

Short report

High frequency of two mutations in codon 778 in exon 8 of the ATP7B gene in Taiwanese families with Wilson disease

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Abstract

The gene for Wilson disease (WD) has been cloned as a P type copper transporter gene (ATP7B). To elucidate the possible genetic mechanism for the diversity of clinical manifestations, we characterised 22 Taiwanese families with WD by microsatellite haplotyping of close DNA markers D13S314-D13S301-D13S316. We also screened for mutations of codon 778 in the transmembrane region. There were at least 15 haplotypes within eight broad subgroups observed among 44 WD chromosomes. Among the 22 unrelated patients, we found that six patients (27%) carried a codon 778 mutation. Nucleotide sequence analysis showed there were two different mutations: the previously reported Arg778Leu mutation in four patients and Arg778Gln, a new mutation, in two patients. The two different mutations of the same codon occurred in two distinct microsatellite haplotypes.

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Key words: ATP7B gene; haplotypes; Taiwan.

Wilson disease (WD) is a rare autosomal recessive disorder of copper transport.¹ Genetic studies showed linkage of the disease locus to the esterase D and retinoblastoma genes^{2,3} and to DNA markers in the 13q14 region.⁴ Microsatellite markers closely flanking the locus have been reported.⁵⁻⁷ Recently, a putative copper transporting P type ATPase (ATP7B) gene has been cloned and is defective in WD.^{8,9} The gene shows remarkable sequence homology to the gene for Menkes disease (ATP7A), another disorder of copper transport.¹⁰⁻¹² The proteins encoded by these genes belong to the P type ATPase family of cation transporters.¹³ Subsequently, more than 25 mutations, including small insertions and deletions, missense, nonsense, and splice site mutations have been described.^{8,9,14} The prevalence of mutations in

the Chinese population has not been extensively studied, although an Arg778Leu* missense mutation has been reported in two Chinese pedigrees from Hong Kong.¹⁴ We have shown linkage of WD to the chromosome 13q14 region, as reported for other populations, suggesting the monogenic nature of the disease.¹⁵ Since the variability of clinical manifestation in the Taiwanese pedigrees with WD^{16,17} might possibly be explained by genetic heterogeneity,^{14,18} we examined the microsatellite haplotypes and screened for the reported Chinese mutation in Taiwanese families. DNA was isolated from leucocytes from 25 patients in 22 families. The diagnosis was established by the detection of Kayser-Fleischer rings or abnormal liver function tests in the presence of low caeruloplasmin levels.¹⁵ Microsatellite (CA) dinucleotide repeats at loci D13S301, D13S314, D13S316, and D13S133 were analysed according to previous reports.^{6,7} Screening for the previously described codon 778 mutation in exon 8 of the ATP7B gene was performed by polymerase chain reaction (PCR) based restriction fragment length polymorphism (RFLP) analysis.¹⁴ The mutations were confirmed by direct nucleotide sequence analysis with an AmpliCycle™ sequencing kit (Perkin Elmer, NJ). The haplotypes carried on the WD chromosomes are listed in the table in the order of prevalence. The A haplotype was significantly higher in patients with WD in Taiwan ($p < 0.015$, χ^2 test). Among the 25 patients tested, eight patients in six (27.3%) pedigrees showed an absence of the *MspI* restriction site in the amplified fragments, suggesting a mutation in codon 778. The nucleotide change was verified by direct sequence analysis. Interestingly, two different mutations were found. Five patients carried a G2273T mutation which resulted in the Arg778Leu mutation, while the other three patients carried a G2273A mutation which resulted in Arg778Gln (figure) in exon 8 of the ATP7B gene. These codon 778 mutations have not been reported in white populations. The frequency of the Arg778Leu mutation in a total of 44 independent chromosomes examined was 11.4%, and that of Arg778Gln was 9.1%. In addition to the missense mutation in exon 8,

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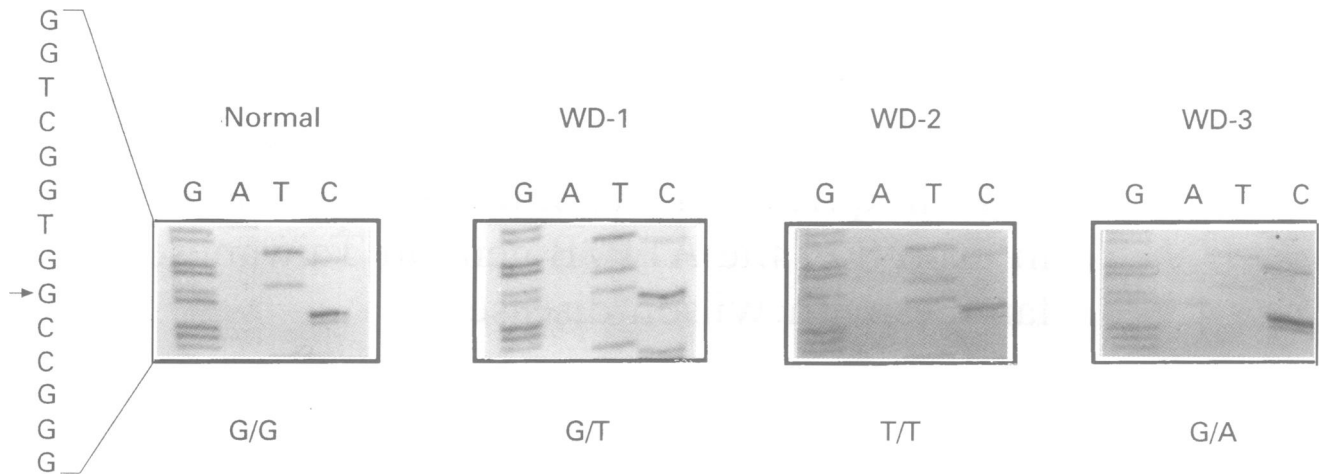
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* Previously described as Arg779Leu. Three erroneous nucleotides at positions 1039, 1044, and 1056 have been deleted from Thomas *et al*¹⁴ resulting in one less amino acid in the designation.



Nucleotide sequence analyses of mutations in codon 778 of the *ATP7B* gene. Substitutions of nucleotide 2273 (arrow) were studied in a normal subject and three patients, a heterozygote (WD-1) and homozygote (WD-2) for G2273T mutation, and a heterozygote (WD-3) for G2273A mutation, respectively.

Common haplotypes in 22 Taiwanese families with WD

	D13S314	D13S301	D13S316	WD chromosomes		Normal chromosomes		p
				No	%	No	%	
A	141 (11)	148 (2)	144 (6)	9		3		
	141 (11)	150 (1)	144 (6)	6		2		
	141 (11)	152 (11)	144 (6)	2		1		
Subtotal				17	38.6	6	13.6	<0.015
B	150 (7)	144 (4)	151 (2)	7		2		
	148 (8)	144 (4)	151 (2)	1		1		
Subtotal				8	18.2	3	6.8	
C	150 (7)	144 (4)	144 (6)	4		3		
	148 (8)	144 (4)	144 (6)	1		2		
	150 (7)	148 (2)	144 (6)	1		3		
	150 (7)	142 (5)	144 (6)	0		1		
Subtotal				6	13.6	9	20.5	
D	148 (8)	144 (4)	147 (4)	4		3		
	148 (8)	142 (5)	147 (4)	1		0		
Subtotal				5	11.4	3	6.8	
E	149 (8)	150 (1)	144 (6)	3	6.8	1	2.3	
F	141 (11)	150 (1)	151 (2)	2		0		
G	147 (9)	140 (6)	151 (2)	2	4.5	1	2.3	
H	148 (8)	150 (1)	151 (2)	1		0		
Others				0		21	47.8	<0.001

Brackets denote designation of alleles according to Thomas *et al.*⁷

there was a previously unreported conservative change (C2250G), suggesting that a normal polymorphism was present in this region. To correlate the haplotypes with mutations, we found that the Arg778Leu mutation was segregating with haplotype D (8-4-4 in D13S314-301-316), while the Arg778Gln was found with haplotype E (8-1-6 in D13S314-301-316). These data strongly support the occurrence of different haplotypes associated with different mutations as suggested previously.¹⁹ We have not yet found the mutation associated with haplotype A, the most frequent haplotype in the Chinese patients with WD. The amino acid changes of codon 778, which is located in the transmembrane domain (Tm 4), might cause disruption of the appropriate anchorage of the transporter in the membrane. Whether Arg778Leu or Arg778Gln play the same role in the pathogenesis of WD requires further biological studies. However, the changes from a basic amino acid (Arg) to a neutral (Leu) or

even to an acidic amino acid (Gln) found in this study predict a dramatic change in the primary and secondary structure of this protein, which could culminate in the impaired copper transport.

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