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Identification of *PKD1L1* Gene Variants in Children with the Biliary Atresia Splenic Malformation Syndrome

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Abstract

Biliary atresia (BA) is the most common cause of end-stage liver disease in children and the primary indication for pediatric liver transplantation, yet underlying etiologies remain unknown. Approximately 10% of infants affected by BA exhibit various laterality defects (heterotaxy) including splenic abnormalities and complex cardiac malformations — a distinctive subgroup commonly referred to as the biliary atresia splenic malformation (BASM) syndrome. We hypothesized that genetic factors linking laterality features with the etiopathogenesis of BA in BASM patients could be identified through whole exome sequencing (WES) of an affected cohort. DNA specimens from 67 BASM subjects, including 58 patient-parent trios, from the NIDDK-supported Childhood Liver Disease Research Network (ChiLDReN) underwent WES. Candidate gene variants derived from a pre-specified set of 2,016 genes associated with ciliary dysgenesis and/or dysfunction or cholestasis were prioritized according to pathogenicity, population frequency, and mode of inheritance. Five BASM subjects harbored rare and potentially deleterious bi-allelic variants in polycystin 1-like 1, *PKD1L1,* a gene associated with ciliary calcium signaling and embryonic laterality determination in fish, mice and humans. Heterozygous *PKD1L1* variants were found in 3 additional subjects. Immunohistochemical analysis of liver from the one BASM subject available revealed decreased PKD1L1 expression in bile duct epithelium when compared to normal livers and livers affected by other non-cholestatic diseases.

Conclusion: WES identified bi-allelic and heterozygous *PKD1L1* variants of interest in 8 BASM subjects from the ChiLDReN dataset. The dual roles for PKD1L1 in laterality determination and ciliary function suggest that *PKD1L1* is a new, biologically plausible, cholangiocyte-expressed candidate gene for the BASM syndrome.

Keywords

Neonatal Cholestasis; Whole Exome Sequencing; Cilia; Laterality; Cholangiocyte

Introduction

Biliary atresia (BA) is a severe neonatal cholangiopathy characterized by progressive fibroinflammatory obliteration of both extra- and intra-hepatic bile ducts, generally leading to cholestasis, portal fibrosis, and, ultimately, biliary cirrhosis. Among children, BA is the most common cause of end-stage liver disease worldwide and the primary indication for liver transplantation, yet its etiology (or etiologies) remains unknown (1–4). Hypotheses regarding the pathogenesis of BA include perinatal viral infections or toxins targeting cholangiocytes, chronic inflammatory or autoimmune-mediated bile duct injury, and mutations in specific genes that regulate hepatobiliary development (1). In the biliary atresia splenic malformation (BASM) syndrome, which accounts for ~10% of infants affected with BA, the coexistence of one or more major congenital malformations within a wide spectrum of laterality defects (heterotaxy) along with abnormal biliary tract development suggests a role for genetic contributions to the etiology of the BASM syndrome (5–7).

A developmental cause of BA was first proposed in an early case collection (8) while support for a potential genetic contribution comes not only from pre- and perinatal evidence of biliary tract dysgenesis in BA (9), but also from several familial case reports of BA (10). However, given the general lack of heritability of BA within families and reports of twin discordance (11–13), recent studies have explored non-genetic etiologies underlying BA, including viral, toxin or immune-mediated mechanisms (2, 14). Nevertheless, the genetic determinants of heterotaxy syndromes often involve mutations in genes essential for ciliary structure or function (15, 16), providing support to re-focus efforts exploring potential roles for variants in key ciliary genes in the pathogenesis of the BASM syndrome (6, 7).

A growing list of genes involved in bile duct development and function have been implicated in the pathogenesis of biliary tract diseases including BA, several of which cause structural and functional alterations in primary cilia (17, 18). Non-motile primary cilia are present in many cell types including the apical surface of biliary epithelium (19, 20). Acting as cellular antennae that sense a wide variety of molecules in the extracellular environment, primary cilia play an integral role in the regulation and transmission of downstream intracellular signaling pathways (15). Moreover, the establishment of normal L-R patterning in vertebrate embryogenesis is dependent on both sensory and motile ciliary functions (15, 16). Support for the potential contribution of ciliary dysfunction in BA comes from several sources: (1) cholangiocyte cilia in BA livers are significantly shorter, abnormal in their orientation, and less abundant compared with other cholestatic disorders and normal livers; (2) mutations in a small number of ciliopathy and laterality genes, including *CFC1*, *NODAL*, and *ZIC3,* have been suggested as a possible etiology for BA in some patients; and (3) several well-characterized mutations in ciliopathy genes lead to developmental biliary tract diseases known as cholangiociliopathies — Caroli syndrome, ductal plate malformations, congenital hepatic fibrosis and polycystic liver diseases (21–25).

Given the central role of cilia in critical developmental pathways of hepatobiliary biogenesis as well as L-R determination during embryogenesis leading to heterotaxy, we hypothesized that ciliary dysgenesis and/or dysfunction may be involved in the pathogenesis of the BASM syndrome. To explore this hypothesis, we performed whole exome sequencing (WES) in carefully phenotyped BASM subjects enrolled in a large multicenter longitudinal North American study of pediatric cholestatic liver diseases, focusing our analysis on a subset of 2,016 genes related primarily to ciliary structure and function.

Materials and Methods

Subject Selection.

Participants enrolled between May 2004 – August 2016 in either of two prospective observational cohort studies (A Prospective Database of Infants with Cholestasis [PROBE; NCT00061828] or Biliary Atresia Study in Infants and Children [BASIC; NCT00345553]) within the NIDDK-supported Childhood Liver Disease Research Network (ChiLDReN, http://childrennetwork.org) were eligible for inclusion. At the time of selection, 2,001 BA participants, of which 1,488 had DNA available for genetic analyses, including 618 complete child-parent trios, were enrolled in ChiLDReN consortia across 18 clinical sites in the United States and Canada. The diagnosis of BA in each participant was confirmed by review of pertinent diagnostic liver biopsy, radiologic and surgical reports. BA participants for whom DNA was available in the ChiLDReN biorepository, and with at least one reported laterality defect (i.e., splenic abnormalities, intestinal malrotation, abdominal heterotaxy, vascular abnormalities, or congenital heart disease), as defined by the National Birth Defects Prevention Study, or cystic renal dysplasia, were included (26, 27). Sixty-seven BASM participants (58 child-parent trios and 9 duos [child and one parent]) underwent WES. Of note, the clinical phenotype of participants' parents and siblings are not collected in PROBE or BASIC, thus obviating knowledge of familial cardiac, hepatic or laterality abnormalities.

WES and Data Analyses.

DNA from each trio was sequenced using standard Illumina protocols and NimbleGen SeqCap technology at two sites, The Human Genome Sequencing Center at the Baylor College of Medicine (Houston, TX) and the Northwest Genomics Center (Seattle, WA), with a mean coverage depth of 50–60X in the targeted region. The raw sequence reads were assembled relative to the Genome Reference Consortium Human GRCh38 (hg38) using the PEMapper software tool and PECaller was used to identify single nucleotide variants (SNVs), insertions and deletions (INDELs) (28). These unique variants were functionally annotated using Bystro (https://bystro.io/) and ANNOVAR which report the variant's type, functional classification (nonsense, replacement, silent, 5' or 3' UTR, splice junction or other intronic, intergenic), presence in the Single Nucleotide Polymorphism Database (dbSNP), and measures of evolutionary conservation (29, 30).

To explore the potential contribution of ciliary dysgenesis and/or dysfunction underlying the BASM phenotype, we used a pre-specified list of 2,016 Genes of Interest (GOI; Table S1) derived principally from 2 large comprehensive data sets dedicated to ciliomic studies as well as the Emory Genetics Laboratory (EGL) Ciliopathies Sequencing Panel, the EGL Neonatal and Adult Cholestasis Sequencing Panel, and a collated list of putative BA candidate susceptibility genes reported in the literature (18, 23, 27, 31) (Figure 1).

Variants in genes included among the 2,016 GOI were then subjected to further duo and trio analyses using a custom gene prioritization algorithm (Figure S1). In addition to standard quality controls, our candidate gene prioritization method included analysis of variants in coding regions of a gene (i.e. exonic and splicing donor/acceptor sites) weighted towards identification of protein-truncating variants (stop gain/loss, start loss, or frameshift), missense variants, canonical splice-site variants, and in-frame INDELs. Furthermore, we prioritized variants in individuals by preferentially selecting rare variants with a minor allele frequency (MAF) <1% and those with a Combined Annotation Dependent Depletion (CADD) score >10, indicating that the variant is amongst the top 10% of deleterious variants in the human genome, or predicted to negatively impact protein function using the *in silico* Sorting Intolerant From Tolerant (SIFT) algorithm (32, 33). Bi-allelic assignation included variants with CADD scores <10, when an allele with a CADD score >10 was initially identified by application of the screening algorithm. Finally, participants' and their parents' variants were systematically categorized based on four different modes of inheritance: (1) germ-line *de novo* mutations; (2) recessive homozygous genotypes, which were heterozygous in both parents; (3) compound heterozygous; and (4) hemizygous X chromosome variants.

Liver Histology and Immunohistochemistry.

Standard hematoxylin and eosin staining was employed in formalin-fixed paraffin-embedded liver tissue. Immunohistochemical (IHC) staining was performed on a Bond Rx automated staining system (Leica Biosystems) utilizing the Bond Refine polymer staining kit (Leica Biosystems). Primary anti-human PKD1L1 antibody incubation (Atlas HPA022424, 1:200 dilution) or anti-human K7 (Dako M7018, 1:100 dilution) proceeded for 1 hour at room temperature, and antigen retrieval was performed with E1 (PKD1L1) and E2 (CK7) (Leica Biosystems) retrieval solution for 20min. Slides were rinsed and dehydrated through a series of ascending concentrations of ethanol and xylene, prior to the placement of coverslips.

Results

Participant Demographics and Clinical Information.

Sixty-seven BASM ChiLDReN participants were included in the current study. There was a slight female predominance with racially and ethnically diverse backgrounds generally reflective of the overall demographics of the ChiLDReN cohort of BA participants (Table 1). The Kasai hepatoportoenterostomy was performed in 61 participants at an average age of 59 days (range: 21 – 122 days) and 61% of participants had undergone liver transplantation at the time of selection (See Table 1 and Figure S2 for detailed outcomes). At the time of selection, 27% of participants were alive with native liver, similar to previously published series (4,5). Review of PROBE and BASIC case report forms indicated that the majority of BASM participants in this study exhibited at least two laterality features (Figure 2A). The most common abnormalities of L-R patterning were splenic abnormalities (primarily polysplenia and less frequently asplenia) and intestinal malrotation, each present in more than half of the participants. Other common anomalies were congenital heart disease, abdominal heterotaxy, various vascular malformations, and renal anomalies (Figure 2B). The range and distribution of anomalies in this cohort were comparable to previously published collections of BASM patients (6, 7, 26).

WES and Systematic Analyses of Ciliopathy Genes.

Applying quality control filters to the collated list of 2016 ciliopathy and cholestatic GOIs (Table S1), led to the identification of 226,058 variants in 67 BASM probands. After utilizing our candidate gene prioritization method, 12,190 variants remained and served as the basis for detailed stratifications and inter-subject analyses (Figure S1). No pathogenic variants were identified in genes underlying the cholangiociliopathies (e.g., *PKHD1*, *PKD2*), neonatal sclerosing cholangitis (*DCDC2*), or neonatal cholestasis (e.g., *ABCB11*, *ABCB4*, *CFTR, JAG1*), suggesting exclusion of non-BA cholestatic diagnoses and adequacy of clinical phenotyping. Furthermore, no significant pathogenic variants in previously proposed BA candidate genes *CFC1*, *FOXA2*, *INVS*, *NODAL*, and *ZIC3* were identified (see Discussion). However, prioritizing bi-allelic variants led to the identification of several new and promising candidate genes, not previously linked to BA or the BASM subgroup. In particular, *PKD1L1*, which encodes the polycystin 1-like 1 protein (a member of the polycystin protein family), had potentially pathogenic bi-allelic and heterozygous variants in multiple BASM participants. Given the role of PKD1L1 in ciliary calcium signaling and laterality determination, its interactions with PKD2, and as a cause of complex congenital heart disease in children, further investigations of *PKD1L1* variants in this BASM cohort were pursued (34–37).

PKD1L1 Gene Variants and Clinical Features of Heterotaxy.

A total of 910 *PKD1L1* variants were found in the 67 BASM participants (Table S2). Employing our candidate gene prioritization algorithm led to the identification of bi-allelic *PKD1L1* variants in 5 participants (Subjects 1, 2, 3, 4, and 5), and heterozygous variants of interest in 3 additional participants (Subjects 6, 7, and 8; Tables 2 and S2). Figure 3 schematically depicts the exonic location of the 9 *PKD1L1* variants in this report. MAFs for these 9 variants ranged from 0.0004% - 0.6072%. Of the 9 *PKD1L1* variants (6 missense, 2 splice-site, and 1 chain-terminating), 8 have yet to be associated with a disease phenotype. The splice site mutation found in Subject 3 (c.6473+2_6473+3delTG) was recently reported as a homozygous variant in 2 siblings with heterotaxy and severe congenital heart disease (35). The variants c.T2399C (p.I800T) and c.C6949T (p.R2317W) were both present in homozygous form in Subject 1, and present as heterozygous alleles in Subjects 2, 6, and 7 (Tables 2 and S2). Subject 2 was compound heterozygous for p.I800T and c.T7121C (p.I2374T). Subjects 3 and 4 had an allelic variant occurring near a splice junction on one allele, and a missense variant on the other allele. Subject 5 was compound heterozygous for a protein-truncating variant c.C7937G (p.S2646X) and a missense variant c.G8266A (p.D2756N). The variant p.R2317W was found in heterozygous form in 2 additional participants (Subjects 6 and 7); a second potentially pathogenic allelic variant was not readily identifiable in either individual (Table S2). Taken together, 5 participants in this study possessed rare and potentially pathogenic bi-allelic variants in a new candidate gene for BASM — *PKD1L1.*

The protein domains of PKD1L1 have not been mapped in fine detail, but exhibit similarities to portions of existing members of the greater PKD gene family (34, 36–38). The PKD1L1 protein is predicted to be 2,849 amino acids in length, with a large external N-terminal domain comprising approximately half the length of the protein (with PKD and REJ regions), followed by 11 transmembrane domains and ending in a short coiled-coil region at the C-terminus. Four of the 5 participants (Subjects 1, 2, 4, and 5) had at least 1 PKD1L1 coding variant in, or near, the proposed C-terminal coiled-coil region, necessary for interactions with PKD2 (36, 37). Three participants (Subjects 1, 2, and 3) had PKD1L1 variants in the large external N-terminal REJ region. Thus, in addition to the 2 splice site variants, there were 6 potentially deleterious PKD1L1 missense variants and 1 chain-terminating variant in 5 BASM subjects.

Polysplenia, intestinal malrotation, abdominal heterotaxy, and vascular anomalies were each present in 4 of the 5 individuals with bi-allelic *PKD1L1* variants (Table 2). Polysplenia, abdominal heterotaxy, and vascular anomalies were jointly present in 3 individuals (Subjects 1, 2, and 5) while the concurrence of polysplenia, intestinal malrotation, and vascular anomalies was also reported in 3 individuals (Subjects 2, 3, and 5). Congenital heart disease/heterotaxy was reported in 2 subjects (Subject 1 – dextrocardia; Subject 5 – atrioventricular discordance, left atrial isomerism, left ventricular outflow obstruction, peripheral pulmonary and mitral valve stenosis). Although the clinical manifestations of heterotaxy in these 5 BASM participants overlapped, there were no clear genotype-phenotype-outcome correlations between individual laterality or hepatic features and specific *PKD1L1* variants. Three of the 5 individuals (60%) with potentially pathogenic bi-allelic *PKD1L1* variants underwent liver transplantation – Subjects 2, 3, and 5 at ages 10, 0.5 and 5 years, respectively. Subjects 1 and 4 are surviving with their native livers at ages 7 and 13 years, respectively. Comparatively, 65% of individuals without notable *PKD1L1* variants from this cohort underwent liver transplantation at an average age of 26 months (range: 3 – 136 months). The limited sample size of this study precludes exploration of connections between *PKD1L1* variants and timing of liver transplantation.

PKD1L1 Expression in Human Bile Duct Epithelium.

Protein function and gene expression data for human PKD1L1 are limited. In liver tissue obtained from a normal 3-day-old infant and 2 patients affected by other non-cholestatic liver diseases (carbamoyl phosphate synthetase deficiency and hepatoblastoma), PKD1L1 is strongly expressed in cholangiocytes (Figure 4 B-C, G), while expression is absent in liver tissue from a patient with Alagille syndrome with paucity of interlobular bile ducts (Figure 4D). Liver tissue from one BASM subject with bi-allelic *PKD1L1* variants was available from the ChiLDReN biorepository for immunohistochemical analysis – Subject 1. Notably, the biliary ductal plate profiles in this subject at the time of Kasai portoenterostomy revealed weak or absent expression of PKD1L1 (Figure 4A). Taken together, this is the first demonstration of PKD1L1 in human liver tissue with expression limited exclusively to bile duct epithelium.

Discussion

To date, a firmly established etiology of BA remains elusive. Rather, multiple factors have been implicated in the perinatal biliary injury characteristic of BA that results in progressive intra-and extra-hepatic cholangiopathy evident soon after birth (1, 39). Until this study, a large cohort of BASM patients has not undergone extensive investigation utilizing next-generation sequencing, a technology that has identified disease-causing variants across a diverse spectrum of pediatric and adult diseases in various fields (40). The application of WES to this cohort of 67 BASM participants, with a focused exploration of variants within a pre-specified list of 2,016 genes associated with ciliopathies, hepatobiliary development, and cholestasis, led to the identification of *PKD1L1* as a new candidate gene for BASM (Figure 3, Tables 2 and S2). Pathogenic variants in several previously-proposed BASM candidate genes, (i.e., *CFC1, FOXA2, INVS, NODAL,* and *ZIC3)* were not observed (Table S3) (41). In primary cilia, PKD1L1 heterodimerizes with PKD2L1 to form a transmembrane ciliary calcium channel that ultimately influences downstream Hedgehog signaling (36, 37, 42). Moreover, PKD1L1-PKD2 heterodimers are required during embryonic development to establish normal L-R patterning, and leads to heterotaxy when this pairing is disrupted (36, 37). These molecular interactions have yet to be explored in cholangiocytes which express PKD1L1 (Figure 4), a crucial step which will help establish the normal functioning of PKD1L1 in bile duct epithelium and the consequences of potentially pathogenic variations in the *PKD1L1* gene.

Support for *PKD1L1* as a plausible candidate gene for BASM includes recently discovered roles for *PKD1L1* variants in humans with heterotaxy and mouse models of laterality (35, 36, 43). Vetrini *et al.* reported the first series of patients with mutations in *PKD1L1* who presented with heterotaxy and severe congenital heart disease (35). Notably, the homozygous *PKD1L1* splice-site mutation, c.6473+2_6473+3delTG, from that report was present in one allele of Subject 3 (Table 2). This BASM participant had polysplenia and intestinal malrotation without reported abdominal heterotaxy or congenital heart disease. It is not known if any of the 3 individuals from 2 families presented by Vetrini *et al.* had splenic or biliary tract abnormalities.

In both mice and Medaka fish, several *Pkd1l1* point mutants exhibit disruption of L-R patterning during embryologic development (36–38). However, as in the human report, the presence of any biliary tract abnormalities in these animal models is unknown, perhaps due to the low survival rates of progeny with heterotaxy due to dysfunctional *Pkd1l1* mutations. Intriguingly, only ~35–45% of mice with homozygous *Pkd1l1* mutations exhibited heterotaxy, attesting to the variable consequences and penetrance of *PKD1L1* variants on subsequent L-R patterning defects, even in well-defined mouse and zebrafish genetic backgrounds. The incomplete penetrance observed in human and animal models of heterotaxy is likely the result of fundamental aspects of randomization inherent in L-R determination during embryogenesis (44). Extending this observation to the BASM syndrome, it is possible that the lack of apparent Mendelian inheritance of BASM within families could be due to reduced penetrance and/or variable expressivity of each specific gene variant during embryologic development. In addition, there may be undiagnosed or clinically-insignificant features of heterotaxy in family members, knowledge of which is currently unavailable within this dataset.

In the BASM subgroup, which accounts for ~10% of infants affected with BA, the coexistence of major congenital anomalies and a wide array of laterality defects along with biliary tract dysmorphogenesis suggests underlying genetic abnormalities linking the altered embryologic development of these structures (45). First classified as BASM by Davenport *et al.* in 1993 (5), various aspects of heterotaxy have been associated with individual cases of BA as early as 1892 (8) and 1941 (46) as well as in more recent case series (6, 7, 26). The diverse assortment of thoracic and abdominal anomalies of L-R asymmetry observed in patients with the BASM syndrome overlaps phenotypically with an emerging class of genetic disorders broadly categorized as ciliopathies; however, support for inclusion of BASM as a ciliopathy has, to date, been limited (16, 25). With functional validation and identification of additional ciliopathy genes like *PKD1L1*, BA, and BASM specifically, may ultimately be classified as a cholangiociliopathy (25).

In addition to *PKD1L1* gene variants as a potential contributor to the BASM syndrome, it is possible that reduced or temporally-modified *PKD1L1* RNA expression could play a role. Tsai *et al.* reported a heterozygous deletion of *FOXA2* in a child with BA, intestinal malrotation, and an interrupted inferior vena cava whose father had situs inversus and polysplenia, but not BA (41). The potential link between FOXA2 and *PKD1L1* gene expression stems from activation of *Pkd1l1* transcription by Foxa2 in mice (47). We did not identify candidate deleterious *FOXA2* gene variants in our cohort of 67 BASM participants (Table S3).

Whether *PKD1L1* variants reported in this study are relevant to the BASM syndrome awaits detailed functional and developmental analyses. Molecular functional validations of *PKD1L1* mutations as a cause of cholangiopathy are needed and will likely require multi-faceted molecular and cellular approaches to investigate and assign normal, and variant, gene and domain functions. We have shown that PKD1L1 is expressed in normal human bile duct epithelium with reduced expression in Subject 1 (Figure 4). From a histological perspective, it is unknown to what degree PKD1L1 is expressed in patients with isolated BA, BASM patients with and without *PKD1L1* gene variants, and other human cholangiopathies, especially since ciliary structures are often blunted in BA bile duct epithelium(22). Moreover, studies of PKD1L1's function will require consideration and incorporation of known heterodimer partners PKD2L1 and PKD2, underscoring the complexities likely to follow detailed explorations of its role in primary ciliary function in cholangiocytes.

In conclusion, a WES-based exploration of variants in a robust ciliopathy gene collection led to the identification of rare and potentially deleterious bi-allelic variants in the *PKD1L1* gene in 5 of 67 BASM participants from the ChiLDReN database. We believe that *PKD1L1,* whose gene product is functionally relevant in primary ciliary calcium signaling, and whose loss-of-function results in heterotaxy in humans and various animal models, emerges as an etiologic candidate gene for BASM, and possibly select cases of BA without splenic malformation. The concept that disordered cholangiocyte ciliary structure and function produces the BASM syndrome seems biologically plausible, particularly given the established role of cilia in sensing and modulating biliary flow and composition, transmission of intracellular signals, and contribution to cholangiopathies (19, 20, 48). Future studies of *PKD1L1* in heterotaxy and BA patients and their families, as well as laboratory-based validations in specific cell and animal-based models, will be needed to determine specific mechanistic roles for *PKD1L1* in biliary tract development, cholangiocyte structure and ciliary signaling.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

BA	biliary atresia
BASM	biliary atresia splenic malformation
WES	whole exome sequencing
ChiLDReN	Childhood Liver Disease Research Network
L-R	left-right
PROBE	A Prospective Database of Infants with Cholestasis
BASIC	Biliary Atresia Study in Infants and Children
SNV	single nucleotide variant
INDELs	insertions/deletions
dbSNP	Single Nucleotide Polymorphism Database
GOI	gene of interest
EGL	Emory Genetics Laboratory
MAF	minor allele frequency
CADD	Combined Annotation Dependent Depletion
SIFT	Sorting Intolerant from Tolerant

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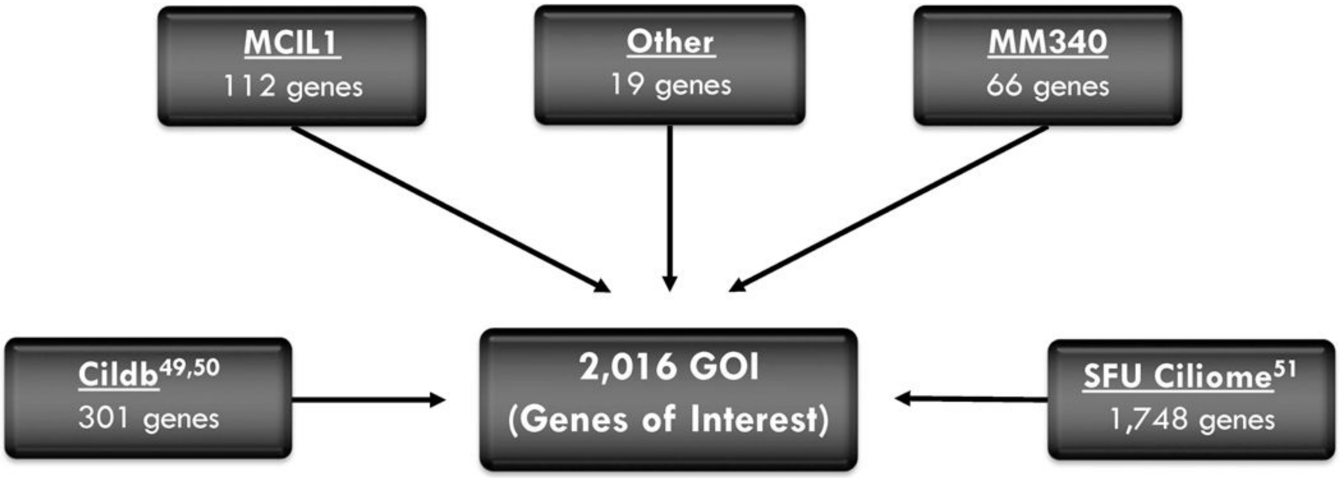

Figure 1. Construction of the set of 2,016 genes of interest (GOI).

To explore the potential contribution of ciliary and ciliopathy gene variants underlying the BASM phenotype, a collated set of ciliopathy and biliary GOI was derived from 4 large comprehensive data sets: (1) Cildb – a knowledgebase for centrosomes and cilia (27, 49); (2) Simon Fraser University (SFU) Ciliome Database – a summary of ciliomic studies (31); (3) MCIL1 – Emory Genetics Laboratory Ciliopathies Sequencing Panel; and (4) MM340 – Emory Genetics Laboratory Neonatal and Adult Cholestasis Sequencing Panel.

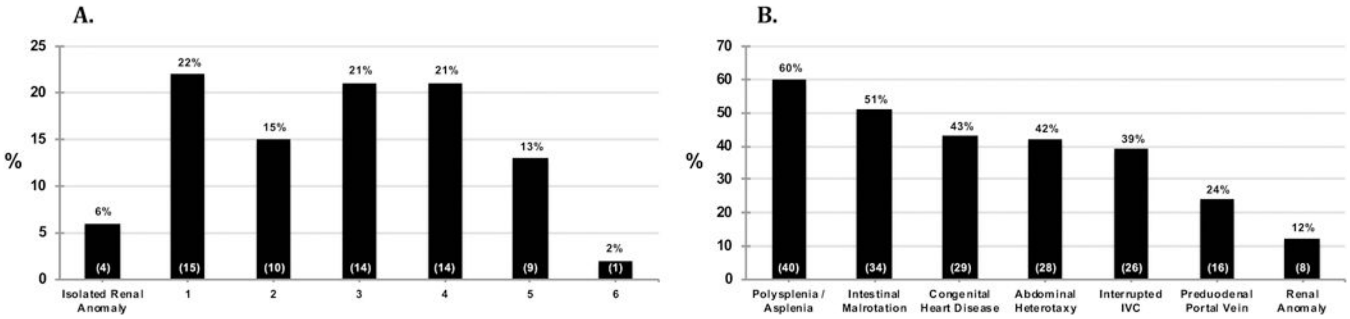

Figure 2.

A.) The number of reported laterality features per participant. Representation of the number of laterality features (1–6) per participant expressed as a percentage of the total cohort (*n* in parentheses). Note that the majority of participants (48) had two or more laterality defects. Four of the 67 participants had isolated renal anomalies. **B.) The 6 categorical types of reported laterality features.** The most common reported abnormalities of left-right patterning in the 67 BASM participants expressed as a percentage of the total cohort (*n* in parentheses). The majority had splenic abnormalities or intestinal malrotation, with substantial overlap in the types of anomalies between participants. Abbreviation: IVC, inferior vena cava.

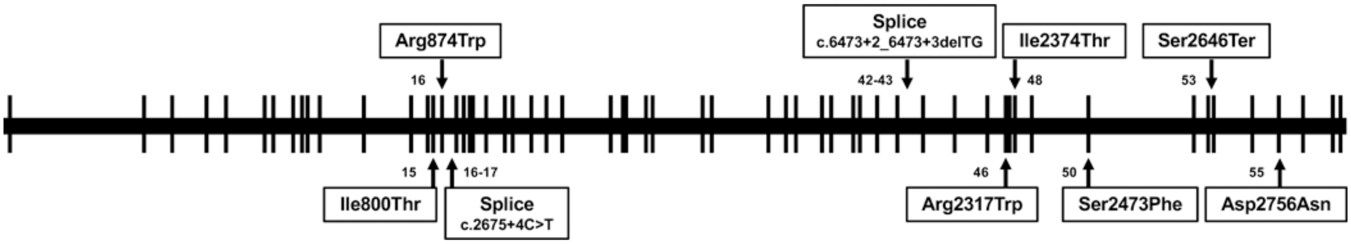

Figure 3. Schematic depiction of the *PKD1L1* variants in this report.

Exonic regions affected by each variant are noted among the 58 *PKD1L1* exons. Adapted from (50).

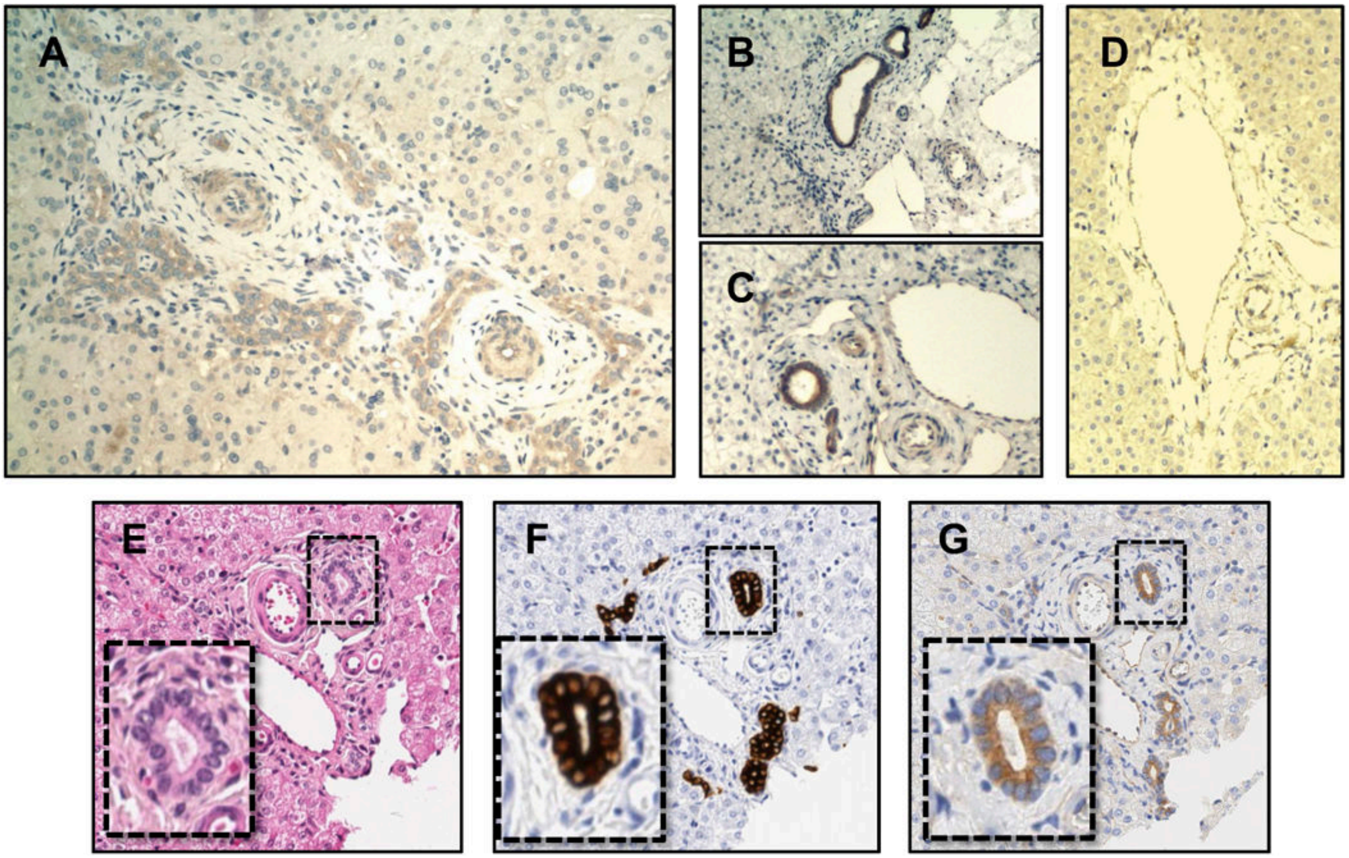

Figure 4. PKD1L1 expression in bile duct epithelium.

Immunohistochemical detection of PKD1L1 in human liver tissue from: ***A)*** Subject 1; and representative regions from patients with ***B)*** carbamoyl phosphate synthetase deficiency; ***C)*** hepatoblastoma; and ***D)*** Alagille syndrome (with absence of portal tract bile ducts). *E-G* are from serial sections of liver tissue from a normal 3-day-old infant: ***E)*** hematoxylin and eosin staining; immunostaining with ***F)*** keratin 7 and ***G)*** PKD1L1. Bile duct profiles are highlighted and enlarged in *E-G*. Note robust PKD1L1 expression in cholangiocytes from two livers affected by hepatocellular disease and normal pediatric liver tissue (*B*, *C*, *G*), absence in Alagille syndrome (*D*), and weak/absent expression in Subject 1(*A*).

Table 1.

Socio-demographic and clinical characteristics of the 67 BASM participants.

Category	n (%)
ChiLDReN Study	
BASIC	43 (64)
PROBE	24 (36)
Sex	
Female	37 (55)
Male	30 (45)
Race/Ethnicity	
White	43 (64)
Hispanic	10 (15)
Asian	6 (9)
Black	4 (6)
Multiracial	4 (6)
Kasai HPE	
Yes Mean: 59 days Range: 21–122 days	61 (91)
No	6 (9)
Status	
Liver Transplantation[*]	41 (61)
SNL	18 (27)
Unknown	6 (9)
Died[*]	3 (4)

[*] Note: One subject died after transplant.

Abbreviations: BASIC, Biliary Atresia Study in Infants and Children; BASM, biliary atresia splenic malformation; ChiLDReN, Childhood Liver Disease Research Network; HPE, hepatoportoenterostomy; PROBE, A Prospective Database of Infants with Cholestasis; SNL, survival with native liver. See Supplemental Figure S2 for a more detailed delineation of status at enrollment.

Table 2.

Clinical and sequence features of the five BASM participants with bi-allelic *PKD1L1* gene variants.

Subject	Polysplenia	Intestinal Malrotation	Abdominal Heterotaxy	Congenital Heart Disease	Vascular Anomaly	***PKD1L1* Variant**	Variant Type	HGVS Nomenclature	RefSNP	CADD	MAF	Notes
1	X	–	X	X	X	Arg2317Trp Ile800Thr	Hom Hom	c.6949C>T c.2399T>C	rs139293796 rs148011149	31 23	0.1856% 0.0211%	Probable consanguinity
2	X	X	X	–	X	Ile800Thr Ile2374Thr	Het (P) Het (M)	c.2399T>C c.7121T>C	rs148011149 rs776420484	23 8	0.0211% 0.0012%	–
3	X	X	–	–	X	Arg874Trp Splice Site*	Het (P) Het (M)	c.2620C>T c.6473+2_6473+3delTG	rs139858574 rs528302390	33 23	0.0154% 0.0365%	* Previously reported pathogenic mutation
4	–	X	X	–	–	Splice Site Ser2473Phe	Het (P) Het (M)	c.2675+4C>T c.7418C>T	rs143005953 rs140456142	4 23	0.6072% 0.1027%	–
5	X	X	X	X	X	Asp2756Asn Ser2646Ter	Het (#) Het (M)	c.8266G>A c.7937C>G	rs770832954 rs752673990	24 36	0.0004% 0.0004%	# Presumed paternal inheritance (duo) or de novo

Abbreviations: BASM, biliary atresia splenic malformation; CADD, Combined Annotation Dependent Depletion; Het, heterozygous; HGVS, Human Genome Variation Society; Hom, homozygous; MAF, minor allele frequency (from gnomAD); M, maternal; P, paternal.