Recessive Mutations in *KIF12* Cause High Gamma-Glutamyltransferase Cholestasis

Aysel Ünlüsoy Aksu,[1] Subhash K. Das,[2] Carol Nelson-Williams,[3] Dhanpat Jain,[2,4] Ferda Özbay Hoşnut,[1] Gülseren Evirgen Şahin,[1] Richard P. Lifton,[3,5,6] and Silvia Vilarinho[2,4]

Undiagnosed liver disease remains an unmet medical need in pediatric hepatology, including children with high gamma-glutamyltransferase (GGT) cholestasis. Here, we report whole-exome sequencing of germline DNA from 2 unrelated children, both offspring of consanguineous union, with neonatal cholestasis and high GGT of unclear etiology. Both children had a rare homozygous damaging mutation (p.Arg219* and p.Val204Met) in kinesin family member 12 (*KIF12*). Furthermore, an older sibling of the child homozygous for p.Val204Met missense mutation, who was also found to have cholestasis, had the same homozygous mutation, thus identifying the cause of the underlying liver disease. *Conclusion*: Our findings implicate rare homozygous mutations in *KIF12* in the pathogenesis of cholestatic liver disease with high GGT in 3 previously undiagnosed children. (*Hepatology Communications* 2019;3:471-477).

Undiagnosed liver disease remains an unmet medical need in pediatric and adult hepatology, including cholestasis of unknown etiology. We[1-3] and others[4-6] have shown the utility of unbiased whole-exome sequencing (WES) in diagnosing patients with atypical presentations of known liver disorders as well as in identifying novel Mendelian forms of liver diseases. Identification of underlying genetic defects provides new insight into the role of specific genes in the maintenance of normal liver function as well as disease pathogenesis; this finding also identifies potential new therapeutic options for affected patients.

Cholestasis results from impaired bile drainage, leading to stasis and retention of substances in the blood circulation that are normally excreted into bile. For the past 2 decades, application of human genetics and genomics tools to patients with cholestatic liver diseases of unknown etiology has uncovered new genetic causes of cholestasis, resulting in the identification of new disease mechanisms.[4,7-11] Here, we report 3 children from 2 unrelated consanguineous families with cholestasis and high gamma-glutamyltransferase (GGT) of unclear etiology, who were found to harbor homozygous damaging mutations in kinesin family member 12 (*KIF12*). KIF12 belongs to the large kinesin superfamily of microtubule-associated molecular motors,[12] which are known to play critical roles in intracellular transport of organelles and function of the mitotic spindle in cell division.[13-15]

Patients and Methods

HUMAN SUBJECTS

This study was performed in accordance with the Dr. Sami Ulus Children's Hospital and Yale University institutional review board standards. All patients and/or their parents provided written informed consent.

DNA ISOLATION, EXOME CAPTURE, AND SEQUENCING

Genomic DNA of the probands, their unaffected parents, and siblings (when available) was isolated from peripheral blood leukocytes using standard procedures. WES of genomic DNA from the probands was performed using the xGen capture kit (Integrated DNA Technologies, Inc., Skokie IL) followed by 100 base paired-end sequencing on the Illumina HiSeq platform (San Diego, CA).

EXOME ANALYSIS

WES data were aligned to the reference human genome (build 19), and variants were called using GATK.[16,17] Allele frequencies of identified variants were determined in the Genome Aggregation Database (gnomAD), which spans more than 123,000 exomes and approximately 15,500 genomes from unrelated subjects of various ethnicities with or without a diverse array of specific human diseases, including National Heart, Lung, and Blood Institute Exome Variant Server, 1000 Genomes, and the Exome Aggregation Consortium.[18] Variants with minor allele frequency 0.01 or less were annotated using Annovar,[19] and the impact of missense variants was predicted using MetaSVM.[20]

SANGER SEQUENCING OF GENOMIC DNA

Sanger sequencing of the identified *KIF12* mutation (p.Val204Met) was performed by polymerase chain reaction (PCR) amplification of genomic DNA of the proband and her parents using forward primer 5′-CTGCCCTAAACCTGGCATGA-3′ and reverse primer 5′-AGCAGAGCCACATCCCTTTC-3′. Sanger sequencing of the identified *KIF12* mutation (p.Arg219*) was performed by PCR amplification of genomic DNA of the proband and his parents and siblings using forward primer 5′-CCCAACTTTGACCCAGTCCA-3′ and reverse primer 5′-GGGAAGGTTGGGTCTTGAGG-3′. Nomenclature of the *KIF12* variants are based on the National Center for Biotechnology Information reference sequence NM_138424.

ORTHOLOGS OF KIF12

Full-length orthologs of KIF12 protein sequences from several species were obtained from GenBank, and protein sequences were aligned using the Clustal Omega algorithm.

ARTICLE INFORMATION:

From the [1]Department of Pediatric Gastroenterology, Hepatology and Nutrition, University of Health Sciences, Dr. Sami Ulus Maternity and Child Health and Diseases Training and Research Hospital, Ankara, Turkey; [2]Department of Internal Medicine, Section of Digestive Diseases, Yale University School of Medicine, New Haven, CT; [3]Department of Genetics, Yale University School of Medicine, New Haven, CT; [4]Department of Pathology, Yale University School of Medicine, New Haven, CT; [5]Yale Center for Mendelian Genomics, Yale University School of Medicine, New Haven, CT; [6]Laboratory of Human Genetics and Genomics, Rockefeller University, New York, NY.

ADDRESS CORRESPONDENCE AND REPRINT REQUESTS TO:

Silvia Vilarinho, M.D., Ph.D.
Departments of Internal Medicine (Digestive Diseases) and
Pathology, Yale University School of Medicine
333 Cedar Street, LMP1080

New Haven, CT 06510
E-mail: silvia.vilarinho@yale.edu
Tel: +1-203-737-6063
Fax: +1-203-737-1755

Results

CLINICAL, LABORATORY, AND LIVER HISTOLOGY FEATURES OF THE 3 CHILDREN WITH UNEXPLAINED CHOLESTASIS

Patient 1 is a 22-month-old girl offspring of a first-cousin union who presented with neonatal cholestasis of unclear etiology at approximately 1 month of age (Fig. 1A). She was born full term without immediate complications and had no family history of liver disease in her parents. Physical examination was remarkable for jaundice and hepatomegaly. Laboratory tests were remarkable for elevated GGT, high direct hyperbilirubinemia, elevated transaminases, elevated alpha-fetoprotein (AFP) level, and hypercholesterolemia (Table 1 and Supporting Fig. S1). Initial abdominal ultrasound showed unremarkable liver parenchyma and grade 1 left hydronephrosis. Abdominal magnetic resonance imaging (MRI) showed a mild increase in left renal pelvic anterior–posterior diameter to 6 mm and no other significant abnormalities. Liver biopsy at 2.5 months of age showed bridging fibrosis with early nodule formation, mixed portal inflammatory infiltrate, multinucleated giant hepatocytes, extensive hepatocellular and canalicular cholestasis, ductular reaction, and bile duct loss (Fig. 1B). At 8 months of age, laboratory tests showed slightly elevated serum bile acids and down-trending GGT, total bilirubin, transaminases, AFP, triglycerides, and total cholesterol (Table 1 and Supporting Fig. S1). AFP level was normalized by 13 months of age. Most recent laboratory tests at 22 months of age showed persistent elevated GGT and elevated transaminases (Supporting Fig. S1).

Patient 2.1 is a 21-month-old male offspring of a second-cousin union who also presented with neonatal cholestasis of unclear etiology at 2 months of age (Fig. 1A and Table 1). He has two siblings, and his mother had a spontaneous abortion. He was born full term without immediate complications and had no family history of liver disease in his first-degree family members. Like patient 1, physical examination was remarkable for jaundice and hepatomegaly, and laboratory tests revealed elevated GGT, alkaline phosphatase, total bilirubin, transaminases, AFP, triglycerides, and total cholesterol (Table 1 and Supporting Fig. S1). At

approximately 3 months of age, the patient underwent MRI cholangiogram that showed mild increase in the right renal pelvic anterior–posterior diameter but no biliary dilatation, strictures, or other liver-related abnormalities. Liver biopsy performed at 3.5 months of age showed biliary pattern of cirrhosis with mixed portal inflammatory infiltrate, mild ductular reaction, pseudo-acini formation, duct loss, and nodule formation (Fig. 1B). At 4 months of age, serum bile acids were measured and found to be elevated (Table 1). Follow-up laboratory tests showed resolution of hyperbilirubinemia and normalization of AFP and serum lipid panel by 6 months of age, whereas GGT, alkaline phosphatase, and liver enzymes remained persistently elevated (Supporting Fig. S1). Upper endoscopy at 21 months of age revealed no esophageal varices.

Patient 2.2 is a 9-year-old boy and an older sibling of patient 2.1 who was followed for prolonged jaundice of unclear etiology until 2 months of age. Physical examination was unremarkable. Liver function tests were remarkable for elevated GGT, alkaline phosphatase, transaminases, and serum bile acids (Table 1). Recent abdominal ultrasound showed heterogeneous liver and splenomegaly. Follow-up abdominal MRI showed liver heterogeneity consistent with chronic liver disease with no biliary dilations or strictures and caliectasis of the upper pole of the left kidney. Upper endoscopy showed no esophageal varices or portal hypertensive gastropathy. No liver biopsy was obtained.

Thus far, all 3 patients have shown international normalized ratio and albumin levels within normal limits and no signs of liver decompensation.

HOMOZYGOUS DAMAGING MUTATIONS IN KIF12 IN PROBANDS

We performed WES of germline DNA isolated from peripheral leucocytes of one proband of each family. Targeted bases were sequenced by a mean of 25 independent reads, with 91% of targeted bases having more than eight independent reads (Supporting Table S1).

Because the probands are both the offspring of consanguineous union, we sought rare homozygous variants (minor allele frequency ≤ 0.01 in public databases) that are predicted to result in loss of function of the encoded protein (i.e., nonsense, frameshift,
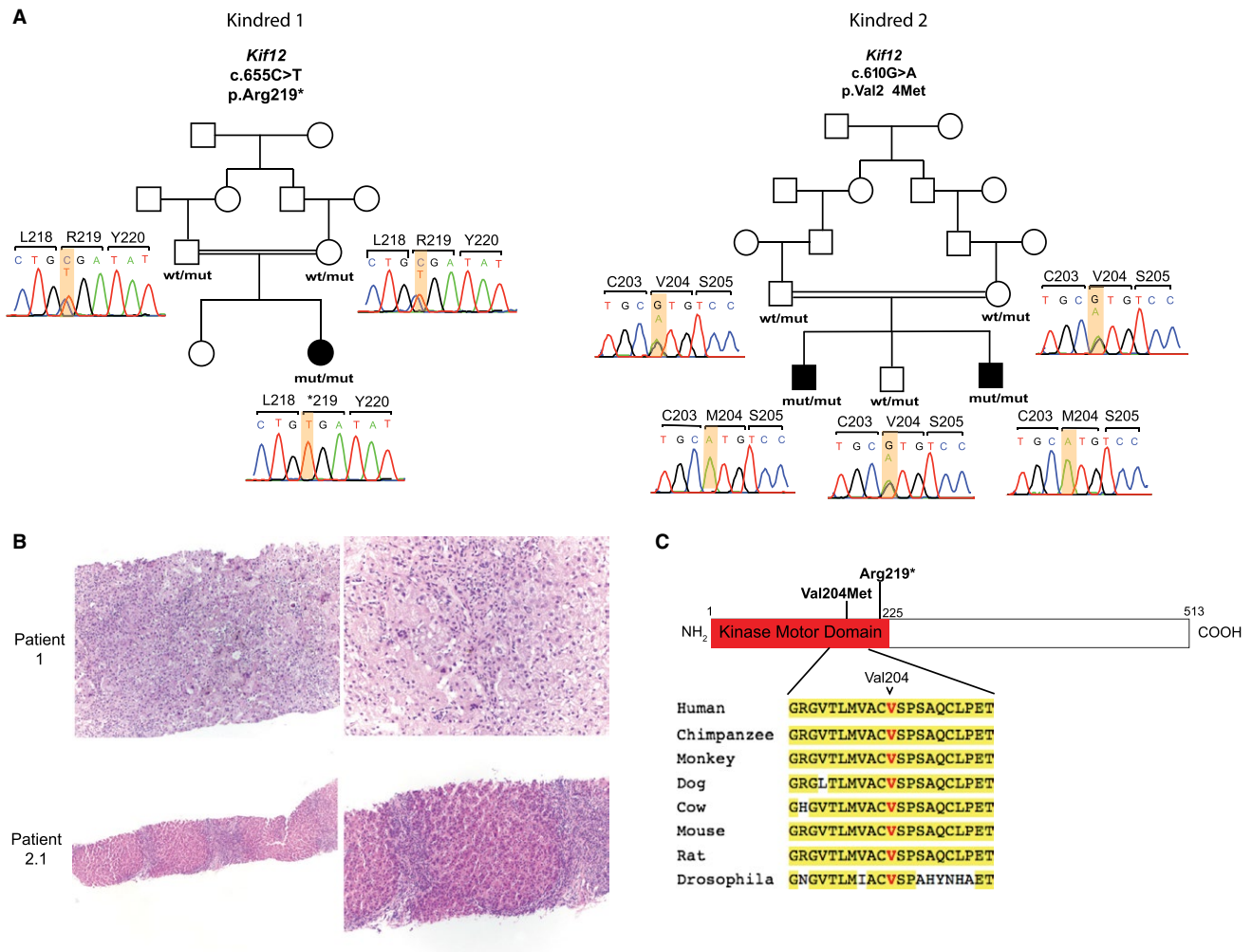
FIG. 1. Cholestasis due to recessive mutations in *KIF12* identified in 3 children. (A) Homozygous mutations in *KIF12* identified in 3 children with cholestasis of unclear etiology. Pedigrees of 2 consanguineous families (shown with a double line) with cholestasis of unclear etiology are depicted. Affected and unaffected subjects are shown as black-filled and white-filled symbols, respectively. Corresponding Sanger sequencing chromatograms of the 3 cases, their parents, and 1 sibling are depicted and denoted as wild type and mutant. Mutations (p.Arg219* in kindred 1 and p.Val204Met in kindred 2) are homozygous in affected subjects and heterozygous in unaffected parents and sibling. (B) In patient 1, lower magnification of liver parenchyma (upper-left image, 100x) shows early nodule formation, mixed portal inflammation, and mild ductular reaction. Higher magnification (upper-right image, 200x) shows lobular disarray with thickened cords, numerous multinucleated giant hepatocytes, and extensive hepatocellular and canalicular cholestasis. In the center, there is a portal area showing bile duct loss and mixed inflammatory infiltrate. In patient 2.1, lower magnification of liver parenchyma (lower-left image, 40x) shows biliary pattern of cirrhosis with nodule formation, mixed portal inflammation, and mild ductular reaction. Higher magnification (lower-right image, 100x) also shows nodules with thickened cords, pseudo-acini formation, ductular reaction, and bile duct loss. (C) Schematic representation of human KIF12 protein with kinase motor domain depicted in red, where both mutations (shown in bold) are located. Conservation of Val204 position across KIF12 orthologs is shown. Amino acids that are identical to the human protein are highlighted in yellow. Abbreviations: mut, mutant; wt, wild type.

and splice-site variants) or are predicted to be damaging missense variants by MetaSVM. Consistent with consanguinity, this analysis identified eight and six rare autosomal homozygous genotypes in the probands, respectively. Notably, both probands had a rare homozygous mutation in the kinase motor domain of *KIF12* (Fig. 1A,C and Table 2), and there was no other gene with rare variants in both kindreds. Patient 1 is homozygous for a variant encoding a p.Arg219* (chr9:116857353, G>A) premature termination. This variant was extremely rare in public databases, with no homozygotes and a minor allele frequency of

TABLE 1. SUMMARY OF DEMOGRAPHICS AND GENETIC, CLINICAL, AND LABORATORY FINDINGS OF THE CHILDREN WITH HIGH GGT CHOLESTASIS AND RECESSIVE MUTATIONS IN KIF12

Patient Number	Ethnicity (Consanguinity)	Age of Onset	Presenting Clinical Features	Homozygous Mutation	GGT (U/L)	ALP (U/L)	Fasting Serum Bile Acids (NR < 10 µmol/L)	Total/Direct Bilirubin (NR < 2/< 0.5 mg/dL)	AST/ALT (NR < 47/< 39 U/L)	Current Status/ Age
1	Syrian (yes)	1 month	Cholestasis (high GGT)	p.Arg219*	998 (NR < 204)	732 (NR = 60-321)	12	4.1/3.5	147/109	Alive with native liver/22 months
2.1	Turkish (yes)	2 months	Cholestasis (high GGT)	p.Val204Met	521 (NR < 204)	703 (NR = 110-302)	38.3	4.5/3.8	105/87	Alive with native liver/21 months
2.2	Turkish (yes)	9 years	Genotype led to recognition of cholestasis	p.Val204Met	62 (NR < 23)	422 (NR = 110-341)	33.5	0.7/0.2	84/88	Alive with native liver/10 years

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; and NR, normal range.

3.233×10^{-5} in the gnomAD database (Table 2).[18] Patient 2 is homozygous for a missense variant encoding p.Val204Met (chr9:116857398, C>T), which is a highly conserved position across orthologues and is predicted to be a damaging mutation by MetaSVM (Fig. 1C and Table 2). This variant was also extremely rare, with no homozygotes and an allele frequency of 2.1×10^{-5} in the gnomAD database (Table 2). Sanger sequencing in each case confirmed the homozygous mutation in probands and the heterozygous carrier state of their consanguineous parents (Fig. 1A). In addition, a sibling of patient 2, a 9-year-old male, also had cholestasis with elevated GGT (Table 1). He too was homozygous for the p.Val204Met mutation (Fig. 1A). From the pedigree structures, we infer that the probability of finding all affected members of these 2 kindreds to have rare homozygous variants at the same locus by chance to be 1 in 5,012 (logarithm of the odds score 3.7), indicating significant evidence of linkage. Together, these findings provide strong evidence that these mutations are the cause of cholestasis and chronic liver disease in these families.

Discussion

Our findings implicate rare homozygous mutations in *KIF12* in the pathogenesis of cholestatic liver disease with high GGT in 3 previously undiagnosed children. First, the genetic evidence is strong, with significant evidence of genetic linkage of rare damaging mutations in *KIF12* that segregate with the disease in 2 consanguineous kindreds. Second, the clinical features and liver histologies obtained at early age from patients 1 and 2.1 share very similar liver parenchyma features, such as advanced fibrosis, mixed inflammatory infiltrate, ductular reaction, and bile duct loss. Third, *Kif12* modulates biliary phenotype severity, including cholangitis, and cystic kidney disease,[21] in a well-characterized murine model of autosomal dominant polycystic kidney disease. In this model, the presence and absence of a biliary phenotype varies based on the genetic background; the presence of a strain-specific 15 base-pair insertion within the kinase motor domain of *Kif12*[21] produces the biliary phenotype. Fourth, the hepatocyte nuclear factor 1 beta, a transcription factor that is important in biliary epithelium formation,[22] regulates Kif12 expression in cell-based and mouse models,[23] providing support

TABLE 2. RARE HOMOZYGOUS VARIANTS IN *KIF12* IDENTIFIED IN THE PATIENTS

Patient Number	Chr:Position (hg19)	Nucleotide Change	AA Change	MetaSVM Score (Prediction)*	NHLBI	1000G	ExAC	gnomAD
1	9:116857353	G > A	p.Arg219*	Truncating	0	0	1.73×10^{-5}	3.233×10^{-5}
2.1 2.2	9:116857398	C > T	p.Val204Met	0.478 (D)	0	1.9968×10^{-4}	2.58×10^{-5}	2.092×10^{-5}

*MetaSVM scores missense variants on a scale from –2 to 3, with scores greater than 0 predicted to be damaging.
Abbreviations: 1000G, 1000 Genome database; AA, amino acid; Chr, chromosome; D, damaging; ExAC, Exome Aggregation Consortium database; and NHLBI, National Heart, Lung, and Blood Institute Exome Sequencing Project database.

for a potential role of Kif12 in bile duct epithelial cell function.

Interestingly, all 3 children were found to have mild renal pelvic abnormalities on imaging studies with unremarkable kidney function. Within this small cohort of patients, it is also remarkable that, although 2 of the affected individuals presented with jaundice and marked cholestasis within the first few months of life, patient 2.2 has been overall asymptomatic during his first 10 years of life despite a history of prolonged neonatal jaundice, and it was the knowledge of his genotype that led to recognition of his "silent" underlying cholestatic liver disease.

Of note, recessive mutations in doublecortin domain containing protein 2 encoded by *DCDC2*, a microtubule-associated protein, are known to cause neonatal sclerosing cholangitis (which presents with high GGT).[5,6] Other well-characterized genetic disorders presenting with high GGT cholestasis in children comprise MDR3 deficiency and Alagille syndrome. Although the latter exhibit typical extrahepatic manifestations that are absent in our patients, MDR3 deficiency is a cholangiopathy with variable rate of progression and liver histological features reminiscent of the findings seen in the KIF12 mutant patients described here.

Lastly, during the final stages of preparation of this manuscript, an independent study was published online describing 4 children from 3 unrelated families with high-GGT cholestasis of unclear etiology, and no extrahepatic abnormalities who were found to harbor recessive mutations in *KIF12*.[24] Two of the 4 affected children underwent liver transplantation at 6 years and 10 months of age, respectively.[24] The latter harbors the aforementioned p.Val204Met missense mutation identified in our patients 2.1 and 2.2. Hence, the full clinical spectrum of this new

cholestatic liver disease due to recessive mutations in *KIF12* will be determined by the study of additional patients and the longitudinal follow-up of all described patients. Future functional studies will be key to elucidate the role of KIF12 in biliary function in humans, with the goal of potentially identifying novel therapeutic targets for the treatment of cholestasis.

Collectively, this study highlights the critical importance of using unbiased genomic analysis in the diagnosis of children with liver disease of indeterminate etiology, such as idiopathic cholestasis, which cannot be determined through conventional approaches such as biochemical, imaging, and histological tests.

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Author names in bold designate shared co-first authorship.

Supporting Information

Additional Supporting Information may be found at onlinelibrary.wiley.com/doi/10.1002/hep4.1320/suppinfo.