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Brief Report

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Transcript selection for the genetic diagnosis of *KIF12*-associated progressive familial intrahepatic cholestasis

Ye Cheng [1,2,3,†], Yi-Qiong Zhang [3,†], Bing-Xuan Wei [1], Lian Chen [4], Qing-He Xing [1,2,*] and Jian-She Wang [3,5,*]

[1]Children's Hospital of Fudan University and Institutes of Biomedical Sciences of Fudan University, Shanghai, P. R. China
[2]Shanghai Center for Women and Children's Health, Shanghai, P. R. China
[3]The Center for Pediatric Liver Diseases, Children's Hospital of Fudan University, Shanghai, P. R. China
[4]Department of Pathology, Children's Hospital of Fudan University, Shanghai, China
[5]Shanghai Key Laboratory of Birth Defects, Shanghai, P. R. China

*Corresponding authors. Jian-She Wang, The Center for Pediatric Liver Diseases, Children's Hospital of Fudan University, 399 Wanyuan Road, Minhang District, Shanghai 201102, P. R. China. Tel: +86-21-64931171; Fax: +86-21-64931901; Email: jshwang@shmu.edu.cn; Qing-He Xing, Institute of Biomedical Sciences, Fudan University, 131 Dong-an Road, Xuhui District, Shanghai 200032, P. R. China. Tel: +86-21-54237615; Fax: +86-21-54237615; Email: qhxing@fudan.edu.cn

†Ye Cheng and Yi-Qiong Zhang are co-first authors of this paper.

Introduction

Cholestatic jaundice in infancy occurs in ~1 in 2,500 term infants and 25% of cases are genetically determined [1]. An accurate diagnosis is crucial for clinicians to provide timely and personalized management. Despite the increasing application of whole-exome sequencing (WES), the genetic diagnosis of some patients remains unclear.

During genetic testing, one reference human genome sequence is selected for variant annotation and interpretation. Although GRCh38 was published, GRCh37 never lost its popularity in laboratories worldwide. This is mainly due to the insufficient annotations and the resistance to changing existing pipelines [2].

Progressive familial intrahepatic cholestasis (PFIC) represents a group of autosomal recessive inherited diseases characterized by intrahepatic cholestasis [3]. According to different pathogenic genes, the PFICs are classified into 12 types in Online Mendelian Inheritance in Man (OMIM). Kinesin family member 12 (*KIF12*) encodes a member of the kinesin superfamily of microtubule-associated molecular motors, whose deficiency can cause PFIC8 (OMIM: #619662). Here, we report on a pair of twin patients who presented with high gamma-glutamyl transpeptidase (GGT) cholestasis in whom the interpretation of WES results differed between references GRCh37 and GRCh38.

Case report

Patients II:2 and II:3 were boy–girl twins born in the 38th week of pregnancy. They were from a non-consanguineous Chinese family, and their parents and elder sister were healthy with normal serum biochemistry (Supplementary Figure 1A and Supplementary Table 1). Patient II:2 presented with jaundice at the age of 1 month and 26 days. At 2 months old, he was sent to the local hospital for high GGT cholestasis (Supplementary Table 1) and treated using ursodeoxycholic acid. At 3 months old, his jaundice had subsided but he still had high levels of transaminases and GGT. He was admitted to our hospital at 21 months old for hepatosplenomegaly with normal liver indices except for an elevated GGT value. Brain magnetic resonance imaging suggested bilateral mastoid effusion. His liver biopsy showed mild fibrosis, minimal inflammation, and proliferated bile ducts (Supplementary Figure 1B). Ursodeoxycholic acid was continuously taken and the liver indices completely normalized at the age of 30 months. At the age of 5 years, he stopped taking ursodeoxycholic acid for 4 months and the transaminase and GGT elevated, so he took it again.

Proband II:3 presented with jaundice at 3 days after birth. At the age of 50 days, she was sent to the local hospital due to high GGT cholestasis (Supplementary Table 1) and treated using ursodeoxycholic acid. Her bilirubin level gradually normalized, while transaminase and GGT remained elevated when she was referred to our hospital at the age of 21 months. Ultrasound revealed hepatosplenomegaly and slowed portal vein flow (≤17.7 cm/s). A radiographic examination showed osseous abnormalities in the metaphysis of the bilateral radius and tibia. Her liver biopsy was similar to that of Proband II:2 (Supplementary Figure 1B). She has been taking ursodeoxycholic acid and, by 31 months old, her transaminase had gradually normalized. At the age of 5 years, her GGT remained elevated. We performed immunohistochemistry for KIF12 on the twins and a normal control but no specific changes were found (Supplementary Figure 1C).

WES was performed in the twins and their unaffected parents. Genetic analysis based on GRCh37 (seen in the Supplementary Methods) revealed two *KIF12* compound heterozygous variants: NM_138424: c.1182 + 1G>A and NM_138424: c.-115-142_-115-

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Table 1. FPKM of different *KIF12* transcripts, *ABCC2*, and *ABCB11* detected in our two patients and control samples

Transcript/gene	II:2	II:3	HBV ($n=4$)	Normal adults ($n=5$)
NM_138424	0	0.47	0.54 ± 0.45	0
NM_001388308	58.65	59.03	2.51 ± 1.04	1.03 ± 0.24
KIF12	58.65	59.50	3.05 ± 0.63	1.03 ± 0.24
ABCC2	108.43	84.76	68.70 ± 6.54	68.76 ± 36.53
ABCB11	56.72	67.94	65.03 ± 19.68	56.67 ± 17.13

FPKM = fragments per kilobase of exon model per million mapped fragments, HBV = patients infected with hepatitis B virus.

136dupCGCCGCA. According to the American College of Medical Genetics and Genomics guidelines, these variants could be classified as likely pathogenic (PVS1 + PM2) and uncertain significance (PM2 + BP7), respectively. However, the canonical transcript of *KIF12* is NM_138424 in GRCh37 and NM_001388308 in GRCh38. When using NM_001388308 as the reference, these two variants (NM_001388308: c.1596 + 1G>A and NM_001388308: c.269_275dupCGCCGCA: p.Leu93AlafsTer79) could both be classified as likely pathogenic (PVS1 + PM2). Due to this inconsistency, we further performed mRNA sequencing in 11 samples (two patients, four children infected with hepatitis B virus and five normal adults; shown in Supplementary Methods). mRNA sequencing showed that the expression of NM_001388308 was significantly greater than that of NM_138424 in the liver ($P = 0.025$) (Table 1) and the number of reads carrying the mutant allele was similar to that of the reference allele (Supplementary Figure 2B and C). The frameshift variant NM_001388308: c.269_275dupCGCCGCA: p.Leu93AlafsTer79 could be detected and the variant NM_001388308: c.1596 + 1G>A could result in the skipping of exon 16, causing the in-frame deletion of 35 amino acids (Supplementary Figure 2B and C). Furthermore, we found that the expressions of *KIF12* in two patients were greater than that in the controls (Table 1), while the expressions of *ABCC2* and *ABCB11* were similar. Thus, these two patients could be clearly diagnosed with PFIC8.

Discussion

In this study, we have reported one pair of boy–girl twins with infantile high GGT cholestasis carrying compound heterozygous variants on *KIF12*. The analysis of WES data using GRCh37 did not offer a definite diagnosis. By using GRCh38 and mRNA sequencing in two patients and nine controls in the reanalysis, we confirmed that NM_001388308 was the major transcript in the liver and the diagnosis of PFIC8 was clear.

To date, a total of 13 PFIC8 patients have been reported and all were diagnosed based on NM_138424 [4–6]. Compared with the transcript NM_001388308, NM_138424 lacks exons 1, 2, and 4 (Supplementary Figure 2A). Nonetheless, their diagnoses were still accurate when using NM_138424 since none of the variants in these 13 patients was located at these exons.

Regarding our two patients, the expressions of alleles carrying two variants were similar and upregulated. Immunohistochemistry showed stronger KIF12 staining in livers of patients with *KIF12* variants than that of controls, implying that these two variants might escape nonsense-mediated mRNA decay or alter the pattern of protein translation or degradation, which resulted in the accumulation of mutant protein. In 2008, a similar pattern was observed and proposed by Kossack *et al.* [7] in the gene encoding the receptor for both luteinizing hormone and choriogonadotropin. Additionally, the increase in expression could also be resulting from a feedback regulatory mechanism due to the loss of normal KIF12 protein. More studies are needed to verify these assumptions. Furthermore, although the difference in the canonical transcript of KIF12 between GRCh37 and GRCh38 complicates the genetic diagnosis, these two references are still suitable for most gene analyses.

In conclusion, we found that NM_001388308 should be used as the reference transcript for *KIF12* variant annotation and interpretation.

Authors' contributions

Q.H.X. and J.S.W. conceived and designed the project. Y.C. and Y.Q.Z. collected the data. Y.C., Y.Q.Z., B.X.W., and C.L. analysed and interpreted the data. Y.C. and Y.Q.Z. drafted the manuscript. All authors read and approved the final manuscript.

Supplementary Data

Supplementary data is available at *Gastroenterology Report* online.

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Conflict of Interest

None declared.

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