



Genetic and Epigenetic Susceptibility in Biliary Atresia: Developmental Pathways, Molecular Alterations, and Clinical Implications

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Received: 28 October 2024, **Accepted:** 17 November 2024, **Published:** 20 November 2024

ABSTRACT

Background: Biliary atresia arises through heterogeneous developmental, genetic and acquired processes, and isolated associations do not yet explain the variable clinical phenotype. **Objective:** To critically integrate evidence available through 2024 while identifying mechanisms relevant to pediatric hepatology and limitations of current translational studies. **Methods:** This narrative review integrates published clinical, genomic and experimental evidence, distinguishing human associations from functional findings. **Results:** Regulatory mechanisms appear interconnected across epithelial maturation, immune injury and matrix remodeling. Biomarker development remains limited by small heterogeneous samples, varying comparator groups and inadequate independent validation. Proposed therapeutic targets frequently derive from experimental systems rather than pediatric clinical trials. **Conclusion:** More informative research will combine age-appropriate clinical phenotyping, cell-specific molecular methods, independent replication and outcomes that matter to children.

Keywords: biliary atresia, genetic susceptibility, epigenetics, bile duct development

INTRODUCTION

The classic clinical description of biliary atresia conceals considerable biological variation. Some infants have associated defects of laterality, while others present with apparently isolated progressive cholestasis. Understanding this difference requires examination of inherited susceptibility, developmental processes and secondary responses to tissue injury. The available literature offers credible leads, but a statistically linked region or changed gene-expression profile does not by itself establish a mechanism of disease. [1]

The evidence base is uneven. Cohort studies, human biopsy analyses, cell experiments and model organisms answer different questions and cannot be combined as if they offered the same level of inference. This review gives particular attention to where observations were made, whether the comparator resembles the clinical diagnostic problem, and how securely an association has been replicated. The aim is to identify research approaches that connect candidate mechanisms to clinically meaningful pediatric outcomes. [1]

Biliary atresia as a developmental susceptibility disorder

Biliary atresia should be understood as a clinical phenotype with more than one biological route to the same final pattern of duct obstruction. A patient with laterality defects differs from an infant whose biliary tree apparently formed normally before a perinatal inflammatory event. This heterogeneity complicates the interpretation of genome-wide studies that combine all cases. Rather than search for one deterministic mutation, a more realistic model considers developmental vulnerability together with environmental or immune triggers. Molecular studies need careful clinical phenotyping if they are to identify mechanisms rather than merely population associations. [1]

The architecture of the newborn biliary tree is still undergoing functional adaptation when biliary atresia becomes clinically apparent. Cholangiocytes must establish epithelial polarity, coordinate transport of bile constituents, and preserve a barrier between the duct lumen and the surrounding portal tissue. Failure at any one level can magnify the toxicity of retained bile constituents and expose the epithelium to inflammatory signals. This developmental perspective explains why a relatively short period of injury may produce extensive duct obstruction in infancy. It also cautions against assuming that a lesion identified after birth necessarily originated at that moment. Clinical recognition usually follows jaundice or acholic stools, whereas pathogenic events may predate both signs. A convincing mechanism should therefore explain timing, tissue



specificity, and progression rather than merely demonstrate that a biomarker differs between affected and unaffected infants. [2]

The clinical significance of biliary atresia as a developmental susceptibility disorder depends on whether the proposed mechanism holds across phenotypes. Consistent case definitions, ancestry-aware analysis and independent functional replication are necessary before a candidate is used to stratify infants. Tissue obtained late in the course may reveal a strong molecular response without revealing the initiating process. A useful investigation should state whether its objective is discovering inherited risk, explaining an injury pathway or predicting progression, because the required controls differ. [3]

Embryonic bile duct formation and remodeling

Development of the intrahepatic ducts begins with the ductal plate, followed by selective remodeling and organization of patent tubular structures. Extrahepatic duct morphogenesis follows a related but distinct developmental program. Signaling interactions between hepatoblasts, portal mesenchyme and adjacent epithelial populations influence branching and polarity. Variants that disturb this coordinated process may leave the newborn bile duct vulnerable without producing complete anatomic absence at birth. A developmental explanation is particularly plausible for cases accompanied by other congenital anomalies, but cannot automatically account for the common isolated phenotype. [4]

Biliary epithelial cells are specialized for secretion, sensing, and controlled contact with the duct lumen. Their apical junctions limit uncontrolled movement of bile constituents into portal tissue, while the cytoskeleton preserves cell shape during changing luminal flow. The primary cilium detects mechanical and chemical information and participates in signals that organize epithelial behavior. Reports of abnormal cilia in biliary atresia support the possibility that the duct epithelium is vulnerable before complete obstruction develops. Nevertheless, structural abnormalities in diseased tissue can also be consequences of chronic inflammation and altered bile flow. Assigning causality requires comparison with age-matched cholestatic conditions, assessment of early specimens, and functional testing rather than reliance on late-stage microscopic differences alone. [5]

The clinical significance of embryonic bile duct formation and remodeling depends on whether the proposed mechanism holds across phenotypes. Consistent case definitions, ancestry-aware analysis and independent functional replication are necessary before a candidate is used to stratify infants. Tissue obtained late in the course may reveal a strong molecular response without revealing the initiating process. A useful investigation should state whether its objective is discovering inherited risk, explaining an injury pathway or predicting progression, because the required controls differ. [6]

Phenotype-specific susceptibility

Anatomical classification describes the level of obstruction, whereas etiological subgroups require information on associated malformations, family history and time of clinical onset. Biliary atresia splenic malformation syndrome is informative because defects in left–right patterning suggest a developmental pathway. Yet a classification based solely on splenic findings may miss related laterality anomalies. Genetic cohorts should record cardiac anatomy, situs and intestinal rotation, alongside duct morphology. This permits better analysis than treating every infant with biliary atresia as biologically interchangeable. [7]

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The clinical significance of phenotype-specific susceptibility depends on whether the proposed mechanism holds across phenotypes. Consistent case definitions, ancestry-aware analysis and independent functional replication are necessary before a candidate is used to stratify infants. Tissue obtained late in the course may reveal a strong molecular response without revealing the initiating process. A useful investigation should state whether its objective is discovering inherited risk, explaining an injury pathway or predicting progression, because the required controls differ. [9]

Genome-wide association and statistical interpretation

Genome-wide association studies identify genomic intervals correlated with disease risk, not necessarily a causal gene or mechanism. A lead variant can act as a marker for several linked changes, and the nearest gene may not be the functional target. Follow-up requires replication in independent populations, expression analysis in relevant tissues and a plausible functional result. Rare disease sample sizes make estimates sensitive to population structure and choice of controls. Risk allele



associations should not be presented as clinical tests unless the absolute predictive value has been demonstrated. [10]

Histopathology remains valuable because it reveals the relationship between bile duct injury, ductular proliferation, portal inflammation, and fibrosis. However, diagnostic and prognostic use are distinct. A finding that helps distinguish biliary obstruction from other infantile cholestatic disorders may not independently predict progression after surgery. Likewise, advanced fibrosis at presentation may reflect delayed recognition, a rapidly progressive phenotype, or both. Comparative studies should report age, duration of symptoms, bilirubin concentration, and prior treatments to avoid conflating severity with causation. A pathological feature must be interpreted within the patient trajectory, not simply scored as present or absent. The same principle applies to experimental biomarkers measured in late samples. [11]

The clinical significance of genome-wide association and statistical interpretation depends on whether the proposed mechanism holds across phenotypes. Consistent case definitions, ancestry-aware analysis and independent functional replication are necessary before a candidate is used to stratify infants. Tissue obtained late in the course may reveal a strong molecular response without revealing the initiating process. A useful investigation should state whether its objective is discovering inherited risk, explaining an injury pathway or predicting progression, because the required controls differ. [12]

The chromosome 10q24 susceptibility region

The 10q24 region attracted attention through human genetic studies linking common variants near ADD3 and XPNPEP1 with biliary atresia. Subsequent replication and experimental analyses strengthened interest in the region. Nevertheless the molecular consequences may vary by tissue, allele background and developmental stage. γ -Adducin participates in cytoskeletal integrity and epithelial organization, making disrupted duct function plausible. This review treats the locus within a wider network of susceptibility findings rather than assuming that any single expression direction fully explains human disease. [13]

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GPC1 and extracellular developmental signals

Glypican-1 participates in extracellular regulation of growth factor signaling and has been implicated through human genomic evidence coupled with zebrafish experiments. Such cross-species findings are valuable because a gene association gains credibility when disturbed function produces a biliary phenotype. Yet a zebrafish duct defect may resemble only part of human biliary atresia. Species differences, genetic compensation and developmental timing must be assessed when translating those experiments into an explanation for fibro-obliterative disease in infants. [16]

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The clinical significance of *gpc1* and extracellular developmental signals depends on whether the proposed mechanism holds across phenotypes. Consistent case definitions, ancestry-aware analysis and independent functional replication are necessary before a candidate is used to stratify infants. Tissue obtained late in the course may reveal a strong molecular response without revealing the initiating process. A useful investigation should state whether its objective is discovering inherited risk, explaining an injury pathway or predicting progression, because the required controls differ. [18]

EFEMP1 and matrix-associated susceptibility



The identification of a susceptibility signal within EFEMP1 broadened genetic investigation beyond the original 10q24 region. A matrix-associated candidate raises questions about the interaction between epithelial stability and extracellular remodeling. The presence of a statistically associated locus does not establish that abnormal matrix deposition is the first event. Functional studies should distinguish a primary effect on duct formation from amplification of fibrosis after obstruction. Replication across populations and assessment of allele-specific expression remain important steps toward determining its biological contribution. [19]

Fibrosis develops when extracellular matrix production exceeds the tissue's ability to remodel and resolve it. In biliary atresia, activated myofibroblast populations arise in an environment shaped by cholestasis, inflammatory cytokines, ductular signaling, and changes in tissue stiffness. Portal fibroblasts and hepatic stellate cells can contribute to this matrix-producing response, although their relative roles vary by model and disease stage. Collagen accumulation distorts portal architecture, increases resistance to blood flow, and ultimately contributes to the complications of cirrhosis. The severity of fibrosis may therefore reflect both the duration of obstruction and the biology of tissue repair. A static collagen score provides important prognostic information but does not fully capture whether the fibrogenic process remains active. [20]

The clinical significance of efemp1 and matrix-associated susceptibility depends on whether the proposed mechanism holds across phenotypes. Consistent case definitions, ancestry-aware analysis and independent functional replication are necessary before a candidate is used to stratify infants. Tissue obtained late in the course may reveal a strong molecular response without revealing the initiating process. A useful investigation should state whether its objective is discovering inherited risk, explaining an injury pathway or predicting progression, because the required controls differ. [21]

ARF6 and intracellular trafficking

ARF6 is a small GTPase that influences membrane trafficking, actin remodeling and cellular polarity. Human association and experimental model data have suggested a contribution to biliary atresia susceptibility. Altered trafficking might affect apical surface organization or the response of cholangiocytes to injury, but multiple pathways could generate a similar duct phenotype. Functional claims therefore require more than a knockdown-associated abnormality. Quantitative assessment of bile flow, localization of polarity proteins and targeted rescue are useful complements to simple morphological observation. [22]

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Laterality genes and ciliary pathways

Variants involving genes such as CFC1 and PKD1L1 are especially relevant to infants with biliary atresia and laterality defects. Establishing the left–right body axis depends on ciliary signaling during embryogenesis. Disruption may coincide with cardiac, splenic and intestinal abnormalities. These associations strengthen developmental models but do not imply that every infant with a duct lesion has a motile ciliary disorder. Genetic counseling must acknowledge variable penetrance, uncertainty surrounding individual variants and the limits of current testing. [25]

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Structural genomic variation and rare familial disease

Copy number changes and unusual familial presentations may reveal susceptibility mechanisms that are not captured by common-variant studies. A rare deletion affecting a developmental regulator can produce multiple organ findings and illuminate the role of a pathway. Such examples are mechanistically valuable even when they explain few population cases. Clinical interpretation should integrate segregation data, phenotype concordance and functional evidence. Describing every rare variant as pathogenic without these supporting observations risks overstating the contribution of genomic testing to clinical diagnosis. [28]

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The clinical significance of structural genomic variation and rare familial disease depends on whether the proposed mechanism holds across phenotypes. Consistent case definitions, ancestry-aware analysis and independent functional replication are necessary before a candidate is used to stratify infants. Tissue obtained late in the course may reveal a strong molecular response without revealing the initiating process. A useful investigation should state whether its objective is discovering inherited risk, explaining an injury pathway or predicting progression, because the required controls differ. [30]

DNA methylation in duct development

DNA methylation regulates the accessibility of genomic regions and can change during cell differentiation. Experimental evidence linking disrupted methylation to biliary defects supports an epigenetic contribution to disease susceptibility. Tissue-level methylation differences in affected infants, however, may reflect inflammation, altered cellular composition or advanced fibrosis. Studies that measure changes after diagnosis cannot determine whether the epigenetic alteration preceded duct injury. Cell-specific assessment and comparison with other cholestatic disorders are needed to support a causal interpretation. [31]

Bulk tissue analysis has identified many inflammatory and fibrotic pathways, but it combines signals from epithelial, immune, stromal, and vascular populations. A rise in a particular transcript can result from higher expression within cells or simply from increased abundance of cells that already express it. Single-cell and spatial transcriptomic methods offer ways to separate these explanations and place cell states within the altered liver architecture. Studies through 2024 have begun to reveal heterogeneous immune and fibrogenic neighborhoods rather than a single uniform hepatic response. These findings refine biological hypotheses, but computational interaction maps are predictions requiring experimental validation. The presence of complementary ligand and receptor transcripts does not prove that signaling occurred. [32]

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Histone regulation and chromatin state

Histone modifications and chromatin remodeling influence the transcriptional programs through which progenitor cells adopt hepatocyte or cholangiocyte identities. These systems also respond to oxidative stress and inflammatory cues. A disease-associated chromatin signature could therefore encode developmental vulnerability or an acquired response to obstruction. Pediatric biopsy samples are particularly challenging because stage of disease and tissue composition vary. Integration with transcriptional and cellular data can help separate altered regulatory state from the simple presence of more fibroblasts or immune cells. [34]

Ductular reaction is a prominent response to cholestatic damage and reflects proliferation or expansion of cells with biliary



characteristics around portal tracts. It may contribute to restoration of drainage pathways, but persistent expansion also occurs alongside inflammation and scar formation. The coexistence of repair-associated structures with severe fibrosis illustrates why a morphological finding can have both adaptive and maladaptive interpretations. Distinguishing these possibilities requires attention to cell lineage, differentiation state, and local signals. Conventional histology can document proliferation and architectural disturbance but cannot by itself show whether a new ductular cell originated from a cholangiocyte, progenitor-like population, or another hepatic lineage. Translational models should test the functional contribution of these cell states. [35]

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Noncoding RNAs as epigenetic modulators

MicroRNAs and long noncoding RNAs may modify gene expression and communicate with broader chromatin and signaling networks. Their roles are not interchangeable: some affect messenger RNA stability, whereas others influence transcription or molecular scaffolding. The existence of a regulatory interaction in cell culture does not establish that it determines neonatal disease susceptibility. Molecular reviews should state whether an observation comes from patient tissue, a model organism or a perturbation experiment. This distinction is essential when considering an RNA regulator as a therapeutic target or screening marker. [37]

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Environmental triggers in genetically susceptible infants

The sporadic distribution of biliary atresia and experimental toxic-injury models have prompted investigation of environmental exposures. Bilatresone-associated cholangiocyte injury illustrates that a chemical can preferentially damage the extrahepatic bile duct under experimental conditions. Such a model provides a mechanistic tool, not proof that a specific toxin causes most human cases. Oxidative defense, duct maturation and host genetic background could change susceptibility to a shared exposure. Studies of environmental effects should avoid assuming causation from clustering or animal phenotypes alone. [39]

An environmental contribution has long been considered because biliary atresia is usually sporadic and shows geographic variation. Experimental work with the naturally occurring compound bilatresone established that a toxic exposure can injure extrahepatic cholangiocytes in a tissue-selective manner. The importance of this model is not that it identifies a confirmed human toxin, but that it demonstrates a biologically plausible pathway from chemical stress to epithelial dysfunction. Effects on glutathione-dependent protection and cellular integrity make oxidative injury a testable mediator of damage. Human exposure to an equivalent agent has not been established, and laboratory doses cannot be converted into assumptions about infants. The translational question is whether oxidative stress is a common final mechanism across otherwise different initiating insults. [40]

The clinical significance of environmental triggers in genetically susceptible infants depends on whether the proposed mechanism holds across phenotypes. Consistent case definitions, ancestry-aware analysis and independent functional replication are necessary before a candidate is used to stratify infants. Tissue obtained late in the course may reveal a strong molecular response without revealing the initiating process. A useful investigation should state whether its objective is discovering inherited risk, explaining an injury pathway or predicting progression, because the required controls differ. [41]

Immune response as a modifier of penetrance



Innate and adaptive immune activation can extend epithelial injury and contribute to progressive obliteration. Genetic variation might affect how strongly an infant responds to an initial insult, while epigenetic alterations can emerge during sustained inflammation. Separating inherited risk from downstream immune changes is difficult when samples are obtained after symptomatic disease has developed. Longitudinal specimens or well-defined animal models may clarify the sequence. The strongest causal account will connect genotype to a specific cellular defect and then to a reproducible disease-relevant outcome. [42]

Viral infection is among the proposed initiating or amplifying influences, but the evidence does not support assigning one pathogen as the cause in all infants. Biliary epithelial cells can respond to viral nucleic acids through pattern-recognition pathways, producing interferons and chemokines that recruit local immune cells. These responses are protective in ordinary infection but may become damaging when they persist or are exaggerated in a small developing duct. Experimental viral models have been crucial for mapping inflammatory cascades, yet they cannot be treated as direct replicas of every human phenotype. Interpretation of viral detection also requires care: positive testing near surgery may indicate concurrent infection, prolonged shedding, or secondary vulnerability rather than a primary etiological event. [43]

The clinical significance of immune response as a modifier of penetrance depends on whether the proposed mechanism holds across phenotypes. Consistent case definitions, ancestry-aware analysis and independent functional replication are necessary before a candidate is used to stratify infants. Tissue obtained late in the course may reveal a strong molecular response without revealing the initiating process. A useful investigation should state whether its objective is discovering inherited risk, explaining an injury pathway or predicting progression, because the required controls differ. [44]

Multimic phenotyping and disease subgroups

Single-cell transcriptional methods and spatial tissue assays can reveal which cells carry a particular molecular signal. Combining such approaches with genotyping and epigenomic profiling may identify subgroups obscured by conventional case definitions. Integration is difficult, however, because liver tissue is usually obtained at different disease stages and surgical ages. Computational clusters need independent biological validation before being called new etiological categories. The useful endpoint is a set of reproducible mechanisms linked to prognosis or treatment response, not a proliferation of unsupervised labels. [45]

Bulk tissue analysis has identified many inflammatory and fibrotic pathways, but it combines signals from epithelial, immune, stromal, and vascular populations. A rise in a particular transcript can result from higher expression within cells or simply from increased abundance of cells that already express it. Single-cell and spatial transcriptomic methods offer ways to separate these explanations and place cell states within the altered liver architecture. Studies through 2024 have begun to reveal heterogeneous immune and fibrogenic neighborhoods rather than a single uniform hepatic response. These findings refine biological hypotheses, but computational interaction maps are predictions requiring experimental validation. The presence of complementary ligand and receptor transcripts does not prove that signaling occurred. [46]

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Clinical translation, genetic counseling and prevention

A susceptibility variant is not synonymous with a diagnostic marker. Biliary atresia is uncommon, and even a robust relative-risk association may have poor predictive value in population screening. Molecular results could eventually refine risk classification or identify pathways for intervention, but they cannot replace direct evaluation of an infant with conjugated jaundice. Counseling should distinguish established syndromic disease genes from common risk loci and research-only candidates. Priority should be given to equitable multicenter validation and to preserving the urgency of clinical referral. [48]

The next phase of research should focus on establishing temporal order. Most available human liver samples are collected after clinical suspicion has already developed, making it difficult to reconstruct the earliest events. Well-annotated biospecimen collections, developmental reference datasets, and links to surgical and long-term outcomes would improve interpretation. Analyses should retain separate categories for embryonic-associated, cystic, isolated, and infection-associated presentations where data permit. Standardized control groups and replication across populations can reduce overinterpretation of small molecular discoveries. Experimental systems remain valuable for causal testing but should be judged by how faithfully they reproduce the relevant human stage rather than by visual resemblance alone. [49]

The clinical significance of clinical translation, genetic counseling and prevention depends on whether the proposed



mechanism holds across phenotypes. Consistent case definitions, ancestry-aware analysis and independent functional replication are necessary before a candidate is used to stratify infants. Tissue obtained late in the course may reveal a strong molecular response without revealing the initiating process. A useful investigation should state whether its objective is discovering inherited risk, explaining an injury pathway or predicting progression, because the required controls differ. [50]

CONCLUSION

Biliary atresia susceptibility arises from several interacting developmental and acquired processes. Genome-wide signals, syndromic genes and epigenetic changes provide complementary clues, but none presently offers a complete explanation or replaces prompt clinical evaluation. Coordinated genotype, tissue and longitudinal phenotype studies are needed to transform these findings into clinically useful risk classification.

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