Ming LI, 2013[44]	Thesis	The Second Xiangya Hospital of Central South University	36	21	57	18.57	13.4	57	NA	NA	NA	NA	NA	88.80	51.13	57
Li-Ming Jiang, 2013[45]	Journal article	Shanghai Children's Medical Center, Shanghai Jiaotong University School of Medicine	3	1	4	8	3.74	4	0	4	0	0	4	132.50	39.61	4
Juan Geng et al., 2013[46]	Journal article	Shanghai Children's Medical Center	3	1	4	7.5	4.5	4	2	1	1	0	4	135	82.00	4
Yu Liu et al., 2015[47]	Journal article	Third Military Medical University	0	6	6	28.50	13.06	6	0	6	0	0	6	NA	NA	NA
Hai-Yun Zhang, 2014[48]	Thesis	Anhui University of Chinese Medicine	355	255	610	NA	NA	NA	NA	NA	276	NA	580	NA	NA	NA
Dong-Feng Zhang, 2015[49]	Thesis	The First Affiliated Hospital of Zhengzhou University	16	12	28	21.14	8.93	28	NA	15	NA	NA	28	120.11	20.05	28
Rui Hua et al. , 2016[50]	Journal article	The First Hospital of Jilin University	18	11	29	21	12.3	29	1	14	4	10	29	75.36	68.16	28
Shan-Shan Peng, 2016[51]	Thesis	The First Affiliated Hospital of Jilin University, the Second Xiangya Hospital of Central South University	22	13	35	24	NA	35	NA	NA	NA	NA	NA	60	NA	35
Ying-Ying Yu, 2017[52]	Thesis	Shandong province	NA	NA	NA	NA	NA	NA	1	3	2	0	6	NA	NA	NA
Hao Yu, 2017[53]	Thesis	The Second Affiliated Hospital Zhejiang University School of Medicine	NA	NA	NA	16	11.3	51	NA	NA	NA	NA	NA	32.3	23.1	50
Nan Cheng et al., 2017[54]	Journal article	Anhui University of Chinese Medicine	NA	NA	NA	14.75	7.69	583	NA	NA	NA	NA	NA	71.9	51.0	583
Xiao Heng et al.9, 201[55]	Journal article	The Third Xiangya Hospital of Central South University (from May 2011 to November 2017)	4	1	5	23	9.06	5	0	1	0	4	5	72.06	58.16	5
Own experiment	/	The Children's Hospital of Zhejiang University School of Medicine	7	4	11	15	13.76	11	0	8	3	0	11	NA	NA	NA

Abbreviations:
A/O = Asymptomatic or other symptom
H = Hepatic
N = Neurological
NA = Not available

(1) Whether studies included the gray literature or not
(2) Whether studies were subject to any restriction by the participant's country or province in the study or not

We pooled the effect in each subgroup and overall subgroup by assuming a random-effects model and tested the heterogeneity by forest plot, Q-test, and I^2 statistic. Subsequently, a Q-test was conducted to assess if there is a difference in the effect sizes between subgroups.

Meta-regression

When including data from more than 10 studies, we conducted univariate random-effects meta-regression with predefined predictors (year of publication). Meta-regression analysis was performed to address heterogeneity by checking for a possible association of predefined factors (year of publication) with effect size differences. Bubble plots were generated to visualize the results of the meta-regression analysis. The slope of the regression line represented the rate of change in effect sizes as a predefined predictor. In addition, the R^2 index has presented the percentage of heterogeneity variation, and the test of the predefined predictor (year of publication) showed the influence of the pooled effect size.

Sensitivity analysis

A sensitivity analysis was conducted by excluding studies from the meta-analysis one by one to examine the robustness of the conclusions. Then the remaining data were pooled and analyzed using the random effect model. Forest plots, Q-test, and the I^2 statistics were calculated to check for heterogeneity.

Publication bias

Publication bias among the included studies was evaluated using a funnel plot between the effect size and its standard error. A funnel plot with asymmetrical distribution indicated publication bias. We used the contour-enhanced funnel plot to find the possible causes of funnel plot asymmetry among the included studies, with contour lines corresponding to different levels of statistical significance ($P = 0.01.\ 0.05,\ 0.1$). When further analysis showed that the studies appear to be missing in areas of statistical low statistical significance it indicated that the funnel plot asymmetry is due to publication bias. Conversely, the studies were perceived to be missing are areas of high statistical significance ($P > 0.05$), indicating the cause of the funnel plot asymmetry may be due to other factors, such as poor methodologic quality or true heterogeneity.[30]

In addition, we applied Egger statistical test for continuous data and the Harbord method for dichotomous data to assess the possibility of publication bias and represented with a regression line.[31-33] When no publication bias was present, the points would scatter around a regression line that ran through the origin. We considered there to be publication bias if the intercept of the regression line deviated from 0 with a *P* value of less than 0.10.

Results

Results of R778L mutation in 22 patients

A total of 22 patients in unrelated families were included with their relevant clinical data (Supplementary Table 3). Eighteen patients (81.8%) presented with symptoms of liver disease or relevant laboratory results. Four patients (18.2%) presented with neuropsychiatric symptoms. In total, 11 patients (50%) had R778L mutation (three patients were homozygous and eight were heterozygous for the R778L mutation), whereas 11 (50%) had other mutations at both alleles. Thus, the R778L mutation was present in 31.8% of the alleles in patients with WD.

Selection process of included studies

The initial search retrieved 54 publications from PubMed, 569 from EMBASE, 27 from Cochrane Library, 78 from Web of Science, and 406 from CNKI (Supplementary Table 2). After removing duplicates, 943 papers remained for the first round of screening. After reviewing the titles and abstracts of all the papers, 802 irrelevant publications were excluded, and 141 articles were then assessed for eligibility. After the first stage, 119 articles were excluded including: (1) articles with less than two patients with R778L mutation (n = 16); (2) articles that lacked the specific R778L mutation data in patients (n = 11) or clinical data such as the age, sex, CP value, or phenotypic manifestations at presentation (n = 59); (3) a conference, meeting abstract, or literature review or review (n = 26); (4) articles with all patients from the same family in the study (n = 5); (5) articles that repeated the study with the same patients (n = 1); and (6) only one Korean paper; the remaining 22 papers were all from China (n = 1). Therefore, 22 articles met all the inclusion criteria for further analysis. Data derived from our clinical patients were also included in this meta-analysis. Total of 23 studies were used for the final analysis (Fig 1).

Characteristics of included studies

Tables 1 and 2 provided a detailed description of the key characteristics for the included 23 studies.

Briefly, the 22 studies were all from China. For further classification, due to the unequal geographic distribution of the Chinese population and participating hospitals in which the patients were diagnosed, we used conventional geographic classifications to divide the Chinese mainland into northern and southern parts by Qinling mountain and the Huaihe river (Fig 2).

We thus got six studies from northern China (i.e., Hebei, Henan, Jilin, Liaoning, Shandong, Shanxi provinces), 14 studies from southern China (i.e., Anhui, Chongqing, Guangdong, Guizhou, Jiangsu, Hubei, Hunan, Shanghai, Sichuan, and Zhejiang provinces), and three studies from both areas. Regarding the reference type, we noticed that 10 studies were journal articles, 12 were thesis, and one study was a personal file. The latter two types belonged to gray literature, which was defined by the Cochrane Handbook for Systematic Reviews of Interventions as the literature that was not formally published in sources such as thesis and ongoing reports.[56]

Quality of included studies

The methodologic quality of the included articles was assessed according to Newcastle-Ottawa Scale, and the scores ranged from 7 to 9, indicating that the quality of selected studies was high (Table 3).

Meta-analysis results

Of the 23 studies in this systematic review, since the clinical variables included the age of onset, sex, the phenotype at first presentation of WD, and CP concentration, we analyzed the variables separately. The following paragraphs discuss the association between the R778L mutation in *ATP7B* and the above-mentioned variables.

Correlation between R778L mutation and the age of onset in WD

There were 19 studies (1674 patients) included for the analysis of R778L mutation and the age of onset. The common-effect model was applied since there was no significant heterogeneity ($I^2 = 0\%$; $P = 0.80$). Age was significantly less in patients with R778L mutation in WD when compared with those without R778L (SMD = -0.18; 95% CI: −0.28 to -0.08, $P = 0.0004$) (Fig 3).

Visual assessments of the funnel plots (Supplementary Figure 1A) and the Egger test (Supplementary Figure 1B) showed no evidence of publication bias for results ($P = 0.75$).

Correlation between R778L mutation and sex in WD

Among the 19 studies (1888 patients) with information on sex, there was no significant difference between R778L mutation and

TABLE 2
Characteristics of the Included Studies About R778L Mutation

Reference	Reference Type	Province/Hospital	Participants			Onset Age (years)			Symptom Onset					Serum CP (mg/L)		
			♂	♀	*n*	Mean	S.D.	*n*	A/O	H	N	H + N	*n*	Mean	S.D.	*n*
Sheng Ye, 2003[34]	Thesis	Zhejiang province	NA	NA	NA	8.27	2.28	15	NA	NA	NA	NA	NA	NA	NA	NA
Yan-Hong Gu et al., 2005[35]	Journal article	Shandong, Jiangsu, Anhui, Liaoning, Hebei, and Sichuan provinces	NA	NA	NA	8.97	2.76	17	1	9	7	0	17	NA	NA	NA
Ai-Hong Qi, 2006[36]	Thesis	Hubei and Henan provinces	7	4	11	15	4.2	11	0	10	1	0	11	53.83	36.54	11
Jing-Fan Yang et al., 2006[57]	Journal article	The First Affiliated Hospital of Sun Yat-sen University	2	1	3	17.33	4.03	3	0	0	2	1	3	NA	NA	NA
Zhong-Sheng Zhang et al., 2008[38]	Journal article	Guizhou province	5	1	6	22	4.44	6	0	4	2	0	6	58.6	10.7	6
Ming-Ran Sun, 2010[39]	Thesis	The First Hospital of Jilin University	6	4	10	18.8	9.23	10	0	5	0	5	10	NA	NA	NA
Nan Cheng, 2010[40]	Thesis	The Hospital of Anhui University of Chinese Medicine	37	32	69	14.17	6.3	69	0	NA	42	NA	69	47.81	25.29	69
Ye-Qing Huang, 2011[41]	Thesis	The First Affiliated Hospital/School of Clinical Medicine of Guangdong Pharmaceutical University	15	17	32	13.19	7.72	32	NA	17	NA	NA	32	67.3	20.6	32
Ying-Qian Li, 2011[42]	Thesis	The First Affiliated Hospital of Medicine of Shanxi University	2	3	5	NA	NA	NA	0	3	2	0	5	NA	NA	NA
Shao-Juan Gu et al., 2013[43]	Journal article	The Third Xiangya Hospital of Central South University	2	2	4	17	4.44	4	0	2	2	0	4	41.4	15.59	4
Ming-Ming LI, 2013[44]	Journal article	The Second Xiangya Hospital of Central South University	19	12	31	17.45	12.13	31	NA	NA	NA	NA	NA	121.27	167.20	31
Li-Ming Jiang, 2013[45]	Journal article	Shanghai Children's Medical Center, Shanghai Jiao Tong University School of Medicine	2	7	9	7.33	3.3	9	0	9	0	0	9	58.38	50.53	8
Juan Geng et al., 2013[46]	Journal article	Shanghai Children's Medical Center	4	3	7	7.29	3.24	7	6	0	1	0	7	105.71	59.00	7
Yu Liu et al., 2015[47]	Journal article	Third Military Medical University	3	1	4	22.5	5.68	4	0	2	2	0	4	NA	NA	NA
Hai-Yun Zhang, 2014[48]	Thesis	Anhui University of Chinese Medicine	401	235	636	NA	NA	NA	NA	NA	333	NA	636	NA	NA	NA
Dong-Feng Zhang, 2015[49]	Thesis	The First Affiliated Hospital of Zhengzhou University	14	10	24	22.83	12.85	24	NA	13	NA	NA	24	120.11	20.05	24
Rui Hua et al. ,2016[50]	Journal article	The First Hospital of Jilin University	19	12	31	21.23	10.65	31	3	13	3	12	31	48.06	38.89	31
Shan-Shan Peng, 2016[51]	Thesis	The First Affiliated Hospital of Jilin University, the Second Xiangya Hospital of Central South University	18	12	30	22.5	NA	30	NA	NA	NA	NA	NA	34	NA	30
Yingying Yu, 2017[52]	Thesis	Shandong province	NA	NA	NA	NA	NA	NA	1	2	1	0	4	NA	NA	NA
Hao Yu, 2017[53]	Thesis	The Second Affiliated Hospital Zhejiang University School of Medicine	NA	NA	NA	13.98	9.24	72	NA	NA	NA	NA	NA	28.48	14.93	69
Nan Cheng et al., 2017[54]	Journal article	Anhui University of Chinese Medicine	NA	NA	NA	13.46	6.4	340	NA	NA	NA	NA	NA	54.78	31.71	340
Xiao Heng et al, 2019[55]	Journal article	The Third Xiangya Hospital of Central South University (from May 2011 to November 2017)	2	0	2	21	11.5	2	0	1	0	1	2	72.06	58.16	2
Own experiment	/	The Children's Hospital of Zhejiang University School of Medicine	6	5	11	6.7	1.49	11	0	10	1	0	11	NA	NA	NA

Abbreviations:
A/O = Asymptomatic or other symptom
H = Hepatic
N = Neurological
NA = Not available

FIGURE 2. The geographic distribution of the included studies. Qinling mountain-Huaihe river (red line) marked the boundary between north and south of China. The color version of this figure is available in the online edition.

non-R778L mutation in patients with WD (ORs = 1.07, 95% CI: 0.89 to 1.29, $P = 0.32$). In addition, heterogeneity was not found among these studies ($I^2 = 14.3\%$, $P = 0.28$) (Fig 4).

The funnel plot of OR did not show obvious asymmetry (Supplementary Figure 2A), and there was no significant publication bias according to Harbord test ($P = 0.45$, Supplementary Figure 2B).

Correlation between R778L mutation and phenotype at the first presentation in WD

When analyzing all the included studies, the hepatic (15 studies, 384 patients; OR = 1.37, 95% CI: 0.87 to 2.16, $P = 0.17$), the neurological (12 studies, 371 patients; OR = 0.79, 95% CI: 0.48 to 1.30, $P = 0.35$), the mix (four studies, 87 patients; OR = 1.04, 95% CI: 0.42 to 2.53, $P = 0.93$), and the asymptomatic or others (six studies, 151 patients; OR = 1.98, 95% CI: 0.49 to 7.96, $P = 0.34$) phenotypes were not associated with R778L mutation in WD (Fig 5A-D). There was no heterogeneity in all phenotypes ($I^2_{hepatic} = 18\%$, $P_{hepatic} = 0.26$; $I^2_{neurological} = 19\%$, $P_{neurological} = 0.26$; $I^2_{mix} = 0\%$, $P_{mix} = 0.87$; $I^2_{asymptomatic/others} = 0\%$, $P_{asymptomatic/others} = 0.75$). Furthermore, we found no publication bias for the results of all phenotypes (Harbord test, $P_{hepatic} = 0.79$, $P_{neurological} = 0.39$, $P_{mix} = 0.25$, $P_{asymptomatic/others} = 0.26$) (Supplementary Figure 3A-H).

Correlation between R778L mutation and CP concentration in WD

In the 13 eligible studies (1553 patients), there was a significant association between the R778L mutation and the lower level of CP concentration (SMD = −0.21, 95% CI: −0.40 to −0.02, $P = 0.03$), yet heterogeneity was found in these studies ($I^2 = 51\%$, $P = 0.02$) (Fig 6A).

Subgroup analysis. The difference between the subgroups regarding the region was not statistically significant ($P_{region} = 0.50$) (Supplementary Figure 4A), whereas the difference between the subgroups regarding reference type was significant ($P_{reference\ type} = 0.02$) (Fig 6B). When the reference type was limited to nongray literature, the result of the association was in conformity with the finding in our meta-analysis (SMD = −0.38, 95% CI: -0.51 to −0.25, $P < 0.0001$). When the reference type was limited to gray literature, the result of the association disappeared (SMD = −0.10, 95% CI -0.31 to 0.10, $P = 0.32$) (Fig 6B).

Meta-regression. In a visual inspection of the bubble plot for the year of publication, we found that the slope of estimated regression was close to flat (Supplementary Figure 4B) and none of the true heterogeneity was explained by the year of publication ($R^2 = 0$).

Sensitivity analysis. Sensitivity analysis revealed that the statistically significant result was lost when excluding seven studies sequentially (Fig 6C) (Supplementary Table 4).

A funnel plot revealed asymmetry, indicating the pooled results might be influenced by the publication bias (Supplementary Figure 4C). However, the Egger linear regression test showed that

TABLE 3
Quality Assessment for All Included Studies Using the Newcastle-Ottawa Scale

Study	Selection				Comparability	Exposure			Score
	Adequate Definition of Cases	Representativeness of the Cases	Selection of Controls	Definition of Controls	Comparability of Cohorts on the Basis of the Design or Analysis	Ascertainment of Exposure	Same Method of Ascertainment for Cases and Controls	Nonresponse Rate	
Sheng Ye, 2003[34]	*	*	*	*	*	*	*	*	8
Yan-Hong Gu et al., 2005[35]	*	*	*	/	*	*	*	*	7
Ai-Hong Qi, 2006[36]	*	*	*	*	*	*	*	*	8
Jing-Fan Yang et al., 2006[37]	*	*	*	*	*	*	*	*	8
Zhong-Sheng Zhang et al., 2008[38]	*	*	*	*	*	*	*	*	8
Ming-Ran Sun, 2010[39]	*	*	*	*	**	*	*	*	9
Nan Cheng, 2010[40]	*	*	*	*	**	*	*	*	9
Ye-Qing Huang, 2011[41]	*	*	*	*	**	*	*	*	9
Ying-Qian Li, 2011[42]	*	*	*	*	**	*	*	*	9
Shao-Juan Gu et al., 2013[43]	*	*	*	*	*	*	*	*	8
Ming-Ming LI, 2013[44]	*	*	*	*	**	*	*	*	9
Li-Ming Jiang, 2013[45]	*	*	*	*	*	*	*	*	8
Juan Geng et al., 2013[46]	*	*	*	*	**	*	*	*	9
Yu Liu et al., 2015[47]	*	*	*	*	**	*	*	*	9
Hai-Yun Zhang, 2014[48]	*	*	*	*	**	*	*	*	9
Dong-Feng Zhang, 2015[49]	*	*	*	*	**	*	*	*	9
Rui Hua et al., 2016[50]	*	*	*	*	*	*	*	*	8
Shan-Shan Peng, 2016[51]	*	*	*	*	*	*	*	*	8
Ying-Ying Yu, 2017[52]	*	*	*	*	*	*	*	*	8
Hao Yu, 2017[53]	*	*	*	*	*	*	*	*	8
Nan Cheng et al., 2017[54]	*	*	*	*	**	*	*	*	9
Xiao Heng et al, 2019[55]	*	/	*	*	*	*	*	*	7
Our experiment	*	/	*	*	*	*	*	/	7

/, Zero score; *, one point; **, two points.

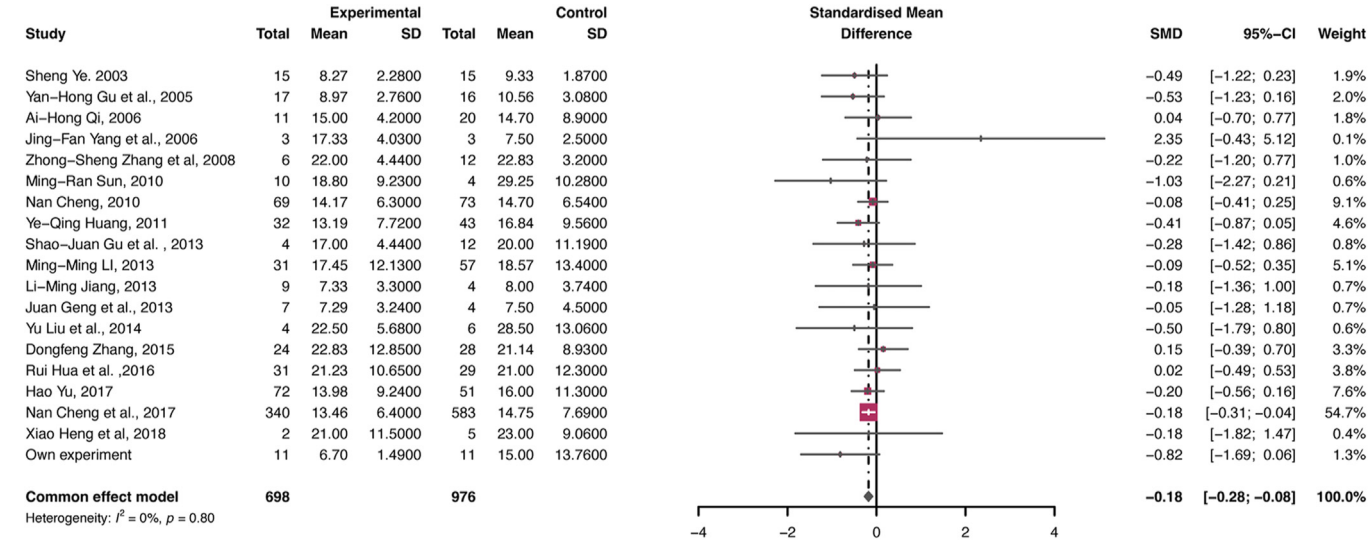

FIGURE 3. Forest plot showing the association between R778L mutation and the age of onset in patients with WD. SMD of included studies indicated age was less in patients with R778L mutation in WD based on the common-effects model. CI, confidence interval; SMD, standardized mean difference; WD, Wilson disease. The color version of this figure is available in the online edition.

there was no potential publication bias ($P = 0.51$) (Supplementary Figure 4D).

Discussion

In the current study, patients with WD in China with R778L mutation were associated with early onset of WD and a lower level of CP than the patients with other mutations at both alleles, suggesting this mutation may play more severe effects on the function of *ATP7B*.[57] The results were partly consistent with the findings that patients with homozygous R778L mutation were younger and with a lower level of CP.[58] However, we did not separate the homozygous and compound heterozygous mutations of R778L in this meta-analysis because of the unavailable information. Interestingly, another mutation of H1069Q that was frequently reported in European patients was suggested to be associated with late presentation and neurological presentation.[59] H1069Q is located in the N-domain; it disrupts the catalytic activity of the P-type ATPase domain and allows excretion of some copper, resulting in the slow accumulation of toxic metal, and leading to a later age at presentation. Although the R778L is located in the TMD4 domain, its mutation would disrupt the subcellular localization and the transportation of *ATP7B* proteins. Recent work suggested that R778L exhibited a higher vulnerability against excessive copper supplementation, resulting in more sensitive cytotoxicity against copper.[60,61] These data suggested that the R778L mutation may result in the copper overloaded and a quick buildup of this potentially toxic metal, consequently leading to earlier age at presentation. Moreover, the mutation could also inhibit the synthesis of CP,4[62] resulting in a lower level of CP. Notably, heterogeneity was

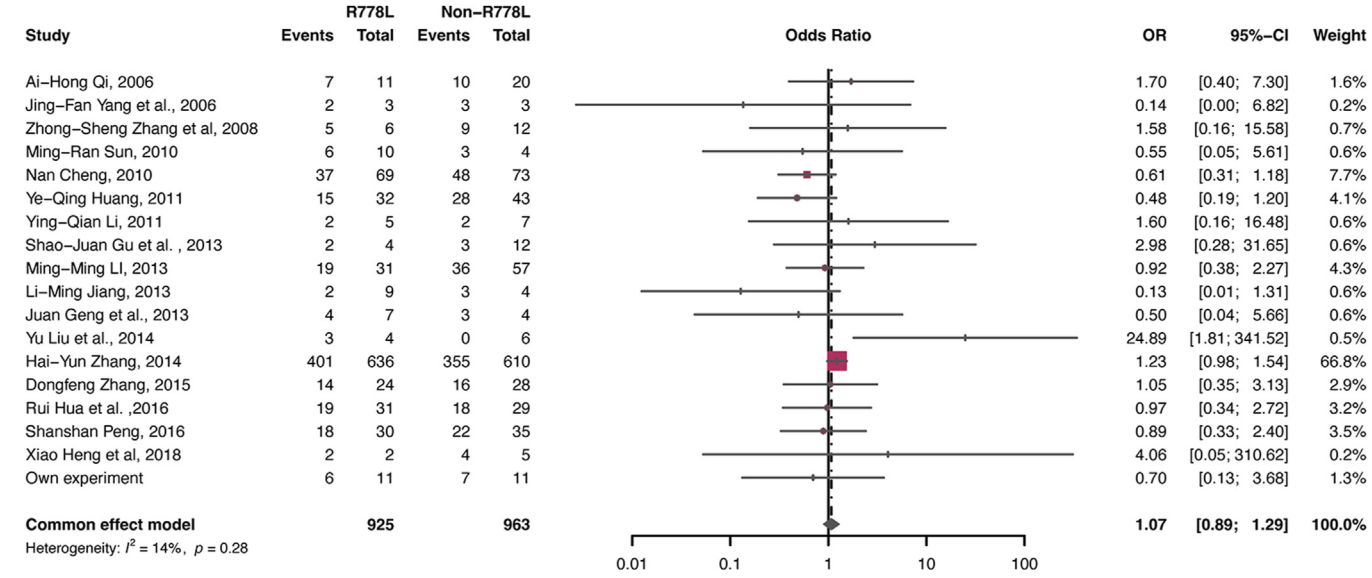

FIGURE 4. Forest plot showing the association between R778L mutation and sex in patients with WD. The OR of included studies indicated there was no association between sex and R778L mutation in WD based on the common-effects model. CI, confidence interval; OR, odds ratio; WD, Wilson disease. The color version of this figure is available in the online edition.

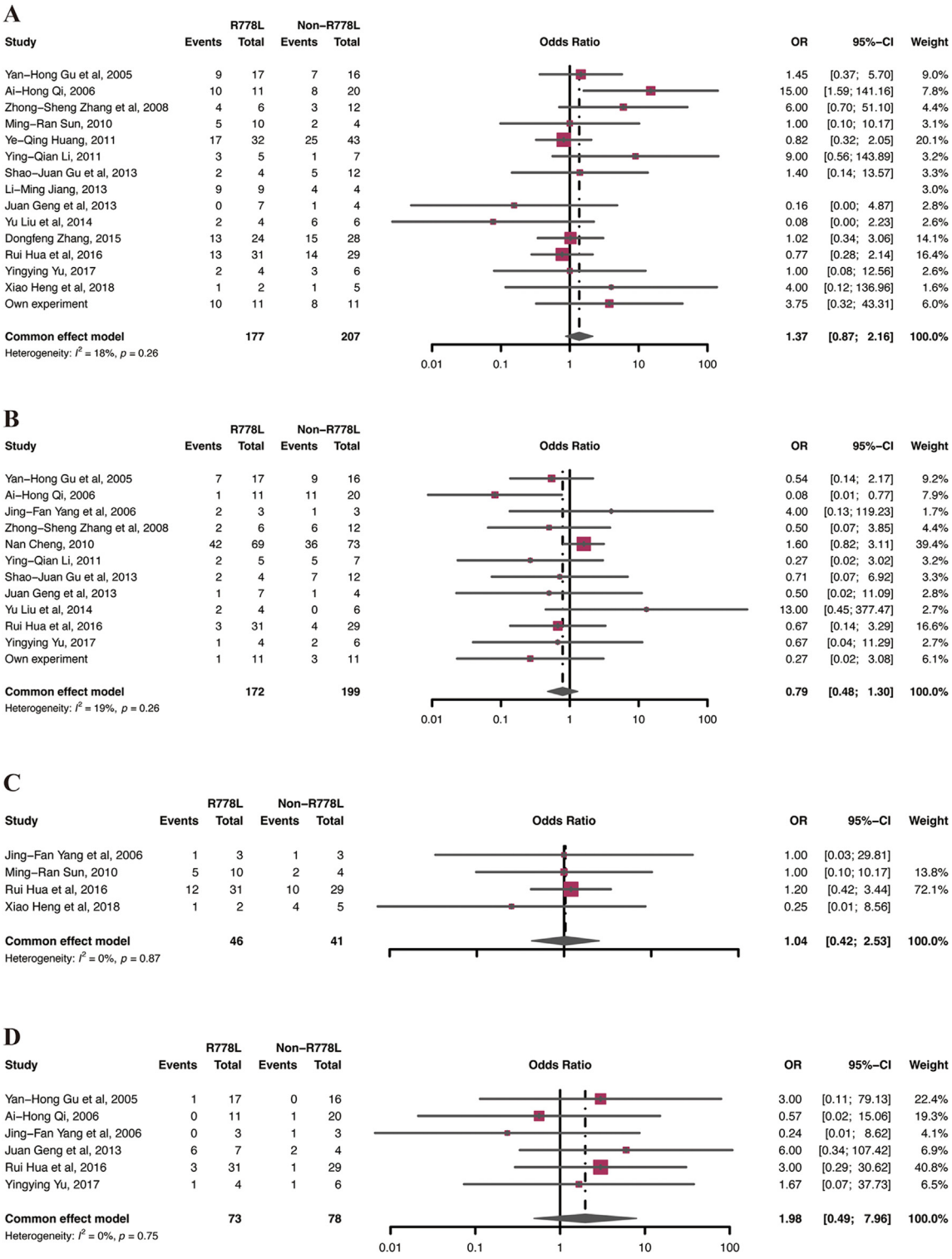

FIGURE 5. Forest plot showing the association between R778L mutation and first presentation in patients with Wilson disease (WD). (A) Forest plot showing the association between R778L mutation and the hepatic presentation in patients with WD. The odds ratio (OR) of included studies indicated there was no association between hepatic presentation and R778L mutation in WD based on the common-effects model. (B) Forest plot showing the association between R778L mutation and the neurological presentation in patients with WD. The OR of included studies indicated there was no association between neurological presentation and R778L mutation in WD based on the common-effects model. CI, confidence interval. (C) Forest plot showing the association between R778L mutation and the mix presentation in patients with WD. The OR of included studies indicated there was no association between mix presentation and R778L mutation in WD based on the common-effects model. (D) Forest plot showing the association between R778L mutation and the asymptomatic/others presentation in patients with WD. The OR of included studies indicated there was no association between asymptomatic/others presentation and R778L mutation in WD based on the common-effects model. The color version of this figure is available in the online edition.

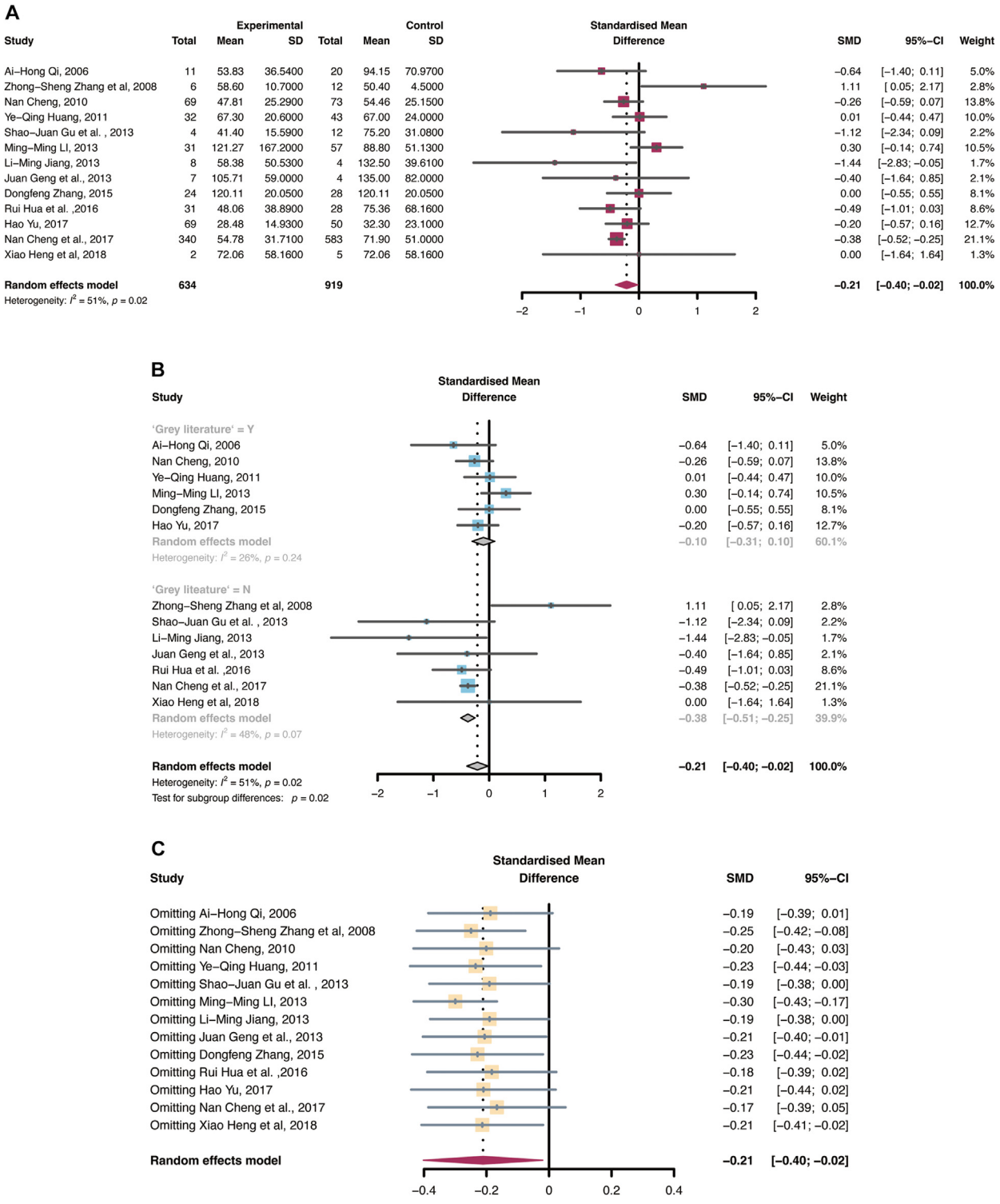

FIGURE 6. The meta-analysis and further analyses showing the association between R778L mutation and ceruloplasmin (CP) concentration in patients with Wilson disease (WD). (A) Forest plot showing the association between R778L mutation and CP concentration in patients with WD. Standardized mean difference (SMD) of included studies indicated the level of CP was lower in patients with R778L mutation in WD based on the random-effects model. CI, confidence interval. (B) Forest plot showing the association between R778L mutation and CP concentration based on the reference type. The difference between the subgroups regarding the reference type was statistically significant ($P = 0.02$). (C) Funnel plot showing the sensitivity analysis of the association between R778L mutation and CP concentration. The meta-analysis result was lost after omitting seven studies sequentially. The color version of this figure is available in the online edition.

found among the included studies. The association of R778L and lower CP only existed in the published journal articles but not in the gray literature group. It might be due to the gray literature providing the data not indicated in the published literature, resulting in the provision of the evidence of negative results in this association. Sensitivity analysis suggested that the R778L mutation of lower CP was less conclusive. More studies were required to confirm the association of R778L and lower CP.

We did not find a significant correlation between R778L mutation and first presentation or sex. However, in Wu's study, R778L homozygotes were associated with hepatic presentation. We could not individually investigate the homozygotes of R778L in this meta-analysis, which might bias the results. However, a recent study in a large cohort of patients with WD[63] did not show any significant association of sex or presentation with R778L in both genotypes. Furthermore, Ferenci's study suggested that the presentation is associated with sex rather than with mutation itself4[64]; it implicated the robustness of our meta-analysis.

In conclusion, our meta-analysis supported that patients with WD in China with R778L had a lower level of CP concentration and were younger at the time of onset, which might be valuable in the diagnosis or treatment of WD in clinical practice.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declaration of Competing Interest

All authors disclosed no relevant relationships.

Acknowledgments

We sincerely appreciate the generous contribution of the patient.

Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.pediatrneurol.2023.04.026.

References

1. Sandahl T, Laursen T, Munk E, Vilstrup H, Weiss K, Ott P. The prevalence of Wilson disease. An update. Hepatology. 2019;71:722–732.
2. Wallace DF, Dooley JS. *ATP7B* variant penetrance explains differences between genetic and clinical prevalence estimates for Wilson disease. Hum Genet. 2020;139:1065–1075.
3. Xie J-J, Wu Z-Y. Wilson's disease in China. Neurosci Bull. 2017;33:323–330.
4. Gao J, Brackley SM, Mann JP. The global prevalence of Wilson disease from next-generation sequencing data. Genet Med. 2019;21:1155–1163.
5. Ferenci P, Członkowska A, Stremmel W, et al. EASL clinical practice guidelines: Wilson's disease. J Hepatol. 2012;56 3:671–685.
6. Członkowska A, Litwin T, Dusek P, et al. Wilson disease. Nat Rev Dis Primers. 2018;4:21.
7. Bull PC, Thomas GR, Rommens JM, Forbes JR, Cox DW. The Wilson disease gene is a putative copper transporting P–type ATPase similar to the Menkes gene. Nat Genet. 1993;5:327–337.
8. Lutsenko S, Barnes NL, Bartee MY, Dmitriev OY. Function and regulation of human copper-transporting ATPases. Physiol Rev. 2007;87:1011–1046.
9. Stenson PD, Mort M, Ball E v, et al. The Human Gene Mutation Database (HGMD®): optimizing its use in a clinical diagnostic or research setting. Hum Genet. 2020;139:1197–1207.
10. Ferenci P. Regional distribution of mutations of the *ATP7B* gene in patients with Wilson disease: impact on genetic testing. Hum Genet. 2006;120:151–159.
11. Yang G-M, Wang R-M, Xia N, Zheng Z, Dong Y, Wu Z-Y. Prevalent pathogenic variants of *ATP7B* in Chinese patients with Wilson's disease: geographical distribution and founder effect. Genes (Basel). 2021;12:336.
12. Bae SH, Kim JW, Seo JK. Haplotype analysis and possible founder effect at the R778L mutation of the *ATP7B* gene in Korean patients with Wilson's disease. Korean J Hepatol. 2009;15:309–319.
13. Chang IJ, Hahn SH. The genetics of Wilson disease. Handb Clin Neurol. 2017;142:19–34.
14. Li K, Zhang W, Lin S, et al. Mutational analysis of *ATP7B* in north Chinese patients with Wilson disease. J Hum Genet. 2013;58:67–72.
15. Jia S, Zhou D, Ou X, Huang J. Progress in molecular mechanism of hepatolenticular degeneration induced by *ATP7B* gene mutation. Zhonghua Gan Zang Bing Za Zhi. 2020;28:188–192.
16. Zhou X, Yin H, Wang C, Liu Z, Zheng B, Jin Y. Phenotype and genotype analysis of 55 children patients with Wilson's disease. Zhonghua Gan Zang Bing Za Zhi. 2020;28:603–607.
17. Liu X-Q, Zhang Y-F, Liu T-T, et al. Correlation of *ATP7B* genotype with phenotype in Chinese patients with Wilson disease. World J Gastroenterol. 2004;10:590–593.
18. Li M, Ma J, Wang W, Yang X, Luo K. Mutation analysis of the *ATP7B* gene and genotype–phenotype correlation in Chinese patients with Wilson disease. BMC Gastroenterol. 2021;21:339.
19. Gu Y-H, Kodama H, Du S-L. Apolipoprotein E genotype analysis in Chinese Han ethnic children with Wilson's disease, with a concentration on those homozygous for R778L. Brain Dev. 2005;27:551–553.
20. Kalita J, Somarajan BI, Misra UK, Mittal B. R778L, H1069Q, and I1102T mutation study in neurologic Wilson disease. Neurol India. 2010;58:627–630.
21. Li X, Lu Z, Lin Y, et al. Clinical features and mutational analysis in 114 young children with Wilson disease from South China. Am J Med Genet. 2019;179:1451–1458.
22. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17:405–423.
23. Xue Z-R, Yu L, Jiang P-F, Chen H-Y. A systematic review and meta-analysis of the R778L mutation in *ATP7B* with Wilson's disease. PROSPERO 2021 CRD42021275791. Available at:https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42021275791. Accessed December 10, 2021.
24. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. Int J Surg. 2010;8:336–341.
25. Wells GA, Shea B, O'Connell D, et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. Available at: https://www.ohri.ca//programs/clinical_epidemiology/oxford.asp; Accessed January 1, 2014.
26. Cohen J. Chapter 1 - the concepts of power analysis. In: Cohen J., editor. Statistical Power Analysis for the Behavioral Sciences. Academic Press, New York; 1977. p. 1–17.
27. Schünemann HJ, Brożek JL, Guyatt GH, Oxman AD, The GRADE Working Group. GRADE Handbook for Grading Quality of Evidence and Strength of Recommendations. Canada: McMaster University and Evidence Prime Inc; 2013.
28. Cochran WG. Some methods for strengthening the common χ^2 tests. Bioethics. 1954;10:417.
29. Higgins JPT, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med. 2002;21:1539–1558.
30. Peters JL, Sutton AJ, Jones DR, Abrams KR, Rushton L. Contour-enhanced meta-analysis funnel plots help distinguish publication bias from other causes of asymmetry. J Clin Epidemiol. 2008;61:991–996.
31. Harbord RM, Egger M, Sterne JAC. A modified test for small-study effects in meta-analyses of controlled trials with binary endpoints. Stat Med. 2006;25:3443–3457.
32. Egger M, Smith GD, Schneider M, Minder CE. Bias in meta-analysis detected by a simple, graphical test. BMJ. 1997;315:629–634.
33. Peters JL, Sutton AJ, Jones DR, Abrams KR, Rushton L. Comparison of two methods to detect publication bias in meta-analysis. JAMA. 2006;295:676–680.
34. Sheng Y. Exon mutation analysis of Wilson disease [D]. Zhejiang University; 2003.
35. Gu Y-H, Kodama H, Du S-L. Apolipoprotein E genotype analysis in Chinese Han ethnic children with Wilson's disease, with a concentration on those homozygous for R778L. Brain Dev. 2005;27:551–553.
36. Aihong Q. Study on the gene diagnosis and the genotype-phenotype correlation of Wilson disease. [D]; Huazhong University of Science and Technology; 2006.
37. Jingfan Y, Xiuling L, Shouzhong M, et al. 7 Novel mutations detected in 74 patients with Wilson's disease [J]. Chin J Neurol. 2006:673–677.
38. Zhongsheng Z, Yingwu Z, Zhu Xu, et al. Study on the Exon mutation of *ATP7B* gene with Wilson Disease[J]. Guizhou Med J. 2008:963–966.
39. Mingran S. ATP7B mutation analysis in Wilson disease patients with liver injuries [D]. Jilin University; 2010.
40. Nan C. Study on hot regions and high frequency spots of WD gene mutations and gene diagnosis for special types of. In: WD[D]. Southern Medical University; 2010.
41. Yeqing H. Study on the relationship of the *ATP7B* gene mutation and clinical phenotype in WD patients [D]; Guangdong Pharmaceutical University, 2011.
42. Yingqian L. *ATP7B* gene mutation analysis in the patients with Wilson disease [D]. Shanxi Medical University; 2011.

43. Shaojuan G, Huarong Y, Yong Q, et al. Novel ATPase Cu(2+) transporting beta polypeptide mutations in Chinese families with Wilson's disease [J]. PloS one. 2013;8(7).
44. Mingming L. The mutation of ATP7B gene and clinical correlation in Chinese WD patients [D]; Central South University, 2013.
45. Liming J, Xiaoqing Z, Yilei Z, et al. Detection of *ATP7B* gene mutation in patients with Wilson's disease [J]. J Diagn Concepts Practice. 2013;12:406–409.
46. Geng J, Wang J, Yao R E, et al. Identification of one novel and nine recurrent mutations of the ATP7B gene in 11 children with Wilson disease [J]. World J Pediatr. 2013;9:158–162.
47. Liu Y, Zhou H, Guo H, et al. Genetic and Clinical Analysis in a Cohort of Patients with Wilson's Disease in Southwestern China [J]. Archives of Medical Research. 2015;46(2).
48. Haiyun Z. Study on mutations spectrum and relationships analysis on genetypes and TCM syndrome types of Chinese WD patients [D]. Anhui University of Chinese Medicine; 2014.
49. Dongfeng Z. Study of the relationship between clinical manifestation and clinical type, genotype and phenotype, and mutation types and efficacy of penicillamine in Wilson disease [D]; Zhengzhou University 2015.
50. Hua R, Hua F, Jiao Y, et al. Mutational analysis of ATP7B in Chinese Wilson disease patients [J]. Am J Transl Res. 2016;8:2851–2861.
51. Shanshan P. Phenotype-genotype correlations in patients with Wilson's disease [D]. Jilin University; 2016.
52. Yingying Y. Clinical analysis and gene detection of 10 HLD children patients [D]. Qingdao University; 2017.
53. Hao Y. Mutational characterization of ATP7B in Southeastern Chinese patients with Wilson's disease and analysis of the special phenotype [D]. Zhejiang University; 2017.
54. Cheng N, Wang H, Wu W, et al. Spectrum of ATP7B mutations and genotype-phenotype correlation in large-scale Chinese patients with Wilson Disease [J]. Clin Genet. 2017;92:69–79.
55. Heng X, Sheng D, Xiong D, et al. Mutation Analysis of the ATP7B Gene in Seven Chinese Families with Wilson's Disease [J]. Digestion. 2019;99.
56. Higgins J., Green S., editors Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 [updated March 2011]. The Cochrane Collaboration; 2011. Available at: www.handbook.cochrane.org.
57. Forbes JR, Cox DW. Functional characterization of missense mutations in *ATP7B*: Wilson disease mutation or normal variant? Am J Hum Genet. 1998;63:1663–1674.
58. Wu Z, Wang N, Lin MT, Fang L, Murong S, Yu L. Mutation analysis and the correlation between genotype and phenotype of Arg778Leu mutation in Chinese patients with Wilson disease. Arch Neurol. 2001;58 6:971–976.
59. Stapelbroek JM, Bollen CW, van Amstel JKP, et al. The H1069Q mutation in *ATP7B* is associated with late and neurologic presentation in Wilson disease: results of a meta-analysis. J Hepatol. 2004;41:758–763.
60. Kim D, Kim S, Ryu JL, et al. Human embryonic stem cell-derived Wilson's disease model for screening drug efficacy. Cells. 2020;9:872.
61. Wei R, Yang J, Cheng C-W, et al. CRISPR-targeted genome editing of human induced pluripotent stem cell-derived hepatocytes for the treatment of Wilson's disease. JHEP Rep. 2022;4, 100389.
62. Lalioti V, Sandoval I, Cassio D, Duclos-Vallée J. Molecular pathology of Wilson's disease: a brief. J Hepatol. 2010;53:1151–1153.
63. Zhang S, Yang W, Li X, et al. Clinical and genetic characterization of a large cohort of patients with Wilson's disease in China. Transl Neurodegener. 2022;11:13.
64. Ferenci P, Stremmel W, Członkowska A, et al. Age and sex but not *ATP7B* genotype effectively influence the clinical phenotype of Wilson disease. Hepatology. 2019;69:1464–1476.