EMERGING TOPIC CONFERENCE

The Genomics Revolution

September 20–21, 2019
Arlington, VA

Program Chairs:
Richard J. Thompson, MD, PhD
Kathleen M. Loomes, MD, FAASLD

AASLD
AMERICAN ASSOCIATION FOR THE STUDY OF LIVER DISEASES

Schedule-at-a-Glance and Meeting Locations

Wi-Fi Network: Westin Meeting

Wi-Fi Password: Psav2019

Friday, September 20, 2019

6:30 AM – 4:20 PM	Registration	Atrium
7 AM – 8 AM	Breakfast	Jefferson 3
8 AM – 4:20 PM	General Session	Jefferson 1 & 2
9:45 AM – 10:05 AM	Break	Atrium
11:30 AM – 1:00 PM	Lunch	Jefferson 3
2:15 PM – 2:30 PM	Break	Atrium

Saturday, September 21, 2019

6:30 AM – 11 AM	Registration	Atrium
7 AM – 8 AM	Breakfast	Jefferson 3
8 AM – 11:15 AM	General Session	Jefferson 1 & 2
9:20 AM – 9:40 AM	Break	Atrium

– Notice –

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2019 Emerging Topic Conference

The Genomics Revolution: Changing Our Approach to Diagnostics, Management and Research in Adult and Pediatric Liver Disease

September 20 – 21, 2019

The Westin Crystal City

Arlington, VA

Program Chairs**:** Richard J. Thompson, MD, PhD, FAASLD and Kathleen M. Loomes, MD, FAASLD

Learning Objectives:

Upon completion of this activity, participants will be able to:

- Better understand how they can use genomic data to answer research questions.
- Use genomic testing in the diagnosis and management of liver disease
- Get insight into the existing datasets that can be used in research and clinical care

This activity was planned in the context of the following ACGME/IOM/IPEC competencies, Patient Care and Procedural Skills, Provide Patient-centered Care, Medical Knowledge, Work in Interdisciplinary Teams, Roles/Responsibilities, Employ Evidence-based Practice, Teams and Teamwork, Professionalism, Utilize Informatics

Accreditation and Designation Statements

Continuing Medical Education (CME)

The American Association for the Study of Liver Diseases (AASLD) is accredited by the Accreditation Council for Continuing Medical Education (ACCME) to provide continuing medical education for physicians. AASLD designates this live activity for a maximum of 9.25 *AMA PRA Category 1 Credits*™. Physicians should claim only the credit commensurate with the extent of their participation in the activity.

American Board of Internal Medicine Maintenance of Certification (MOC)

Successful completion of this CME activity, which includes participation in the evaluation component, enables the participant to earn up to 9.25 MOC points in the American Board of Internal Medicine's (ABIM) Maintenance of Certification (MOC) program. Participants will earn MOC points equivalent to the amount of CME credits claimed for the activity. It is the CME activity provider's responsibility to submit participant completion information to ACCME for granting ABIM MOC points.

American Board of Pediatrics Maintenance of Certification (MOC)

Successful completion of this CME activity, which includes participation in the activity, with individual assessments of the participant and feedback to the participant, enables the participant to earn 9.00 MOC points in the American Board of Pediatrics' (ABP) Maintenance of Certification (MOC) program. It is the CME activity provider's responsibility to submit participant completion information to ACCME for the purpose of granting ABP MOC points.

Claiming CME Credits

Physicians and other health care professionals seeking 9.25 *AMA PRA Category 1 Credits*™ for this live continuing medical education activity must complete an evaluation by **Monday, October 21, 2019**. A link to the CME and MOC evaluation will be emailed to attendees after the conference. Upon evaluation completion, you will be able to view/print your certificate online.

ABIM MOC Points
Physicians seeking ABIM MOC points must complete the CME evaluation and the MOC evaluation by **Monday, October 21, 2019**. Requests for MOC after this date will not be honored. The MOC evaluation is included in the CME evaluation that will be emailed to all attendees and will remain live until the deadline.

MOC points will be reported to the ABIM by the end of October 2019 for attendees who successfully complete the MOC evaluation.

Disclosures
This live educational activity has been planned in accordance with AASLD and ACCME Standards of Commercial Support by members of the Emerging Topic Conference faculty and the Clinical Research Committee and Governing Board.

As an accredited provider, AASLD requires individuals involved in the planning of continuing medical education (CME) activities to disclose all financial relationships, including those of their spouse or partner, with a commercial interest within the past 12 months. A commercial interest is defined as any entity producing, marketing, re-selling, or distributing health care goods or services consumed by, or used on, patients. All conflicts of interest are resolved prior to participation.

Statement on off-label and investigational use: Speakers are asked to make a reasonable effort to identify during their presentation any discussion of off-label or investigative use or application of a product or device.

Financial disclosures will appear at the beginning of each session and are provided below.

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Conference Agenda

Friday, September 20, 2019

7:00 am	**Breakfast**
Session I: Big Projects *Moderators: Richard J. Thompson, MD, PhD, FAASLD*	
8 am – 8:05 am	Introduction
8:05 am – 8:25 am	Personalizing Care Through Genomics: Genomic Medicine Programs of the National Human Genome Research Institute (NHGRI) *Teri A. Manolio, MD, PhD*
8:25 am – 8:45 am	Exome Sequencing in Gene Discovery and Diagnosis *Nancy Spinner, PhD*
8:45 am – 9:05 am	Creating Collaborative Databases *Steven Harrison, PhD*
9:05 am – 9:25 am	The Promise and Pitfalls of Genetic Testing *Lisa S. Parker, PhD*
9:25 am – 9:45 am	Panel Discussion/Q&A
9:45 am – 10:05 am	**Break**
Session II: Genetic Liver Diseases *Moderators: Kathleen M. Loomes, MD, FAASLD*	
10:05 am – 10:25 am	Genetic Cholestasis in Children *Laura Bull, PhD*
10:25 am – 10:40 am	IDENTIFICATION OF ABCC12 AS A NOVEL CAUSATIVE GENE IN PROGRESSIVE FAMILIAL INTRAHEPATIC CHOLESTASIS AND BILE DUCT PAUCITY *Chunyue Yin, PhD*
10:40 am – 11 am	Genetic Cholestasis in Adults *Catherine Williamson, PhD*
11 am – 11:20 am	Genetic Determinants of Cholangiopathies *Richard J. Thompson, MD, PhD, FAASLD*
11:20 am – 11:45 am	Panel Discussion/ Q&A
11:45 am – 1 pm	**Lunch and Poster Session**
Session III: Genetics of Common Disease *Moderators: Binita M. Kamath, MBBChir*	
1 pm – 1:20 pm	Genetics of Drug Induced Liver Injury *Merrie Mosedale, PhD*
1:20 pm – 1:40 pm	Genetics of NAFLD *Quentin M. Anstee, MBBS, PhD, FRCP*
1:40 pm – 2 pm	Genetic Etiology of Liver Cancer *Silvia M. Vilarinho, MD, PhD*
2 pm – 2:30 pm	Panel Discussion / Q&A
2:30 pm – 2:50 pm	**Break**
Session IV: Genetic Contribution to Complex Disease *Moderators: Nancy Spinner, PhD*	

2:50 pm – 3:10 pm	Genetic Susceptibility to Biliary Atresia *Saul J. Karpen, MD, PhD, FAASLD*
3:10 pm – 3:30 pm	Genetic Modifiers in Inherited Liver Disease *Kathleen M. Loomes, MD, FAASLD*
3:30 pm – 3:50 pm	Genetics of Autoimmune Liver Disease *Gideon Hirschfield, FRCP, PhD*
3:50 pm – 4:10 pm	Epigenetics and Disease Phenotypes *Kimberly D. Tremblay, PhD*
4:10 pm – 4:45 pm	Panel Discussion/Q&A

Saturday, September 21, 2019

7 am	**Breakfast**
Session V: Beyond Sequence Variation *Moderators: Kathleen M. Loomes, MD, FAASLD*	
8 am – 8:20 am	miRNA as a Biomarker of Acute Rejection in Liver Transplant *Brendan James Keating, PhD*
8:20 am – 8:40 am	The Role of microRNA's in the Pathophysiology of Liver Disease *Justin L. Mott, MD, PhD*
8:40 am – 9 am	Precision Medicine in CF *Joseph Zabner, MD*
9 am – 9:20 am	Panel Discussion / Q&A
9:20 am – 9:40 am	**Break**
Session VI: Targeted Interventions *Moderators: Richard J. Thompson, MD, PhD, FAASLD*	
9:40 am – 10 am	Personalized Disease Models Using iPS Cell Derived Models *Binita M. Kamath, MBBChir*
10 am – 10:15 am	A Truncating Mutation of TJP2 in Human Hepatocytes Derived from Induced Pluripotent Stem Cells Reduces Barrier Function and Alters Cellular Polarity *Akihiro Asai, MD, PhD*
10:15 am – 10:35 am	Novel Therapeutics in Liver Disease *Gyongyi Szabo, MD, PhD, FAASLD*
10:35 am – 10:55 am	Application of Genomic Data to Clinical Practice *Stephen L. Guthery, MD*
10:55 am – 11:15 am	Panel Discussion /Q&A
11:15 am – 11:30 am	Wrap-Up *Kathleen M. Loomes, MD, FAASLD and* *Richard J. Thompson, MD, PhD, FAASLD*
11:30 am	**Adjourn**

SPEAKER SUMMARIES

Teri A. Manolio, MD, PhD
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Personalizing Care Through Genomics: Genomic Medicine Programs of the National Human Genome Research Institute (NHGRI)

The growing availability of reliable, cost-effective genetic testing and increasing knowledge about the influence of genetic variation on human health have spurred the implementation of genomic medicine into clinical care. The National Human Genome Research Institute (NHGRI) defines genomic medicine as an emerging medical discipline that involves using genomic information about an individual as part of their clinical care, and the health outcomes and policy implications of that clinical use. Genomic medicine is already advancing diagnosis, treatment, and prevention in the fields of oncology, pharmacology, rare and undiagnosed diseases, and infectious diseases. Yet many barriers remain, including lack of familiarity and understanding by patients and clinicians, limited evidence of efficacy, scarcity of genomics expertise, lack of access and high cost of genetic testing, limited availability of genomic data in populations not of European ancestry, and difficulties in integrating genomic results into electronic medical records. Many of these barriers represent research opportunities and gaps, which NHGRI has attempted to fill through a combination of large consortia-driven programs and smaller investigator-initiated projects.

Six large-scale research and dissemination initiatives, including the Undiagnosed Diseases Network (UDN), the Newborn Sequencing in Genomic Medicine and Public Health (NSIGHT) program, the Clinical Sequencing Evidence-Generating Research (CSER) consortium, the Electronic Medical Records and Genomics (eMERGE) network, the Implementing Genomics in Practice (IGNITE) network, and the Clinical Genome Resource (ClinGen), along with a growing number of individual research and training programs, comprise NHGRI's genomic research portfolio. Key research questions being addressed in these programs include the value of exome and genome sequencing in undiagnosed diseases, the impact of incidental genomic findings on patients' subsequent clinical care, the importance and ease of collection of online patient-reported family history information, the value of pharmacogenetic variation in drug selection and dosing, the potential for identifying persons at high risk of monogenic diseases from electronic medical record data alone, and the benefits and risks of predictive genomic testing for both monogenic and polygenic conditions. Key concepts to be understood include the major types of disease-related genomic variation and how to test for them, the evidence needed to infer a particular variant is disease-causing, the need for truly informed consent and genetic counseling both before and after genetic testing is performed, and readily available sources of information on genetic variants and related diseases.

NHGRI's genomic medicine research program is intended to speed the evaluation and incorporation (where appropriate) of genomic technologies and findings into routine clinical care, and to ensure that the resulting findings are applicable and accessible to patients of diverse ancestral backgrounds. Actual adoption of successful approaches in the clinic will depend upon the willingness, interest, and energy of professional societies, practitioners, patients, and payers to promote the responsible use of these approaches and share their experiences in doing so.

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Exome Sequencing in Gene Discovery and Diagnosis

Since the finalization of the first draft of the human genome sequence in 2001, there has been explosive growth in identification of new disease genes, increasing knowledge of normal human variation, advances in understanding the mutations that cause human disease and widespread technological advances that allow faster and cheaper analysis of the genome for both research and diagnostics. The number of conditions that are amenable to genomic diagnostics has risen from a few hundred in 2012 to more than 10,000 in 2018, with the number of genes that can be tested now over 10,000. This is a result of the change from sequencing methods that target genes individually (Sanger sequencing) to methods that allow evaluation of many (or all) genes simultaneously using Next-Generation or massively parallel sequencing, which came into clinical usage around 2012 (Biesecker LG 2012). Currently, single gene tests are utilized when a clinical diagnosis is strongly suspected and the physician knows which gene they want to examine. When there are a number of disorders in the differential diagnosis and the clinician wants to evaluate all simultaneously, targeted panels are the diagnostic tool of choice, where from 2 to over 500 genes can be sequenced and analyzed simultaneously. When, the differential diagnosis is unclear or too broad, or the disorder is highly heterogeneous, sequencing of the entire "exome" is the preferred choice (Bowdin et al., 2016).

Exome sequencing is the sequencing of a small percentage of the human genome (about 1%), that contains all of the protein coding regions (Ng et al., 2009). These regions can be captured and sequenced, minimizing costs by limiting the amount of sequencing. This tool, first introduced in 2009 has been highly powerful in diagnosis of known disease genes, and has been used to identify novel candidate genes for many disorders since its introduction. Clinical exome sequencing has become the standard sequencing-based test for children with clinical presentations that do not fit a known disorder, suspected diagnoses for which there is no clinically available diagnostic test, clinical presentations with poorly understood etiology and highly heterogeneous clinical presentations such as mitochondrial disorders, intellectual disability or clinical features that are not consistent with a known disorder (Abou Tayoun et al., 2016). Exome sequencing has variable diagnostic rates, ranging from 15 to over 50% depending on the presenting clinical features (Adams and Eng 2018).

While the success of exome sequencing is very strong, there are also challenges that the medical community continues to struggle with. The two primary challenges are the identification of variants of uncertain significance ("VUS") and secondary findings (Richards et al., 2015, Petersen et al., 2018). VUS are genetic changes in a gene that cannot be interpreted at the present time, usually because there is too little information in the literature to determine if they are a rare benign variant, or a rare disease-causing variant. Correct interpretation and counseling for these variants is difficult and frustrating for both clinicians and patients. Secondary findings are genomic variants that are associated with a clinical finding that is unrelated to the reason for which the patient was studied, but nevertheless clinically significant. These can include mutations associated with cancer predisposition or the risk for disorders that will occur later in life, and again, counseling for these disorders can be highly sensitive and require specialized knowledge and clinical skills (Bowdin et al., 2016).

Lessons from exome sequencing will be applied to understanding of disorders of the liver, with examples demonstrating how exome sequencing has served as a bridge between clinical diagnostics and research propelling our understanding of the genetic basis of liver disease.

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Creating Collaborative Databases

In recent years, resources have become available to provide knowledge on variants to the genomics community, such as the ClinVar repository hosted by the National Center for Biotechnology Information (NCBI) and resources provided by the Clinical Genome Resource (ClinGen) such as curated datasets.

ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) is a freely accessible, public archive of reports of the relationships among genomic variants and phenotypes (1). To facilitate evaluation of the clinical significance of each variant, ClinVar aggregates submissions of the same variant, displays supporting data from each submission, and determines if the submitted clinical interpretations are conflicting or concordant (2). Given the rarity of most variants of clinical relevance, it is imperative that genomic variant classifications and supporting evidence are shared in a public, centralized database such as ClinVar. Data sharing improves our understanding of genomic variation and improves patient care activities that rely on this information. Sharing variant classifications with ClinVar allows laboratories to identify classification differences and work towards consensus, providing more accurate and consistent results to patients. Studies of clinical laboratory ClinVar submitters have shown data sharing is a successful approach to prioritizing variant reassessment and resolving classification differences (3). Additionally, ClinVar now accepts and encourages submissions from clinical providers providing their own interpretation of the variant ('provider interpretation') or from groups such as patient registries that primarily provide phenotypic information from patients ('phenotyping only').

The goal of the Clinical Genome Resource (ClinGen, http://www.clinicalgenome.org) is to develop an authoritative central resource that defines the clinical relevance of genes and genomic variants for use in precision medicine and research (4). ClinVar is an integral resource for ClinGen's curation activities and for archiving the results. ClinGen activities rely on ClinVar for the deposition and retrieval of variants and their clinical interpretation. A core goal of ClinGen is expert interpretation of variants, which is accomplished by convening Variant Curation Expert Panels (VCEPs) that focus on a gene or group of genes (5). The VCEPS are tasked with providing specifications to the ACMG/AMP guidelines for their individual genes or diseases, interpreting variants according to these rules, and publishing the interpretations in ClinVar.

ClinGen has also developed a framework to define and evaluate the clinical validity of gene-disease pairs across a variety of Mendelian disorders to help the community differentiate clinically valid relationships from less well-substantiated relationships (6). This framework provides a semiquantitative measurement for the strength of evidence of a gene-disease relationship that correlates to a qualitative classification: "Definitive," "Strong," "Moderate," "Limited," "No Reported Evidence," or "Conflicting Evidence." Within the ClinGen structure, classifications derived with this framework are reviewed and confirmed or adjusted based on clinical expertise of appropriate disease experts. This evidence-based, systematic method to assess the strength of gene-disease relationships will facilitate more knowledgeable utilization of genomic variants in clinical and research settings.

In summary, sharing of variant classification rationale and community-driven expert curation of genes and variants will help the community move toward more consistent variant classifications, improving the care of patients with, or at risk for, genetic disorders.

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The Promise and Pitfalls of Genetic Testing

As understanding and technologies for genetic analysis have developed, the clinical promise and the ethical liabilities associated with genetic testing have evolved as well. This talk will examine ethical issues that accompany genetic testing / genomic sequencing for a range of liver-related conditions. Ethical concerns vary somewhat in accordance with different features or types of genetic analysis—e.g., targeted testing vs. genome sequencing, diagnostic vs. therapeutic vs. predictive/preventive goals, analyzing mono/oliog/polygenetic conditions, research vs. clinical care contexts.

Historically, hemochromatosis served as the poster child of conditions for which genetic testing presented clinical benefit, and even potential and even social and ethical benefit, with almost no ethical risks. Genetic testing for alpha-1 antitrypsin deficiency disease presents a slightly more complicated psychosocial, and thus ethical, picture; however, there are clearly potential clinical benefits that result in a positive risk:benefit ratio. Discussion of these two rather straightforward indications for genetic testing provides an initial framework for mapping the ethical terrain—the points and perspectives to consider—regarding genetic testing.

The ethical terrain becomes more complicated as we turn from these early uses of genetic testing for liver disease to different targets and more recent use of genomic sequencing. The range of points to consider expands to include:

- individual, familial, and social concerns
- consequences, rights, and special responsibilities arising from relationships
- material (health-related, clinical, economic), psychological, social, and dignitary or moral risks/benefits
- matters of individual values and preferences vs. social and institutional policies
- professional concerns (e.g., about liability, professional judgment), individual or patient concerns, institutional interests, and societal interests
- ethical considerations intensified in the context of genetics and the usual clinical ethics issues
- considerations related to particular (patient/research) populations—e.g., pediatrics, people with impaired decisional capacity, indigenous peoples, and racial, ethnic, or isolated minorities
- research ethics vs. clinical ethics
- health policy and social policy issues, including the implications of genomic medicine (and more broadly, precision medicine) for private and for-profit healthcare, health insurance, and other types of insurance
- relevance of genetic components of disease for other ethical considerations (e.g., the implications of genetic components of liver disease for transplantation ethics)
- clinical responses to direct-to-consumer genetic testing
- nonclinical uses of genetic information

A 20-minute talk cannot even characterize all the points to consider, let alone address these points adequately. Nevertheless, this talk will illuminate some of the more complex ethical issues that arise.

Recommended Readings

1. **Ethics, genetics, & liver disease:**
   van Leeuwen DJ and Bernat JL. Ethical, Social and Legal Implications of Genetic Testing in Liver Disease *Hepatology* 2006; 43:1195-1201. PMID: 16729331; DOI: 10.1002/hep.21206
2. **Ethical implications of the scientific complexity of genomic and precision medicine:**
   Batten JN. How Stratification Unites Ethical Issues in Precision Health. *AMA Journal of Ethics* 2018; 20(9):E798-803. doi: 10.1001/amajethics.2018.798
3. Paul C, Adams PC, Reboussin DM, Barton JC. Hemochromatosis and Iron-Overload Screening in a Racially Diverse Population. *New England Journal of Medicine* 2005; 352:1769-1778. DOI: 10.1056/NEJMoa041534
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5. **Pediatric genetic testing and screening**:
   American Academy of Pediatrics Committee on Bioethics and Committee on Genetics, and The American College of Medical Genetics and Genomics Social, Ethical, Legal Issues Committee. Ethical and policy issues in genetic testing and screening of children. *Pediatrics* 2013 Mar; 131(3):620-2. doi: 10.1542/peds.2012-3680. Epub 2013 Feb 21. PMID: 23428972; DOI: 10.1542/peds.2012-3680
6. Berg JS, Agrawal PB, Bailey DB, et al. Newborn Sequencing in Genomic Medicine and Public Health. *Pediatrics* 2017; 139(2):e20162252
7. **Direct-to-consumer genetic testing:**
   Brothers KB and Knapp EE. How Should Primary Care Physicians Respond to Direct-to-Consumer Genetic Test Results? *AMA Journal of Ethics.* 2018; 20(9):E812-818. doi: 10.1001/amajethics.2018.812
8. Loike JD. Opinion: Consumer DNA Testing Is Crossing into Unethical Territories. *The Scientist*. August 16, 2018.
9. Nitkin K. Five Things to Know about Direct-to-Consumer Genetic Tests. *DOME*. March/April 2018.
10. **Implications for transplantation:**
    Singhvi A, Welch AN, Levitsky J, MD, Singhvi D, and Gordon EJ. Ethical Considerations of Transplantation and Living Donation for Patients with Alcoholic Liver Diseases. *AMA Journal of Ethics.* 2016; 18(2):163-173. doi: 10.1001/journalofethics.2017.18.2.sect1-1602

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Genetic Cholestasis in Children

In just over 20 years, our understanding of the genetic underpinnings of pediatric cholestasis has dramatically increased. This presentation will focus on progressive familial intrahepatic cholestasis (PFIC) and related disorders. In 1998, increasing availability of efficient genetic approaches allowed the 1st 3 genes implicated in PFIC to be identified, all appearing to demonstrate autosomal recessive inheritance. Mutations in some cases of 'low-gGT' PFIC and benign recurrent intrahepatic cholestasis (BRIC) were reported in *FIC1/ATP8B1,* and other low-gGT PFIC patients were found to carry mutations in *BSEP/ABCB11*(refs). Together, mutations in these 2 genes account for the majority of low-gGT PFIC cases. Mutations in *MDR3/ABCB4* were identified in some patients with high-gGT PFIC (refs). At the time, these PFIC loci were labelled chronologically as PFICs 1-3.

In subsequent years, we have learned much about the phenotypes present in patients with these deficiencies, and additional, rarer genetic forms of PFIC and related disorders have been identified. ATP8B1 is a type-4 P-type ATPase, expressed in many tissues, that functions as a phospholipid flippase, moving phospholipid between leaflets of the plasma membrane. Absence of ATP8B1 function appears to disrupt the normal asymmetric distribution of lipids between the 2 membrane leaflets, with a variety of consequences. Mutations in *ATP8B1* can result in a continuum of disease severity, from PFIC to BRIC, depending upon the severity of the functional consequences of the mutation(s) involved; mild mutations can also be incompletely penetrant. While liver transplantation is sometimes necessary in ATP8B1 deficiency, patients can continue to have extrahepatic disease manifestations, some of which may even worsen post-transplant, as well as developing steatosis in the new liver.

Bile salt export protein (BSEP) is a liver-specific protein that transports bile acids out of the liver into the canaliculus. Loss of BSEP function causes bile acids to accumulate in the liver, causing tissue damage. Similarly to ATP8B1 deficiency, BSEP deficiency can be partial or complete, resulting in a range of phenotypic severity. Even amongst BSEP patients diagnosed with PFIC, a patient's precise *ABCB11* mutation profile can influence response to partial external biliary diversion, as well as the age at which liver transplantation becomes necessary. Severe BSEP deficiency typically results in more rapid progression of liver disease than seen in severe FIC1 deficiency, and BSEP deficiency patients have an increased risk of developing hepatocellular carcinoma. In contrast to ATP8B1 deficiency, BSEP deficiency is a liver-specific disorder, so liver transplantation corrects the primary disorder. Patients can nevertheless have complications after transplantation, such as formation of antibodies to BSEP, with consequent greater risk of rejection.

MDR3 transports phosphatidylcholine (PC) from the inner leaflet of the plasma membrane into the canaliculus, where PC plays an important role in protecting cholangiocytes from the detergent effects of bile acids. MDR3 deficiency is therefore a form of cholangiopathy. In contrast to ATP8B1 and BSEP deficiencies, people can have significant liver disease even if they only carry one mutated mutated ABCB4 allele, so MDR3 deficiency cannot be considered a purely autosomal recessive condition.

BSEP deficiency is the most common genetic form of PFIC, and together, ATP8B1 and BSEP deficiencies appear to account for the majority of low-gGT PFIC cases. However, a significant proportion of patients diagnosed with PFIC have no mutations detected in either of these genes. In recent years, technological advances, including whole exome sequencing, have decreased the cost and personnel time of genetic studies, beginning to allow identification of additional, rarer genetic etiologies of PFIC. Recessive mutations in *TJP2* have been found in patients with disease along a continuum from relatively mild (hypercholanemia) to PFIC.[1, 2] Biallelic mutations in *FXR/NR1H4* have also been identified as a rare genetic etiology of PFIC.[3] Other very recently identified examples of rare genetic etiologies of cholestasis include biallelic mutations in *UNC45A* implicated in a syndrome including cholestasis, and homozygous loss of *USP53* in patients with cholestasis and hearing loss from an extended family.[4, 5] In some such cases, the evidence has not yet accumulated sufficiently to definitively identify a given gene as a Mendelian cholestasis gene, but rather, as a strong candidate gene.

A phenomenon that has arisen repeatedly in recent years is that of phenotype extension- i.e. mutations are found in a patient with a primary diagnosis of cholestasis, in a gene previously implicated in a disorder in which hepatic involvement was not previously reported, or was considered minor or a secondary consequence of disease. One example is *MYO5B*, in which biallelic mutations had previously been identified in some cases of microvillus inclusion disease; more recently, *MYO5B* mutations been reported in some patients with cholestasis, in the absence of clinically significant intestinal disease.[6, 7] A second example is that of *HNF1*β, in which monoallelic mutations had been identified in a rare form of diabetes, but which have now also been reported in patients with cholestasis.[8]

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Genetic Cholestasis in Adults

As our understanding of the mechanisms underlying bile acid homeostasis has advanced, insights have been gained about the genetic etiology of cholestasis in adults as well as children. It is becoming clear that cholestasis and hypercholanemia can be caused by genetic variation that influences a number of processes including biliary transport, cell to cell interaction and nuclear receptors that control homeostatic pathways. There are a large number of genetic variants now reported in biliary transporter genes, e.g. ABCB4, ABCB11, ATP8B1. In addition to causing severe childhood cholestasis, mutations in these biliary transport proteins, and likely in others, e.g. ABCC2, can result in late onset disease. There is a spectrum of associated phenotypes, including drug-induced cholestasis, cholelithiasis and intrahepatic cholestasis of pregnancy. Similarly mutations in TJP2 may be responsible for adult disease. Pedigree studies are elucidating mutations in a number of new genes, some with associated phenotypes, e.g. hearing loss or bleeding.

In addition to more penetrant mutations, there is evidence for coding and non-coding SNPs in cholestasis genes that influence disease susceptibility. The use of large sample resources will enable detailed investigation of the genetic architecture of cholestasis syndromes. Adult-onset cholestasis may be precipitated by drugs, e.g. the combined oral contraceptive or antibiotics. For intrahepatic cholestasis of pregnancy (ICP), elevated serum concentrations of estrogens or progesterone metabolites in affected women are believed to unmask susceptibility to disease. The genetic etiology of cholestasis in different individuals is likely to influence severity and associated phenotypes, including the risk of dyslipidemia, cholelithiasis, hepatic fibrosis and susceptibility to malignancy. It is known that women with ICP have increased risks of serious biliary disease in later life, underlining the importance of clinical follow-up of individuals with cholestasis-associated genetic variation. The impact of treatments to reduce the severity of cholestasis should be evaluated.

ICP is also associated with poor pregnancy outcomes, e.g. spontaneous preterm birth and stillbirth, that are more prevalent in pregnancies where maternal serum bile acids are high. There is also evidence that the 16 year-old children of mothers with ICP have increased risks of adiposity and dyslipidemia, indicating the importance of optimising maternal treatment. As for other forms of adult-onset cholestasis, an understanding of the underlying genetic etiology of maternal cholestasis will enable consideration of appropriate treatment to improve long-term outcomes.

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Genetic Determinants of Cholangiopathies

Damage to the bile ducts can occur due any number of causes. Whatever the initiator of the damage, bile ducts are an intrinsically hostile environment. This is largely because they contain bile; an intrinsically damaging fluid. The initial damage to bile ducts can be caused by extrinsic factors such infection, autoimmunity, or ischaemia. It can be caused by abnormalities in the structure of the bile ducts themselves, or it can be due to changes in the bile within them.

Cholangiopathies are further complicated by the fact that bile ducts exhibit heterogeneity, anatomically and functionally, along the length of the biliary tree.

Primary biliary cholangitis, primary sclerosing cholangitis and autoimmune sclerosing cholangitis are primary inflammatory conditions affecting the biliary tree. They are being discussed elsewhere. However, they do not appear to be associated with high-penetrance genetic variants but do have HLA alleles associated with risk of disease.

The best characterised disease, with intrinsic abnormality of bile duct structure, is claudin-1 deficiency. As might be expected with a loss of this integral tight junction, there is paracellular leak of bile acids and consequent periductular inflammation. The more remarkable finding is that the patients appear to only have liver and skin phenotypes. TJP2 deficiency is discussed separately, but this claudin-associated protein causes a form of cholestasis and not a cholangiopathy.

Abnormalities of bile content underlie many cholangiopathies. The most obvious example is MDR3 deficiency, where a reduction in biliary phospholipids leads to reduced micelle formation and increased free bile acids in bile. This has a direct damaging effect on biliary epithelium. Micelles require a low pH for stability. This is normally maintained by a combination of chloride channels and anion exchangers. CFTR is one of several chloride channels in biliary epithelium, where it has been shown to regulate anion exchange. CFLD is most likely a cholangiopathy with aetiology overlapping that of MDR3 deficiency.

Primary cilia are present on many epithelia, including cholangiocytes. A rapidly growing list of ciliopathies include liver phenotypes. Primary cilia are sensory organelles, sensing luminal contents; using mechanical or chemical sensing mechanisms. Ciliopathy related gene products can be involved in cilial structural proteins, those responsible for transport within the cilium or the sensing mechanisms. In all cases the consequence is a loss of feedback of luminal contents, in one or more tissue. Some of the secondary signalling mechanisms are understood, but the exact consequences on biliary structure, bile composition or flow and not yet clear.

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Genetics of Drug-Induced Liver Injury

Drug-Induced Liver Injury

Drug-induced liver injury (DILI) is broadly divided into two categories: “intrinsic” and “idiosyncratic” [1]. Intrinsic DILI is dose-dependent, typically occurs within days of dosing, and is usually predicted in nonclinical or early clinical studies. As a result, there are few drugs that cause intrinsic DILI in therapeutic use today. One example of an approved intrinsic toxicant is acetaminophen. Sub-toxic exposures of acetaminophen can generally be safely tolerated, but essentially all patients will develop liver injury if they receive a sufficiently high dose, thus “the dose makes the poison”. In contrast, idiosyncratic DILI lacks a clear dose-response relationship and typically occurs weeks to months after starting drug exposure. These reactions are unpredictable and can be severe making them the most problematic type of ADR for patients, health care providers, drug developers, and drug regulators. With a true idiosyncratic toxin, only a small fraction of the total patients exposed to drug (typically 1 in greater than 10,000 patients) are susceptible to clinically significant liver injury, even when receiving high doses. Susceptibility is thought to reflect an individual’s unique influence on multiple risk factors, therefore “the host makes the poison”. There is significant evidence to support that genetic variation contributes to DILI risk.

Genetic Risk Factors

The strongest genetic associations for DILI susceptibility have been identified in the human leukocyte antigen (HLA) region of the genome. It is hypothesized that certain HLA molecules can present neoantigens that are formed as a result of exposure to a given drug, resulting in an adaptive immune attack on the liver [2]. HLA associations are largely drug specific. One of the most robust associations identified to date has been with DILI due to the antibiotic flucloxacillin and *HLA-B*57:01* (OR of 80.6) [3]. Subsequent mechanistic studies have provided biological plausibility for this association by demonstrating flucloxacillin-dependent proliferation of T-lymphocytes isolated from the blood of patients who had experienced flucloxacillin DILI as well as drug naive healthy volunteers expressing *HLA-B*57:01* [4]. Polymorphisms in a variety of drug metabolizing enzymes and transporter (DMET) genes have also been associated with susceptibility to various hepatotoxic drugs [2]. The most robust DMET association is between variants in N-acetyltransferase 2 (*NAT2*) and increased risk of isoniazid-induced DILI. NAT2 helps to detoxify acetlyhdyrazine, a potentially harmful metabolite of isoniazid, thus toxic metabolites may accumulate in individuals with low NAT2 activity. Finally, some associations with DILI susceptibility have also been identified in non-HLA/DMET genes that would likely modulate a DILI response [2]. These include polymorphisms in genes associated with antioxidant, apoptosis, and immune responses. A recent manuscript describes the identification of missense variant in the protein tyrosine phosphatase, nonreceptor type 22 gene (*PTPN22*) as a general risk factor for idiosyncratic DILI as opposed to DILI associated with a specific drug [5]. PTPN22 plays numerous roles in T-cell receptor signaling including regulatory T-cell function and variation in this gene has been linked to autoimmune disorders.

Current Methods

Like other areas of genetics, early progress in the field was advanced via candidate gene studies [2]. With the decreasing cost of PCR and increasing availability of genomic data, investigators could more easily and affordably genotype SNPs in a small number of candidate

genes hypothesized to play a role in drug response. This is how many of the associations with SNPs in DMET genes were identified. Unfortunately, this approach has several limitations including: 1) a focus on a particular gene or gene variants that have a biologically plausible relationship to the trait and exclusion of all other genes; 2) a failure to account for population structure and the possibility of confounding due to ancestry; and 3) a general lack of statistical rigor [6]. Not surprisingly, many of the published studies identifying DMET risk alleles by the candidate gene approach have not been replicated in other DILI cohorts, calling into question the true strength of these associations [2]. Genome-wide association studies (GWAS) in which millions of SNPs are genotyped simultaneously in an effort to test for the association of all common genetic variation allow for a more comprehensive and unbiased scan of the genome. Established GWAS standards ensure the appropriate conduct and interpretation of these studies. As the cost for high-throughput genotyping technologies came down, the application of GWAS to DILI expanded and a number of very strong associations were identified, almost exclusively in the HLA region of the genome, again indicating the importance of immune response in the pathogenesis of disease [7]. The current gold standard for genetic studies in DILI utilizes GWAS genotyping and further imputation of SNPs and HLA types to identify associations, which are subsequently validated by sequencing-based approaches[5, 8, 9].

New Approaches

Much variation in DILI susceptibility remains unexplained [1]. Given the low incidence of serious events, one logical explanation is that DILI risk is driven by rare variants (minor allele frequency; MAF<1%). It has recently been demonstrated that as much as 40% of the functional variability in drug response can be attributed to rare variants [10], polymorphisms that would not be detected without sequencing-based approaches. Next-generation sequencing studies of entire patient genomes or of the whole exome are now being performed. Another possibility is that DILI is a polygenic trait with multiple variants contributing different amounts to overall susceptibility. Newer approaches like the genome-wide polygenic scores [11] may be more useful estimating risk. Finally, epigenetic changes may also play a role, particularly in the liver which is sensitive to environmental exposures [12]. New technologies to integrate epigenetic analyses into transcriptomic studies have begun to uncover the extent and dynamic nature of the perturbations resulting from xenobiotic exposure. However, epigenetic analyses will likely require access to liver tissue, a difficult resource to obtain in clinical studies.

Clinical Samples

Idiosyncratic DILI is an extremely rare event and the diagnosis is one of exclusion [1]. As a result, an ongoing challenge in the area of DILI genetics has been the collection of sufficient numbers of well-phenotyped cases for conducting GWAS [2]. In many published studies this is reflected as small samples sizes collected from a single site, often with diagnostic and/or causality criteria that may allow for the inclusion of liver injury cases that are not drug related and/or are not attributed to the correct drug. Fortunately, there are a growing number of national and international registries (DILIGEN, iDILIC, EUDRAGENE, the Spanish DILI Cohort, etc) that enroll DILI patients and use expert review of available clinical information for causality assessment [13]. In the US, the drug-induced liver injury network (DILIN) has collected GWAS genotyping data and additional DNA from over 1600 subjects who have experienced clinically significant liver injury due to drugs as well as herbal and dietary supplements (dilin.org). The DILIN, often in collaboration with other registries, has conducted several genetic studies[5, 8, 13, 14], and makes data and biospecimens available to other investigators. Because DILI has a very low prevalence, ancestry-matched general population samples are commonly used as study controls.

Nonclinical Tools

Given the limited availability of clinical cases, lack of true treatment-matched controls, and inability to conduct prospective studies, researchers have sought nonclinical tools to evaluate the impact of genetic variation on DILI susceptibility. The goal of these studies is to identify variation that influences the early events in DILI that facilitate an adaptive immune attack such as direct hepatocellular stress, the release of danger signals, and activation of the innate immune system [1]. Because these processes that can occur in the absence of overt injury, endpoints must be appropriately selected and frequently include gene expression profiling, functional changes, and high-content imaging. One highly promising approach has been the use of genetically diverse mouse populations which support pharmacogenomic analysis to identify risk factors and mechanisms associated with toxicity susceptibility [15]. There are also several efforts underway to develop an *in vitro* platform containing hepatocytes from genetically diverse mice to enable a more rapid and cost-effective implementation of the approach. Finally, mouse-based approaches are paving the way for human studies, which can also be performed *in vitro* using primary human hepatocytes from large numbers of random donors or induced, pluripotent stem-cell derived hepatocytes from even larger numbers of random donors and importantly, DILI patients.

Clinical Implementation

To date, even the most robust HLA risk allele associations have not led to clinically accepted risk management strategies. This is in part because the associations found identify a relatively large subpopulation at increased risk of idiosyncratic DILI, but the majority of those who carry the allele can in fact take the drug safely [1]. There has been one attempt to introduce genetic testing for the management of DILI risk: Novartis' lumiracoxib, a COX-2 inhibitor withdrawn from world-wide markets due to DILI estimated to occur in less than 1:10,000 treated patients [16]. A retrospective GWAS of DNA samples obtained in the clinical trials demonstrated *HLA-DRB1*1501* as highly sensitive for identifying patients at risk for developing DILI [16]. However, over 25% of the subjects in the clinical trials carried this allele, and the vast majority could be treated safely. Furthermore, there was not a clear unmet need for the drug. Nonetheless, the effectiveness of genotyping in reducing the risk of lumiracoxib DILI was not challenged, and the FDA remains a strong advocate for the use of precision medicine [17]. With the right drug and indication, genotyping as a means of managing DILI risk will surely make it into the clinic in the future. In the meantime, genotyping is likely to assist clinicians in the establishment of a DILI diagnosis. To this end, DILI-associated genotypes for many drugs are now available on the LiverTox website (livertox.nlm.nih.gov).

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Genetics of NAFLD

Non-Alcoholic Fatty Liver Disease (NAFLD) are the most common cause of chronic liver disease worldwide. NAFLD is a complex, multifactorial and multistage disease encompassing steatosis (hepatic triglyceride content (HTGC) greater than 5%), steatohepatitis, fibrosis, cirrhosis and, in some cases, HCC [1]. It is characterized by substantial inter-patient variation in disease progression and outcomes: approximately 40% of NAFLD patients exhibit progressive liver fibrosis whist the remaining 60% exhibit stable disease or some degree of regression during long-term follow-up [2]. The reasons for these variations remain incompletely understood, but NAFLD is best considered *complex disease trait* where environmental factors (e.g. dietary constituents, intestinal microbiota), acting upon a susceptible polygenic background comprising multiple subtle inter-patient variations, lead to a disease phenotype and ultimately determine disease progression [3].

Three strands of evidence suggest that there is a significant heritable component to NAFLD: familial aggregation; twin studies (greater concordance between monozygotic twins than dizygotic twins); and inter-ethnic differences in susceptibility [3]. As disease susceptibility is due to the combined effects of multiple relatively common causative polymorphisms each of which makes a small overall contribution to disease risk [4]. As no single gene is sufficient to determine outcome, clear patterns of inheritance are not seen within kinships.

Technical advances with the development of SNP arrays has paved the way for the genome-wide association study (GWAS) [5,6]. Whilst such techniques are not a panacea, and a substantial proportion of disease heritability remains elusive, the non-hypothesis driven nature of GWAS means that many of the loci identified are novel and might not otherwise have been identified. This however also implies that much work is required to validate the results and explore the underlying pathophysiological mechanisms.

Key variants that have been associated with NAFLD severity include: the non-synonymous *PNPLA3* variant (rs738409 c.444 C>G, p.I148M)[7,8]; the variant within *TM6SF2* (rs58542926 c.449 C>T, p.E167K)[9-11]; a variant near *MBOAT7* (rs626283 C>G) [12]; and most recently, a variant near HSD17B13 (rs6834314 A>G)[13,14].

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Genetic Etiology of Liver Cancer

Cancer is a genetic disease that largely arises from acquired somatic genetic alterations. DNA replication is an essential part of cell division during which alterations in cell's DNA may occur, and in some circumstances are not repaired. These genetic alterations are called somatic events as they occur after conception and therefore are not present in germ line cells and are not passed on to the offspring.

Over the last decade, advances in human genomics through next generation sequencing technology have created an unprecedent opportunity to identifying cancer driver genes and oncogenic pathways in a diverse set of human malignancies, including liver cancer. Next generation sequencing (NGS), also known as massively parallel sequencing or deep sequencing, have several different applications, such as whole-genome sequencing (WGS), whole-exome sequencing (WES), targeted gene(s) sequencing and RNA-sequencing, among others. WES and WGS studies have uncovered genetic alterations that are exclusively present in malignant cells and not in non-tumor tissue of the same individual. These somatic genetic events include single nucleotide variants (such as premature termination, splice-site or missense variants), small nucleotide insertions and deletions (indels), copy number variants (CNVs) and structural variations. Somatic gene alterations that contribute to tumor evolution at any stage, from cancer origin to metastatic disease are defined as cancer driver mutations, whereas genetic alterations with no role in tumor pathogenesis and therefore happened by chance are designated passenger mutations.

The most common liver cancer is hepatocellular carcinoma (HCC). HCC is the second leading cause of cancer-related death in men, affecting > 700,000 lives / year worldwide(1) and being the fastest growing cancer in the U.S. up to 2030. Approximately 85% of HCCs occur in a background of cirrhosis. The most frequent mutations detected in HCC occur in the promoter of *TERT*, which encodes telomerase reverse transcriptase (2, 3). These mutations are recognized as an early genetic event to immortality and proliferation of hepatocytes resulting in dysplastic nodules. Additional frequently mutated genes in HCC are *CTNNB1* and *TP53*, each being present in approximately one-third of cases. Furthermore, mutations in genes involved in (i) chromatin remodeling, (ii) PI3K/mTOR signaling, (iii) RAS/MAPK signaling, (iv) JAK/STAT signaling and (v) oxidative stress pathways have also been described as contributors to HCC.

On the other hand, approximately 15% of HCCs occur in patients without underlying liver fibrosis/cirrhosis. These cases include malignant transformation of hepatocellular adenomas, most of which have been associated to mutations in exon 3 of *CTNNB1*. Combining deep phenotyping with WES, we studied the genomic events underlying the hepatocellular adenoma-carcinoma transition, vascular invasion and brain metastasis(4). Moreover, the application of RNA-sequencing to fibrolamellar HCC, which typically affects adolescents and young adults with no prior history of liver disease, uncovered a DNAJB1-PRKACA chimeric transcript providing a novel insight into the molecular pathogenesis of this rare liver cancer(5).

In children, HCC occurs rarely and represents the second most common primary liver malignancy. It is associated with vertically transmitted hepatitis B infection, inherited metabolic diseases (e.g. tyrosinemia type I, glycogen storage disease type I, alpha-1-anti-trypsin deficiency, MPV17-hepatocerebral mitochondrial depletion syndrome) and cholestatic

liver diseases, such as biliary atresia, Alagille syndrome, and progressive familial intrahepatic cholestasis type 2 – PFIC2 (6, 7). WES of genomic DNA extracted from HCC tissue resected from a PFIC2 patient revealed somatic driver mutations in *CTNNB1* and *NFE2L2* genes, and clonality analysis suggested that *CTNNB1* mutation occurred earlier during carcinogenesis whereas *NFE2L2* mutation was acquired later(6).

Lastly, it is noteworthy that the genetic alterations of cholangiocarcinoma, also known as bile duct cancer, differ from those discussed above. Cholangiocarcinoma has frequent mutations in *KRAS*, *BRAF*, *BAP1*, *SMAD4*, *IDH1* and *IDH2*. However, current genomics data suggest a potential continuum among HCC with stem cell features, mixed hepato-cholangiocarcinoma and cholangiocarcinoma, but further studies are required(2).

Collectively, with the advent of next generation sequencing technology, we are now well-equipped to understand at the molecular level why an individual develop liver cancer and therefore aim to deliver the right targeted treatment to the right patient, which approach is in perfect alignment with the Precision Medicine Initiative launched by President Obama in January 2015.

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Genetic Susceptibility to Biliary Atresia

Biliary atresia (BA) is a disease that has eluded major discoveries of etiology and pathophysiology for decades. (1) Since BA is a disease that is present prenatally (2, 3), with cholestasis noted soon after birth (4), a genetic etiologic component is likely, although a common contribution has yet to reveal itself. (5) Consideration of immunologic, toxic and viral contributions have all been suggested to contribute to BA, but firm evidentiary support has yet to be produced for these three categories. Genetic contributions using strict Mendelian approaches have been mostly unrewarding as well, although select GWAS or case reports of non-stratified BA patients has yielded some genes of potential interest (e.g., CFC1, ADD3, EFEMP1, GPC1 and others). (6-8)

If modern genomic technologies can be applied to discovery a genetic etiology of BA, then the highest probability of gene discovery should focus upon the ~ 10-20% of BA patients with developmental laterality defects (polysplenia, situs inversus, malrotation, cardiac structural defects)—collectively known as BA Splenic Malformation (BASM) (9, 10). These patients, with the combination of features of heterotaxy suggesting an embryologic phenotype along with the pan-biliary cholangiopathy of BA, are prime candidates to apply unbiased next generation DNA sequencing. Given the large collection of clinically-phenotyped BA patients with accompanying biospecimens in the PROBE (NCT NCT00061828 and BASIC (NCT00345553) studies of the NIH-supported network ChiLDReN network, exome sequencing was employed to explore potential functional variants in a panel of 2016 ciliopathy genes in 67 BASM patients. A filtering mechanism for rare and likely damaging bi-allelic variants led to the discovery of 5 subjects with significant mutations in one gene—*PKD1L1*. (11) Lab-based validation studies are underway to determine the potential functional significance of this ciliary, cholangiocyte-expressed gene, which has been recently associated with significant heterotaxic disorders in humans. (12-14)

PKD1L1 is an attractive candidate for those subjects with BASM, which would place it as a cholangiociliopathy, similar to those due to deficiencies in *PKHD1*, *DCDC2*, and other disorders. (15, 16) However, it is likely that additional patients' DNA will need to be studied for PKD1L1 and other gene variants to see if there are other genes in linked pathways, that may lead to BA. This frustrating disease will need a broad range of analytical as well as lab-based studies to determine roles for exome-based candidate genes, or combinations of gene variants, to determine a fuller explanation of their roles in the genetic underpinnings of the developmental cholangiopathy known as BA.

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Genetic Modifiers in Inherited Liver Disease

With advances in genetic testing, it is now possible to identify a molecular etiology in many cases of chronic liver disease, especially those presenting in childhood. However, in many cases, there is not a clear genotype-phenotype correlation and severity of disease may be determined at least partially by other genetic modifiers.

Alagille syndrome (ALGS) is an autosomal dominant disorder characterized by bile duct paucity, cholestasis and involvement of other organ systems including cardiac, skeletal, vascular, renal and ophthalmologic. ALGS is associated with mutations in the Notch ligand *JAG1* in about 94% of cases and the receptor *NOTCH2* in 2 to 3%. Since the disease was first described, it has been recognized that the clinical manifestations of ALGS are extremely variable, ranging from very mild to severe and life threatening. Within families, individuals carrying the same mutation in *JAG1* or *NOTCH2* often demonstrate remarkably different clinical phenotypes, raising the possibility that genetic modifiers play a role in determining clinical severity. This was first studied in a mouse model in which loss of the protein O-glucosyltransferase Rumi (encoded by *Poglut*) resulted in more severe bile duct morphogenesis defects in the *Jag1* mutant animals (1). Rumi is responsible for O-glucosylation of the EGF repeats within the Notch receptor, and its loss resulted in less efficient Jag1-Notch signaling during bile duct development. In a more recent study, Tsai et al performed a Genome Wide Association Study on a cohort of ALGS patients with known *JAG1* mutations and either mild or severe liver disease (2). They identified a locus that suggested genome wide significance upstream of the thrombospondin 2 (*THBS2*) gene. Further, they showed that thrombospondin 2 is expressed in bile ducts and periportal regions in the mouse liver and can directly inhibit JAG1-NOTCH2 interactions. In ALGS, *THBS2* is proposed to modify the liver disease phenotype through direct interference with Notch signaling. Further studies will be necessary to confirm the role of *THBS2* as a genetic modifier in mouse and/or zebrafish models.

Another inherited liver disease with variable phenotype and possible genetic modifiers is alpha one antitrypsin deficiency (A1AT). A1AT deficiency is an autosomal recessive disorder in which loss of the protease inhibitor A1AT results in early emphysema. One particular missense mutation in the A1AT disease gene *SERPINA1* (designated protease inhibitor phenotype PI ZZ) causes abnormal folding of the protein and accumulation within the endoplasmic reticulum (ER) of hepatocytes. In some individuals, this accumulation of abnormally folded protein leads to progressive liver disease of variable severity. It has long been hypothesized that individual genetic variation in elimination of abnormally folded proteins within cells and protective mechanisms could lead to mild or severe liver disease phenotypes. For example, Pan et al found that a single nucleotide polymorphism suppressed expression of the ER mannosidase I, impairing the cell’s ability to break down PI Z polymers (3). In this setting, ER stress is increased, leading to more severe liver disease.

Similarly, there is not a strong genotype-phenotype correlation in Wilson disease that can fully explain the severity of liver disease presentation. A recent report correlates a truncated variant in *HSD17B13*, previously reported to be protective in NAFLD, with a mild liver disease phenotype in Wilson disease (4). *HSD17B13* encodes hydroxysteroid 17-beta dehydrogenase 13, a novel lipid droplet-associated protein that is involved in regulation of lipid biosynthesis.

These and other examples of genetic modifiers of inherited liver disease will be discussed.

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Genetics of Autoimmune Liver Disease

Autoimmune liver diseases are rare but impactful. They are seen across all ages, in all heritages, and impact men and women. Conceptually disease is broadly classified as autoimmune liver disease wherein either the hepatocyte is the principal target (hepatitis) or where the biliary epithelium is the focus of injury (cholestatic). There are also patients in whom overlapping features of injury are evident; this is more poorly described but taken as a reflection of shared mechanisms of disease. The three core autoimmune liver diseases that are generally encountered in clinic are named primary biliary cholangitis (PBC), primary sclerosing cholangitis (PSC), and autoimmune hepatitis. IgG4 related diseases can include autoimmune biliary and/or hepatic presentations, but is much rarer, and likely has more distinct etiology.

Since the precise causes of autoimmune liver disease remain unknown, diagnosis in practice remains one of exclusion, alongside identifying characteristic features (but not necessarily exclusive ones) in laboratory markers, autoantibody profiles, imaging or histology. Across the autoimmune liver diseases it is also clear that individuals, as well as family members, have a greater predisposition to other autoimmune diseases. Rare familial presentations have been reported, as have higher prevalence rates in certain populations e.g. PBC in First Nation communities in Canada.

Patho-physiologically these chronic inflammatory disorders are considered to arise as a result of a coalescence of risk factors, that collectively lead to chronic inflammatory and fibrotic liver disease, with insufficient reparative responses to prevent liver damage. The diseases are variable in their clinical course, and it is clearly recognised that there is a spectrum of severity, but without clarity as to why that is per se. Without knowing precise disease triggers the risk factors for disease are classically considered to be genetic, environmental (including the host microbiome) and epigenetic. Fundamentally the expression of disease balances all of these risk factors, with appreciation that at different stages of disease, the relative contribution of different risks may be distinct. This heterogeneity of disease course means clearly understanding the nature of the disease phenotype being studied in any report is key i.e. an analysis of genetic risk factors for disease diagnosis, is distinct to an evaluation of gene variation associated with disease progression or symptom burden.

Focusing on how genetic risk factors explain these diseases has contributed to the overall understanding of autoimmune liver disease and its presentation, but to date has as yet not contributed to patient care or treatment per se. The approach taken to genetic analysis in autoimmune liver disease has been to study each disease individually, as well as alongside closely related conditions such as inflammatory bowel disease or other autoimmune diseases such as type 1 diabetes, celiac disease or autoimmune rheumatologic conditions. Technologies applied to gene studies have varied but most informative data of late has arisen from genome wide association studies, as well as high-density genotyping efforts. Some rare familial presentations of autoimmune liver disease have also been investigated with whole genome or exome sequencing efforts, but the bulk of our appreciation of genetics in autoimmune liver disease reflects the impact of common genetic variation on disease susceptibility, as opposed to ultra-rare paradigms highlighted in unique families or individuals.

The common genetic variants identified to date can be segregated into HLA associations and non-HLA associations. These associations seem distinct between autoimmune liver diseases, but conceptually, and specifically for some, are not distinct as compared to non-hepatic autoimmune diseases that are seen more frequently in patients. Non-HLA associations have proven to be varied and whilst giving some direction in pathophysiology, still remain quite poorly described. In PBC for example the non-HLA associations are more distinct with clear supporting evidence for a predisposing role for the IL-12-STAT4-Th1 pathway. Such pathway signals have been less evident in PSC or AIH for example. Whilst the genetic signatures have highlighted immune regulation and antigen presentation in disease aetiopathogenesis, it has proven much harder to always directly link genetic variation with biology; particularly for the biliary autoimmune diseases, striking in its absence are genetic risk loci specifically focused on the biliary epithelium for example. Additionally because disease is a reflection of environmental triggers in genetically predisposed hosts, alongside an ultimate balance of injury against repair, to date direct therapeutic implications for genetic findings have been hard to traverse.

Nevertheless concepts raised about genetic risks factors in autoimmune liver disease, have helped, alongside other approaches, to define aspects of the jigsaw of risks that are relevant to the clinical presentation and outcome for patients living with autoimmune liver diseases.

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Epigenetics and Disease Phenotypes

The human genome, first published in 2001, revealed that 97% of the 3.2 billion base pairs do not encode gene sequences. We now know that such regions are involved in regulating gene expression and do so largely by utilizing reversible epigenetic modification of both the DNA sequences themselves and of the histone proteins that are intimately associated with the DNA. Herein we discuss several types of epigenetic modifications: DNA methylation, a covalent modification that typically occur on cytosine residues that are part of CpG dinucleotides, histone modifications, specifically acetylation of Lysine 27 of Histone H2 (H3K27ac) a modification present at loci associated with active chromatin and changes in nucleosome positioning. We will assess the associations that recently uncovered between disease outcomes and epigenetic modifications. Specifically we will review two studies. The first examines the epigenetic changes underlying the increased risk of HCC for patients that have undergone successful treatment of HCV and the second study utilizes a drug that induces demethylation to identify novel therapeutic targets that may be used to treat HCC.

Significant changes in H3K27ac have been noted after HCV infection and these changes are correlated with corresponding changes in gene expression at the RNA and protein level. It has been known that epigenetic changes persist after successful HCV treatment and it is believed that such changes may underlie the increased risk these patients have for HCC. A recent study utilized a variety of human liver samples including those without HCV infection, those with active infection and those successfully treated for HCV, to identify to identify HCV-induced epigenetic modifications[1]. To understand how such changes result in increased HCC risk, independent of other confounding factors such as fibrosis or inflammation, humanized mouse livers were produced with cells that had been infected with HCV. Intriguingly, the authors identify certain acetylation marks are associated with HCC risk, including acetylation of the SPHK1 gene as well as expression of this gene. These results suggest that high expression of this gene may be predictive of HCC formation.

Because increased DNA methylation (hypermethylation) is a common hallmark of cancer, DNA methyltransferase inhibitors (DNMTis) have been used as treatments. One mechanism underlying DMNTs role as a cancer treatment is the re-activation of tumor suppressors that are otherwise silenced by DNA hypermethylation. A recent study, designed to test the efficacy of a more stable second generation of DNMTi (Guadecitabine or SGI-110) was performed to systematically identify its direct targets in HCC[2]. These studies, performed by treating several human HCC cell lines with SGI-110, examine the transcriptional profiles as well as the methylation and nucleosome accessibility changes that occur after drug treatment. The clinical relevance of each cell line was first examined by comparing the cell line to the Cancer Genome Atlas (TGCA) HCC clinical data. The authors demonstrated that each of the cell lines has a unique methylation profile that are representative of a different subtype of primary HCC tumor based on the TGCA data. Furthermore each cell line showed similar phenotypic responses to SGI-110 treatment, including reduced proliferation. While drug treatment led to the expected hypomethylation, it produced both increases and decreases in gene expression. Genes that were activated in response to the drug included the expected tumor suppressor genes. Such genes displayed increased nucleosome accessibility and reduced methylation in the

corresponding gene's promoter. Genes that were down regulated after drug treatment included oncogenes and these genes were associated with decreased nucleosome accessibility as well increased gene body methylation. Novel effects were also noted. This includes the decreased activity of components of the polycomb repressive complex 2 (PRC2) genes via demethylation of their gene bodies and a methylation-independent reactivation of the PRC2 targets. Furthermore, the drug induces an activation of endogenous retroviruses and an enhanced immune response. Together the qualities of this drug suggest that is could be useful in treating HCC and reveal potential therapeutic targets.

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miRNA as a Biomarker of Acute Rejection in Liver Transplant

Liver transplant is the only treatment option for end-stage liver disease. Standard immunosuppression therapy (IST) involves calcineurin inhibitor (CNI)-based therapies which are iterated d at achieving and maintaining therapeutic trough levels. Clinical evidence of allograft dysfunction is usually indicated by elevated bilirubin and/or liver enzymes, but patients must undergo invasive liver biopsy to confirm the diagnosis of acute rejection. Long-term exposure to IST places transplant recipients at high risk for chronic kidney disease, new onset diabetes after transplant (NODAT), cardiovascular complications, infections, and malignancies (1). Reduction of the dose of IST to the lowest level sufficient to prevent allograft rejection in each individual would be a significant advance in the prevention of IST-related side effects. There is no current method to personalize IST on a case-by-case basis. IST minimization is currently guided by clinical monitoring with risk of developing allograft dysfunction and acute cellular rejection (ACR) with need to intensify IST when ACR is diagnosed. Therefore, minimization strategies aimed at personalized IST would greatly benefit from biomarkers that are predictive of successful minimization, and for the prediction and diagnosis of ACR.

Micro-RNAs (miRNAs) are attractive potential biomarkers for the management of IST and diagnosis of ACR. Circulating miRNAs in cell-free fractions of blood were first described in 2008 as a novel class of biomarkers for the diagnosis of cancer and other diseases. These small, non-coding RNA molecules, which are typically 22 nucleotides, function as transcriptional and post-transcriptional regulators of gene expression by acting as translational inhibitors or by degrading mRNA transcripts. Circulating miRNA are highly promising biomarkers because of their stability over time and under a variety of conditions and the ease by which they can be measured. (2-5). Altered miRNA expression has been shown to associate with inflammatory diseases and with regulation of immune responses in a number of tissues. They are observed to leak into the peripheral circulatory system from solids organs and may reflect specific patterns of injury or recovery in disease processes (6-7). Since their discovery, circulating miRNA have been reported to be sensitive biomarkers in a number of liver injury and disease states. In particular, liver-specific miR-122 has now taken a leading role as a sensitive and specific biomarker of liver injury, and has been proposed as an excellent candidate to complement the current gold standard in the diagnosis of liver injury – ALT (8). Biomarker studies in the liver transplant setting to date have proposed several miRNA signatures of hepatic injury and rejection.

We recently demonstrated that miRNA signatures that were diagnostic and prognostic of ACR and IST minimization (18). We performed miRNA profiling in 318 serum samples from 69 liver transplant recipients enrolled in the Immune Tolerance Network immunosuppression withdrawal and Clinical Trials in Transplantation (CTOT)-03 studies. We quantified serum miRNA at clinically indicated and/or protocol biopsy events (n=130). The trajectory of ACR diagnostic miRNAs during IST reduction were also evaluated in sera taken at predetermined intervals during IST minimization prior to, and at clinically indicated liver biopsy (n=119). The levels of 31 miRNAs were significantly associated with ACR diagnosis with two miRNAs differentiating ACR from non-ACR (AUC = 90%, 95%CI = 82%-96%), and predicted ACR events up to 40 days prior to biopsy proven rejection. The most differentially expressed miRNA were low in blood of healthy individuals, but highly expressed in liver tissue, and detectable in the periphery.

Integration of differentially expressed serum miRNA with concordant liver biopsy mRNA revealed pathways with known roles in transplant rejection. Analyses of miRNA and mRNA datasets from the same timepoint showed that the two predominate signatures evident in these acute rejection samples versus appropriate controls were (a) liver damage and (b) immune activation. Further statistical analyses showed that the ACR signals associated by the initial 31 miRNAs could be regressed to two miRNAs (one related to liver injury and the other related to immune activation). We also show a signature of 6 miRNA which predict which individuals in the AWISH study can successfully minimize IST.

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The Role of microRNAs in the Pathophysiology of Liver Disease

Introduction:
MicroRNA function largely depends on binding to proteins to form a ribonucleoprotein complex that then uses the microRNA sequence to add specificity to the regulation of gene expression. The RNA-induced silencing complex, RISC, binds the microRNA in a manner independent of the sequence, positioning the bases so they may anneal to an mRNA target. Binding to the target is then dependent on the sequence of the microRNA, but is also sensitive to other conditions. MicroRNAs also have RISC-independent functions, such as cell-cell signaling. RISC-mediated functions involve reduction, or in some cases silencing, of the target gene product. All of these mechanisms are active in liver health and disease.

Cell-cell signaling:
MicroRNAs can be released from cells, usually in extracellular vesicles such as exosomes or microvesicles. While unbound RNA has a circulating life span of seconds to minutes, microRNAs that are tightly bound in protein complexes or inside lipid vesicles are protected. These released and protected microRNAs can be delivered to other cells. There is strong evidence that microRNAs can be taken into cells, delivered for degradation to the lysosome, and before destruction bind in a sequence-specific manner to toll-like receptors (TLR-7/8) initiating NF-kB signaling. This signaling pathway does not require large amounts of microRNA to enter the cytoplasm of the target cell, but rather allows for a receptor-mediated function that can amplify a small amount of transferred RNA (ligand).

Canonical microRNA signaling:
For most microRNA:mRNA interactions, the result of microRNA regulation of an mRNA is a modest decrease in the mRNA level and in the expression of the cognate protein. This process is repeated for all targets of the microRNA, allowing for a complex outcome based on fine-tuning gene expression of hundreds of targets. MicroRNA functions seem to have cell-type specific effects, where the same microRNA targets a separate set of mRNAs depending on the host cell. The effect of microRNAs in liver pathophysiology can be exemplified in cholangiocarcinoma where microRNAs alter tumor cell apoptosis and migration. The microRNAs are not the initial source of disease, but mitigate or exacerbate the disease process.

MicroRNA mediated silencing:
Other microRNA:mRNA interactions lead to robust knockdown of target genes. This can occur similar to siRNA-mediated depletion. Though less common, this interaction has the potential to silence one or a few genes. Such a mechanism does not preclude having the microRNA also act as a subtle dampener of expression of other targets. Thus, there is the opportunity for the cell to use microRNAs to blunt expression of a spectrum of proteins and also silence a few. When this occurs, it is important to identify if major functional changes are through the one target, or the many. Our research has found that miR-10b has the potential to silence some targets and subdue others.

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Precision Medicine in CF

Summary Not Available at Time of Print

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Personalized Disease Models Using Induced Pluripotent Stem Cell Derived Models

The paucity of effective therapies for many liver diseases, especially those of biliary origin, is largely a consequence of a lack of in vitro systems to faithfully model liver disease. Currently available cellular and animal model systems have substantial limitations. Fresh human liver tissue is difficult to access and primary cells are difficult to isolate and maintain in culture. Cultured cell lines of hepatic origin are cancer-derived or immortalized and have limited functions and physiologic responses, as compared to primary cells. Finally, animal models often fail to recapitulate disease phenotypes. The advent of human induced pluripotent stem cell (iPSC) technology is poised to overcome these limitations and generate truly personalized models of liver disease. iPSC technology provides a platform for generating specific liver cells or liver organoids from patients with different genetic diseases, or for gene editing of normal iPSC-derived cells. This technology may be suitable for regenerative medicine in the future, but utilizing this technology for personalized disease modeling and assaying drug toxicity is a current reality.

Hepatocytes from iPSCs: Current differentiation strategies include a stepwise induction of definitive endoderm, generation of hepatic progenitor cells and final maturation to hepatocyte-like cells by addition of specific cocktails of growth factors in culture(1, 2). These protocols result in high efficiency differentiation and the production of hepatocyte-like cells that express albumin, cytochrome p450 enzymes and alpha-1-antitrypsin (A1AT), in addition to displaying some functional activities such as LDL uptake, urea metabolism, glycogen production, and inducible cytochrome P450 activity. However, some concerns remain over iPSC-derived hepatocyte maturation due to the persistent expression of AFP and lack or reduced activity of more mature cytochrome P450 isoforms. Although these concerns may limit regenerative medicine opportunities, the utilization of these cells to develop personalized disease models is very exciting (see below).

Cholangiocytes from iPSCs: Differentiation of cholangiocytes from iPSCs has been reported relatively recently, as compared to hepatocyte differentiation. Sampaziotis and colleagues used growth factors with activin and retinoic acid to induce differentiation of bipotential hepatoblasts into early cholangiocyte-like cells(3). These cells were grown in 3D conditions using matrigel to promote maturation, which resulted in the formation of cystic organoids and branching tubular structures.These structures express mature biliary markers and exhibit a range of cholangiocyte functions such as increased proliferation in response to VEGF, secretion of Rhodamine 123 via MDR1 and active export of a fluorescent bile acid. We followed a similar approach to differentiating cholangiocytes from iPSCs with the addition of a co-culture step to stimulate the Notch signaling pathway(4). Hepatoblasts were co-cultured with OP9 cells which express Jag1 and these chimeric aggregates were cultured in a mixture of collagen and matrigel and exposed to HGF, EGF and TGFβ. As with the protocol described above, the cells organized in cyst and duct structures containing a single layer of epithelial-like cells with an internal lumen and expressed cholangiocyte markers, cilia and evidence of apicobasal polarity.

Liver organoids from iPSCs: Liver organoids can be generated from iPSCs, embryonic stem cells, and adult stem cells, though iPSCs are the most accessible and amenable to scale-up

methods. The use of a 3D system allows for cell-to cell contact and better recapitulates the cellular organization of liver tissue. Takebe and colleagues generated a liver bud using iPSCs that are differentiated into hepatic endoderm(5). This monolayer of hepatic endoderm was then co-cultured with human umbilical vein endothelial cells and human mesenchymal stem cells on matrigel coated plates. Under these conditions, the cells self-assembled to generate 3D clusters described as liver buds since they resemble the human liver bud stage during development. When transplanted into NOD/SCID mice, the liver buds connected with host vessels and became vascularized, improving their maturation and human-specific metabolites were identified in the serum of the mice. This early iPSC-derived liver organoid lacked biliary structures. More recently, investigators simultaneously induced endoderm and a small amount of mesoderm resulting in concomitant differentiation of hepatic and biliary lineages and hepatobiliary organoids which have notably mature functional ability(6).

iPSC-derived Disease Models: Several groups have applied iPSC technology to model liver diseases. Rashid and colleagues generated iPSC-derived hepatocytes from patients with A1AT deficiency, familial hypercholesterolemia and Glycogen Storage Disease Type 1a(7). The investigators recapitulated key cellular aspects of the disease phenotypes such as the retention of A1AT polymers in the endoplasmic reticulum, the impaired ability to incorporate LDL, and the accumulation of intracellular glycogen, respectively. Zhang and colleagues demonstrated that iPSC-derived hepatocytes from a patient with Wilson disease had abnormal cytoplasmic localization of the ATP7B protein and defects in copper export(8). A further example of the success of this approach and one that highlights the ability to personalize disease models with this technology, is a study by Tafaleng, in which the investigators generated iPSC-hepatocytes from patients with A1ATD with and without early- onset liver disease, and compared their characteristics(9). Interestingly, iPSC-hepatocytes from A1ATD patients without liver disease were more efficient at degrading the mutant misfolded A1AT Z protein than iPSC-derived hepatocytes from A1ATD patients with significant liver disease. This suggests that this iPSC technology can be used to model individual variation in clinical presentation and progression of metabolic liver disease.

iPSC-derived cholangiocytes have also been used to model monogenic cholangiopathies. Sampaziotis used iPSC-derived organoids from a patient with polycystic liver disease (PLD) to model, in vitro, the effect of Ocreotide, which is used clinically to reduce cyst size in PLD patients(3). Both Sampaziotis and Ogawa have shown that iPSC-derived cholangiocytes from cystic fibrosis (CF) patients (ΔF508) demonstrate impaired cyst swelling and CFTR function, that can be partially corrected by the addition of clinically approved small corrector molecules(3, 4). Fiorotto et al used iPSC-derived polarized cholangiocyte monolayers to model human CF liver disease and identified aberrant innate immune activation in response to LPS that seems to be crucial to the development of liver disease, in addition to the secretory defect(10).

These successes have occurred with applying iPSC-technology to modeling monogenic liver diseases. Modeling complex liver disease remains a major challenge. It is likely that co-culture methods with different cells types derived from iPSCs from the same patient and organoid systems will be needed to model complex diseases such as primary sclerosing cholangitis.

Gene editing of iPSC-derived Liver Cells As a Therapy: Gene-editing technology such as with CRISPR/Cas9 offers the possibility to correct disease-causing mutations in iPSC-derived liver cells or organoids. iPSCs have the distinct advantage that they can be derived from a specific patient and thereby carry individual mutations and maintain the same genetic background of that individual. After gene correction the healthy isogenic cell lines can be differentiated into the specific liver cell and in the future, may be used for gene-replacement

therapies. Targeted gene correction of iPSCs from a patient with A1ATD was first reported by Yusa(11). Using zinc finger nucleases they corrected a point mutation in the AAT locus. ELISA analysis revealed the absence of mutant polymeric AAT and efficient secretion of normal monomeric AAT in the culture supernatant of corrected AATD- iPS-HLCs. Zhang et al, used a self-inactivating viral vector to correct a specific point mutation in the ATP7B gene responsible for Wilson disease and restored functional deficits in the iPSC-derived hepatocytes(8). Using CRISPR/Cas9 technology Guan et al were able to correct a JAGGED1 mutation in iPSCs derived from patients with Alagille syndrome, by reverting a mutation in Jag1, and ameliorating a biliary phenotype(12).

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Novel Therapeutics in Liver Disease

Lessons learned from the success of drug development for HCV lends optimism for building on basic science discoveries and translating those to potential therapies in other types of liver diseases. The current active areas in pharmacologic studies involve clinical trials in NASH, cholestatic liver diseases, chronic HBV infection and alcoholic hepatitis. While the vast majority of NASH clinical trials in the past focused on testing single agents, lessons learned from human and animal studies indicate that novel combination therapies that attack more than one of the key elements of NASH pathogenesis and disease progression might be more effective than the previously tested single agents. In addition, the list of new drug targets in NASH has also expanded and new drug candidates affecting metabolism (PPARa, GLP1RA, FGF19, FGF21, ACC1 and FASN), inflammation (CCR2/CCR5, ASK1, VAP1) and fibrogenesis (integrin inhibitors) are under development. In cholestatic liver diseases, agents under development in clinical trials focus on protection of cholangiocytes against bile acid toxicity through the beneficial effects of farnesoid X nuclear receptor (FXR) activation. Previous clinical trials using obeticholic acid (OCA) showed little or no benefit in PBC; studies in PSC are still ongoing. Novel therapies in cholestatic liver disease now focus on transmembrane G-protein-coupled receptor 5 (TGR5), peroxisome proliferator-activated receptor (PPAR) and pregnane X receptor (PXR) modulation. Drug development in HBV infection has become very active with direct-acting antivirals (DAAs) and host targeting agents that inhibit viral replication by modulating host cell function including immunomodulation. It has been realized that effective approaches in chronic HBV infection will likely require suppression of viral replication in combination with a host targeting agent. Alcoholic hepatitis, a neglected major liver disease, is gaining attention in therapeutic clinical trials. Based on translational research, various targets in inflammatory cascade activation such as IL-1inhibition/IL-1 receptor antagonist, agents that interfere with LPS (bovine cholostrum) or improve gut barrier function (IL-22), or augment liver regeneration (GCSF) are now in early clinical trials. A groundbreaking approach with small interfering RNA (siRNA) delivery now is FDA approved for the treatment of acute hepatic porphyria (AHP). This siRNA approach opens new opportunities in the treatment of single genetic diseases. Other means of altering genes is under investigation using adeno-associated viral (AAV) vectors for gene delivery for liver-directed gene therapy in monogenic liver diseases. Other novel approaches in the early translational phase of discovery target key elements of epigenetic modulations in disease at the level of DNA methylation and histone modification. The recent discovery of extracellular vesicles (EV) opened a new avenue for discovery for using EVs or the small EVS, exosomes, as delivery vehicles in therapeutic approaches in liver diseases given the easy uptake of EVs by hepatocytes and other liver cell types.

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Application of Genomic Data to Clinical Practice

In this talk we will review how genomic data are used in Mendelian disease diagnostics, how phenotypic data are incorporated into machine learning algorithms, and how genomic data are used in metagenomics.

Genomic data in Mendelian disease discovery and diagnostic testing. Mendelian disorders are diseases governed by a single locus and follow a specific inheritance pattern (i.e. autosomal recessive, autosomal dominant etc). Mendelian disorders are generally caused by genetic mutations that are highly penetrant: that is, if inherited, they confer an extremely high risk (100% in many cases) of disease. When a genetic variant is identified in a patient, a key question is one of causation: does this genetic variant(s) cause this patient's disease? To establish causation, a genetic testing laboratory or a disease discovery research program use databases such as ClinVar. ClinVar contains information about the relationship between a genetic variant and a phenotype. It is easily queried and frequently provides actionable diagnostic information.

Information to establish causality may be absent in ClinVar or the information may be incomplete. In this case, disease causation can be *inferred* using other genomic data and tools. These tools leverage the observation that Mendelian disease-causing genetic variants generally share several characteristics: They are a) rarely observed in human populations because of the effects of negative selection; b) they result in amino acid substitutions that are predicted to be damaging; and c) they are evolutionarily conserved across species. The Genome Aggregation Database (gnomAD) contains more than 100,000 genomic sequences and provides genetic variant frequency information in world-wide human populations (1). Software tools such as SIFT (2), PolyPhen (3, 4), and CADD (5) generate "damaging scores" based on information from amino acid substitution matrices and/or evolutionary conservation. Thus, if a genetic variant is "rare" in gnomAD and predicted to be "damaging" by SIFT, PolyPhen, and/or CADD, the genetic variant is more likely to be disease causing. Several software packages such as VAAST (6-8) and gene.iobio (9), aggregate frequency data, damaging scores, and conservation scores into a single analytical package to allow the clinician to assign causality. These tools also provide the clinical investigator a framework with which to prioritize genetic variants for experimental validation. Such software tools can provide an accurate diagnosis from millions of sequence variants in mere minutes.

Phenotype information and machine learning. A clinician's description of a phenotype is critical in interpreting a genetic test result. Some databases collate clinical information about genetic disorders into searchable tools, such as the Online Mendelian Inheritance in Man (www.omim.org). Other efforts have focused on developing structured databases (i.e. ontologies) that incorporate phenotypic information into machine learning algorithms to enhance diagnostic rates and/or to improve the rate of disease discovery (10). The Human Phenotype Ontology is one example (11), and Phevor (12) is an example of a machine learning algorithm that uses the HPO to prioritize potential disease causing variants.

Genomic data for metagenomics. Metagenomics relies on recovering all nucleic acid sequences—RNA, DNA, pathogen and host-- from a biological sample. Sequence data from these experiments are then queried against multiple databases to identify the source of the sequence and quantify the number of sequence reads. These databases contain reference sequences from bacteria, fungi, viruses and host mRNA. Analytical tools, such as Taxonomer (13), query these databases to paint a portrait of the pan-microbial community and its accompanied host response in a patient sample. While metagenomic techniques are widely used for disease discovery, diagnostic testing using metagenomic techniques are now

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POSTER PRESENTATIONS

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GENOMIC SHIFT IN LIVER INJURY/REPAIR AND FIBROSIS IN HIV PATIENTS AFTER DUAL OF CCR5/CCR2 BLOCKADE

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Background: Liver injury is common among HIV infected person with rapid progression to fibrosis and hepatocellular carcinoma. In this study, we evaluated the effect of Cenicriviroc (CCV) a dual CCR5/CCR2 blocker on the expression of markers of fibrosis and inflammation before and after 48 weeks of treatment in HIV patients infected with HIV R5-tropic virus.

Methods: All subjects were treated with either CVC (200 mg) or efavirenz (EFV) and a backbone of tenofovir/emtricitabine for 48 weeks. We evaluated seven subjects in the CVC group and six in the EFV (control) group. Expression of a group of 84 fibrosis and immunoregulatory genes was assessed using a target chip approach (RT2 Profiler PCR Array, Qiagen). Significant fold-change (up and down regulation) after Bonferroni correction was compared at baseline and following 48 weeks of exposure to the study medications.

Results: At baseline, there were no significant gene expression differences between the CVC and the EFV groups. HIV suppression was equivalent in both treatment groups. After 48 weeks of treatment, eleven genes were significantly down regulated in the CVC group compared to the control drug group. We noticed significant down regulation of PLAT, ITGAV, CAV1, IL10, CCL11, CCL3 and IL4 genes in addition to the significant down regulation of ECM modifying genes MMP1, COL1A2, and the inflammatory modulating genes IL13, IL1A. Significant up regulation of the latent transforming growth factor beta binding protein 1 (LTBP1) was also observed after CVC exposure.

Conclusion: In HIV-infected subjects treated with CVC, there was a significant shift in ECM modifying genes and inflammatory modulating genes when compared to the control drug efavirenz despite equivalent suppression of HIV. The use of CVC for 48 weeks preferentially Up-regulates the TGFB latent factor LTBP1 with associated down regulation of members of the integrin family and TGFB signaling pathway genes that implies inhibition of TGFB release and the shift in ECM remodeling due to TGFB associated liver injury and inactivation of this pro-fibrogenic pathway. The lower expression of IL10, CCL11 and CCL3 further indicates a shift towards lower T-cell migration and activation. Differential expression of these liver fibrosis regulatory mechanisms Indicate a genomic shift towards reversal of liver injury and fibrosis after CCR5/CCR2 blockade.

Disclosure: Nothing to disclose.

2
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VALIDATION OF A PROTOCOL BASED DIAGNOSTIC APPROACH FOR IN-BORN ERRORS OF METABOLISM PRESENTING AS METABOLIC LIVER DISEASE

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Background: Metabolic liver diseases are still considered as a ‘rare’ diagnosis, but scenario is changing fast with emerging awareness. With recent advances and wider availablility of newer techniques, many of these are now amenable to diagnosis and optimum management. Though the logistics involved are still beyond reach of a significant proportion of our population, a stepwise and protocol based methodological approach with simple diagnostic tests can help point towards a probable diagnosis, helping to avoid unnecessary and costly workup. This study reviews the validity of these protocol-based diagnostic approach.

Methods: All suspected cases of inborn error of metabolism were diagnosed, on the basis of established protocols being followed in the department. These patients were than subjected to clinical exome study, TATA box sequence for Gilbert and suspected Wilson Disease cases were screened for 5 common mutations. In order to validate the protocols, results were than correlated with protocol based diagnosis.

Results: This study included 329 children: Progressive familial intrahepatic cholestasis suspected in 21, 12 cases confirmed, no known mutation could be identified in 6 patients, 1 as cystic fibrosis, 1 as hereditary hemmorhagic telengiectasia and 1 as infantile CPT II deficiency. Two suspected to have bile acid synthetic defect and confirmed on. All 13 cases of suspected Glycogen storage disease confirmed. One case of galactossemia and 3 cases of tyrosinemia also confirmed. Among 3 cases of suspected lysosomal storage disease, diagnosis matched in 1 case of Cholestryl ester storage disorder and 1 case of Niemann pick disease, while the third case suspected of having Gaucher’s was found to be having mutation of unknown significance. Heriditery fructose intolerance was confirmed in 3 of the 5 suspected cases. Three cases of PILBD turned out to be Alagille syndrome. Fifty eight cases out of 85 suspected cases of Wilson disease studied were found to be harbouring at least one of the 5 common mutations studied. In 3 cases diagnosis was uncertain and so were the mutations of unknown significance. Crigler Najjar syndrome was suspected in 2 cases however mutation did not match. A total of 195 pediatric subjects with unconjugated hyperbilirubinemia were investigated for probable GS during 2011-2018. Of these, 170 subjects confirmed as having GS on TATA box analysis. Of the 329 suspected cases of MLD as analysed by the protocols, 265 were confirmed to have the same diagnosis. As can be seen in the table, the sensitivity, specificity, PPV, NPV and diagnostic accuracy of protocol for non Wilsonian MLD as evaluated by Exome sequencing is 100%, 50%, 68.52%, 100%, 76.06% repectively. The overall sensitivity, specificity, PPV, NPV and diagnostic accuracy of protocols are 100% (95%CI 98.62-100), 58%, (95%CI 41.04-58.9), 80.55% (95%CI 77.69-83.12), 100% and 83.72%(95%CI 79.69-87.2)

respectively.

Conclusion: The protocol based diagnostic approach are good screening test with a sensitivity of 100%, specificity of 58 % and a diagnostic accuracy of 83.72%. Hence to avoid work up and economic burden on the patient's, we should use diagnostic algorithmic approach for indicator of a diagnosis, which can be confirmed on genetic analyses

Table : Evaluation of the protocol based diagnostic test

Evaluation	Exome sequencing for non Wilsonian MLD	Common Mutations for Wilson Disease	TATA box for Gilbert disease	All 3 types of genetic analysis
Sensitivity	100% (95%CI 90.5-100)	100% (95%CI 93.84-100)	100% (95%CI97.8-100)	100% (95%CI 98.62-100)
Specificity	50% (95%CI 32.43-67.57)	50% (95%CI 36.08-63.92)	50% (95%CI 35.5-64.47)	58% (95%CI 41.04-58.9)
PPV	68.52%(95%CI 60.86-75.28)	68.24%(95%CI 62.2-73.72)	87.18%(95%CI 83.75-89.97)	80.55%(95%CI 77.69-83.12)
NPV	100%	100%	100%	100%
Diagnostic Accuracy	76.06% (95%CI64.46-85.39)	75.89% (95%CI 66.90-83.47)	88.64% (95%CI 83.68-92.51)	83.72% (95%CI 79.69-87.2)

Disclosure: Nothing to disclose.

3

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A TRUNCATING MUTATION OF TJP2 IN HUMAN HEPATOCYTES DERIVED FROM INDUCED PLURIPOTENT STEM CELLS REDUCES BARRIER FUNCTION AND ALTERS CELLULAR POLARITY

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Institution(s): (1)Cincinnati Children's Hospital Medical Center

Background: Progressive Familial Intrahepatic Cholestasis (PFIC) is a category of neonatal liver disease caused by genomic mutations in genes related to bile flow. Homozygous mutations in TJP2, which encodes a tight junction scaffold protein, have been recognized to cause PFIC type 4.1,2 The molecular mechanisms responsible for liver injury in patients with PFIC4 remain largely unknown. We developed a human induced pluripotent stem cell (iPSCs)-based disease modeling system to investigate the impact of TJP2 deficiency on hepatocellular function. We hypothesized that induced hepatocytes from iPSCs carrying TJP2 mutations recapitulate the pathologic mechanisms, including reduced barrier function and irregular cellular polarity.

Methods: We generated iPSCs from a patient with PFIC4 (iPSCPFIC4), who carries a homozygous truncating mutation, and from healthy donors (iPSCNL) by an episomal transfection of Yamanaka factors into peripheral blood cells. We also edited the genome of iPSCNL with CRISPR/Cas9 to generate isogenic iPSCs carrying homozygous truncating mutations of TJP2 gene (iPSCTJP2-KO). We induced hepatic differentiation into iPSCs by sequential stimulation of the cells with ActivinA, Wnt3a, BMP4, FGF2, and HGF on the Transwell membrane with upper and lower chambers. The morphological features of the induced hepatocytes (iHep) were analyzed via transmission electron microscopy and immunofluorescent staining. Hepatocytic excretory function was determined by albumin secretion measured by ELISA, bile acid transport by tracking bile acids from the lower to the upper chamber. Barrier function was determined by a fluorescent labelled dextrose “leak” assay. Hepatocyte injury was detected by an ATP-based cellular viability assay.

Results: A truncating homozygotic mutation (H164Pfs*19) of TJP2 was introduced into iPSCs with CRISPR editing, followed by confirmation of Sanger sequencing. Each iPSC line (iPSCNL, iPSCPFIC4, iPSCTJP2-KO) differentiated into iHep (iHepNL, iHepPFIC4, iHepTJP2-KO). Morphology of iHepPFIC4 and iHepTJP2-KO showed features similar to those observed in liver tissue of patients with PFIC4. Transmission electron microscopy revealed diminished microvilli on the apical membrane and broad desmosome structure at the cell-cell junction. By immunostaining, iHepNL were noted to express ZO1 at the juxtaposition of the apical and lateral membrane and BSEP on the apical membrane. In contrast, iHepPFIC4 and iHepTJP2-KO retained ZO1 on the middle of lateral membrane and BSEP expression was diminished. F-actin accumulated on the apical membrane in iHepNL, but was irregularly distributed in iHepPFIC4

and iHepTJP2-KO. The secretion rates of albumin into culture media was comparable. When exposed to taurocholate (TCA), iHepNL transported the bile acid from the lower to upper chamber in 48 h, while iHepTJP2-KO did not. When cultured with TCA, iHepTJP2-KO exhibited a significant leak of dextrose conjugated fluorescent probes from one chamber to the other. TCA exposure at high concentration (500microM) for 24 h reduced cell viability of iHepTJP2-KO compared to iHepNL.

Conclusion: A truncating mutation of TJP2 induced irregular cellular polarity and reduced barrier function, resulting in increased susceptibility to cell injury when exposed to high-level bile acids.

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Disclosure: Nothing to disclose.

4
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RARE VARIANTS IN GENES THAT REGULATE BILE ACID HOMEOSTASIS IN A PAEDIATRIC NAFLD COHORT

Authors: Anil Dhawan; Emer Fitzpatrick; Pierre Foskett; Sandra Strautnieks(1); Teresa Brunetti; Richard Thompson(2)

Institution(s): (1)King's College Hospital; (2)King's College London

Background: Bile acids (BA) are steroid molecules synthesized in the liver from cholesterol via two different enzymatic pathways. Recently, the role of BA within glucose and lipid metabolism has been investigated, with a particular interest in the pathophysiology of non-alcoholic fatty liver disease (NAFLD). BA homeostasis is largely regulated by the farnesoid X receptor (FXR) which, once activated, suppresses BA synthesis and uptake by hepatocytes and also reduces hepatic lipogenesis. Susceptibility to development of NAFLD is complex and undoubtedly mediated, to a large extent, by environmental factors. The genetic predisposition in NAFLD is also now well recognized. We hypothesized that genetic variants in the BA metabolic pathway may be present in children with NAFLD.

Methods: Our cohort is represented by children with a biopsy-proven diagnosis of NAFLD with no coexistence of other liver diseases. Data and blood samples were collected using the King's Paediatric Liver Biobank. Liver biopsies were scored by a consultant hepatohistopathologist according to Brunt/Kleiner criteria. A next generation sequencing (NGS) custom panel of 135 genes was designed with online Agilent SureDesign tool, based on literature reviews and pathway recognition: 22 of these genes are involved in the BA transport and metabolism. Agilent SureSelect QXT kit was used for the generation of the library and the preparation of the samples.

Results: DNA was extracted from blood samples of 100 children (Male=62; Female=38) with liver fibrosis at different stages. Median age was 13 years (11, 14) with a median BMI z-score of 1.97 (1.59, 2.25). Overall 878 variants were found across the cohort. Among 591 missense variants, 106 missense variants were found within the BA genes. 8 rare variants with a very low minor allele frequency (MAF) in the general population were found in CYP8B1 (rs563690413; MAF: 0.00002 and rs372472190; MAF: 0.00014), FGFR4 (rs569265847; MAF: 0.00007), ABCA1 (rs138056193; MAF: 0.00006), ABCC2 (rs186620377; MAF: 0.00006), NR1H4 (rs747025458; MAF: 0.00001), ABCC4(rs150633056; MAF: 0.000004) and HNF4A (rs768495780; MAF: 0.000008). The functional effect of these missense variants was evaluated using in silico software (Polyphen-2; CADD score). With a CADD score above 20, the variants rs563690413 in CYP8B1, rs569265847 in FGFR4, rs186620377 in ABCC2, rs150633056 in ABCC4 and rs768495780 in HNF4A showed a possible damaging effect on the function of the corresponding protein. The MAF in our population was 0.49 (28% homozygous) for the variant rs738409 in PNPLA3 (MAF general population: 0.27), 0.48 (24% homozygous) for the variant rs1260326 in GCKR (MAF general population: 0.63) and 0.22 (6% homozygous) for

the variant rs58542926 in TM6SF2 (MAF general population: 0.06).

Conclusion: Eight genetic variants in BA genes have been found in a cohort of children with severe NAFLD. Further studies, through assessment of mRNA and protein expression and in vitro models, are needed to validate the function of the variants in early onset severe NAFLD. The understanding of the genetic contribution of BA genes to NAFLD might lead to a personalised approach to the treatment of patients.

Disclosure: Nothing to disclose.

5

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EXPLORING THE METABOLIC CONSEQUENCES OF CARBAMOYL PHOSPHATE SYNTHASE I DOWNREGULATION IN HEPATOCELLULAR CARCINOMA THROUGH NETWORK RECONSTRUCTION

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Institution(s): (1)Imperial College London

Background: Hepatocellular carcinoma (HCC) is the fourth highest cause of cancer-related mortality. HCC is associated with various genetic alterations, some involved in metabolic reprogramming. Carbamoyl Phosphate Synthase I (CPS1) downregulation is known to play a role in hepatocarcinogenesis through an increased glutamine availability for de novo pyrimidine biosynthesis. This study aimed to construct a network combining information from the Kyoto Encyclopedia of Genes and Genomes (KEGG) database, Human Metabolome Database (HMDB) and Human Protein Atlas (HPA) to carry out metabonomic analyses to confirm the role of CPS1 in HCC.

Methods: KEGG, HMDB and HPA were used to reconstruct a network of relevant pathways, demonstrating the relationships between genes and metabolites using the MetaboSignal package in R. The concentrations of metabolites displayed within the network that were most confidently matched to features from liquid chromatography-mass spectrometry (LC-MS) data from the UK HCC Biomarker Study, which consisted of 198 participants, compared between HCC (n= 114), cirrhosis (n=30) and healthy control (n=54) cohorts using Wilcoxon's Rank Sum Tests.

Results: Following extensive filtering, a network of 18 metabolites, 28 metabolic genes and 1 signalling gene was visualised. Seven features from four LC-MSdatasets were matched to metabolites found on the network at varying confidence levels. No statistically significant differences were observed for any feature between the healthy controls and the HCC cohort (p-values ranging from 0.219 to 0.702) or between the cirrhosis and the HCC cohorts (p-values ranging from 0.224 to 0.996).

Conclusion: Information from different databases was collated for the first time to form an informative network that integrated different '-omics' approaches. Despite the lack of statistically significant findings between the healthy control, cirrhosis and HCC cohorts for the metabolites tested, the study paved the way for further research by acting as a template to investigate the relationships between genes and metabolites, explore their potential roles in various diseases and aid the development of new screening and treatment methods through network reconstruction.

Reference(s):
Ally A, Balasundaram M, Carlsen R, Chuah E, Clarke A, Dhalla N, et al. Comprehensive and Integrative Genomic Characterization of Hepatocellular Carcinoma. Cell. 2017; 169 (7): 1327-1341.e23. Available from: http://dx.doi.org/10.1016/j.cell.2017.05.046

Disclosure: Nothing to disclose.

6

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A SPECTRUM OF LATE ONSET LIVER DISEASE IN PATIENTS WITH VARIANTS IN ABCB4 GENE

Authors: Pierre Foskett; Deepak Joshi; Sandra Strautnieks; Richard Thompson(1); Jeremy Nayagam(2)

Institution(s): (1)Institute of Liver Studies; (2)King's College Hospital

Background: There has been a dramatic increase in our understanding of the genetics underlying hereditary cholestatic disorders. Through work on patients with progressive familial intrahepatic cholestasis (PFIC), a variant in genes vital to the transport of bile acids and stability of the canalicular membrane has been identified in most PFIC patients. Variants in the ABCB4 gene, which encodes the major phospholipid transporter (MDR3), is associated with PFIC 3, where patients typically present in childhood, with severe protein deficiency and complete loss of function from both alleles. A smaller cohort of patients with late onset disease and variants in ABCB4 have been described, in most cases the variants are predicted to be less damaging or patients are heterozygous. Our aim was to characterise patients with late onset disease with variants in ABCB4, including detailed clinical phenotype and genotype.

Methods: A prospectively assembled database of patients who underwent sequencing of cholestasis related genes from 1/1/14-31/12/18 was used. Patients with variants in ABCB4 and aged >18 years were included, those with onset of liver disease <18 or tested as family screening were excluded. Clinical details were collected from medical records. Next Generation Sequencing of targeted genes was undertaken using Illumina MiSeq machines.

Results: 338 patients were tested and variants in *ABCB4* were identified in 44 (13%). For those with variants: median age at onset was 33 years, 66% were female. Clinical phenotypes included: chronic cholestasis in 73%; pregnancy related liver dysfunction in 52%; gallstones in 39%; recurrent cholestasis in 7%; and drug induced liver injury in 2%. 8 patients (20%) also had a clinical label of another cause of liver disease.
The allele count for *ABCB4* was 62 (allele frequency in tested population 9.2%). 7 patients were homozygous for *ABCB4*, 10 had two or more variants, 21 were heterozygous. 17 patients had nonsense variants, 41 exonic variants and 7 intronic variants. 15 patients had variants in the region encoding ABC transporter.
4 patients have undergone liver transplantation: 3 had a nonsense and a missense variant; 1 was homozygous for a missense variant.

Conclusion: We report a cohort with a spectrum of late onset liver disease and variants in ABCB4 gene. The majority were heterozygous for variants and it is unlikely that these variants are behaving like true recessive mendelian inheritance alleles. The genetic variants are unlikely to fully explain the development of liver disease and it is predicted that reduced protein levels manifest as a slower progression of liver disease or a predisposition to further hepatic insult. It appears that the degree of protein loss is important to clinical presentation. Although further work in the field is required, testing ABCB4 provides valuable information in late onset disease.

Disclosure: Nothing to disclose.

7

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DELETION OF GSTM1 IS ASSOCIATED WITH INCREASED INJURY IN NAFLD

Authors: Joon-Yong Cung; Stephen Hewitt; David E. Kleiner; Kris Ylaya(1); Amy Huang; Nevitt Morris; Maren Podszun; Yaron Rotman; Kristin Valdez(2)

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Background: Oxidative stress is thought to play an important role in the transition of non-alcoholic fatty liver disease (NAFLD) from steatosis to steatohepatitis (NASH). In the liver, oxidative stress is mitigated by antioxidant enzymes, including several glutathione S-transferases (GSTs). GSTM1 belongs to the mu class of GSTs and plays a role in the detoxification of electrophilic compounds and products of oxidative stress. A structural deletion of the GSTM1 gene is common (as high as 50% in Caucasians) and the resulting null genotype (GSTM1-null) leads to a complete loss of function. It was suggested that GSTM1-null associates with higher NAFLD risk but its impact on NAFLD severity and in vivo hepatic oxidative stress is unknown. We aimed to determine the hepatic histological, transcriptomic and oxidative stress features associated with GSTM1-null.

Methods: GSTM1 was genotyped in a cohort of adult NAFLD patients (n=21) using a TaqMan copy number assay. Liver biopsies were scored using the NASH-CRN scoring system and stained for 4-hydroxynonenal (4-HNE, n=21) as a marker of lipid peroxidation using a cell-based calibrator. RNA sequencing was performed on liver samples (n=19). Mallory bodies were assessed on ubiquitin immunostains and scored semiquantitatively from 0 to 4.

Results: The genotypic frequency of GSTM1-null was 48%. Patients with GSTM1-null had no difference in hepatic steatosis but had higher scores for hepatocyte ballooning (0.4 vs 1.0, p=0.02), Mallory bodies (0.45 vs 1.9, p=0.02) and a higher NAFLD Activity Score (3.0 vs 4.2, p=0.04) than patients with the intact gene. Quantitative 4-HNE staining showed numerically higher results (p=0.17) in patients with GSTM1-null. RNA Sequencing revealed a significant (FDR q<0.1) 9.5-fold increase of CHIT1 and 2.9-fold increase of TREM2 expression in GSTM1-null patients; both genes have been previously associated with hepatic inflammation and injury. We further observed a 5-fold increase in ALDH3A1, which is known to detoxify 4-HNE.

Conclusion: In this pilot study, a GSTM1-null genotype was associated with increased histological hepatocyte injury and a consistent gene expression signature. The upregulation of ALDH3A1 is likely a compensatory mechanism for the increased oxidative stress and may be responsible for attenuating the rise in 4-HNE adducts. Overall, our data suggests a protective role for GSTM1 in patients with NAFLD.

Disclosure: Nothing to disclose.

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EFFECTS OF CYP2B6 ALLELIC VARIANTS, INFECTION STATUS, LIVER FIBROSIS AND STEATOSIS ON METHADONE PHARMACOKINETICS

Authors: Evan Kharasch(1); Lawrence Brown; Anthony McLeod(2); Lindsay Chakan; Arpan Dharia; Yuxin Ding; Marianthi Markatou; Gene Morse; Andrew Talal(3); Charles Venuto(4)

Institution(s): (1)Duke University Medical Center; (2)START Treatment & Recovery Centers; (3)University at Buffalo; (4)University of Rochester

Background: Methadone is highly effective for treatment of opioid use disorder (OUD) and pain management. However, the narrow therapeutic index and inter-individual variability in disposition create dosing challenges. While overdose can lead to toxicity and death, sub-therapeutic doses can potentiate withdrawal. Recent data establish that CYP2B6 is the enzyme responsible for methadone metabolism to 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) instead of CYP3A4. CYP2B6 alleles encode variant enzymes with loss-of-function (LOF) (CYP2B6*6, CYP2B6*16, CYP2B6*18) and gain-of-function (CYP2B6*4) compared to wild-type
(CYP2B6*1). Methadone metabolism by the variant gene product CYP2B6.6 is less than, while CYP2B6.4 is greater than, that by CYP2B6.1. The frequency of CYP2B6 alleles in a population consisting largely of African-Americans (AA), many of whom are also infected with HIV and/or hepatitis C virus (HCV), and influence on methadone disposition, has not been evaluated.

Methods: Pre-dose (trough) plasma was collected from 96 adults on stable daily, oral methadone for OUD. We measured (R)- and (S)-methadone and (R)- and (S)-EDDP concentrations as well as methadone metabolism-(logarithm of (R) and (S)-EDDP/methadone concentration ratio). We performed transient elastography to assess hepatic fibrosis and steatosis, and assessed the CYP2B6 alleles principally responsible for methadone metabolism. We utilized a multivariate linear mixed effects model to analyze the effects of multiple predictors (sex, body mass index (BMI), CYP2B6 genotype, concomitant medication) on plasma methadone metabolism. Significance was set at $p<0.05$.

Results: Participants were largely male (58%), minority (61% AA, 28% Caucasian) and non-Hispanic (68%). 41% were HCV mono-infected, 41% were uninfected, and 18% were HIV/HCV co-infected. Modeling results reveal that female has a significant effect ($p=0.035$) on (R)-methadone metabolism but has borderline effect on (S)-methadone metabolism ($p=0.071$). LOF alleles are highly significant on (S)-methadone metabolism ($p=0.012$) and have borderline effect on (R)-methadone metabolism ($p=0.066$). BMI also has borderline significant effect ($p=0.084$) on (R)-methadone metabolism. Methadone metabolism appears to be decreased in males and individuals with LOF alleles. Liver stiffness has no significant effect on methadone metabolism.

Conclusion: In the age of precision medicine, genetic analysis is essential to delivering individualized treatments. It is crucial to include minority populations in genetic studies as some alleles are race specific. Our results suggest that sex and CYP2B6 genotype should be incorporated into multivariate models, along with other predictors (e.g. BMI), to create dosing algorithms. The development of a methadone dosing algorithm should facilitate methadone delivery as well as improve patient satisfaction with methadone prescription and prevent overdose.

Demographics

	HCV-mono (n= 39)	HIV/HCV (n=18)	Uninfected (n=39)	Total (n=96)
Sex				
Male	28 (72%)	11 (61%)	17(44%)	56 (58%)
Female	11 (28%)	7 (39%)	22 (56%)	40 (42%)
Race				
Black or African American	22 (56%)	9 (50%)	28 (72%)	59 (61%)
Caucasian	13 (33%)	8 (44%)	6 (15%)	27 (28%)
Others	4 (11%)	1 (6%)	5 (13%)	10 (11%)
Ethnicity				
Non-Hispanic or Latino	24 (62%)	10 (56%)	31 (79%)	65 (68%)
Hispanic or Latino	15 (38%)	8 (44%)	8 (21%)	31 (32%)
Age, years (Median, IQR)	58 (13)	60.5 (13)	53 (11)	56 (14)
BMI, kg/m^2 (Median, IQR)	26 (7)	23 (3)	29 (9)	25 (8)

Abbreviation: IQR-interquartile range; BMI-body mass index; HCV-hepatitis C virus;

Disclosure: Nothing to disclose.

9
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IDENTIFICATION OF ABCC12 AS A NOVEL CAUSATIVE GENE IN PROGRESSIVE FAMILIAL INTRAHEPATIC CHOLESTASIS AND BILE DUCT PAUCITY

Authors: Jillian Ellis; Ammar Husami; Alexander Miethke; Mary Mullen; Duc-Hung Pham; Alexander Valencia; Lingfen Xu; Chunyue Yin; Kejian Zhang(1); Kimberley Evason(2)

Institution(s): (1)Cincinnati Children's Hospital Medical Center; (2)University of Utah

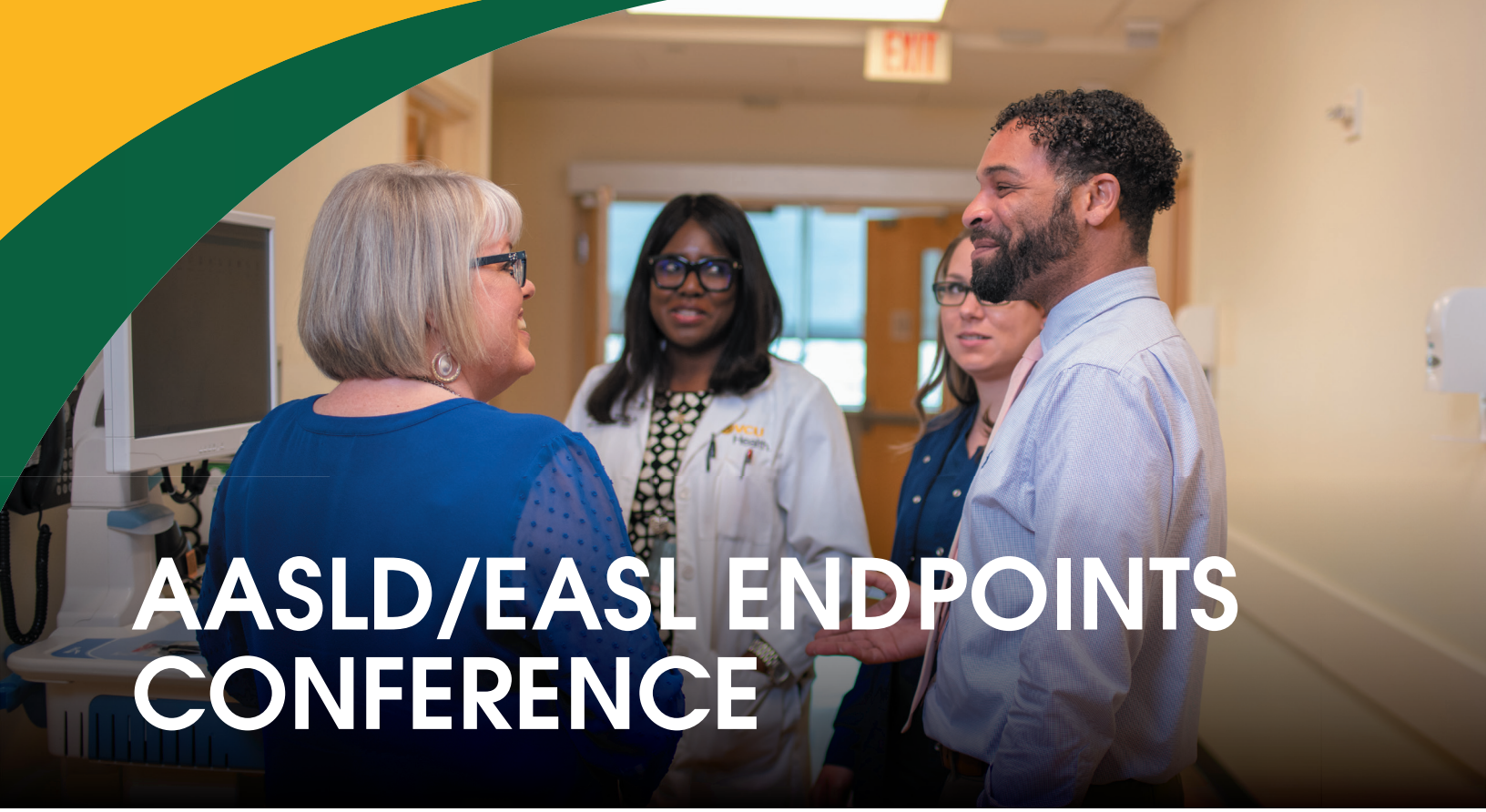
Background: Progressive familial intrahepatic cholestasis (PFIC) is a group of autosomal-recessive disorders affecting up to 1/50,000 newborns. The genetic basis for 30% of PFIC disorders has been assigned to mutations in genes encoding canalicular transporters involved in bile formation. We hypothesize that next-generation sequencing of DNA from children with low GGT cholestasis who lack mutations in known PFIC genes will identify novel causative genes for PFIC.

Methods: We sequenced 91 children with idiopathic low GGT chronic cholestasis of which 50 were enrolled into the observation Childhood Liver Disease and Education Network (ChiLDReN). Consequences of global deletion of the candidate gene were studied in zebrafish and C57BL6 mice subjected to CRISPR/Cas9 gene editing.

Results: We identified one patient with bi-allelic truncating mutations in the gene ABCC12 encoding the ATP-binding cassette (ABC) transporter MRP9, and three additional patients with compound heterozygous mutations in the same gene. Immunohistochemistry and western blot revealed conserved expression of ABCC12 protein in the cholangiocytes in human, mouse, and zebrafish. ABCC12 deficiency was associated with progressive bile duct paucity and accompanied by elevation in serum biomarkers of hepatocellular injury (ALT) and cholestasis (bile acid/ALP levels) in both model organisms. Studies in zebrafish mutants revealed that the ductopenic phenotype was due to cholangiocyte death, rather than impaired proliferation or switch of cholangiocyte fate. Lack of ABCC12 in mouse cholangiocytes conferred susceptibility to bile acid induced liver injury. Challenge with 1% cholic acid admixed to the chow for 7 days resulted in increased bile duct proliferation (as assessed by image analysis of CK19+ area) and higher serum total bilirubin levels in Abcc12-/- mice compared with age and gender matched wild type mice.

Conclusion: Our work connects ABCC12 with PFIC for the first time, thus providing a proof of concept for addressing unmet needs in the fields of pediatric hepatology and genomic research: the discovery of novel genes associated with chronic cholestasis syndromes using massive parallel sequencing technology, and the validation of their biological function in model organisms. Furthermore, it identifies a potential therapeutic target to attenuate bile acid induced cholangiocyte injury.

Disclosure: Nothing to disclose.



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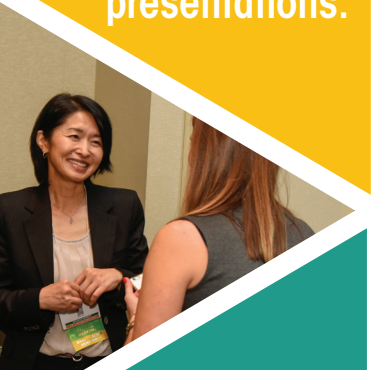


Networking.

Highlights of The Liver Meeting® 2019 include:

- **Trainee Day** on **Friday, November 8**
- **Postgraduate Course** on **Saturday, November 9:** Precision Hepatology in Clinical Practice
- **Basic Science Symposium** on **Saturday, November 9:** Current Trends in Liver Regeneration and Repair
- **Associates Day** on **Sunday, November 10**
- **SIG Programming:** Programs sponsored by our SIGs to drill down on highly specialized topics in hepatology.

July 17	**Advance Registration for AASLD Fellow Members**
July 24	**Advance Registration for AASLD Members**
July 31	**General Advance Registration**
October 11	**Advance Registration Deadline**

Register Online at: **aasld.org/livermeeting**

The Liver Meeting® will offer many opportunities to claim CME, CE and MOC this year. ***Stay tuned for more information on continuing education.***

AASLD
THE LIVER MEETING®
NOVEMBER 8-12 2019 BOSTON

2019 Postgraduate Course

Precision Hepatology in Clinical Practice

Saturday, November 9 | 8 am-5 pm

Hepatology practice is undergoing major changes fueled by the discovery of disease biomarkers and digital strategies to stage and monitor clinical courses. Once glimpses of a possible future, minimally invasive tests and electronic resources are increasingly available in the clinic. In this Postgraduate Course, experts review how these advances improve care protocols and allow for precise, even customized clinical approaches.

Program Chairs

Jorge A. Bezerra
MD, FAASLD

Andrew P. Keaveny
MD, FRCPI, FAASLD

Rebecca G. Wells
MD

▶ Learn more at **aasld.org/PGcourse**

AASLD
THE LIVER MEETING®
NOVEMBER 8-12 2019 BOSTON

2019 Basic Science Symposium

Current Trends in Liver Regeneration and Repair

Saturday, November 9 | 8 am–5 pm

This liver is constantly exposed to external and internal damaging factors, yet it can regenerate and repair itself. With the help of novel tools, we now know more about the mechanisms of this process. At this symposium, experts report new research on liver regeneration and repair and how this knowledge may facilitate clinical applications to rescue and restore liver function.

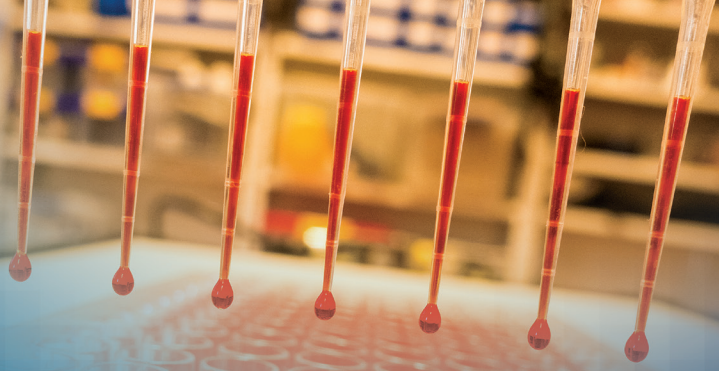
Program Chairs

Xiao-Ming Yin
MD, PhD, FAASLD

George K. Michalopoulos
PhD, FAASLD

▶ Learn more at **aasld.org/BSS**

AASLD FOUNDATION | KNOWLEDGE PRACTICE CURE®



CALL FOR APPLICATIONS

2020 Research & Career Development Awards

AASLD Foundation is investing **even more** in **innovative hepatology research** and in the **people who study and treat liver disease.**

Funding available for:

- Early to mid-career investigators pursuing liver-related research.
- Front-line providers seeking hepatology-focused training to adequately treat and care for liver disease patients.
- Travel to educational meetings, including the Abstract Travel Awards and the Emerging Liver Scholar Resident Travel Awards.*

*These awards have different deadlines. To find out more and to apply, refer to the "Travel Awards" section at **aasldfoundation.org/awards**

Application Deadlines

Research Awards:
DECEMBER 4, 2019
(for funding beginning July 1, 2020)

Bridge Award:
CYCLE 1: OCTOBER 5, 2019
(for funding beginning February 1, 2020)
CYCLE 2: JANUARY 15, 2020
(for funding beginning May 1, 2020)

Advanced/Transplant Hepatology Award and NP/PA Clinical Hepatology Fellowship:
JANUARY 15, 2020
(for funding beginning July 1, 2020)

Questions? Contact awards@aasld.org

SPECIAL FUNDING ANNOUNCEMENT

Additional funding is available for research in
Autoimmune Liver Diseases
See **aasldfoundation.org/autoimmunefunding** for eligible categories and restrictions.

LEARN MORE AND APPLY AT
aasldfoundation.org/awards

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