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An Adult Case of Benign Recurrent Intrahepatic Cholestasis Due to *MYO5B* Deficiency

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Abnormalities in *MYO5B*, which encodes an unconventional myosin Vb, not only cause microvillus inclusion disease but also cholestatic liver disease, including benign recurrent intrahepatic cholestasis (BRIC). However, *MYO5B*-related cholestasis has not yet been reported in Japan. In this study, we present the case of a female patient in her thirties, who had developed jaundice, without diarrhea, in the first year after birth. The jaundice spontaneously subsided and occasionally recurred. Whole-exome sequencing identified two pathogenic variants in *MYO5B*: a nonsense mutation (c. G1124A: p. W375X) and a missense mutation (c.C2470T: p.R824C). Therefore, the patient was diagnosed with *MYO5B*-associated BRIC. This is the first reported case of cholestasis with a defined *MYO5B* defect in Japan.

Key words: *MYO5B*, mutations, BRIC, cholestasis

INTRODUCTION

Genetic abnormalities in the molecules associated with bile acid transport can cause cholestasis [1, 2]. Progressive familial intrahepatic cholestasis (PFIC) is a disease that develops during an individual's childhood and requires liver transplantation. This autosomal recessive disease is attributed to biallelic mutations in familial intrahepatic cholestasis 1 (FIC1, *ATP8B1*), bile salt export pump (BSEP, *ABCB11*), as well as multidrug resistance protein 3 (MDR3, *ABCB4*), referred to as PFIC 1, 2, and 3, respectively. Patients with PFIC 1 or 2 exhibit low serum gamma-glutamyl transferase (GGT) levels, whereas those with PFIC 3 exhibit elevated GGT levels.

In contrast, benign recurrent intrahepatic cholestasis (BRIC), a milder form of PFIC, manifests as occasional jaundice that subsides spontaneously. BRIC is caused by biallelic mutations in *ATP8B1* or *ABCB11*, which affect protein function to a lesser extent than that due to PFIC-causing mutations.

Recent studies have identified PFIC-causing mutations in three more genes: *TJP2* (PFIC4), *NR1H4* (PFIC5), and *MYO5B* (PFIC6) [1, 2]. *MYO5B* encodes the unconventional myosin Vb, an actin-based motor involved in plasma membrane recycling via its interaction with Rab GTPases [3]. In 2008, an *MYO5B* mutation was identified as a cause of microvillus inclusion disease (MVID), which is characterized by intractable watery diarrhea during the early days of life [4]. Several researchers have reported patients with MVID presenting with low-GGT cholestasis [5–7]. Notably, two groups identified *MYO5B* mutations in patients with low-GGT cholestasis without enteropathy [8, 9]. Thus, *MYO5B*-related diseases have a wide phenotypic spectrum, from MVID to low-GGT cholestasis [10, 11].

To the best of our knowledge, a case of MVID or cholestasis associated with a defined *MYO5B* defect has not been reported in Japan. In this study, for the first time, we describe a Japanese case of BRIC without diarrhea in a patient who has biallelic *MYO5B* mutations.

CASE REPORT

A female patient presented jaundice in her first year after birth (Fig. 1). The jaundice spontaneously subsided and occasionally recurred. A liver biopsy performed at the age of 5 years revealed chronic inactive hepatitis with fibrosis; however, a definite diagnosis was not obtained. Thereafter, she manifested intermittent jaundice and elevated serum transaminase levels. From the available data, we found that she had experienced an icteric episode more than 10 times. Notably, her serum GGT levels were normal throughout the clinical course. She did not complain of diarrhea. Subsequently, the patient was diagnosed with low-GGT BRIC and was referred to Juntendo University Urayasu Hospital in her thirties to seek a genetic diagnosis. The blood chemistry results revealed a slight elevation in bilirubin levels; the total and direct bilirubin were 3.5 and 2.7 mg/dl, respectively (at X years old in Fig. 1). Her serum transaminase levels were also elevated, with aspartate aminotransferase (AST) and alanine aminotransferase

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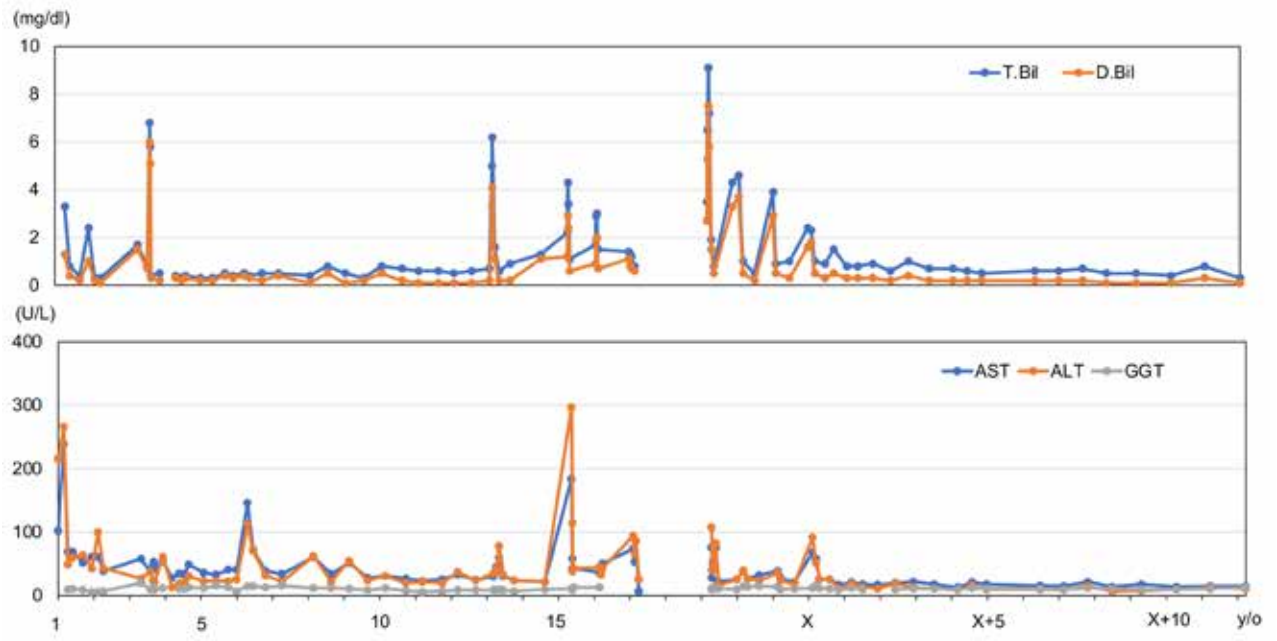

Fig. 1 Clinical course of the patient
Changes in the total serum and direct bilirubin levels (upper panel), as well as the AST, ALT, and GGT levels (lower panel) throughout her life. Data between 17 and X-3 years old are not available.

Table Mutations identified in the patient

gene	rsID	variant	AA change	gnomAD_ genome	ToMMo 38KJPN	ACMG classification	PolyPhen-2	SIFT
MYO5B	rs777038090	c.C2470T	R824C	0.000013	NA	Uncertain Significance	Probably damaging	Deleterious
MYO5B	rs965419129	c.G1124A	W375X	NA	NA	Pathogenic	NA	NA
JAG1	rs758687380	c.A3038T	H1013L	0.000046	0.006	Benign	Probably damaging	Deleterious

The values of the gnomAD_genome and ToMMo 38KJPN indicate allele frequency in the global and Japanese populations, respectively. Abbreviations: ACMG, American College of Medical Genetics; NA, not available.

(ALT) at 76 U/L and 108 U/L, respectively. Serum alkaline phosphatase (ALP) levels were slightly elevated (611 U/L), whereas GGT levels were normal (11 U/L). Other laboratory tests and complete blood counts were normal, except for a positive antinuclear antibody test (1:160). Bile duct obstruction was ruled out using abdominal ultrasonography. Her parents did not have any liver disease. Her younger sister presented with episodic jaundice, but a genetic diagnosis had not been made.

After obtaining written informed consent, DNA was extracted from the patient's peripheral blood and analyzed via whole-exome sequencing (WES, NovaSeq, Illumina Japan, Tokyo, Japan). This study was approved by the Institutional Review Board for Clinical Research, Tokai University (21R282).

Three potentially pathogenic variants were detected in the PFIC-related genes, two of which were found in *MYO5B* and one in *JAG1*, being heterozygous (Table). These variants were confirmed using Sanger sequencing (Fig. 2).

c.C2470T in *MYO5B* is a missense mutation resulting from a cysteine substitution for arginine at codon 824 (p.R824C). Prediction tools Polyphen2 and SIFT described the impact of this substitution on protein function as 'Probably damaging' and 'Deleterious', respectively. c.G1124A of *MYO5B* is a nonsense mutation that generates a truncated protein (W375X). Both are extremely rare variants, with no carriers in Japan.

The *JAG1* c.A3038T variant causes the substitution of histidine for leucine at codon 1013 (p:H1013L). The prediction tools indicate the influence of this variation as 'Probably damaging' or 'Deleterious;' however, this variation is categorized as 'Benign' in the American College of Medical Genetics and Genomics (ACMG) classification. Although the impact of the *JAG1* c.A3038T variant is unclear, we diagnosed the patient with *MYO5B*-associated BRIC due to the presence of two pathogenic *MYO5B* mutations.

DISCUSSION

The patient experienced several episodes of intermittent jaundice since early stages of life, which is a typical manifestation of BRIC. Until recently, *ATP8B1* or *ABCB11* were thought to be responsible for BRIC occurrence. While these two genes are associated with

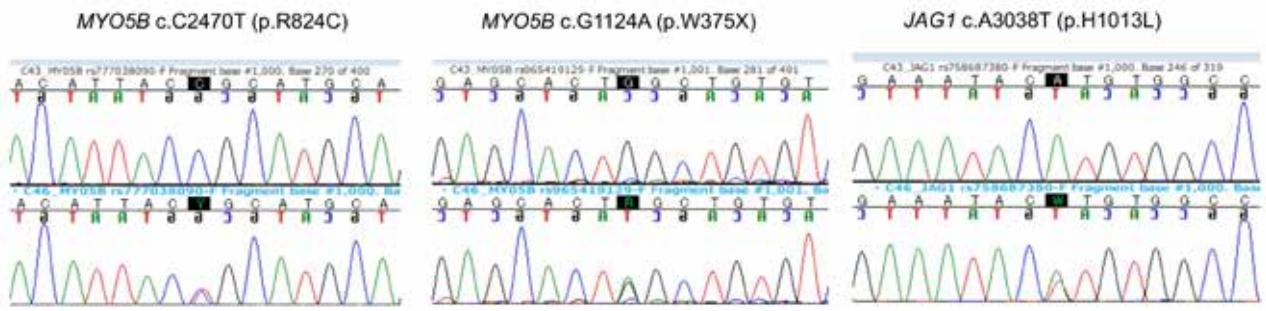

Fig. 2 Chromatograms indicating three mutations: c.G1124A:p.W375X and c.C2470T:p.R824C in *MYO5B*, and c.A3038T:p.H1013L in *JAG1*.

BRIC and PFIC, the BRIC-causing mutations affect protein functions less severely than the PFIC-causing ones [12]. In 2017, *MYO5B* was listed on the low-GGT BRIC-causing gene list for the first time. It was first identified as the gene responsible for MVID, of which three phenotypes due to *MYO5B* mutations are known: MVID, intrahepatic cholestasis (IC), and MIXED, which includes both phenotypes. These phenotypes appear to be determined by residual protein function and mutation location. Aldrian *et al*. investigated the genotype–phenotype relationship in 78 mostly Caucasian patients with *MYO5B*-related disease, including 29 patients with MVID, 19 with IC, and 30 with MIXED [10]. Among the patients with MVID, 52%, 17%, and 31% carried biallelic nonsense (null/null), heterozygous truncating and missense (null/missense), and biallelic missense (missense/missense) mutations, respectively. In contrast, none of the patients with IC (0%) carried null/null mutations, whereas 42% and 58% of the patients carried null/missense and missense/missense mutations, respectively. Patients with the MIXED phenotype exhibited characteristics intermediate to those in the MVID and IC groups; 40%, 23%, and 37% of the patients carried null/null, null/missense, and missense/missense mutations, respectively. A recent Chinese study that included 130 patients reported similar results [11]. Interpretation of the MIXED phenotype is difficult because total parenteral nutrition due to intractable watery diarrhea in the early days of life could cause cholestasis, which is indistinguishable from IC. Collectively, the complete lack of myosin Vb function causes MVID but not IC phenotypes. Furthermore, the location of missense mutations seems different between patients with MVID and IC. Mutations are frequently found in the motor domain of the MYO5B protein of patients with MVID, whereas mutations outside the motor domain are common in patients with IC. The motor domain is critical for myosin Vb function because of its ability to bind actin and hydrolyze ATP [11]. The IC-causing genotypes appeared to contain at least one non-null mutation outside the motor domain. The reason for this observation remains unknown; nevertheless, RAB11a-mediated gain-of-toxic-function has been indicated. Myosin Vb plays an important role in vesicular trafficking by binding to the RAB proteins. The complete loss of this protein causes intestinal failure (MVID). Meanwhile, milder mutations that possess the residual binding capacity to RAB11a might cause cholestasis by acting as decoys [13].

Our patient carried a null (c.G1124A:p.W375X) and a missense mutation (c.C2470T:p.R824C). The missense mutation, located in the IQ domain, has only been detected in patients with IC [10, 11]. Therefore, the BRIC phenotype without MVID observed in our case is not contradictory to the literature.

The Jagged1 protein encoded by *JAG1* is involved in Notch signaling. Mutations in *JAG1* are associated with Alagille syndrome, an autosomal dominant disorder that affects multiple organs including the liver, heart, kidneys, eyes, vertebrae, and skin [14]. Our patient carried a missense mutation in *JAG1* (c.A3038T:p.H1013L), in addition to the *MYO5B* mutations. This variant was predicted as 'Probably damaging' by Polyphen-2 and 'Deleterious' by SIFT; however, its association with Alagille syndrome has not been reported. Therefore, its contribution to the occurrence of cholestasis in our case was unlikely.

Our patient had two *MYO5B* mutations. In this study, we could not determine whether these two mutations existed in *cis* or *trans* because the distance between them was too long to be analyzed and samples from the parents were not available. To date, no patients carrying a monoallelic mutation have been reported in the context of *MYO5B*-related diseases. Therefore, our case was likely to be compound heterozygous for c.C2470T:p.R824C and c.G1124A:p.W375X.

A significant proportion of patients with *MYO5B*-related disease have severe diarrhea and/or cholestasis. According to the large cohort study including 114 patients, approximately 30% of the patients died at a median age of 1 year and 20% received single or multi-organ transplants [10]. In general, BRIC patients have a good prognosis without specific treatment, as does our patient.

The younger sister of this patient also presented with episodic jaundice. We think that this sister would likely have BRIC with the same genetic background with our patient. Unfortunately, there was no opportunity to perform genetic analysis for the sister.

To the best of our knowledge, no cases of *MYO5B*-related cholestasis, including BRIC, have been reported in Japan. This is the first documentation of *MYO5B*-related BRIC in the country.

The cluster of PFIC-causing genes has expanded beyond known loci, such as *ATP8B1*, *ABCB11*, and *ABCB4*. In the present case, we identified the *MYO5B* mutations using WES. This method appears to be a

highly useful tool for identifying the genes responsible for hereditary cholestatic diseases.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ETHICS STATEMENT

This study was approved by the Institutional Review Board for Clinical Research, Tokai University (approval number: 21R282).
Approval of the research protocol: April 7, 2017
Informed Consent: Written informed consent was obtained from the patient.

List of abbreviations

ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; ACMG, American College of Medical Genetics and Genomics; BRIC, benign recurrent intrahepatic cholestasis; GGT, gamma-glutamyl transferase; IC, intrahepatic cholestasis; MVID, microvillus inclusion disease; PFIC, progressive familial intrahepatic cholestasis; WES, whole-exome sequencing.

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