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Wilson's Disease In Japan
-Nationwide Survey of Clinical Features and Molecular Analysis In Japanese Patients-

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INTRODUCTION

Wilson's disease (hepatolenticular degeneration), an autosomal recessive disorder, was described by Wilson in 1912. Wilson's disease (WD) is characterized by copper toxicity, believed to result from the loss of the ability to (i) export copper from liver to bile and (ii) incorporate copper into ceruloplasmin in the liver. Copper builds to toxic levels in the liver and other tissues, including brain, kidney and cornea. The resulting liver cirrhosis and/or neurological degeneration are fatal if not treated with copper-chelating agents. Walshe, in 1956, introduced an effective treatment with chelating agents, such as D-penicillamine. Although the basic defect was not known, the pathogenesis of Wilson disease is based on two fundamental disturbances of copper metabolism. Defective biliary excretion leads to the accumulation of copper in the liver, with progressive liver dysfunction and subsequent overflow to brain causing a loss of coordination and involuntary movements. Deposition in the cornea produces Kayser-Fleischer rings and accumu-lation in other sites may cause renal tubular damage (secondary, Fanconi syndrome), osteoporosis, arthropathy, cardiomyopathy and hypoparathyroidism.

Inheritance of Wilson's disease is autosomal recessive. The incidence is 1 in 30,000 to in 100,000 live-birth. The gene is located at chromosome 13q14.3. The protein belongs to copper transporting P-type ATPase family, similar to that of Menkes disease, which is an X-linked disorder in copper transport.

A candidate gene (MNK) for Menkes disease was cloned in January 1993. The gene encodes a putative copper-transporting ATPase, similar to previously identified prokaryotic heavy metal transporters. The identification of the MNK gene as a potential copper-transporting P-type ATPase protein and its broad profile of expression in most tissues except liver led to the suggestion that Wilson's disease may be caused by a defect in a liver-specific copper transporter. Using slightly different strategies for positional cloning, this hypothesis has been confirmed by the isolation of a candidate gene for Wilson's disease at the end of 1993.

Expression studies of the WD gene have revealed a transcript of approximately 7.5 kb, expressed predominantly in liver, kidney and placenta. A much lower level of the transcript has also been detected in heart, brain, lung, muscle and pancreas. The full-length cDNA sequence obtained by Cox and colleagues has revealed a predicted 1411 amino acid protein, which is a member of a cation-transporting P-type ATPase sub-

family and is highly homologous to the MNK gene product. The overall identity between the MNK and WD gene products is 56%, but greater identity is observed in the phosphate domain (78 %), the transduction-phosphorylation domains (89 %), the ATP-binding domain (79%) and the N-terminal domain containing the six potential copper-binding domains (65%). However, potential structural differences have been found, such as a small hydrophobic region in the fourth copper-binding domain.

Although various data regarding the expression profiles of the two genes can clarify some speculations, it is the immunocytological, functional and physiological studies of these two gene products that should clarify the mechanisms of copper transport within the cell and between tissues and eventually lead to a better understanding of Menkes disease and Wilson's disease pathogenesis. Interestingly, potential animal models, such as the mottled mouse and the brindled mouse for Menkes disease and the Long-Evans Cinnamon rat, the toxic milk mouse and the Bedlington terrier for Wilson's disease, have been described. Investigation of these animal models by searching for phenotype-specific mutations may provide further insight into copper metabolism and appropriate models to develop new therapies.

Nationwide Survey of Clinical Feature of Wilson's Disease In Japan

This study on Wilson's disease in Japanese was aimed at analysis of the clinical features, estimation of the exact prevalence rate and early detection of this disease by mass-screening.

To study the clinical features of patients with Wilson's disease, we sent out questionnaires to 5,228 clinics, and hospital departments or divisions of pediatrics, child neurology, neurology, psychiatry, internal medicine and gastroenterology all over Japan. This questionnaire included information on the age of disease onset, sex, family history, clinical types of Wilson's disease, first symptoms, serum ceruloplasmin and copper concentrations, treatment-lag, methods of treatment and management of the patients.

In the 5 years from January 1990 to December 1994, 673 cases were collected and investigated. Return rate of the questionnaires was 41.2%. The prevalence rate of Wilson's disease was found to be about 1:30,000 to 1:34,000 live-births in Japan. Sex ratio of males to females was about 55 to 45 and the peak age of disease onset was 12 years. The hepatic type of Wilson's disease, including the fulminant and severe hemolytic type, was 52.2 %, hepato-neurologic type 18%, neurologic type 13.5% and presymptomatic type 14.3% (Table 1).

Table 1. Distribution of the Age of Disease-onset of Each Type of Wilson's Disease

Type at onset	0-5	6-10	11-15	16-20	20-30	31	Total numbers of patients (%)
Hepatic	12	131	165	18	5	5	336(49.5)
fulminant	1	14	14	0	0	0	29(4.3)
with hemolysis	0	16	16	3	0	0	35(5.2)
Hepato-neurologic	1	21	43	18	30	8	121(18.0)
Neurologic	0	9	41	18	15	8	91(13.5)
Presymptomatic	35	49	11	1	0	0	96(14.3)
unknown	0	8	10	6	3	2	29(4.3)
	48	218	270	61	23		673(100)

Table 2. Serum Ceruloplasmin Level of Patients with Wilson's Disease and Their Parents

Distributions of serum Cp level (mg/100 ml)	Number of patients with Wilson's disease	Parents of Wilson's disease Patients		
		Father	Mother	Ather & mother
0-4.9	316 (59.5)	1 (1.0)	2 (1.0)	3 (1.0)
5-9.9	147 (27.7)	5 (2.7)	7 (3.6)	12 (3.2)
10-14.9	42 (7.9)	23 (12.6)	18 (9.2)	41 (10.9)
15-19.9	13 (2.4)	59 (32.4)	49 (25.1)	108 (28.6)
20-24.9	5 (1.0)	41 (22.5)	61 (31.3)	102 (27.1)
25.0	8 (2.0)	53 (29.1)	58 (29.7)	111 (29.4)
Total	531 (100)	182 (100)	195 (100)	377 (100)

The youngest patient of presymptomatic type detected by family screening was 18 months old and the youngest case with only slightly elevated ser um AST and ALT was 2 years old. He was a presymptomatic case detected by chance. The most common age of patients presented clinically of the hepatic type was 9 years old, and of the neurologic type 13 years old. Twenty-nine cases of fulminant hepatic failure were investigated, but 18 died within a few weeks. Only 8 cases are still alive. Partial liver transplantation had been performed in 3 patients, 2 were successful, but 1 died. In 97% of patients with Wilson's disease in Japan, serum ceruloplasmin concentrations were reduced to below 20 mg/100 ml (Table 2).

We investigated about 30 cases of presymptomatic Wilson's disease and 30 symptomatic cases. We measured the serum GOT (AST) and GPT (ALT) levels, serum ceruloplasmin concentration (S-Cp), urinary Cu excretion (U-Cu), Cu content in the liver and Cu content in the bile juice. We obtained liver specimens by liver biopsy and measured the Cu concentrations by ICP-MS.

The serum AST and ALT levels were normal in many cases of under 3 years old children. The ratio of serum active-Cp to total-Cp decreased. The urinary copper excretion per body weight increased and was higher than the critical level (1.5) in cases of over 4 years old children. The ratio of urinary copper excretion to urinary creatinine increased and was higher than the critical level (0.2) in cases of over 5 years old children. All presymptomatic patients had high copper contents in their livers, in excess of 200 μg/g wet tissue. The ratio of biliary Cu total bile acid decreased significantly in all types of Wilson's disease, in contrast to the control subjects.

Based on our analysis of the serum AST and ALT levels and urinary copper excretion, liver dysfunction due to Wilson's disease appeared to occur at 3 years old and particularly at 5 years old. We suggest that hepatocytes undergo necrosis due to the toxicity of accumulated Cu at around this age, and urinary Cu excretion becomes increased. We conclude that the present examinations provide a useful tool for the diagnosis of presymptomatic patients with Wilson's disease.

We found that Wilson's disease in Japan has an incidence rate of 1 in 30,000 to 1 in 34,000 live-birth. This is similar to the worldwide prevalence of Wilson's disease which is about 1: 23,000.

In spite of various methods of treatments, many patients in our survey still die of fulminant hepatic failure with severe hemolysis, severe hepatic damage and severe neurologic deterioration with irreversible brain dysfunction.

Our results showed that we should establish methodology useful for mass-screening of Wilson's disease at the presymptomatic stage as ear-

ly as possible. Early detection is extremely useful for treatment of Wilson's disease to improve morbidity and mortality.

Molecular Analysis and Diagnosis in Japanese Patients with Wilson's Disease

The Wilson's disease gene (ATP7B) was cloned in 1993 and encodes a putative copper-transporting P-type ATPase. More than 70 disease-specific mutations have been reported. The major mutations include the deletion or insertion of one or two base pairs and point mutations (nonsense and missense). This study reports the results of mutation analysis performed on Japanese Wilson's disease patients and their families and also proposes the establishment of a molecular diagnosis system for this disease.

A total of 23 Japanese patients with Wilson's disease, including 20 unrelated patients and one family (parents and siblings) were examined in this study. Twenty-five normal healthy volunteers were also examined as control subjects.

The diagnosis of these patients was based on the clinical symptoms and/or laboratory findings, including the serum ceruloplasmin levels, copper levels in serum and urine and hepatic copper content. However, a definitive diagnosis on two patients from one family was performed based on a gene analysis. Various clinical types of disease were observed, including five hepatic, two fulminant, seven neurological or hepato-neurological, seven presymptomatic and two unknown types.

We identified 13 mutations, including one insertion, four deletions, one instance of exon skipping and seven missense mutations in the coding region (Table 3). The deletion mutations cause frameshifts. These mutations thus alter the reading frame of ATP7B, which then results in either the absence of the protein product or the production of a shortened non-functional protein. The 2514deletion (del)A and 3541delA mutations are also expected to lead to frameshifts and to result in the synthesis of a truncated protein. The novel missense mutation aspartate (Asp)1223 asparagine (Asn), which resides in the ATP hinge region, is non-conservative and changes an acidic amino acid to a neutral hydrophobic residue. To assess the possibility that this missense substitution may indicate a polymorphism, we tested for this substitution in a normal subject of the same origin. This mutation was not detected among the 50 normal alleles.

The allele frequency of each mutations is also shown in Table 1. The Arg778Leu and 2874delC mutations are quite common *ATP7B* mutations in Japanese patients, occurring in more than 50% of these alleles. No family was found to show consanguinity. Eleven of 20 patients have a homozygous gene mutation (data not shown). The ratio of the patients who are genetic homozygotes was

Table 3. Mutations Identified in ATP7B and Their Allele Frequency

Mutations	Exon	No. alleles (/40)	Frequency (%)
Missense			
Arg778Leu	8	10	25
Ala875Val	11	3	7.5
Arg920Gly	12	1	2.5
Thr1030Ile	14	1	2.5
Gly 1035Val	14	1	2.5
Gly 1187Ser	16	1	2.5
Asp1223Asn	17	1	2.5
Frameshift			
2302insC	8	3	7.5
2514delA	10	1	2.5
2662delG	11	3	7.5
2874delC	13	12	30
3541delA	16	1	2.5
Exon skipping			
1711-5 T→G	5	2	5.0

Arg, arginine; Leu, Ieucine; Ala, alanine; Val, valine; Gly, glycine; Thr, threonine; Ile, isoleucine; Ser, serine; Asp, aspartate; Asn, asparagine; ins, insertion; del, deletion.

also found to be higher than in other reports.

None of the observed mutations, except for 2302insertion(ins)C, have been previously detected in either European or North American patients. We conclude that the mutation spectrum of Wilson's disease may thus indicate a population-dependent pattern. Based on the population-dependent manner of the occurrence of ATP7B gene mutations, it may be possible to establish a molecular diagnosis system. A molecular diagnosis system is considered to be very effective for making a definitive diagnosis in very young patients and for also detecting carriers.

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