Original Article

Phenotypes and Chronic Organ Damage May Be Different among Siblings with Wilson's Disease

Shinsuke Yahata[‡1], Seitetsu Yung[1], Mari Mandai[2], Takakazu Nagahara[2], Daisaku Kuzume[3], Hiroshi Sakaeda[3], Shinya Wakusawa[4], Ayako Kato[5], Yasuaki Tatsumi[5], Koichi Kato[5], Hisao Hayashi*[5], Ryohei Isaji[6], Yoji Sasaki[6], Motoyoshi Yano[7], Kazuhiko Hayashi[8], Masatoshi Ishigami[8] and Hidemi Goto[8]

[1]*Department of Gastroenterology, Hyogo Prefectural Kakogawa Medical Center, Kakogawa, Hyogo, Japan;* [2]*Department of Gastroenterology and Hepatology, Tottori Prefectural Kousei Hospital, Kurayoshi, Tottori, Japan;* [3]*Departments of Neurology and Gastroenterology, Chikamori Hospital, Kochi, Japan;* [4]*Medical Laboratory Sciences, Nagoya University Graduate School of Medicine, Higashi-ku, Nagoya, Japan;* [5]*Department of Medicine, Aichi Gakuin University School of Pharmacy, Chikusa-ku, Nagoya, Japan;* [6]*Department of Gastroenterology, Konan Kosei Hospital, Takaya-cho, Konan, Aichi, Japan;* [7]*Department of Gastroenterology, Yokkaichi Municipal Hospital, Yokkaichi, Mie, Japan;* [8]*Department of Gastroenterology and Hepatology, Nagoya University Graduate School of Medicine, Showa-ku, Nagoya, Japan*

Abstract

Background and Aims: Cloning of ATP7B provided evidence that Wilson's disease is a hepatic copper toxicosis with a variety of extrahepatic complications. Affected siblings with the same genetic background and exposure to similar environmental factors may be a good model for the study of genotype-phenotype correlation. ***Methods:*** Twenty-three affected siblings in 11 families were selected from a database. The first phenotypes were determined according to the international proposal. The final types of chronic organ damage were re-evaluated for life-long management. ***Results:*** Phenotypes were identical in 5 of the families and different in 6 of the families. The acute hepatic phenotype *H1* was found in 3 younger siblings and 1 older sibling. All survived an acute episode of hemolysis with underlying chronic liver disease. One also presented complication with neurological disease. The neurological phenotype *N1* with neuropsychiatric symptoms and hepatic disease was found in 2 aged siblings of 1 family, in an older sibling in 3 families and in the oldest sibling in 1 family. Phenotypes in siblings were mainly split by either *H1* occurring in random order or age-dependent *N1*. Types of chronic organ damage were identical in 8 of the families and different in 3 of the families. The same combination of chronic liver disease was found in 6 families and chronic liver disease complicated with neurological disease in 2 families. Split organ damage in siblings was found when an older sibling was complicated by neurological disease. There was no reverse combination of a younger sibling being complicated by neurological disease in any of the families. ***Conclusion:*** Phenotype combinations of siblings were mainly modified by externally-induced hemolytic episodes, while chronic organ damage in siblings was split by age-dependent neurological complications.

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Introduction

The cloning of *ATP7B*, responsible for Wilson's disease (WD), provided evidence that WD is a primary hepatic disease caused by toxic copper retained in the liver.[1–3] In addition, various forms of extra-hepatic organ damage occur in patients. They include an acute hemolytic episode of transient jaundice and anemia, neuropsychiatric disorders, and Kayser-Fleischer (KF) rings.[4–7]

WD first appears with diverse clinical features, resulting in a delayed diagnosis and poor prognosis. To promote an early diagnosis and consequently improved prognosis, an international study group for WD (ISGW) proposed the first clinical features consisting of acute hepatic (*H1*), chronic hepatic (*H2*) and neurological (*N1*, etc.) phenotypes, and a scoring system for the definite diagnosis of WD.[8] The phenotyping of ISGW has been proposed to simplify the diverse clinical features for diagnosis at the initial presentation, but it does not describe the chronic organ damage of WD which will require life-long treatment. Chronic liver disease, designated as phenotype *H2* in the ISGW proposal, is a fundamental feature of WD.[9] Neurological disease is a major chronic complication of patients with phenotype *N1*,[10] and hemolytic episodes are major acute complications of patients with phenotype *H1*.[11]

Affected siblings who have the same genetic background and exposure to similar environmental factors, including

Keywords: Hemolysis; Liver disease; Neurological disease; Wilson's disease.
Abbreviations: WD, Wilson's disease; KF, Kayser-Fleischer; ISGW, international study group of Wilson's disease; *H1*, acute hepatic phenotype; *H2*, chronic hepatic phenotype; *N1*, neurological phenotype with neuropsychiatric symptoms and hepatic disease; *WD*, uncomplicated chronic liver disease of Wilson; *N*, neurological disease of Wilson; *WD* with *N*, chronic liver disease complicated with neurological disease of Wilson; M, male; F, female.
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***Correspondence to:** Hisao Hayashi, Department of Medicine, Aichi Gakuin University School of Pharmacy, 1-100 Kusumoto-cho, Chikusa-ku, Nagoya 464-8650, Japan. Tel: +81-52-757-6779, Fax: +81-52-757-6799, E-mail: hhayashi@dpc.agu.ac.jp
[‡]**Current address:** Division of Community Medicine and Career Development, Kobe University Graduate School of Medicine, 2-1-5, Arata-cho, Hyogo-ku, Kobe 652-0032, Japan

foods and drinking water, during their childhood growth in a familial setting may be a good model for the study of the genotype-phenotype correlation of WD, which has been the main clinical issue since the cloning of *ATP7B*.[12–16] In this retrospective study, we evaluated the clinical features of 23 affected siblings in 11 families using the phenotypes of the ISGW proposal at the first appearance[8] and the final diagnosis based on chronic organ damage for the life-long treatment of patients.[9,11]

Methods

This study was approved by the review boards evaluating research involving human subjects at the Aichi Gakuin University School of Pharmacy (Nagoya, Japan) and participating hospitals. Informed consent for *ATP7B* analysis was then obtained from each patient according to the study protocols approved.

Two or more siblings with WD found in a family were selected from our database covering the last 20 years. All plural siblings referred to our institutes underwent *ATP7B* analysis for the final diagnosis of WD.[17] Homozygotes or compound heterozygotes of *ATP7B* responsible for copper toxicosis were diagnostically definitive for WD. In the patients for who genetic diagnosis was incomplete, the scoring system proposed by ISGW[8] was applied for the final diagnosis of WD.

According to the ISGW proposal,[8] the clinical features at the initial manifestation were classified into *H1*, *H2* and *N1* phenotypes. Briefly, the phenotype *H1* causes acute jaundice due to hepatitis-like illness or Coombs-negative hemolysis in a previously apparently healthy subject, the phenotype *H2* is any type of chronic hepatic disease, and the phenotype *N1* refers to chronic hepatic disease associated with neuropsychiatric symptoms being present at diagnosis. Information on the chronic liver diseases of our patients was complete at the time of *ATP7B* examination.

The chronic organ damage of patients for their life-long management was classified into two types: uncomplicated chronic liver disease of Wilson (*WD*) and chronic liver disease complicated with neurological disease of Wilson (*WD* with *N*).[9,11] Phenotype combinations at the first appearance and final combinations of chronic organ damage renewed for life-long management were investigated in the 23 affected siblings of the 11 unrelated families.

Results

The clinical features, *ATP7B* mutations, first phenotypes and final diagnoses of the 23 siblings affected with WD in the 11 families are summarized in Table 1. Twenty-one affected siblings of 10 families were either homozygous or compound heterozygous for the *ATP7B* mutation responsible for WD. The diagnosis of WD in the 2 heterozygous siblings was confirmed by the clinical characteristics of hypoceruloplasminemia, KF rings and copper contents in the liver and urine.[8]

According to the phenotypes of ISGW,[8] the identical combination of *H2/H2* was found in 4 families and *N1/N1* in 1 family, while the different combination of *H1/H2* was found in 2 families, *H1/N1* in 2 families, *H2/N1* in 1 family, and *H2*/*H2*/*N1* in 1 family.

There were 4 siblings affected by phenotype *H1*. They comprised 2 each of the phenotype combinations *H1*/*H2* and *H1*/*N1*. All the patients survived their acute episodes with conservative treatment and short-term anti-copper regimens. The phenotype *H1* first appeared in a younger sibling in 3 of the families, and in 1 older sibling in 1 family. Re-evaluation of the *H1* phenotypes at the recovery stage showed that *WD* remained in 3 of the survivors and *WD* with *N* in 1 survivor. The phenotype *H2* or *WD* was incidentally found in 4 unrelated patients during the investigation of their biochemical liver damage of unknown etiology. Subsequent family studies identified another sibling with phenotype *H2*, being affected with *WD*. There were 6 siblings affected by phenotype *N1*, and they comprised *H1*/*N1* in 2 families, *H2*/*H2*/*N1* in 1 family, *H2*/*N1* in 1 family, and *N1*/*N1* in 1 family.

The final organ damage combinations of siblings renewed for their life-long management were *WD*/*WD* in 6 families, *WD*/*WD* with *N* in 2 families, *WD*/*WD*/*WD* with *N* in 1 family, and *WD* with *N*/*WD* with *N* in 2 families. In the 3 families with split organ damage combination, neuropsychiatric symptoms appeared age-dependently in the older sibling in 2 of the families and in the oldest sibling in 1 family. The identical organ damage of *WD* with *N* was found in the relatively aged siblings after *ATP7B* study of family members. The probands were both younger siblings because the genetic study was the first for the older siblings with neuropsychiatric symptoms.

Discussion

The study results obtained from the plural siblings with the same genetic backgrounds and exposure to similar environmental factors before their late teens provided important information regarding the genotype-phenotype correlation of WD. Different from other genetic diseases, the phenotype combinations of the affected siblings with WD were significantly modified by the phenotype *H1* complicated with self-limiting hemolysis. The phenotypes at the first manifestation and chronic organ damage requiring lifelong anti-copper management were different in 6/11 and 3/11 family members, respectively.

Physiologically, the liver takes up dietary copper from the portal blood, synthesizes cuproproteins in hepatocytes, and secretes excess copper into the bile via the essential role of the hepatic copper transporter ATP7B.[4] Dysfunction of ATP7B causes a sequence of the copper-induced chronic liver diseases of steatohepatitis, chronic hepatitis and cirrhosis in patients.[5,6] Therefore, the liver disease stages may be age-dependent in most siblings with the same genetic background and similar environmental factors.

Copper is a hemolytic agent.[18] The hemolytic episode of phenotype *H1* may occur in WD patients with excess copper stored in the liver and other organs when these organs are incidentally damaged, releasing the toxic copper into the systemic circulation.[4–6] Triggers inducing copper overflow may include infectious agents, toxins and drugs. It may be incidental to whether or not either a younger or older sibling is affected by such an external agent. Therefore, the phenotype *H1* in a sibling with WD could be determined by an environmental factor rather than the age and genetic background.[9,11]

When the uncomplicated liver disease of Wilson is designated *WD*, the neurological disease designated *N* is a major extra-hepatic complication of *WD*.[9,10] Non-ceruloplasmin-bound copper accumulates increasingly in the brain of WD patients along with the progression of copper-induced liver disease.[4–6] This process may also be age-dependent in affected siblings. In fact, in our family studies, an older sibling with *WD* was the first to be complicated by *N*, followed by the identification of *WD* in a younger sibling. When *WD* with

Table 1. Clinical features, *ATP7B* mutations, first phenotypes and final diagnoses of the 23 affected siblings in 11 families

Family	Siblings	Age	Sex	*ATP7B*-1	*ATP7B*-2	First phenotypes	Final diagnosis
1	1[#1]	6	F	2333G>T	2333G>T	*H1*	*WD*
	2	10	M	2333G>T	2333G>T	*H2*	*WD*
2	1[#1]	6	M	2333G>T	2621C>T	*H2*	*WD*
	2	17	M	2333G>T	2621C>T	*H2*	*WD*
3	1	10	F	2871delC	2871delC	*H2*	*WD*
	2	14	F	2871delC	2871delC	*H2*	*WD*
	3[#1]	17	M	2871delC	2871delC	*N1*	*WD* with *N*
4	1	12	F	2871delC	3809A>G	*H2*	*WD*
	2[#1]	17	M	2871delC	3809A>G	*N1*	*WD* with *N*
5	1	13	M	2871delC	3643G>T	*H2*	*WD*
	2[#1]	17	M	2871delC	3643G>T	*H1*	*WD*
6	1[#1]	16	M	1708-5T>G	3809A>G	*H2*	*WD*
	2	18	M	1708-5T>G	3809A>G	*H2*	*WD*
7	1[#1]	16	M	1708-5T>G	1708-5T>G	*H2*	*WD*
	2	18	M	1708-5T>G	1708-5T>G	*H2*	*WD*
8	1[#1]	31	M	2298_2299insC	2755C>G	*N1*	*WD* with *N*
	2	37	M	2298_2299insC	2755C>G	*N1*	*WD* with *N*
9	1[#2]	32	F	2871delC	–	*H1*	*WD*
	2[#2]	35	M	2871delC	–	*N1*	*WD* with *N*
10	1	38	M	1846C>T	1846C>T	*H2*	*WD*
	2[#1]	41	M	1846C>T	1846C>T	*H2*	*WD*
11	1[#1]	40	M	2659delG	4007T>C	*H1*	*WD* with *N*
	2	47	M	2659delG	4007T>C	*N1*	*WD* with *N*

[#1] A proband in the affected siblings; [#2] Both siblings visited a hospital on the same day.

Abbreviations: M, male; F, female; *H1*, acute hepatic phenotype; *H2*, chronic hepatic phenotype; *N1*, neurological phenotype with neuropsychiatric symptoms and chronic hepatic disease; *WD*, uncomplicated chronic liver disease of Wilson; *N*, neurological disease of Wilson; *WD* with *N*, chronic liver disease complicated with neurological disease of Wilson.

Note: Two siblings were affected in 10 families, and 3 siblings in 1 family. A younger sibling was the proband in 6 families, while either older or the oldest sibling being the proband in 4 families. Two affected siblings with different phenotypes were referred to hospital on the same day. Nine affected siblings of 4 families were homozygous, 12 affected siblings of 6 families were compound heterozygous, and 2 affected siblings of 1 family were heterozygous for the *ATP7B* mutation responsible for WD. All 4 patients with the phenotype *H1* survived their acute episodes with underlying chronic diseases.

N in an older sibling had been missed due to various reasons, a younger sibling presented with *WD* with *N*. However, the reverse split combination of a younger sibling with *WD* with *N* and an older sibling with *WD* was not found in our case study.

Such reverse split, suggesting involvements of the genes other than *ATP7B*, epigenetic factors and environmental agents, was reported in neuropsychiatric disorders of relatively aged patients. A male patient with digenic mutations in *PRNP* and *ATP7B* presented with a severe neuropsychiatric disorder, while his older sister with *ATP7B* mutations alone had asymptomatic liver disease of WD.[16] Two females from two monozygotic twins were first complicated by neurological disease at the ages of 26 and 36 years respectively; yet, their siblings remained asymptomatic for more than 2 and 3 years respectively.[14] Two male siblings, at the ages of 16 and 28 in a single family, and a male patient, at the age of 32 in another family, first presented with neurological symptoms, followed by the identification of older female siblings with asymptomatic WD.[12] Backgrounds other than *ATP7B* may cause a reverse split of the neurological complications in relatively aged pairs of affected siblings, probably because of the prolonged period of exposure to these factors.

Conclusions

The phenotype combinations of the siblings with WD in our case series were significantly modified by acute hemolytic episodes, and split chronic organ damage was determined by the age-dependent neurological complications.

Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Conception of study objective and design (SK, HH), collection of data (MM, TN, DK, HS, RI, YS, MY, KH, MI), analysis of data (AK, YT, KK), revising the article for important intellectual content (SY, SW, HG).

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