

# Relationship between genetic mutations and clinical phenotypes in patients with Wilson disease

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## Abstract

To study the relationship between genotype and clinical phenotype of major gene mutation sites in patients with Wilson disease (WD).

Clinical and laboratory data were collected from 40 children with WD admitted to the hospital by high-pass sequencing. The basic clinical data of patients included the following: age, sex, first symptom, K-F ring, clinical classification, serum Ceruloplasmin (CP), 24 hours urine copper. High Frequency Mutations were identified in WD patients: Exon 8, Ar9778Leu, and study the relationship between high frequency mutation and clinical phenotype.

The mutation frequency of 2333G>T(Arg778Leu) in Exon 8 was the highest (48%). The mutation frequency of Exon 13 at 2975C>T site was 29%. The age ( $t=0.296$ ,  $P=.768$ ), sex ( $\chi^2=0.005$ ,  $P=.944$ ), first symptom ( $\chi^2=0.480$ ,  $P=.449$ ), K-F ring ( $\chi^2=0.321$ ,  $P=.17$ ), clinical classification ( $\chi^2=20.064$ ,  $P>.969$ ), serum CP levels ( $t=0.007$ ,  $P=.897$ ) had no significant difference between Arg778Leu mutation group and non-Arg778Leu mutation group. Twenty-four-hour urinary copper levels ( $t=12.134$ ,  $P<.001$ ) in the Arg778Leu mutation group were higher than those in the Arg778Leu mutation group.

Arg778Leu mutation is associated with 24 hours urinary copper. The study of the association between the type of gene mutation and the clinical phenotype has important implications for the occurrence regularity, pathogenesis, and disease progression in patients with WD.

**Abbreviations:** CP = ceruloplasmin, WD = Wilson disease.

**Keywords:** clinical phenotype, copper metabolism, gene mutation, Wilson disease

## 1. Introduction

Wilson disease (WD) is an autosomal recessive single-gene disease caused by a mutation in the ATP7B gene,<sup>[1]</sup> characterized by a reduction of copper in Bile excretion. Excessive copper

deposits in organs, mainly in the liver and brain, causing liver, nerve, and mental symptoms.<sup>[2]</sup> The incidence of this disease is about 1/100,000 to 1/30,000.<sup>[3]</sup> WD is one of monogenic genetic diseases which can be treated effectively, and early diagnosis is very important. The early clinical manifestations of most patients are concealed or varied, WD course of disease developed quickly, if not timely intervention treatment is fatal.<sup>[4]</sup> The association between Wd genotypes and clinical phenotypes, both complex and diverse, has long been a hot topic in the WD. However, because of the low prevalence of WD itself, specific genotypes tend to be rarer. In this study, 40 WD patients with second-generation gene sequencing were used to collect the basic clinical data: Onset Age, sex, clinical typing, serum Ceruloplasmin (CP), 24 hours urine copper, K-F ring, etc. According to the result of gene detection, the high frequency mutation site of exon 8, 2333G>T(Arg778Leu), was screened out in WD patients. The high frequency mutation was used as the main index of genotype to study the relationship between gene mutation and clinical phenotype.

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QZ, MB, and KZ are co-first authors.

Ethics approval and consent to participate Ethical approval was obtained from the Regional Ethical Review Board in Jiangsu Province (2013/101-22), as was written informed consent from all included patients.

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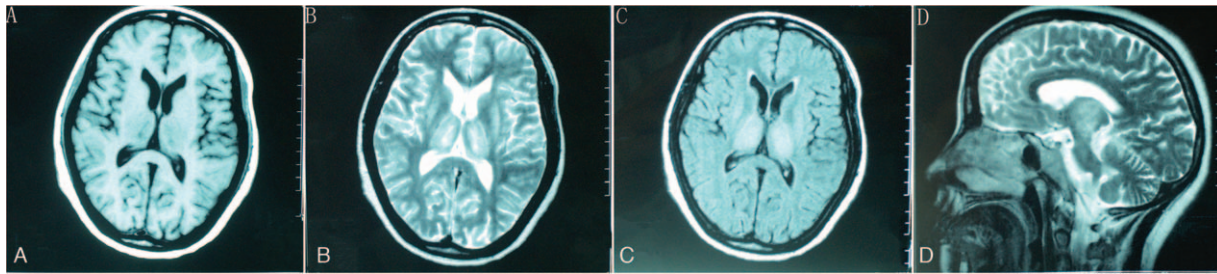
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## 2. Materials and methods

### 2.1. Clinical data

The clinical and laboratory data of 40 patients with WD determined by qualcomm sequencing were collected from January 2013 to December 2018 in Jiangsu Province. There were 24 males and 16 females. The age of onset was 5 to 35 years, among which 17 cases were  $\leq 15$  years old. The study was approved by the local ethics committee and all patients were informed by themselves or their guardians.



**Figure 1.** Magnetic Resonance Imaging of the brain in brain type Wilson's disease patients.

## 2.2. Diagnostic criteria and clinical classification

The diagnosis was based on the following criteria: signs of liver disease or neurological impairment; a decrease in serum CP (CP) ( $<0.2$  g/L); urine copper (24 hours)  $>100$   $\mu$ g; and the presence of a K-F ring under a slit lamp. The diagnosis is in accordance with the guidelines for the diagnosis and treatment of WD developed by the neurology branch of the Chinese Medical Association, which classify WD as liver type, brain type (Fig. 1), other type and mixed type.<sup>[5,6]</sup>

## 2.3. Genetic testing

Peripheral venous blood was collected and genomic DNA was extracted by Genomic DNA Extraction Kit (QIAamp DNA Blood MiniKit, Qiagen, Germany). Twenty-one exons of ATP7B gene were amplified, the primer sequence was referenced,<sup>[7]</sup> the PCR products were detected and recorded by automatic capillary (QIA xcel Advanced, Germany). The PCR product was purified and sequenced by Illumina HiSeq X-Ten platform (San Diego, USA). The results were compared with the normal sequence by Chromas software to find the mutation point.

## 2.4. Observation index

The clinical data of 40 patients including age, sex, first symptom, K-F ring, clinical classification, serum CP, 24 hours urine copper, liver function, abdominal color doppler ultrasound, abdominal CT, and Magnetic Resonance Imagine of the brain were analyzed retrospectively. Based on our DNA results from 40 WD patients, we found that it was consistent with a small number of hot-spot mutations, with other mutations showing sporadic distribution. The mutation frequency of 2333G>T in Exon 8 (Arg778Leu)

was found to be the highest (48%). Therefore, the high frequency mutation site was used as the main genotype index to study the relationship between high frequency mutation and clinical phenotype.

## 2.5. Statistical analysis

SPSS22.0 statistical analysis software (SPSS Inc., Chicago, IL) was used for statistical analysis. All the quantitative data were expressed by mean  $\pm$  standard deviation ( $\bar{x} \pm s$ ), and the statistical analysis of the count data was performed by  $\chi^2$  test.  $P < .05$  was considered statistically significant.

## 3. Results

### 3.1. Analysis of high frequency mutation sites

Table 1 shows the top 10 mutation sites for mutation frequency. The dominant mutation type was base substitution, which suggested that base substitution was the dominant mutation type in WD patients. The mutation frequency of 2333G>T (Arg778Leu) in Exon 8 was the highest (48%). The mutation frequency of Exon 13 at 2975C>T site was 29%.

### 3.2. Comparison of age, sex, first symptom and K-F ring between Arg778Leu mutation group and non-Arg778Leu mutation group

The age ( $t=0.296$ ,  $P=.768$ ), sex ( $\chi^2=0.005$ ,  $P=.944$ ), first symptom ( $\chi^2=0.480$ ,  $P=.449$ ), K-F ring ( $\chi^2=0.321$ ,  $P=.17$ ) had no significant difference between Arg778Leu mutation group and non-Arg778Leu mutation group (Fig. 2).

**Table 1**  
Analysis of mutation sites in the first 10 mutation frequencies.

Mutation site	Mutation type	Mutation result	Nucleotide change	Amino acid change	Functional domain of protein	Exons	Heterozygous or homozygous mutation
2333G>T	Substitution	Missense	CGG-CTG	Arg778Leu	TM4	8	Heterozygous
2975C>T	Substitution	Missense	CCC-CTC	Pro992Leu	bet TM6/Ph	13	Heterozygous
2755C>T	Substitution	Missense	CGG-TGG	Arg919Trp	bet Td/TM5	12	Heterozygous
3443T>C	Substitution	Missense	ATT-ACT	Ile1148Thr	ATPloop	16	Heterozygous
2604delC	Deletion	Premstop	ACCCGGA	Gly869GlufsX4ACCCGGA	bet Td/TM5	11	Heterozygous
2621C>T	Substitution	Missense	GCG-GTG	Ala874Val	bet Td/TM5	11	Heterozygous
2333G>A	Substitution	Missense	CGG-GAG	Arg778Gln	TM6	8	Heterozygous
2304dupC	Duplication	Premstop	CCCCATG	Met769HisfsX26	TM4	8	Heterozygous
3809A>G	Substitution	Missense	AAT-AGT	Asn1270Ser	ATP hinge	18	Heterozygous
2804C>T	Substitution	Missense	ACG-ATG	Thr935Met	TM5	12	Heterozygous

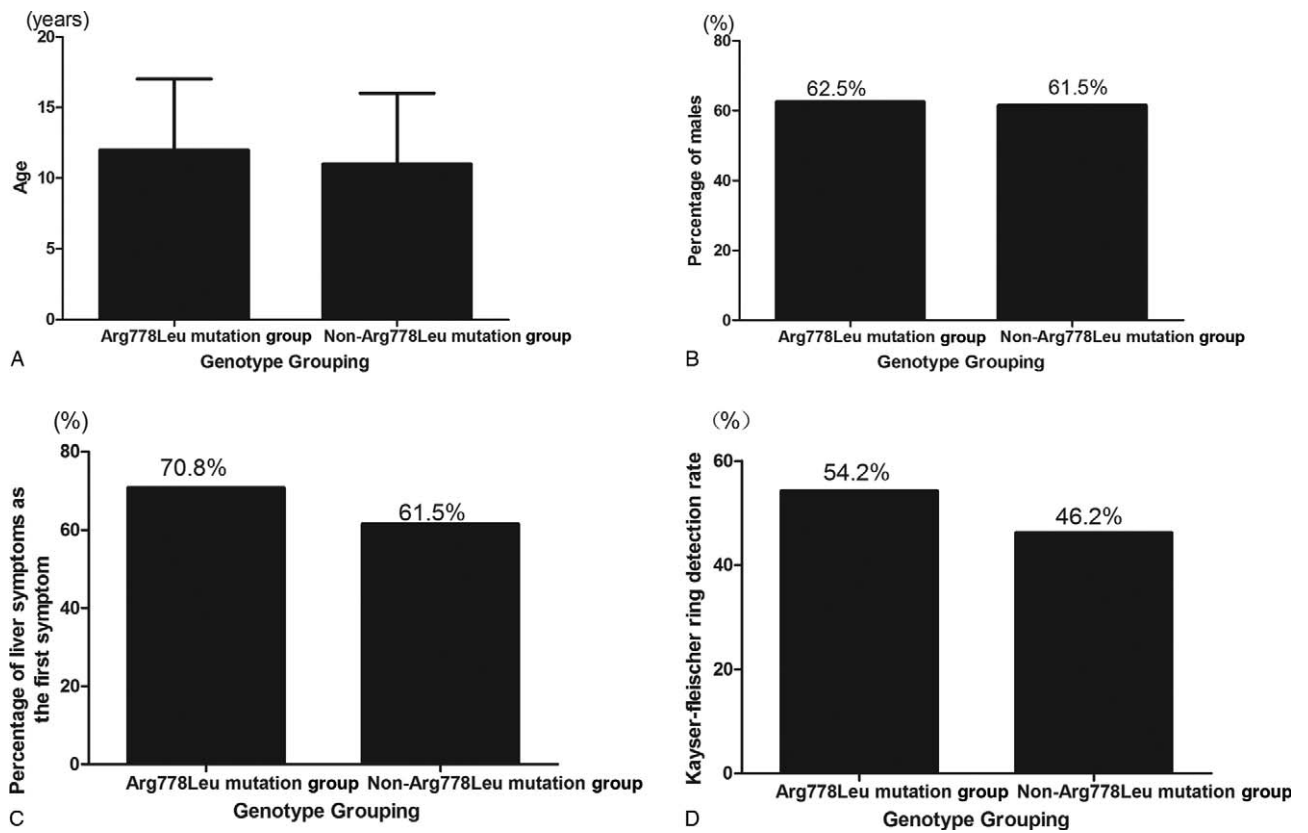


Figure 2. Comparison of age, sex, first symptom and K-F ring between Arg778Leu mutation group and non-Arg778Leu mutation group.

**3.3. Relationship between genotype and clinical typing of high frequency gene mutation sites in patients with WD**

Compared with the clinical classification of the patients with Arg778Leu mutation and the patients non-Arg778Leu mutation, the liver type patients with Arg778Leu mutation were higher than the patients non-Arg778Leu mutation, and the brain type and other type patients with non-Arg778Leu mutation were higher than the patients with Arg778Leu mutation. But there was no significant difference ( $\chi^2=20.064, P>.969, \text{Fig. 3}$ ).

**3.4. Association of genotype with CP and 24hours urinary copper in patients with high frequency gene mutation in WD**

Serum CP levels in the Arg778Leu mutation group were higher than those in the NON- Arg778Leu mutation group, but there

was no significant difference between the two groups ( $t=0.007, P=.897$ ). Twenty-four-hour urinary copper levels in the Arg778Leu mutation group were higher than those in the non-Arg778Leu mutation group. The statistical differences between the 2 groups were significant ( $t=12.134, P<.001, \text{Fig. 4}$ ).

**4. Discussion**

WD is a genetic mutation that causes a disorder in the P type copper transport enzyme (ATP7B), called genetic disorder. Most of the mutations of ATP7B were heterozygous, fewer homozygous mutations and more common missense mutations. The ATP7B gene is located in the 4-band 3 sub-band of the region 1 of the short arm of the chromosome 13 (13q14.3), and contains 21 exons and 20 introns. So, there is a lot of variation. More than

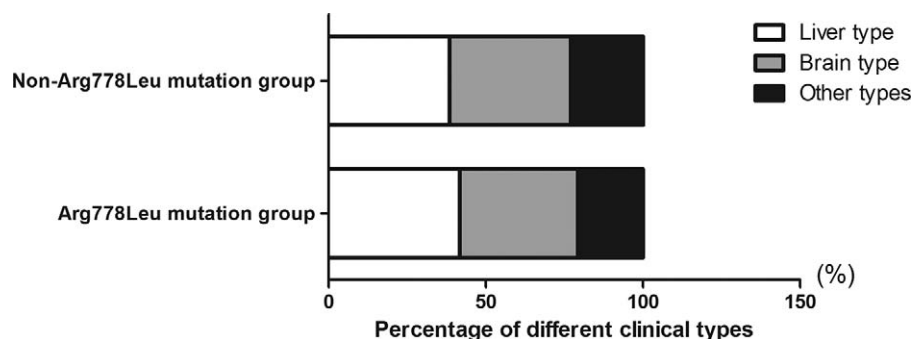


Figure 3. Relationship between genotype and clinical typing of high frequency gene mutation sites in patients with WD.

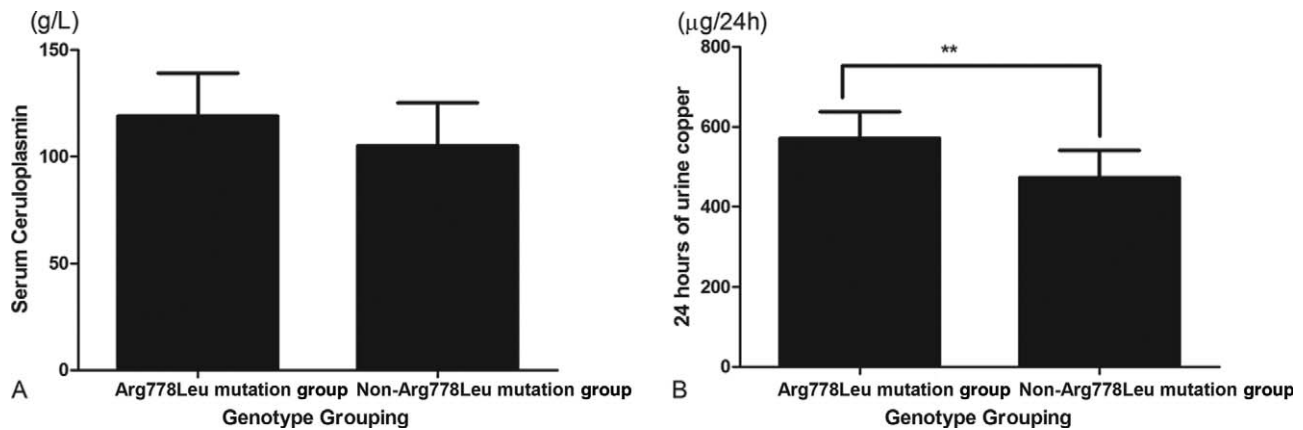


Figure 4. Association of genotype with Ceruloplasmin and 24 hours urinary copper in patients with high frequency gene mutation in WD.

500 mutations have been counted in the human genome database, of which more than 120 have been reported in the Chinese population. The ATP7B enzyme is a 1465 amino acid protein that contains 6 copper binding domains and 8 transmembrane domain.<sup>[8]</sup> According to the current research on ATP7B gene mutation, the main ATP7B gene mutation type and frequency are not the same in different regions.<sup>[9]</sup> Point Mutation was the main exon mutation of ATP7B gene in Europe, and His1069Gln was the high frequency mutation, the mutation rate was 35% to 45%.<sup>[10]</sup> The 2333 locus of Exon 8, Arg778Leu, is the most important mutation of ATP7B, which has been found in Asia.<sup>[11]</sup> The ATP7B gene of 40 clinically diagnosed patients was amplified and sequenced. The mutation frequency of 2333G>T (Arg778Leu) in Exon 8 was the highest. The 2333 Locus Arg778Leu mutation in Exon 8 is the most common mutation locus in Chinese WD. Our results are consistent with this finding. And the missense mutation of this site has changed the structure of the transmembrane region of ATP7B gene and seriously affected the function of WD Protein.<sup>[12]</sup> Chloe et al showed that the allele frequency of Pro992Leu in Exon 13 of WD patients was as high as 13.4% in the coastal areas of southern China.<sup>[13]</sup> This study also demonstrated a high frequency of mutations at the 2975C→T locus in Exon 13 of the ATP7B gene in patients with WD, with a mutation rate of 29%.

With the discovery of more and more mutation sites, some scholars began to speculate whether the type of gene mutation in WD patients is related to clinical phenotype. So, the study of the relationship between the type of gene mutation and the clinical phenotype has become a hot topic in the WD. The clinical manifestations of WD patients are highly heterogeneous, the genotype-phenotype correlation is uncertain, the onset age is broad, the clinical manifestations are diverse, and may be related to a specific genotype.<sup>[14]</sup> Race, environment, copper tolerance and genetic factors all affect the gene mutation to produce different phenotypes.<sup>[15]</sup> At present, it is reported that the mutation of Exon 13 Pro992Leu is closely related to the clinical phenotype of liver type. There was no significant correlation between Pro992Leu Mutation and clinical type, sex, K-F ring, serum CP concentration, Alanine transaminase concentration, 24 hours urine copper content and age of onset.<sup>[16]</sup> The effect of Arg778Leu Mutation on ATP7B gene and clinical phenotype is still controversial in China. The Arg778Leu mutation in Chinese population is associated with liver type, and some studies have

found no association between Arg778Leu mutation and clinical type, sex, etc.<sup>[17]</sup> The results showed that the mutation of Pro992Leu involved the Ch / Tm6region, which resulted in the structural change of Transmembrane region, and then affected the transport of copper. A national study published in 2016 included 1222 WD patients from across the country.<sup>[18]</sup> The sample size was by far the largest in the country, but only 88 mutations were detected, the first three being p. R778L (32.4%), p. P992L (18.5%) and p. S406A (1.84%). Some researchers have found that WD patients with nonsense and frameshift mutations have significantly lighter symptoms and slower disease progression than those with missense mutations. Thomas found that the type of mutation affected the age of onset and the major affected organs.<sup>[19]</sup> Stapelbroek found that His1069Arg homozygous mutation was associated with age at onset and neurological symptoms in patients with WD.<sup>[20]</sup> Shah Ab et al showed that the clinical phenotype of His1069Gln mutation in Exon 14 was not significantly associated with the mutation compared with other WD patients.<sup>[21]</sup> Our study found a statistically significant difference between the Arg778Leu Mutation and the non-Arg778Leu mutation in 24 hours urinary copper ( $t=12.134$ ,  $P<.001$ ), the 24 hours urinary copper level in the Arg778Leu mutation group was lower than that in the non-Arg778Leu mutation group. There was no significant difference between Arg778Leu mutation and non-Arg778Leu mutation in clinical type, sex, age, first symptom, K-F ring, serum CP. So, it is suggested that Arg778Leu mutation is associated with 24 hours urinary copper.

## 5. Conclusions

Therefore, there is no consensus on the correlation between genotype and clinical phenotype in WD patients. Wu et al found that Arg778Leu mutation affected the age of onset in patients with WD, and that the age of onset in patients with homozygous mutation was significantly lower than that in patients with heterozygous mutation. Furthermore, Serum CP levels in homozygous mutation of Arg778Leu were lower than those in heterozygous of Arg778Leu.<sup>[22]</sup> Some studies also found no significant association between the high frequency mutation Arg778Leu and the onset age, clinical type, sex, K-F ring, serum CP concentration and 24 hours urinary copper volume.<sup>[23]</sup> Because of the diversity of genetic mutations and clinical

manifestations of WD, and the disease in different ethnic groups and regions have significant differences in genetic mutations, therefore, there is no unified understanding of the correlation between gene and clinical phenotype. But the study of the association between the type of gene mutation and the clinical phenotype has important implications for the occurrence regularity, pathogenesis, and disease progression in patients with WD. And it also lays the foundation for more effective treatments.

### Author contributions

**Data curation:** Keyu Zhu, Jing Wang.

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**Writing – original draft:** Qingwen Zhu, Keyu Zhu.

**Writing – review & editing:** Qingwen Zhu, Keyu Zhu.

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