Systematic Analysis and Insights Into the Mutation Spectrum and Ethnic Differences in ATP7B Mutations Associated With Wilson Disease

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ABSTRACT

BACKGROUND: *ATP7B* (ATPase copper transporting beta gene) is constituted of 21 exons, and codes for a 1465 amino acid. The protein of ATP7B plays an key role of copper metabolism. Many previous reports indicated that mutations in *ATP7B* are well known to cause defective copper transporting copper-transporting ATPase 2 protein leading to the accumulation of copper, resulting the Wilson disease.

OBJECTIVES: The meta-analysis was performed to comprehensive gain a thorough grasp of the spectrum of genetic variations.

DESIGN: A meta-analysis was conducted according to the guiding of PRISMA. aiming to assess the diversity and frequency of mutations in the *ATP7B* gene, with an emphasis on mutations located within specific exons.

DATA SOURCES AND METHODS: The dataset of detected mutations within their positions, types as well as nomenclature, were recorded from previous studies (spanning the year from 2013 to 2023). The analysis focused on exon-specific variations and their prevalence across different populations.

RESULTS: A total of 40 studies were enrolled into current data analysis. Our comprehensive study revealed a variety of mutations, most notably over 50% of single nucleotide changes described, distributed over the 21 exons of the gene. Focusing on the exon 8, itisplayed the most diversity of mutations, with 18 studies identifying 53 unique variants, the majority of which were missense mutations (81.13%). Additionally, the variations c.2333G>A/T (p.R778Q/L), c.2305A>G (p.M769V), c.2336G>A (p.W779*), and c.2304dupC (p.M769HfsX26) are reported in many populations. The weighted mean of variants' proportion was used to calculate the pooled estimate of these percentages, which were 14.19% for c.2333G>A/T (p.R778Q/L), 2.70% for c.2305A>G (p.M769V), 1.42% for c.2336G>A (p.W779*), and 2.33% for c.2304dupC (p.M769HfsX26)..

CONCLUSION: This design demonstrate to identify the spectrum of *ATP7B* gene's mutations, especially exon 8, offering important insights into the prevalence and significance of exon 8 mutations. Understanding the mutation in the *ATP7B* gene offers insights into the mechanisms behind WD and guides strategies for diagnosis and treatment.

KEYWORDS: ATP7B, Wilson disease, missense, mutation, meta-analysis

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Introduction

Wilson disease (WD, OMIM: #277900) is a rare autosomal recessive disorder characterized by impaired metabolism of copper, which causes copper to accumulate and become toxic, affecting the liver and brain among other organs.[1-3] The Wilson disease was first described in the year of by Dr. Samuel Alexander Kinnier Wilson, a British neurologist, who observed neurological symptoms and liver disease in affected patients.[1,4] Symptoms of WD present in childhood, adolescence or adulthood with a wide range of clinical manifestations. A greenish-brown corneal opacity, called as the Kayser-Fleisher ring, has been reported as a key ophthalmologic characteristic presents in WD. Additionally, the accumulation of copper in the liver leads to the damage of cellular, resulting in liver disease. Copper buildup also affects the brain, leading to many neurological diseases, including tremors, dystonia, and mood disorders.[5,6]

As the molecular level, the gene of *ATP7B* (OMIM: #606882), responsible for Wilson's disease, was identified by Dr. John H. W. Wason in 1993.[7] The homozygous or compound heterozygous in the coding region and the promoter of *ATP7B*, located on chromosome 13q14.3, has been reported to be responsible for this pathology in more than 95% case.[1,8,9] *ATP7B* gene contains 21 exons and 20 introns, and encodes for a 1465-aminoacid protein—a copper-transporting ATPase (Cu-ATPase) across the plasma membrane, which is synthetized in the endoplasmic reticulum and highly located in the trans-Golgi network of hepatocytes.[1,3,7,8] The structure of ATP7B protein, are described below, to receive copper ions within the cell, transport them through specific cellular compartments, and ultimately facilitate their incorporation into copper-dependent proteins or their elimination from the cell, maintaining copper homeostasis. ATP7B has a P-type ATPase architecture of conserved domains: a phosphatase domain (A-domain), a phosphorylation domain (P-domain), ATP-binding domain (N-domain), and 8 transmembrane domains (M-domain) form an intramembranous Cu channel.[7,10,11] This multi-domain has the distinctive structural feature: a large cytosolic N-terminal tail that contains 6 70-aa long independently folded Cu (I) binding domains (metal binding domains, MBDs, namely MBD1 to MBD6).[12] The function of MBD

Table 1. Search strategies.

NUMBER	SEARCH ITEM
#1	"Hepatolenticular degeneration"[MeSH] OR "Wilson disease"[tw] OR "Wilson's disease*" [tw] or WD [tw]
#2	"P-type ATPases"[Mesh] OR "Copper-Transporting ATPases" [MeSH] OR "ATP7B" [tw] OR "P-type ATPases"[tw] OR "Copper-Transporting ATPases" [tw]
#3	"Mutation"[Mesh] OR "Missense"[tw] OR "Nonsense"[tw] OR "Frameshift"[tw]
#4	#1 AND (#2 OR #3)

has been reported as receiving Cu^+ from a cytoplasmic Cu^+ chaperone antioxidant protein 1 and participating in subsequent Cu^+ transport across the membrane.[11] MBDs are found in the N-terminal with MBD1 being the first domain from the N-terminus and MBD6 being the domain nearest to the region of ATP7B that spans the membrane.[11] ATP7B serves a function in the export of copper from cells into bile and delivers copper for the functional synthesis of ceruloplasmin with the energy of ATP hydrolysis.[1,2,8,13]

Recent years have seen tremendous progress in our knowledge of characterizing the mutations of *ATP7B* gene related to WD and their effects on protein function. Mutations in the ATP7B gene may impair protein function, leading to copper accumulation in the liver and brain as well as improper ceruloplasmin synthesis.[14] Thur far, more than 1,000 variations of ATP7B associated with WD have been reported from various countries (The Human Gene Mutation Database (HGMD®). Available at: http://www.hgmd.cf.ac.uk/ac/index.php, Accessed: 17th Dec 2023). Due to the wide-spectrum of phenotypic variations in symptomatology, combined with the rarity of Wilson's disease, it can lead to misdiagnosis or delayed diagnosis.[12] Promptly identifying the disorder presents, particularly in its early stages when symptoms may be nonspecific, challenges for clinicians. Therefore, for an accurate and timely diagnosis, it becomes essential to combine clinical evaluation, genetic analysis to identify ATP7B mutations, and biochemical testing for copper levels.

The purposes of current study is to gain a thorough grasp of the spectrum of genetic variations as well as to consolidate and analyze existing data from various sources, including clinical studies and genetic databases, to identify patterns, prevalence. Overall, understanding the mutation in the ATP7B gene offers insights into the mechanisms behind WD and guides strategies for diagnosis and treatment.

Materials and Methods

Search strategy and data extraction

The comprehensive and systematic search were carried out following the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines.[15,16] Using the following separating and combining keywords or MeSH terms: Hepatolenticular degeneration, Wilson, Wilson disease, WD, ATP7B, P-type ATPases, Copper-Transporting ATPases, on databases of PubMed, EMBASE, Cochrane Library, and Web of Science, a protocol-driven systematic search was carried out for articles reporting on the mutations of *ATP7B* associated with WD (Table 1). Additional studies were also identified via the references listed in articles.

Studies were considered eligible when they met all of the following inclusion criteria if the article met: (1) in English; (2) a case-control design or cohort design; (3) report of the mutation of *ATP7B*; (4) report of mutations' frequencies. Exclusion criteria were as follow: (1) non-English publications; (2) non-article researches; (3) no report of ATP7B's mutations; (4) without available data for analysis.

For each edibility study, the relevant data were independently extracted by 2 authors. The disagreements were resolved through the discussion with the third author or members of our research team. The relevant data were extracted from each study according to data form, including following information: first author's name, year of publication, country where the study was performed, sample types, methods, types of mutation, and frequencies.

Data analysis: Schematic representation of the ATP7B *gene*

The compiled dataset of identified mutations, encompassing their positions and types to ensure consistency in mutation nomenclature, is entirely derived from previous researches. The mutation frequency of each distinct mutation can be determined by dividing the count of each specific mutation by the total number of observations in the dataset, expressed as a percentage. To identify the highest frequencies, the rank of the mutations was calculated based on their occurrence percentages and selected those with the highest values. In order to generate a schematic representation of the mutation in the ATP7B gene, all known mutations, systematically collected, are organized based on their locations within the gene sequence.

Assessment of risk, and other statistical analysis in included studies

The mutation type with the highest frequency, determined through previous studies, will be assessed for its potential to

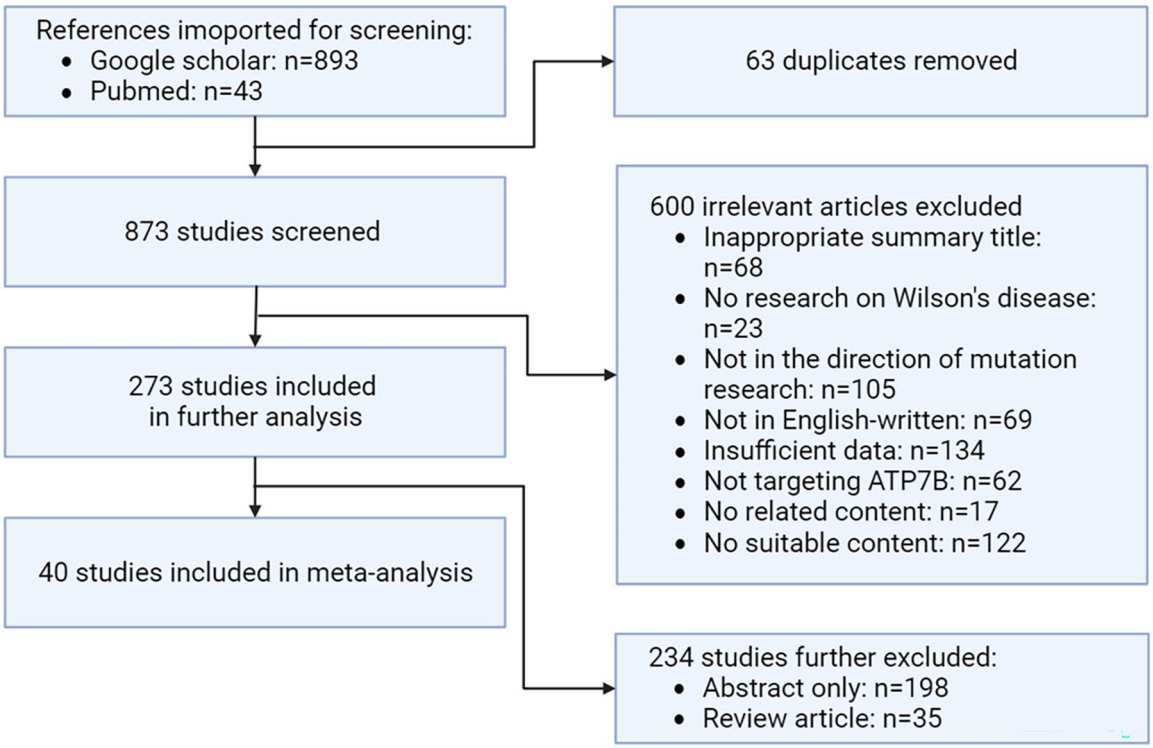


Figure 1. Study flow diagram for the selection of studies.

cause disease. We performed the data analysis for its frequencies, pooling odd ratios (OR) or relative risk (RR) by using software of MedCalc®, by MedCalc Software Ltd (https://www.medcalc.org/). In current study, the heterogeneity among the included studies was estimated by the Cochran Q test, and I^2 statistics. The cut-off value of $P = .05$ for Q test and I^2 were used to test the heterogeneity between the studies. The scale of I^2 value was classified as follow: $I^2 < 25\%$: no heterogeneity; $25\% \leqslant I2 \leqslant 50\%$: moderate heterogeneity; and $I^2 > 50\%$: strong heterogeneity.[17] In the case of no heterogeneity, a fixed-effects model was applied to systematically analyses, while a random-effects model was applied.

Results

Schematic representation of the ATP7B *gene*

In total, 936 studies, including 893 studies from Google scholar, 43 studies from Pubmed, were identified through the initial search in electronic databases. Following the removal of studies that did not meet the inclusion criteria, 40 studies published from 2001 to 2023 were included in the analysis that is being conducted (Figure 1).

The data of *ATP7B* gene was created by extracting the information of mutation from 40 studies. As a summarization, the mutation analysis allowed the detection of 463 mutations that existed in ATP7B. They are dispersed among the 21 exons of the *ATP7B* gene, from exon 1 to exon 21 (Table 2). Most remarkably, among these exons, exon 8 contains the greatest variety of mutations, followed by exon 18, 2, 14. Conversely, exon 1 had only one mutation (Figure 2). Most of previously identified variants were found on the transmembrane region, metal binding domains, and ATP binding site.

Characteristic of research involving exon 8-relevant mutations

Among 40 studies, 18 studies pertaining to mutations in exon 8 of ATP7B were isolated for further analysis. Most of identified variants were missense mutation, counting for 81.13% (43 of 53 cases), followed by frame-shift counting for 13.21% (7 of 53 cases), silent mutation counting for 3.77% (2 of 53 cases), non-sense mutation counting for 7.55% (4 of 53 cases). Among the frame-shift mutations, the frequencies of deletion, duplication, and insertion were 3.77% (2 of 53 cases), 1.89% (1 of 53 cases), and 7.55% (4 of 53 cases), respectively. Therefore, for a prompt diagnosis, especially in cases of hepatic failure disease, the mutation on exon 8 should be given priority analysis in patients who are suspected of having Wilson disease. Then, the allele and patient frequencies of each mutation on were identified. The mutation of c.2333G>A (p.R778Q), c.2333G>T (p.R778L), c.2305A>G (p.M769V), c.2336G>A (p.W779*), c.2304dupC (p.M769HfsX26) have been reported at least 5 different studies (Table 3). In previous studies, the share of the allelic frequency of the c.2333G>A (p.R778Q), c.2333G>T (p.R778L), c.2305A>G (p.M769V), c.2336G>A (p.W779*), c.2304dupC (p.M769HfsX26) variants, amounting 0.44 to 3.24, 0.01 to 29.67, 0.08 to 2.85, 0.02 to 8.57, 0.03 to 8.57, respectively, prevailed in China, India, France, and western countries, in patients with WD, was almost detected in a homozygous state. Additionally, based on the database of

Table 2. Variants distribution detected throughout the entire coding region of *ATP7B* gene.

EXONS	NUCLEOTIDE CHANGE (AMINO ACID CHANGE)	NUMBER
1	del_E1 (p.L1373P)	1
2	(p.G85V); c.314C>A (p.S105Stop); c.525dupA(p.V176SfsX28); c.1057delC (p.Q353RfsX10); c.1168 A>G(p.I 390V); c.1216 T>G (p.S406A); c.1063C>A (p.Q355X); c.695C>T (p.P232L); c.406A>T (p.R136W); c.1145C>G (p.S382C); c.199A>G (M67V); c.406A>G (R136G); c.172_173insC (A58A-fs); c.365_c.372dup8 (E122G-fs); c.448_452del5 (E150H-fs); c.892delC (Q298K-fs); c.561T>A (Y187X); c.813C>A (C271X); c.331C> T (p.Q111*); c.676C> T (p.R226W); c.503T> C (p.L168P); c.778dupC (p.Q260P-fs*10); c.865C> T (p.Q289*); c.122delA (p.N41M-fs*26); c.122A>G (p.N41S); c.379del (p.E127K-fs*26); c.524_525delAA (p.K175S-fs*28); c.560_561del (p.Y187S-fs*16); c.1136delG (p.G379A-fs*2); c.1242_1243del (p.R414S-fs*9); c.268_271del (p.K90FfsX10); c.367delG (p. A123PfsX30); c.588C>A (p.D196E); c.592A>G (p.R198G); c.695delC (p.P232QfsX30); c.994G>T (p.E332X); c.813delC (p.C271WfsX3); c.1050G>A (p.P350P); c.1122C>G (p.V374V); c.122A>G (p. N41S); c.172_173insC (p.A58fs*19); c.220_222delA (p.K74fs*9); c.841C>T (p.Q281*); c.1048delC (p. P350fs*12); c.1442C>G (p.S481*); c.813C>A (p.C271*);	46
3	c.3556G> A (C431S-fs); c.1470C>A (p.C490Stop); c.1531C>T (p.Q511Stop); c.1366 G>C (p.V456 L); c.1518-21delAGAA (p.E507fs); c.1369C> T (p.Q457*); c.1512dupT (p.N505*); c.1403_1416del (p.A468GfsX33); c.1426G>A (p.A476T); c.1449_1456del (p.R483SfsX20); c.1475T>C (p.L492S); c.1448_1455del (p.R483SfsX20); c.1448_1455del (p.R483Sfs X19);	13
4	c.1607T>C (p.V536A); c.1621G>A (p.E541K); c.1658C> A (p.A553E); c.1705_1707 + 10del (p.T569del); c.1544G>T (p.G515V); c.1545delT (p.G515GfsX9); c.1552_1553delTC (p.S518RfsX15); c.1639C>T (p.Q547X); c.1630C>T (p.Q544*);	9
5	c.1739delA (p. H580Pfs); c.1849delG (p.D617Ifs); c. 1847G> A (p.R616Q); c.1846C> T (R616W); c.1789G>A (p.V597I); c.1840G>T (p.G614C); c.1771G>C (G591S); c.1783G>A (A595T); c.1740del (p.H580Q-fs*3); c.1745_1746del (p.I582R-fs*25); c.1772G> A (p.G591D); c.1811C> A (p.A604D); c.1760C>T (p.T587M) ;c.1782T>A (p.Y594X); c.1803delC (p.S602AfsX46); c.1817T>G (p.V606G); c.1820dupA (p.F608VfsX2); c.1846C>T (p.R616Y); c.1746dup (p.E583Rfs*25); c.1831 G> A (p.E611K); c.1847G>A (p.R616Q); c.1817T>G (p.V606G);	22
6	c.1934T>G (p.M645R); c.1875-1876insAATT (p.G626fs); c.1915C>T (p.H639Y); c.1875-1876insAATT (p.G626fs); c.1922T>C (p.L641S); c.1877G> C (p.G626A); c.1925A>G (p.D642G); c.1924G>T (p. D642Y); c.1877G>C (p.G626A); c.1924G>C (p.D642H);	10
7	c.1995G> A (p.M665I); c.2078C> G (p.S693C); c.2097_2099del (p.F700del); c.2120A>G (p.Q707R); c.1958C>A (p.S653Y); c.2120A>G (p.Q707R); c.2111C>T (p.T704I); c.2060_2062delACA (N687I-fs); c.2071G>A (p.G691R); c.1950G>A (p.W650Term); c.2038C>T (p.Q680X); c.2043delC (p.S681SfsX15); c.2078C>G (p.S693C); c.1973 T> C (p.L658P); c.2075T>C (p.L692P); c.1969A>C (p.S657R); c.1971dupC (p.M658fS); c.2063T>G (p.I688S); c.2007_2013delATATGCT (p.I669I-fs);	19
8	c.2123T>C (p.L708P); c.2304delC (p.M769CfsX38); c.2304dupC (p.M769HfsX26); c.2305A>G (p.M769V0); c.2336G>A (p.R779X); c.2293G>C (p.D765H); c.2129G>C (G710A); c.2128G>A (G710S); c.2279C>T (P760L); c.2293G>A (D765N); 2299insC (P767P-fs); c.2305A>G (M769V); c.2337G>A (W779X); c.2294A>G (p.D765G); c.2332C >T (p.R778W); c.2333G >A (p.R778Q); c.2333G >T (p.R778L); c. 2310C>G (p.L700L); c.2332C > G (p.R778G); c.2304insC (p.M769fs); c.2327T >C (p.L776P); c.2267C>G (p.A756G); c.2267C>T (p.A756V);; c.2192T>C (p.V731A); c.2234T>C (p.L745P); c.2297C>T (T766M); c.2136G>A (W712X); c.2131G > A (p.G711R); c.2303C > T (p.P768L); c.2337G > C (p.W779C); c.2156A>G (p.Y719C); c.2157C>A (p.Y719X); c.2192T>A (p.V731E); c.2195T>C (p.L732P); c.2223T>A (p.Y741X); c.2251G>T (p.A751S); c.2261A>G (p.E754G); c.2341G>A (p.E781K); c.2333C>A (p.L745I); c.2312C > A (p.F771Y); c.2246 T > A (p.V749E); c.2441A>T (p.T762S); c.2145C>T (p.A756A); c.2268G>A (p.A756A); c.2292C>T (p.F764F); c.2195T>A (p.L732H); c.2204T>G (p.L735R); c.2131G>T (p.G711W); c.2264A>G (p.K755R); c.2305insC (p.M769H-fsX26); c.2316_2317insCTCTTTGTG (p.V772insLFV); 2308C>T (p.L770F); c.2297C>G (p.T766R)	53
9	c.2383C>T (p.L795F); c.2366C>G (S789X); c.2390C>T (p.S797F); c.2375 T> C (p.L792P); c.2435delA (p.N812fs*2);	5
10	2464insA; c.2532delA; c.2507G>A (p.G836E); c.2549C> T (p.T850I0; c.2564C>A (p.S855Y); c.2495 A>G (p.K832R); c.2532delA (p.V845fs); c.2494A>C (p.K832Q); c.2564C>T (S855F); c.2605G>A (G869R); c.2495_2496insG (K832K-fs); c.2513delA (K838S-fs); c.2532delA (p.V845S-fs*28); c.2570T> C (p.I857T); c.2506G> A (p.G836R); c.2512A>G (p.K838E); c.2455C>T (p.Q819X); c.2525A>G (p.D842G); c.2464dupA (p.M822NfsX32); c.2495A>G (p.K832R); c.2534T>C (p.V845A); c.2567T>G (p.L856R); c.2513delA (p.K838S-fsX34);	23
11	c.2672G>T (p.G891V); p.V890M; c.2621C> T (p.A874V); c.2191T>G (p.V864G); c.2668G>A (p.V890M); c.2620G> C (p.A874P); c.2621C>T (p.A874V); c.2662A>C (p.T888P); c.2590_2593dup (p.T865SfsX3); c.2605G>A (p.G869R); c.2582delC (A861A-fs); c.2637_2650de (p.G881*); c.2587C>T (p.P863S); c.2648_2649del (p.V883AfsX3); c.2705 T> C (p.L902P); c.2583C>T (p.A861A); c.2633A>C (p. N878T); c.2730G>T (p.K910N);	18

(Continued)

Table 2. (Continued)

EXONS	NUCLEOTIDE CHANGE (AMINO ACID CHANGE)	NUMBER
12	p.T935M; p.R919G; c.2817G> T (p.W939C); c.2807T>A (p.L936X); c.2816G>A (p.W939X); c.2755C> G (p.R919Q); c.2755C>T (p.R919W); c.2761A>C (p.S921R); c.2810delT (p.V937GfsX5); c.2828G>A (p.G943D); c.2855 G>A (p.R952K); c.2755C>G (p.R919G); c.2790_2792del (p.I930del); c.2810delT (p.V937fs); c.2806T>G (p.L936V); c.2752G> A (p.D918N); c.2740C>T (p.Q914X); c.2794_2795insGT (p.S932CfsX4); c.2810delT (p.V937GfsX5); c.2827G>A (p.G943S); c.2848G>T (p.V950F); c.2853_2856del (p.Q951HfsX15); c.2785 A>G (p.I929V); c.2763T>A (p.S921R);	25
13	c.3008C> T (p.A1003V); C.2906G>A (R969Q); c.2975C>T (P992L); c.2924C>A (p.S975W); c.2939G>A (p.C980Y) c.3007G>A (p.A1003T); c.3029A>C (p.K1010T); c.2930T>C (p.T977M); c.2962G>C (p.G988R); c.2986_3008del23insTATGTGG (p.M996fs); c.2924C>A (p.S975Y); c.2939G>A (p.C980Y); c.2975C>T (p.R1319Q); c.2972C>T (p.T991M); c.3051G>A (p.M1017I); c.2932G>A (V978M); c.3059A>G (p.K1020R); c.3008C>T (p.A1003V); c.3010C>T (p.Q1004X); c.3044T>C (p.L1015P); c.3053C>T (p.A1018V); c.2930C> A (p.T977K); c.2943T>G (I981S); p.P922H; c.2998G>A (p.G1000R); c.3053C>T (p.A1018V); c.2998G>A (p.G1000R);	27
14	c.3207C>A (p.H1069Q); c.3190G> A (p.E1064K); c.3140A>T (p.D1047V); c.3155C>T (p.P1052L); c.3236G>T (p.C1079F); c.3140delA (p.D1047fs); c.3236G>T (p.C1079F); c.3074T>G (p.M1025R); c.3104G>T (p.G1035V); c.3208C>T (p.P1070S); c.3061A>G (p.I1021V); c.3121C>T (p.R1041W); c.3173C>T (p.A1058V); c.3221C>T (p.A1074V); c.3091A>G (T1031A); c.3182G>A (G1061E); c.3083_3085delinsG (p.K1028S-fs*40); c.3201T> G (p.S1067R); c.3074T> A (p.M1025K); c.3083A>G (p.K1028R); c.3087delT (p.G1030AfsX91); c.3089G>A (p.G1030D); c.3098C>T (p.T1033I); c.3104G>T (p.G1035V); c.3122G>C (p.R1041P); c.3121C>T (p.R1041W); c.3209C>G (p.P1070R); c.3221C>T (p.A1074V); c.3124delG (p.V1042Cfs*79); c.3077 T> A (p.F1026Y); c.3079 G> C (p.D1027H); c.3188C>T (p.A1063V); c.3157dupC (p.L1053PfsX16); c.3088G>A (p.G1030S); c.3079G>A (p.D1027N); c.3190G>A (p.E1064K); c.3089G>A (p.G1030D); c.3190G> A (p.E1064K); c.3233C>G (Y1078X); c.3182G>A (p. G1061E); c.3086C>T (p.T1029I);	41
15	c.3402del C (p.A1135Qfs); c.3244G>T (p.E1082X); 3400delC (P1134P-fs); c.3316G>A (p.V1106I); c.3284A>C (p.Q1095P); c.3316G>A (p.V1106I); c.3282C>G (F1094L); c.3301G>A (G1101R); c.3295G> A (p.G1099S); c.3316G> C (p.V1106L); c.3263T>C (p.L1088S); c.3271T>C (p.C1091R); c.3274A>C (p.T1092P); c.3311G>A (p.C1104Y); c.3368C>T (p.P1123L); c.3377_3378delAC (p.H1126PfsX3); c.3310 T> C (p.C1104R); c.3284 A>C (p.Q1095P); c.3402delC (p.A1135QfsX13); c.3247C>T (p.L1083F); c.3403G> A (p.A1135T); c.3376C> T (p.H1126Y); c.3301G>A (p. G1101R);	23
16	c.3556G> A (p.G1186S); c.3426G> C (p.Q1142H); c.3443T> C (p.I1148T); c.3446G>A (p.G1149E); c.3532A>G (p.T1178A); c.3419 T>C (p.V1140A); c.3472_3482delGGTTTAACCAT (p.G1158fs); c.3350-3353delAGCG (p.E1117fs); p.V1146M; c.3451C>T (p.R1151C); c.3517G>A (p.E1173K); c.3517G>A (p.E1173K); c.3459G>T (p.W1153C); c.3556G>C (G1186R); c.3547G> A (p.A1183T); c.1924G> C (p.D642H); c.3426G>C (p.Q1142H); c.3451C>T (p.R1151C); c.3452G>A (p.R1151H); c.3517G>A (p.G1173K); c.3473G>T (p.G1158V0; c.3506T>C (p.M1169T);	22
17	c.3646G>A (p.V1216M); c.3682A>T (p.R1228Stop); c.3659C>T (p.T1220M); c.3593T>C (p.V1198A); c.3644A>G (D1215G); c.3694A>C (p.T1232P); c.3563T>G (p.L1188R); c.3587A>G (p.D1196G); c.3605C>G (p.A1202G); c.3653T>C (p.L1218P); c.3634A>G (p. M1212V); c.3655A>T (p.I1219F); c.3620A>G (p.H1207R);	13
18	c.3818C>T (p.P1273L); c.3318C>T (p.P1273L); c.3890T>A (p.V1297D0; c.3809A>T (p.N1270S); c.3842G>A (p.G1281D); c.3877G>A (p.E1293K) c.3889G>A (p.V1297I); c.3833C->T (p.A1278V); c.3851_3876del (p.G1285Efs); c.3884C>T (p.A1295V); c.3700del (p.V1234LfsX96); c.3724G>A (p.E1242K); c.3749C>G (p.A1250G); c.3796G>A (p.G1266R); c.3892G>C (p.V1298L); c.3741C>G (H1247Q); c.3832G>A (A1278T); c.3895C>T (L1299F); 3809A>G (p. N1270S); c.3800A>C (p.D1267A0; c.3796G> A (p.G1266R0; c.3820G> A (p.A1274T); c.3842G> A (p.G1281D); c.3843dup (p.V1282K-fs*22); c.3845dupT (p.A1283G-fs*21); c.3700delG (p.V1234LfsX96); c.3715G>T (p.V1239F); c.3733C>G (p.P1245A); c.3741C>G (p.H1247Q); c.3766_3767dupCA (p.Q1256PfsX75); c.3791T>C (p.M1264T); c.3802G>A (p.G1268R); c.3818C>T (p.P1273L); c.3818C>A (p.P1273Q); c.3848C>T (p.A1283V0; c.3859G>A (p.G1287S); c.3896T>G (p.L1299R); c.3901_3902insA (p.R1301KfsX3); c.3842G> A (p.G1281D); c.3796 G> C (p.G1266R); c.3895C> T (p.L1299F); c.3818C>T (p. P1273L); c.3948delG (p.T1317fs); c.3797G>A (p.G1266E); c.3843dupT (p.V1282Cfs*22); c.3741C>G (p. H1247Q); c.3767A>G (p.Q1256R);	47
19	c.3955C>T (p.R1319Stop); c.3809A>G (p.N1270S); c.3960G>C (p.R1320S); c.3956G>A (p.R1319Q); c.3925G>C (A1309P); c.3938T>C (L1313P); c.3962T>A (I1321K); c.3955C>T (R1319X); c.3982G>A (p.A1328T); c.4003G>C (p.G1335R); c.4014T>A (p.I1338I); c.4006delA (p. I1336fs); c.3965G>C (p.R1322P); c.4022G>A (p.G1341D); c.4006delA (p.I1336Y-fsX57);	16
20	c.4022G> A (p.G1341D); 4085delCC (S1362F-fs); c.4112T> C (p.L1371P); c.4051C>T (p.Q1351X); c.4084T>G (S1362A); c.4088C>T (S1363F); c.4092_4093del (p.S1365C-fs*12); c.4057T>C (p.W1353R); c.4059G>A (p.W1353X); c.4114C>T (p.Q1372X); c.4064G>T (p. G1355V); c.4114C>T (p.Q1372Term); c.4112C>T (p.I1371P); c.4088C>T (p.S1363F);	14
21	c.4301C> T (p.T1434M); c.4176dup (p.K1393EfsX15); c.4292C>A (p.S1431Y); c.4295C>T (p.S1432F); c.4277G>T (S1426I); c.4144G> T (p.E1382*); c.4162delG (p.A1388RfsX5); c.4272T>G (p.Y1424X); c.4251A>G (p.T1417T); c.4374_4375delCA (p.R1459GfsX2); c.4295C>T (p.S1432F);	11

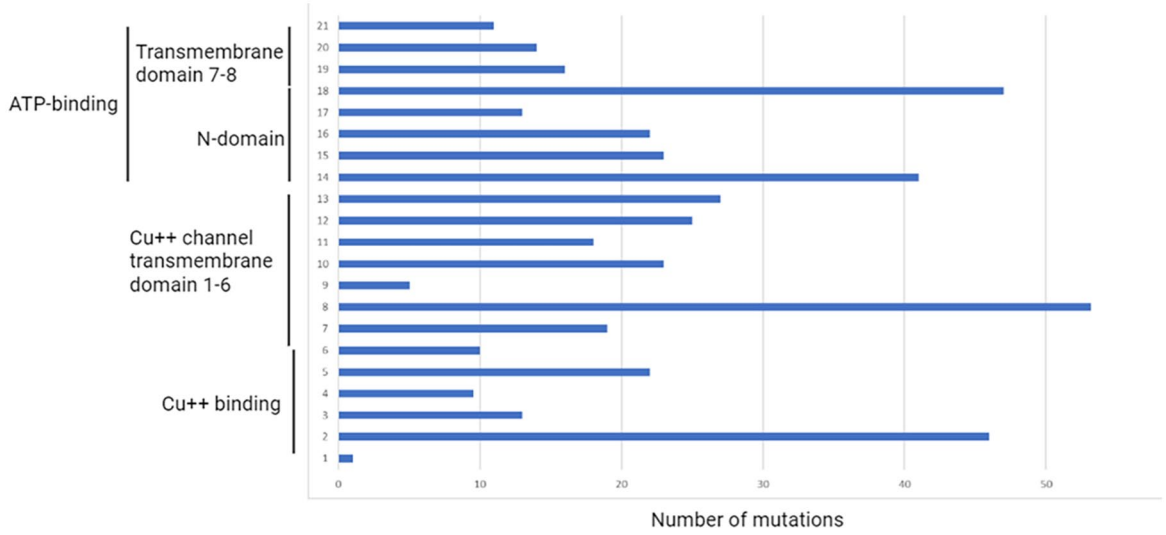


Figure 2. Number of mutation in exon 1 to 21 of *ATP7B* gene.

ClinVar, those variants were classified as pathogenic variants (last evaluated by 2024). This classification underscores the clinical significance of these variants in the development of Wilson Disease.

Variants c.2333G>A>T (p.R778Q/L), c.2305A>G (p.M769V), c.2336G>A (p.W779), c.2304dupC (p.M769HfsX26) in WD patients*

Of the 18 studies in this systematic screening, there were 10 studies (2847 alleles) included for the analysis of variants c.2333G>A/T (p.R778Q/L) mutation. The random effect model was applied for calculating the weighted mean of proportion of variants c.2333G>A/T (p.R778Q/L) mutation since there was a heterogeneity across studies ($Q = 655.17$, $P < .001$, $I^2 = 98.63\%$, 95%CI for $I^2 = 98.21$-98.94). The weighted mean of proportion of variants c.2333G>A/T (p.R778Q/L) mutation was 14.19% (Figure 3A). This was not statistically significant as evidenced by Egger's test ($P = .45$), which confirmed the results were not influenced by publication bias. Asking for the variant c.2305A>G (p.M769V), 6 studies (2220 alleles) were included for analyzing. The random effect model was applied to calculate the weighted mean of proportion of variants c.2305A>G due to a heterogeneity across studies ($Q = 141.86$, $P < .001$, $I^2 = 96.48\%$, 95%CI for $I^2 = 94.34$-97.81). The weighted mean of proportion of variants c.2333G>A/T (p.R778Q/L) mutation was 2.70% (Figure 3B). The $P = .36 > .05$ of Egger's test indicated the results were not influenced by publication bias. Only 4 studies (1916 alleles) are included the calculating the weighted mean of proportion of variants. The weighted mean of proportion of variants c.2336G>A (p.W779*) was 1.42% by applying the random effect model ($Q = 24.60$, $P < .001$, $I^2 = 87.81\%$, 95%CI for $I^2 = 71.09$-94.86) (Figure 3C). The results were not influenced by publication bias since the $p = .12 > .05$ of Egger's test. For the study of c.2304dupC (p.M769HfsX26) mutation, 6 studies (1804 alleles) were included for systematically analyzing. Due to the significant heterogeneity among studies ($Q = 10.92$, $P = .03$, $I^2 = 63.36\%$, 95%CI for $I^2 = 3.27$-86.12), the random effect model was used to calculate the weighted mean of proportion of variation. 2.33% was the weighted mean of the fraction of variants with the mutation c.2304dupC (p.M769HfsX26; Figure 3D). According to Egger's test ($P = .45$), which verified publication bias had no effect on the results, this was not statistically significant. These value represents the pooled estimate of the c.2333G>A/T (p.R778Q/L), c.2305A>G (p.M769V), c.2336G>A (p.W779*) and c.2304dupC (p.M769HfsX26) mutation prevalence of 14.19%, 2.70%, 1.42%, 2.33% taking into account the different sample sizes and variances of the included studies. This pooled estimate is essential for understanding the broader implications of the c.2333G>A/T (p.R778Q/L), c.2305A>G (p.M769V), c.2336G>A (p.W779*) and c.2304dupC (p.M769HfsX26) mutation across diverse populations and study settings.

Discussion

The gene of *ATP7B* has been reported to be pivotal in studies of WD related to the metabolism of copper.[1,12,14,26] This study comprehensively highlighted the mutation of *ATP7B* in WD patients by an analysis of 40 studies from 2001 to 2023. According our systematic analysis, various mutations, notably more than 50% described single nucleotide variations, dispersed among the gene's 21 exons, were identified. Most detected variants were located in crucial regions such as the transmembrane domain, metal-binding domains, and ATP-binding site, highlighting their potential impact on the gene's function and the progression of Wilson disease. The mutation profile of previous studies highly presented most of our identified variants located on the exon 8, which exhibited the highest mutation diversity, 18 studies identified 53 distinct variants, predominantly missense mutations (81.13%), followed by exons 18, 2, and 14, with exon 1 showing the least variability.

Table 3. Characteristics of studies included in the meta-analysis.

STUDY	GEOGRAPHICAL LOCATION	SOURCE OF SAMPLE	METHOD	MUTATION		CASE/ALLELE (DISEASE)	
				NUCLEOTIDE	AMINO ACID	N	P (%)
Bem et al[18]	Brazil	W.blood	PCR, DNA sequencing	c.2123T>C	p.L708P	35	1.42
	Brazil	W.blood	PCR, DNA sequencing	c.2304delC	p.M769CfsX38	35	1.42
	Brazil	W.blood	PCR, DNA sequencing	c.2304dupC	p.M769HfsX26	35	4.28
	Brazil	W.blood	PCR, DNA sequencing	c.2305A>G	p.M769V	35	1.42
	Brazil	W.blood	PCR, DNA sequencing	c.2336G>A	p.W779*	35	4.28
Lepori et al[19]	Italy	Pe.Blood	PCR, DNA sequencing	c.2293G>C	p.D765H	118	nt
	Caucasian	Pe.Blood	PCR, DNA sequencing	c.2304insC	p.M769fs	118	3.80
Caca et al[20]	Germany	Pe.Blood	PCR, DNA sequencing	c.2129G>C	G710A	84	0.60
	Germany	Pe.Blood	PCR, DNA sequencing	c.2128G>A	G710S	84	0.60
	Germany	Pe.Blood	PCR, DNA sequencing	c.2279C>T	P760L	84	0.60
	Germany	Pe.Blood	PCR, DNA sequencing	c.2293G>A	D765N	84	0.60
	Germany	Pe.Blood	PCR, DNA sequencing	2299insC	P767P-fs	84	3.57
	Germany	Pe.Blood	PCR, DNA sequencing	c.2305A>G	M769V	84	1.79
	Germany	Pe.Blood	PCR, DNA sequencing	c.2337G>A	W779X	84	2.38
Wei et al[21]	China	Pe.Blood	PCR, DNA sequencing	c.2294A>G	p.D765G	103	0.49
	China	Pe.Blood	PCR, DNA sequencing	c.2332C>T	p.R778W	103	0.49
	China	Pe.Blood	PCR, DNA sequencing	c.2333G>A	p.R778Q	103	0.49
	China	Pe.Blood	PCR, DNA sequencing	c.2304dupC	p.M769Hfs26	103	0.49
	China	Pe.Blood	PCR, DNA sequencing	c.2333G>T	p.R778L	103	18.93
Lu et al[22]	China	W.blood	PCR, DNA sequencing	c.2310C>G	p.L700L	3	50.0
	China	W.blood	PCR, DNA sequencing	c.2333G>T/A	p.R778L/Q	3	50.0
Gromadzka et al[23]	Poland	Nt	PCR, DNA sequencing	c.2337G>A	p.W779X	142	1.1

(Continued)

Table 3. (Continued)

STUDY	GEOGRAPHICAL LOCATION	SOURCE OF SAMPLE	METHOD	MUTATION		CASE/ALLELE (DISEASE)	
				NUCLEOTIDE	AMINO ACID	N	P (%)
	Poland	Nt	PCR, DNA sequencing	c.2332C>G	p.R778G	142	1.1
	Poland	Nt	PCR, DNA sequencing	c.2304insC	p.M769fs	142	0.7
	Poland	Nt	PCR, DNA sequencing	c.2327T>C	p.L776P	142	0.7
	Poland	Nt	PCR, DNA sequencing	c.2293G>A	p.D756N	142	0.4
Xu et al[24]	China	Pe.Blood	PCR, DNA sequencing	c.2267C>G	p.A756G	34	1.47
	China	Pe.Blood	PCR, DNA sequencing	c.2333G>T	p.R778L	34	36.76
Huang et al[25]	China	Blood	Illumina paired-end sequencing	c.2333G>T	p.R778L	19	10.52
	China	Blood	Illumina paired-end sequencing	c.2267C>T	p.A756V	19	2.63
	China	Blood	Illumina paired-end sequencing	c.2304dupC	p.M769Hfs*25	19	2.63
Coffey et al[26]	United Kingdom	W.blood	PCR, DNA sequencing	c.2192T>C	p.V731A	178	0.02
	United Kingdom	W.blood	PCR, DNA sequencing	c.2234T>C	p.L745P	178	0.02
	United Kingdom	W.blood	PCR, DNA sequencing	c.2336G>A	p.R779*	178	0.02
	United Kingdom	W.blood	PCR, DNA sequencing	c.2305A>G	p.M769V	178	0.14
	United Kingdom	W.blood	PCR, DNA sequencing	c.2332C>T	p.R778Y	178	0.04
Mukherjee et al[27]	India	Nt	PCR, DNA sequencing	c.2128G>A	G710S	398	0.25
	India	Nt	PCR, DNA sequencing	c.2293G>A	D765N	199	0.5
	India	Nt	PCR, DNA sequencing	c.2297C>T	T766M	199	0.25
	India	Nt	PCR, DNA sequencing	c.2333G>A	R778Q	199	0.75
	India	Nt	PCR, DNA sequencing	c.2298_2299insC	P767P-fs	199	1.51
	India	Nt	PCR, DNA sequencing	c.2136G>A	W712X	199	0.25
Couchonnal et al[28]	France	Serum	MLPA, sequencing	c.2131G>A	p.G711R	113	0.88
	France	Serum	MLPA, sequencing	c.2123T>C	p.L708P	113	0.44

(Continued)

Table 3. (Continued)

STUDY	GEOGRAPHICAL LOCATION	SOURCE OF SAMPLE	METHOD	MUTATION		CASE/ALLELE (DISEASE)	
				NUCLEOTIDE	AMINO ACID	N	P (%)
	France	Serum	MLPA, sequencing	c.2303C>T	p.P768L	113	0.44
	France	Serum	MLPA, sequencing	c.2337G>C	p.W779C	113	0.44
	France	Serum	MLPA, sequencing	c.2304dupC	p.M769H-fs*26	113	2.21
	France	Serum	MLPA, sequencing	c.2332C>G	p.R778W	113	1.77
	France	Serum	MLPA, sequencing	c.2293G>A	p.D765N	113	1.32
	France	Serum	MLPA, sequencing	c.2128G>A	p.G710S	113	0.88
	France	Serum	MLPA, sequencing	c.2297C>G	p.T766R	113	0.88
	France	Serum	MLPA, sequencing	c.2305A>G	p.M769V	113	0.88
	France	Serum	MLPA, sequencing	c.2336G>A	p.W779*	113	0.88
	France	Serum	MLPA, sequencing	c.2333G>A	p.A778Q	113	0.46
Dong et al[29]	China	Blood	PCR, DNA sequencing	c.2156A>G	p.Y719C	632	0.08
	China	Blood	PCR, DNA sequencing	c.2157C>A	p.Y719X	632	0.24
	China	Blood	PCR, DNA sequencing	c.2192T>A	p.V731E	632	0.08
	China	Blood	PCR, DNA sequencing	c.2195T>C	p.L732P	632	0.08
	China	Blood	PCR, DNA sequencing	c.2223T>A	p.Y741X	632	0.08
	China	Blood	PCR, DNA sequencing	c.2251G>T	p.A751S	632	0.08
	China	Blood	PCR, DNA sequencing	c.2261A>G	p.E754G	632	0.08
	China	Blood	PCR, DNA sequencing	c.2267C>G	p.A756G	632	0.08
	China	Blood	PCR, DNA sequencing	c.2341G>A	p.E781K	632	0.08
	China	Blood	PCR, DNA sequencing	c.2128G>A	p.G710S	632	0.08
	China	Blood	PCR, DNA sequencing	c.2294A>G	p.D765G	632	0.24
	China	Blood	PCR, DNA sequencing	c.2297C>T	p.T766M	632	0.16

(Continued)

Table 3. (Continued)

STUDY	GEOGRAPHICAL LOCATION	SOURCE OF SAMPLE	METHOD	MUTATION		CASE/ALLELE (DISEASE)	
				NUCLEOTIDE	AMINO ACID	N	P (%)
	China	Blood	PCR, DNA sequencing	c.2304dupC	p.M769H-fsX26	632	0.87
	China	Blood	PCR, DNA sequencing	c.2305A>G	p.M769V	632	0.08
	China	Blood	PCR, DNA sequencing	c.2308C>T	p.L770F	632	0.08
	China	Blood	PCR, DNA sequencing	c.2316_2317insCTCTTTGTG	p.V772insLFV	632	0.08
	China	Blood	PCR, DNA sequencing	c.2332C>T	p.R778W	632	0.16
	China	Blood	PCR, DNA sequencing	c.2333G>T	p.R778L	632	29.67
	China	Blood	PCR, DNA sequencing	c.2333G>A	p.R778Q	632	1.98
	China	Blood	PCR, DNA sequencing	c.2336G>A	p.W779*	632	0.08
Zhang and Teng[30]	China	Pe.Blood	PCR, DNA sequencing	G2333T	p.R778L	68	25.74
	China	Pe.Blood	PCR, DNA sequencing	c.2333C>A	p.L745I	68	0.73
	China	Pe.Blood	PCR, DNA sequencing	c.2305A>G	p.M769V	68	0.73
	China	Pe.Blood	PCR, DNA sequencing	c.2299insC	P767P-fs	68	0.73
Rangaraju et al[31]	India	Blood	PCR, DNA sequencing, SSCP	c.2312C>A	p.F771Y	13	11.54
	India	Blood	PCR, DNA sequencing, SSCP	c.2246T>A	p.V749E	13	19.23
	India	Blood	PCR, DNA sequencing, SSCP	c.2441A>T	p.T762S	13	15.39
Xu et al[32]	China	Pe.Blood	PCR, DNA sequencing	c.2145C>T	p.Y715Y	12	4.17
	China	Pe.Blood	PCR, DNA sequencing	c.2268G>A	p.A756A	12	4.17
	China	Pe.Blood	PCR, DNA sequencing	c.2292C>T	p.F764F	12	4.17
Li et al[33]	China	Pe.Blood	PCR, DNA sequencing	c.2145C>T	p.Y715Y	114	0.88
	China	Pe.Blood	PCR, DNA sequencing	c.2195T>A	p.L732H	114	0.44
	China	Pe.Blood	PCR, DNA sequencing	c.2195T>C	p.L732P	114	0.44
	China	Pe.Blood	PCR, DNA sequencing	c.2267C>G	p.A756G	114	0.44

(Continued)

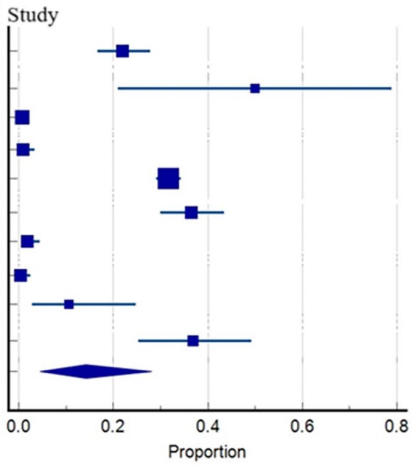
Table 3. (Continued)

STUDY	GEOGRAPHICAL LOCATION	SOURCE OF SAMPLE	METHOD	MUTATION		CASE/ALLELE (DISEASE)	
				NUCLEOTIDE	AMINO ACID	N	P (%)
	China	Pe.Blood	PCR, DNA sequencing	c.2305insC	p.M769H-fsX26	114	0.44
	China	Pe.Blood	PCR, DNA sequencing	c.2310C>G	p.L770L	114	21.1
	China	Pe.Blood	PCR, DNA sequencing	c.2333G>T	p.R778L	114	21.5
	China	Pe.Blood	PCR, DNA sequencing	c.2333G>A	p.R778Q	114	0.44
Singh et al[34]	Europe, Middle Eastern, Hungary, Indian	Pe.Blood	SSCP, ASP	c.2128G>A	p.G710S	113	0.4
	Pakistan	Pe.Blood	SSCP, ASP	c.2131G>T	p.G711W	113	7.0
	Indian	Pe.Blood	SSCP, ASP	c.2204T>G	p.L735R	113	0.8
	Indian	Pe.Blood	SSCP, ASP	c.2255T>G	p.V752G	113	0.4
	Italy	Pe.Blood	SSCP, ASP	c.2267C>T	p.A756V	113	0.8
	England	Pe.Blood	SSCP, ASP	c.2297C>T	p.T766M	113	0.8
	India, Spain	Pe.Blood	SSCP, ASP	c.2303C>T	p.P768L	113	0.4
	Taiwan, China, India	Pe.Blood	SSCP, ASP	c.2333G>A	p.R778Q	113	1.7
Shim et al[35]	Korea	Serum	PCR, DNA sequencing	c.2264A>G	p.K755R	104	0.48
	Korea	Serum	PCR, DNA sequencing	c.2297C>T	p.T766M	104	0.48
	Korea	Serum	PCR, DNA sequencing	c.2333G>T	p.R778L	104	36.5
	Korea	Serum	PCR, DNA sequencing	c.2304_2305insC	p.M769H-fsX26	104	1.92

Abbreviations: Nt, no description; Pe.Blood, peripheral blood; MLPA, multiplex ligation-dependent probe amplification; SSCP, single-stranded conformation polymorphism; ASP, allele-specific PCR.

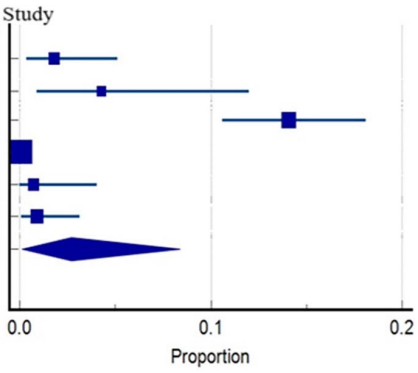
	Alleles (n)	Proportion (%)	95% CI	Weight (%)
Li et al., 2013	228	21.930	16.74 - 27.89	10.29
Lu et al., 2014	12	50.000	21.09 - 78.91	8.28
Mukherjee et al., 2014	398	0.754	0.15 - 2.19	10.35
Wei et al., 2014	206	0.971	0.12 - 3.46	10.27
Dong et al., 2016	1264	31.646	29.09 - 34.29	10.41
Shim et al., 2018	208	36.538	29.99 - 43.48	10.27
Singh et al., 2019	226	1.770	0.48 - 4.47	10.29
Couchonnal et al., 2021	226	0.442	0.011 - 2.44	10.29
Huang et al., 2022	38	10.526	2.94 - 24.81	9.60
W.-Q. Xu et al., 2023	68	36.765	25.39 - 49.33	9.95
Total (random effects)	2874	14.192	4.52 - 28.07	100.00

A



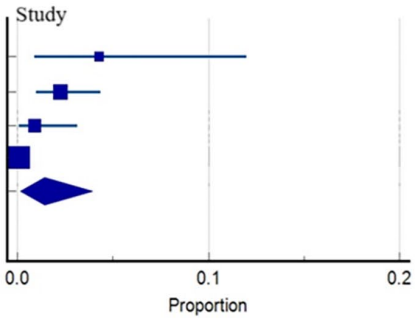
Study	Alleles (n)	Proportion (%)	95% CI	Weight (%)
Caca et al., 2001	168	1.786	0.37 - 5.13	16.63
Bem et al., 2013	70	4.286	0.89 - 12.02	15.42
Coffey et al., 2013	356	14.045	10.61 - 18.09	17.15
Dong et al., 2016	1264	0.0791	0.00 - 0.44	17.50
Zhang & Teng, 2016	136	0.735	0.02 - 4.03	16.42
Couchonnal et al., 2021	226	0.885	0.11 - 3.16	16.88
Total (random effects)	2220	2.701	0.13 - 8.38	100.00

B



Study	Alleles (n)	Proportion (%)	95% CI	Weight (%)
Bem et al., 2013	70	4.286	0.89 - 12.02	18.46
Coffey et al., 2013	356	2.247	0.98 - 4.38	26.93
Couchonnal et al., 2021	226	0.885	0.11 - 3.16	25.28
Dong et al., 2016	1264	0.0791	0.00 - 0.44	29.33
Total (random effects)	1916	1.417	0.16 - 3.91	100.00

C



	Alleles (n)	Proportion (%)	95% CI	Weight (%)
Bem et al., 2013	70	4.286	0.89 - 12.02	13.02
Wei et al., 2014	206	2.913	1.08 - 6.23	22.44
Huang et al., 2022	38	2.632	0.07 - 13.8	8.54
Couchonnal et al., 2021	226	2.212	0.72 - 5.09	23.21
Dong et al., 2016	1264	0.870	0.44 - 1.55	32.79
Total (random effects)	1804	2.328	1.01 - 4.17	100.00

D

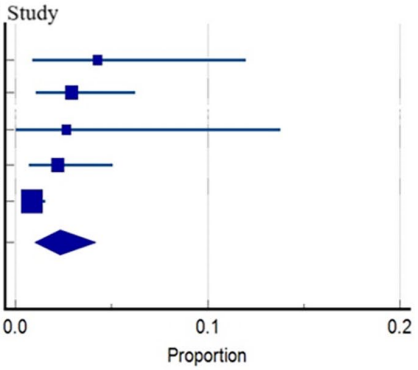


Figure 3. Forest plot showing the proportion of (A) c.2333G>A/T (p.R778Q/L); (B) c.2305A>G (p.M769V); (C) c.2336G>A (p.W779*), and (D) c.2304dupC (p.M769HfsX26) in patients with WD.

Exon 8 encodes part of the transmembrane domain 4 (Tm4) of the ATP7B protein.[9] Tm4 spans the cellular membrane, allowing the protein to embed within the lipid bilayer and form a pathway for copper ion transport. The transmembrane domains are essential for the function of ATP7B protein in moving copper ions across cellular membranes. Therefore, mutations occurred in exon 8 can disrupt the structure of the Tm4 domain, impairing the ability to effectively transport copper of ATP7B

protein, resulting in the clinical manifestations of Wilson disease.

Focusing on exon 8, which exhibited the highest mutation diversity, 18 studies identified 53 distinct variants, predominantly missense mutations, which constituted 81.13% (43 out of 53 cases). Other notable types included frame-shift, silent, and non-sense mutations. These figures underscore the predominance of missense mutations and the relative rarity of other mutation types in exon 8. In this study, we pointed out the notably frequent variants of as c.2333G>A/T (p.R778Q/L), c.2305A>G (p.M769V), c.2336G>A (p.W779*), and c.2304dupC (p.M769HfsX26) being reported in different populations, with notable prevalences in China, India, France, and Western countries. Based on the calculation of weighted mean of variants' proportion, the pooled estimate of these percentages of 14.19% for c.2333G>A/T (p.R778Q/L), 2.70% for c.2305A>G (p.M769V), 1.42% for c.2336G>A (p.W779*), and 2.33% for c.2304dupC (p.M769HfsX26), provided critical insights into the prevalence and impact of exon 8 mutations. Understanding these proportions is vital for grasping the broader implications across diverse populations and study settings, emphasizing the importance of exon 8 in genetic studies related to Wilson Disease. Therefore, the critical nature of exon 8 mutations is further emphasized by their relevance in diagnosing hepatic failure diseases, particularly Wilson Disease. The method of DNA extraction and Polymerase Chain Reaction (PCR) sequencing were widely used to identify these mutations. This technique amplifies specific DNA sequences, making it possible to detect even minor genetic variations with high precision.

The significance of mutation types has been reported that it can be used as the element for WD prognosis, as well as influence response to treatment of WD in some cases. Notably, the correlation between the mutations of *ATP7B* gene and clinical parameters has a few reports, and also remains controversial.[34] This is how studies are biased. Despite extensive research, studies have yielded inconsistent results regarding how specific mutations influence disease onset, severity, and progression. This controversy arises from several factors, including the genetic diversity across different populations, which can affect mutation prevalence and clinical expression. WD patients, who presented a phenotype of chronic liver disease, on chelation therapy, and carried at least one loss-of-function variant in the *ATP7B* gene, tended to display a worse prognosis during long-term follow-up.[36] This pointed out the importance of identifying genetic mutations of *ATP7B* gene in WD patients with hepatic involvement to better predict the progression of disease as well as diagnose the WD. The p.R778L mutation in Chinese population was associated with liver types, and some studies have no significant association between the p.R778L mutation and clinical type, sex, age.[37] One of the best known examples of the genotype-phenotype correlation in WD patients is the p.H1609Q mutation in East German patients with WD.[20] In their study, patients carried the homozygous mutation of p.H1609Q have symptoms started later than compound heterozygous mutation or no mutation. Moreover, they displayed more frequently neurologic symptoms and Kayser-Fleischer rings. The status of mutation did not significantly correlate with liver biopsy findings, serum ceruloplasmin levels or Cu-assay results. Molecular therapies of WD targeting molecular interactions to the mutant protein ATP7B have been studied. For instance, the p.H1609Q mutation was reported promoting the interaction between ATP7B and HSP70, resulting in the mutant promoted the accumulation of the endoplasmic reticulum degradation of mutant ATP7B protein led to the copper accelerating in hepatic cells. Domperidone is an HSP70 inhibitor that effectively counteracts the beginning of WD by enhancing the trafficking and function of ATP7B–H1069Q by preventing its access to the HSP70 proteostasis network.[38] Tang et al (2023) developed a WD cell model within a p.R778L mutation. Importantly, they reported that both necroptosis and autophagy are critically involved to the pathogenesis of WD. In this model of a p.R778L mutation, ULK1- and ATG16L1-mediated autophagy inhibits RIPK3- and MLKL-mediated necroptosis.[39] Their finding suggested that the possibility of mutation-specific treatment approaches, in which patients with specific ATP7B mutations, such p.R778L, might benefit from targeting processes like autophagy.

The strength of our study focused on the comprehensive approach to analyze the mutations dispersed among the gene's 21 exons across previous multiple studies. By utilizing a methodical screening procedure, noteworthy patterns and rates of mutations were detected, guaranteeing a comprehensive comprehension of their frequency and therapeutic consequences. Additionally, the application of a random effects model in our meta-analysis allowed us to account for heterogeneity among studies, providing more accurate pooled estimates. The lack of publication bias, confirmed through Egger's test, further strengthens the validity of our results. Overall, our study offers a robust and detailed examination of exon 8 mutations, contributing valuable insights to the field of genetic research on Wilson Disease. However, the limitation of current study, the heterogeneity among the included studies, which may have impacted the accuracy of the pooled estimates. The results' applicability to different ethnic populations may be limited by the omission of articles written in languages other than English. Subtle biases in research selection or mutation nomenclature may still exist, despite efforts to assess publication bias having been made.

Conclusion

In current study, the results of our comprehensive analyzing presented the knowledge of mutations, especially in exon 8, functioned as the transmembrane domain, occurred on *ATP7B*. Consequently, the variations of c.2333G>A/T (p.R778Q/L), c.2305A>G (p.M769V), c.2336G>A (p.W779*), and c.2304dupC (p.M769HfsX26) as hotspot mutations on exon 8 for the present study.

Declaration

Ethics approval and consent to participate

None provided.

Consent for publication

N/A.

Author Contributions

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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Competing interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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