Zebrafish Models of Biliary Atresia and Other Infantile Cholestatic Diseases

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Abstract Zebrafish are a powerful tool for the study of diseases that appear during development. Over the past decade, several zebrafish models of diseases that affect the developing liver in infants have been generated, including models of the most common identifiable cause of neonatal cholestasis, biliary atresia (BA). While none of these models exactly duplicate the disorder, studies on the zebrafish models have uncovered potentially important features of BA such as an importance of epigenetic changes and an association with specific mutations. In addition, other models replicate other cholestatic disorders, and there are zebrafish models of other pediatric liver diseases as well. Continued efforts with these models, and future models that take advantage of emerging technologies, will uncover additional facets of disease pathogenesis and may identify novel, more effective therapies.

Keywords Zebrafish model · Biliary atresia · Neonatal cholestasis · Epigenetic changes · Cholestatic disorders · Disease pathogenesis

Introduction

Over the past decade, zebrafish have emerged as an intriguing model for the study of liver diseases that primarily affect children. As zebrafish are a developmental

model, they would seem to be well suited to this role, as most of these diseases first appear in infancy, when the liver and bile ducts continue to develop, and thus are likely to have a developmental component. While the etiologies of many of these disorders are not known, the zebrafish models have allowed us to uncover mechanisms important in mediating the defects in the fish. This has in turn yielded insight into disease pathogenesis, and our continued efforts using the models described below should uncover further aspects of pathogenesis and may suggest novel treatments.

Liver Disease in Children

To date, the greatest advances in our understanding of pediatric liver disease using zebrafish have come from efforts to model cholestatic disease. Cholestasis is defined as poor bile flow and is most frequently caused by obstruction or malformation of the biliary tree. Cholestasis manifests as jaundice and is associated with poor growth and malabsorption of fat-soluble vitamins that most frequently leads to excess bleeding from vitamin K deficiency. Bile is necessary for normal digestion of fat and excretion of toxins, but is itself toxic to liver cells, as accumulation of bile components in the liver results in inflammation and fibrosis. Over time, this can lead to liver failure and cirrhosis, which untreated can lead to life-threatening sequelae such as severe infection and profound bleeding.

Approximately 1 in 3,000 infants presents with cholestasis, and the list of potential causes for infantile cholestasis is exhaustive [1]. Broadly, the causes include infections, metabolic disorders, and genetic and/or developmental disorders. The most common identifiable cause of infantile cholestasis is biliary atresia (BA), an idiopathic

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disorder that affects $\sim 1/12,000$ infants [2]. BA is characterized by a fibro-inflammatory destruction of the extrahepatic biliary tree and is also associated with intrahepatic bile duct proliferation that is probably secondary to the obstruction. If left untreated, there is progressive biliary cirrhosis, resulting in profound malnutrition and other complications, leading to death. Ten to 20 percent of patients with BA have a syndromic form of the disease, with other anomalies, most commonly involving defects in left–right asymmetry such as polysplenia and intestinal malrotation [3, 4]. The only treatment for BA is a surgical procedure (the Kasai portoenterostomy) that is not particularly effective, as many children with BA eventually require liver transplantation [5]. Although there have been considerable efforts to better understand BA, identification of an etiology remains elusive, and thus studies in zebrafish may provide some insight into pathogenesis.

While recent studies of BA have demonstrated possible genetic susceptibility, and some syndromic BA patients may have an underlying genetic disorder, a unifying genetic cause of BA is unlikely. In contrast, several genetic causes of cholestatic disease have been characterized, such as Alagille syndrome, arthrogryposis-renal dysfunction-cholestasis (ARC) syndrome, and North American Indian childhood cirrhosis (NAIC). Of note, these disorders have all been modeled in zebrafish (see below). Patients with these disorders often have defects in other organs. In Alagille syndrome, there is a wide heterogeneity of findings, but many patients have heart, skeletal, eye, and/or vascular defects [6]. In ARC, there are characteristic kidney, joint, and skin findings, as well as the liver defects [7]. NAIC is quite rare, but is characterized by intrahepatic findings similar to BA without extrahepatic obstruction [8]. The zebrafish models of these rare disorders provide opportunities for studying pathogenic mechanisms in a model organism.

Other genetic disorders that affect the developing liver lead to developmental biliary anomalies and are associated with liver fibrosis, and are also associated with the formation of kidney cysts [9]. Like patients with syndromic BA, patients with the cystic kidney diseases may have defects in left–right asymmetry. These disorders have been described mostly in genes associated with cilia, and patients with these ciliopathies may have a variety of developmental defects affecting multiple organ systems, including the liver [10]. There are several zebrafish models with cystic kidneys [11, 12], but there has not yet been extensive examination of liver phenotypes in these models.

In addition to the cholestatic disorders that affect the developing liver, there are also several metabolic disorders that affect children. While many of these also affect other organs, often the primary derangement is manifest most clearly in the liver. Symptoms of these disorders vary

widely, but the liver disease can present as cholestasis or as acute liver failure (ALF). In ALF, the metabolic derangement typically results in hepatocyte death, which leads to an inflammatory response and continued destruction, resulting in impairment of liver function. ALF in infants and children can progress rapidly, requiring emergent liver transplantation or even leading to death [13]. Treatment of these disorders in patients requires quickly addressing the cause, if possible. Thus, animal models of these disorders may yield important understanding of underlying mechanisms that could lead to more effective treatments.

There are of course multiple disorders that affect the liver in older children, but most of these disorders are also seen in adults. In addition to the many viral infections that affect both adults and children, conditions such as autoimmune hepatitis, Wilson's disease, alpha-1-antitrypsin deficiency, and non-alcoholic steatohepatitis (NASH) are seen in older children. In fact, NASH is now the most common chronic liver disease affecting US children [14]. Several zebrafish models of these disorders have been established, but as these disorders are not unique to children, they will be discussed elsewhere.

Zebrafish Models of BA

Ideally, a zebrafish model of BA would have defects in the extrahepatic biliary tree that resemble the fibroinflammatory destruction seen in patients and would also have intrahepatic bile duct proliferation, activation of inflammatory pathways such as interferon-gamma, fibrogenesis, and the potential to be associated with left–right asymmetry defects. The most frequently utilized mouse model of BA, derived by injection of a strain of rotavirus from rhesus macaque (RRV) into mouse pups [15], has many of these features, but is of course derived from an infectious model and thus may not be relevant to all forms of BA in patients. Zebrafish offer a more facile model system, in which the study of multiple mechanisms that lead to a BA-like phenotype may lead to uncovering more universal factors in BA pathogenesis. To date, no zebrafish model possesses all of the above features of BA (though neither does the mouse model), but the existing models that do share elements with the disease have yielded some insights into pathogenesis.

The first zebrafish model to address BA directly was the mutant *duct-trip*, in which there is a mutation in the gene encoding *S*-adenosylhomocysteine hydrolase (AHCY) [16]. While the liver of homozygous *ahcy*^{−/−} larvae had several abnormalities, there was a striking absence of intrahepatic bile ducts and reduction in the size of the gallbladder [17•]. We found that inhibition of DNA methylation, either with the chemical inhibitor

5-azacytidine or by injecting morpholinos directed against *dnmt1* (DNA methyltransferase I), led to an identical biliary phenotype. While this alone was not enough to draw a parallel to BA, analysis of gene expression changes uncovered an activation of interferon-gamma target genes [17•] and a decrease in promoter methylation of *ifng* family genes as well [18]. Others had shown that interferon-gamma was upregulated in patients with BA [19], and studies in the mouse BA model showed that activation of interferon-gamma was critical for development of the disease phenotype [20]. Thus, inhibition of DNA methylation in zebrafish led to defects in the biliary tree and activation of interferon-gamma, which could be considered similar to BA. We examined DNA methylation in samples from patients with BA, which demonstrated lower levels of DNA methylation in bile duct cells in the BA patients [17•], further supporting a connection between the disorder and this zebrafish model. In addition, this last finding provided a potentially novel mechanism by which BA could arise in patients, as DNA methylation can be elicited by viruses, toxins, environmental effects, and genetically. Thus, establishment of this zebrafish model of BA, while not exactly duplicating all of the features of BA in patients, did uncover novel features of the disorder that may yield clues to pathogenesis.

Similarly, studies of potential genetic influences on BA using zebrafish have yielded models that share some features with the disease, enhancing our understanding of pathogenesis. Rare patients with defined genetic disorders have developed BA, and thus defects in the causative genes may be at least partially leading to the disease. We identified a patient with Mowat-Wilson syndrome, which is typically characterized by neurological deficits, who also had BA. Mowat-Wilson syndrome is caused by a mutation in *ZFX1B*, a transcription factor associated with SMAD signaling. Using morpholinos, we knocked down *zfx1b* in zebrafish, which led to developmental biliary defects [21]. While this certainly does not demonstrate a causative role for *ZFX1B* in BA, it does suggest a potential importance of this gene and the pathway in general in bile duct formation and thus a possible role in mediating pathogenesis of BA and other cholestatic diseases.

Several groups have examined potential genetic influences on BA in genomewide association studies, uncovering possible roles for *GPC1*, *ADD3*, and *XPNPEP1* [22•, 23–25], among others. We utilized a morpholino antisense oligonucleotide-mediated knockdown of the glypican *gpc1* to examine the effect of inhibition of *gpc1* in biliary development, which demonstrated both extra- and intrahepatic biliary defects and an activation of Hedgehog signaling [22•]. Examination of GPC1 in patient samples showed a loss of staining in all BA samples examined, as well as a decrease in staining in other causes of cholestasis

[22•]. Glypicans had been shown to potentially act as a “sink” for Hedgehog ligand [26], and others had shown activation of Hedgehog in patients with BA [27], so our findings supported a role for this signaling pathway in the pathogenesis of BA and suggested a possible connection between a loss of GPC1 and activation of Hedgehog. Hedgehog signaling is important in fibrogenesis as well as in developmental pathways and is intimately associated with primary cilia [10], which have also been implicated in BA [28] and other cholestatic disorders [10]. Importantly, we were able to reverse the defects elicited by *gpc1* knockdown by inhibiting Hedgehog activity [22•], again supporting *gpc1* knockdown as a BA model and potentially identifying treatment strategies for patients.

While most work to date on BA in zebrafish has focused on the biliary tract, more recently developed tools may lead to further examination of the importance of hepatic stellate cells (HSCs) and fibrogenesis. Yin et al. developed a transgenic reporter line in which HSCs are visible, allowing these cells to be tracked during development. Moreover, these investigators determined that inhibition of pathways such as VEGF and retinoic acid receptor signaling decreased the number of HSCs, while inhibition of retinoid X receptor signaling increased the number of HSCs [29•]. These studies show the potential utility of such a reporter line in further studies on the importance of HSCs in liver disease in general and may specifically be able to examine the importance of these cells in diseases such as BA as well.

As mentioned above, abnormalities in primary cilia have been noted in patients with BA. Because primary cilia play an important role in Hedgehog signaling and in the establishment of left–right asymmetry, the link between primary cilia and BA is intriguing. While primary cilia have not yet been identified in developing cholangiocytes in zebrafish, abnormalities in pathways linked to primary cilia such as Hedgehog signaling and glypicans do affect biliary development in zebrafish. These pathways, along with cilia, are strongly linked to cell polarity pathways [30–32], and numerous models with abnormalities in cell polarity demonstrate developmental biliary defects. A few years ago, Sakaguchi et al. [33] demonstrated that disruption of the endothelial cells in the developing zebrafish liver led to abnormal hepatocyte apicobasolateral polarity, which in turn led to defects in intrahepatic bile duct development. Subsequent work has shown that mutation of specific genes associated with cell polarity such as *cldn15lb* (claudin 15-like b) leads to intrahepatic biliary defects, further supporting an importance of the development of apicobasolateral cell polarity in biliary development [34•].

In addition to apicobasolateral cell polarity, planar cell polarity (PCP), which mediates polarity within the epithelial sheet and is orthogonal to apicobasolateral polarity,

also appears important in bile duct development in zebrafish [35]. This report also showed that the knockdown of PCP family members led to sidedness defects somewhat similar to those seen in patients with syndromic BA. Interestingly, Garnaas et al. [36] observed a similar phenotype in zebrafish in which the retinoic acid receptor gamma b (*rargb*) is targeted by morpholino antisense oligonucleotides. These models suggest the involvement of other pathways in biliary development and organ sidedness, thus possibly also being involved in syndromic BA and perhaps more globally in BA in general.

While the connection between BA and cell polarity is not completely clear, recent reports have shown that mice with haploinsufficiency of *sox17* demonstrate loss of cholangiocyte polarity that results in shedding of cells into the duct lumen and consequent obstruction [37]. Such a scenario in BA patients, in which loss of cell polarity leads to accumulation of cell debris and a resulting obstruction, would be consistent with several of the above features noted in zebrafish models and in patients. Demonstration of abnormalities in Hedgehog signaling or in other inflammatory pathways in the zebrafish polarity models would further support such a model. Clear extrahepatic defects such as obstruction of the extrahepatic ducts and/or gallbladder would also strengthen the case for an importance of polarity pathways in BA pathogenesis.

Very recently, an intriguing zebrafish model of BA has managed to replicate the extrahepatic defects. Over the past several decades, droughts in New South Wales have been associated with outbreaks of a BA-like illness in lambs and calves, thought to be a result of their mothers grazing on *Dysphania* species [38]. Investigators utilized zebrafish to screen extracted fractions of *Dysphania* for compounds that resulted in biliary defects and eventually isolated a single active compound that when given to zebrafish results in extrahepatic defects. Interestingly, administration of this compound to cultured cholangiocytes results in defects in primary cilia, and treatment of cholangiocyte spheroids results in marked loss of epithelial polarity, suggesting that the compound may affect cell polarity (Pack et al. personal communication). These studies further support a potential importance of polarity pathways in mediating biliary defects in zebrafish and thus possibly mediating BA pathogenesis; further studies of the targets of this compound could yield valuable clues regarding BA.

The above models approximate BA in zebrafish. No model has yet been able to replicate all of the features of BA, such as extrahepatic atresia, intrahepatic duct proliferation, increased fibrosis, and association with laterality defects. Several of the models get some or most of these features, though, and clearly we have learned and will probably continue to learn about disease pathogenesis from these models. The models have uncovered mechanisms by

which important pathways, such as cell polarity, Hedgehog signaling, and inflammatory pathways may be influenced by genetic or epigenetic changes, resulting in abnormalities in the biliary tree, inflammation, and fibrogenesis (Fig. 1). As mentioned above, one of the great strengths of zebrafish is the ability to construct multiple models, and the combination of all of the above models may uncover additional facets of disease pathogenesis.

Models of Other Cholestatic Diseases in Zebrafish

Because none of the BA models perfectly recapitulates the human disease, it seems somewhat arbitrary to divide models of cholestasis in zebrafish into BA and non-BA categories. The models discussed in this section, though, were not intended to model BA. Nevertheless, these animal models can inform our understanding not only of the intended diseases, but for disease mechanisms of cholestasis in general. This may in turn allow us to increase our understanding of any cholestatic disease, including BA.

One of the first cholestatic diseases to be modeled in zebrafish was Alagille syndrome, which in patients is characterized by bile duct paucity, cardiac defects, vertebral defects, facial dysmorphism, and other anomalies [6]. Discovery of the causative gene led to recognition of the Jagged/Notch pathway as an important mediator of biliary development [39, 40]. Morpholino-mediated knockdown of Jagged and Notch orthologs in zebrafish led to establishment of an Alagille syndrome phenocopy, supporting the importance of this pathway in biliary development in zebrafish [41]. More recent studies on Jagged/Notch signaling in zebrafish biliary development have shown that there is continued importance of Notch signaling during biliary remodeling [42], similar to mammalian biliary remodeling [39, 40]. These studies support further use of zebrafish to model Alagille syndrome and to study the role of jagged/notch signaling.

Another early model of cholestatic disease was uncovered in a mutagenesis screen for hepatomegaly. While the underlying causes of hepatomegaly in this screen varied, of relevance to cholestasis was a model of choledochal cyst formation in *nf2* (*merlin*) mutants [43]. Choledochal cysts are cysts involving the bile ducts, typically the extrahepatic ducts, that often present as cholestasis and can appear in infancy [1]. There is occasionally overlap between BA and the presence of a choledochal cyst [44]. The *nf2* mutant remains one of the only genetic models of extrahepatic defects in zebrafish. Work on this pathway in other systems has shown that Nf2 is an important part of the Hippo pathway, regulating growth and organ size [45]. Further studies on *nf2* in zebrafish could help understand the importance of this pathway in extrahepatic biliary

Fig. 1 Pathways implicated by studies on zebrafish models of pediatric cholestatic diseases. A schematic diagram depicting the pathways important in biliary development and in mediating biliary defects uncovered in zebrafish models of cholestatic diseases. Shown are pathways such as Jagged/Notch, primary cilia with Hedgehog signaling, polarity pathways, intracellular trafficking, and the

cytoskeleton. The effects of these pathways on nuclear and other targets are noted by the *dark arrows*, while the interrelationships between these pathways are depicted by the *gray arrows*. Nuclear targets are mediated by these pathways and are depicted, or are affected by changes in DNA methylation, also noted

development and may also show an importance of this pathway in patients with choledochal cysts and perhaps in BA as well.

In the same initial report on *nf2*, another biliary mutant was identified, caused by mutation of the intracellular trafficking gene *vps18* [43]. Interestingly, investigators had identified the related *VPS33B* as the causative gene for arthrogryposis-renal dysfunction-cholestasis (ARC) syndrome, a rare disorder associated with the eponymous conditions [7]. We used morpholinos to knock down *vps33b*, which resulted in biliary defects [46], and others identified *vps39* mutants in zebrafish that also demonstrated liver defects [47]. More recently, we reported characterization of *pk* mutants, which demonstrate biliary and pigmentation defects [48•], similar to the phenotype of many of the *vps* mutants. The causative gene of *pk* is *atp6ap2*, which is important in mediating intracellular compartment pH and thus also important in intracellular trafficking pathways [49]. Thus, there are several models of abnormal biliary development in zebrafish in which intracellular trafficking pathways are affected, while there are at least two intracellular trafficking genes identified as disease-causing in patients [7, 50]. Continued exploration of the importance of this pathway in mediating biliary development and disease may demonstrate a more universal importance of

trafficking in biliary disease, as trafficking plays an important role in mediating cell polarity.

Central to the importance of cell polarity and primary cilia in biliary defects is increasing understanding of the mechanisms by which ciliopathies result in liver disease. The ciliopathies are a broad category of diseases, caused by genes important in cilia formation, in which patients have a characteristic set of findings that include liver and kidney cysts, brain abnormalities, and sidedness defects [10]. Liver defects can also include the ductal plate malformation, thought to be an arrest of biliary development, and relatively rapid progression of liver fibrosis [51]. While understanding the pathogenesis of these disorders is of itself important, the overlap between these disorders and some features of BA is intriguing and suggests some possible shared mechanisms. Several years ago, we determined that biliary development was abnormal in *vhnl1* (*hnf1b*) mutants [52], in which there are also kidney cysts [53]. *vhnl1*, or *Hnf1b*, is not generally considered a ciliopathy gene, however, as it encodes a transcription factor important in hepatobiliary development [54]. More recently, we have examined other kidney cyst mutants, and there are severe abnormalities in biliary development as well as activation of fibrogenesis genes (Cui and Matthews unpublished data). Others have shown

conservation of these pathways in mediating liver cysts as well, as morpholino-mediated knockdown of several ciliopathy genes leads to liver cyst formation [55•]. These investigators stressed the utility of these models in future drug screening studies, which could also of course increase our understanding of the pathogenesis of these disorders as well.

Clear genetic causes of cholestasis and biliary defects in infants include Alagille syndrome, ARC syndrome, ciliopathies, and North American Indian childhood cirrhosis (NAIC). NAIC is a condition of particular interest, in that in patients it is indistinguishable from BA in terms of symptoms and the intrahepatic defects, but without extrahepatic defects [8, 56]. Recently, a zebrafish model of NAIC was reported, utilizing morpholinos to knockdown the causative gene, *cirh1a* [57]. The intrahepatic defects seen in this model appeared to be mediated by p53-mediated cell death, suggesting a potential importance of this pathway in NAIC.

The above models have established the utility of zebrafish in studying genetic causes of cholestasis. There is remarkable conservation of the importance of these molecular pathways. Further study of these pathways in the zebrafish models should increase our understanding of the

human disorders and may help uncover treatments for these disorders as well.

Models of Acute Liver Failure and Metabolic Disease

To date, most models of acute liver failure in zebrafish have been generated as tools to study liver regeneration, but these models could serve to examine the disease process as well. There is hepatocyte death in larvae with homozygous mutation of *tomm22*, which encodes a mitochondrial import gene [58], and in larvae engineered to overexpress the nitroreductase enzyme in the liver, which leads to hepatocyte death after treatment with metronidazole [59]. Use of other toxins, such as the well-studied liver toxin acetaminophen, also leads to hepatocyte death in larval zebrafish, and studies have uncovered potentially novel pathways and treatments for reversing the effects of acetaminophen [60].

The mutant *duct-trip*, described above, demonstrates an ALF phenotype in addition to the biliary defects. This phenotype is not elicited by inhibition of DNA methylation and is rescued by inhibition of TNF α [16]. The ALF phenotype in *duct-trip* was associated with mitochondrial

Table 1 Summary of models described in this review, showing manipulated gene or toxin, phenotype, pathway, and method

Gene	Disease/phenotype	Pathway/target	Method	References
<i>Ahcy</i>	Biliary atresia	Methylation	Mutation	[16, 17•]
<i>dnmt1</i>	Biliary atresia	DNA methylation	Morpholino knockdown	[17•]
azacytidine	Biliary atresia	DNA methylation	Chemical treatment	[17•, 18]
<i>zfhx1b</i>	Biliary atresia	SMAD signaling	Morpholino knockdown	[21]
<i>gpc1</i>	Biliary atresia	Hedgehog	Morpholino knockdown	[22••]
<i>Claudin15bl</i>	Biliary atresia	Polarity	Mutation	[34•]
<i>Prickle1a</i>	Biliary atresia (syndromic)	Planar cell polarity	Morpholino knockdown	[35]
<i>Rargb</i>	Biliary atresia (syndromic)	Polarity/Retinoic acid	Morpholino knockdown	[36•]
Unknown	Biliary atresia	Unknown – cilia?	<i>Dysphania</i> exposure	Pers. comm.
<i>Jagged/Notch</i>	Alagille syndrome	Notch	Morpholino knockdown	[41, 42]
<i>nf2</i>	Choledochal cyst	Hippo	Insertional mutagenesis	[43]
<i>vps33b</i>	ARC syndrome	Intracellular trafficking	Morpholino knockdown	[46]
<i>vps18</i>	Cholestasis/ARC-like	Intracellular trafficking	Insertional mutagenesis	[43]
<i>vps39</i>	Cholestasis/ARC-like	Intracellular trafficking	Mutation	[47]
<i>atp6ap2</i>	Cholestasis/ARC-like	Intracellular compartment pH	Mutation	[48•]
<i>hnf1b</i>	Ciliopathy-like	Hepatobiliary development	Insertional mutagenesis	[52]
<i>pkhd1</i>	Ciliopathy	Polarity/cilia	Morpholino knockdown	Unpub. data
<i>cirh1a</i>	North American Indian childhood cirrhosis	p53	Morpholino knockdown	[57]
<i>hegl</i>	Cholestasis?	Apicobasolateral polarity (indirectly)	Mutation	[33]

References are provided

abnormalities and fatty liver, and there is considerable overlap between ALF and hepatic steatosis in other zebrafish models [61] and in patients, particularly those with ALF secondary to mitochondrial disorders. Supporting this mechanism, we have elicited hepatic steatosis in larval zebrafish using the mitochondrial toxin valinomycin (Sapp et al. submitted). These models, similar to those described above, have not been used directly to examine ALF, but there would seem to be an opportunity here to address the pathogenesis of early ALF using the above models.

Conclusions

We have presented several models of infantile cholestatic and metabolic diseases in zebrafish (Table 1). The pathogenesis of many of these disorders, including the most common identifiable infantile cholestatic disorder, is not well understood, and zebrafish models offer an intriguing method to better understand these diseases. The models utilize frequently touted advantages of zebrafish, such as the ease of genetic manipulation, rapid development time, and the relative ease of establishing transgenic lines. Moreover, the existence of multiple models allows for the comparison of models to uncover similarities that may be important in the underlying pathogenic mechanisms.

This analysis has uncovered a potential importance of trafficking and polarity pathways in mediating biliary defects, which as detailed above is supported by work in mammalian models and in patients that show involvement of the pathways in disease. In addition to the importance of polarity pathways in cholestatic diseases such as BA, the zebrafish models have also uncovered a potential importance of DNA methylation in BA. Future studies, in zebrafish, mammalian models, and in patients, will help to solidify these findings and will of course continue to uncover interesting facets of disease pathogenesis.

Future studies on cholestatic disease using zebrafish, as with other models in zebrafish, will likely be tremendously exciting. New technologies that allow for more facile engineering of mutants, such as TALENs and CRISPR/Cas, will allow us to examine the roles of multiple genes and pathways in zebrafish models. Continued close work with our geneticist colleagues will prove fruitful as well, as using the same technologies we can examine the potential importance of specific mutations and advance the nascent field of “personalized medicine.” We can also take advantage of our multiple models to uncover possible treatments for these disorders. These continued translational efforts should lead to great advances in the understanding of pediatric cholestatic diseases.

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Compliance with Ethics Guidelines

Conflict of Interest Randolph Matthews and Zenobia Cofer declare they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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