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MicroRNAs in Pediatric Cholestatic Liver Diseases: Molecular Regulation, Diagnostic Biomarkers, and Therapeutic Perspectives

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ABSTRACT

Background: Infantile cholestatic liver disease develops within a rapidly changing regulatory environment, yet tissue-specific molecular signals are difficult to interpret and have not entered routine diagnosis. 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: microRNAs, neonatal cholestasis, biliary atresia, circulating biomarkers

INTRODUCTION

The clinical and molecular study of pediatric cholestasis occupies a distinctive position: the affected organ is still maturing while serious injury develops. Gene-expression studies can identify candidates that connect epithelial dysfunction, inflammation and fibrosis, yet the origin and timing of the observed signals are often uncertain. These features require a synthesis that distinguishes upstream regulation from tissue responses observed after the onset of cholestasis. [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]

Why microRNAs matter in the infant liver

MicroRNAs are suited to the biology of neonatal liver disease because a modest change in gene regulation may affect several cell populations at once. Hepatocytes, cholangiocytes, Kupffer cells and activated mesenchymal cells do not respond to injury through isolated pathways. The relevance of a particular microRNA therefore depends on cell type, maturation and the timing of the insult. Pediatric cholestatic disease offers an opportunity to examine these interactions during rapid organ growth, but also creates a risk of mistaking age-dependent physiological variation for a disease-specific signal. [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]

For why micrornas matter in the infant liver, interpretation should separate the initiating susceptibility from changes driven by persistent cholestasis. Sample collection at referral, surgery and recovery would offer substantially different biological views. Age matching, documentation of inflammatory burden and clear comparison with other pediatric cholestatic illnesses would make any suggested relationship more credible. These steps also allow negative findings to be informative rather than being attributed automatically to insufficient sample size or assay sensitivity. [3]

Small RNA synthesis and target recognition

Most canonical microRNAs arise from longer primary transcripts processed by the nuclear Drosha–DGCR8 machinery and cytoplasmic Dicer. Mature sequences are loaded into Argonaute-containing silencing complexes and generally interact with partially complementary messenger RNAs. One microRNA can influence many targets, while an individual transcript may be regulated by several microRNAs. Experimental evidence of sequence complementarity alone is insufficient to establish biological control in a diseased liver. Target abundance, transcript isoforms, cellular localization and rescue experiments determine whether a predicted interaction carries functional weight. [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]

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Growth-dependent patterns of hepatic expression

A liver sample collected shortly after birth is not equivalent to a sample obtained months later. Metabolic maturation, expansion of bile acid transport, changes in nutritional input and the developing immune environment can alter the baseline small-RNA profile. This is particularly important for studies comparing cholestatic infants of different ages or different stages of fibrosis. Age matching is preferable to statistical adjustment alone when the distributions barely overlap, and reference groups should include children with other causes of conjugated jaundice rather than healthy older participants only. [7]

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Cholangiocyte specification, polarity and bile ducts

Biliary epithelial differentiation requires coordination between transcriptional regulators, cytoskeletal organization and signals from surrounding portal mesenchyme. Changes in post-transcriptional regulation could influence duct branching, barrier function or regenerative response, but these outcomes need not occur through the same microRNA. Many experiments use immortalized epithelial cells, which lack neonatal duct architecture and physiological bile flow. Findings from such systems should be interpreted alongside organoid, animal and patient-tissue evidence before a direct explanation for infantile duct obstruction is proposed. [10]

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MicroRNAs and bile acid homeostasis

Cholestasis is not simply a structural obstruction. Accumulated bile acids modify transcription, oxidative stress, metabolism and innate immune signaling. Small RNAs could affect this adaptive network by changing the translation of transporters, nuclear receptor pathway components and proteins involved in cell survival. Yet the direction of regulation may reverse as injury progresses. A microRNA that protects a hepatocyte in an acute model might accompany advanced damage in human serum because injured cells release it. This difference between intracellular function and extracellular concentration is central to clinical interpretation. [13]

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. [14]

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Inflammation-associated microRNA networks

Portal inflammation in biliary disorders combines innate and adaptive signals, including chemokine production and recruitment of lymphoid and myeloid cells. Small RNAs can act within inflammatory cells or change the epithelial messages that recruit them. Bulk liver measurements therefore mix transcriptional information from cells whose proportions vary greatly between patients. A higher apparent expression may indicate immune infiltration rather than induction within cholangiocytes. Cell separation and spatially resolved methods can resolve this distinction more convincingly than an additional unstratified biopsy series. [16]

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. [17]

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Fibrogenic signaling and extracellular matrix

Progressive liver fibrosis reflects both persistent injury and active matrix production. The transition from quiescent to activated hepatic stellate cells and the behavior of portal fibroblasts are regulated by networks that include TGF-β and inflammatory mediators. MicroRNAs may shift these networks, but experimental antifibrotic effects cannot establish benefit for infants with established duct obliteration. A therapeutic intervention would need to preserve bile drainage and avoid impairing necessary epithelial repair. Fibrosis markers must also be distinguished from measures of portal hypertension or long-term native liver survival. [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]

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Epithelial plasticity and the ductular reaction

The ductular reaction is often described as evidence of biliary injury, yet it also contains a reparative component. Its composition changes with age, inflammatory activity and the degree of obstruction. Small RNA signatures measured in ductular-rich tissue might therefore reflect cell expansion or attempted repair rather than direct initiation of disease. This possibility explains why the same molecule may correlate with fibrosis while its manipulation in cell culture has a protective effect. Interpretation requires paired morphology and expression analyses, ideally with identification of the expressing cell population. [22]

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. [23]

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Candidate circulating markers in biliary atresia

Studies of circulating microRNAs have reported signals including the miR-200 family and selected small RNAs identified through array or sequencing approaches. Such findings are promising for distinguishing biliary atresia from other causes of infantile cholestasis, but diagnostic estimates depend strongly on the comparison population. Healthy controls are a weak substitute for age-matched infants with clinically similar jaundice. Testing also needs to account for hemolysis, collection tubes, extraction methods and sample handling. A marker that performs well in a discovery cohort may not retain the same accuracy in everyday referral practice. [25]

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. [26]

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MicroRNA expression across pediatric cholestatic phenotypes

Neonatal cholestasis includes anatomical obstruction, inherited transporter disorders, developmental duct paucity, infection and metabolic illness. Their shared phenotype does not imply a shared molecular signature. A candidate signal in biliary atresia should be compared with progressive familial intrahepatic cholestasis, Alagille syndrome and other relevant differential diagnoses wherever sample availability allows. These disorders differ in the distribution of epithelial injury, hepatic inflammation and bile acid toxicity. Pooling them as a single control group may conceal clinically important misclassification and reduce transportability of diagnostic models. [28]

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Cell-free RNA compartments and stability

Circulating RNA does not exist in one homogeneous compartment. Small RNAs may travel in extracellular vesicles or in complexes with proteins, and proportions can vary with tissue injury and specimen processing. Serum and plasma should not be treated as interchangeable matrices without a bridging study. Equally important, common normalization strategies can become unstable in cholestatic samples if a proposed housekeeping sequence changes with inflammation or age. Absolute quantification and external spike-in controls address different problems, and neither alone resolves cell-of-origin uncertainty. [31]

The intestinal microbiome may affect hepatic inflammation through microbial products, bile acid metabolism, and immune development. Biliary obstruction changes the enterohepatic circulation, potentially altering intestinal conditions at the same time that disease-related factors influence microbial communities. Observational studies have reported associations between fecal microbiome profiles and bile flow after portoenterostomy. Such associations are biologically interesting but bidirectional: successful drainage can alter the intestinal milieu, just as microbial products may modify the inflammatory response. Cross-sectional differences cannot settle which process came first. Antibiotic use, feeding patterns, age, geographic setting, and hospitalization further complicate interpretation and must be incorporated into study design. [32]

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Sequencing, assays and analytical reproducibility

Discovery platforms measure many transcripts simultaneously and are vulnerable to batch effects, multiple testing and low-read-count artifacts. Candidate selection should be documented before independent validation. Quantitative PCR adds technical precision for chosen microRNAs but does not repair selection bias introduced during discovery. Reporting should include sequencing depth, quality thresholds, missing-value handling, hemolysis assessment and prespecified normalization. Assays that cannot be repeated in another laboratory are not ready to guide an infant toward surgery, even when their initial receiver operating characteristic curves appear impressive. [34]

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. [35]

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Diagnostic modelling beyond a single marker

A molecular measurement becomes clinically useful only when it changes a decision. A circulating signature could potentially be combined with stool color, direct bilirubin, γ-glutamyl transferase and ultrasonographic findings. The test should be evaluated at the point in care where a diagnostic decision is actually made, not retrospectively after the final diagnosis is known. Calibration and sensitivity at clinically acceptable false-negative rates matter more than an isolated area under the curve. Consequences of delay must be incorporated when choosing thresholds for expedited specialist assessment. [37]

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Postoperative and prognostic signatures

Restoration of bile flow after portoenterostomy creates a different biological setting from untreated neonatal obstruction. Changes in microRNA abundance may reflect successful drainage, persistent portal inflammation or liver regeneration. Associations with bilirubin clearance or fibrosis should be tested with serial specimens and consistent time points. A prognostic assay must provide information beyond established clinical predictors and should demonstrate stable performance across differing surgical ages. Studies based on a single postoperative sample cannot establish whether an altered signature preceded an adverse outcome or followed it. [40]

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Therapeutic modulation and delivery barriers

RNA mimics and antagonists provide experimental methods to alter gene regulation, but delivery is an unusually important obstacle in pediatric cholangiopathies. Effective hepatocyte uptake does not ensure equivalent cholangiocyte exposure. Dosing must consider developmental pharmacology, immune effects and unintended suppression of multiple transcripts. Disease models should test functional bile flow and tissue architecture rather than molecular target engagement alone.

Before clinical translation, a proposed agent needs a convincing therapeutic window and a strategy for identifying infants most likely to benefit. [43]

Kasai portoenterostomy addresses the anatomical barrier to bile drainage, but it does not directly reverse the inflammatory and fibrotic processes already established within the liver. This explains the appeal of postoperative adjuvant treatment while also defining the standards by which such treatment should be judged. Trials of corticosteroids have provided important evidence, but the overall balance of benefits and adverse events remains dependent on regimen and population, and no universal molecular treatment has been established. A mechanistic target must be evaluated within contemporary surgical and supportive care rather than against untreated historical cohorts. Clinical equipoise should be explicit when potentially immunosuppressive agents are considered in young infants. [44]

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Clinical study design and responsible interpretation

Useful future studies will enroll consecutive infants with conjugated jaundice, measure small RNAs before definitive intervention and include a representative range of alternative diagnoses. Independent validation should be separated in time or geography from discovery. Investigators should report missing specimens, assay failures, adjusted effect estimates and the clinical consequences of false classification. Biological replication across more than one sample type can strengthen mechanistic claims, although it does not substitute for prospective diagnostic evaluation. Evidence must distinguish association, prediction and causality throughout. [45]

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. [46]

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Research agenda for pediatric translation

The next phase of small-RNA research should connect cell-specific biology with a realistic pediatric clinical pathway. Paired tissue and blood profiles could identify whether candidate circulating molecules originate from biliary epithelium, inflammatory cells or injured hepatocytes. Longitudinal collections extending from diagnosis through postoperative follow-up would clarify temporal relationships. A common assay protocol and common comparator definitions would permit multicenter validation. The most valuable success would not be a longer list of differentially expressed transcripts, but a reproducible measurement that improves timely care. [48]

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CONCLUSION

MicroRNAs offer a plausible connection between the developmental biology of the infant liver and its response to obstructive and inflammatory injury. Current evidence is more convincing for identifying candidate mechanisms and exploratory markers than for routine clinical application. The next studies should prioritize independent pediatric cohorts, rigorous handling of circulating RNA and a clear decision pathway for testing.

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