Received: 26 October 2023 | Revised: 5 March 2024 | Accepted: 8 April 2024

DOI: 10.1111/liv.15945

Liver INTERNATIONAL WILEY

ORIGINAL ARTICLE

Comorbidities, mortality and metabolic profile in individuals with primary biliary cholangitis—A Phenome-Wide-Association-Study

Paul-Henry Koop[1] | Constanze Schwenzer[1] | Jan Clusmann[1] | Mara S. Vell[1] | Julius Jaeger[1] | Wenfang Gui[1] | Christian Trautwein[1] | Alexander Koch[1] | Tony Bruns[1] | Carolin V. Schneider[1,2] | Kai Markus Schneider[1]

[1]Department of Internal Medicine III, Gastroenterology, Metabolic Diseases and Intensive Care, University Hospital RWTH Aachen, Aachen, Germany

[2]The Institute for Translational Medicine and Therapeutics, The Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA

Correspondence
Kai Markus Schneider, Department of Internal Medicine III, Gastroenterology, Metabolic Diseases and Intensive Care, University Hospital RWTH Aachen, Pauwelsstr. 30, 52074 Aachen, Germany.
Email: kmschneider@ukaachen.de

Funding information
Federal Ministry of Education and Research; Ministry of Culture and Science of the German State of North Rhine-Westphalia

Handling Editor: Dr. Alessio Aghemo

Abstract

Background and Aims: Primary biliary cholangitis (PBC) is a chronic, immune-mediated liver disease that can lead to fibrosis and cirrhosis. In this cohort study, we aimed to investigate morbidity and mortality in conjunction with metabolomic changes of PBC in a UK population-based cohort.

Methods: 454 participants with PBC and 908 propensity score (age, sex, BMI, ethnicity) matched controls without liver disease were included in the study. A subset of participants with PBC and controls were analysed for their metabolomic profile. Further, PBC-associated comorbidities were investigated by PheWAS analysis. Lastly, we assessed causes of death in individuals with PBC using a Fine and Grey competing-risks regression model.

Results: Compared to the control group, various pathways associated with the metabolism of amino acids, lipids, and liver biochemistry were significantly enriched in individuals with PBC. We found reduced levels of S-HDL-cholesterol and Glycoprotein Acetyls in individuals with PBC as well as an association with diseases of the circulatory system. Notably, PBC individuals had a higher prevalence of digestive diseases, autoimmune diseases, cardiovascular diseases, anaemias, mental disorders, and urinary tract infections compared to the control group. Strikingly, the overall mortality was almost three times higher in the PBC group compared to the control group, with diseases of the digestive system accounting for a significant elevation of the death rate. A subsequent analysis, enhanced by propensity score matching that included the APRI score, demonstrated that the observed morbidity could not be exclusively attributed to advanced hepatic disease.

Conclusions: Our study provides a detailed perspective on the morbidity of individuals with PBC. The exploration of potential effects of disease state on morbidity suggest that early detection and early treatment of PBC could enhance patient prognosis

Paul-Henry Koop and Constanze Schwenzer share the first authorship.

Carolin V. Schneider and Kai Markus Schneider share the last authorship.

and prevent the onset of comorbid diseases. Finally, the metabolomic alterations could represent a link between the pathophysiological processes underlying PBC development, progression, and associated morbidity.

KEYWORDS
HDL, HPheWAS, metabolism, morbidity, mortality, PBC

1 | INTRODUCTION

Primary biliary cholangitis (PBC) is a chronic immune-mediated cholestatic liver disease characterised by a slow progression leading to the destruction of the small intrahepatic bile ducts, with a notable female predominance of approximately 90%.[1] The disease spectrum ranges from asymptomatic early stages to severe complications such as fatigue, pruritus, ascites, hepatic encephalopathy, and oesophageal variceal haemorrhage in advanced stages.[2] The aetiology of PBC is multifactorial, involving genetic predisposition, environmental factors, and immune-mediated mechanisms, including epigenetic alterations, dysregulated mucosal immunity, and impaired biliary epithelial cell function.[3] Despite the recognition of genetic contributions to autoimmunity in PBC,[4] identified genetic variants account for only 16% of the disease's total heritability,[5] highlighting the significant role of environmental factors and metabolic pathways in its pathogenesis.[6–9] Current first-line treatment with ursodeoxycholic acid (UDCA) has been suboptimal, with a notable proportion of patients not responding adequately.[10] Recent research has focused on identifying novel biomarkers, including autoantibodies, genetic polymorphisms, metabolomics and microRNA, to enhance our understanding of PBC pathogenesis and improve patient stratification.

This study aims to elucidate the complex interplay between genetic, environmental, and metabolic factors in PBC by conducting a comprehensive analysis of comorbidities, lipidomic, and metabolic profiles on a population level. In smaller study cohorts the metabolic profile has already been analysed to determine the serum metabolomes of individuals with PBC, particularly compared to other cholestatic diseases showing disease-specific metabolomic alterations.[11] However, to the best of our knowledge, we are the first to provide a comprehensive picture of PBC comorbidities by PheWAS together with the lipidomic and metabolic profile on a population-based level.

We hypothesise that a detailed examination of these aspects will reveal novel insights into the pathogenesis of PBC, uncover mechanistic links between PBC and its associated comorbidities, and identify potential biomarkers for disease progression. Ultimately, our objectives are to improve the understanding of PBC's underlying mechanisms, enhance patient care through more precise and individualised follow-up procedures, and identify potential therapeutic targets for intervention.

Keypoints

Here, we provide insights into novel and established associations of primary biliary cholangitis, an immune-mediated cholestatic liver disease, with a variety of other diseases. Moreover, we describe alterations in serum lipidomics and metabolomics, which could represent a link between the pathophysiological processes underlying PBC development, progression and associated morbidity.

2 | METHODS

2.1 | Study cohort

The 'UK Biobank' (UKB) is a community-based cohort study conducted in the United Kingdom at 22 participating centres. The baseline examinations were carried out from 2006 to 2010 and recruited 502.505 volunteers aged 37 to 73 years. All participants gave informed consent for data linkage to medical reports. At the baseline assessment (2006–2010), the participants provided demographic and physical measures as well as laboratory measurements. Ongoing inpatient hospital records beginning in 1996 were used to identify diagnoses according to ICD-9 and 10 codes as it was performed in other studies for PBC disease utilising the UK biobank. This research has been conducted using the UK Biobank Resource under Application Number 71300.

2.2 | Case definition

PBC was defined as the presence of K74.3 in the lifetime ICD-10 diagnoses or coding as the primary cause of death in the *National Death Registries*.

2.3 | Disease progression and severity

To assess the baseline extent of disease progression or severity, specifically in terms of liver fibrosis and cirrhosis, we also included the *aspartate aminotransferase* (*AST*) *to platelet ratio index* (APRI score), calculated as described by Wei et al.[12,13] Through the matching of our cohort based on the APRI score, our objective is to provide more

comprehensive insights into the distinctive characteristics of PBC in contrast to other biliary disorders.

2.4 | Case–control matching

We performed 2 propensity score matchings. The first one only on age, sex, BMI and self-reported ethnic background and the second one additionally on the individual APRI score and the use of cholesterol lowering medication at baseline (Field ID 6177). The second matching resulted in 1057 participants with 356 individuals with PBC, examined using also a logistic multivariate regression model (Figure 3B).

After the logistic-regression-based propensity score estimation with k-nearest-neighbour (k-NN) allocation, two iterations were performed, resulting in a 2:1 balance of controls over cases, using the *PsmPy* (*0.3.13*) package in a Python 3.9.5 environment. The propensity score was calculated using age, sex, BMI and self-reported ethnic background in both matchings, as well as the APRI score and the use of cholesterol lowering medication (data field 6177) in the second one. In total, 1362 participants were enrolled in further analysis.

2.5 | PheWAS analysis

The coding for clinical diagnoses in our study followed the WHO's International Classification of Diseases 10th and 9th generation (ICD-10). For each participant, ICD codes from the electronic health records (EHR) were collated and duplicates removed. We converted the ICD codes of the 1362 enrolled participants into 457 associated Phe-Codes using the pyPheWAS package.[14] A series of case–control tests were performed by fitting multiple logistic regression models, two for every PheCode of interest, on the binary outcome of PBC or control, using the participant's PheCodes as covariates. The influence of the analysed PheCode was then evaluated by evaluating the beta and testing for statistical significance. To ensure statistical power, we only included PheCodes that appeared at least 20 times in the matched cohort. To further reduce the influence of age, sex, BMI and self-reported ethnic background after propensity score matching,[15] these factors were used as patient-specific covariates in every regression of the PheWAS. Thereby, the regression models are corrected for age, sex, BMI and self-reported ethnic background, as well as the APRI score and the use of cholesterol lowering medication if also matched on.[13]

2.6 | Metabolomics

To dissect the metabolic alterations associated with *PBC*, we analysed 143 metabolites that were measured via nuclear magnetic resonance spectroscopy in a subset of 204 individuals diagnosed with PBC and 451 controls. These 655 study participants were analysed by a logistic multivariate regression model (Figure 1A). For we had to drop some participants when correcting for the APRI in the comparative analysis, additionally corrected for the APRI, this includes 195 participants with PBC and 406 controls (Figure 1B).

2.7 | Mortality analysis

The UK Biobank receives death notifications (age at death and primary ICD diagnosis that led to death) through linkage to National Death Registries. End of follow-up was defined as death or end of hospital inpatient data collection in January 2023. Causes of death included all Malignancies (C00-C97), Malignant neoplasm of the palate (C5), Malignant neoplasm of liver and intrahepatic bile ducts (C22), Malignant neoplasm of bronchus and lung (C34), Mental and behavioural disorders (F), Inflammatory disease of the central nervous system (G), Disease of the circulatory system (I), Rheumatic aortic stenosis (I6), Diseases of the respiratory system (J), Diseases of the digestive system (K), Liver diseases (K7), Fibrosis and cirrhosis of the liver (K74), Other inflammatory liver disease (K75) and Other disease of the biliary tract (K83).

2.8 | Statistical analysis

All continuous variables were analysed by unpaired, two-tailed t-tests or non-parametric Mann–Whitney *U* test, and by an appropriate multivariable regression-model. The results are presented as mean ± standard deviation (normal distribution) or median [IQR] (non-normal distribution). All categorical variables were displayed as relative (%) frequencies, and the corresponding contingency tables were analysed using the Chi-square test. Odds/hazard ratios (ORs/HRs) were presented with their corresponding 95% or 90% confidence intervals (CI) given in brackets. HRs were calculated using Cox proportional hazard regression models. For competing risk analyses (Fine and Grey model),[16] we tabulated the numbers of deaths per ICD-10 code and compared them with those who were deceased of other causes as well as survivors. Multivariable logistic regression was performed to test for independent associations. The PheWAS analysis was performed using an adjusted version of the *pyPheWAS* python package.[14] Differences were considered to be statistically significant when $p \leq 0.05$ and adjusted for multiple testing using the *Bonferroni* method.[17] The data were analysed using SPSS Statistics version 26 (IBM; Armonk, NY, USA) and *statsmodels* (*v0.14.0*), *SciPy* (*1.10.1*),[18] *tableone* (*0.7.12*)[19] in a python 3.9 environment. For visualisation, we used Prism version 8 (GraphPad, LaJolla, CA, USA), matplotlib (v3.5.1)[20] and seaborn (v0.12.2).[21]

3 | RESULTS

3.1 | General characteristics of the study population

The prevalence rate of PBC was 91 per 100.000 for the UKB cohort in total. Our study included 453.627 participants from the UKBiobank, resulting in a 2:1 propensity-score-matched cohort of

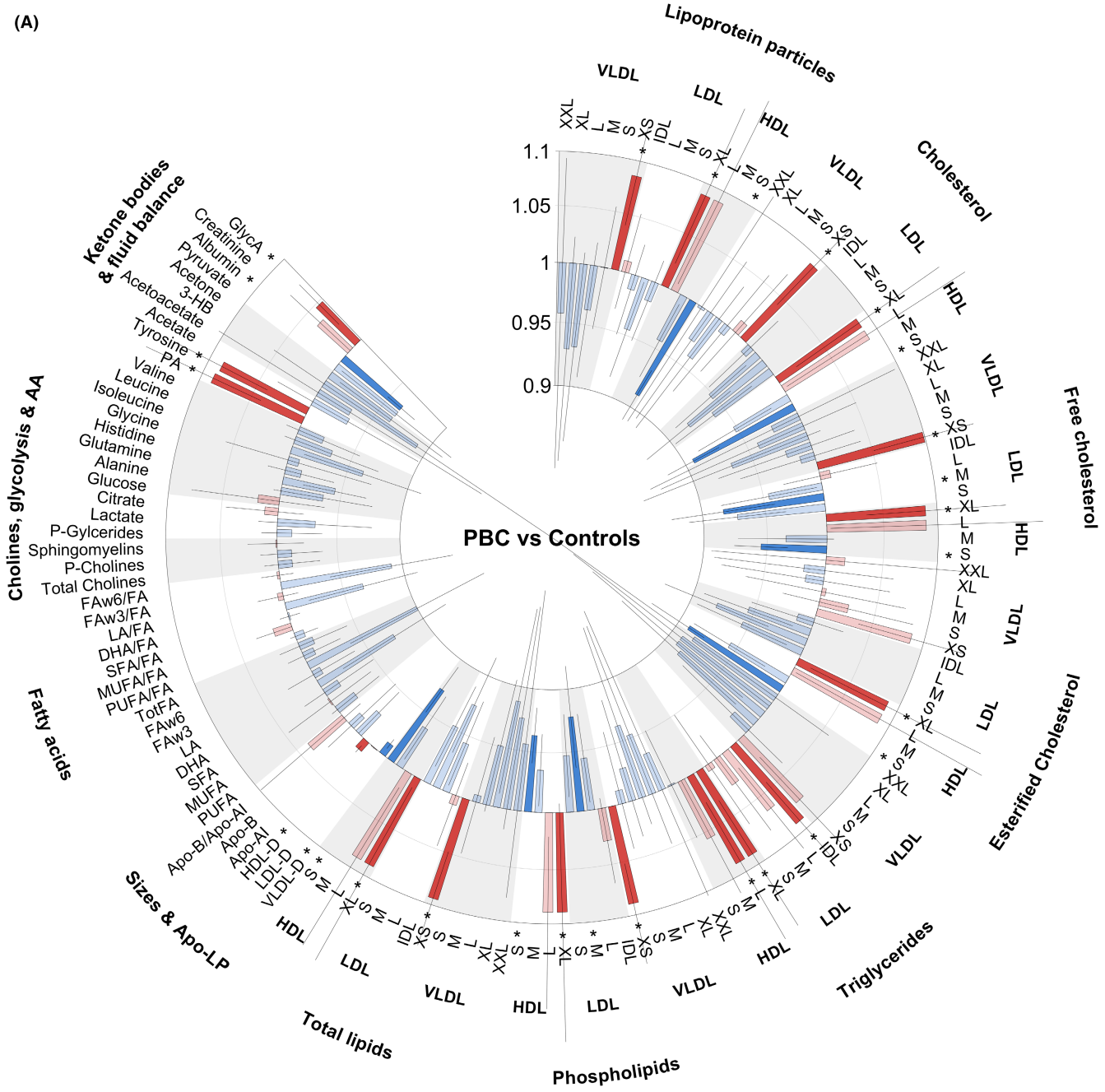

FIGURE 1 Circle plots for lipidomic analysis for PBC participants compared to controls. Lipidomic parameters were measured via nuclear magnetic resonance spectroscopy (NMR). Hazard ratios (with 95% confidence intervals) are presented per 1-SD higher metabolic biomarker on the natural log scale. (A) Regression using dataset of 1362 participants, matched on age, sex, BMI, self-reported ethnic background, with 454 PBC individuals and is corrected by age, sex, BMI and self-reported ethnic background. Out of these participants, metabolomic data were available for 451 controls and 204 participants with PBC. (B) Regression performed on the dataset matched on age, sex, BMI, self-reported ethnic background, use of cholesterol lowering medication and the APRI, hence on 195 participants with PBC and 406 controls. The correction and matching of APRI results in a more balanced presence of liver impairment in the compared populations and therefore elucidates the effects more unique for PBC as one specific cause of hepatic cirrhosis or fibrosis. *$p < 0.05$. DHA, docosahexaenoic acid; FA, fatty acids; FAw3, omega-3 fatty acids; FAw6, omega-6 fatty acids; HDL-D, high-density lipoprotein particle diameter; LA, linoleic acid; LDL, low-density lipoproteins; LDL-D, low-density lipoprotein particle diameter; LP, lipoprotein; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; VLDL-D, very low-density lipoprotein particle diameter; (original code by Diego J Aguilar-Ramirez).

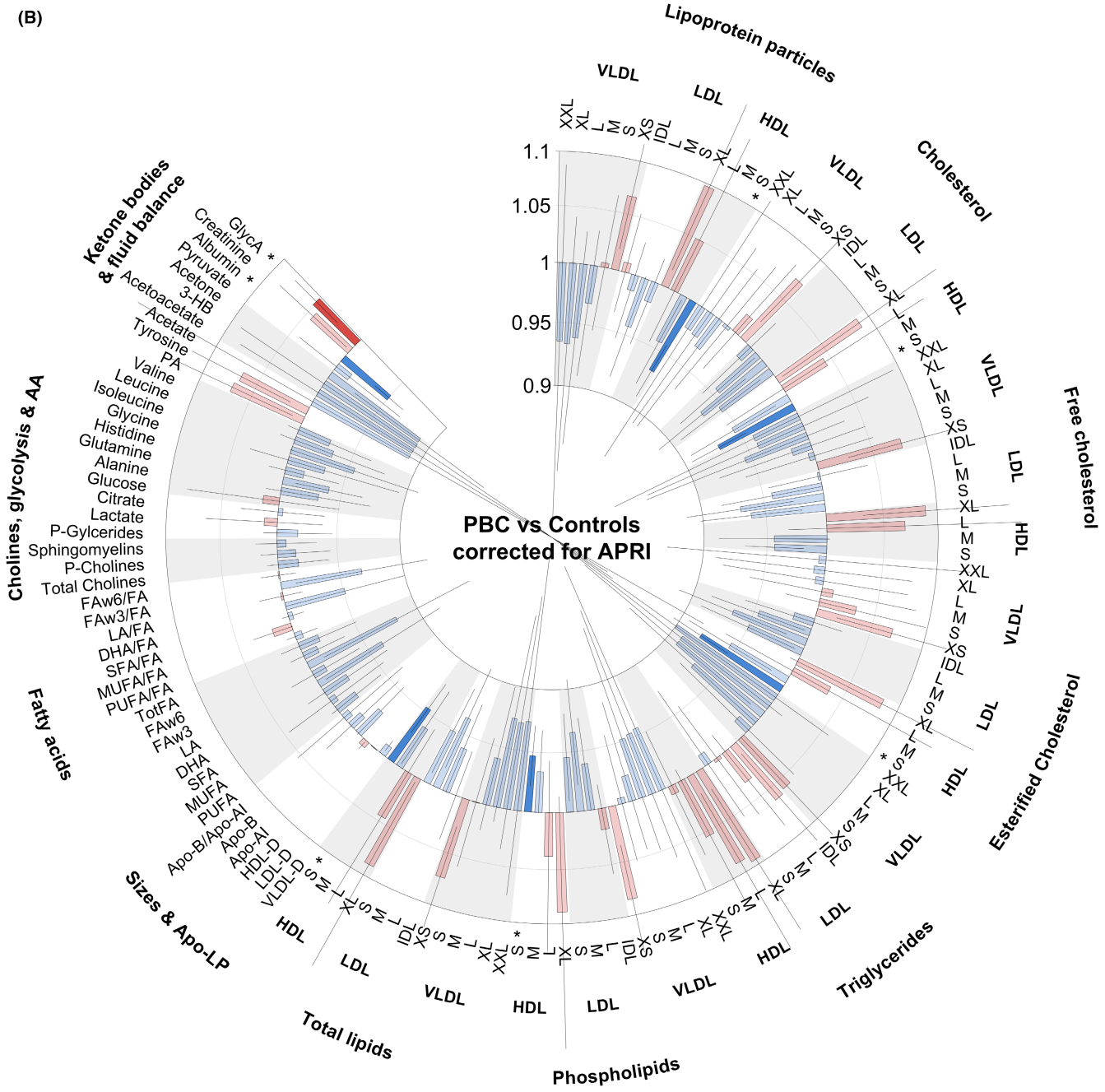

FIGURE 1 (Continued)

1362 participants, while 454 (33.3%) were diagnosed with primary biliary cholangitis. After the matching, age, sex, self-reported ethnic background and BMI were well-balanced between the PBC and healthy control group, ensuring adequate comparability (Tables 1 and S1). When incorporating the APRI score within the matching process, we were able to balance the cases and controls across all covariates except for APRI. Notably, the degree of similarity for APRI far surpassed that observed in the unmatched cohort. The vast majority, consistent with the overall demographics of the UKBiobank, reported a Caucasian ethnic background. (97.2%—control group and 98.3%—PBC group) (Table 1).

3.2 | Serum biochemical changes in PBC individuals

Statistical comparisons of liver biochemistry showed significantly elevated levels of cholestasis parameters and transaminases in the PBC group compared to healthy controls (Alkaline phosphatase (U/L) [PBC: 201.2 ±; C: 86.3 ± 23.11; $p < 0.001$]; y-glutamyltransferase (U/L) [PBC: 168.8 ± 185.3; C: 34.1 ± 34.5; $p < 0.001$]; total bilirubin (μmol/L) [PBC: 10.2 ± 8.6; C: 8.2 ± 3.6; $p < 0.001$]; aspartate aminotransferase (U/L) [PBC: 42.8 ± 29.9; C: 25.7 ± 12.9; $p < 0.001$] and alanine aminotransferase (U/L) [PBC:

TABLE 1 Descriptive statistics comparing controls and individuals with PBC.

	Missing	Overall	PBC	Control	*p* value
Count, *n* (%)		1362	454 (33.3)	908 (66.7)	
Sex, *n* (%)					
Female	0	1099 (80.7)	366 (80.6)	733 (80.7)	1.000
Male		263 (19.3)	88 (19.4)	175 (19.3)	
Ethnicity, *n* (%)					
Asian or Asian British	0	5 (0.4)	3 (0.7)	2 (0.2)	0.563
Mixed		17 (1.2)	4 (0.9)	13 (1.4)	
Other ethnic group		9 (0.7)	3 (0.7)	6 (0.7)	
White		1326 (97.4)	444 (97.8)	882 (97.1)	
Black or Black British		3 (0.2)		3 (0.3)	
Do not know		1 (0.1)		1 (0.1)	
Prefer not to answer		1 (0.1)		1 (0.1)	
BMI, mean (SD) [kg/m^2]	0	27.7 (5.1)	28.1 (5.2)	27.5 (5.1)	0.056
Age, mean (SD) [y]	0	59.2 (7.1)	59.1 (7.1)	59.3 (7.1)	0.565
Platelets, mean (SD) [109/L]	70	257.0 (67.3)	247.5 (74.8)	261.8 (62.7)	0.001
Albumin, mean (SD) [g/L]	176	44.5 (3.0)	43.2 (3.2)	45.1 (2.6)	<0.001
ALP, mean (SD) [U/L]	83	125.1 (118.2)	201.2 (180.3)	87.5 (23.7)	<0.001
ALT, mean (SD) [U/L]	84	27.5 (22.2)	39.3 (31.0)	21.7 (12.5)	<0.001
AST, mean (SD) [U/L]	93	31.4 (21.7)	42.8 (29.9)	25.7 (12.9)	<0.001
CRP, mean (SD) [mg/L]	85	3.8 (5.1)	5.8 (6.0)	2.8 (4.3)	<0.001
Cystatin C, mean (SD) [mg/L]	83	1.0 (0.2)	1.1 (0.3)	0.9 (0.1)	<0.001
GammaGT, mean (SD) [U/L]	91	78.3 (126.7)	168.8 (185.3)	34.1 (34.5)	<0.001
IGF-1, mean (SD) [nmol/L]	91	19.6 (6.0)	17.2 (6.3)	20.7 (5.5)	<0.001
Bilirubin (total), mean (SD)	92	8.9 (5.8)	10.2 (8.6)	8.2 (3.6)	<0.001
Cholesterol lowering medication users (%)	0	10	10	10	0.278
APRI, mean (SD)	127	0.4 (0.4)	0.5 (0.7)	0.3 (0.1)	<0.001
APRI high, *n* (%)					
No	0	1231 (90.4)	345 (76.0)	886 (97.6)	<0.001
Yes		131 (9.6)	109 (24.0)	22 (2.4)	

Note: The results shown here describe the population after knn-based propensity score matching (2:1; controls over cases). Quantitative variables are displayed as the mean (standard deviation), and categorical variables as the count (relative frequency). For all variables matched for (age, sex, BMI, self-reported ethnic background), a sufficient matching result could be achieved, for the groups do not significantly differ. Based on the available baseline characteristics, the APRI was calculated.

Abbreviations: ALT, alanine-aminotransferase; ALP, alkaline phosphatase; APRI, aspartate aminotransferase (AST) to platelet ratio index; Gamma-GT, gamma-glutamyl-transferase; IGF-1, insulin-like growth factor 1; UDCA, ursodeoxycholic acid.

39.3 ± 31; C: 21.7 ± 12.5; $p < 0.001$]). Additionally, we observed higher levels of C-reactive protein in the PBC group [PBC: 5.8 ± 6; C: 2.8 ± 4.3; $p < 0.001$], suggesting increased inflammatory activity in individuals with PBC. Above that, we also analysed lower levels of insulin-like growth factor (nmol/L) in study participants of the PBC group compared to the control group [PBC: 17.2 ± 6.3; C:20.7 ± 5.5; $p < 0.001$]. Moreover, we found lower albumin levels (g/dL) in individuals with PBC as compared to matched controls [PBC: 43.2 ± 3.2; C: 45.1 ± 2.6; $p < 0.001$]. Significantly higher levels of Cystatin C (mg/dL) in PBC cases were documented [PBC: 1.1 ± 0.3; C: 0.9 ± 0.1; $p < 0.001$] indicative of impaired renal function (Table 1).

3.3 | Changes in lipidomics and metabolomics in PBC individuals

Alterations in serum metabolomics and lipidomics might link PBC pathophysiology with its systemic comorbidities. We therefore analysed the metabolomic profile of 204 individuals with PBC with 451 matched controls. EDTA plasma samples were analysed using high-throughput nuclear magnetic resonance spectroscopy (NMR). The biomarkers investigated included detailed measures of cholesterol metabolism, fatty acid composition, and various low molecular weight metabolites. In order to make a statement about lipoprotein subclasses, the lipid composition and concentration were measured

considering triglycerides, phospholipids, total cholesterol, cholesterol esters, free cholesterol, and total lipid concentration within each subclass. Real-time monitoring of the measurement consistency within and between spectrometers throughout the UK Biobank samples was performed to ensure quality control. The results are reported as relative risk with standard error (SE).

Comparing both groups, we found 28 metabolites related to the metabolism of lipids and amino acids, which were significantly altered in individuals with PBC compared to healthy controls after the correction for multiple testing by the Bonferroni method. Lipidomic and metabolomic parameters were measured via NMR. It is well established that chronic liver diseases lead to lipidomic changes in individuals with chronic liver disease. In our lipidomic analysis, we found decreased free cholesterol in LDL (L-LDL 0.95 [0.92–1.0], $p=0.03$; M-LDL 0.92 [0.88–0.96], $p<0.001$; S-LDL 0.93 [0.89–0.97], $p<0.001$) and total lipids in LDL (L-LDL 0.96 [0.92–0.99], $p=0.025$; M-LDL 0.93 [0.88–0.97], $p=0.001$; S-LDL 0.95 [0.91–0.98], $p=0.006$). Above that, we found on the one hand elevated triglycerides in HDL (VL-HDL 1.15 [1.08–1.23], $p<0.001$; L-HDL 1.2 [1.12–1.28], $p<0.001$) and total lipids in HDL (VL-HDL 1.24 [1.14–1.36], $p<0.001$), as well as elevated cholesterol in large HDL (VL-HDL 1.19 [1.1–1.28], $p<0.001$), but on the other hand decreased levels of cholesterol in small HDL (S-HDL 0.91 [0.88–0.93], $p<0.001$). In general, a bigger average diameter of HDL particles seems to be associated with PBC independently of the investigated subfraction (Figure 1A).

We next sought to evaluate whether the observed lipidomic alterations were associated with already manifested chronic liver diseases or might be more specific changes for PBC. Therefore, we analysed the lipidomic profile of PBC individuals regarding liver disease severity and therefore matched and corrected additionally on the APRI (Figure 3B). After this, 7 investigated changes remained significant after correction for multiple testing (Bonferroni). Cholesterol in small HDL particles remained significantly reduced in PBC individuals (S-HDL 0.93 [0.91–0.95], $p<0.001$), as well as cholesteryl esters in small HDL (0.92 [0.89–0.95], $p<0.001$). While some of the observed lipidomic alterations might therefore be a consequence of liver cirrhosis, the concentration of small HDL particles (0.93 [0.91–0.96], $p<0.001$) seems to be associated with PBC more specifically (Figure 1B).

Lastly, we also analysed potential alterations in levels of amino acids and additional biomarkers. Indeed, we found significantly elevated levels of tyrosine (1.09 [1.04–1.14], $p<0.001$), phenylalanine (1.11 [1.05–1.17], $p<0.001$) and glycoprotein acetyls (1.05 [1.02–1.07], $p<0.001$). Lower levels of albumin (0.94 [0.92–0.95], $p<0.001$) were significantly associated with PBC individuals compared to healthy controls (Figure 3A). *Changes in* glycoprotein acetyls and albumin concentration also remained significantly associated after the incorporation of the APRI (1.05 [1.02–1.08], $p<0.001$ and 0.95 [0.93–0.96], $p<0.001$) (Figure 1B).

After including the APRI Score in the matching and correction (Figure 1B), we identified that altered levels of glycoprotein acetyls and albumin remained significantly associated with PBC (1.05 [1.02–1.08], $p<0.001$ and 0.95 [0.93–0.96], $p<0.001$). Albumin levels are, among other serum biomarkers, used as independent prognostic factors, particularly to predict hepatic adverse events.[20] Moreover, glycoprotein acetyls have recently been regarded as a novel inflammatory biomarker of cardiovascular risk.[22]

3.4 | Morbidity associated with primary biliary cholangitis

Next, we sought to investigate whether the observed metabolomic and lipidomic alterations may also reflect in comorbidities of PBC. Interestingly, we observed that PBC predisposes to many comorbidities concerning not only the digestive system but also distant organ systems, when compared to healthy matched control participants and corrected for the use of cholesterol lowering medication, as shown in Figures 2 and 3. Individuals with PBC had, besides liver-related diseases and direct symptoms of portal hypertension, also significantly more often a comorbid diagnosis with digestive diseases such as gastritis and duodenitis (OR 4.211 [3.006–5.9]), gastroesophageal reflux disease (GERD) (OR 2.847 [2.064–3.927]) or gastric ulcer (OR 6.066 [3.171–11.605]). Above that, we found an association with diseases of the circulatory system, including Raynaud's syndrome (OR 14.694 [5.17–41.764]), heart failure—not otherwise specified (NOS) (OR 1.973 [1.218–3.196]), essential hypertension (OR 2.428 [1.886–3.125]) and coronary atherosclerosis (OR 2.507 [1.67–3.765]). Furthermore, sicca syndrome proves to be a typical complication of PBC (OR 39.877 [9.869–161.128]). Additionally, our study cohort shows higher prevalence of genitourinary diseases for instance chronic renal failure (OR 3.522 [2.128–5.831]), acute renal failure (OR 3.197 [2.165–4.721]) and urinary tract infection (OR 2.182 [1.533–3.107]).

Thrombocytopenia (OR 7.824 [3.524–17.368]), lymphadenitis (OR 8.018 [3.231–19.898]), iron deficiency anaemia (OR 5.47 [3.699–8.09]) and other anemias (OR 4.173 [2.989–5.827]) are examples for diseases of the haematopoietic system which are associated with PBC (Figure 3).

Comorbidities concerning mental disorders (altered mental status, tobacco use disorder), endocrine, neurological, musculoskeletal (osteoporosis, rheumatoid arthritis) and infectious diseases (i.e., septicemia and *E. coli* infections) were also documented. Lastly, we also found an overrepresentation of PheCodes related to the respiratory system (pneumonia, pleurisy) in individuals with PBC. All significantly associated comorbidities of PBC individuals found in our study cohort are shown in Figures 2 and 3.

3.5 | The influence of the disease stage on comorbidities

A significant number of the identified complications relate to advanced chronic liver disease. Therefore, we used the APRI to account for the degree or presence of hepatic cirrhosis or fibrosis. We calculated the APRI for every patient based on their baseline laboratory

test results. Considering all participants with PBC in our study cohort, 109 (24%) had an APRI ≥ 0.54 indicative of liver impairment. In the control group, only 22 (2.4%) had an APRI ≥ 0.54. We repeated PheWAS analyses on the APRI-matched cohort and observed that the probability of additional symptoms, especially cirrhosis of liver without mention of alcohol (OR 90.491 [26.896–304.455]), ascites (non-malignant) (OR 13.567 [5.918–31.101]) or oesophageal bleeding (varices/haemorrhage) (OR 52.901 [15.964–175.3]) as major manifestations of portal hypertension. Moreover, not only sicca syndrome, Raynaud's syndrome, hypovolemia, osteoporosis, chronic and acute renal failure remained significantly associated with PBC (Figure 4).

To illuminate the comorbidities associated with an APRI ≥ 0.54, we performed two more analyses. First, we aimed to identify the PheCodes associated with a high APRI within the individuals with PBC. In this PheWAS, we found a significant association of a high APRI with splenomegaly (OR 5.413 [2.367–12.381]) and oesophageal bleeding (varices/haemorrhage) (OR 4.446 [2.656–7.441]). All these PheCodes imply a more severe impairment of the hepatic function and portal hypertension (Figure S3).

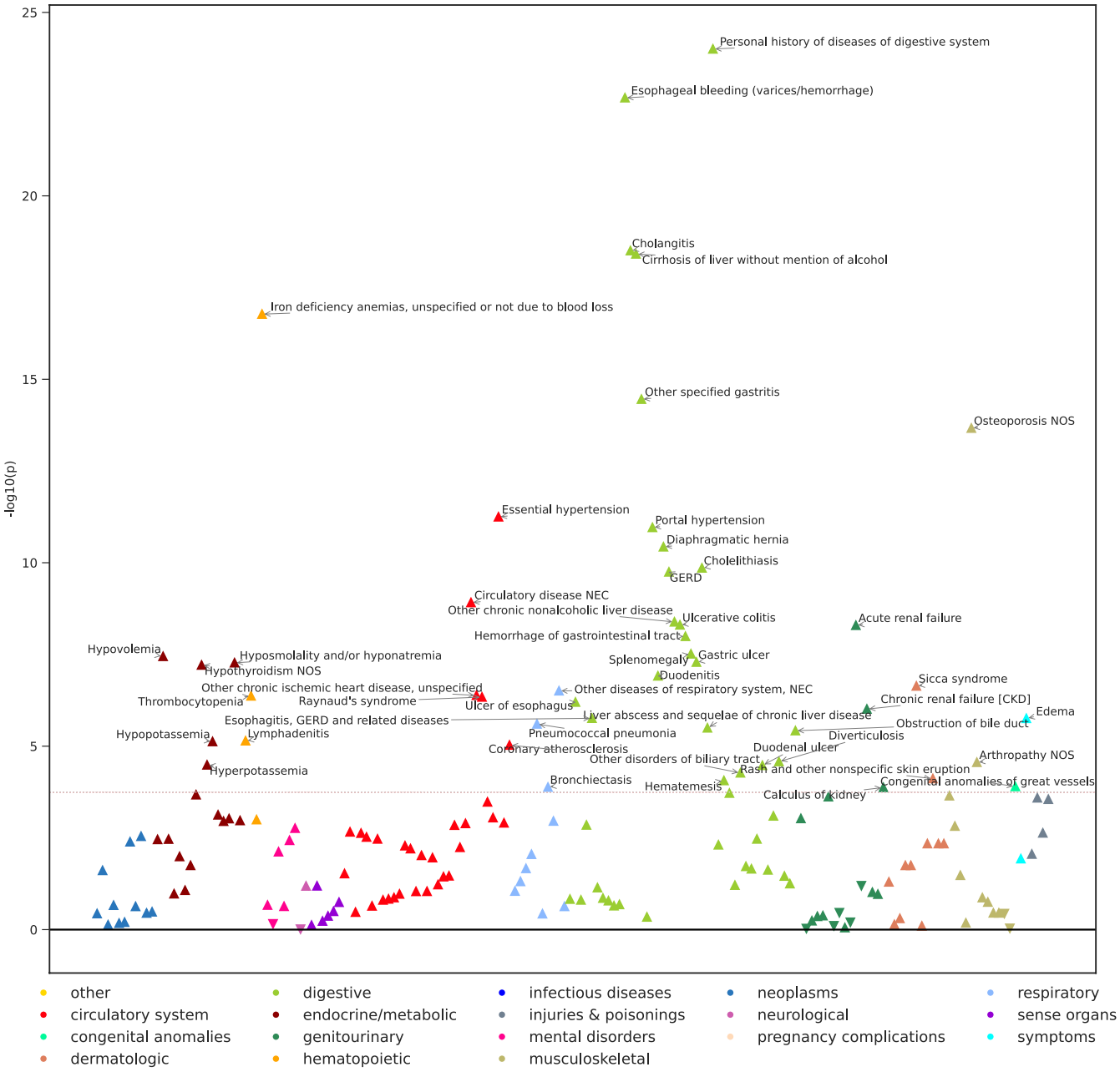

FIGURE 2 Manhattan plot of sex, age, BMI and the self-reported ethnic background adjusted −log10 (*p* values) for all selected PheCodes comparing their occurrence in PBC individuals vs. propensity-matched control population. Highlighted are associations with *p* values ≤0.05 (corrected for multiple testing by Bonferroni-correction to the threshold 0.00021 (red dotted line)). Upwards/downwards pointing triangular markers refer to PheCodes, that are over- or underrepresented, respectively, in PBC individuals compared to controls. NOS, not otherwise specified.

Variable	OR [CI95]	p-value
circulatory system		
Raynaud's syndrome	14.694 (5.17 to 41.764)	4.60e-07
Other chronic ischemic heart disease, unspecified	2.953 (1.942 to 4.49)	4.12e-07
Circulatory disease NEC	2.674 (1.947 to 3.671)	1.22e-09
Coronary atherosclerosis	2.507 (1.67 to 3.765)	9.36e-06
Essential hypertension	2.428 (1.886 to 3.125)	5.64e-12
congenital anomalies		
Congenital anomalies of great vessels	3.872 (1.938 to 7.737)	1.26e-04
dermatologic		
Sicca syndrome	39.877 (9.869 to 161.128)	2.30e-07
Rash and other nonspecific skin eruption	6.465 (2.563 to 16.306)	7.68e-05
digestive		
Portal hypertension	119.972 (30.147 to 477.436)	1.10e-11
Cirrhosis of liver without mention of alcohol	97.234 (35.651 to 265.192)	3.85e-19
Liver abscess and sequelae of chronic liver disease	93.482 (13.854 to 630.772)	3.19e-06
Cholangitis	61.249 (24.909 to 150.605)	3.13e-19
Esophageal bleeding (varices/hemorrhage)	29.637 (15.219 to 57.712)	2.13e-23
Obstruction of bile duct	28.17 (6.84 to 116.023)	3.80e-06
Other disorders of stomach and duodenum	20.38 (10.736 to 38.69)	3.05e-20
Splenomegaly	18.613 (6.613 to 52.39)	3.06e-08
Other disorders of liver	15.036 (8.066 to 28.03)	1.47e-17
Ascites (non malignant)	11.366 (6.144 to 21.026)	9.66e-15
Duodenal ulcer	9.806 (3.335 to 28.834)	3.35e-05
Ulcerative colitis	9.506 (4.469 to 20.22)	4.97e-09
Other chronic nonalcoholic liver disease	7.623 (3.873 to 15.002)	4.11e-09
Ulcer of esophagus	6.304 (3.055 to 13.008)	6.32e-07
Other specified gastritis	6.168 (3.921 to 9.704)	3.51e-15
Gastric ulcer	6.066 (3.171 to 11.605)	5.14e-08
Hematemesis	5.752 (2.401 to 13.781)	8.68e-05
Personal history of diseases of digestive system	5.527 (3.988 to 7.66)	9.96e-25
Hemorrhage of gastrointestinal tract	4.689 (2.763 to 7.956)	1.02e-08
Other disorders of biliary tract	4.447 (2.156 to 9.174)	5.37e-05
Gastritis and duodenitis	4.211 (3.006 to 5.9)	6.51e-17
Duodenitis	3.913 (2.361 to 6.488)	1.22e-07
Cholelithiasis	3.731 (2.496 to 5.579)	1.39e-10
Noninfectious gastroenteritis	2.971 (2.019 to 4.371)	3.27e-08
Esophagitis, GERD and related diseases	2.968 (1.9 to 4.636)	1.74e-06
GERD	2.847 (2.064 to 3.927)	1.79e-10
Dysphagia	2.564 (1.585 to 4.149)	1.25e-04
Diaphragmatic hernia	2.563 (1.939 to 3.387)	3.70e-11
Constipation	2.025 (1.432 to 2.865)	6.69e-05
Diverticulosis	1.853 (1.39 to 2.471)	2.64e-05
endocrine/metabolic		
Other disorders of metabolism	7.766 (2.839 to 21.242)	6.53e-05
Hyperpotassemia	6.236 (2.63 to 14.787)	3.26e-05
Hypovolemia	4.178 (2.512 to 6.948)	3.58e-08
Hypopotassemia	3.985 (2.176 to 7.298)	7.52e-06
Hyposmolality and/or hyponatremia	3.852 (2.37 to 6.263)	5.35e-08
Hypothyroidism NOS	2.495 (1.792 to 3.474)	6.17e-08
genitourinary		
Calculus of kidney	4.694 (2.123 to 10.378)	1.34e-04
Chronic renal failure [CKD]	3.522 (2.128 to 5.831)	9.76e-07
Acute renal failure	3.197 (2.165 to 4.721)	5.09e-09
Urinary tract infection	2.182 (1.533 to 3.107)	1.50e-05
hematopoietic		
Lymphadenitis	8.018 (3.231 to 19.898)	7.16e-06
Thrombocytopenia	7.824 (3.524 to 17.368)	4.29e-07
Iron deficiency anemias, unspecified or not due to blood loss	5.47 (3.699 to 8.09)	1.68e-17
Other anemias	4.173 (2.989 to 5.827)	4.97e-17
infectious diseases		
Hepatitis NOS	13.986 (4.224 to 46.311)	1.57e-05
Postoperative infection	4.054 (2.106 to 7.801)	2.78e-05
Bacterial infection NOS	4.011 (2.32 to 6.935)	6.58e-07
Candidiasis	3.602 (1.96 to 6.62)	3.68e-05
E. coli	2.889 (1.708 to 4.888)	7.66e-05
Septicemia	2.78 (1.726 to 4.479)	2.63e-05
injuries & poisonings		
Poisoning by analgesics, antipyretics, and antirheumatics	2.603 (1.608 to 4.214)	9.90e-05
mental disorders		
Other mental disorder	3.289 (2.521 to 4.29)	1.70e-18
musculoskeletal		
Osteoporosis NOS	4.62 (3.12 to 6.841)	2.15e-14
Other disorders of bone and cartilage	3.933 (2.016 to 7.675)	5.93e-05
Arthropathy NOS	1.804 (1.369 to 2.376)	2.77e-05
neoplasms		
Benign neoplasm of other parts of digestive system	2.587 (1.618 to 4.136)	7.17e-05
Benign neoplasm of colon	1.948 (1.395 to 2.721)	9.07e-05
other		
Complications of surgical and medical procedures	4.472 (2.266 to 8.826)	1.58e-05
Symptoms concerning nutrition, metabolism, and development	3.932 (2.516 to 6.143)	1.82e-09
respiratory		
Pleurisy; pleural effusion	4.084 (2.646 to 6.303)	2.08e-10
Bronchiectasis	3.228 (1.77 to 5.885)	1.31e-04
Other diseases of respiratory system, NEC	3.072 (1.998 to 4.721)	3.11e-07
Pneumococcal pneumonia	2.748 (1.804 to 4.185)	2.49e-06
symptoms		
Malaise and fatigue	3.617 (2.053 to 6.373)	8.58e-06
Edema	3.512 (2.098 to 5.878)	1.76e-06
Fever of unknown origin	3.443 (1.991 to 5.955)	9.70e-06
Abdominal pain	3.089 (2.362 to 4.041)	1.82e-16
Nausea and vomiting	2.652 (1.851 to 3.8)	1.06e-07

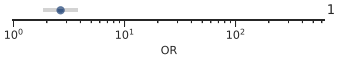

FIGURE 3 The overrepresented PheCodes in individuals with PBC, adjusted for age, sex, BMI and ethnic background. Odds ratios (ORs) and 95% confidence intervals (CI) of the 77 significantly associated PheCodes are presented. Only PheCodes that remained significant after adjustment for multiple testing (Bonferroni method) are displayed and have thereby a (*p* value of ≤0.00021). No negative association remained significant after the correction for multiple testing, therefore, all PheCodes shown here have a positive association with PBC compared to controls. The opacity of the displayed dots corresponds to the *p* value within the ranges of significant associations. NEC, not elsewhere classified; NOS, not elsewhere specified.

Finally, we aimed to identify comorbidities that were associated with a high APRI within our APRI-matched cohort. In this context, a high APRI was strongly associated, besides liver-related PheCodes and direct consequences of portal hypertension, with, for instance, varicose veins (OR 14.493 [6.161–34.091]), thrombocytopenia (OR 13.668 [6.615–28.242]) and fever of unknown origin (OR 4.528 [2.396–8.557]). As expected, a high APRI was associated with many other liver-related PheCodes besides primary biliary cirrhosis (OR 5.638 [3.472–9.157]), hinting at its lack of specificity for PBC (Figure 5 and Table 1).

3.6 | Mortality in PBC individuals

All-cause mortality was almost three times higher in the general PBC cohort (HR 2.62 [1.97–3.49]; $p<0.001$) than in the control group. We examined the causes of death in individuals with PBC and found diagnoses related to the digestive system (HR 32.34 [7.74–135.1]; $p<0.001$) and malignancies (HR 1.83 [1.21–2.79]; $p=0.004$), especially with hepatic origin, to be the main driver of this increased mortality. Diseases of the respiratory (HR 1.51 [0.6–3.8]; $p=0.38$) and the circulatory system (HR 1.27 [0.55–2.94]; $p=0.54$) were not significantly more common in the group of participants with PBC (Table 2).

4 | DISCUSSION

The development of primary biliary cholangitis is known to be influenced by environmental, ethnic and metabolic factors. In this study, we provided a comprehensive overview of the lipidomic and metabolomic profiles of PBC individuals as well as comorbidities. A comparison of epidemiological data from recent decades, covering a period of thirty-five years (1972–2007), shows much lower prevalence rates (1.91–40.2 per 100 000 inhabitants) but also a clear majority of females (92%). Above that, in recent decades, individuals with PBC were diagnosed at an older age and with reduced disease severity.[8,23] The global prevalence of PBC is rising with highest rates found in the Western world, particularly Northern Europe and North America.[24] This development may be due to improved awareness of the disease and advances in diagnostic tools, but might also be influenced by changes in environmental factors that may act as a trigger in susceptible hosts. Currently, serum liver tests and immune serology are used to identify individuals with PBC. Although the diagnosis is made based on the presence of serological AMAs and elevated alkaline phosphatase, these criteria are not pathogenetic, which complicates the final diagnosis and emphasises the need for further feasible diagnostic tools. Our analysis shows an elevation of aspartate aminotransferase and alanine aminotransferase (U/L) as indicators for liver injury and alkaline phosphatase (U/L) and y-glutamyltransferase (U/L) as indicators for cholestasis ($p<0.0001$). Consistent with our results, a statistical comparison of liver biochemistry in a recent study from 2022 also depicts a significant ($p<0.001$) elevation of transaminases and cholestasis parameters.[25] On the contrary, other studies reported a non-significant elevation in transaminases, total bilirubin, serum albumin and serum creatinine.[11,24–26] These differences are likely related to different study sizes, statistical power and populations at interest.

Changes in the lipidomic profile of PBC individuals are a repeatedly described phenomenon.[11,26–28] The pathogenesis is described as complex, while an impaired release of bile acids into the intestine in PBC individuals and a resulting restriction in dietary cholesterol solubilisation and micelle formation is suggested, thereby leading to a reduction of intestinal cholesterol absorption. However, we observed decreased levels of HDL-cholesterol, especially in the smaller diameter HDL. This may be due to impaired hepatocyte function. The severity of cirrhosis was assumed to influence the lipidomic profile, leading to lower values of HDL-cholesterol in advanced stages.[26] While HDL-cholesterol is found to be increased in individuals with early disease stages, decreased levels related to concomitant *lecithin cholesterol acyltransferase* (LCAT) deficiency were seen in advanced stages.[29]

We found a threefold increased occurrence of coronary atherosclerosis (OR 2.507 [1.67–3.765]) and other chronic ischemic heart disease—not otherwise specified (NOS) (OR 2.953 [1.942–4.49]) in PBC individuals with accordingly reduced levels of S-HDL cholesterol and increased levels of glycoprotein acetyls, which has been proposed as a potential new prognostic marker for cardiovascular events.[22,30] Therefore, our data suggest that PBC is associated with an increased risk for cardiovascular events.[31,32] However, these results are contradictory to other studies showing an enrichment of HDL-cholesterol and accordingly no significant or even a protective influence of PBC on cardiovascular disease.[26,27] While individual studies have found an association between PBC and cardiovascular events[32] and reduced cardiac function,[33] most studies do not find a specific link.[26] We hypothesised that these conflicting findings might be related to the presence of advanced liver disease in our PBC cohort. Therefore, we decided to include APRI in the matching process to validate our observations after correction for stronger hepatic impairment. When additionally matching and correcting for APRI (Figure 4), we still observe a strong association between PBC and diseases of the circulatory system. This suggests that not only chronic liver disease in general, but PBC in particular,

Variable	OR [CI95]	p-value
circulatory system		
Other forms of chronic heart disease	7.142 (2.718 to 18.766)	6.65e-05
Heart failure NOS	3.551 (1.926 to 6.549)	4.93e-05
Essential hypertension	2.522 (1.888 to 3.368)	3.84e-10
Circulatory disease NEC	2.514 (1.715 to 3.685)	2.28e-06
Angina pectoris	2.452 (1.538 to 3.908)	1.63e-04
Nonspecific chest pain	2.014 (1.405 to 2.886)	1.37e-04
congenital anomalies		
Congenital anomalies of great vessels	6.271 (2.483 to 15.837)	1.03e-04
dermatologic		
Sicca syndrome	14.875 (5.621 to 39.368)	5.43e-08
Cellulitis and abscess of foot, toe	4.286 (2.129 to 8.629)	4.58e-05
Cellulitis and abscess of arm/hand	4.286 (2.129 to 8.629)	4.58e-05
Cellulitis and abscess of leg, except foot	4.286 (2.129 to 8.629)	4.58e-05
digestive		
Cirrhosis of liver without mention of alcohol	90.491 (26.896 to 304.455)	3.39e-13
Esophageal bleeding (varices/hemorrhage)	52.901 (15.964 to 175.3)	8.47e-11
Liver abscess and sequelae of chronic liver disease	23.375 (5.387 to 101.423)	2.57e-05
Obstruction of bile duct	18.162 (5.214 to 63.26)	5.27e-06
Ascites (non malignant)	13.567 (5.918 to 31.101)	7.24e-10
Other disorders of stomach and duodenum	13.259 (6.641 to 26.471)	2.35e-13
Other chronic nonalcoholic liver disease	9.423 (4.207 to 21.106)	4.97e-08
Other disorders of liver	7.678 (3.897 to 15.129)	3.84e-09
Ulcerative colitis	7.024 (3.293 to 14.982)	4.56e-07
Abdominal hernia	5.644 (2.367 to 13.461)	9.52e-05
Ventral hernia	5.465 (2.26 to 13.215)	1.64e-04
Duodenitis	4.811 (2.641 to 8.765)	2.86e-07
Diaphragmatic hernia	3.957 (2.835 to 5.523)	6.09e-16
Personal history of diseases of digestive system	3.638 (2.555 to 5.18)	7.80e-13
Cholelithiasis	3.37 (2.093 to 5.426)	5.74e-07
Constipation	3.105 (1.999 to 4.823)	4.60e-07
Esophagitis, GERD and related diseases	3.077 (1.855 to 5.106)	1.35e-05
Hemorrhage of gastrointestinal tract	3.06 (1.747 to 5.358)	9.12e-05
Gastritis and duodenitis	2.939 (2.006 to 4.304)	3.09e-08
Noninfectious gastroenteritis	2.753 (1.778 to 4.262)	5.58e-06
GERD	2.745 (1.92 to 3.924)	3.03e-08
Other specified gastritis	2.546 (1.637 to 3.96)	3.39e-05
endocrine/metabolic		
Hyperpotassemia	9.126 (2.854 to 29.18)	1.93e-04
Hypovolemia	5.56 (2.964 to 10.427)	8.94e-08
Hyposmolality and/or hyponatremia	4.022 (2.11 to 7.669)	2.36e-05
Hypothyroidism NOS	3.151 (2.145 to 4.63)	5.04e-09
genitourinary		
Chronic renal failure [CKD]	7.252 (3.887 to 13.53)	4.77e-10
Cyst of kidney, acquired	6.571 (2.554 to 16.904)	9.42e-05
Acute renal failure	3.577 (2.2 to 5.816)	2.78e-07
Urinary tract infection	2.557 (1.68 to 3.89)	1.16e-05
hematopoietic		
Iron deficiency anemias, unspecified or not due to blood loss	4.988 (3.239 to 7.681)	2.98e-13
Other anemias	3.319 (2.256 to 4.883)	1.14e-09
infectious diseases		
Hepatitis NOS	37.948 (5.745 to 250.654)	1.60e-04
Septicemia	3.928 (2.079 to 7.422)	2.51e-05
E. coli	3.866 (2.035 to 7.346)	3.64e-05
Bacterial infection NOS	3.296 (1.815 to 5.986)	8.93e-05
injuries & poisonings		
Poisoning by analgesics, antipyretics, and antirheumatics	3.105 (1.799 to 5.359)	4.70e-05
mental disorders		
Tobacco use disorder	2.972 (1.909 to 4.627)	1.41e-06
Other mental disorder	2.653 (1.96 to 3.589)	2.59e-10
musculoskeletal		
Osteoporosis NOS	5.26 (3.295 to 8.397)	3.52e-12
Osteoarthrosis NOS	2.677 (1.662 to 4.312)	5.16e-05
Arthropathy NOS	2.142 (1.559 to 2.943)	2.58e-06
other		
Complications of surgical and medical procedures	6.356 (2.75 to 14.692)	1.52e-05
Symptoms concerning nutrition, metabolism, and development	2.571 (1.6 to 4.131)	9.52e-05
respiratory		
Bronchiectasis	4.193 (2.008 to 8.758)	1.36e-04
Pneumococcal pneumonia	3.907 (2.349 to 6.497)	1.51e-07
Pleurisy; pleural effusion	3.573 (2.172 to 5.876)	5.28e-07
Pneumonia	3.434 (1.937 to 6.09)	2.42e-05
Chronic airway obstruction	2.91 (1.723 to 4.914)	6.48e-05
symptoms		
Edema	4.335 (2.202 to 8.532)	2.19e-05
Malaise and fatigue	3.977 (2.037 to 7.764)	5.25e-05
Abdominal pain	3.278 (2.41 to 4.458)	3.80e-14
Nausea and vomiting	3.09 (2.037 to 4.687)	1.12e-07

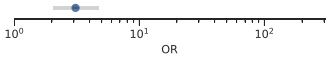

FIGURE 4 Associated comorbidities with PBC, when matched on APRI, sex, age, BMI, self-reported ethnic background and the use of cholesterol lowering medication at baseline. To further reduce the influence of these confounders, they are also used as covariates in the PheWAS. Shown are the ORs of the significantly associated comorbidities with a Bonferroni corrected $p \leq 0.00024$. The opacity of the displayed dots corresponds to the p value within the ranges of significant associations. GERD, gastroesophageal reflux disease; NEC, not elsewhere classified; NOS, not elsewhere specified.

Variable	OR [CI95]	p-value
circulatory system		
Varicose veins	14,493 (6,161 to 34,091)	9.00e-10
Disease of tricuspid valve	7,372 (3,058 to 17,77)	8.58e-06
Nonrheumatic mitral valve disorders	4.89 (2.42 to 9.883)	9.80e-06
Mitral valve disease	4.675 (2.265 to 9.651)	3.04e-05
Circulatory disease NEC	2.422 (1.554 to 3.774)	9.28e-05
digestive		
Splenomegaly	13.218 (6.152 to 28.397)	3.68e-11
Portal hypertension	13.042 (7.953 to 21.388)	2.52e-24
Liver abscess and sequelae of chronic liver disease	10.209 (5.092 to 20.469)	5.90e-11
Liver replaced by transplant	9.24 (4.279 to 19.952)	1.50e-08
Blood in stool	8.155 (3.417 to 19.465)	2.27e-06
Cirrhosis of liver without mention of alcohol	7.806 (4.972 to 12.254)	4.23e-19
Other disorders of liver	7.652 (4.562 to 12.833)	1.23e-14
Duodenal ulcer	6.802 (2.719 to 17.017)	4.17e-05
Other disorders of stomach and duodenum	6.062 (3.795 to 9.682)	4.59e-14
Primary biliary cirrhosis	5.638 (3.472 to 9.157)	2.73e-12
Ascites (non malignant)	5.587 (3.137 to 9.951)	5.14e-09
Abnormal results of function study of liver	4.718 (3.04 to 7.323)	4.56e-12
Hemorrhage of gastrointestinal tract	3.385 (1.852 to 6.189)	7.45e-05
Personal history of diseases of digestive system	3.223 (2.136 to 4.863)	2.44e-08
Cholangitis	3.149 (1.931 to 5.137)	4.33e-06
Other specified gastritis	3.013 (1.809 to 5.016)	2.24e-05
Cholelithiasis	2.991 (1.806 to 4.956)	2.09e-05
Gastritis and duodenitis	2.741 (1.764 to 4.261)	7.43e-06
endocrine/metabolic		
Hyperpotassemia	5.428 (2.351 to 12.536)	7.45e-05
Hyposmolality and/or hyponatremia	4.171 (2.349 to 7.407)	1.10e-06
genitourinary		
Acute renal failure	3.126 (1.909 to 5.12)	5.90e-06
hematopoietic		
Thrombocytopenia	13,668 (6,615 to 28,242)	1.64e-12
Neutropenia	4.832 (2.209 to 10.57)	7.97e-05
Other anemias	3.993 (2.592 to 6.152)	3.42e-10
Iron deficiency anemias, unspecified or not due to blood loss	3.183 (1.985 to 5.104)	1.54e-06
infectious diseases		
Chronic hepatitis	7.629 (3.915 to 14.863)	2.36e-09
respiratory		
Pulmonary collapse; interstitial and compensatory emphysema	5.883 (2.976 to 11.629)	3.44e-07
symptoms		
Fever of unknown origin	4.528 (2.396 to 8.557)	3.32e-06
Edema	4.435 (2.338 to 8.413)	5.12e-06

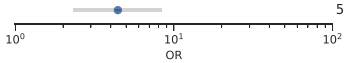

FIGURE 5 Results of the PheWAS analysis including only individuals from the matched cohort; fitted for the presence of a high APRI over or equal to 0.54. This analysis therefore describes the PheCodes associated with an elevated APRI within our matched cohort. Only PheCodes with a significant association after Bonferroni correction ($p \leq 0.00014$) are shown here. The opacity of the displayed dots corresponds to the *p* value within the ranges of significant associations. NEC, not elsewhere classified.

might be a significant risk factor for subclinical and clinical cardiovascular disease, although the exact mechanisms are not yet fully understood.[34] Importantly, in this regard, our findings are also conflicting to the UK PBC guidelines, which state that there is no robust evidence to suggest that ischemic heart disease or other forms of atherosclerotic disease are seen at increased frequency in individuals with PBC.[35–37]

Above that, we also observe that individuals with PBC are more susceptible to the development of other diseases including digestive diseases, autoimmune diseases, anaemias, mental disorders,

TABLE 2 Multivariable Hazard ratios for overall and cause-specific mortality after a mean follow-up of 13.6 years among individuals with PBC and controls.

Cause of death	ICD10	Controls (%), total $n = 908$	PBC (%), total $n = 454$	HR	KI_2.5	KI_97.5	p value	Total count
Overall mortality	Death	86 (9.47)	104 (22.91)	**2.62**	**1.97**	**3.49**	**<0.001**	190
Malignancies	C	46 (5.07)	41 (9.03)	**1.83**	**1.21**	**2.79**	**0.004**	87
Malignant neoplasm of palate	C5	10 (1.1)	3 (0.66)	0.63	0.18	2.21	0.47	13
Malignant neoplasm of liver and intrahepatic bile ducts	C22	0 (0.0)	11 (2.42)				<0.001	11
Malignant neoplasm of bronchus and lung	C34	8 (0.88)	6 (1.32)	1.61	0.55	4.65	0.38	14
Mental and behavioural disorders	F	1 (0.11)	2 (0.44)	3.75	0.29	49.18	0.31	3
Inflammatory disease of the central nervous system	G	3 (0.33)	2 (0.44)	1.38	0.23	8.44	0.73	5
Disease of the circulatory system	I	14 (1.54)	9 (1.98)	1.27	0.55	2.94	0.57	23
Rheumatic aortic stenosis	I6	6 (0.66)	4 (0.88)	1.32	0.37	4.7	0.67	10
Diseases of the respiratory system	J	11 (1.21)	8 (1.76)	1.51	0.6	3.8	0.38	19
Diseases of the digestive system	K	2 (0.22)	31 (6.83)	**32.34**	**7.74**	**135.1**	**<0.001**	33
Liver diseases	K7	1 (0.11)	22 (4.85)	**45.09**	**6.05**	**336.37**	**<0.001**	23
Fibrosis and cirrhosis of the liver	K74	0 (0.0)	17 (3.74)				<0.001	17
Other inflammatory liver disease	K75	0 (0.0)	4 (0.88)				<0.001	4
Other disease of the biliary tract	K83	0 (0.0)	5 (1.1)				<0.001	5

Note: All analyses were corrected for age, sex, BMI and self-reported ethnic background. Hazard ratios with $p \leq 0.00041$ are stated in bold font.
Abbreviation: HR, Hazard ratio.

and urinary tract infections.[23] The association with urinary tract infections is in line with the previously hypothesised infectious aetiology of PBC, which is also supported by a significantly elevated C-reactive protein level in our analysis.[38,39] Recent studies found an even stronger association between UTI and PBC and suggested that this association may be causal if UTI precedes PBC.[38] Yang et al. (2022) observe that exposure to E.coli in the susceptible host, which is most commonly responsible for urinary tract infections, is the basis for the antimitochondrial antibody (AMA) response due to molecular mimicry and, thereby, the first step to the development of human PBC.[39]

PBC belongs, together with primary sclerosing cholangitis (PSC), to the group of immune-mediated cholestatic liver diseases.[40] Already in 1982, Culp et al. described that eighty-four per cent of the individuals with PBC had at least one associated autoimmune disease, and 41% had two or more such diseases in addition to primary biliary cholangitis.[41] Indeed, PBC is reported to be associated with CREST syndrome in 1%–6% of cases[42] and symptoms of sicca complex were found in 65% of PBC cases.[43] In our study cohort, we found a strong association between sicca syndrome (OR 39.877 [9.869–161.128]) and Raynaud's syndrome (OR 14.694 [5.17–41.764]). When matched for APRI score (Figure 4), autoimmune diseases became even more prevalent in individuals with PBC. As autoimmunity is strongly influenced by genetic background, the predisposition to PBC in families with a history of autoimmune diseases needs to be further investigated. Infrequent associations including inflammatory bowel disease (IBD)[44,45] and celiac disease[46] have been previously observed. However, the strong association between PBC and IBD in our study might also be explained by mislabeling of PBC.

By matching additionally on ARPI (Figure 4), we see that some comorbid diseases, for instance, ulcerative colitis (OR 2.02 [0.9–3.14]), gastritis and duodenitis (OR 1.71 [1.16–2.27]), and urinary tract infections (OR 1.61 [0.99–2.23]) stay associated with PBC also when comparing with an APRI matched cohort. In PBC, fibrosis is associated with a progressive obstruction of the biliary system, leading to impaired enterohepatic circulation of bile acids and strong alterations in bile acid composition.[7] Altered bile acid composition and impaired flow into the intestine may disturb intestinal homeostasis and thereby contribute to the pathogenesis of the aforementioned inflammatory intestinal diseases.[5]

There are recent studies investigating the influence of morbidities on the course of PBC. Iron deficiency anaemia and other anaemias are, for instance, known to have implications on the course of PBC, particularly in the stage of cirrhosis and were identified as a predictor of adverse outcomes, with increased mortality and occurrence of acute-on-chronic liver failure.[41,47] Regarding the survival and risk factor for developing extrahepatic malignancies in primary biliary cirrhosis (PBC) limited information is available.[48] While we observe an increased risk from diagnoses related to malignancies, Floreani et al. find equal results regarding survival for PBC individuals with and without extrahepatic malignancies.[48] Above that, the mean survival after the diagnosis of

PBC was equal with and without extrahepatic autoimmunity conditions in recent studies.[48,49] This gives evidence to suggest that the increased all-cause mortality rate in our PBC group is, at least partly, a consequence of (mis)-coding. When particularly individuals in advanced disease stages are assigned to the PBC group, it is likely that mortality rates increase respectively. As detection of PBC is often difficult, individuals in early disease stages are not included. Therefore, mortality rates might be increased due to a selection bias.

4.1 | Strength and limitations

In this case–control study, we analysed the metabolomic and lipidomic profile, investigated comorbidities, and evaluated causes and predictors of death in individuals with PBC. Due to our large study cohort, we were able to validly address these points of interest. This study is the first that analyses comorbidities using PheWAS analysis in a large cohort of PBC individuals. Although there is data concerning GWAS and PheWAS Analyses in individuals with cirrhosis,[50] we find only scarce information related to the metabolomic profile in PBC. By applying PheWAS analysis, we were able to define the comorbidities of PBC at the population level. Weaknesses of the study include the fact that individuals were recruited only in the UK. This might lead to a distorted depiction of etiologic factors as the incidence and prevalence of PBC are known to vary considerably worldwide.[51] Indeed, we observe a higher prevalence rate compared to other studies, which is most likely an effect of geographically limited data acquisition. Therefore, large population-based studies are necessary to build up a better understanding of PBC in a global context. Additionally, our study only offers a descriptive overview of potential relationships without providing proof for mechanistic processes.

Furthermore, as mentioned above, potential mislabeling of ICD codes of study participants is an unavoidable weakness of this study design. It is hardly distinguishable whether associations between diseases are based on valid linkages or a consequence of concomitant or incorrect coding. We found a strong association between PBC and IBD, e.g., ulcerative colitis. The frequent occurrence of ulcerative colitis in individuals with primary sclerosing cholangitis has been known for decades and confirmed in multiple studies.[52,53] Hence, we see a certain risk that study participants, suffering from primary sclerosing cholangitis, were inadvertently mislabeled as individuals with PBC. However, an exclusion of participants with concomitant diagnoses could potentially mask associations and lead to an attrition bias. In order to avoid distortion of data, we decided not to exclude specific groups in order to give a full picture of our findings, thereby accepting potential miscoding. Nevertheless, this study is the first to analyse alterations in lipidomics and metabolomics in a study cohort of this size, thereby providing a better understanding of primary biliary cholangitis, its development, prognosis, and morbidities.

In conclusion, this study provides insight into morbidity and metabolomic and lipidomic profiles of PBC individuals in a large UK population-based cohort. Although we do not analyse the pathological mechanisms behind our findings, we provide comprehensive data which might improve patient counselling and inform future studies.

AUTHOR CONTRIBUTIONS

K.M.S., C.V.S. and P.H.K. had full access to all the data in the study and took responsibility for the integrity of the data and the accuracy of the data analysis. P.H.K. and Co.V.S. analysed the data overseen by C.V.S. and K.M.S. Co.V.S., and P.H.K. conceptualised and drafted the manuscript. J.C., T.B., A.K., J.J., M.S.V. and C.T. gave important intellectual input, C.V.S and K.M.S. supervised the work. All the authors agreed to submit the manuscript, read, edited and approved the final draft and take full responsibility of its content.

ACKNOWLEDGEMENTS

C.V.S. was supported by a grant from the Interdisciplinary Centre for Clinical Research within the Faculty of Medicine at the RWTH Aachen University (PTD 1-13/IA 532313), the Junior Principal Investigator Fellowship program of RWTH Aachen Excellence strategy and the NRW Rueckkehr Programme of the Ministry of Culture and Science of the German State of North Rhine-Westphalia (MKW). K.M.S was supported by the Federal Ministry of Education and Research (BMBF) and the Ministry of Culture and Science of the German State of North Rhine-Westphalia (MKW) under the Excellence strategy of the federal government and the Laender as well as the NRW Rueckkehr Programme of the Ministry of Culture and Science of the German State of North Rhine-Westphalia (MKW). T.B., C.V.S and K.M.S were supported by the CRC 1382 project A11 and B09 funded by Deutsche Forschungsgesellschaft (DFG, German Research Foundation)—Project-ID 403224013—SFB 1382". The authors gratefully acknowledge the computing time provided to them at the NHR Centre NHR4CES at RWTH Aachen University (project number: rwth1414). This study was funded by the Federal Ministry of Education and Research and the state governments participating based on the resolutions of the GWK for national high-performance computing at universities (www.nhr-verein.de/unsere-partner). Open Access funding enabled and organized by Projekt DEAL.

CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from UK Biobank. Restrictions apply to the availability of these data, which were used under license for this study. Data are available from the author(s) with the permission of UK Biobank.

ORCID

Paul-Henry Koop https://orcid.org/0009-0004-2846-703X
Jan Clusmann https://orcid.org/0000-0003-2925-8438

Mara S. Vell https://orcid.org/0000-0002-3211-7823
Julius Jaeger https://orcid.org/0000-0002-1122-5331
Christian Trautwein https://orcid.org/0000-0003-2762-8247
Tony Bruns https://orcid.org/0000-0002-5576-6914
Carolin V. Schneider https://orcid.org/0000-0002-6728-9246
Kai Markus Schneider https://orcid.org/0000-0002-0047-3794

REFERENCES

1. Kumagi T, Heathcote EJ. Primary biliary cirrhosis. *Orphanet J Rare Dis*. 2008;3:1.
2. Harms MH, Lammers WJ, Thorburn D, et al. Major hepatic complications in ursodeoxycholic acid-treated patients with primary biliary cholangitis: risk factors and time trends in incidence and outcome. *Am J Gastroenterol*. 2018;113:254-264.
3. Gulamhusein AF, Hirschfield GM. Primary biliary cholangitis: pathogenesis and therapeutic opportunities. *Nat Rev Gastroenterol Hepatol*. 2020;17:93-110.
4. Örnolfsson KT, Olafsson S, Bergmann OM, Gershwin ME, Björnsson ES. Using the Icelandic genealogical database to define the familial risk of primary biliary cholangitis. *Hepatology*. 2018;68:166-171.
5. Liu Q, He W, Tang R, Ma X. Intestinal homeostasis in autoimmune liver diseases. *Chin Med J*. 2022;135:1642-1652.
6. Schneider KM, Kummen M, Trivedi PJ, Hov JR. Role of microbiome in autoimmune liver diseases. *Hepatology*. 2023. doi:10.1097/HEP.0000000000000506
7. Reshetnyak V-I. Concept on the pathogenesis and treatment of primary biliary cirrhosis. *World J Gastroenterol*. 2006;12:7250-7262.
8. Poupon R. Primary biliary cirrhosis: a 2010 update. *J Hepatol*. 2010;52:745-758.
9. Yang R, Zhao Q, Hu D-D, Xiao X-R, Huang J-F, Li F. Metabolomic analysis of cholestatic liver damage in mice. *Food Chem Toxicol*. 2018;120:253-260.
10. Kuiper EMM, Hansen BE, de Vries RA, et al. Improved prognosis of patients with primary biliary cirrhosis that have a biochemical response to ursodeoxycholic acid. *Gastroenterology*. 2009;136:1281-1287.
11. Bell LN, Wulff J, Comerford M, Vuppalanchi R, Chalasani N. Serum metabolic signatures of primary biliary cirrhosis and primary sclerosing cholangitis. *Liver Int*. 2015;35:263-274.
12. Wai C-T, Greenson JK, Fontana RJ, et al. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology*. 2003;38:518-526.
13. Trivedi PJ, Bruns T, Cheung A, et al. Optimising risk stratification in primary biliary cirrhosis: AST/platelet ratio index predicts outcome independent of ursodeoxycholic acid response. *J Hepatol*. 2014;60:1249-1258.
14. Kerley CI, Chaganti S, Nguyen TQ, et al. pyPheWAS: a phenome-disease association tool for electronic medical record analysis. *Neuroinformatics*. 2022;20:483-505.
15. Kline A, Luo Y. *PsmPy: A Package for Retrospective Cohort Matching in Python*. IEEE; 2022. doi:10.1109/EMBC48229.2022.9871333
16. Austin PC, Steyerberg EW, Putter H. Fine-gray subdistribution hazard models to simultaneously estimate the absolute risk of different event types: cumulative total failure probability may exceed 1. *Stat Med*. 2021;40:4200-4212.
17. Bonferroni C. Teoria statistica delle classi e calcolo delle probabilita. *Statisticians of the Centuries*. R Instituto Superiore di Scienze Economiche e Commerciali di Firenze; 1936.
18. Virtanen P, Gommers R, Oliphant TE, et al. SciPy 1.0: fundamental algorithms for scientific computing in python. *Nat Methods*. 2020;17:261-272.
19. Pollard TJ, Johnson AEW, Raffa JD, Mark RG. Tableone: an open source python package for producing summary statistics for research papers. *JAMIA Open*. 2018;1:26-31.
20. Hunter JD. Matplotlib: a 2D graphics environment. *Comput Sci Eng*. 2007;9:90-95.
21. Waskom M. seaborn: statistical data visualization. *J Open Source Softw*. 2021;6:3021.
22. Chiesa ST, Charakida M, Georgiopoulos G, et al. Glycoprotein acetyls: a novel inflammatory biomarker of early cardiovascular risk in the young. *J Am Heart Assoc*. 2022;11:e024380.
23. Marschall H-U, Henriksson I, Lindberg S, et al. Incidence, prevalence, and outcome of primary biliary cholangitis in a nationwide Swedish population-based cohort. *Sci Rep*. 2019;9:11525.
24. Myers RP, Shaheen AAM, Fong A, et al. Epidemiology and natural history of primary biliary cirrhosis in a Canadian health region: a population-based study. *Hepatology*. 2009;50:1884-1892.
25. Ma Z-H, Wang X-M, Wu R-H, et al. Serum metabolic profiling of targeted bile acids reveals potentially novel biomarkers for primary biliary cholangitis and autoimmune hepatitis. *World J Gastroenterol*. 2022;28:5764-5783.
26. Loaeza-Del Castillo AM, Gaytán-Santillán A, López-Tello A, et al. Patterns of serum lipids derangements and cardiovascular risk assessment in patients with primary biliary cholangitis. *Ann Hepatol*. 2019;18:879-882.
27. Zhang Y, Hu X, Chang J, et al. The liver steatosis severity and lipid characteristics in primary biliary cholangitis. *BMC Gastroenterol*. 2021;21:395.
28. Walker DI, Juran BD, Cheung AC, et al. High-resolution exposomics and metabolomics reveals specific associations in cholestatic liver diseases. *Hepatol Commun*. 2022;6:965-979.
29. Wah-Suarez MI, Danford CJ, Patwardhan VR, Jiang ZG, Bonder A. Hyperlipidaemia in primary biliary cholangitis: treatment, safety and efficacy. *Frontline Gastroenterol*. 2019;10:401-408.
30. Connelly MA, Otvos JD, Shalaurova I, Playford MP, Mehta NN. GlycA, a novel biomarker of systemic inflammation and cardiovascular disease risk. *J Transl Med*. 2017;15:219.
31. Ungprasert P, Wijarnpreecha K, Ahuja W, Spanuchart I, Thongprayoon C. Coronary artery disease in primary biliary cirrhosis: a systematic review and meta-analysis of observational studies. *Hepatol Res*. 2015;45:1055-1061.
32. Zöller B, Li X, Sundquist J, Sundquist K. Risk of subsequent coronary heart disease in patients hospitalized for immune-mediated diseases: a nationwide follow-up study from Sweden. *PLoS One*. 2012;7:e33442.
33. Jones DEJ, Hollingsworth K, Fattakhova G, et al. Impaired cardiovascular function in primary biliary cirrhosis. *Am J Physiol Gastrointest Liver Physiol*. 2010;298:G764-G773.
34. Francque SM, van der Graaff D, Kwanten WJ. Non-alcoholic fatty liver disease and cardiovascular risk: pathophysiological mechanisms and implications. *J Hepatol*. 2016;65:425-443.
35. Crippin JS, Lindor KD, Jorgensen R, et al. Hypercholesterolemia and atherosclerosis in primary biliary cirrhosis: what is the risk? *Hepatology*. 1992;15:858-862.
36. European Association for the Study of the Liver. Electronic address: easloffice@easloffice.eu, European Association for the Study of the Liver. EASL clinical practice guidelines: the diagnosis and management of patients with primary biliary cholangitis. *J Hepatol*. 2017;67:145-172.
37. Solaymani-Dodaran M, Aithal GP, Card T, West J. Risk of cardiovascular and cerebrovascular events in primary biliary cirrhosis: a population-based cohort study. *Am J Gastroenterol*. 2008;103:2784-2788.
38. Varyani FK, West J, Card TR. An increased risk of urinary tract infection precedes development of primary biliary cirrhosis. *BMC Gastroenterol*. 2011;11:95.

39. Yang Y, Choi J, Chen Y, et al. E. Coli and the etiology of human PBC: antimitochondrial antibodies and spreading determinants. *Hepatology*. 2022;75:266-279.
40. Sarcognato S, Sacchi D, Grillo F, et al. Autoimmune biliary diseases: primary biliary cholangitis and primary sclerosing cholangitis. *Pathologica*. 2021;113:170-184.
41. Culp KS, Fleming CR, Duffy J, Baldus WP, Dickson ER. Autoimmune associations in primary biliary cirrhosis. *Mayo Clin Proc*. 1982;57:365-370.
42. Floreani A, Franceschet I, Cazzagon N. Primary biliary cirrhosis: overlaps with other autoimmune disorders. *Semin Liver Dis*. 2014;34:352-360.
43. Mang FW, Michieletti P, O'Rourke K, et al. Primary biliary cirrhosis, sicca complex, and dysphagia. *Dysphagia*. 1997;12:167-170.
44. Núñez FP, Castro F, Mezzano G, Quera R, Diaz D, Castro L. Hepatobiliary manifestations in inflammatory bowel disease: a practical approach. *World J Hepatol*. 2022;14:319-337.
45. Liberal R, Gaspar R, Lopes S, Macedo G. Primary biliary cholangitis in patients with inflammatory bowel disease. *Clin Res Hepatol Gastroenterol*. 2020;44:e5-e9.
46. Callichurn K, Cvetkovic L, Therrien A, Vincent C, Hétu P-O, Bouin M. Prevalence of celiac disease in patients with primary biliary cholangitis. *J Can Assoc Gastroenterol*. 2021;4:44-47.
47. Rashidi-Alavijeh J, Nuruzade N, Frey A, et al. Implications of anaemia and response to anaemia treatment on outcomes in patients with cirrhosis. *JHEP Rep*. 2023;5:100688.
48. Floreani A, Spinazzè A, Caballeria L, et al. Extrahepatic malignancies in primary biliary cirrhosis: a comparative study at two European centers. *Clin Rev Allergy Immunol*. 2015;48:254-262.
49. Floreani A, Franceschet I, Cazzagon N, et al. Extrahepatic autoimmune conditions associated with primary biliary cirrhosis. *Clin Rev Allergy Immunol*. 2015;48:192-197.
50. Chen VL, Chen Y, Du X, Handelman SK, Speliotes EK. Genetic variants that associate with cirrhosis have pleiotropic effects on human traits. *Liver Int*. 2020;40:405-415.
51. Tanaka A. Current understanding of primary biliary cholangitis. *Clin Mol Hepatol*. 2021;27:1-21.
52. Wang M-H, Friton JJ, Rebert N, et al. Novel genetic risk variants and clinical predictors associated with primary sclerosing cholangitis in patients with ulcerative colitis. *Clin Transl Gastroenterol*. 2023;14:e00615.
53. Han IS, Baek DH, Hong SM, et al. Incidence and adverse clinical events of primary sclerosing cholangitis with ulcerative colitis. *Int J Color Dis*. 2023;38:175.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Koop P-H, Schwenzer C, Clusmann J, et al. Comorbidities, mortality and metabolic profile in individuals with primary biliary cholangitis—A Phenome-Wide-Association-Study. *Liver Int*. 2024;44:2038-2053. doi:10.1111/liv.15945