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Pathogenesis and Therapeutic Advances in Cholelithiasis: From Gut Microbiota Regulation to the Frontiers of Precision Medicine

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ABSTRACT

Cholelithiasis, or gallstone disease, has long been associated with cholesterol oversaturation and biliary stasis. The gut microbiota and metabolic dysbiosis play an important role in the disease's development. This study explores the role of gut microbial community structure and metabolic derangements in gallstone formation and clinical consequences. This review integrates current literature on microbiome studies, multi-omics characterization, and precision medicine techniques in cholelithiasis. Gut dysbiosis disrupts bile acid balance, promotes inflammation, and facilitates cholesterol crystallization. Disease-associated microbial signatures include reduced diversity, increased Proteobacteria, and impaired bile salt hydrolase activity. New diagnostic technologies, such as Artificial Intelligence (AI)-based imaging and exosome biomarkers, aid in early detection. New molecular pathway medicines, such as Farnesoid X Receptor (FXR) agonists, customized probiotics, and phage therapy, have the potential to go beyond standard surgical treatments. Shortcomings include a lack of longitudinal data, limited efficacy of targeted drug delivery, and complexities in multi-omics integration. Combining gut microbiota information with metabolic and precision diagnostic approaches opens up new possibilities for individualized cholelithiasis prevention and therapy. Multidisciplinary and computational improvements are required to properly incorporate these findings into clinical practice.

Keywords: *Cholelithiasis, Gut Microbiota, FXR agonists, Metabolic dysbiosis, Bile acid metabolism, Precision medicine*

1. INTRODUCTION

Cholelithiasis, or gallstone disease, is a frequent gastrointestinal disease that consists of the formation of calculi in the bile ducts or gallbladder. Prevalent in 20% of the world's adult population, it is very much associated with lifestyle, dietary habits, and metabolic disorders. In most patients, it is silent but potentially can cause major complications including pancreatitis, biliary colic, and cholecystitis (Georgescu et al., 2022). Cholelithiasis has an incidence in 10-20% of adults worldwide, with cholesterol and pigment stones common in Western and Asian populations. Postoperative sequelae such as cholecystitis and pancreatitis are indications for surgery and recurrence, which presents a clinical problem. High costs of health care and diagnostic imaging burden have made tailored interventions a necessity. Figure 1 demonstrates the anatomy of the biliary apparatus, with the liver, gallbladder, cystic duct, and common bile duct about the duodenum. The gallstones develop in the gallbladder and can block bile flow and thus cause pain and gastrointestinal issues. The inset indicates the organs' positions in the human abdomen for reference.

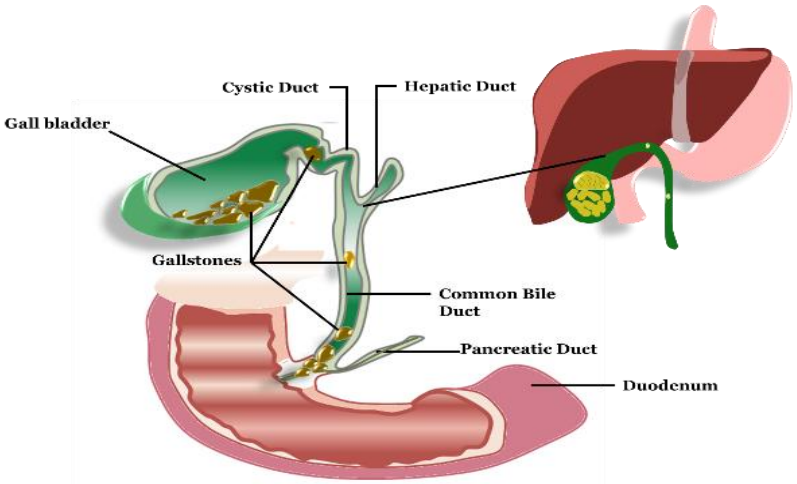

Figure 1: Gallstone formation and biliary anatomy

The portal vein acts as a mediator in the active, two-way communication network known as the gut-liver axis. The axis is used for the transfer of microbial metabolites, endotoxins, and bile acids and involves gut microbiota in biliary and hepatic physiology. Dysregulation of the axis has increasingly been found to be involved in hepatobiliary disease, including cholelithiasis (Liu et al., 2025). The cholesterol metabolism hypothesis neglects stone heterogeneity and recurrence. A microbiota-metabolism-immunity paradigm unifies host-microbe interaction, immune reaction, and metabolic processes in the pathogenesis of gallstones. The multidisciplinary model provides new avenues for prevention and therapy, urging systems biology in clinical science (Xu et al., 2022; Hu et al., 2023). Gut microbiota contains trillions of microbes needed for nutrient metabolism, immune system modulation, and barrier function. Metabolic homeostasis is based on an equilibrated microbial community. Dysbiosis, or alteration of microbial composition, has been implicated in obesity, insulin resistance, and dyslipidemia, all of which are recognized risk factors for the formation of gallstones. The liver produces bile acids, which the gut microbiota chemically alters by deconjugation and dehydroxylation. Microbial transformation modulates the structure of the bile acid pool and regulates FXR and TGR5 receptor signaling (Liu et al., 2023). Dysbalance in bile acid metabolism, commonly due to dysbiosis of the microbiota, can cause cholesterol supersaturation and gallstone nucleation. There have been new developments in next-generation sequencing that have found certain microbial signatures among patients with gallstone disease. These patients are found to have reduced microbial diversity, a reduced Firmicutes/Bacteroidetes ratio, and higher Proteobacteria abundance. Certain taxa like Enterobacteriaceae and *Clostridium spp.* are involved in gallstone pathogenesis facilitation (Li et al., 2023; Batra, 2024). Metabolic dysbiosis, with deranged lipid and glucose metabolism along with gut microbial disturbance, is a key mechanism in the pathogenesis of cholelithiasis. It can occur before clinical presentation, implying a possible role for gut microbiota as an early biomarker or therapeutic target in gallstone disease. Elucidation of the gut microbiota's role in cholelithiasis presents new possibilities for prevention and treatment of the disease. Dietary therapy, fecal microbiota transplantation (FMT), probiotics, and prebiotics are under investigation as means of restoring the microbiome and lowering the risk of gallstones (Liu et al., 2023). Precision medicine, nanomedicine, and AI are transforming the treatment of cholelithiasis. Personalized treatment, early diagnosis, and targeted treatment are made possible by these technologies. The outcome can be improved tremendously by combining therapy with molecular pathology, enabling the possibility for a patient-oriented strategy. The aim is to investigate how metabolic abnormalities and the composition of the gut microbial population contribute to the development of gallstones and their clinical effects.

2. MULTIDIMENSIONAL PATHOGENESIS OF CHOLELITHIASIS

The pathogenesis of cholelithiasis is multifactorial, and classic mechanisms include cholesterol supersaturation and bile stasis. New evidence emphasizes the role of gut microbiota, genetic susceptibility, and multi-omics variation in bile chemistry and gallbladder mechanics. These integrated results allow a more accurate model of disease initiation, progression, and therapeutic intervention. Table 1 summarizes the heterogeneous and integrated mechanisms of gallstone formation.

Table 1: Summary of pathogenetic mechanisms in cholelithiasis

Mechanisms	Descriptions
Classical Mechanisms	Cholesterol supersaturation, bile stasis
Microbiota-Mediated Mechanisms	Microbial bile acid transformation, immune modulation (Costa et al., 2024)

Multi-Omics Insights	Molecular profiling of gallbladder and bile
Genetic & Epigenetic Factors	Gene mutations (ABCG5/G8) and DNA methylation (SLC10A2)

2.1 Classical Mechanisms

The traditional understanding of gallstone formation centers on the supersaturation of cholesterol in bile, leading to crystal nucleation. Impaired gallbladder motility and bile stasis facilitate the growth of these crystals into stones. Disruptions in enterohepatic circulation reduce bile acid concentration, destabilizing bile and promoting precipitation. Risk factors such as obesity, rapid weight loss, and estrogen use exacerbate these imbalances. While the framework explains many cholesterol stones, it doesn't address pigment stones or recurrence after treatment. Thus, classical mechanisms serve as a foundational layer, supplemented by newer biological insights.

2.2 Microbiota-Mediated Mechanisms

The importance of gut bacteria in gallbladder health and bile acid metabolism is being highlighted by a new study. Microbial enzymes alter bile synthesis and secretion through activating the FXR and TGR5 signaling pathways, which transform primary bile acids into secondary bile acids, including deoxycholic acid (DCA) and lithocholic acid (LCA) (Kim et al., 2024). Butyrate and other short-chain fatty acids work through GPR43 to reduce inflammation in gallbladder tissue. Conversely, pathogens like *Klebsiella pneumoniae* can induce Th17-mediated inflammation and biliary fibrosis. Dysbiosis can also impair bile salt hydrolase activity, favoring cholesterol accumulation. These discoveries suggest microbiota modulation as a therapeutic strategy. The gut-liver axis is recognized as central in gallstone pathogenesis (Wang et al., 2025).

2.3 Multi-Omics Insights into Mechanisms

Multi-omics technologies have revolutionized understanding of gallstone disease. Metabolomics has identified biomarkers of dysregulated Sterol O-Acyltransferase 2 (SOAT2)-mediated cholesterol esterification and phospholipid imbalance. Single-cell sequencing has revealed epithelial subpopulations, such as LGR5+ stem-like cells, prone to lithogenic transformation. Spatial transcriptomics has shown localized activation of IL-33/ST2 signaling around stones, illuminating microenvironmental influences. These tools provide high-resolution, cell-type-specific, and spatial data that link molecular events to clinical phenotypes. The phenotypes also help uncover therapeutic targets and predict disease progression. Integration of omics layers is a powerful approach to dissecting the complexity of cholelithiasis.

2.4 Genetic and Epigenetic Contributions

Genetic predisposition plays a crucial role in gallstone development. Mutations in ABCG5/G8, particularly rs11887534 in East Asians**,** grow hepatic cholesterol secretion into bile. Epigenetic alterations, such as DNA methylation of SLC10A2, impair bile acid reabsorption, further promoting lithogenesis. These factors interact with conservation exposures and gut microbiota, contributing to population-specific disease patterns. Genome-wide association studies have also identified other candidate loci linked to gallstone risk. Understanding these mechanisms enables genetic screening and modified prevention strategies. The epigenetic directive offers a reversible therapeutic target, especially relevant for modifying gene expression inclined by diet and microbiota.

3. PRECISION MEDICINE-DRIVEN INNOVATIONS IN DIAGNOSIS AND THERAPY

Precision medicine is developing the diagnosis and treatment of gallstone disease through AI-enhanced imaging, liquid biopsy biomarkers, and targeted molecular therapies. Emerging interferences such as FXR agonists, inflammasome inhibitors, and microbiota variation shift the focus from symptomatic relief to disease prevention. Meanwhile, minimally invasive tools like the SpyGlass Direct Visualization (SpyGlass DS) system and recreating methods such as biodegradable scaffolds and gallbladder organoids offer personalized, less offensive care with improved outcomes.

3.1 Precision Diagnostics

Advancements in imaging and molecular diagnostics are improving the accuracy of gallstone disease exposure and classification:

AI-Based Radiomics: AI based radiomics models are revolutionizing the field of imaging analysis in gallstone complications. More specifically, in acute gallstone pancreatitis, radiomics techniques apply sophisticated machine-learning (ML) algorithms to derive high-dimensional quantitative features from routine Computed Tomography (CT) imaging data (Ma et al., 2024). These characteristics, frequently unseen by the naked eye, encompass texture, shape, and intensity parameters that are associated with the severity of the disease. Analyses have shown that these models predict accurately the severity of inflammation, necrosis, and systemic complications. The method enables early stratification of patients into risk groups by clinicians, allowing effective and tailored management interventions with improvement in outcomes and a decreased chance of complications or inappropriate interventions.

Ultrasound Elastography: Ultrasound elastography is a significant breakthrough in non-invasive imaging in hepatobiliary diseases. The modality quantitates tissue mechanical stiffness or elasticity with shear wave propagation or strain imaging methods. In gallbladder disease, elastography can measure gallbladder wall fibrosis, a significant surrogate of chronic inflammation and risk of malignancy. Unlike conventional ultrasound, which offers only anatomy, elastography provides functional information regarding tissue integrity, which enables more sophisticated clinical decision-making. Its non-invasive character makes it particularly valuable for serial testing, tracking the progression of disease, and even the mitigation of the need for invasive testing modalities like endoscopic ultrasound or biopsy (Ma et al., 2024).

Liquid Biopsy with Exosomal lncRNA H19: Liquid biopsy represents a new arena in precision diagnosis, providing a minimally invasive method of gaining access to molecular information from body fluids. One of the most promising biomarkers being studied is the long non-coding RNA H19 (lncRNA H19), which is packaged inside circulating exosomes tiny extracellular vesicles that carry RNA, proteins, and lipids. Recent study indicates that increased concentrations of exosomal lncRNA H19 in peripheral blood could be a marker for the early formation of gallstones or related pathologies like biliary inflammation or dysplasia. Detection of such biomarkers would enable early diagnosis before overt radiographic changes or clinical symptoms. In addition, the technique has the potential for real-time monitoring of disease and prediction of recurrence (Ma et al., 2024), which could make it a useful tool in longitudinal patient care and personalized medicine.

Integrating these diagnostic tools enables earlier intervention, personalized surveillance, and more informed clinical decision-making, eventually improving patient outcomes and reducing pointless surgical involvements.

3.2 Targeted Therapeutic Strategies

Novel therapies are being developed to target specific molecular pathways involved in gallstone formation and associated inflammation:

FXR Agonists: The nuclear receptor known as the farnesoid X receptor, or FXR, is essential for maintaining bile acid homeostasis. FXR activation regulates the expression of genes involved in metabolism, transport (e.g., BSEP, MRP2), and bile acid production (e.g., CYP7A1). The cholesterol saturation index (CSI) of bile has been successfully decreased in preclinical animals by both synthetic FXR agonists (such as obeticholic acid) and natural ligands (such as chenodeoxycholic acid). By enhancing bile acid feedback inhibition and stimulating cholesterol efflux, FXR agonists prevent cholesterol crystal nucleation, a critical early process in gallstone development. In addition FXR signaling has anti-inflammatory actions on the liver and gallbladder, generating a more favorable microenvironment and lowering the risk of gallstone complications (Zhang et al., 2023). Current clinical trials are exploring the translational value of these chemicals in humans, specifically in individuals with metabolic syndrome or nonalcoholic fatty liver disease (NAFLD), who are at increased risk of gallstone formation.

NLRP3 Inflammasome Inhibitors: An essential intracellular sensor of cellular stress and damage, the NLRP3 (NOD-like receptor family, pyrin domain-containing 3) inflammasome coordinates the innate immune response by activating caspase-1 and releasing pro-inflammatory cytokines such as IL-1β and IL-18. Crystal precipitation and cholesterol supersaturation in bile can operate as danger-associated molecular patterns (DAMPs) in gallstone disease, triggering the NLRP3 inflammasome in immunological and biliary epithelial cells. Chronic inflammation and tissue remodeling contributes to gallbladder malfunction and the development of stones easier (Zhang et al., 2023). MCC950, a selective and potent inhibitor of NLRP3, has shown in preclinical models the capacity to inhibit cascade of inflammation, lowering gallstone burden and inflammatory pathology significantly. Its specific mechanism of action renders it a candidate for further therapeutic potential in gallstone prevention and chronic cholecystitis.

Microbiota-Based Therapies: Gut and biliary microbiota are being increasingly valued as crucial regulators of bile acid composition and pathogenesis of gallstones. Dysbiosis is defined as a disturbance of microbial populations and can result in shifted bile salt hydrolase (BSH) activity to favor lithogenic bile and secondary toxic bile acids (Zhang et al., 2023). Genetically engineered probiotics that have improved BSH enzymes are being developed to maximize bile acid deconjugation, thus restoring homeostasis and minimizing lithogenic bile potential. Besides, bacteriophage therapy is becoming a very specific approach to eradicate and target pathogenic bacteria involved in gallstone disease, including enterotoxigenic Bacteroides fragilis strains. These targeted therapies have the double benefit of regulating bile acid metabolism and maintaining healthy microbial populations, lowering inflammation, and reverting the pathogenic alterations to gallstone development. Combining microbiota-related treatments with dietary and lifestyle interventions can provide a comprehensive and individualized solution to gallstone disease prevention.

These therapies are an advance over symptom management to prevention and disease modification and aim to maximize efficacy with the least side effects.

3.3 Minimally Invasive and Regenerative Techniques

Innovative procedures and biomaterials are reforming treatment options for gallstone disease:

- **SpyGlass DS System**: The SpyGlass DS Direct Visualization System makes it possible to treat troublesome bile

duct stones with electrohydraulic lithotripsy guided by peroral cholangioscopy. Clinical studies have established its efficacy in achieving complete stone removal with reduced procedure times and fewer endoscopic conferences compared to conventional methods (Murabayashi et al., 2020).

- **Biodegradable Magnesium Alloy Scaffolds**: Biodegradable stents made from magnesium alloys, such as WE43, are being developed to prevent biliary strictures while promoting tissue healing. These scaffolds degrade over time, removing the need for stent removal and reducing long-term problems (Ni et al., 2024).
- **Human-Derived Gallbladder Organoids**: Organoids derived from human gallbladder tissue serve as models for drug screening and toxicity assessment. These 3D culture systems closely mimic the in vivo environment, allowing for more correct evaluation of therapeutic agents and personalized medicine methods.

These advancements represent the convergence of engineering and medicine, offering precision actions that minimize trauma and reduce recurrence rates in gallstone disease management.

4. NATIONAL AND INTERNATIONAL RESEARCH HIGHLIGHTS & COLLABORATIVE INNOVATION

China combines traditional medicine with genomics and big data to promote precision gallstone treatment. AI and nanomedicine are refining diagnostics and therapies worldwide. Cross-regional partnerships speed up biomarker verification and fair treatment advancements.

4.1 Chinese Research Strengths

China has been able to integrate traditional medicine with contemporary scientific approaches to treat gallstone disease. Conventional herbal drugs like Rheum palmatum extract suppressed cholesterol stone formation via the FXR/AMPK pathway, which demonstrates the promise of traditional medicine in the contemporary therapeutic context. A traditional herbal formulation has shown potential to suppress microbial β-glucuronidase activity, thereby modulating bile acid metabolism and alleviating biliary obstructive diseases in animal models (Wang et al., 2015).

The Chinese National Cholelithiasis Registry Research (NCRS) validates large-scale genotype-phenotype correlation to enable precision care. Previous studies have demonstrated associations of considerable strength between ABCB4 polymorphisms (rs1202283 and rs2230028) and gallstone disease in Han-Chinese populations, implying genetic susceptibilities to guide targeted interventions (Zhan et al., 2016).

These initiatives underscore the promise of coupling cultural heritage with cutting-edge research. The robust big data infrastructure in China and extensive clinical networks are spurring discoveries and facilitating real-world trials of novel treatments.

4.2 Global Technological Frontiers

Worldwide, investigators are evolving gallstone treatment knowledge. Nanomedicine innovations, such as liposome-encapsulated ursodeoxycholic acid (UDCA), offer targeted delivery to gallbladder tissues, enhancing therapeutic efficacy while minimizing systemic side effects. AI models like DeepGalStone integrate clinical, omics, and imaging data to support personalized therapy decisions, improving diagnostic accuracy and patient stratification.

4.3 Cross-Regional Collaboration Directions

Collaborative studies between Eastern and Western institutions is crucial to uncover global patterns in gallstone disease. Multicenter cohorts enable the review of genetics, diet, and microbiota in diverse populations, providing insights into disease etiology and progression. Joint development of AI-powered full-cycle platforms from screening to treatment and follow-up can standardize care and reduce disparities. Cross-regional integration of biobanks, imaging data, and registries accelerates biomarker validation and fosters innovation. Such collaborations promote resource sharing and equitable healthcare advancements, facilitating the global adoption of precision medicine approaches for gallbladder disease.

4.4 Key Recent Studies on Gallstones and Gut Microbiota

The illustration of various microbial profiles in the bile, gallstones, and feces of afflicted patients in Table 2 highlights the critical function of microbiota in gallstone disease. Bacterial genes and species, including Streptococcus and Bacteroides, are involved in gallstone disease (GD) pathogenesis. Altered gut microbiota and bile acid metabolism after bariatric surgery increase the risk of cholelithiasis. ML models, particularly Convolutional neural networks (CNNs), exhibit encouraging accuracy in GD diagnosis based on imaging data. In combination, these breakthroughs provide new perspectives for targeted prevention, diagnosis, and treatment approaches.

Table 2: Summary of Recent Studies on Microbiota, Gallstone Disease, and Diagnostic Advancements

References	Data	Analysis/ Methods	Results
(Zhang et al., 2025)	Bile and gallstone samples from 16 individuals with pigmented gallstone disease (PGS) were subjected to 16S rRNA sequencing.	Found important bacterial genera and genes (uidA, pldA, plc); compared the microbial makeup of bile and gallstones; studied characteristics of bile resistance.	Microbial diversity varied among patients.
(Ding et al., 2023)	Using the Illumina HiSeq technology, metagenomic next-generation sequencing (mNGS) was used to evaluate human fecal samples from individuals with GD.	The composition and function of the gut microbiota in GD patients and healthy people were compared using bioinformatics methods.	GD patients had higher levels of Bacteroidetes (phylum), Bacteroides (genus), and Thetaiotaomicron (species).
(Komorniak et al., 2024)	Cholelithiasis (gallstones) is common after bariatric surgery, possibly due to rapid weight loss.	Overview of the study on gallstone development after surgery, risk factors, and preventative techniques.	Gallstone development can result from changes in the makeup of bile acids and gut flora.
(Ahmed et al., 2024)	Gallstone disease is common and linked to obesity, rapid weight loss, diabetes, and genetics. Complications include cholecystitis, cholangitis, pancreatitis, and GB cancer.	Systematic review following PRISMA guidelines. Searched PubMed, Cochrane, Scopus, and Embase up to April 2024. Used MeSH terms and Boolean operators. Included human studies using AI/ML with imaging.	ML models show promise for diagnosing gallstones, but further validation is needed to ensure reliability across clinical settings.

5. CHALLENGES AND FUTURE PROSPECTS

Determining the causal relationships between changes in the gut microbiota and gallstone development is a major challenge in gallstone study. Gallstone development is induced by FMT from individuals with cholesterol gallstones into mice that are resistant to gallstones, according to recent studies, which links certain bacterial taxa, including *Desulfovibrionales*, to the pathophysiology (Hu et al., 2022). Gallstone pathogenesis is further supported by the discovery of causal links between certain gut microbial taxa and gallstone illness by Mendelian randomization analysis (Hu et al., 2024). Another limitation is the lack of long-term follow-up data on the natural history of asymptomatic gallstones. Most available studies are short-term and lack multi-omics analysis, limiting understanding of disease recurrence and progression. A 20-year prospective cohort study with multi-omics analysis would be ideal to define the natural history of asymptomatic gallstones and inform risk stratification and management guidelines. These limitations call for collaborative, longitudinal, and multidisciplinary efforts. Technical limitations continue to exist despite recent developments in nanomedicine in gallbladder-targeted drug delivery. Current gallbladder-targeted nanodrugs have low penetration efficiency, with less than 5% of the delivered dose reaching the target site, thus limiting their clinical utility. Moreover, integration and analysis of multi-omics data present daunting challenges due to their size, heterogeneity, and noise. Recent developments, including the OCEAN framework, have been shown to overcome such challenges by enabling flexible feature set aggregation for multi-omics analysis (Ebrahimpoor et al., 2024). In addition, AI-based multi-omics integration models are being explored to enable causal inference and real-time decision support in intricate biological systems (Wu & Xie, 2024). Investment in computational infrastructure and interdisciplinarity training is needed to overcome these bottlenecks and advance precision medicine in gallstone disease to the next level. Emerging technologies offer promising paths for the prevention and monitoring of gallstone disease. CRISPR-Cas9 technology delivers a platform to engineer gut microbes that resist lithogenic transformation, although the method remains in the preclinical stage. Additionally, the development of wearable Raman spectroscopy devices for real-time bile composition monitoring is being discovered, which could enable dynamic risk calculation and timely interventions (Wang et al., 2024). Translating these revolutions from bench to bedside could require rigorous validation, regulatory approval, and cost-effectiveness analyses. However, they could hold the potential to significantly enhance anticipation and disease management approaches for cholelithiasis.

6. CONCLUSION

The cholelithiasis has changed from simplistic models to complex, system-level frameworks. The complex change is driven by insights from microbiome science, multi-omics, bioinformatics, and materials engineering. Precision medicine approaches are enabling personalized diagnostics and therapies that address the root causes of gallstone formation. However, to fully realize these benefits will require overcoming scientific, technical, and translational challenges. Multidisciplinary relationships and international data sharing could be vital. To move forward, integrating diverse revolutions holds the key to revolutionizing the anticipation and treatment of gallstone disease worldwide. A key limitation of existing studies is the absence of longitudinal human studies correlating gallstone progression with alterations in gut microbiota. Most results are drawn from small cohorts or animal models, and thus have limited generalizability. Large-scale, multi-omics analyses need to be addressed in future studies to design targeted microbial therapies.

REFERENCES

[1] Georgescu, D., Ionita, I., Lascu, A., Hut, E.F., Dragan, S., Ancusa, O.E., Ionita, M., Calamar-Popovici, D., Georgescu, L.A. and Lighezan, D.F., 2022. Gallstone disease and bacterial metabolic performance of gut microbiota in middle-aged and older patients. *International Journal of General Medicine*, pp.5513-5531.https://doi.org/10.2147/IJGM.S350104

[2] Liu, Y., Li, H., Sun, T., Sun, G., Jiang, B., Liu, M., Wang, Q., Li, T., Cao, J., Zhao, L. and Xiao, F., 2025. The gut microbiome and metabolome characteristics of patients with cholesterol gallstones suggest the preventive potential of prebiotics. *iMeta*, *4*(1), p.e70000.https://doi.org/10.1002/imt2.70000

[3] Xu, Y., Jing, H., Wang, J., Zhang, S., Chang, Q., Li, Z., Wu, X. and Zhang, Z., 2022. Disordered gut microbiota correlates with altered fecal bile acid metabolism and post-cholecystectomy diarrhea. *Frontiers in microbiology*, *13*, p.800604.https://doi.org/10.3389/fmicb.2022.800604

[4] Hu, J., Tang, J., Zhang, X., Yang, K., Zhong, A., Yang, Q., Liu, Y., Li, Y. and Zhang, T., 2023. Landscape in the gallbladder mycobiome and bacteriome of patients undergoing cholelithiasis with chronic cholecystitis. *Frontiers in Microbiology*, *14*, p.1131694.https://doi.org/10.3389/fmicb.2023.1131694

[5] Liu, X., Qi, X., Han, R., Mao, T. and Tian, Z., 2023. Gut microbiota causally affects cholelithiasis: a two-sample Mendelian randomization Research. *Frontiers in cellular and infection microbiology*, *13*, p.1253447.https://doi.org/10.3389/fcimb.2023.1253447

[6] Li, W., Ren, A., Qin, Q., Zhao, L., Peng, Q., Ma, R. and Luo, S., 2023. Causal associations between human gut microbiota and cholelithiasis: a Mendelian randomization Research. *Frontiers in Cellular and Infection Microbiology*, *13*, p.1169119.https://doi.org/10.3389/fcimb.2023.1169119

[7] Batra, P., 2024. Synergy of gut microbiome and bile acids in inducing cholelithiasis. *Journal of Advanced Scientific Research*, *15*(08), pp.1-9.https://doi.org/10.55218/JASR.2024150801

[8] Liu, Q., Zheng, L., Wang, Y., Huang, Z., Zhu, J., Fang, M., Xie, L., Ding, C., Gu, Y., Xu, D. and Jin, H., 2023. Primary choledocholithiasis occurrence and recurrence are synergetically modulated by the bile microbiome and metabolome alternations. *Life Sciences*, *331*, p.122073.https://doi.org/10.1016/j.lfs.2023.122073

[9] Costa, C.J., Nguyen, M.T.T., Vaziri, H. and Wu, G.Y., 2024. Genetics of gallstone disease and their clinical significance: a narrative review. *Journal of Clinical and Translational Hepatology*, *12*(3), p.316.http://dx.doi.org/10.14218/JCTH.2023.00563

[10] Kim, S., Seo, S.U. and Kweon, M.N., 2024, July. Gut microbiota-derived metabolites tune host homeostasis fate. In *Seminars in Immunopathology* (Vol. 46, No. 1, p. 2). Berlin/Heidelberg: Springer Berlin Heidelberg.https://doi.org/10.1007/s00281-024-01012-x

[11] Wang, X., Fang, Y., Liang, W., Cai, Y., Wong, C.C., Wang, J., Wang, N., Lau, H.C.H., Jiao, Y., Zhou, X. and Ye, L., 2025. Gut–living translocation of pathogen Klebsiella pneumoniae promotes hepatocellular carcinoma in mice. *Nature Microbiology*, pp.1-16.https://doi.org/10.1038/s41564-024-01890-9

[12] Ma, Y., Yue, P., Zhang, J., Yuan, J., Liu, Z., Chen, Z., Zhang, H., Zhang, C., Zhang, Y., Dong, C. and Lin, Y., 2024. Early prediction of acute gallstone pancreatitis severity: a novel machine learning model based on CT features and open access online prediction platform. *Annals of Medicine*, *56*(1), p.2357354.https://doi.org/10.1080/07853890.2024.2357354

[13] Zhang, X., Wang, Z., Zheng, Y., Yu, Q., Zeng, M., Bai, L., Yang, L., Guo, M., Jiang, X. and Gan, J., 2023. Inhibitors of the NLRP3 inflammasome pathway as promising therapeutic candidates for inflammatory diseases. *International Journal of Molecular Medicine*, *51*(4), p.35.https://doi.org/10.3892/ijmm.2023.5238

[14] Murabayashi, T., Ogawa, T., Koshita, S., Kanno, Y., Kusunose, H., Sakai, T., Masu, K., Yonamine, K., Miyamoto, K., Kozakai, F. and Endo, K., 2020. Peroral cholangioscopy-guided electrohydraulic lithotripsy with

a SpyGlass DS versus a conventional digital cholangioscope for difficult bile duct stones. *Internal Medicine*, *59*(16), pp.1925-1930.https://doi.org/10.2169/internalmedicine.4463-20

[15] Ni, D.J., Yang, Q.F., Nie, L., Xu, J., He, S.Z. and Yao, J., 2024. The past, present, and future of endoscopic management for biliary strictures: technological innovations and stent advancements. *Frontiers in Medicine*, *11*, p.1334154.https://doi.org/10.3389/fmed.2024.1334154

[16] Wang, B., Sun, M.Y., Long, A.H., Cao, H.Y., Ren, S., Bian, Y.Q., Lu, X., Gu, H.T., Liu, C.H. and Liu, P., 2015. Yin-Chen-Hao-Tang alleviates biliary obstructive cirrhosis in rats by inhibiting biliary epithelial cell proliferation and activation. *Pharmacognosy Magazine*, *11*(42), p.417. https://doi.org/10.4103/0973-1296.153098

[17] Zhan, L., Pan, Y.Z., Chen, L., Zhang, H., Zhang, H., Song, J., Tzeng, C.M. and Sun, C.Y., 2016. Prevalence of ABCB4 polymorphisms in gallstone disease in han-Chinese population. *American Journal of Translational Research*, *8*(2), p.1218.'

[18] Zhang, R., Chen, C., Zheng, S., Zhang, J., Chen, W. and Chen, Z., 2025. Preliminary Research of biliary microbiota and identification of bacterial species associated with pigmented gallstone formation. *Frontiers in Cellular and Infection Microbiology*, *15*, p.1532512.https://doi.org/10.3389/fcimb.2025.1532512

[19] Ding, L., Wang, S., Jiang, W., Miao, Y., Liu, W., Yang, F., Zhang, J., Chi, W., Liu, T., Liu, Y. and Wang, S., 2023. Identification of intestinal microbial community in gallstone patients with metagenomic next-generation sequencing. *Diagnostics*, *13*(16), p.2712.https://doi.org/10.3390/diagnostics13162712

[20] Komorniak, N., Pawlus, J., Gaweł, K., Hawryłkowicz, V. and Stachowska, E., 2024. Cholelithiasis, Gut Microbiota, and Bile Acids after Bariatric Surgery—Can Cholelithiasis Be Prevented by Modulating the Microbiota? A Literature Review. *Nutrients*, *16*(15), p.2551.https://doi.org/10.3390/nu16152551

[21] Ahmed, A.S., Ahmed, S.S., Mohamed, S., Salman, N.E., Humidan, A.A.M., Ibrahim, R.F., Salim, R.S., Elamir, A.A.M., Hakim, E.M., Humidan Jr, A.A.M. and Salim, R., 2024. Advancements in Cholelithiasis Diagnosis: A Systematic Review of Machine Learning Applications in Imaging Analysis. *Cureus*, *16*(8).https://doi.org/10.7759/cureus.66453

[22] Hu, H., Shao, W., Liu, Q., Liu, N., Wang, Q., Xu, J., Zhang, X., Weng, Z., Lu, Q., Jiao, L. and Chen, C., 2022. Gut microbiota promotes cholesterol gallstone formation by modulating bile acid composition and biliary cholesterol secretion. *Nature communications*, *13*(1), p.252.https://doi.org/10.1038/s41467-021-27758-8

[23] Hu, X., Binxu, Q., Shao, G.Z., Huang, Y. and Qiu, W., 2024. Gut microbiota, circulating metabolites, and gallstone disease: a Mendelian randomization Research. *Frontiers in microbiology*, *15*, p.1336673.https://doi.org/10.3389/fmicb.2024.1336673

[24] Ebrahimpoor, M., Menezes, R., Xu, N. and Goeman, J.J., 2024. OCEAN: Flexible Feature Set Aggregation for Analysis of Multi-omics Data. *arXiv preprint arXiv:2410.19523*.https://doi.org/10.48550/arXiv.2410.19523

[25] Wu, Y. and Xie, L., 2024. AI-driven multi-omics integration for multi-scale predictive modeling of causal genotype-environment-phenotype relationships. *arXiv preprint arXiv:2407.06405*.https://doi.org/10.1016/j.csbj.2024.12.030

[26] Wang, H., Gong, J., Chen, J., Zhang, W., Sun, Y. and Sun, D., 2024. Intestinal microbiota and biliary system diseases. *Frontiers in Cellular and Infection Microbiology*, *14*, p.1362933.https://doi.org/10.3389/fcimb.2024.1362933