

# Clinical and brain MR imaging effectively influences the ATP7B genotype of Neurologic Wilson patients

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## Research article

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# Abstract

## Objective

To understand the relationship between the two types of mutations in patients with Wilson disease (WD) and clinical practice, and to search for the clinical biological markers of the two types of mutations.

## Methods

The hospitalized patients, who were in the affiliated hospital of neurology institute of anhui university of traditional Chinese medicine from May 2014 to May 2019, with p. arg778leu or p. pro992leu homozygous mutation type of neurologic WD, were selected and underwent demographic, clinical manifestations, serological indicators and brain MR imaging(MRI) data were analyzed to compare the differences of the two mutant types of neurologic WD.

## Results

The group of 103 patients with neurologic WD join in this research, including p.A rg778Leu mutant WD 65 cases and p.P ro992Leu mutant WD 38 cases. The two types of mutations in the WD demographic, clinical manifestation and most serological index indifference, and brain MRI findings have significant differences, especially the p.A rg778Leu mutant WD damage the thalamus( $\chi^2 = 17.834$ ,  $P < 0.001$ ), midbrain( $\chi^2 = 12.579$ ,  $P < 0.001$ ) and pons( $\chi^2 = 10.605$ ,  $P = 0.001$ )p.P ro992Leu mutant WD have obvious difference, the results of multivariate analysis were also different ( $P < 0.05$ ).

## Conclusions

The demography, clinical features and serology of neurologic WD have nothing to do with its gene mutation type, and the MRI manifestations of brain are related to its gene mutation type, among which the ATP7B gene p.arg778leu mutation is more likely to involve thalamus, midbrain and pons.

## Background

Wilson disease (WD) is an autosomal recessive disorder of human copper metabolism[1–2], caused by pathogenic variants in the copper-transporting gene ATP7B, leads to intracellular copper accumulation, causing damage to many organs, especially the brain[3–4]. With the development of medical technology, the diagnosis problem of WD has been basically solved[5]. With the application and development of liver transplantation, the curative effect of patients with liver type WD or severe liver type WD has been greatly improved[6–7], but the treatment of neurologic WD has always been a difficult problem for clinicians to solve.

Complicated clinical symptoms of neurologic WD, diverse brain damage sites, high death and disability rate, seriously affect the quality of life of WD patients[8–11]. At present, the classic drugs of WD are penicillamine and trientine, but in the treatment of neurologic WD, 25–40% of the neurological symptoms

are aggravated and difficult to recover, which seriously affects the prognosis[12–14]. So far, the treatment of WD is a difficult problem that clinicians are eager to solve, although numerous studies have confirmed that the brain damage of WD may have other mechanisms[15–17], however, ATP7B gene mutation is the root cause, and other mechanisms should be secondary, so studying the relationship between ATP7B gene mutation type and clinical practice is the most direct and most likely to reveal the treatment problems of cerebral WD.

The ATP7B gene mutation type of WD has obvious regional and ethnic differences, for example, the ATP7B gene mutation type in Europe is mostly H1069Q(46.9%), followed by p767p-fs (2.85%)[18]; His1070Gln(28%) is the most common ATP7B mutation in the Americas, followed by Gly1267Lys(10%)[19], but in Asia, p.arg778leu (31.8%) was the most common ATP7B mutation, followed by p.p992leu (18.54%)[20]. Therefore, it is of great significance to study the relationship between the two most common ATP7B genotype of neurologic Wilson patients and the clinical and brain MRI.

## Methods

### Study Participants

The study enrolled 103 patients, who were diagnosed with WD at the affiliated hospital of neurology institute of anhui university of traditional Chinese medicine, from May 2014 to May 2019.

### Diagnostic criteria

The criteria used for recruitment to this study, as is standard for WD diagnoses, were as follows[14, 21–22].

- (1) Family heredity: parental consanguinity, having a sibling with WD, or having a sibling who died from unexplained liver disease.
- (2) Presence of an extra-corticospinal tract symptom such as dystonia, parkinsonism, chorea, torsion spasm, or liver symptoms.
- (3) Kayser–Fleisher rings visible to the naked eye or with slit-lamp examination.
- (4) Ceruloplasmin level < 2.16  $\mu\text{mol/L}$  or serum copper oxidase level < 0.20 ODU (optical density units).
- (5) Urinary copper excretion > 1.6  $\mu\text{mol}$  every 24 hours.
- (6) Hepatic copper concentration via needle biopsy > 250  $\mu\text{g/g}$ .

Participants fulfilling both criteria #1 and #4 or both criteria #2 and #4 were diagnosed with symptomatic WD. Participants fulfilling both criteria #3 and #5, only criterion #4, or only criterion #6, were diagnosed with asymptomatic WD. Only symptomatic WD patients were included in the cohort of this study.

# The standard set

(1) Accord with the diagnostic criteria of WD [14, 21–22].

(2) Clinical symptoms of brain injury were mainly presented, such as dystonia, tremor, and choreoathetosis, et al, and MRI of brain showed evidence of injury at one or more sites [22].

(3) Genetic examination revealed homozygous mutations in the ATP7B gene p.arg778leu or p.pro992leu(Fig. 1) [20].

## Study Measures and Group Analysis

Retrospective statistics on demography, clinical manifestations, serological indicators and craniocerebral MRI data of patients with cerebral WD who met the inclusion criteria were conducted to compare the differences between the two mutant types of neurologic WD.

## Standard protocol approvals, registrations and patient consents

Informed consent was obtained from each patient or his/her parents.

## Statistical Analyses

The description of classified data is represented by sample size (%), and the comparison between data is represented by chi-square test (or exact probability method). The description of the quantitative data with normal distribution to  $\bar{X} \pm S$ , analysis using two independent sample t-test; Quantitative data that do not conform to normal distribution are expressed by M (P25, P75) and analyzed by non-parametric test. Multivariate logistic regression analysis was carried out for single-factor significant variables, and the regression analysis results were drawn into a forest map by using R software. This analysis was mainly conducted in SPSS16.0, Graphpad 5.0 and R software.

## Results

### 1. Comparison Of the ATP7B genotype with neurologic WD patients and clinical characteristics

In this study, a total of 103 patients with neurologic WD were enrolled, including 65 cases of p. arg778leu genotype WD and 38 cases of p. pro992leu genotype WD, 65 cases of males and 38 cases of females. There was no difference in the demography and clinical manifestations of p. arg778leu and p. pro992leu genotypes WD, and the specific situation was shown in Table 1.

Table 1

Comparison Of the ATP7B genotype with Neurologic WD patients and clinical characteristics

	p. Arg778Leu	p.Pro992Leu	$\chi^2/Z$	P
Gender (%)			0.000	0.993
Male	41 (63.1)	24 (63.2)		
Female	24 (36.9)	14 (36.8)		
Disage (M (P <sub>25</sub> , P <sub>75</sub> ))	15.0 (11.5, 19.0)	16.0 (11.8, 19.3)	0.798	0.425 <sup>b</sup>
Longdis (M (P <sub>25</sub> , P <sub>75</sub> ))	2.0 (1.0, 4.0)	2.0 (1.8, 3.0)	0.612	0.540 <sup>b</sup>
Firstsign				0.056 <sup>a</sup>
Dystonia	42 (64.6)	15 (39.5)		
Tremor	14 (21.5)	16 (42.1)		
Choreoathetosis	3 (4.6)	4 (10.5)		
Mental symptoms	6 (9.2)	3 (7.9)		
B ultrasonic Liver			1.303	0.254
Fatty liver	11 (16.9)	10 (26.3)		
Liver cirrhosis	54 (83.1)	28 (73.7)		
Gallbladder			0.299	0.861
Normal	25 (38.5)	16 (42.1)		
Cholecystitis	29 (44.6)	17 (44.7)		
Gallstone	11 (16.9)	5 (13.2)		
Spleen			2.718	0.099
Normal	10 (15.4)	11 (28.9)		
Enlargement	55 (84.6)	27 (71.1)		
Kidney				0.378 <sup>a</sup>
Normal	25 (38.5)	20 (52.6)		

Note: a,Fisher's exact probability method finds P;b Nonparametric test for z-value M (P<sub>25</sub>, P<sub>75</sub>) Median (lower quartile, upper quartile).

	p. Arg778Leu	p.Pro992Leu	$\chi^2/Z$	P
Nephrosis	33 (50.8)	15 (39.5)		
Kidney stone	7 (10.8)	3 (7.9)		
Kayser-Fleischer rings				0.763 <sup>a</sup>
(+)	56 (86.2)	34 (89.5)		
(-)	9 (13.8)	4 (10.5)		
Note: a,Fisher's exact probability method finds P;b Nonparametric test for z-value M (P25, P75) Median (lower quartile, upper quartile).				

## 2.Comparison Of the ATP7B genotype with neurologic WD patients and brain MRI

The MRI findings of the two genotypes of WD showed no differences in the lesion of putamen, globus pallidum, caudate nucleus, cerebral cortex and cerebellum, but there were significant differences in the WD of p.arg778leu genotype in thalamus ( $\chi^2 = 17.834$ ,  $P < 0.001$ ), middle brain ( $\chi^2 = 12.579$ ,  $P < 0.001$ ), pons( $\chi^2 = 10.605$ ,  $P = 0.001$ ) and encephalatrophy ( $\chi^2 = 4.186$ ,  $P = 0.041$ ) compared with that of p.pro992leu genotype(Fig. 2), as shown in Table 2.

Table 2  
Comparison Of the ATP7B genotype with neurologic WD patients and brain MRI

	p. Arg778Leu(%)	p.Pro992Leu(%)	$\chi^2$	P
Putamen			0.272	0.602
Normal	11 (16.9)	8 (21.1)		
Abnormal	54 (83.1)	30 (78.9)		
Globus pallidus				0.098 <sup>a</sup>
Normal	63 (96.9)	33 (86.8)		
Abnormal	2 (3.1)	5 (13.2)		
Caudate nucleus				0.066 <sup>a</sup>
Normal	60 (92.3)	30 (78.9)		
Abnormal	5 (7.7)	8 (21.1)		
Thalamus			17.834	< 0.001
Normal	56 (86.2)	18 (47.4)		
Abnormal	9 (13.8)	20 (52.6)		
Midbrain			12.579	< 0.001
Normal	34 (52.3)	33 (86.8)		
Abnormal	31 (47.7)	5 (13.2)		
Pons			10.605	0.001
Normal	30 (46.2)	30 (78.9)		
Abnormal	35 (53.8)	8 (21.1)		
Cerebral cortex				1.000 <sup>a</sup>
Normal	57 (87.7)	34 (89.5)		
Abnormal	8 (12.3)	4 (10.5)		
Encephalatrophy			4.186	0.041
Normal	52 (80.0)	36 (94.7)		
Note: a,Fisher's exact probability method finds P.				

	p. Arg778Leu(%)	p.Pro992Leu(%)	$\chi^2$	P
Abnormal	13 (20.0)	2 (5.3)		
Cerebellum				0.707 <sup>a</sup>
Normal	61 (93.8)	35 (92.1)		
Abnormal	4 (6.2)	3 (7.9)		
Note: a,Fisher's exact probability method finds P.				

### 3.Comparison Of the ATP7B genotype with neurologic WD patients and serological indicator

Serological comparison of the two genotypes of WD showed no significant difference in serum copper biochemistry, liver function and renal function except ALT(Z /t = 2.239<sup>b</sup>,P = 0.025), as shown in Table 3.

Table 3

Comparison Of the ATP7B genotype with neurologic WD patients and serological indicator

	p. Arg778Leu	p.Pro992Leu	Z /t	P
	M (P <sub>25</sub> , P <sub>75</sub> )	M (P <sub>25</sub> , P <sub>75</sub> )		
biochemical copper Cu	2.2 (1.7, 3.5)	2.3 (1.8, 3.3)	0.062 <sup>b</sup>	0.951
Sco	0.03 (0.02, 0.04)	0.03 (0.02, 0.04)	1.224 <sup>b</sup>	0.221
CP	46.8 (37.0, 57.3)	44.3 (35.0, 54.5)	0.670 <sup>b</sup>	0.503
Liver function TBIL	13.4 (10.1, 21.8)	15.2 (12.0, 18.3)	0.342 <sup>b</sup>	0.733
DBIL	4.2 (2.4, 5.9)	3.8 (2.8, 4.8)	0.383 <sup>b</sup>	0.702
TP	66.75 ± 5.35	68.61 ± 6.11	1.613 <sup>a</sup>	0.110
ALB	43.8 (40.9, 46.5)	45.2 (43.8, 47.9)	2.239 <sup>b</sup>	0.025
GLB	23.0 (20.2, 25.1)	22.8 (20.4, 24.9)	0.232 <sup>b</sup>	0.816
ALT	24.0 (16.0, 38.0)	24.5 (12.5, 39.0)	0.417 <sup>b</sup>	0.677
Kidney function BUN	4.7 (3.5, 5.5)	4.9 (4.0, 5.3)	0.263 <sup>b</sup>	0.792
Cr	48.3 (40.6, 70.2)	50.9 (39.5, 62.6)	1.063 <sup>b</sup>	0.288
Note: a,T value obtained by t test of two independent samples;b□Z value obtained by nonparametric test.				

## 4. Multivariate analysis Of the ATP7B genotype with neurologic WD patients

Multivariate logistic regression analysis of univariate significant variables showed no difference in serological index ALB(P = 0.068) and encephalatrophy (P = 0.392) of craniocerebral MRI, but there were statistically significant differences in thalamus (P < 0.001), midbrain (P = 0.043) and pons (P = 0.004), as shown in Table 4. At the same time, R software was used to draw the regression analysis results into a forest map (Fig. 3).

Table 4  
Multivariate analysis Of the ATP7B genotype with neurologic WD patients

	$\beta$	OR (95% CI)	P
Thalamus	2.581	13.214 (3.534, 49.413)	< 0.001
Midbrain	-1.368	0.255 (0.068, 0.956)	0.043
Pons	-1.874	0.154 (0.043, 0.551)	0.004
Encephalatrophy	-0.749	0.473 (0.085, 2.630)	0.392
ALB	0.115	1.122 (0.992, 1.270)	0.068

## Discussion

WD patients nervous/mental symptoms as the main clinical manifestations accounted for about 40% ~ 50%[23], brain MRI abnormalities found rate at about 64.2%[24], nervous/mental symptoms as the main symptoms of neurologic WD patients is relatively heavy, serious impact on the prognosis of patients, WD guidelines explicitly pointed out that in 2008 the United States[14], flooding copper drugs for patients with severe brain injury symptoms of WD already exists tend to work hard, prognosis is poorer, therefore, the treatment of patients with cerebral WD is still a problem clinicians need to be addressed[25]. In this study, 1222 cases of WD patients with ATP7B gene sequencing results have been reported[20], with 88 mutation forms. The most common mutation type is p.arg778leu (31.8%), followed by p.pro992leu (18.54%), etc. Therefore, the study on the relationship between the mutation types of p.arg778leu and p.pro992leu of ATP7B gene and clinical and cerebral MRI is of great significance for the diagnosis and treatment of cerebral WD.

Since the pathological gene of WD has been confirmed, many studies have been trying to explain the individual difference of WD's disease by using genetics, such as the influence of gender on WD[24]. Recently, it was reported that WD age and gender have nothing to do with the mutation type of gene [18]. In this study, the mutation types of p.arg778leu and p.pro992leu of ATP7B gene were not related to gender and age, and there was no difference in the initial symptoms of brain injury, liver, gallbladder, spleen and kidney injury, and K-F rings of cornea. This study further confirmed that the demography and clinical manifestations of WD were not related to the mutation type of gene.

Craniocerebral MRI examination is an important examination of WD [26–29], and numerous brain MRI examinations related to WD all suggest that the putamen is the most common site of injury [22, 27, 30], and brain injury is related to its disease course and age [22]. There was no correlation between ATP7B mutation type and brain MRI, and the data in this study showed no difference between the two kinds of mutations, but there were significant differences in the WD induced by p.arg778leu genotype in thalamus ( $\chi^2 = 17.834$ ,  $P < 0.001$ ), midbrain ( $\chi^2 = 12.579$ ,  $P < 0.001$ ), pons ( $\chi^2 = 10.605$ ,  $P = 0.001$ ) and

encephalatrophy ( $\chi^2 = 4.186$ ,  $P = 0.041$ ) compared with that of p.p992leu genotype. It suggests that the different brain injury sites may be the specific marker of ATP7B gene mutation type, which needs to be confirmed by more studies.

Serological indicators of WD have important value in diagnosis and efficacy evaluation [31–34], but the correlation with gene mutation types has not been reported. Single-factor analysis of blood copper biochemistry, liver function and kidney function in the data of this group indicated that other indicators had nothing to do with gene mutation type except ALB, and further multi-factor analysis found that ALB had nothing to do with gene mutation type, so serological indicators of WD had nothing to do with gene mutation type.

Finally, for single factor meaningful variables multivariable logistic regression analysis, regression analysis and the result was drawn into the forest with R software diagram (Fig. 1), found that brain atrophy and propagated has nothing to do with gene mutation type, but the thalamus ( $P < 0.001$ ), the midbrain bridge ( $P = 0.043$ ) and brain ( $P = 0.004$ ) related to gene mutation type, whether to prompt the thalamus, midbrain and bridge brain damage is ATP7B gene p.A rg778Leu mutant of specific performance, subject to further research.

The defect of this study is that it only studied the two most common gene mutation types and clinical and brain MRI studies in China, without a comprehensive study on the gene mutation types in China, and no clinical and brain MRI comparison and meta-analysis with the most common mutation types in Europe and America.

## Conclusions

The demography, clinical features and serology of neurologic WD have nothing to do with its gene mutation type, and the MRI manifestations of brain are related to its gene mutation type, among which the ATP7B gene p.arg778leu mutation is more likely to involve thalamus, midbrain and pons.

## Abbreviations

WD: Wilson disease; ATP7B: copper-transporting P-type ATPase; MRI: MR imaging.

## Declarations

### Ethics approval and consent to participate

This study was approved by the ethics committee of Anhui University of Chinese Medicine and all participants signed informed consent.

### Consent for publication

Not applicable.

## Availability of data and materials

The datasets are available from the corresponding author on reasonable request.

## Competing interests

All authors declare they have no conflicts of interest.

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There are no Funding Sources in this study.

## Authors' Contributions

X Y and J W designed this study and J W obtained funding. J W performed this study and wrote manuscript. J W and X Y analyzed data and revised manuscript. Y H and M Y recruited and screened participants. All authors read and approved the final version of the manuscript.

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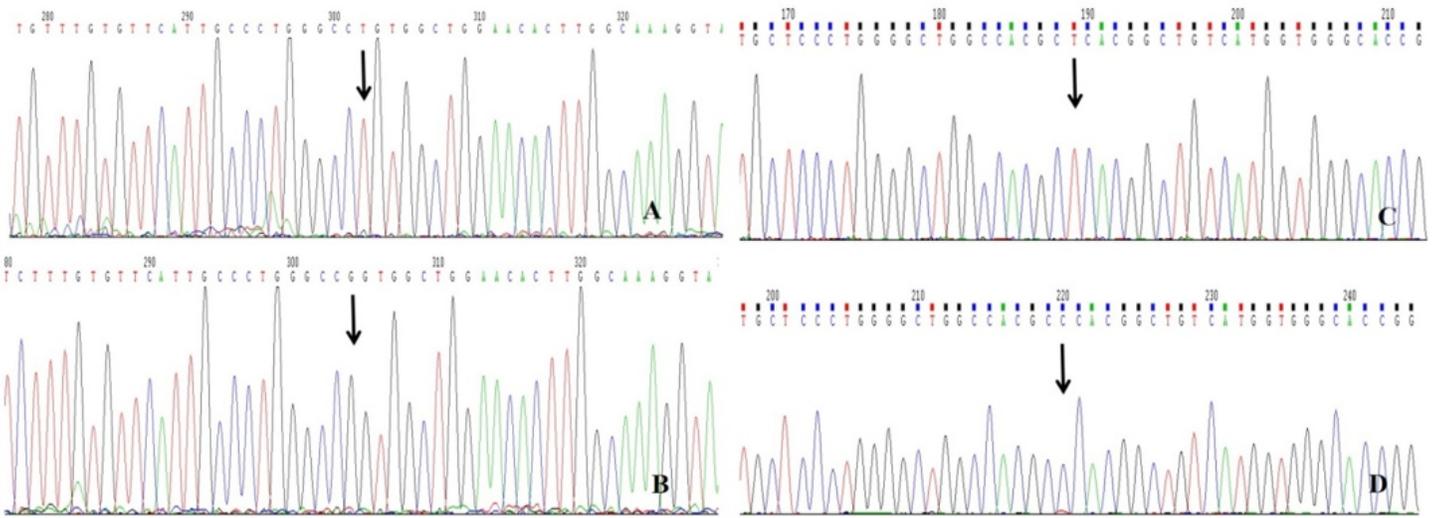
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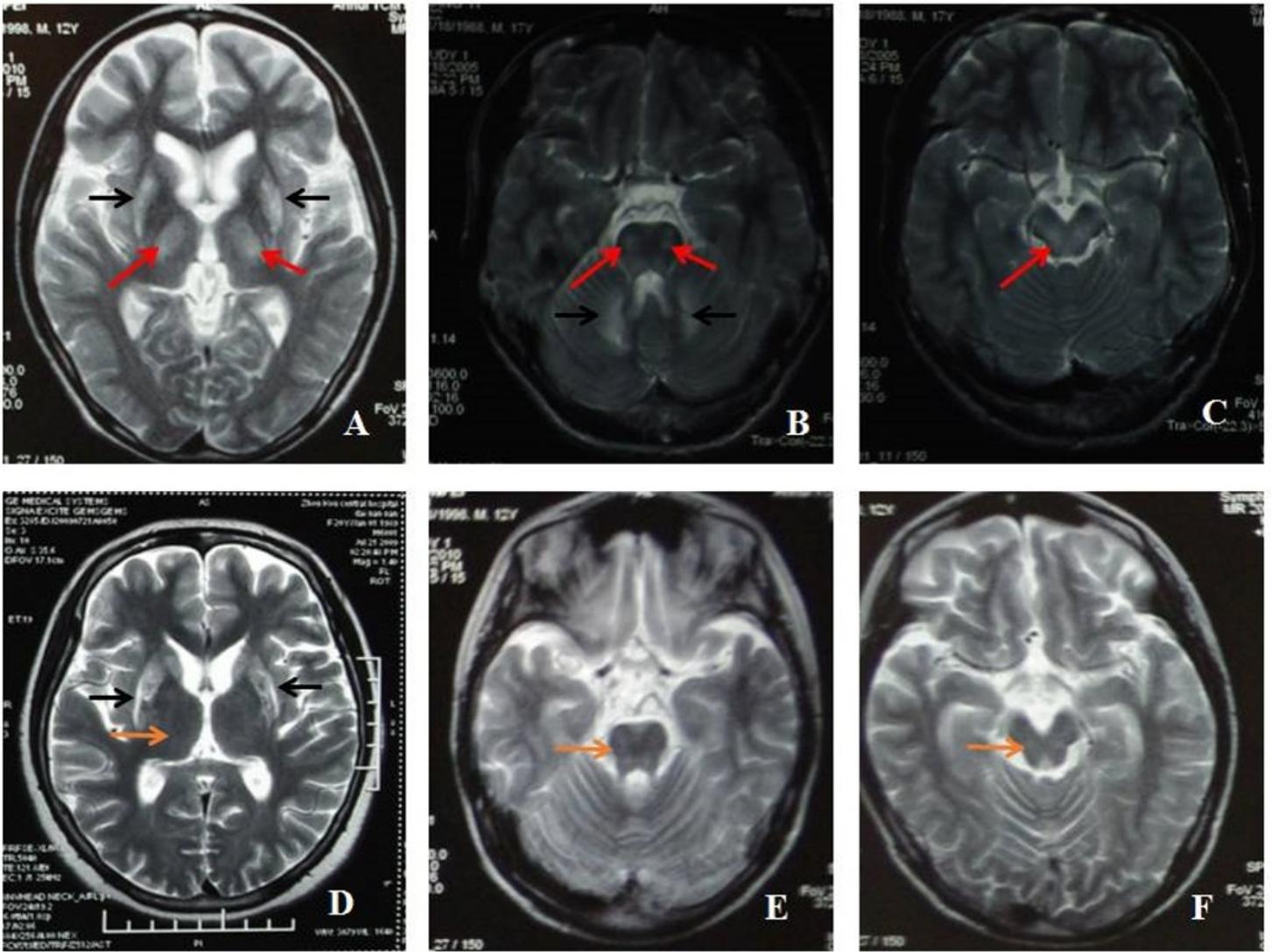
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## Figures



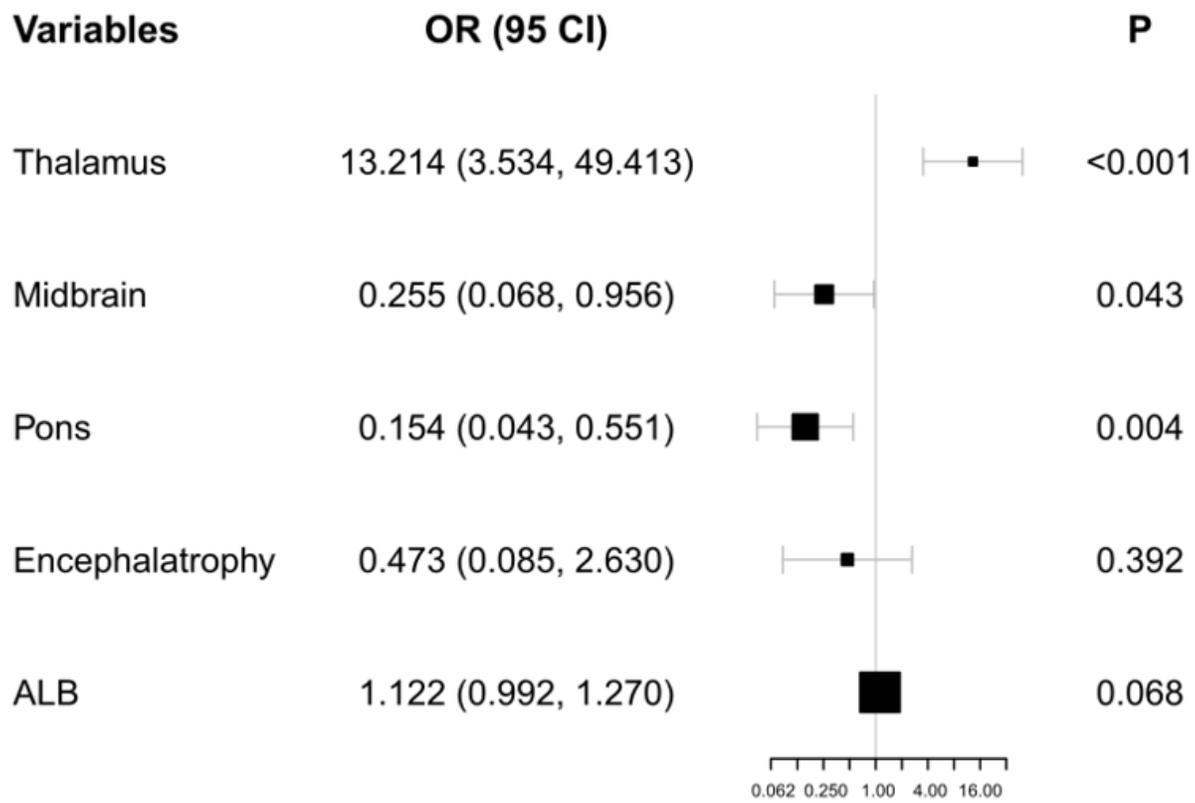
**Figure 1**

A, the homozygous mutation of Arg778Leu (c.2333G >t) in EXON8; B, the normal control; C, the homozygous mutation of p.Pro992leu (c.2975c >t) in EXON13; D, the normal control. (Black arrow)



**Figure 2**

A-C was the homozygous mutation patient of Arg778Leu (c.2333G >t) in EXON8, D-F was the homozygous mutation patient of p.Pro992leu (c.2975c >t) in EXON13. A, putamen lesions (black arrows) and thalamic lesions (red arrows); B, pons lesions (red arrows) and cerebellar lesions (black arrows); C, midbrain lesions (red arrow); D, putamen lesion (black arrows) and normal thalamus (yellow arrow); E, normal pons (yellow arrow); and F, normal midbrain (yellow arrow).



**Figure 3**

Multi-factor analysis of forest plots in the ATP7B genotype with neurologic WD patients