Liver International Communications

WILEY

Liver INTERNATIONAL COMMUNICATIONS

BRIEF REPORT OPEN ACCESS

Abcb4 Haploinsufficiency Sensitises Mice to a Diet-Induced PSC-Like Hepatobiliary Phenotype With Gallstone Formation

Eric L. Bell | Youhwa Jo | Jennifer K. Truong | Nathan O. Fuller | John P. Miller | Alastair S. Garfield | Robert O. Hughes

Rectify Pharmaceuticals, Boston, Massachusetts, USA

Correspondence: Eric L. Bell (ebell@rectifypharma.com)

Received: 18 January 2026 | **Revised:** 2 March 2026 | **Accepted:** 7 March 2026

Keywords: Abcb4 | bile composition | Mdr2 | MDR3 | PSC | toxic bile

ABSTRACT

ABCB4 translocates phospholipids (PL) into bile to buffer the toxicity of bile acids (BA) and free cholesterol (CHOL). While recessively inherited *ABCB4* deficiency causes childhood Progressive Familial Intrahepatic Cholestasis type 3, haploinsufficiency has been linked to adult-onset hepatobiliary diseases. To model this partial defect, we challenged phenotypically normal *Abcb4*$^{+/-}$ mice, which have a toxic biliary BA/PL ratio, with diets supplemented with different lipids. Within 1 week, *Abcb4*$^{+/-}$ mice fed a lithogenic diet rapidly developed severe phenotypes of cholestasis (elevated ALP and sTBA) and hepatic inflammation (increases in ALT and AST), as well as accelerated cholesterol calculi formation. By 6 weeks, animals on diet developed florid cholangitis (ductular reaction, immune cell infiltration, elevated cytokines) and peri-portal fibrosis. This model demonstrates that a partial deficit in biliary PL secretion under conditions of environmental stress can act as a critical predisposing factor for adult-onset hepatobiliary diseases like Primary Sclerosing Cholangitis. This model is suitable for evaluating therapeutics targeting abnormal bile composition.

1 | Introduction

Hepatobiliary diseases, ranging from rare monogenic disorders to common chronic liver conditions, represent a significant global health burden [1]. A growing body of evidence highlights the critical role of bile composition homeostasis in maintaining the health and integrity of the biliary tree [2]. Bile acids (BAs) are essential for lipid absorption, but their intrinsic detergent properties render them cytotoxic if left unbuffered. The ATP-binding cassette (ABC) transporter *ABCB4/MDR3* (*Abcb4/Mdr2* in mouse), expressed on the hepatocyte canalicular membrane, performs a vital floppase activity translocating phospholipids (PL), primarily composed of phosphatidylcholine, into the bile canaliculus. This PL forms protective mixed micelles with BAs and cholesterol (CHOL), which neutralises the BAs' detergent/cytotoxic effect and CHOL's inflammatory action in the biliary system, and during transport to the gut, where BAs promote the absorption of fats and lipid-soluble vitamins [3].

Disruption of these mixed micelles, particularly a deficiency in ABCB4 and biliary PL secretion, results in the presence of unbuffered, monomeric BAs and crystallization of free CHOL that damage the sensitive biliary epithelium, leading to cholangiopathy and progressive liver injury. The importance of

Abbreviations: ABC, ATP-binding cassette; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BA, Bile acid; CA, cholic acid; Cd11b, Integrin alpha M; CHOL, cholesterol; Ck19, cytokeratin 19; Col1a1, alpha-1 chain of type I collagen; Col1a2, alpha-2 chain of type I collagen; Cxcl-1, C-X-C motif chemokine ligand 1; HET, heterozygous; ICP, intrahepatic cholestasis of pregnancy; Itgb6, Integrin beta 6; LCA, lithocholic acid; LD, lithogenic diet; LPAC, low phospholipid-associated cholelithiasis; Mcp-1, monocyte chemoattractant protein 1; Mip1a, macrophage inflammatory protein 1α; Mip-2, macrophage inflammatory protein 2; PFIC3, progressive familial intrahepatic cholestasis type 3; PL, Phospholipid; PSC, primary sclerosing cholangitis; PSR, picro-Sirius red; sTBA, serum total bile acid; Timp-1, tissue inhibitor of metalloproteinases 1; Tnf-1, tumour necrosis factor a; Vcam, vascular cell adhesion molecule; WT, wild-type.

ABCB4-mediated PL secretion is underscored by Progressive Familial Intrahepatic Cholestasis type 3 (PFIC3), a severe monogenic childhood disease caused by bi-allelic pathogenic variants in *ABCB4*, which results in a congenital deficiency of biliary PL [4]. This condition directly links toxic bile composition to bile duct injury and subsequent secondary cholestasis.

Beyond childhood cholestasis, recent clinical and genetic studies have revealed a broader role for *ABCB4* variants in predisposing adults to common and rare complex hepatobiliary diseases. Heterozygous pathogenic variants in *ABCB4* are associated with conditions like intrahepatic cholestasis of pregnancy (ICP) and low phospholipid-associated cholelithiasis (LPAC) [5, 6]. Furthermore, whole-genome sequencing and cohort studies have linked specific *ABCB4* variants with increased risk of gallstone disease, bile duct carcinoma and cirrhosis [7]. Multiple cohort studies of adult cholestatic patient populations identified the presence of pathogenic variants in cholestatic genes, including *ABCB4* [8–11]. This collective evidence strongly supports the concept that even a partial deficit in ABCB4 function and the resultant shift toward toxic bile composition can act as a critical predisposing factor in the development and progression of adult-onset hepatobiliary disease phenotypes, including Primary Sclerosing Cholangitis (PSC) [12].

While the *Abcb4* knockout mouse provides a robust model for PFIC3, representing a near-complete loss of biliary PL secretion [13], it does not accurately reflect the clinical bile composition observed in adult patients with partial functional deficiencies [12, 14]. To investigate the mechanisms underpinning rare adult-onset diseases driven by subclinical or partial defects in bile composition, there is a clear need for translational models that effectively simulate a milder yet clinically significant toxic bile phenotype. The *Abcb4* heterozygous (*Abcb4*$^{+/-}$) mouse, which maintains partial PL secretion [13], provides an ideal candidate. By stressing the phenotypically normal *Abcb4*$^{+/-}$ mouse with dietary BAs, CHOL, and fat, severe phenotypes that replicate key features of adult-onset hepatobiliary diseases including PSC and LPAC can be induced. Such a model establishes a much-needed preclinical platform to test therapeutics targeting the modulation of bile composition.

2 | Methods

2.1 | Mouse Studies

FVB.129P2-Abcb4tm1Bor/J (strain# 002539) were purchased and bred with wild-type FVB/NJ (strain# 001800) and genotyped at Jackson Laboratories. Eight- to ten-week-old female mice were used in all experiments with $N = 5–12$ per group. The lithogenic diet (LD) studies were executed at Physiogenex (Toulouse, France) in accordance with the Guide for the Care and Use of Laboratory Animals (revised 1996 and 2011, 2010/63/EU) and French laws. The LD consisted of 15% fat, 1.25% CHOL, 0.5% cholic acid (CA) and was purchased from Research Diets (D12336). The 0.5% CA and 0.3% lithocholic acid (LCA) diet studies were executed at Pharmaron (Beijing, China) in accordance with Animal Use Protocol Number: IVP-PD/PK-09132024. The 0.5% CA and 0.3% LCA diets were custom-made at Research Diet by adding LCA (Sigma L6250) or Sodium Cholate (Sigma C6445) to the base grain diet (LabDiet 5002).

2.2 | Biochemical Assays

The CA and LCA diet studies used the Total Bile Acid Assay Kit (RB11302, Sjodax) and the Alkaline Phosphatase Assay Kit (KB10311, Sjodax) on the TBA-120FR biochemical analyser (Canon Medical Systems).

LD study measurements for ALT, ALP,and AST were performed using a chemical analyser Horiba Pentra 400 machine and related Pentra assay kits (kit for ALT: A11A01627; kit for ALP: A11A01626; kit for AST: A11A01629; Horiba France SAS, Longjumeau, France) using 4 μL, 20 μL and 20 μL of serum, respectively. Serum TBA was quantified from 2 μL of serum using the Abcam Kit, AB239702, with a TECAN Infinite 200Pro and Magellan software.

2.3 | Gene Expression

Liver tissues were frozen with liquid nitrogen and ground into powder from which genomic DNA-free RNA was extracted using Rneasy Plus mini Kit (Qiagen 74 134). Isolated RNA was converted to cDNA with the High-Capacity RNA-to-cDNA Kit (Invitrogen 4 387 406). Using TaqMan FAST Gene Expression Master Mix (Invitrogen 4 444 557), 0.5uL of cDNA was analysed using the following Thermo Fisher Taqman probe sets: *Col1a1* (Mm00801666_g1), *Col1a2* (Mm05726851_s1), *Timp-1* (Mm01341361_m1), *Itgb6* (Mm01269869_m1), *Gapdh* (Mm99999915_g1), *Gusb* (Mm01197698_m1) and *TBP* (Mm01277041_m1). Relative expression was calculated using the ΔΔCt method using the average of *Gapdh*, *Gusb* and *Tbp* as housekeeping genes.

2.4 | Cytokines

Liver tissues were homogenised in RIPA lysis buffer supplemented with protease and phosphatase inhibitors (Thermo Fisher 89 900, 78 445) in TissueLyser II with steel beads at 30 Hz for 3 min. Samples were mixed at 4°C for 30 min, centrifuged and the protein concentration of the lysate was determined with BCA (Thermo Fisher 23 227). 300 μg of liver tissues was added to V-PLEX Proinflammatory panel 1 mouse (MSD K15048D) and V-PLEX Cytokine panel 1 mouse (MSD K15245D), and manufacturer instructions were followed.

2.5 | Histology

Liver was formalin-fixed for 24 h, transferred to ethanol and then embedded in paraffin. Representative 4-μm thick sections were stained by single brightfield immunohistochemistry for CK19 (Abcam ab133496), CD11b (Abcam ab133357), VCAM (Abcam ab134047) or Picro Sirius red. Whole liver section histomorphometric measurements were generated with the software from Visiopharm (Hoersholm, Denmark) using a semi-automated approach. Algorithms were prepared for the morphometric measurement of each signal. The algorithm was generated with

the linear Bayesian segmentation tool in the software package, which was further refined through training on a subset of liver sections from 5 animals.

2.6 | Statistical Analysis

GraphPad Prism software was used to analyse data for statistical significance. Outliers were removed by the ROUT method [15] and then distribution was determined; a two-tailed *t*-test was used to determine significance. Group means and SEM are presented.

3 | Results

We reasoned that *Abcb4*$^{+/-}$ mice, which have reduced biliary PL content [13], would be susceptible to bile duct injury and form the foundation of a model for toxic bile-induced adult-onset hepatobiliary disease. Bile isolated from phenotypically normal *Abcb4*$^{+/-}$ mice contained half the amount of PL compared to wild-type (WT) mice (Figure 1A left HET = 17 0.3 mM ± 1.1 vs. WT = 35.2 mM ± 3.6), while BA levels were equivalent between WT and *Abcb4*$^{+/-}$ mice (Figure 1A middle HET = 70.0 mM ± 5.0 vs. WT = 76.0 mM ± 5.7) as previously published [13]. There was a significant increase in the ratio of BA/PL in the *Abcb4*$^{+/-}$ mice, indicating a more toxic bile composition creating a more permissive environment for biliary injury (Figure 1A right HET = 4.1 ± 0.2 vs. WT = 2.2 ± 0.1).

3.1 | Cholic Acid Diet and Lithocholic Acid Diet Challenge of Abcb4$^{+/-}$ Animals

Initially, both WT mice and *Abcb4*$^{+/-}$ mice were fed a diet supplemented with either 0.5% cholic acid (CA) or 0.3% of the more hydrophobic lithocholic acid (LCA) and monitored over a period of three to 4 weeks for increases in the levels of alkaline phosphatase (ALP) and serum total BA (sTBA). The CA diet failed to increase ALP levels in either WT or *Abcb4*$^{+/-}$ mice (Figure 1B left WT = 131.7 U/L ± 16.8 vs. 135.2 U/L ± 5.7; HET = 124.9 U/L ± 6.5 vs. 132.5 U/L ± 7.4). An increase in sTBA over time was observed; however, there was no difference between WT and *Abcb4*$^{+/-}$ mice on this diet (Figure 1B right WT 3 week no CA = 3.2 μM ± 0.4 vs. WT 3 week with CA = 34.4 μM ± 10.9; HET 3 week no CA = 4.6 μM ± 0.9 vs. HET 1 week with CA = 17.3 μM ± 5.6 vs. HET 2 week with CA = 21.7 μM ± 5.1 vs. HET 3 week with CA = 36.0 μM ± 15.6). In mice fed the 0.3% LCA diet, there was not a significant difference between WT and *Abcb4*$^{+/-}$ in ALP or sTBA levels (Figure 1C ALP: WT 4 week = 108.5 U/L ± 13.6 vs. HET 4 week 205.7 U/L ± 42.7; sTBA WT 4 week = 12.9 μM ± 5.3 vs. HET with LCA = 19.0 μM ± 3.5). These data indicate that moderate levels of additional CA or LCA alone were not sufficient to induce significant hepatobiliary injury over a 3–4-week period in animals with half the amount of Abcb4 and biliary PL present in WT animals.

3.2 | Lithogenic Diet Challenge of Abcb4$^{+/-}$ Animals

To explore the impact of additional stress on the hepatobiliary system, both WT and *Abcb4*$^{+/-}$ mice were fed a LD, which is a western diet with 15% fat,1.25% CHOL and 0.5% CA. This diet induces hepatobiliary injury and CHOL crystallization/gallstones (calculi) in WT animals as early as 4 to 8 weeks after change to the diet, depending on strain background [16–18]. Notably, after as little as 1 week on the LD diet, the *Abcb4*$^{+/-}$ mice demonstrated increases in both ALP and sTBA relative to WT animals (Figure 1D, top ALP: WT 1 week = 124.0 U/L ± 13.0 and WT 6 week = 127.8 U/L ± 5.2 vs. HET 1 week = 267.7 U/L ± 23.0 and HET 6 week = 579.8 U/L ± 105.8; sTBA WT 1 week = 5.4 μM ± 1.0 and WT 6 week = 10.5 μM ± 2.8 vs. HET 1 week = 35.7 μM ± 7.3 and HET 6 week = 96.3 μM ± 30.1). In addition, markers indicating hepatic inflammation such as alanine transaminase (ALT) and aspartate amino transferase (AST) were preferentially elevated in *Abcb4*$^{+/-}$ mice compared to WT mice as early as 1 week (Figure 1D bottom ALT: WT 1 week = 231.4 U/L ± 31.5 and WT 6 week = 248.0 U/L ± 36.7 vs. HET 1 week = 485.8 U/L ± 64.9 and HET 6 week = 1122.8 U/L ± 252.3; AST: WT 1 week = 362.0 U/L ± 38.2 and WT 6 week = 358.6 U/L ± 58.6 vs. HET 1 week = 696.4 U/L ± 147.1 and HET 6 week = 954.0 U/L ± 234.0). These data demonstrated that the additional stress from the increased fat and CHOL in combination with the 0.5% CA was sufficient to selectively induce a hepatobiliary phenotype in the presence of altered bile composition in the *Abcb4*$^{+/-}$ mice.

3.3 | Hepatobiliary Phenotypes Induced by Lithogenic Diet Challenge of Abcb4$^{+/-}$ Animals

Upon necropsy, the livers of *Abcb4*$^{+/-}$ mice were significantly increased in weight relative to body weight, when compared to WT mice (Figure 2A left WT 1 week = 5.4 ± 0.1 and WT 6 week = 6.6 ± 0.2 vs. HET 1 week = 6.7 ± 0.1 and HET 6 week = 11.2 ± 0.4). This increase was apparent as early as 1 week on the diet and was further exaggerated the longer the animals remained on the LD diet. Because pathogenic *ABCB4* variants are associated with LPAC [6], some PSC patients have low biliary PL [12], and 1 in 4 PSC patients present with cholelithiasis [19]. The incidence of crystal formation in the gall bladders in *Abcb4*$^{+/-}$ and WT animals was scored (Figure 2A center), Although WT animals did not present with any incidence of calculi for the first 2 weeks on diet, *Abcb4*$^{+/-}$ animals rapidly formed calculi as early as 1 week on diet (40% of animals), and all *Abcb4*$^{+/-}$ animals presented with calculi after only 2 weeks on diet. The observation of calculi in the gallbladder raises the possibility that calculi are also forming in intrahepatic bile ducts and damaging the liver.

To characterise the extent of liver damage occurring in the *Abcb4*$^{+/-}$ mice, we evaluated markers of ductular reaction, cholangitis and fibrosis using immunohistochemistry. Following 6 weeks on the LD diet, *Abcb4*$^{+/-}$ animals displayed elevated levels of Ck19 and Vcam, indicating ductular epithelial cell injury, activation and proliferative reaction consistent with the presence of ductular reaction (Figure 2B Ck19 WT = 0.44% ± 0.03 vs. HET = 4.03% ± 0.75; Vcam WT = 2.09% ± 0.26 vs. HET = 7.99% ± 1.19). Moreover, increased Cd11b staining indicated the presence of infiltrating neutrophils, a hallmark of the cholangitis in PSC (Figure 2B WT = 0.31% ± 0.04 vs. HET = 0.74% ± 0.14) [20, 21]. Corroborating the presence of cholangitis, levels of liver cytokines Cxcl-1, Tnf-α, Mcp-1, Mip1α and Mip-2 were all elevated in *Abcb4*$^{+/-}$ mice compared to WT mice (Figure 2B Cxcl-1 WT = 1491.4 pg/g ± 268.0 vs. HET = 3203.5 pg/g ± 431.3; Tnf-α WT = 2269.3 pg/g ± 333.1

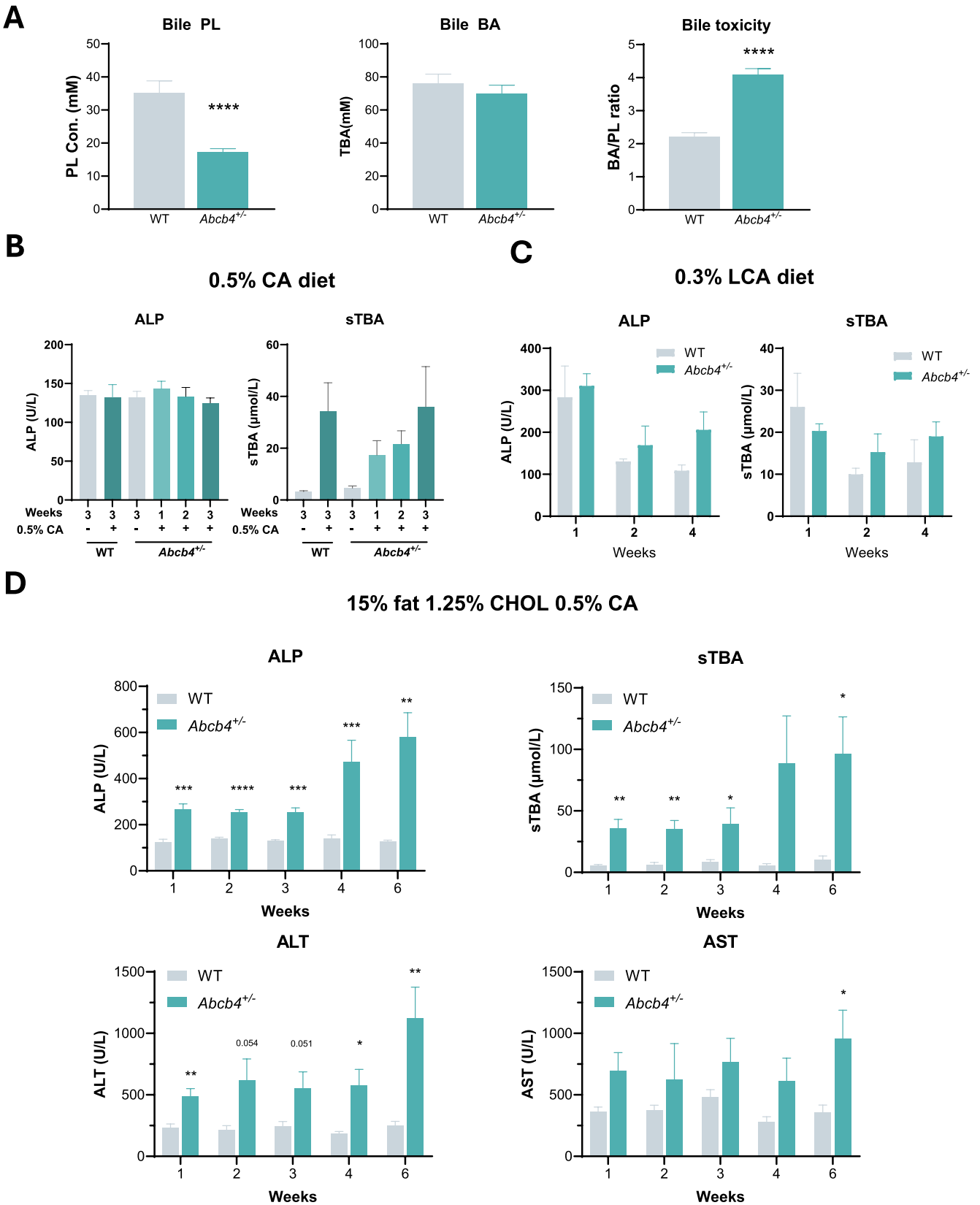

FIGURE 1 | *Abcb4* haploinsufficiency predisposes to hepatic and cholestatic injury in response to diet. (A) Bile concentration of phospholipid (left) and bile acid (middle), and the ratio of BA to PL (right) from WT and *Abcb4*$^{+/-}$mice ($N = 8$ WT, 8 *Abcb4*$^{+/-}$). (B) Serum levels of cholestatic indicators ALP and BA from WT and *Abcb4*$^{+/-}$mice fed regular diet or diet supplemented with 0.5% cholic acid (CA) ($N = 6$ WT, 10 *Abcb4*$^{+/-}$). (C) Serum levels of ALP and BA from WT and *Abcb4*$^{+/-}$ mice fed a regular diet or a diet supplemented with 0.3% lithocholic acid (LCA) ($N = 5$ at 1 and 1 weeks, $N = 10$ at 4 weeks). (D) Time course of serum levels of cholestatic (*top*, ALP and BA) and hepatotoxic (*bottom*, ALT and AST) from WT and *Abcb4*$^{+/-}$ mice fed LD for up to 6 weeks ($N = 5$).

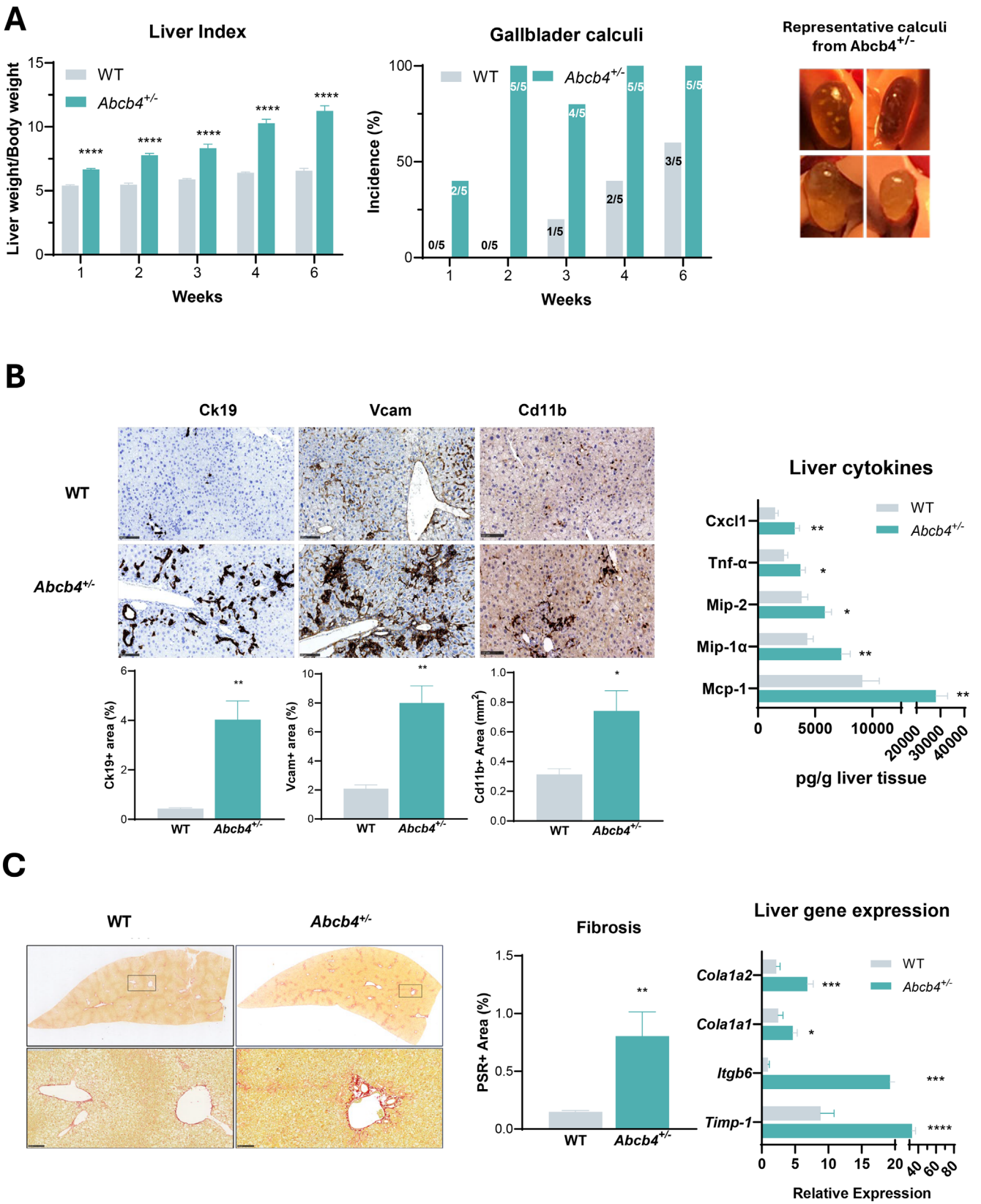

FIGURE 2 | Development of hepatobiliary phenotypes is accelerated in the *Abcb4*$^{+/-}$ mice. (A) Liver weight to body weight (*left*) and incidence of gallbladder calculi (*middle and right*) for WT and *Abcb4*$^{+/-}$ mice fed LD after 1 week through 6 weeks ($N = 5$). (B) Representative images (*top left*) and quantitation of (*bottom left*) immunohistochemistry for makers of ductular reaction (Ck19, Vcam) and cholangitis (CD11b) from WT and *Abcb4*$^{+/-}$ animals on LD diet for 6 weeks ($N = 5$). (*right*) Protein levels of inflammatory modulators from livers of WT and *Abcb4*$^{+/-}$ mice fed LD diet for 6 weeks ($N = 6$WT, *Abcb4*$^{+/-}$). (C) Picro Sirius red staining (left) of livers from WT and *Abcb4*$^{+/-}$ mice on LD diet for 6 weeks ($N = 5$). Quantitation of staining (*center*) and gene expression for markers of fibrosis (*right*) ($N = 6$WT, *Abcb4*$^{+/-}$).

vs. HET = 3696.2 pg/g ± 441.4; Mip-2 WT = 3815.9 pg/g ± 529.6 vs. HET = 5836.1 pg/g ± 591.8; Mip-1α WT = 4308.1 pg/g ± 517.6 vs. HET = 7277.8 ± 784.4; Mcp-1 WT = 9113.7 ± 1479.4 vs. HET = 28067.5 ± 4908.6). *Abcb4*$^{+/-}$ mice also demonstrated elevated fibrosis and collagen deposition in the portal region with initial spreading into the septum (Figure 2C PSR WT = 0.15% ± 0.01 vs. HET = 0.80 ± 0.21). Transcript levels of fibrogenic genes *Col1a1*, *Col1a2*, *Timp-1* and *Itgb6* were also elevated in *Abcb4*$^{+/-}$ mice (Figure 2C Col1a1 WT = 2.5 ± 0.7 vs. HET = 4.7 ± 0.7; Col1a2 WT = 2.2 ± 0.6 vs. HET 6.8 ± 0.9; Timp-1 WT = 8.84 ± 2.0 vs. HET = 33.0 ± 3.9; Itgb6 WT = 0.9 ± 0.2 vs. HET = 19.3 ± 4.1). Taken together, these data indicate that biliary PL plays an important role in maintaining hepatobiliary homeostasis and that a reduction in those homeostatic levels predisposes to hepatobiliary injury.

4 | Conclusion

The current study demonstrates that haploinsufficiency of the canalicular PL transporter ABCB4 significantly sensitises mice to a secondary dietary injury, providing a robust, translational model to explore how altered toxic bile composition acts as a critical predisposing factor in rare adult-onset hepatobiliary disease.

While biallelic inheritance of *ABCB4*-deficient alleles causes PFIC3, the association of *ABCB4* haploinsufficiency with human conditions like ICP and LPAC reveals that even a partial deficit in biliary PL secretion is pathogenic. By subjecting *Abcb4*$^{+/-}$ mice to a LD, we preferentially induced a severe hepatobiliary phenotype compared to WT animals on the same diet. The pathology encompassed a rapid and preferential increase in peripheral markers of cholestasis and hepatotoxicity; accelerated CHOL crystallization/calculi formation; histological evidence of cholangitis including ductular reaction, inflammatory cell infiltration and elevated pro-inflammatory cytokines and significant peri-portal fibrosis.

The combined features of cholestasis, cholangitis, calculi formation and fibrosis observed in the stressed *Abcb4*$^{+/-}$ mice closely resemble key pathological traits found in complex adult diseases, notably PSC [12, 19]. We propose that the resulting reduction of protective PL in bile [12] makes the biliary epithelium highly vulnerable to monomeric BAs and CHOL crystals [19], promoting ductular reaction and subsequent progression to chronic inflammation and fibrosis. This model strongly suggests that toxic bile composition is present in, and actively promotes the progression of, heterogeneous adult cholangiopathies like PSC.

Given the clear correlation between human genetics, phenotypic associations and the induced pathology, the *Abcb4*$^{+/-}$ combined with a LD model establishes a vital preclinical platform for interrogating disease mechanisms and for testing novel therapeutics designed to modulate bile composition as a treatment strategy for PSC and other rare adult-onset hepatobiliary diseases.

Author Contributions

E.L.B., Y.J., J.K.T. and J.P.M. performed the research and data analysis, E.L.B. and A.S.G. designed the research study; E.L.B., N.O.F., J.P.M. and R.O.H. wrote the paper.

Acknowledgements

We would like to thank Dan Crawford, Pol Boudes and the rest of the Rectify team for valuable input on this work.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

Conflicts of Interest

E.L.B., N.O.F., J.P.M., A.S.G., R.O.H. hold equity in Rectify Pharmaceuticals.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1. H. Devarbhavi, S. K. Asrani, J. P. Arab, Y. A. Nartey, E. Pose, and P. S. Kamath, "Global Burden of Liver Disease: 2023 Update," *Journal of Hepatology* 79 (2023): 516–537.

2. J. S. Fleishman and S. Kumar, "Bile Acid Metabolism and Signaling in Health and Disease: Molecular Mechanisms and Therapeutic Targets," *Signal Transduction and Targeted Therapy* 9 (2024): 97.

3. J. L. Boyer and C. J. Soroka, "Bile Formation and Secretion: An Update," *Journal of Hepatology* 75 (2021): 190–201.

4. J. M. L. de Vree, E. Jacquemin, E. Sturm, et al., "Mutations in the MDR3 Gene Cause Progressive Familial Intrahepatic Cholestasis," *National Academy of Sciences of the United States of America* 95 (1998): 282–287.

5. P. H. Dixon, N. Weerasekera, K. J. Linton, et al., "Heterozygous MDR3 Missense Mutation Associated With Intrahepatic Cholestasis of Pregnancy: Evidence for a Defect in Protein Trafficking," *Human Molecular Genetics* 9, no. 8 (2000): 1209–1217, https://doi.org/10.1093/hmg/9.8.1209.

6. H. Wang, P. Portincasa, M. Liu, and D. Wang, "Genetic Analysis of ABCB4 Mutations and Variants Related to the Pathogenesis and Pathophysiology of Low Phospholipid-Associated Cholelithiasis," *Genes* 13, no. 6 (2022): 1047, https://doi.org/10.3390/genes13061047.

7. D. Gudbjartsson, H. Helgason, S. Gudjonsson, et al., "Large-Scale Whole-Genome Sequencing of the Icelandic Population," *Nature Genetics* 47 (2015): 435–444, https://doi.org/10.1038/ng.3247.

8. J. S. Nayagam, P. Foskett, S. Strautnieks, et al., "Clinical Phenotype of Adult-Onset Liver Disease in Patients With Variants in ABCB4, ABCB11, and ATP8B1," *Hepatology Communications* 6 (2022): 2654–2664.

9. C. Dröge, M. Bonus, U. Baumann, et al., "Sequencing of FIC1, BSEP and MDR3 in a Large Cohort of Patients With Cholestasis Revealed a High Number of Different Genetic Variants," *Journal of Hepatology* 67 (2017): 1253–1264.

10. M. Ziol, V. Barbu, O. Rosmorduc, et al., "ABCB4 Heterozygous Gene Mutations Associated With Fibrosing Cholestatic Liver Disease in Adults," *Gastroenterology* 135 (2008): 131–141.

11. D. Degiorgio, A. Crosignani, C. Colombo, et al., "ABCB4 Mutations in Adult Patients With Cholestatic Liver Disease: Impact and Phenotypic Expression," *Journal of Gastroenterology* 51 (2016): 271–280.

12. S. Mohajeri, T. Bezabeh, O. B. Ijare, et al., "In Vivo 1H MRS of Human Gallbladder Bile in Understanding the Pathophysiology of Primary Sclerosing Cholangitis (PSC): Immune-Mediated Disease Versus Bile Acid-Induced Injury," *NMR in Biomedicine* 32 (2019): e4065.

13. J. J. M. Smit, A. H. Schinkel, R. P. J. O. Elferink, et al., "Homozygous Disruption of the Murine MDR2 P-Glycoprotein Gene Leads to a Complete Absence of Phospholipid From Bile and to Liver Disease," *Cell* 75 (1993): 451–462.

14. O. Rosmorduc, R. Poupon, and B. Hermelin, "MDR3 Gene Defect in Adults With Symptomatic Intrahepatic and Gallbladder Cholesterol Cholelithiasis," *Gastroenterology* 120 (2001): 1459–1467.

15. H. J. Motulsky and R. E. Brown, "Detecting Outliers When Fitting Data With Nonlinear Regression – A New Method Based on Robust Nonlinear Regression and the False Discovery Rate," *BMC Bioinformatics* 7 (2006): 123.

16. P. M. Nishina, J. Verstuyft, and B. Paigen, "Synthetic Low and High Fat Diets for the Study of Atherosclerosis in the Mouse," *Journal of Lipid Research* 31 (1990): 859–869.

17. B. Khanuja, Y. C. Cheah, M. Hunt, et al., "Lith1, a Major Gene Affecting Cholesterol Gallstone Formation Among Inbred Strains of Mice," *National Academy of Sciences of the United States of America* 92 (1995): 7729–7733.

18. D. Q. Wang, B. Paigen, and M. C. Carey, "Phenotypic Characterization of Lith Genes That Determine Susceptibility to Cholesterol Cholelithiasis in Inbred Mice: Physical-Chemistry of Gallbladder Bile," *Journal of Lipid Research* 38 (1997): 1395–1411.

19. K. Said, H. Glaumann, and A. Bergquist, "Gallbladder Disease in Patients With Primary Sclerosing Cholangitis," *Journal of Hepatology* 48 (2008): 598–605.

20. C. Zimmer, E. von Seth, M. Buggert, et al., "A Biliary Immune Landscape Map of Primary Sclerosing Cholangitis Reveals a Dominant Network of Neutrophils and Tissue-Resident T Cells," *Science Translational Medicine* 13, no. 599 (2021): eabb3107, https://doi.org/10.1126/scitranslmed.abb3107.

21. M. Takeuchi, P. T. Vidigal, M. T. Guerra, et al., "Neutrophils Interact With Cholangiocytes to Cause Cholestatic Changes in Alcoholic Hepatitis," *Gut* 70 (2021): 342–356.