

## Association of *ATP7B* Mutation Detection Rate with Biochemical Characteristics in Korean Patients with Wilson Disease

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**Abstract.** Wilson disease (WD) is an autosomal recessive disorder caused by mutations in the *ATP7B* gene, yet many patients have either one mutation, or no mutation. We investigated whether the mutation detection rate is associated with any biochemical characteristics of WD. In a study of 71 patients, we used PCR-sequencing to screen for *ATP7B* mutations in 7 exons (exons 8, 10, 11, 14, 15, 16, and 18) covering 95% of known mutations in Korean patients with WD. We also investigated serum concentrations of various biochemical analytes. Data were analyzed by linear association test and one-way ANOVA. Based on the number of detected *ATP7B* mutations, a significant difference in serum ceruloplasmin concentration was found among the 3 groups ( $p < 0.001$ ). Serum ceruloplasmin concentration averaged  $3.32 \pm 1.74$ ,  $10.8 \pm 5.50$ , and  $14.9 \pm 3.88$  mg/dl (mean  $\pm$  SD) in the 25, 20, and 26 patients with two, one, and no *ATP7B* mutations, respectively. We observed 82.9% and 16.7% of mutant allele frequency in WD patients with ceruloplasmin concentration  $< 10$  mg/dl and 10-20 mg/dl, respectively ( $p < 0.001$ ). Thus serum ceruloplasmin concentrations among WD patients differed according to the number of *ATP7B* mutations detected.

**Keywords:** Wilson disease, *ATP7B* mutation, ceruloplasmin

### Introduction

Wilson disease (WD; MIM #277900) is a disorder of copper (Cu) metabolism [1]. Liver disease, neurologic presentations, and psychiatric disturbances are caused by an accumulation of Cu. The age of affected individuals varies from 3 to  $> 50$  yr. Worldwide prevalence of WD appears to be about 30 per 1 million, with a gene frequency of 0.56% and a carrier frequency of 1 in 90 [2,3]. However, the incidence of WD in Korea may be higher than that in Western populations, given the report of a Korean family with WD in two consecutive generations [4].

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Diagnosis of WD usually depends on detection of low serum Cu and ceruloplasmin concentrations, increased urinary Cu excretion, presence of Kayser-Fleischer rings in the cornea, and/or increased hepatic Cu concentration. No single feature is completely reliable, but a diagnosis can usually be made provided that WD is suspected [2,3,5]. There is no worldwide consensus on a definitive diagnostic tool, but elevated liver Cu concentration is important for confirmation of WD [6,7]. WD is caused by mutations in the *ATP7B* gene, which encodes a Cu-transporting P-type ATPase. More than 379 probable disease-causing mutations from populations worldwide have been reported [8]. Because WD is an autosomal recessive disorder, most WD patients have two *ATP7B* mutations. Mutation analysis may be useful in cases with unclear liver disease [9]. However, many WD patients have either one *ATP7B* mutation

or no mutation. Direct sequencing of all 21 *ATP7B* exons and their adjacent introns from 120 Korean WD patients showed a mutation detection rate of 75.0% (180/240), and about 95% of mutations were identified within 7 exons (exons 8, 10, 11, 14, 15, 16, and 18) [10]. About 25% of mutant alleles had not been previously identified in Korean patients with WD [10,11]. With an autosomal recessive disorder, patients with either one mutation or no mutation in the *ATP7B* gene may have point mutations in the other 14 exons, a large deletion or duplication, mutations in promoter regions, presence of gene rearrangements, or possible mutations in Cu-transport chaperone genes, such as *ATOX1* and *COMMD1* [12-14]. However, there is no evidence of a large deletion or duplication in WD patients with either one *ATP7B* mutation or no mutation (unpublished data). Therefore, we tried to uncover differences in the biochemical characteristics of patients with WD according to the number of *ATP7B* mutations detected.

## Materials and Methods

Seventy-one patients were recruited for the present study (median age 21.0 yr, range 4-49 yr). Sixty-nine patients were unrelated and two were sisters of two patients. Patients had received a diagnosis of WD based on clinical, biochemical, and molecular analyses; at least two positive findings were observed for the following characteristics: presence of Kayser-Fleischer rings in the cornea, increased Cu concentration in 24-hr urine or liver tissue, and decreased serum ceruloplasmin. In addition, many patients presented with hepatic symptoms, signs of increased serum alanine aminotransferase (ALT) or aspartate aminotransferase (AST) activities, and neurologic

symptoms, such as movement disorders. Various biochemical parameters were analyzed, including ceruloplasmin, Cu, ALT, AST, alkaline phosphatase, and albumin, as well as viral markers, including HBs Ag, anti-HBs Ab, and anti-HCV using either the Hitachi 7600-110 analyzer (Hitachi, Tokyo, Japan), Roche Modular E170 analyzer (Roche Diagnostics, Basel, Switzerland), or the ADVIA Centaur immunoassay system (Siemens Healthcare Diagnostics, Deerfield, IL, USA). This study was approved by the Institutional Review Board at Samsung Medical Center.

We used PCR-sequencing to investigate molecular defects in the *ATP7B* gene in patients. Genomic DNA was extracted from peripheral blood using a Wizard genomic DNA purification kit (Promega, Madison, WI) after obtaining informed consent from either patients or their parents. Mutation screening was performed for 7 exons (exons 8, 10, 11, 14, 15, 16, and 18) representing 95% of exons containing mutations found in Korean patients with WD [10]. Forward and reverse primers for genomic DNA sequencing were designed to amplify the 7 coding exons (primer sequences available upon request). Sequencing was carried out using the BigDye terminator cycle sequencing kit, and products were resolved on an ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Data were analyzed by linear association test and one-way ANOVA. All significance tests were two-tailed, and p values <0.05 were considered significant. All statistical analyses were carried out using the SPSS 12.0 statistical software (SPSS, Chicago, IL, USA).

## Results

We identified 70 *ATP7B* mutations in Korean patients with suspected WD. When patients were classified into three groups according to the number of *ATP7B* mutations, 25 patients had two mutations (homozygous or compound heterozygous), and 20 had one mutation. The other 26 patients had no mutation in 7 *ATP7B* exons (Table 1). Patients with

Table 1. Biochemical findings in patients with suspected Wilson disease.

<i>ATP7B</i> mutations	No. of subjects		Serum ceruloplasmin† (mg/dl)	Urine Cu* (µg/day)	Liver Cu* (µg/g dry wt)	Serum AST* (U/L)	Serum ALT* (U/L)	Serum albumin* (g/dl)
		reference range:	20-60	<38	<50	<40	<40	3.5-5.2
2	25	Mean	3.32	443.7	293.5	64.0	113	3.99
		SD	1.74	419.3	380.2	40.3	103	0.42
		Range	1.7-7.8	89-1525	32-857	14-140	12-371	2.8-4.5
1	20	Mean	10.8	368.6	226.2	65.3	125	4.16
		SD	5.50	539.5	287.5	43.9	93.1	0.45
		Range	2.1-18.3	14-1697	39-919	12-164	8-297	2.6-4.8
0	26	Mean	14.9	192.7	125.5	70.0	112	3.92
		SD	3.88	217.8	108.4	77.9	152	0.72
		Range	3.5-19.9	43-982	34-304	16-331	12-615	2.0-4.9

† p < 0.001; \* not significant

HBs Ag or anti-HCV were not observed. With the exception of ceruloplasmin, other clinical or biochemical characteristics among the three groups were not significantly different. Serum ceruloplasmin concentrations (mean  $\pm$  SD, mg/dl) were  $3.32 \pm 1.74$ ,  $10.8 \pm 5.50$ , and  $14.9 \pm 3.88$  in patients with two, one, and no *ATP7B* mutations, respectively. Significant differences were observed in serum ceruloplasmin concentrations among the three patient groups ( $p < 0.001$ ) (Fig. 1).

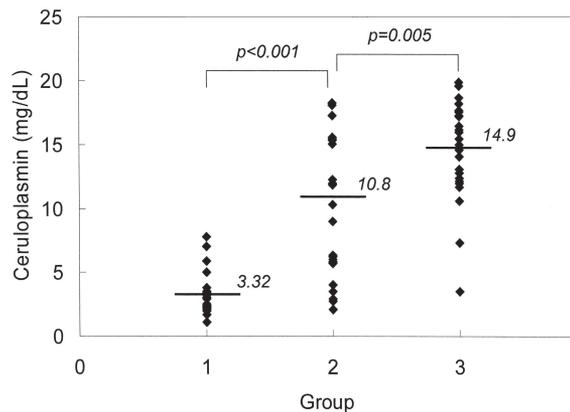


Fig. 1. Serum ceruloplasmin concentrations in WD patients. Patients with two, one, and no *ATP7B* mutations were classified into groups 1, 2 and 3, respectively. Mean ceruloplasmin concentrations in groups 1, 2, and 3 were 3.32, 10.8, and 14.9 mg/l, respectively. There was a significant difference of ceruloplasmin concentrations between groups 1 and 2 ( $p < 0.001$ ) and between groups 2 and 3 ( $p = 0.005$ ).

A significant difference was observed in mutant allele frequency according to serum ceruloplasmin concentrations in patients with WD (Table 2). We observed 82.9% and 16.7% mutant allele frequency in WD patients with ceruloplasmin concentrations  $< 10$  and  $10$ - $20$  mg/dl, respectively ( $p < 0.001$ ).

## Discussion

A scoring system based on a combination of clinical symptoms and laboratory test results was proposed for assessment of the diagnostic accuracy of WD [15]. However, even the most sensitive and specific laboratory tests are secondary to astute clinical suspicion in the search for WD [16]. Correct diagnosis of WD requires strong clinical suspicion and adequate laboratory testing. About 85% of 516 Korean patients with WD have demonstrated hepatic symptoms [17], similar to the results of the present study. Because hepatitis B is endemic to Korea, HBs Ag and anti-HBs Ab tests are preferred for Korean patients with hepatic symptoms [18]. WD should be suspected in patients with abnormal liver function who test positive for anti-HBs Ab. Although elevated urinary Cu excretion resulting from release of Cu from necrotic hepatocytes has been reported in other forms of acute liver failure, urinary Cu excretion is significantly elevated in patients with fulminant WD [19].

In comparison to other available tests, including serum alkaline phosphatase, bilirubin, and aminotransferases, conventional WD tests utilizing serum ceruloplasmin and/or serum Cu concentration are less sensitive and specific for identification of patients with acute liver failure due to WD [20]. Ceruloplasmin deficiency can also be seen in other pathological conditions, including Menkes' disease, protein calorie malnutrition, nephrotic syndrome, protein-losing enteropathy, acquired Cu deficiency, severely impaired hepatic synthetic function, and hereditary aceruloplasminemia [16]. However, ceruloplasmin deficiency is a key finding in the diagnostic approach to WD because there is no single definitive test for diagnosis of WD. A

Table 2. The *ATP7B* mutant allele frequency was associated with serum ceruloplasmin concentration in WD patients.

	Ceruloplasmin $< 10$ mg/dl			Ceruloplasmin $10$ - $20$ mg/dl		
No. of <i>ATP7B</i> mutations	2	1	0	2	1	0
No. of patients	25	8	2	0	12	24
No. of total mutant alleles	50 (12*)	8 (6*)	0	0	12 (5*)	0
Mutant allele frequency (%)†	82.9			16.7		

†  $p < 0.001$ ; \* No. of unique mutant alleles

ceruloplasmin concentration of <20 mg/dl is the conventional diagnostic cutoff for WD. One report demonstrated a ceruloplasmin concentration of <10 mg/dl in 72.7% of patients with WD, but the ceruloplasmin concentration in 9.1% of patients with WD was >21 mg/dL [21]. However, another study recently reported that modification of the decision threshold of serum ceruloplasmin from 20 mg/dl to 14 mg/dl increases diagnostic accuracy for Wilson disease [22]. In the present study, all WD patients with two *ATP7B* mutations had serum ceruloplasmin concentrations <10 mg/dL. Ceruloplasmin concentrations in WD patients with two *ATP7B* mutations were severe and attenuated compared to patients with no mutation.

The 2006 Mayo Clinic study on use of dried blood spots for WD screening concluded that while screening for WD by testing for blood ceruloplasmin appears desirable, larger studies would be needed to determine sensitivity, specificity, effectiveness, and efficacy of screening [23]. Therefore, patients with mild, attenuated ceruloplasmin concentrations who also have other characteristics of WD require thorough evaluation in order to determine whether they are patients or carriers of WD. In particular, we found a wide distribution of serum ceruloplasmin concentrations in 20 patients with one *ATP7B* mutation. In these patients, WD may be attributed to a point mutation in an uncovered exon or to another pathogenic mechanism. These patients presented with ceruloplasmin reduction and no other condition.

If left untreated, WD is a potentially lethal disorder. Early diagnosis of WD is important, because early administration of D-penicillamine can prevent the need for transplantation in most cases [24]. Some anticopper drugs, including penicillamine, zinc, and trientine, have been used, and various management protocols are available to WD patients, depending on the type and severity of symptoms [25]. Cu chelation requires life-long compliance; therefore, prompt, accurate diagnosis of WD is important. WD carriers often have no clinical symptoms, so no treatment is necessary.

In summary, we investigated the relationship between biochemical and molecular characteristics in WD patients. Ceruloplasmin concentrations differed significantly, depending on the number of

*ATP7B* mutations, and mutation detection rate was associated with ceruloplasmin concentration. Because only a limited number of patients were studied, these results contribute little to the use of ceruloplasmin for screening purposes. However, these findings suggest that another mechanism may be the cause of WD in patients with less than two *ATP7B* mutations. Further study will be necessary to confirm or refute this hypothesis.

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