Advancing diagnosis and management of liver disease in adults through exome sequencing

Melanie Zheng,[a] Aaron Hakim,[b,c] Chigoziri Konkwo,[a] Aimee M. Deaton,[d] Lucas D. Ward,[d] Alnylam Human Genetics,[d] Marina G. Silveira,[a] David N. Assis,[a] AnnMarie Liapakis,[a] Ariel Jaffe,[a] Z. Gordon Jiang,[b] Michael P. Curry,[b] Michelle Lai,[b] Michael H. Cho,[c] Daniel Dykas,[e] Allen Bale,[e] Pramod K. Mistry,[a] and Silvia Vilarinho[a,f,*]

[a]Department of Internal Medicine, Section of Digestive Diseases, Yale School of Medicine, New Haven, CT, USA
[b]Division of Gastroenterology and Hepatology, Beth Israel Deaconess Medical Center, Boston, MA, USA
[c]Channing Division of Network Medicine, Brigham and Women's Hospital, Boston, MA, USA
[d]Alnylam Pharmaceuticals, Boston, MA, USA
[e]Department of Genetics, Yale School of Medicine, New Haven, CT, USA
[f]Department of Pathology, Yale School of Medicine, New Haven, CT, USA

eBioMedicine
2023;95: 104747
Published Online 9 August 2023
https://doi.org/10.1016/j.ebiom.2023.104747

Summary

Background Whole-exome sequencing (WES) is an effective tool for diagnosis in patients who remain undiagnosed despite a comprehensive clinical work-up. While WES is being used increasingly in pediatrics and oncology, it remains underutilized in non-oncological adult medicine, including in patients with liver disease, in part based on the faulty premise that adults are unlikely to harbor rare genetic variants with large effect size. Here, we aim to assess the burden of rare genetic variants underlying liver disease in adults at two major tertiary referral academic medical centers.

Methods WES analysis paired with comprehensive clinical evaluation was performed in fifty-two adult patients with liver disease of unknown etiology evaluated at two US tertiary academic health care centers.

Findings Exome analysis uncovered a definitive or presumed diagnosis in 33% of patients (17/52) providing insight into their disease pathogenesis, with most of these patients (12/17) not having a known family history of liver disease. Our data shows that over two-thirds of undiagnosed liver disease patients attaining a genetic diagnosis were being evaluated for cholestasis or hepatic steatosis of unknown etiology.

Interpretation This study reveals an underappreciated incidence and spectrum of genetic diseases presenting in adulthood and underscores the clinical value of incorporating exome sequencing in the evaluation and management of adults with liver disease of unknown etiology.

Funding S.V. is supported by the NIH/NIDDK (K08 DK113109 and R01 DK131033-01A1) and the Doris Duke Charitable Foundation Grant #2019081. This work was supported in part by NIH-funded Yale Liver Center, P30 DK34989.

Keywords: Idiopathic liver disease; Next generation sequencing; Undiagnosed disease; Genomics; Genetic disorder

Introduction

Genomic medicine, or the utilization of a patient's genomic information to personalize their clinical care, has revolutionized the practice of clinical medicine. However, its application has varied across specialties, with oncology and pediatrics leading the way in its incorporation in the clinical armamentarium. While certain areas of internal medicine, such as nephrology[1,2] and cardiology,[3] have also begun to incorporate genomic analysis in routine practice[4]; the use of genomic analysis to evaluate patients with liver disease has lagged. Genetic defects are rarely considered in the diagnosis of adult liver disease, and hepatology clinical practice remains largely centered around broad phenotypic categories. Efforts in the past several years have shown that genetics play a much larger and more nuanced role than

*Corresponding author. Departments of Internal Medicine (Digestive Diseases) and of Pathology, Yale School of Medicine, New Haven, CT, USA.
E-mail address: silvia.vilarinho@yale.edu (S. Vilarinho).
Alnylam Human Genetics is composed by Rachel A. Hoffing, Aaron M. Holleman, Lynne Krohn, Philip LoGerfo, Paul Nioi, Mollie E. Plekan.

Research in context

Evidence before this study

A pilot study has demonstrated the clinical utility of whole-exome sequencing in the diagnosis and management of adults with unexplained liver disease despite a comprehensive work-up.

Added value of this study

This study validates the diagnostic utility of exome analysis in the evaluation of adult patients with liver disease of unclear etiology and particularly in adults with idiopathic cholestasis or hepatic steatosis of unknown cause.

Implications of all the available evidence

We hope that this study will spur clinicians and investigators in the field to pursue genetic investigation of other patients with unexplained liver abnormalities. Moreover, this data provides strong rationale for the development of a clinical practice guidance delineating when and how to incorporate genomic analysis in clinical hepatology.

previously understood, suggesting that genomic medicine is presently overlooked in adult liver disease.[5–8] Addressing this unmet need represents a significant opportunity to advance patient care.[9,10]

Despite recent advances in the treatment of hepatitis B and C, the incidence of chronic liver disease continues to rise globally, accounting for over 120 million individuals suffering from end-stage liver disease and 2 million deaths annually worldwide.[11,12] It is estimated that up to 30% of individuals with cirrhosis and up to 14% of adults listed for liver transplantation have liver disease of unknown cause.[13,14] These patients often undergo multi-year 'diagnostic odysseys' of medical evaluations, investigations, and empirical interventions. Prompt identification of the underpinnings of monogenic liver diseases in adults would promote targeted management that may prevent progression to advanced liver disease and can lessen the burden on patients with undiagnosed disease and the healthcare system thereof, as shown in other areas of clinical medicine.[15] Thus, there is a clear impetus for implementing genomic analysis in diagnostic work-up.

Whole-exome sequencing (WES) facilitates the identification of individual nucleotide changes in protein-coding regions and flanking intronic regions of nearly all 20,000 genes in the human genome, to rapidly uncover rare disease-causing variants. In the evaluation of idiopathic disease, WES strikes a balance between cost, time of analysis, and scope of genetic information collected compared to more limited targeted gene panels or single-gene Sanger sequencing. Our research group first demonstrated the clinical utility of WES in children with liver failure of indeterminate etiology,[16] and subsequently in a pilot study of nineteen adults with unexplained liver disease. This pilot study included one patient found to have familial partial lipodystrophy type 3 due to *PPARG* deficiency,[5] whose WES-facilitated diagnosis led to the initiation of leptin replacement therapy, resulting in improvement in serum triglyceride levels, normalization of liver transaminases, and reduction in insulin requirements.[5] Moreover, in this pilot study, over 25% of these adult patients with liver disease of unknown etiology received an actionable genetic diagnosis.[5] Here, we employed WES to an expanded cohort of fifty-two adult patients with unexplained liver disease spanning two tertiary US academic medical centers.

Methods

Patient cohort

Adults with unexplained liver disease despite a conventional work-up performed by a clinical hepatologist were recruited and evaluated using WES. All patients were characterized by primary liver disease clinical phenotype, presenting age, sex, family history of liver disease, and cirrhosis status (Table 1). Liver disease phenotypes were broadly categorized based on main clinical presentation for hepatology evaluation. Age of presentation was defined as age at exome analysis. Family history of liver disease was based on patient's report. Cirrhosis was determined by either liver biopsy or transient elastography liver stiffness measurement of greater than 12.5 kPa,[17] or otherwise indicated as 'unknown'.

Ethics

This study was performed in accordance with protocols approved by the Yale Human Investigation Committee (HIC#1503015498) and the Beth Israel Deaconess Medical Center Committee on Clinical Investigation (Protocol #: 2019P001102). Written informed consent was obtained in accordance with institutional review board standards.

DNA isolation, exome capture, sequencing, and analysis

Genomic DNA was isolated from peripheral blood or buccal swab samples, and exonic fragments were captured, sequenced, and aligned against the genome reference human build 19 (hg19). Variants were called using GATK[18] and annotated using Annovar.[19] Initial variant filters include minor allele frequency (MAF) < 0.01 for homozygous and compound heterozygous variants and MAF < $2e^{-5}$ for heterozygous variants. Genetic variants in accordance with MAFs outlined above were considered rare and prioritized based on

Characteristic	Total of patients (%)	n of patients with genetic diagnosis (%)
Sex		
Female	31 (60%)	8 (47%)
Male	21 (40%)	9 (53%)
Age of presentation (yo)		
<30	11 (21%)	7 (41%)
30–39	9 (17%)	5 (29%)
40–49	12 (23%)	2 (12%)
50–59	7 (13%)	2 (12%)
60–69	10 (19%)	1 (6%)
70–79	3 (6%)	0 (0%)
Family history		
Yes	14 (27%)	5 (29%)
Absent/unknown	38 (73%)	12 (71%)
Cirrhosis		
Yes	11 (21%)	4 (24%)
No	39 (75%)	13 (76%)
Unknown	2 (4%)	0 (0%)
Primary liver phenotype		
Cholestasis	17 (33%)	8 (47%)
Hepatic steatosis	8 (15%)	4 (23%)
Advanced fibrosis/cirrhosis	10 (19%)	1 (6%)
Elevated transaminases	9 (17%)	2 (12%)
HFE-negative iron overload	2 (4%)	1 (6%)
Other	6 (12%)	1 (6%)

n: number; yo: years-old.

Table 1: **Summary of study population characteristics and demographics.**

their predicted deleteriousness (premature termination, frameshift, and splice-site variants) and protein alteration using a combined annotation dependent depletion (CADD) score ≥20 for missense variants. Candidate variants were then considered using prior reports of pathogenicity in NCBI ClinVar and gene-liver disease relationship (Fig. 1). No enrichment or independent testing of specific genomic regions or mitochondrial genome was performed. Tag single nucleotide polymorphisms (SNPs) were obtained from WES data, along with the same SNPs extracted from the HapMap project, to perform principal component analysis using EIGENSTRAT software to assess the ancestry of the individuals within our cohort (Supplementary Figure S1).

Exome depth of coverage-based copy number variation detection

Copy number variation (CNV) was assessed using exome capture read depth normalized to chromosome read depth across all samples. Comparing normalized sequence depths across samples established a baseline reference depth range for a given exonic region, allowing for significant depth coverage variance to be detected as a potential CNV. CNVs of either deletions or duplications of two or more contiguous exons can be detected with close to 100% sensitivity.[5] Validation of this method was performed using standard, high-resolution array comparative genomic hybridization and exon-array comparative genomic hybridization or PCR.

Statistics

The sub-group of patients given a genetic diagnosis was compared with the sub-group of patients who remain undiagnosed, using presenting age, family history status, and sex. Chi-square test was performed using SPSS Statistics 25 (IBM Corporation, Armonk, NY), and reported with an adjusted p-value according to Bonferroni correction.

UK Biobank analysis

Candidate disease-causing variants identified in undiagnosed patients were searched in exome sequencing data of 454,756 participants in the UK Biobank,[20] accessed through Application 26,041. Ethics oversight of the UK Biobank is through the National Health Service National Research Ethics Service, and all participants provided written informed consent to participate in the study. Variant calling was performed as described previously[21]; variants were first called on hg38 and then converted to hg19 coordinates to match the analysis of patient's samples. Six continental ancestry groups, as defined by the Pan UKBB analysis (https://pan.ukbb.broadinstitute.org/), were used to assigned 414,424 of the exome-sequenced participants to subpopulations. Ancestry assignments from the Pan UKBB analysis and 20 genetic principal components calculated within each population were obtained from the UK Biobank Data Showcase, Return 2442. Candidate variants that were found in at least ten carriers in at least one subpopulation were tested for association with levels of six circulating liver biomarkers, namely alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), albumin, direct bilirubin, and total bilirubin (Bonferroni correction, $p < 0.0083$). In addition, a rare variant in *APOB*, p.Glu1976Ter (rs267599184), was also tested for these liver biomarkers and serum apolipoprotein B levels. For each variant, the association tests were performed within the subpopulation with the most carriers. Association tests were performed in PLINK using the subset of unrelated individuals in the subpopulation tested, using age, sex, and the 20 genetic principal components as covariates. Prior to testing, the liver biomarker values were inverse rank normalized.

Roles of funders

Funders of this study had no role in study design, data collection, data analyses, interpretation, or writing of manuscript.

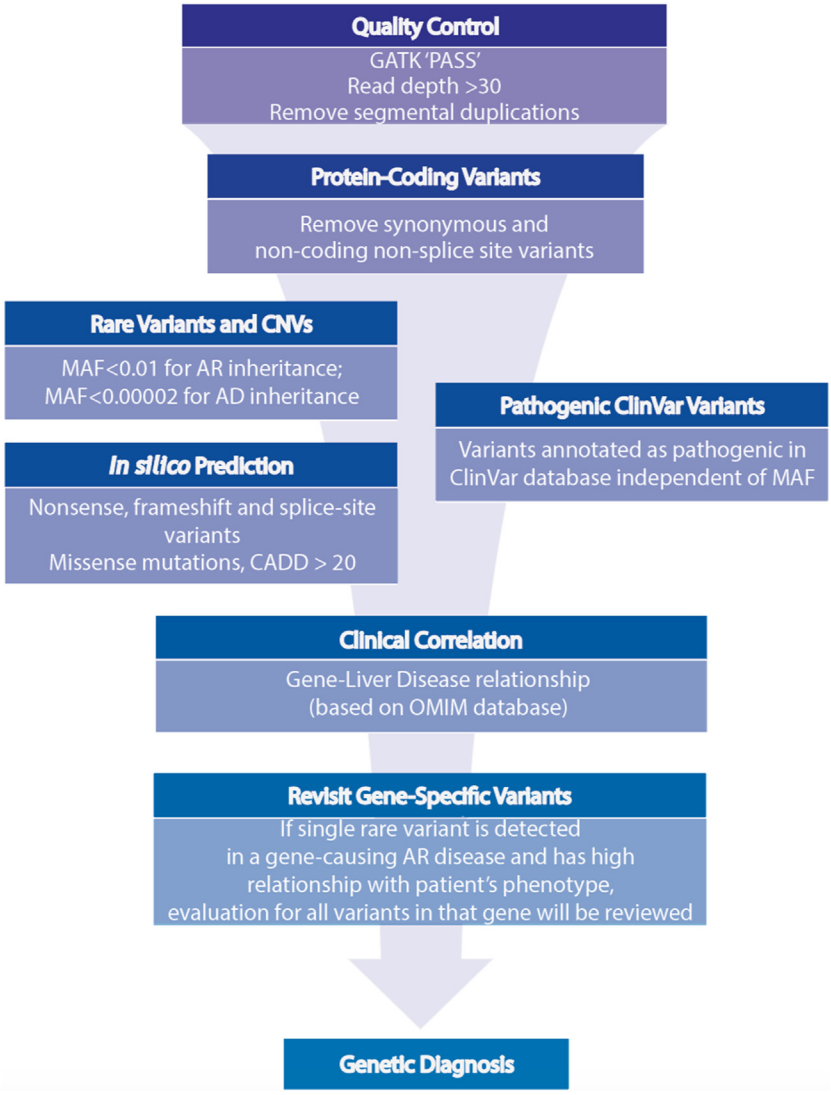

Fig. 1: Flow diagram of variant and candidate gene filtering strategy. AR, autosomal recessive; AD, autosomal dominant; OMIM, Online Mendelian Inheritance in Man.

Results

Cohort overview

We performed WES on genomic DNA from fifty-two adults with unexplained liver disease. Presenting phenotypic categories included cholestasis (17/52, 33%), hepatic steatosis (8/52, 15%), advanced fibrosis/cirrhosis (10/52, 19%), elevated transaminases (9/52, 17%), non-*HFE* iron overload (2/52, 4%), and other (6/52, 12%) (Table 1). Most of the patients (31/52, 60%) were female, and twenty-one individuals (40%) were male. Age at presentation ranged from 23 to 74, with twenty (38%) individuals presenting before the age of 40. Within the cohort, 27% reported a positive family history of liver disease, while the majority reports no family history or being 'unknown'. Principal component analysis revealed a multi-ethnic cohort (Supplementary Figure S1).

WES yielded a definitive or presumed diagnosis in seventeen patients with unexplained liver disease

Using WES analysis, we identified definitive or presumed monogenic disorders in seventeen patients of our cohort of fifty-two adults with liver disease of unknown cause (Table 2) with implications in their management (Table 3). These individuals include eight patients with idiopathic cholestasis, four lean patients with hepatic steatosis of unclear etiology, one patient with cryptogenic decompensated cirrhosis, two patients with unexplained elevated transaminases, one individual with non-HFE iron overload, and one patient

Patient ID	Patient's phenotype			Patient's genotype							Genotype-phenotype correlation consistent with
	Liver phenotype category	Age (yo)	Sex	Gene(s)	*Genomic Variation (hg19) dbSNP*	HGVS consequence	Zygosity	gnomAD MAF (overall)	CADD score	ACMG variant classification	
1	Idiopathic cholestasis	35	M	*ABCB4*	chr7:87041333:C>T rs61730509	p.Ala934Thr	Het	$1.246e^{-3}$	34	Likely pathogenic	Presumed MDR3 deficiency[a]
					chr7:87042985:G>C -	p.Gln911Glu	Het	0	23.2	VUS	
2	Idiopathic cholestasis	49	M	*ABCB4*	chr7:87076396:G>A rs72552778	p.Ser320Phe	Homo	$1.593e^{-4}$	27.5	Pathogenic	MDR3 deficiency
3	Idiopathic cholestasis	23	F	*ABCB4*	chr7:87069645:T>A -	p.Gln477Leu	Het	0	28.9	VUS	LPAC
4	Idiopathic cholestasis	57	F	*ABCB4*	chr7:87035695:T>C rs374676517	p.Tyr1132Cys	Het (cis)	$1.061e^{-5}$	27.9	VUS	ABCB4-related intrahepatic cholestasis of pregnancy
					chr7:87035743:G>T -	p.Ser1116Tyr	Het (cis)	0	27.7	VUS	
5	Idiopathic cholestasis	25	F	*ABCB4*	chr7:87069546:T>C rs375315619	p.Asn510Ser	Het	$1.84e^{-4}$	23.5	Likely pathogenic	ABCB4-related intrahepatic cholestasis of pregnancy
6	Idiopathic cholestasis	28	F	*ABCB11*	chr2:169826656:C>T rs886043807	p.Ala570Thr	Het	$4.026e^{-6}$	34	Pathogenic	BRIC2
					chr2:169830328:A>G rs2287622	p.Val444Ala	Risk Allele	0.5694	15.6	Benign	
7	Idiopathic cholestasis	30	F	*JAG1*	chr20:10653424-10653455del -	p.Phe94Ter	Het	0	N/A	Pathogenic	Alagille syndrome
8	Idiopathic cholestasis	32	M	*ZNHT3, PIGW, ACACA, HNF1B*	Copy number variation	17q12 deletion	Het	0	N/A	Pathogenic	Presumed 17q12 deletion-like syndrome[b]
9	Hepatic steatosis	29	M	*APOB*	chr2:21233814:C>A rs267599184	p.Glu1976Ter	Het	$1.062e^{-5}$	40	Pathogenic	Heterozygous familial hypobetalipoproteinemia
10	Hepatic steatosis	30	M	*APOB*	chr2:21247943-21247944del rs1553385715	p.Lys766fs	Het	0	N/A	Pathogenic	Heterozygous familial hypobetalipoproteinemia
11	Hepatic steatosis	44	M	*LIPE*	chr19:42906196:G>A rs770169189	p.Ser1000Leu	Homo	$3.263e^{-5}$	35	VUS	Familial partial lipodystrophy type 6
12	Hepatic steatosis	23	F	*ABHD5*	chr3:43753257:C>T rs776635558	p.Ala188Val	Het	$1.591e^{-5}$	23.2	VUS	Presumed monoallelic *ABHD5*-related NAFLD[c]
13	Advanced fibrosis/ cirrhosis	61	M	*RTEL1*	chr20:62326972:G>A rs201540674	p.Arg1264His	Het	$1.471e^{-4}$	32	Pathogenic	Presumed telomerase syndrome[d]
14	Elevated transaminases	24	F	*DYSF*	chr2:71708048:G>A rs374203339	p.Val43Met	Het	$7.07e^{-5}$	25	VUS	Presumed dysferlinopathy[e]
					chr2:71886005:C>T rs371227553	c.4756-15C>T	Het	$4.704e^{-4}$	N/A	Likely benign	
15	Elevated transaminases	39	F	*SCARB1*	chr12:125284671:G>A rs74830677	p.Pro376Leu	Homo	$9.304e^{-4}$	27.5	Pathogenic	SCARB1-related hypercholesterolemia
16	Non-HFE iron overload	51	M	*SLC40A1*	chr2:190439920:C>T rs978427853	p.Gly80Ser	Het	0	34	Pathogenic	Hemochromatosis type 4
17	Other (vascular)	23	M	*SMAD3*	chr15:67473779:C>T rs387906850	p.Arg287Trp	Het	0	25.9	Pathogenic	Loeys-dietz syndrome type 3

yo, years-old; chr, chromosome; LPAC, low phospholipid-associated cholelithiasis; BRIC2, benign recurrent intrahepatic cholestasis type 2; VUS, variant of unknown significance. [a]Presumed MDR3 deficiency given that heterozygous variants could not be confirmed to be on different alleles. [b]Presumed 17q12 deletion-like syndrome given that this patient's exact deletion has not been previously reported. [c]Presumed monoallelic *ABHD5*-related non-alcoholic fatty liver disease (NAFLD) given that this variant has not been previously associated with liver disease. [d]Presumed telomerase syndrome given that the allele frequency is above our cut-off for autosomal dominant inheritance; however, this variant is annotated as pathogenic in Clin Var database, and has shown to segregate with idiopathic pulmonary fibrosis/familial interstitial pneumonia (Cogan JD et al., Am J Respir Crit Care Med 2015). [e]Presumed dysferlinopathy given that heterozygous variants could not be confirmed to be on different alleles.

Table 2: **Demographics, clinical presentation, genetic information and diagnoses identified in seventeen subjects of our cohort.**

with a vascular liver disease (Table 2 and Fig. 2). Of these seventeen patients, nine and eight individuals were recruited from each of the two participating medical centers, respectively. Additional evidence of pathogenicity for the candidate variants detected in these seventeen patients was investigated by testing for association with liver traits using exome sequencing data from the UK Biobank; ten variants were sufficiently frequent in the UK Biobank to allow for association testing (Supplementary Table S1).

Patient ID	Genetic diagnosis	Changes to clinical management
1	Presumed MDR3 deficiency	Given that patient is post-transplant, transplant is expected to be curative.
2	MDR3 deficiency	Ursodiol therapy; family counseling; transplant candidacy; consideration for ileal bile acid transporter inhibitors
3	LPAC	Avoidance of cholestasis-triggering drugs/exposures; family counseling; expectant management of potential future pregnancies; maintain regular liver follow-up
4 5	*ABCB4*-related ICP and cholestatic liver disease	Avoidance of cholestasis-triggering drugs/exposures; family counseling; maintain regular liver follow-up
6	BRIC2	Avoidance of cholestasis-triggering drugs/exposures; family counseling; maintain regular liver follow-up
7	Alagille syndrome	Multi-subspecialty referrals: nephrology, cardiology, ophthalmology; family counseling; consideration for ileal bile acid transporter inhibitors; maintain regular liver follow-up
8	Presumed 17q12 deletion-like syndrome	Multi-subspecialty referrals: endocrinology, nephrology; maintain regular liver follow-up
9 10	Heterozygous familial hypobetalipoproteinemia	Family counseling, regular exercise, and maintain BMI <23 for Asians and <25 for non-Asians, maintain routine liver follow-up
11	Familial partial lipodystrophy type 6	Endocrinology referral for adequate therapy; family counseling
12	Presumed monoallelic *ABHD5*-related NAFLD	Family counseling, regular exercise and maintain BMI <23 for Asians and <25 for non-Asians background; maintain regular liver follow-up
13	Presumed Telomerase syndrome	Monitoring of bone marrow function; family counseling
14	Presumed dysferlinopathy	Referral to neuromuscular center; provide prognostication; consideration for future treatment/clinical trial enrollment
15	*SCARB1*-related hypercholesterolemia	Preventative interventions for coronary artery disease, family counseling, avoid further test(s) to evaluate abnormal transaminase levels
16	Hemochromatosis type 4	Consideration for chelation therapy; family counseling and screening
17	Loeys-dietz syndrome type 3	Screening ± intervention for other aneurysms; family counseling and screening

Table 3: **Summary of impact in clinical management in patients whom a genetic diagnosis was attained.**

Over two-thirds of the individuals who attained a genetic diagnosis were being evaluated for cholestasis or hepatic steatosis of unclear etiology

Nearly 50% of individuals with idiopathic cholestasis (8/17) and lean individuals with hepatic steatosis (4/8) were found to have a monogenic liver disease, which was unrecognized until exome sequencing was performed. Hence, the large majority (70%) of patients in whom a genetic diagnosis was attained (12/17) were evaluated by a hepatologist for cholestasis or hepatic steatosis of unclear etiology.

Genetic diagnoses in eight patients with idiopathic cholestasis

We established definitive or likely diagnoses in eight out of seventeen patients with cholestatic liver disease of unknown etiology (Table 1). Patient 1 harbored two rare missense variants in *ABCB4* (chr7:87041333, C>T, NM_000443, c.2800G>A, p.Ala934Thr; and chr7:87042985, G>C, NM_000443, c.2731C>G, p.Gln 911Glu) (Table 2). While familial segregation studies to confirm whether these genetic variants are in *trans* were not possible, it is suspected that the patient suffers from MDR3 deficiency given patient's progression to cirrhosis requiring liver transplantation and explant revealing bile ductopenia. *ABCB4* is a member of the ATP-binding cassette (ABC) transporter family and encodes for the protein MDR3, which plays a role in phospholipid transport from hepatocytes into bile.[22] Deficiency in *ABCB4* leads to chemical composition changes in the bile contributing to the generation of cholesterol stones, and bile salt mediated injury of the biliary tree.[23] In the UK Biobank, one of patient 1's genetic variants in *ABCB4*, p.Ala934Thr, was found in 204 carriers of African ancestry and associated with increased ALT (mean among carriers 24.2 U/L, mean among noncarriers 22.0 U/L; $p = 3.3 \times 10^{-6}$; Supplementary Table S2). Patient 2 was found to have a rare homozygous missense variant in *ABCB4* (chr7:87076396, G>A, NM_000443, c.959C>T, p.Ser320Phe) (Table 2), also consistent with a diagnosis of MDR3 deficiency. In the UK Biobank, patient 2's *ABCB4* variant, p.Ser320Phe, was found in 148 carriers of European ancestry associated with higher ALT (26.3 U/L vs. 23.5 U/L; $p = 3.0 \times 10^{-4}$; Supplementary Table S2), further supporting its pathogenicity.

Patient 3 has a past medical history significant for cholecystectomy at the age of fifteen for cholelithiasis and a presumed diagnosis of primary sclerosing cholangitis (PSC) in the setting of persistent mild cholestasis. However, the patient underwent endoscopic retrograde cholangiopancreatography (ERCP) evaluation, which was unremarkable with no evidence of ductal abnormalities. Since ERCP findings were not consistent with a PSC diagnosis, the patient was evaluated for idiopathic cholestasis using WES. She was found to harbor one rare, heterozygous missense variant in *ABCB4* (chr7:87069645, T>A, NM_000443, c.1430A>T, p.Gln477Leu), and this amino acid position has been shown to represent a critical residue within one of two ABCB4 ATP binding sites,[24]

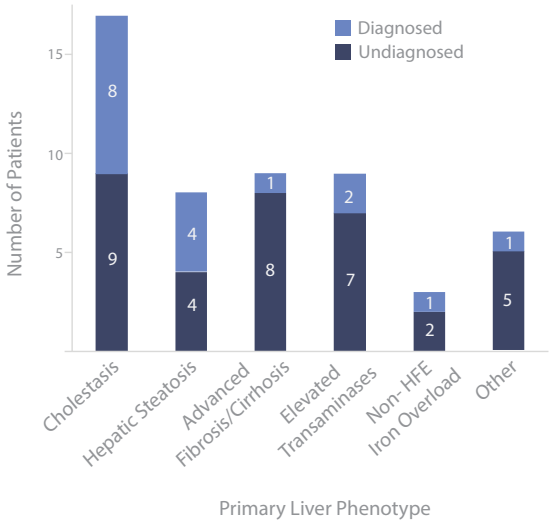

Fig. 2: Proportion of patients in whom a genetic diagnosis was attained versus who remain undiagnosed across the different primary liver phenotype classifications.

supporting the diagnosis of *ABCB4*-related Low-Phospholipid Associated Cholelithiasis (LPAC) (Table 2).[7,25]

Patient 4 had a history of intrahepatic cholestasis of pregnancy (ICP) in each of three consecutive pregnancies, and elevated liver transaminases since teenage years; she is now post-transplant with the explant demonstrating biliary cirrhosis. The patient had a remote history of alcohol use, which may have contributed to the severity of the disease. Interestingly, she was found to harbor two rare heterozygous variants, in *cis*, in *ABCB4* (chr7:87035695, T>C, NM_018849, c.3416A>G, p.Tyr1132Cys and chr7:87035743, G>T, NM_018850, c.3206C>G, p.Ser1116Tyr) (Table 2). Patient 5 had a history of peri-partum hepatitis of unknown etiology and ICP. She was found to have intra- and extra-hepatic bile duct dilation on a right upper quadrant ultrasound as well as narrowing at the hilum with intrahepatic biliary duct dilation on follow-up magnetic resonance cholangiopancreatography (MRCP). ERCP with sphincterotomy noted choledocholithiasis. This patient was found to have one rare, heterozygous variant in *ABCB4* (chr7:87069546, A>G, NM_000443, c.1529A>G, p.Asn510Ser) (Table 2).[26,27] Both patients 4 and 5 were diagnosed with *ABCB4*-related ICP and cholestatic liver disease.

Patient 6 was found to harbor one rare, heterozygous variant in *ABCB11* (chr2:169826656, C>T, NM_003742, c.1708G>A, p.Ala570Thr), which in isolation would not be considered causative for their cholestatic disease. However, this patient also harbors a homozygous common *ABCB11* risk variant (chr2:169830328, A>G, NM_003742, c.1331T>C, p.Val444Ala). This common variant has been associated with drug-induced cholestasis and decreased hepatic bile salt export pump (BSEP) expression *in vivo*[28,29] and associates with increased GGT levels in the UK Biobank (37.9 U/L vs. 37.2 U/L; $p = 1.3 \times 10^{-7}$, Supplementary Table S2). *ABCB11* encodes the ABC transporter BSEP, which is responsible for the transport of taurocholate into bile and determining bile flow and formation.[30] In combination, this rare heterozygous variant (p.Ala570Thr), and the common risk allele (p.Val444Ala) in *ABCB11* were thought to explain this patient's phenotype, and the patient was diagnosed with benign recurrent intrahepatic cholestasis type 2 (BRIC2).

Patient 7 harbors a frameshift variant in *JAG1* (chr20:10653424–10653455del, NM_000214, c.281_312del, p.Phe94Ter) and was diagnosed with Alagille syndrome at 30 years of age (Table 2). Though often diagnosed in early childhood, Alagille syndrome is known to have considerable clinical heterogeneity.[31] This patient has mildly elevated transaminases and GGT, duplication of superior vena cava and a single congenital kidney. Notably, the patient also has a broad forehead, deep-set eyes, and underwent an ophthalmological surgery, which she was unable to specify, at eighteen months of age.

Patient 8 has a past medical history of diabetes mellitus and presented with persistent elevated alkaline phosphatase of unknown etiology, with unrevealing MRCP and liver biopsy findings. He also had a past medical history remarkable for recurrent pyelonephritis and insulin-dependent diabetes mellitus. He was found to have a contiguous deletion in chromosome 17 that spanned fifteen distinct genes including but not limited to *CCL3*, *ZNHIT3*, *PIGW*, *ACACA*, *HNF1B*, and *GPR179*. While this specific deletion has not been previously reported, it overlaps with the region involved in 17q12 deletion syndrome, which is associated with maturity-onset diabetes of the young, elevated liver enzymes, and recurrent urinary tract infections.[32]

Four patients with unexplained hepatic steatosis were found to have a monogenetic disease

We established definitive or likely diagnoses in four out of eight patients with hepatic steatosis on ultrasound imaging and/or liver biopsy in the absence of alcohol overuse or visceral adiposity. Both patients 9 and 10 presented with hepatic steatosis and normal body mass index (BMI). Serum LDL cholesterol levels were 19 and 27 mg/dL (normal range 50–130 mg/dL), and serum triglyceride levels were 91 and 27 mg/dL, respectively (normal range 90–150 mg/dL). Patient 9 harbored a nonsense heterozygous variant in *APOB* (chr2:21233814, C>A, NM_000384, c.5926C>A, p.Glu1976Ter, Table 2) and his circulating APOB level is low (25 mg/dL) consistent with the genetic diagnosis (Table 2). There were ten heterozygous carriers of this nonsense variant in UK Biobank, six of whom had

reportable APOB values with a mean circulating APOB level of 47 mg/dL (mean for 431,551 noncarriers, 103 mg/dL; p = 7.21e^{-8}, Supplementary Table S2); and four of whom had missing APOB values since the value was outside of the reportable limits (40–200 mg/dL). Patient 10 harbored a frameshift variant in *APOB* (chr2:21247942, ATT>A, NM_000384, c.2297_2298del, p.Lys766fs) and his circulating APOB level is also low at 22 mg/dL, consistent with the genetic diagnosis (Table 2). Both patients 9 and 10 were diagnosed with heterozygous *APOB*-related familial hypobetalipoproteinemia (FHBL). Apolipoprotein B is the primary apolipoprotein of chylomicrons, VLDL, IDL, and LDL particles.[33] The low LDL-cholesterol and triglyceride levels in these patients were consistent with the genetic diagnosis, and contrast with the metabolic syndrome derangements that would be expected to correlate with the degree of hepatic steatosis observed.[34]

Patient 11 presented with hepatic steatosis in the setting of normal BMI, F2 fibrosis (transient elastography liver stiffness of 7.7 kPa) and recurrent uric acid nephrolithiasis. He was found to have a novel, homozygous, missense variant (chr19:42906196, G>A, NM_005357, c.2999C>T, p.Ser1000Leu) in the *LIPE* gene and was diagnosed with *LIPE*-related familial partial lipodystrophy type 6 (Table 2). *LIPE* encodes for lipase E, a hormone-sensitive lipase which functions in the rate-limiting step for fatty acid cleavage and mobilization from triglycerides.[35] In the UK Biobank, the patient's genetic variant in *LIPE*, p.Ser1000Leu, is found in 59 carriers of European ancestry and associates with elevated GGT (44.0 U/L vs. 37.3 U/L; p = 0.005; Supplementary Table S2). This diagnosis provided explanation for hepatic steatosis and possibly for uric acid-related nephrolithiasis, since *LIPE*-related familial partial lipodystrophy type 6 has been associated with elevated serum uric acid.[36]

Patient 12 is a lean patient with hepatic steatosis, which was revealed as part of evaluation for mildly elevated transaminases. She was found to harbor a missense variant (chr3:43753257, C>T, NM_016006, c.563C>T, p.Ala188Val) in the gene *ABHD5* supporting the diagnosis of autosomal dominant *ABHD5*-related NAFLD (Table 2). Liver biopsy revealed steatosis with mild focal lobular inflammation and mild portal fibrosis. Macrovesicular steatosis (40%, grade 2) was seen, but Mallory-Denk bodies and satellitosis were not identified. The portal tracts showed mild chronic inflammation without interface hepatitis. While its biological function and mechanism are still unclear, *ABHD5* has been associated with autosomal recessive Chanarin-Dorfman syndrome, a neutral lipid storage disease associated with myopathy, ichthyosis and fatty liver; and rare heterozygous, premature termination, frameshift and missense variants in the same gene have been described as a monoallelic inherited cause of hepatic steatosis.[37]

Molecular diagnosis in one patient with cryptogenic cirrhosis

Patient 13 had a past medical history of gastric MALT lymphoma status post-radiotherapy, decompensated cirrhosis of uncertain etiology and progressive idiopathic pulmonary fibrosis. He was discovered to have a heterozygous missense variant in *RTEL1* (chr20:62326972, G>A, NM_001283009, c.3791G>A, p.Arg1264His), annotated as pathogenic in ClinVar, and was subsequently diagnosed with presumed *RTEL1*-associated telomerase syndrome, possibly explaining his lung and liver fibrosis (Table 2). *RTEL1* encodes a DNA helicase that stabilizes and elongates telomerases during DNA replication.[38] However, we did not detect any difference in liver biomarkers or diagnosis of liver disease among 40 carriers for this variant (p.Arg1264His) in the UK Biobank.

Genetic diagnosis in two patients with persistent elevation of transaminases

We established definitive or likely diagnoses in two patients with isolated hepatocellular injury characterized by persistent elevation of serum transaminase levels. Patient 14 was admitted with creatine kinase levels greater than assay detection and elevated transaminases (ALT = 237 IU/L and AST = 815 IU/L). She was found to have fatty liver on abdominal ultrasound. WES revealed two rare, heterozygous variants in *DYSF* (chr2:71708048, G>A, NM_001130455, c.127G>A, p.Val43Met; chr2:71886005, C>T, NM_001130455, c.4642-3C>T) and she was diagnosed with presumed *DYSF*-related dysferlinopathy (Table 2). The protein dysferlin, encoded by the gene *DYSF*, is highly expressed in muscle tissue, and proposed to be critical for membrane repair in muscle.[39] While familial segregation studies were not possible, this patient's prolonged history of severe muscle cramps and liver biochemical abnormalities can both be explained by her presumed compound heterozygous variants in *DYSF*.

Patient 15 has past medical history significant for hyperlipidemia, coronary artery calcification, and mildly elevated transaminases. She was found to harbor a rare homozygous variant in *SCARB1* (chr12:125284671, G>A, NM_001082959, c.1127C>T, p.Pro376Leu) (Table 2). As a scavenger receptor-encoding gene, *SCARB1* has been implicated in cholesterol regulation and foam cell stabilization in atherosclerosis,[40] and therefore this genotype information can explain her lipid abnormalities and related transaminase abnormalities and coronary artery disease.

Molecular diagnosis in a patient with non-HFE iron overload

Patient 16 has a past medical history significant for iron overload requiring phlebotomy every two weeks. His father and paternal cousin have a history of iron overload requiring regular phlebotomies. Patient 16 was

initially tested for the two most common *HFE* variants, and he was found to have a heterozygous p.H63D, which fell short in explaining the degree of his iron overload. WES was performed and revealed that this patient also harbors a rare heterozygous missense variant in *SLC40A1* (chr2:190439920, C>T, NM_014585, c.238G>A, p.Gly80Ser), solute carrier family 40 member 1, which encodes for ferroportin (Table 2). Hence, he was diagnosed with *SLC40A1*-related ferroportin disease, previously known as hemochromatosis type 4.

A genetic disease in one patient with liver vascular disease of unknown etiology

Patient 17 had a past medical history significant for rupture of hepatic artery aneurysm in childhood requiring emergent liver transplantation. Given the unknown cause for such rare life-threatening presentation, WES was performed in early adulthood and revealed a rare heterozygous missense variant in *SMAD3* (chr15:67473779, C>T, NM_001145104, c.274C>T, p.Arg287Trp), encoding SMAD family member 3 (Table 2). This finding supports the diagnosis of *SMAD3*-related Loeys-Dietz Syndrome type 3, which is associated with arterial aneurysm, tortuosity, and valvular insufficiency. The SMAD family of proteins is involved in TGF-beta signaling. Variants in this family of genes are associated with visceral and aortic aneurysm disease, with one previous report of *SMAD3*-related hepatic artery aneurysm.[41,42]

Patients under 40 years-old appear to be more likely to harbor a genetic diagnosis

The subgroup of patients in whom genetic diagnosis was attained via WES analysis was compared against the subgroup of our cohort who remain undiagnosed. Individuals who presented before the age of 40 were more likely to be diagnosed with a genetic disease as compared to patients who presented after 40 years of age (Fig. 3). On the other hand, the subgroup in which genetic diagnoses were attained did not vary significantly compared to the undiagnosed subgroup regarding known family history of liver disease or sex. Of the seventeen patients with genetic diagnoses, only five had known family history of any liver disease.

Notably, during the revision of this manuscript and as part of our annual re-analysis of undiagnosed cases, one of the individuals with idiopathic liver disease and younger than 40 years of age was found to harbor a rare homozygous splicing variant in *TULP3* (chr12:3046796, G>A, NM_011657, c.925-1G>A, rs761012512, recently implicated in progressive liver, kidney, and heart degeneration. This patient corresponds to the female in family 7 reported in the discovery cohort recently published.[43]

Discussion

This study underscores and validates the clinical utility of WES in the diagnosis and management of adult patients with idiopathic liver disease, and suggests that a genetic disease is more likely to present in adults younger than 40 years of age.[44] Furthermore, our findings align with other studies where the diagnostic utility of WES in adult cohorts was independent of a positive family history.[45,46] WES curtails the number of unexplained liver disease cases and facilitates a precise diagnosis within pre-genomic broad categories of liver disease, enhancing clinical care.

In accordance with our pilot study,[5] more than one-fourth of these individuals had evidence of likely or definitive monogenic disorder as the cause of or significant contributor to their liver dysfunction, which had been occult until exome analysis was considered and performed. For ten variants predicted to be disease-causing with sufficient power to test in the population-based UK Biobank cohort, we found association between genotype and liver biomarker(s) in four of them. This is consistent with data supporting that quantitative association of variants with endophenotypes at the population level may provide further evidence for pathogenicity in Mendelian disease.[47]

Importantly, nearly all patients in whom a genetic diagnosis was attained benefited from changes or refinements to the subsequent management of their disease, including but not limited to targeted therapy, preventative screening, family counseling, consideration for clinical trial enrollment, and additional specialist referrals (Table 3). Additionally, genetic diagnosis contributes to disease prognostication. For instance, patient 1 who had progressed to end-stage liver disease and was post-liver transplant by the time exome sequencing was performed, a genetic diagnosis of MDR3 deficiency can provide reassurance given that transplant is curative.[48] On the other hand, patient 14, with a presumptive diagnosis of dysferlinopathy, was referred to a neuromuscular specialist and received counseling on the natural history of disease progression, which can lead to wheelchair-dependence.

This study features a clinically diverse cohort of patients with the 'unifying' diagnosis of idiopathic liver disease. Given the challenges of defining precise inclusion criteria for undiagnosed liver disease patients, the decision to pursue WES work-up reflected, to some extent, the individual consideration of an experienced hepatologist at a large tertiary academic center in the US. Consistent with preliminary results from our pilot cohort,[5] idiopathic cholestasis and hepatic steatosis of unclear etiology—in the absence of metabolic syndrome or visceral adiposity—represent most participants with unexplained liver disease who attained a genetic diagnosis. Specifically, five patients who presented with cholestasis of unknown etiology were found to harbor rare bi-allelic or monoallelic variants in *ABCB4*. While MDR3 deficiency was initially described as an autosomal recessive disorder, a growing number of studies

report cholestatic and gallbladder related disease in individuals with heterozygous mutations in *ABCB4*.[7,49,50] Our findings represent the basis for further independent studies to investigate the contribution of monogenic diseases to different liver phenotype categories (e.g.: idiopathic cholestasis, lean individuals with hepatic steatosis,[51,52] cryptogenic cirrhosis) with presentation and evaluation during adulthood. Further research should also explore the prevalence and the spectrum of monogenic disease burden in adult patients undergoing evaluation and awaiting liver transplantation. Between 47 and 67% of living donor liver transplants from 2015 to 2019 were among biological relatives. Experts have recently proposed to consider evaluation for hereditary liver disease in biologically related donor-recipient pairs in order to minimize risk in living donor liver transplantation, as identifying these genetic disorders is beneficial to both donor and recipient long-term outcomes.[53]

This study should encourage physicians to consider WES analysis in patients with unexplained liver disease with or without other organ involvement. This is in tandem with the overarching initiatives to practice precision medicine[8,54] and elucidate previously unappreciated incidence and spectrum of monogenic diseases with clinical presentation in adulthood. Patient 7 diagnosed with Alagille syndrome at 30 years-old illustrates the unappreciated phenotypic spectrum of genetic liver diseases, which are traditionally attributed to pediatric ages and therefore poorly investigated in adulthood. This emergent knowledge will lead to greater awareness of adult presentations of monogenic diseases and will shape the teaching and incorporation of genetic diseases in differential diagnosis in internal medicine practice.

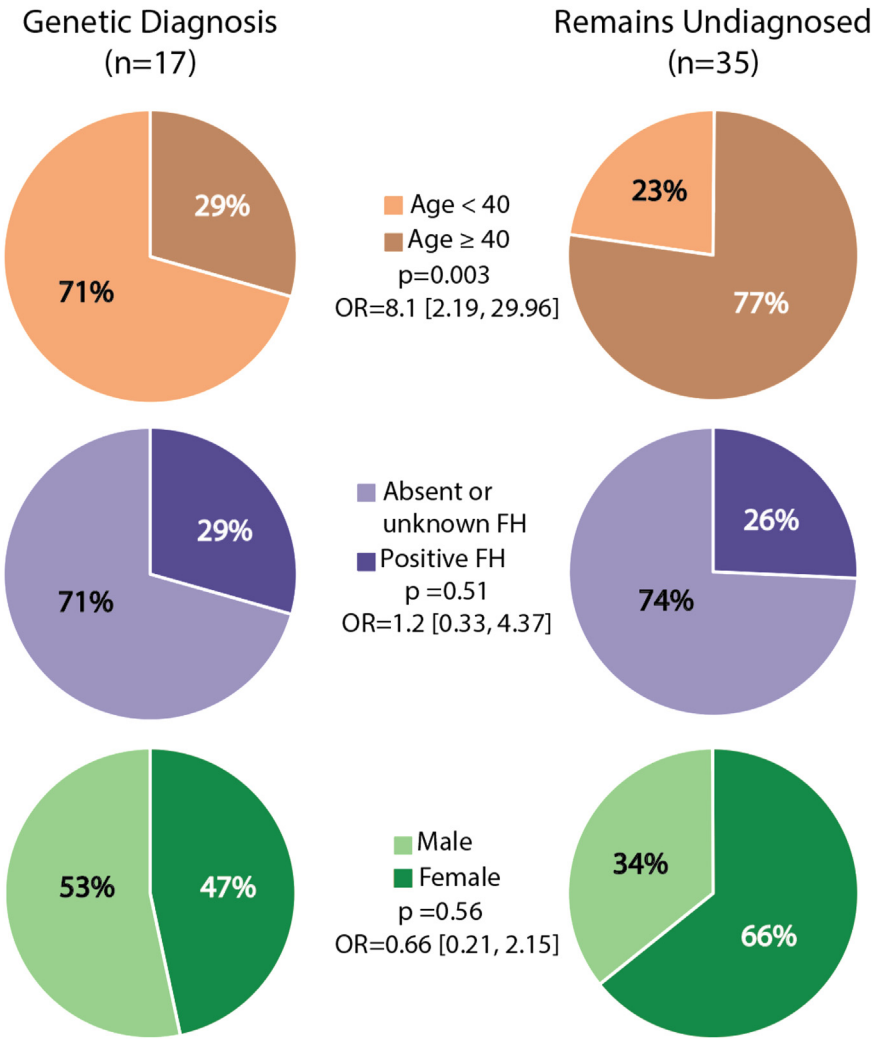

Fig. 3: Proportion of patients in whom a genetic diagnosis was attained versus who remain undiagnosed according to age (< or ≥40 years-old), presence or unknown/absence of family history and sex.

As exome and genome sequencing becomes more frequently used by clinicians in the evaluation of undiagnosed patients,[10] our knowledge of liver disease taxonomy will refine and expand,[9,55,56] in part through reverse phenotyping by genetics. Specifically, it will enable the stratification of adult liver disease into categories informed by genotype with direct implications in emergent personalized genetic-based therapeutic approaches and clinical trial design.[9]

Given the rising accessibility and decreasing cost of genomic technologies, combined with increased understanding and knowledge of gene function, expression,[57] and regulation, genomic medicine is an increasingly important field in advancing patient care in internal medicine, including liver disease.[9,10] Our data, together with other studies, further support the incorporation of exome sequencing, or potentially a comprehensive liver disease-related multi-gene panel, as part of clinical diagnosis and management of adult liver disease.

Contributors

M.Z., A.H., P.K.M., and S.V., participated in study concept and design. M.Z., A.H., C.K., A.M.D., L.D.W., R.A.H., A.M.H., L.K., P.L., P.N., M.E.P., D.D., A.B., S.V., performed research and/or participated in data analysis. M.Z., A.H., M.S., D.N.A., A.L., M.H.N, A.J., Z.G.J., M.P.C., M.L., M.H.C., P.K.M., and S.V., contributed to recruitment and/or ascertainment of participants. M.Z. and S.V. have verified the underlying data, wrote the first draft and all authors critically read and contributed to the final version of the manuscript.

Data sharing statement

Data is available upon reasonable request to corresponding author.

Declaration of interests

A.M.D., R.A.H., A.M.H., L.K., P.L., P.N., M.E.P., and L.D.W., are employees of Alnylam Pharmaceuticals. A.M.D. and L.D.W., also hold stock options in Alnylam Pharmaceuticals. M.G.S., is a principal investigator for clinical studies from Novartis, Gilead, Pliant, Cymabay, Genfit, Target PharmaSolutions. Z.G.J., consults for Olix Pharmaceuticals and receives grants from Gilead Sciences and Pfizer Pharmaceuticals. M.P.C., consults for Mallinckrodt LLC, Alexion Pharmaceuticals, and Albireo Pharma and has also received grants from Sonic Incytes. M.H.C., has received grant support from Bayer. S.V., served as consultant for Albireo Pharma. The other authors do not report any potential conflict of interest.

Acknowledgements

We thank the patients and their families for their invaluable contributions to this study. We thank the staff members of Yale Center for Genomic Analysis (YCGA) for technical and bioinformatic assistance; Yale hepatologist colleagues and Drs. Nezam H. Afdhal, Alan Bonder, Suzanne Chan, Zachary Fricker, Vilas Patwardhan, and Lauren Yang for their efforts to refer patients to this study.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ebiom.2023.104747.

References

1 Lata S, Marasa M, Li Y, et al. Whole-exome sequencing in adults with chronic kidney disease: a pilot study. *Ann Intern Med*. 2018;168(2):100–109.
2 Groopman EE, Marasa M, Cameron-Christie S, et al. Diagnostic utility of exome sequencing for kidney disease. *N Engl J Med*. 2019;380(2):142–151.
3 Leopold JA, Loscalzo J. Emerging role of precision medicine in cardiovascular disease. *Circ Res*. 2018;122(9):1302–1315.
4 Walsh M, West K, Taylor JA, et al. Real world outcomes and implementation pathways of exome sequencing in an adult genetic department. *Genet Med*. 2022;24(7):1536–1544.
5 Hakim A, Zhang X, DeLisle A, et al. Clinical utility of genomic analysis in adults with idiopathic liver disease. *J Hepatol*. 2019;70(6):1214–1221.
6 Drzewiecki K, Choi J, Brancale J, et al. GIMAP5 maintains liver endothelial cell homeostasis and prevents portal hypertension. *J Exp Med*. 2021;218(7):e20201745.
7 Stättermayer AF, Halilbasic E, Wrba F, Ferenci P, Trauner M. Variants in ABCB4 (MDR3) across the spectrum of cholestatic liver diseases in adults. *J Hepatol*. 2020;73(3):651–663.
8 Valenti L, Ronzoni L. Genetics: a new clinical tool for the hepatologist. *Liver Int*. 2022;42(4):724–726.
9 Zheng M, Allington G, Vilarinho S. Genomic medicine for liver disease. *Hepatology*. 2022;76(3):860–868.
10 Chung DH, Zheng M, Bale AE, Vilarinho S. Hepatology genome rounds: an interdisciplinary approach to integrate genomic data in clinical practice. *J Hepatol*. 2023. https://doi.org/10.1016/j.jhep.2023.03.030.
11 Asrani SK, Devarbhavi H, Eaton J, Kamath PS. Burden of liver diseases in the world. *J Hepatol*. 2019;70(1):151–171.
12 Sepanlou SG, Safiri S, Bisignano C, et al. The global, regional, and national burden of cirrhosis by cause in 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet Gastroenterol Hepatol*. 2020;5(3):245–266.
13 Rinaldi L, Nascimbeni F, Giordano M, et al. Clinical features and natural history of cryptogenic cirrhosis compared to hepatitis C virus-related cirrhosis. *World J Gastroenterol*. 2017;23(8):1458–1468.
14 The scientific registry of transplant recipients. https://srtr.transplant.hrsa.gov/default.aspx. Accessed November 16, 2022.
15 Splinter K, Adams DR, Bacino CA, et al. Effect of genetic diagnosis on patients with previously undiagnosed disease. *N Engl J Med*. 2018;379(22):2131–2139.
16 Vilarinho S, Choi M, Jain D, et al. Individual exome analysis in diagnosis and management of paediatric liver failure of indeterminate aetiology. *J Hepatol*. 2014;61(5):1056–1063.
17 Foucher J, Chanteloup E, Vergniol J, et al. Diagnosis of cirrhosis by transient elastography (FibroScan): a prospective study. *Gut*. 2006;55(3):403–408.
18 Van der Auwera GA, Carneiro MO, Hartl C, et al. From FastQ data to high-confidence variant calls: the genome analysis toolkit best practices pipeline. *Curr Protoc Bioinformatics*. 2013;43(1):11.10.1–11.10.33.
19 Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Res*. 2010;38(16):e164.
20 Backman JD, Li AH, Marcketta A, et al. Exome sequencing and analysis of 454,787 UK Biobank participants. *Nature*. 2021;599(7886):628–634.
21 Deaton AM, Dubey A, Ward LD, et al. Rare loss of function variants in the hepatokine gene INHBE protect from abdominal obesity. *Nat Commun*. 2022;13(1):4319.
22 Ruetz S, Gros P. Phosphatidylcholine translocase: a physiological role for the mdr2 gene. *Cell*. 1994;77(7):1071–1081.
23 Shoda J, Oda K, Suzuki H, et al. Etiologic significance of defects in cholesterol, phospholipid, and bile acid metabolism in the liver of patients with intrahepatic calculi. *Hepatology*. 2001;33(5):1194–1205.
24 Olsen JA, Alam A, Kowal J, Stieger B, Locher KP. Structure of the human lipid exporter ABCB4 in a lipid environment. *Nat Struct Mol Biol*. 2020;27(1):62–70.
25 Goubault P, Brunel T, Rode A, Bancel B, Mohkam K, Mabrut JY. Low-phospholipid associated cholelithiasis (LPAC) syndrome: a synthetic review. *J Vis Surg*. 2019;156(4):319–328.
26 Dixon PH, Sambrotta M, Chambers J, et al. An expanded role for heterozygous mutations of ABCB4, ABCB11, ATP8B1, ABCC2 and TJP2 in intrahepatic cholestasis of pregnancy. *Sci Rep*. 2017;7(1):11823.
27 Dixon PH, Wadsworth CA, Chambers J, et al. A comprehensive analysis of common genetic variation around six candidate loci for intrahepatic cholestasis of pregnancy. *Am J Gastroenterol*. 2014;109(1):76–84.
28 Lang C, Meier Y, Stieger B, et al. Mutations and polymorphisms in the bile salt export pump and the multidrug resistance protein 3 associated with drug-induced liver injury. *Pharmacogenet Genom*. 2007;17(1):47–60.

29 Meier Y, Pauli-Magnus C, Zanger UM, et al. Interindividual variability of canalicular ATP-binding-cassette (ABC)-transporter expression in human liver. *Hepatology*. 2006;44(1):62–74.
30 Noé J, Stieger B, Meier PJ. Functional expression of the canalicular bile salt export pump of human liver. *Gastroenterology*. 2002;123(5):1659–1666.
31 McDaniell R, Warthen DM, Sanchez-Lara PA, et al. NOTCH2 mutations cause Alagille syndrome, a heterogeneous disorder of the notch signaling pathway. *Am J Hum Genet*. 2006;79(1):169–173.
32 Bellanné-Chantelot C, Clauin S, Chauveau D, et al. Large genomic rearrangements in the hepatocyte nuclear factor-1beta (TCF2) gene are the most frequent cause of maturity-onset diabetes of the young type 5. *Diabetes*. 2005;54(11):3126–3132.
33 Lee J, Hegele RA. Abetalipoproteinemia and homozygous hypobetalipoproteinemia: a framework for diagnosis and management. *J Inherit Metab Dis*. 2014;37(3):333–339.
34 Haas ME, Pirruccello JP, Friedman SN, et al. Machine learning enables new insights into genetic contributions to liver fat accumulation. *Cell Genom*. 2021;1(3):100066.
35 Holm C, Kirchgessner TG, Svenson KL, et al. Hormone-sensitive lipase: sequence, expression, and chromosomal localization to 19 cent-q13.3. *Science*. 1988;241(4872):1503–1506.
36 Zolotov S, Xing C, Mahamid R, Shalata A, Sheikh-Ahmad M, Garg A. Homozygous LIPE mutation in siblings with multiple symmetric lipomatosis, partial lipodystrophy, and myopathy. *Am J Med Genet A*. 2017;173(1):190–194.
37 Youssefian L, Vahidnezhad H, Saeidian AH, et al. Inherited non-alcoholic fatty liver disease and dyslipidemia due to monoallelic ABHD5 mutations. *J Hepatol*. 2019;71(2):366–370.
38 Vannier JB, Sandhu S, Petalcorin MI, et al. RTEL1 is a replisome-associated helicase that promotes telomere and genome-wide replication. *Science*. 2013;342(6155):239–242.
39 Anderson LV, Davison K, Moss JA, et al. Dysferlin is a plasma membrane protein and is expressed early in human development. *Hum Mol Genet*. 1999;8(5):855–861.
40 Vitali C, Khetarpal SA, Rader DJ. HDL cholesterol metabolism and the risk of CHD: new insights from human genetics. *Curr Cardiol Rep*. 2017;19(12):132.
41 Inman GJ, Nicolás FJ, Hill CS. Nucleocytoplasmic shuttling of Smads 2, 3, and 4 permits sensing of TGF-beta receptor activity. *Mol Cell*. 2002;10(2):283–294.
42 van der Linde D, Verhagen HJM, Moelker A, et al. Aneurysm-osteoarthritis syndrome with visceral and iliac artery aneurysms. *J Vasc Surg*. 2013;57(1):96–102.
43 Devane J, Ott E, Olinger EG, et al. Progressive liver, kidney, and heart degeneration in children and adults affected by TULP3 mutations. *Am J Hum Genet*. 2022;109(5):928–943.
44 Pinto EVF, Lazaridis KN. Individualized medicine comes to the liver clinic. *J Hepatol*. 2019;70(6):1057–1059.
45 Posey JE, Rosenfeld JA, James RA, et al. Molecular diagnostic experience of whole-exome sequencing in adult patients. *Genet Med*. 2016;18(7):678–685.
46 Trakadis Y, Accogli A, Qi B, et al. Next-generation gene panel testing in adolescents and adults in a medical neuropsychiatric genetics clinic. *Neurogenetics*. 2021;22(4):313–322.
47 Halford JL, Morrill VN, Choi SH, et al. Endophenotype effect sizes support variant pathogenicity in monogenic disease susceptibility genes. *Nat Commun*. 2022;13(1):5106.
48 Sticova E, Jirsa M. ABCB4 disease: many faces of one gene deficiency. *Ann Hepatol*. 2020;19(2):126–133.
49 Rosmorduc O, Hermelin B, Boelle PY, Parc R, Taboury J, Poupon R. ABCB4 gene mutation-associated cholelithiasis in adults. *Gastroenterology*. 2003;125(2):452–459.
50 Nayagam JS, Foskett P, Strautnieks S, et al. Clinical phenotype of adult-onset liver disease in patients with variants in ABCB4, ABCB11, and ATP8B1. *Hepatol Commun*. 2022;6(10):2654–2664.
51 Vilarinho S, Ajmera V, Zheng M, Loomba R. Emerging role of genomic analysis in clinical evaluation of lean individuals with NAFLD. *Hepatology*. 2021;74(4):2241–2250.
52 Zheng M, Huang DQ, Konkwo C, et al. Genomic analysis of lean individuals with NAFLD identifies monogenic disorders in a prospective cohort study. *JHEP Rep*. 2023;5(4):100692.
53 Matthew RK, Jackson WE. https://www.myast.org/sites/default/files/5_Recipients%20with%20Inherted%20Disease.pdf.
54 Collins FS, Varmus H. A new initiative on precision medicine. *N Engl J Med*. 2015;372(9):793–795.
55 Vilarinho S, Mistry PK. Exome sequencing in clinical hepatology. *Hepatology*. 2019;70(6):2185–2192.
56 Gao E, Hercun J, Heller T, Vilarinho S. Undiagnosed liver diseases. *Transl Gastroenterol Hepatol*. 2021;6:28.
57 Brancale J, Vilarinho S. A single cell gene expression atlas of 28 human livers. *J Hepatol*. 2021;75(1):219–220.