Note

Identification of Three Novel Insertion/Deletion Mutations in Wilson Disease’s Gene

Yuxin Fan,1,3 Long Yu,1 Yongzhu Han,2 Mingshan Ren,2 Renmin Yang,2 and Shouyuan Zhao1

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INTRODUCTION

Wilson disease (WD) is an autosomal recessive disorder of copper transport first reported by Kinnier-Wilson in 1912 as hepatolenticular degeneration (Wilson, 1912). It is characterized by decreased biliary excretion of copper and reduced copper incorporation into apoceruloplasmin. The disorder has an early onset in children and adolescents with symptoms of cirrhosis, neuronal degeneration of the brain especially in the basal ganglia, Kaiser-Fleischer ring of the cornea and kidney damage. Biochemical diagnosis of the disease is based on low plasma ceruloplasmin, and increased urinary and liver copper. However, some patients may not be biochemically distinguishable from healthy carriers, since ceruloplasmin levels may be normal in 5–10% of patients and low in 15–20% of carriers (Tanner, 1999). Therefore, the diagnosis of the disease at the gene level is necessary in some cases.

The WD gene, which encodes a copper transporting P-type ATPase (ATP7B), was cloned and characterized in 1993 (Bull et al., 1993; Tanzi et al., 1993; Yamaguchi et al., 1993). The ATP7B gene contains 21 exons (22 in the kidney) that span about 80-kb genomic distance and produce a 7.5-kb mRNA (Petrukhin et al., 1994; Tanzi et al., 1993). WD is caused by a number of mutations in the ATP7B gene, some of which appear to be population-specific, while others are found in patients from a variety of different ethnic backgrounds (Thomas et al.,...
WD has considerable phenotypic variation. It is not known why some WD patients mainly present with hepatic symptoms during childhood and others with neurological degeneration in adolescence or adult life. Although many mutations in the ATP7B gene have been identified (Bull et al., 1993; Curtis et al., 1999; Kalinsky et al., 1998; Majumdar et al., 2000; Shah et al., 1997; Tanzi et al., 1993; Thomas et al., 1995), the frequency and distribution of ATP7B gene mutations in Chinese WD patients remain unknown. Most importantly, genotype–phenotype correlations have not been determined for most mutations reported, except the His1069Gln mutation, which is the most common ATP7B mutation in eastern and northern European populations (Thomas et al., 1995).

In the present study, we aimed to identify the mutation spectrum of the ATP7B gene in Chinese WD patients, and to determine genotype–phenotype correlation. In this paper, we report three novel insertion/deletion mutations of the ATP7B gene identified in Chinese population.

**MATERIALS AND METHODS**

**Subjects**

A total of 141 WD patients (86 males and 55 females) was collected from 128 unrelated Chinese WD families with a mean onset age of 16.82 ± 7.31, all of whom were inpatients in the Department of Neurology, Institute of Neurology, First Affiliated Hospital, Anhui College of Traditional Chinese Medicine (TCM). They came from all over the country including 12 different provinces. The diagnosis of these patients was based on the clinical symptoms of WD and biochemical tests including low serum ceruloplasmin and copper concentrations, high urinary copper, and high hepatic copper content. According to the clinical presentations, 141 WD patients were divided into the neurological type (119 cases), the hepatic type (20 cases), the spinal cord type (1 case) and the osteomuscular type (1 case) (Walshe, 1986). The degree of sickness was graded by the modified Goldstein method (Goldstein et al., 1962). The group included 49 patients of grade I, 54 of grade II, 28 of grade III, and 10 of grade IV. The 20 normal controls were healthy volunteers without a WD family history from Fudan University. Informed consents were obtained from each patient and each control concerning gene analysis. Genomic DNA was extracted from peripheral blood samples by the salting-out method as previously reported (Miller et al., 1988) and examined by agarose gel electrophoresis and proven to be larger than 23 kb.

**DNA Amplification and Purification**

PCR primers were designed according to Thomas et al. (1995). Primers amplifying exons 4 (WD4-1: 5'-CCACCAGAGTGTTACAGCC-3' and WD4-2: