Published in final edited form as:

Am J Med Genet A. 2010 April ; 152A(4): 886–895. doi:10.1002/ajmg.a.33332.

Genomic Alterations in Biliary Atresia Suggests Region of Potential Disease Susceptibility in 2q37.3

Melissa Leyva-Vega¹, Jennifer Gerfen², Brian D. Thiel², Dorota Jurkiewicz³, Elizabeth B. Rand¹, Joanna Pawlowska⁴, Diana Kaminska⁴, Pierre Russo⁵, Xiaowu Gai⁶, Ian D. Krantz², Binita M. Kamath⁷, Hakon Hakonarson⁸, Barbara A. Haber¹, and Nancy B. Spinner²

¹Division of Gastroenterology, Hepatology and Nutrition, Children's Hospital of Philadelphia, Philadelphia, PA ²Division of Human Genetics, Children's Hospital of Philadelphia, Philadelphia, PA ³Department of Medical Genetics, The Children's Memorial Health Institute, Warsaw, Poland ⁴Department of Gastroenterology, Hepatology and Immunology, The Children's Memorial Health Institute, Warsaw, Poland ⁵Departments of Pathology and Laboratory Medicine and Pediatrics, The Children's Hospital of Philadelphia and the University of Pennsylvania School of Medicine, Philadelphia, PA ⁶Center for Biomedical Informatics, The Children's Hospital of Philadelphia, Philadelphia, PA ⁷Division of Pediatric Gastroenterology, Hepatology, and Nutrition, Hospital for Sick Children, Toronto, Ontario, Canada ⁸Center for Applied Genomics, Children's Hospital of Philadelphia, Philadelphia, PA

Abstract

Biliary atresia (BA) is a progressive, idiopathic obliteration of the extrahepatic biliary system occurring exclusively in the neonatal period. It is the most common disease leading to liver transplantation in children. The etiology of BA is unknown, although infectious, immune and genetic causes have been suggested. While the recurrence of BA in families is not common, there are more than 30 multiplex families reported and an underlying genetic susceptibility has been hypothesized. We screened a cohort of 35 BA patients for genomic alterations that might confer susceptibility to BA. DNA was genotyped on the Illumina Quad550 platform, which analyzes over 550,000 single nucleotide polymorphisms (SNPs) for genomic deletions and duplications. Areas of increased and decreased copy number were compared to those found in control populations. In order to identify regions that could serve as susceptibility factors for BA, we searched for regions that were found in BA patients, but not in controls. We identified two unrelated BA patients with overlapping heterozygous deletions of 2q37.3. Patient 1 had a 1.76 Mb (280 SNP), heterozygous deletion containing thirty genes. Patient 2 had a 5.87 Mb (1,346 SNP) heterozygous deletion containing fifty-five genes. The overlapping 1.76 Mb deletion on chromosome 2q37.3 from 240,936,900 to 242,692,820 constitutes the critical region and the genes within this region could be candidates for susceptibility to BA.

Keywords

Biliary atresia; copy number variation; deletion 2q37.3

Introduction

Biliary atresia (BA) is a progressive, idiopathic obliteration of the extrahepatic biliary system occurring only in infancy. It is initially treated with a hepatopertoenterostomy, a surgical procedure to allow bile drainage. However, despite this surgical intervention, the majority of patients eventually require liver transplantation. If untreated, patients die within the first two years of life.

The cause of BA is unknown, but is most likely multifactorial. Several mechanisms have been proposed, including a genetic predisposition, an environmental factor such as a virus or toxin, and immune dysregulation. It is likely that several of these processes need to be involved for BA to develop, so that in a genetically predisposed infant, immune dysregulation targeting the biliary tree is triggered by an environmental factor, such as a virus or a toxin. The strongest evidence for a potential environmental trigger comes from three reported outbreaks of BA in crossbred lambs and calves in New South Wales, Australia in 1964, 1988 and 2007 [Harper et al., 1990]. It has been proposed that the pregnant ewes ingested an environmental toxin when grazing on lands previously submerged and that this toxin may have triggered BA. To date, a specific toxin has not been identified.

As is the case in many complex disorders, uncovering genes important in the etiology of BA has not been straightforward. Genetic studies have been hampered by the rarity of this disease and the lack of a systematic reporting mechanism. Nevertheless, there are clues to support the hypothesis that genetic factors contribute to susceptibility. First, the incidence of BA is more common in certain racial and ethnic backgrounds. Epidemiological studies reveal that Asian populations have the highest rates of BA with a rate of 1.46 per 10,000 live births in Taiwan, compared to 0.65-0.85 in the U.S. Other Asian regions such as Japan and Hawaii have double the rate of European countries like the Netherlands [Tiao et al., 2008]. This does not preclude a possible role of the environment in the pathogenesis of BA, but no strong environmental exposure or regional clustering of cases has yet been identified. Second, BA has been associated with certain HLA genotypes [HH et al., 2002; Silveira et al., 1991]. Third, while BA is most often sporadic, there are multiple reports of BA occurring in the same families [Anneren et al., 1998; Cunningham et al., 1988; Gunasekaran et al., 1992; Kobayashi et al., 2008; Lachaux et al., 1988; Nevin et al., 1969; Smith et al., 1991; Whitten et al., 1952], including one report of mother to child transmission of BA [Kobayashi et al., 2008]. The rarity of BA makes twin studies difficult and the lack of zygosity reporting in the majority of twin cases makes interpretation impossible, however, the low concordance overall suggests that BA is not a purely genetic disease. [Hart et al., 1991; Hyams et al., 1985; Khaimin 1969; Morris et al., 1977; Poovorawan et al., 1996; Schweizer et al., 1988; Silveira et al., 1991; Smith et al., 1991; Strickland et al., 1985; Werlin 1981]. Nevertheless, these studies do not rule out the possibility of a genetic susceptibility factor.

In 20-30% of cases, BA is accompanied by additional malformations. 10-15% of these cases have poly- or asplenia and this sub-form of BA is referred to as BASM (Biliary Atresia Splenic Malformation). Patients with BASM often have other anomalies such as cardiovascular defects, situs inversus, intestinal malrotation and anomalies of the portal vein and hepatic artery. Patients with additional anomalies have been considered to have an embryonic form of BA and are generally diagnosed earlier than those with no other anomalies. However, there is overlap across these groups and the utility of these distinctions is not yet clear.

Several potential genetic factors have been investigated in BASM. The *CFC1* gene, which encodes the CRYPTIC protein, is associated with some cases of visceral heterotaxy. In one small study a heterozygous missense mutation leading to the amino acid substitution Ala145Thr on *CFC1* was detected in patients with BASM at a frequency double that of controls [Davit-Spraul et al., 2008]. The authors hypothesized that this polymorphism may represent a genetic predisposition for BASM. Recessive mutations of the *inversin* gene were implicated in mice with BASM [Mazziotti et al., 1999], however, this gene does not seem to be causative in humans. Although much of the focus of the studies on potential genetic mechanisms underlying BA has been on BASM, the majority (70-80%) of patients actually have no other malformations. Most recently a study involving 45 children with BA found specific polymorphisms of the vascular endothelial growth factor (*VEGF*) to be associated with BA [Lee et al., 2009]. In light of the sparse knowledge we have of the development of BA, the identification of a potential rare variant in the present study is especially important.

Recent advances in microarray technologies have revealed unexpected complexity in the human genome and we are now aware that a large percentage of the genome varies with respect to copy number [Redon et al., 2006]. These deletions or duplications (copy number variants or CNVs) can be rare or common, benign or disease-causing, or they may infer risk to complex diseases. Copy number variation has already been associated with susceptibility to several complex diseases, such as age-related macular degeneration, HIV, lupus, and neuroblastoma [Birnbbaum et al., 2009; Fritsche et al., 2009; Kugathasan et al., 2008; Kulkarni et al., 2008; Mamtani et al., 2008; Maris et al., 2008]. In this study we used single nucleotide polymorphism (SNP) genotyping arrays to detect genomic changes in 35 BA patients. Our goal was to identify variants that may serve as susceptibility factors for BA by searching for CNVs that occur in multiple BA patients and not in controls.

Materials and Methods

Thirty-five patients with BA were recruited into the study under a protocol of informed consent, which was approved by the Institutional Review Board of the Children's Hospital of Philadelphia (CHOP). Controls were recruited by the Center for Applied Genomics (CAG) at CHOP from randomized well-child visits throughout the extended CHOP ambulatory healthcare network. 2,026 healthy individuals served as controls and were genotyped by the CAG prior to this study. The control data is publicly available at <http://cnv.chop.edu/>.

Genotyping

DNA was isolated from whole blood using the Puregene DNA Genomic Purification System (Gentra Systems, Minneapolis, MN). Genotyping of cases and controls was performed by the CAG using the Illumina Quad550 BeadChips platform, which includes 550,000 SNPs (Illumina, Inc. San Diego, Ca). The genotyping methods and quality control measures have been previously published [Diskin et al., 2009; Shaikh et al., 2009].

Analysis of Genotype Data

Identification of copy number variation was performed using an automated calling algorithm [Shaikh et al. 2009] as well as visual inspection of the data. We visually inspected the log R ratio and B allele frequency for each CNV using the Illumina BeadStudio 3.1 software. In the cases where the visual inspection did not agree with the automated calling, we used the data generated from visual inspection.

To identify CNVs we selected genomic alterations that were defined by 10 or more SNPs [Elia et al., 2009]. In the study by Elia et al., investigators experimentally validated 200 CNVs and showed 99% accuracy for CNVs greater than or equal to 10 consecutive CNVs.

In addition, Shaikh et al. [2009] reported 97.1% validation between two different arrays in CNV deletions that were at least 10 SNPs in size and 90.5% validation for CNV duplications at least 10 SNPs in size. For this analysis we flagged alterations that were called by a minimum of 10 SNPs and were not present in control populations, which consisted of our in-house cohort of 2,026 healthy individuals and cases reported in the Database of Genomic Variants (DGV). In addition, PubMed, Online Mendelian Inheritance in Man (OMIM), and DatabasE of Chromosomal Imbalance and Phenotype in Humans Using Ensembl Resources (DECIPHER) were used to determine if there were any other reported cases of the identified CNV.

Validation

Fluorescence in situ hybridization (FISH) was carried out using a 2q subtelomere probe (Vysis, Inc.) to confirm the deletions, and a 2q centromere probe to mark chromosome 2.

Sequencing of AGXT

The alanine-glyoxylate aminotransferase (*AGXT*) gene is an 11-exon gene encoded by 10.375 kb of genomic DNA within 2q37.3. Genomic DNA was amplified using primers designed to cover the coding regions and splice sites, as previously described [Monico et al., 2007]. One exception was the amplification of exons 1 and 2 in a single reaction using the following primers (forward primer: 5' GGAAATATTCCAGGCTTTGG 3'; reverse primer: 5' CCAGCTTCAAACAGAGGGAG 3'). PCR products were cleaned using ExoSap-IT (USB, Cleveland, OH) and sequenced at the Nucleic Acid and Protein Core at CHOP.

Clinical Reports and Laboratory Results

Subjects

Thirty-five patients with BA were studied. There were 21 males and 14 females. The racial and ethnic backgrounds were self-defined by parents and included 20 Caucasians, 11 Blacks, 2 Asians, 1 bi-racial Black and Caucasian, and 1 Black and Hispanic. Seven patients had other congenital anomalies in addition to BA: one had BASM without other anomalies, one had BASM and multiple other anomalies (Patient 2), two had intestinal malrotation without splenic anomalies, one had duodenal atresia and an annular pancreas, one had a choledochal cyst, and one had multiple additional issues (mental retardation, hypotonia, seizures, hearing loss, speech delay, obstructive sleep apnea, ptosis, nystagmus, and low set ears) but did not have a splenic malformation.

Genomic analysis

A total of 192 CNVs called by ten or more consecutive SNPs were identified in the entire cohort of BA patients. Twenty-one of these CNVs were non-unique (found in more than one BA patient). Only one of these CNVs was found in cases but not in controls. This CNV was a heterozygous deletion localized to 2q37.3 and found in two unrelated patients.

Patient 1

This male child was born at 40 weeks gestation by spontaneous vaginal delivery to non-consanguineous parents following an uncomplicated pregnancy. His birth weight was 3836 grams. His mother was 37 years of age at the time of his birth and had good prenatal care and no history of medication use during pregnancy. There were no known teratogenic exposures during the pregnancy, although his mother worked for a house cleaning service and was therefore frequently exposed to cleaning agents. He had no siblings and there was a family history of a maternal aunt having had a liver transplant for unknown reasons in her sixth decade of life. At three weeks of age he was referred to our hospital for evaluation of

jaundice and acholic stools. His weight was 4.31 kg and length was 54 cm. On exam, he was non-dysmorphic and had hepatomegaly, but no splenomegaly. His physical examination was otherwise unremarkable. His total and direct bilirubin were elevated at 7.5 mg/dL and 4.0 mg/dL, respectively. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were normal at 30 U/L and 31 U/L, respectively, but his gamma-glutamyl transpeptidase (GGT) was elevated at 270 U/L. Prothrombin Time (PT) and Partial Thromboplastin Time (PTT) were normal at 10.6 seconds and 36.1 seconds, respectively.

Abdominal ultrasound revealed a small gallbladder and an enlarged liver. Hepatobiliary scintigraphy had no excretion. He tested negative for alpha-1 antitrypsin deficiency. To explore the possibility of Alagille Syndrome as a cause of his jaundice he underwent an ophthalmologic exam, which was negative for posterior embryotoxon, and a chest x-ray, which revealed no butterfly vertebrae. A percutaneous liver biopsy at three weeks of age showed patchy portal fibrosis and only minimal bile duct proliferation and was considered equivocal. At five weeks of life he underwent an exploratory laparotomy, which revealed fibrotic extrahepatic ducts and an atretic gallbladder. A cholangiogram could not be performed and he underwent a hepatoportoenterostomy. His post-operative course was complicated by necrotizing enterocolitis, requiring resection of 30 cm of small intestine. He had persistent jaundice and had a liver transplantation at eight months of life. The explant showed portal fibrosis and marked bile duct proliferation consistent with BA.

The patient was recently evaluated at seventeen years of age (Figure 1) and is healthy. He has an elongated face and a broad nasal tip, however, his father has these same features. There were no reports of developmental delays in the patient or his father. He was recently evaluated by neurology for headaches and was found to have a venous hemangioma in the right tentorium. He has no other known medical problems.

A total of five CNVs called by 10 or more consecutive SNPs were detected. These ranged in size from 5,407 bp to 1.76 Mb and between 14 and 280 SNPs. There were three heterozygous deletions and 2 duplications. Of these CNVs, only one was not observed in controls and was also found in another BA patient. This was a 280 SNP, 1.76 Mb heterozygous deletion within chromosome 2q37 (Figures 2A and 3). The 2q37.3 deletion was verified by fluorescence in situ hybridization (Figure 4). His father's array showed an identical deletion and his father has no history of liver disease.

There are 30 genes in the deleted region on 2q37.3. We sequenced the *AGXT* gene, because unlike the other 29 genes, it is expressed only in the liver. Homozygous mutations are associated with autosomal recessive primary hyperoxaluria type I (PH1), but we hypothesized that there may be specific variants in patients with BA that we could identify in our patient. Sequencing of the proband revealed seven polymorphisms (Table II). All of these have been previously described. Of note, there is a haplotype that includes three of the polymorphisms seen in our proband (PIIL, I340M and IVS1+74bp dup), which occurs in 2-28% of the population [Caldwell et al., 2004;Purdue et al., 1990], but 50% of patients with PH1 [Tarn et al., 1997]. Our proband has the minor allele for this haplotype, which includes the variants stated above, although his father (who carries the 2q37.3 deletion, but does not have BA) does not have this haplotype.

Patient 2

This child was born by spontaneous vaginal delivery at 38 weeks gestation and his birth weight was 3030 grams. There were no complications with the delivery. Prenatal history was significant for mother having had a primary varicella infection at 15 weeks gestation for which she was treated with acyclovir. The mother had occasional alcohol consumption

during the pregnancy, but no other known toxin exposures during the pregnancy. There was no history of consanguinity and no family history of liver diseases.

On physical exam he was noted to have dysmorphic facies including a prominent forehead, pointy chin, and asymmetric palpebral fissures. He also had abnormally long fingers, a heart murmur, third and fourth digit syndactyly of the foot, clubfoot, umbilical hernia, and a submucosal cleft palate. On the first day of life he was noted to have jaundice. At 2 days of age his total bilirubin was elevated at 17.3 mg/dl, conjugated bilirubin was unknown, AST was slightly increased (47 U/L), ALT was normal (32 U/L). By 4 weeks of age he had acholic stools and his total bilirubin was 6.8 mg/dL and direct bilirubin was 4.9 mg/dL, AST was elevated at 104 U/L, ALT was 52 U/L, GGT was elevated at 691 U/L. The facial dysmorphism in combination with jaundice led to molecular screening for Alagille syndrome. *JAGGED1* and *NOTCH2* sequencing were normal. In addition, his ophthalmologic exam was negative for posterior embryotoxon and his chest X-ray was negative for butterfly vertebrae.

Ultrasound showed a normal sized liver with normal echogenicity and a small gallbladder. Hepatobiliary scintigraphy at six weeks of age had no excretion. At 7 weeks of life he underwent a laparotomy and was noted to have fibrotic extrahepatic ducts and an atretic gallbladder, diagnostic of BA. He underwent a hepatoportoenterostomy and a liver biopsy was obtained at that time. The biopsy showed marked bridging fibrosis and bile duct plugging. At ten months of life he required liver transplantation due to persistent jaundice. The explant was characterized by cirrhosis with marked bile duct proliferation and bile duct plugging, findings consistent with BA. Now at sixteen months of age he is doing well from a liver standpoint.

Throughout his neonatal course, multiple other medical problems were diagnosed including hypothyroidism, seizures, developmental delay, and polysplenia. Given the presence of multiple congenital anomalies, a karyotype was sent which revealed a 47,XXY karyotype, consistent with Klinefelter Syndrome.

A total of eight CNVs called by 10 or more consecutive SNPs were detected. They ranged in size from 10 to 1,346 SNPs and 9,824 bp and 5.87 Mb. There were 6 heterozygous deletions and 2 duplications. Two of the eight CNVs were not seen in controls. He had a 1,346 SNP, 5.87 Mb heterozygous deletion on chromosome 2q37.3 extending from 236,814,680 to 242,692,820 (Figures 2B and 3). This second CNV that not seen in controls was a 529 SNP 2.9 Mb heterozygous deletion on 2q33.1. Parental DNA was not available.

The heterozygous deletion in Patient 1 on chromosome 2q37.3 is 1.76 Mb long and consists of 280 SNPs. This deletion is encompassed within the much larger deletion in Patient 2. Therefore this 1.76 Mb deletion on chromosome 2q37.3 from 240,936,900 to 242,692,820 constitutes the critical region for a BA susceptibility gene.

Discussion

Here we report two unrelated patients with BA and overlapping heterozygous deletions on chromosome 2q27.3. There are thirty genes in the 1.76 Mb region of overlap. To date there have been no reports of specific deletions or duplications associated with BA. As stated previously, the etiology of BA is largely unknown, therefore, this gene-rich deletion (Table I) is of potential clinical significance.

We hypothesize that this heterozygous deletion may confer susceptibility to BA. Deletion of this region did not occur in any of our 2,026 healthy controls and is not reported in the literature or DGV as a normal variant, yet it occurred in two of our 35 unrelated BA patients.

The fact that the deletion in Patient 1 is inherited from his unaffected father indicates that the deletion alone is not sufficient to cause BA in every case. However, this deletion may contain a susceptibility factor that predisposes to BA upon exposure to an environmental trigger at a critical time in biliary development. This theory was originally proposed by Schreiber and Kleinman who suggested that perhaps BA is the result of a multi-hit pathologic process, in which a viral or toxic insult in a genetically predisposed individual leads to BA [Schreiber et al., 1993].

Our two patients with the 2q37.3 heterozygous deletions shared several phenotypic characteristics related to their liver disease. First, they both clearly had BA. This is an important point to address, because BA can be a challenging diagnosis to make, since it is an evolving process and its histology can mimic that of other cholestatic diseases. To address this issue, a pathologist experienced in childhood liver disorders reviewed the histology slides from both explants and both cases were consistent with BA. In addition, the intra-operative findings of both patients at the time of hepatoportoenterostomy were consistent with BA. These two patients also shared similarities with regard to their clinical course (Table III). Despite having had a hepatoportoenterostomy prior to 8 weeks of age (as is the current general recommendation), both patients went on to require liver transplantation before a year of age. Studies estimate that about 75% of patients who undergo a hepatoportoenterostomy will eventually require liver transplantation [Sokol et al., 2007] and the median age of transplantation is 12 months [Schreiber et al., 2007]. In addition, both patients did well clinically following liver transplantation, as is the case in the majority of BA patients who fail hepatoportoenterostomy and require liver transplantation.

There are a few notable differences between the two cases, including the presence of other congenital anomalies in Patient 2 but not in Patient 1. The explanation for this may lie in the more complicated cytogenetic findings in Patient 2, who has Klinefelter syndrome, in addition to a 2.90 Mb heterozygous deletion on chromosome on 2q33.1 (Figure 2B). This proximal deletion includes *SATB2*, which is involved in palate formation, and may account for the submucosal cleft palate in Patient 2. A 2q32.2q33 deletion syndrome has been described based on analysis of 4 patients [Van Buggenhout et al., 2005]. Although this syndrome involves a larger deletion than that of Patient 2, Patient 2 shares many features with these 4 patients, including failure to thrive, cleft palate, prominent forehead, clubfoot, developmental issues, and seizures. Neither the 2q33.1 deletion nor Klinefelter syndrome has been associated with BA or other forms of liver disease.

There is a recognized syndrome associated with deletion of 2q37.3, but neither of our patients seems to match the phenotype described. In a recent review, 20 patients with deletions including 2q37.3 were reported [Aldred et al., 2004]. These deletions varied from 2.1 to 10.8 Mb, and all included the critical region defined by our study. The phenotypic features reported in these patients varied significantly, but common features included facial dysmorphism and developmental delay (95%), Albright hereditary osteodystrophy (AHO)-like brachymetaphalangism (55%), non-febrile seizures (35%), autistic-like features (35%), and cardiac defects (20%). None of the patients in this cohort had BA. Patient 1 (this study) had none of these features. Patient 2 had seizures and developmental delay, but did not have AHO-like brachymetaphalangism. In addition, the facial features characteristic of 95% of the cohort reported by Aldred et al. were not seen in either of our patients, i.e. round face, flattened nasal bridge, frontal bossing, deep-set eyes, upslanting palpebral fissures, anteverted nares, and thin upper lip.

There are 30 genes in the region of overlap of these two deletions (Table I). Any one of these genes could be a potential candidate for BA susceptibility. We were particularly interested in the *AGXT* gene, which is only expressed in the liver. *AGXT* is found in the

peroxisomes of liver cells. Peroxisomes play a role in the metabolism of toxic substances and fat breakdown. AGXT allows for conversion of glyoxylate to glycine. Absence or decreased level of AGXT leads to an accumulation of glyoxylate and oxalate, which can lead to renal stones. Primary hyperoxaluria type I (PH1) is a rare autosomal recessive disease that occurs due to low or absent levels of AGXT. The phenotype is variable and some patients require liver transplantation. Sequencing of the *AGXT* gene in patient 1 revealed that he has a well-characterized haplotype that results in a reduction of specific activity of AGXT [Lumb et al., 2000]. The significance of this finding in our patient is unclear. We could speculate that the reduced activity of an enzyme that plays a role in detoxification could be important in the context of the hypothesis that a toxin-mediated injury is involved in the pathogenesis of BA. However, we are not aware of an increase in BA frequency in families affected by PH1, who have an increased frequency of this polymorphism.

As we pointed out earlier, there were 192 CNVs greater than 10 SNPs in size in our cohort of 35 BA patients. We chose to focus on the deletion that occurred in more than one patient and did not occur in controls; however, there were other CNVs that may be of potential interest. For example, BA 1 had a 55 SNP 282 kb gain on 11p15.5. This region contains insulin-like growth factor 2 (*IGF-2*). *IGF-2* is reported to be overexpressed in certain forms of BA [Zhang et al., 2004]. A larger study will be useful in helping us determine if this region is affected in other patients with BA.

In summary, in this small study we identified two patients with BA and overlapping large heterozygous deletions on 2q37.3. Although there is no definite proof that BA is related to these deletions, in light of the limited understanding we have of BA and the genes contained in this region, we believe this finding should be further explored. A larger study is needed to determine if this or other regions house potential susceptibility factors for BA. Given the heterogeneity and complexity of BA, it is unlikely that BA is caused by one specific genetic alteration. Instead it is most likely caused by an interplay of environmental and genetic factors. The identification of a susceptibility locus for BA could potentially help focus future studies on the mechanisms underlying the pathogenesis of this disease and bring us closer to the development of new therapies.

Acknowledgments

This study was supported in part by the Fred and Suzanne Biesecker Pediatric Liver Center at the Children's Hospital of Philadelphia and by grant DK53104 to NBS and the NIH T32 Genetics Training Grant (GM008638-13). Genome-wide genotyping was funded by an Institute Development Award to the Center for Applied Genomics from the Children's Hospital of Philadelphia and by a grant from the Cotswold Foundation (HH). We are grateful to the patients and their families who participated in this study. We thank the technical staff in the Center for Applied Genomics and the Clinical CytoGenomics lab at The Children's Hospital of Philadelphia. We also acknowledge Laura Conlin for her input on the interpretation of the array data and David Piccoli for his continuing support. We thank the Center for Biomedical Informatics especially Hongbo Xie for his input in the analysis of array results. We would like to acknowledge Jessi Erlichman for her assistance in providing the phenotypic data.

References

- Aldred MA, Sanford RO, Thomas NS, Barrow MA, Wilson LC, Brueton LA, Bonaglia MC, Hennekam RC, Eng C, Dennis NR, Trembath RC. Molecular analysis of 20 patients with 2q37.3 monosomy: definition of minimum deletion intervals for key phenotypes. *J Med Genet.* 2004; 41:433–439. [PubMed: 15173228]
- Anneren G, Meurling S, Lilja H, Wallander J, von Döbeln U. Lethal autosomal recessive syndrome with intrauterine growth retardation, intra- and extrahepatic biliary atresia, and esophageal and duodenal atresia. *Am J Med Genet.* 1998; 78:306–307. [PubMed: 9677074]

- Birnbaum S, Ludwig KU, Reutter H, Herms S, Steffens M, Rubini M, Baluardo C, Ferrian M, Almeida de Assis N, Alblas MA, Barth S, Freudenberg J, Lauster C, Schmidt G, Scheer M, Braumann B, Berge SJ, Reich RH, Schiefke F, Hemprich A, Potzsch S, Steegers-Theunissen RP, Potzsch B, Moebus S, Horsthemke B, Kramer FJ, Wienker TF, Mossey PA, Propping P, Cichon S, Hoffmann P, Knapp M, Nothen MM, Mangold E. Key susceptibility locus for nonsyndromic cleft lip with or without cleft palate on chromosome 8q24. *Nat Genet.* 2009; 41:473–477. [PubMed: 19270707]
- Caldwell EF, Mayor LR, Thomas MG, Danpure CJ. Diet and the frequency of the alanine:glyoxylate aminotransferase Pro11Leu polymorphism in different human populations. *Hum Genet.* 2004; 115:504–509. [PubMed: 15480793]
- Cunningham ML, Sybert VP. Idiopathic extrahepatic biliary atresia: recurrence in sibs in two families. *Am J Med Genet.* 1988; 31:421–426. [PubMed: 3232705]
- Davit-Spraul A, Baussan C, Hermeziu B, Bernard O, Jacquemin E. CFC1 gene involvement in biliary atresia with polysplenia syndrome. *J Pediatr Gastroenterol Nutr.* 2008; 46:111–112. [PubMed: 18162845]
- Diskin SJ, Hou C, Glessner JT, Attiyeh EF, Laudenslager M, Bosse K, Cole K, Mosse YP, Wood A, Lynch JE, Pecor K, Diamond M, Winter C, Wang K, Kim C, Geiger EA, McGrady PW, Blakemore AI, London WB, Shaikh TH, Bradfield J, Grant SF, Li H, Devoto M, Rappaport ER, Hakonarson H, Maris JM. Copy number variation at 1q21.1 associated with neuroblastoma. *Nature.* 2009; 459:987–991. [PubMed: 19536264]
- Elia J, Gai X, Xie HM, Perin JC, Geiger E, Glessner JT, D'Arcy M, Deberardinis R, Frackelton E, Kim C, Lantieri F, Muganga BM, Wang L, Takeda T, Rappaport EF, Grant SF, Berrettini W, Devoto M, Shaikh TH, Hakonarson H, White PS. Rare structural variants found in attention-deficit hyperactivity disorder are preferentially associated with neurodevelopmental genes. *Mol Psychiatry.* 2009
- Fritsche LG, Freitag-Wolf S, Bettecken T, Meitinger T, Keilhauer CN, Krawczak M, Weber BH. Age-related macular degeneration and functional promoter and coding variants of the apolipoprotein E gene. *Hum Mutat.* 2009; 30:1048–1053. [PubMed: 19384966]
- Gunasekaran TS, Hassall EG, Steinbrecher UP, Yong SL. Recurrence of extrahepatic biliary atresia in two half sibs. *Am J Med Genet.* 1992; 43:592–594. [PubMed: 1605254]
- Harper P, Plant JW, Unger DB. Congenital biliary atresia and jaundice in lambs and calves. *Aust Vet J.* 1990; 67:18–22. [PubMed: 2334368]
- Hart MH, Kaufman SS, Vanderhoof JA, Erdman S, Linder J, Markin RS, Kruger R, Antonson DL. Neonatal hepatitis and extrahepatic biliary atresia associated with cytomegalovirus infection in twins. *Am J Dis Child.* 1991; 145:302–305. [PubMed: 1848391]
- HH AK, El-Ayyouti M, Hawas S, Abdalla A, Al-Tonbary Y, Bassiouny M, Boneberg A, El-Sallab S. HLA in Egyptian children with biliary atresia. *J Pediatr.* 2002; 141:432–433. [PubMed: 12219069]
- Hyams JS, Glaser JH, Leichtner AM, Morecki R. Discordance for biliary atresia in two sets of monozygotic twins. *J Pediatr.* 1985; 107:420–422. [PubMed: 2993572]
- Khaimin VM. Congenital atresia of the bile ducts in twin boys. *Vopr Okhr Materin Det.* 1969; 14:84–85. [PubMed: 5349909]
- Kobayashi K, Kubota M, Okuyama N, Hirayama Y, Watanabe M, Sato K. Mother-to-daughter occurrence of biliary atresia: a case report. *J Pediatr Surg.* 2008; 43:1566–1568. [PubMed: 18675657]
- Kugathasan S, Baldassano RN, Bradfield JP, Sleiman PM, Imielinski M, Guthery SL, Cucchiara S, Kim CE, Frackelton EC, Annaiah K, Glessner JT, Santa E, Willson T, Eckert AW, Bonkowski E, Shaner JL, Smith RM, Otieno FG, Peterson N, Abrams DJ, Chiavacci RM, Grundmeier R, Mamula P, Tomer G, Piccoli DA, Monos DS, Annese V, Denson LA, Grant SF, Hakonarson H. Loci on 20q13 and 21q22 are associated with pediatric-onset inflammatory bowel disease. *Nat Genet.* 2008; 40:1211–1215. [PubMed: 18758464]
- Kulkarni H, Marconi VC, Agan BK, McArthur C, Crawford G, Clark RA, Dolan MJ, Ahuja SK. Role of CCL3L1-CCR5 genotypes in the epidemic spread of HIV-1 and evaluation of vaccine efficacy. *PLoS One.* 2008; 3:e3671. [PubMed: 18989363]
- Lachaux A, Descos B, Plauchu H, Wright C, Louis D, Raveau J, Hermier M. Familial extrahepatic biliary atresia. *J Pediatr Gastroenterol Nutr.* 1988; 7:280–283. [PubMed: 3351711]

- Lee HC, Chang TY, Yeung CY, Chan WT, Jiang CB, Chen WF, Chan HW, Liu HF, Lin M, Lee YJ. Genetic Variation in the Vascular Endothelial Growth Factor Gene is Associated With Biliary Atresia. *J Clin Gastroenterol*. 2009
- Lumb MJ, Danpure CJ. Functional synergism between the most common polymorphism in human alanine:glyoxylate aminotransferase and four of the most common disease-causing mutations. *J Biol Chem*. 2000; 275:36415–36422. [PubMed: 10960483]
- Mamtani M, Rovin B, Brey R, Camargo JF, Kulkarni H, Herrera M, Correa P, Holliday S, Anaya JM, Ahuja SK. CCL3L1 gene-containing segmental duplications and polymorphisms in CCR5 affect risk of systemic lupus erythematosis. *Ann Rheum Dis*. 2008; 67:1076–1083. [PubMed: 17971457]
- Maris JM, Mosse YP, Bradfield JP, Hou C, Monni S, Scott RH, Asgharzadeh S, Attiyeh EF, Diskin SJ, Laudenslager M, Winter C, Cole KA, Glessner JT, Kim C, Frackelton EC, Casalunovo T, Eckert AW, Capasso M, Rappaport EF, McConville C, London WB, Seeger RC, Rahman N, Devoto M, Grant SF, Li H, Hakonarson H. Chromosome 6p22 locus associated with clinically aggressive neuroblastoma. *N Engl J Med*. 2008; 358:2585–2593. [PubMed: 18463370]
- Mazziotti MV, Willis LK, Heuckeroth RO, LaRegina MC, Swanson PE, Overbeek PA, Perlmutter DH. Anomalous development of the hepatobiliary system in the Inv mouse. *Hepatology*. 1999; 30:372–378. [PubMed: 10421642]
- Monico CG, Rossetti S, Schwanz HA, Olson JB, Lundquist PA, Dawson DB, Harris PC, Milliner DS. Comprehensive mutation screening in 55 probands with type 1 primary hyperoxaluria shows feasibility of a gene-based diagnosis. *J Am Soc Nephrol*. 2007; 18:1905–1914. [PubMed: 17460142]
- Morris BD, Nuss D, Winship WS. Biliary atresia in a twin. *S Afr Med J*. 1977; 51:469–470. [PubMed: 860173]
- Nevin NC, Bell M, Frazer MJ, Froggatt P. Congenital extrahepatic biliary atresia in two brothers. *J Med Genet*. 1969; 6:379–381. [PubMed: 5365945]
- Poovorawan Y, Chongsrisawat V, Tanunythawongse C, Norapaksunthorn T, Mutirangura A, Chandrakamol B. Extrahepatic biliary atresia in twins: zygosity determination by short tandem repeat loci. *J Med Assoc Thai*. 1996; 79 1:S119–124. [PubMed: 9071076]
- Purdue PE, Takada Y, Danpure CJ. Identification of mutations associated with peroxisome-to-mitochondrion mistargeting of alanine:glyoxylate aminotransferase in primary hyperoxaluria type 1. *J Cell Biol*. 1990; 111:2341–2351. [PubMed: 1703535]
- Redon R, Ishikawa S, Fitch KR, Feuk L, Perry GH, Andrews TD, Fiegler H, Shapero MH, Carson AR, Chen W, Cho EK, Dallaire S, Freeman JL, Gonzalez JR, Gratacos M, Huang J, Kalaitzopoulos D, Komura D, MacDonald JR, Marshall CR, Mei R, Montgomery L, Nishimura K, Okamura K, Shen F, Somerville MJ, Tchinda J, Valsesia A, Woodward C, Yang F, Zhang J, Zerjal T, Armengol L, Conrad DF, Estivill X, Tyler-Smith C, Carter NP, Aburatani H, Lee C, Jones KW, Scherer SW, Hurles ME. Global variation in copy number in the human genome. *Nature*. 2006; 444:444–454. [PubMed: 17122850]
- Schreiber RA, Barker CC, Roberts EA, Martin SR, Alvarez F, Smith L, Butzner JD, Wrobel I, Mack D, Moroz S, Rashid M, Persad R, Levesque D, Brill H, Bruce G, Critch J. Biliary atresia: the Canadian experience. *J Pediatr*. 2007; 151:659–665. 665, e651. [PubMed: 18035148]
- Schreiber RA, Kleinman RE. Genetics, immunology, and biliary atresia: an opening or a diversion? *J Pediatr Gastroenterol Nutr*. 1993; 16:111–113. [PubMed: 8450373]
- Schweizer P, Kerremans J. Discordant findings in extrahepatic bile duct atresia in 6 sets of twins. *Z Kinderchir*. 1988; 43:72–75. [PubMed: 3291470]
- Shaikh TH, Gai X, Perin JC, Glessner JT, Xie H, Murphy K, O'Hara R, Casalunovo T, Conlin LK, D'Arcy M, Franckelton EC, Geiger EA, Haldeman-Englert C, Imielinski M, Kim CE, Medne L, Annaiah K, Bradfield J, Dabaghyan E, Eckert A, Onyiah CC, Ostapenko S, Otieno FG, Santa E, Shaner JL, Skraban R, Smith RM, Elia J, Goldmuntz E, Spinner NB, Zackai EH, Chiavacci RM, Grundmeier R, Rappaport EF, Grant SF, White PS, Hakonarson H. High-resolution mapping and analysis of copy number variations in the human genome: A data resource for clinical and research applications. *Genome Res*. 2009
- Silveira TR, Salzano FM, Howard ER, Mowat AP. Extrahepatic biliary atresia and twinning. *Braz J Med Biol Res*. 1991; 24:67–71. [PubMed: 1823220]

- Smith BM, Laberge JM, Schreiber R, Weber AM, Blanchard H. Familial biliary atresia in three siblings including twins. *J Pediatr Surg.* 1991; 26:1331–1333. [PubMed: 1812269]
- Sokol RJ, Shepherd RW, Superina R, Bezerra JA, Robuck P, Hoofnagle JH. Screening and outcomes in biliary atresia: summary of a National Institutes of Health workshop. *Hepatology.* 2007; 46:566–581. [PubMed: 17661405]
- Strickland AD, Shannon K, Coln CD. Biliary atresia in two sets of twins. *J Pediatr.* 1985; 107:418–420. [PubMed: 4040965]
- Tarn AC, von Schnakenburg C, Rumsby G. Primary hyperoxaluria type 1: diagnostic relevance of mutations and polymorphisms in the alanine:glyoxylate aminotransferase gene (AGXT). *J Inherit Metab Dis.* 1997; 20:689–696. [PubMed: 9323564]
- Tiao MM, Tsai SS, Kuo HW, Chen CL, Yang CY. Epidemiological features of biliary atresia in Taiwan, a national study 1996–2003. *J Gastroenterol Hepatol.* 2008; 23:62–66. [PubMed: 17725591]
- Van Buggenhout G, Van Ravenswaaij-Arts C, Mc Maas N, Thoelen R, Vogels A, Smeets D, Salden I, Matthijs G, Fryns JP, Vermeesch JR. The del(2)(q32.2q33) deletion syndrome defined by clinical and molecular characterization of four patients. *Eur J Med Genet.* 2005; 48:276–289. [PubMed: 16179223]
- Werlin SL. Extrahepatic biliary atresia in one of twins. *Acta Paediatr Scand.* 1981; 70:943–944. [PubMed: 7198861]
- Whitten WW, Adie GC. Congenital biliary atresia; report of three cases; two occurring in one family. *J Pediatr.* 1952; 40:539–548. [PubMed: 14928160]
- Zhang DY, Sabla G, Shivakumar P, Tiao G, Sokol RJ, Mack C, Shneider BL, Aronow B, Bezerra JA. Coordinate expression of regulatory genes differentiates embryonic and perinatal forms of biliary atresia. *Hepatology.* 2004; 39:954–962. [PubMed: 15057899]

FIGURE 1.

Patient 1 at 17 years of age. While no overt dysmorphic features were identified, the patient does have an elongated face and a broad nasal tip, which were shared with his father. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

FIGURE 2.

Illumina Beadstudio output from our two patients with 2q37.3 deletions.

- A.** Patient 1 has a 1.76 Mb deletion, which can be seen as loss of heterozygosity on the B allele frequency and decreased log₂R ratio.
- B.** The black arrow indicates the 5.87 Mb deletion on 2q37.3 in Patient 2. He also has a 2.90 heterozygous deletion on 2q33.1 which starts at 199,833,395 and ends at 202,729,535, indicated by the white arrow.

FIGURE 3.

UCSC Genome Browser view of chromosome 2q37.3, 236,814,680-242,692,820.

Each horizontal line represents a CNV in one individual. Deleted portion of chromosome 2q37.3 in Patient 1 and Patient 2. Deletions seen in 2,026 controls are shown beneath the patient samples. Note that no controls have deletions spanning this region. Refseq genes in this region are shown towards the bottom of the figure. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

FIGURE 4.

Fluorescence in situ hybridization shows a heterozygous deletion on the telomere of chromosome 2. Chromosome 2 centromere probe is visible on both chromosomes, but 2q telomere probe is missing from one homologue (deletion). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE I

Genes in deleted overlapping 2q37.3 region

Gene Symbol	Role	Highest expression
<i>GPC1</i>	Cell division and growth regulation	heart
<i>CAPN10</i>	Associated with non-insulin-dependent diabetes mellitus (NIDDM)	Ubiquitous
<i>GPR35</i>	G protein coupled receptor	Adult lung, small intestine, colon and stomach
<i>AQP12A/B</i>	Transport water, small neutral molecules, and ions out of and between cells.	pancreas, salivary gland, lung
<i>KIF1A</i>	May play a role in the development of axonal neuropathies resulting from impaired axonal transport.	Brain
<i>AGXT</i>	Hepatic peroxisomal enzyme involved in metabolism of glyoxylate. Primary hyperoxaluria type I is an autosomal recessive disease involving this gene.	Liver
<i>PASK</i>	Intracellular signaling pathways in response to both extrinsic and intrinsic stimuli.	T cells
<i>HDLBP</i>	Binds high density lipoprotein molecules and may function in the removal of excess cellular cholesterol	pancreas, salivary gland, lung
<i>BOK</i>	May play a role in apoptosis	Liver
<i>ATG4B</i>	Involved in autophagy, process by which endogenous proteins and damaged organelles are destroyed intracellularly.	T/B cells, thyroid
<i>ING5</i>	Similar to <i>ING1</i> , a tumor suppressor protein	T/B cells, thyroid
<i>D2HGDH</i>	Encodes D-2hydroxyglutarate dehydrogenase, a mitochondrial enzyme. Mutations in this gene are present in D-2-hydroxyglutaric aciduria, a rare recessive neurometabolic disorder causing developmental delay, epilepsy, hypotonia, and dysmorphic features.	Liver, kidney

There are 18 genes not included in the table, for which function is unknown, these include: *ANKMY1*, *FLJ33590*, *DTYMK*, *THAP4*, *TMEM16G*, *STK25*, *FARP2*, *SNED1*, *RNPEPL1*, *DUSP28*, *C2orf5*, *C2orf54*, *SEPT2*

Table II

Polymorphisms on the non-deleted chromosome in Patient 1 and his father

Exon	Sequence Variant	Proband	Father
1	c.32C>T (Pro11Leu)	T	C
IVS1	c.165+16A>G	G	A
IVS1	IVS1+74 bp	duplication	-
2	c.264 C>T	T	C
IVS2	c.358+13C>T	T	C
IVS6	c.680+17C>T	T	C
10	c.102A>G (Ile340Met)	G	A
11	c.1220C>A (3'UTR)	C	A

TABLE III

Comparison of the two patients with heterozygous deletion of 2q37.3

	Patient 1	Patient 2
Gestational Age	40 weeks	38 weeks
Birth weight	3836 grams	3030 grams
Exposures during pregnancy	Cleaning agents	Varicella Alcohol
Onset of Jaundice	3 weeks	Day 1
Age at Liver biopsy	3 weeks	7 weeks
Liver biopsy results	Patchy portal fibrosis and minimal bile duct proliferation	Marked bridging fibrosis and bile plugging
Age at time of hepatoportoenterostomy	5 weeks	7 weeks
Laboratory Studies (at 3-4 wks)		
Total bilirubin mg/dL	7.5	6.8
Direct bilirubin mg/dL	4.0	4.9
AST U/L	30	104
ALT U/L	31	52
GGT U/L	270	691
Transplant	Yes	Yes
Age at Transplant	8 months	10 months
Explant histology	marked bile duct proliferation and portal fibrosis	marked bile duct proliferation and cirrhosis
Other anatomic anomalies	Venous hemangioma in right tentorium	Dysmorphic facies prominent forehead pointy chin asymmetric palpebral fissures Failure to thrive Abnormally long fingers Syndactyly of 3 rd and 4 th toe Clubfoot Umbilical hernia Submucosal cleft palate Patent foramen ovale Polysplenia
Other medical issues	None	Hypothyroidism Karyotype - XXY
Developmental Issues	None	Developmental delay

	Patient 1	Patient 2
		Seizures
Location of 2q37.3 deletion	240,936,900-242,692,820	236,814,680-242,692,820
Size of 2q37.3 deletion	1.76 Mb 280 SNP's	5.87 Mb 1,346 SNP's