





Protein Phosphatase 1 Regulatory Subunit 3B Genotype at rs4240624 Has a Major Effect on Gallbladder Bile Composition

Ville Männistö ^{1,2*}, Dorota Kaminska ^{3*}, Pirjo Käkälä,⁴ Mikko Neuvonen,⁵⁻⁷ Mikko Niemi,⁵⁻⁷ Marcus Alvarez,⁸ Päivi Pajukanta,⁸⁻¹⁰ Stefano Romeo ¹¹⁻¹³, Max Nieuwdorp,² Albert.K. Groen,² and Jussi Pihlajamäki ^{3,14}

The protein phosphatase 1 regulatory subunit 3B (*PPP1R3B*) gene is a target of farnesoid X receptor (*FXR*), which is a major regulator of bile acid metabolism. Both *PPP1R3B* and *FXR* have been suggested to take part in glycogen metabolism, which may explain the association of *PPP1R3B* gene variants with altered hepatic computed tomography attenuation. We analyzed the effect of *PPP1R3B* rs4240624 variant on bile acid composition in individuals with obesity. The study cohort consisted of 242 individuals from the Kuopio Obesity Surgery Study (73 men, 169 women, age 47.6 ± 9.0 years, body mass index 43.2 ± 5.4 kg/m²) with *PPP1R3B* genotype and liver RNA sequencing (RNA-seq) data available. Fasting plasma and gallbladder bile samples were collected from 50 individuals. Bile acids in plasma did not differ based on the *PPP1R3B* rs4240624 genotype. However, the concentration of total bile acids (109 ± 55 vs. 35 ± 19 mM; $P = 1.0 \times 10^{-5}$) and all individual bile acids (also 7α -hydroxy-4-cholesten-3-one [C4]) measured from bile were significantly lower in those with the AG genotype compared to those with the AA genotype. In addition, total cholesterol ($P = 0.011$) and phospholipid ($P = 0.001$) levels were lower in individuals with the AG genotype, but cholesterol saturation index did not differ, indicating that the decrease in cholesterol and phospholipid levels was secondary to the change in bile acids. Liver RNA-seq data demonstrated that expression of *PPP1R3B*, tankyrase (*TNKS*), *Homo sapiens* chromosome 8 clone RP11-10A14.5 (*AC022784.1* [*LOC157273*]), *Homo sapiens* chromosome 8 clone RP11-375N15.1 (*AC021242.1*), and *Homo sapiens* chromosome 8, clone RP11-10A14 (*AC022784.6*) associated with the *PPP1R3B* genotype. In addition, genes enriched in transmembrane transport and phospholipid binding pathways were associated with the genotype. **Conclusion:** The rs4240624 variant in *PPP1R3B* has a major effect on the composition of gallbladder bile. Other transcripts in the same loci may be important mediators of the variant effect. (*Hepatology Communications* 2021;5:244-257).

The protein phosphatase 1 regulatory subunit 3B gene (*PPP1R3B*) is located on chromosome 8p and encodes the regulatory subunit historically known as G_L. G_L regulates the activity of protein phosphatase 1, which activates glycogen synthase and inactivates glycogen phosphorylase, which

in turn catalyzes the rate-limiting step in glycogenolysis.⁽¹⁾ *PPP1R3B* polymorphisms have been associated with type 2 diabetes and plasma glucose,^(2,3) serum lipids and lipoproteins,^(3,4) and plasma lactate levels.⁽⁵⁾ The meta-analysis of genome-wide association identified *PPP1R3B* as a genome-wide significant loci for

Abbreviations: ABC, adenosine triphosphate binding cassette subfamily C; AC021242.1, *Homo sapiens* chromosome 8 clone RP11-375N15.1; AC022784.1, *Homo sapiens* chromosome 8 clone RP11-10A14.5; AC022784.6, *Homo sapiens* chromosome 8 clone RP11-10A14; AQP, aquaporin; BMI, body mass index; C4, 7α -hydroxy-4-cholesten-3-one; CSI, cholesterol saturation index; CT, computed tomography; eQTL, expression quantitative trait loci; FDR, false discovery rate; FXR, farnesoid X receptor; GDCA, glycodeoxycholic acid; GO, gene ontology; HK, hexokinase; kb, kilobase; LRYGB, laparoscopic Roux-en-Y gastric bypass; mRNA, messenger RNA; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NR1H4, nuclear receptor subfamily 1 group H member 4; PPP1R3B, protein phosphatase 1 regulatory subunit 3B; RNA-seq, RNA sequencing; SLC, solute carrier; TNKS, tankyrase.

Received July 23, 2020; accepted September 29, 2020.

Additional Supporting Information may be found at onlinelibrary.wiley.com/doi/10.1002/hep4.1630/suppinfo.

Supported by the Sigrid Jusélius Foundation (V.M.), Academy of Finland (contract No. 316458 to D.K., 120979 to J.P., 138006 to J.P.), Finnish Diabetes Research Foundation (Kuopio Obesity Surgery Study to J.P.), Kuopio University Hospital (project grant EVO/VTR grants 2005-2019 to J.P.), Finnish Cultural Foundation (to J.P.), University of Eastern Finland (Spearhead Funding to J.P.), Howard Hughes Medical Institute (Gilliam Fellowship to M.A.), and National Institutes of Health (grants HL-095056, HL-28481, and U01 DK105561 to P.P.).

*These authors contributed equally to this work.

hepatic steatosis measured by computed tomography (CT). The variant rs4240624, located 175 kilobase (kb) upstream of *PPP1R3B* and 229 kb downstream of tankyrase (*TNKS*), has been associated with decreased hepatic CT attenuation (the effect allele is a wild-type A allele with allelic frequency of 90%⁽⁶⁾). Similarly, the minor allele of rs4841132 variant (a proxy variant in complete linkage with rs4240624) has been associated with increased hepatic CT attenuation and with an increase in plasma levels of alanine aminotransferase but not with hepatic fat content,^(3,7) as also published for rs4240624.⁽⁶⁾ Several factors can cause a change in hepatic CT attenuation, with increased hepatic fat, which decreases CT attenuation, being the most prevalent, followed by glycogen and iron content, both of which increase attenuation.^(7,8) Interestingly, the preprint of a recent study identifies rs4240624 as the strongest genome-wide genetic determinant of liver volume whereas no association with liver fat or liver iron content was observed.⁽⁹⁾ Overall, the mechanisms for how the *PPP1R3B* polymorphism contributes to liver CT attenuation or liver metabolism are unclear.

It has been suggested that *PPP1R3B* is a target of farnesoid X receptor (*FXR*, also known as nuclear receptor subfamily 1 group H member 4 [*NR1H4*]),⁽¹⁰⁾ which not only is an important regulator of bile acid metabolism but also affects glycogen metabolism.⁽¹¹⁾ On the other hand, bile acid metabolism is linked to both nonalcoholic fatty liver disease (NAFLD)⁽¹²⁾ and obesity.⁽¹³⁾ Therefore, we analyzed the effect of the *PPP1R3B* rs4240624 variant on bile acid composition in individuals with obesity.

Participants and Methods

SUBJECTS

The study cohort consisted of 242 individuals from the Kuopio Obesity Surgery Study⁽¹⁴⁾ with rs4240624 (*PPP1R3B*) genotype and liver RNA sequencing (RNA-seq) data available (Table 1). Of these, 50 individuals had bile acids measured from both plasma and gallbladder bile (Supporting Table S1).

© 2020 The Authors. *Hepatology Communications* published by Wiley Periodicals LLC on behalf of the American Association for the Study of Liver Diseases. This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

View this article online at wileyonlinelibrary.com.

DOI 10.1002/hep4.1630

Potential conflict of interest: Nothing to report.

ARTICLE INFORMATION:

From the ¹Department of Medicine, University of Eastern Finland and Kuopio University Hospital, Kuopio, Finland; ²Department of Experimental Vascular Medicine, Amsterdam UMC, University of Amsterdam, Amsterdam, the Netherlands; ³Institute of Public Health and Clinical Nutrition, University of Eastern Finland, Kuopio, Finland; ⁴Department of Surgery, University of Eastern Finland and Kuopio University Hospital, Kuopio, Finland; ⁵Department of Clinical Pharmacology, University of Helsinki, Helsinki, Finland; ⁶Department of Clinical Pharmacology, HUS Diagnostic Services, Helsinki University Hospital, Helsinki, Finland; ⁷Individualized Drug Therapy Research Program, Faculty of Medicine, University of Helsinki, Helsinki, Finland; ⁸Department of Human Genetics, David Geffen School of Medicine; ⁹Bioinformatics Interdepartmental Program; ¹⁰Institute for Precision Health, University of California Los Angeles, Los Angeles, CA, USA; ¹¹Department of Molecular and Clinical Medicine, University of Gothenburg, Gothenburg, Sweden; ¹²Cardiology Department, Sahlgrenska University Hospital, Gothenburg, Sweden; ¹³Clinical Nutrition Department of Medical and Surgical Science, University Magna Graecia, Catanzaro, Italy; ¹⁴Department of Medicine, Endocrinology, and Clinical Nutrition, Kuopio University Hospital, Kuopio, Finland.

ADDRESS CORRESPONDENCE AND REPRINT REQUESTS TO:

Jussi Pihlajamäki, M.D., Ph.D.
Institute of Public Health and Clinical Nutrition
University of Eastern Finland

70210 Kuopio, Finland
E-mail: jussi.pihlajamaki@uef.fi
Tel.: +358-50-3440187

The study protocol was approved by the Ethical Committee of Northern Savo and followed the Declaration of Helsinki. Written consent was obtained from all study subjects.

CLINICAL AND LABORATORY MEASUREMENTS

Plasma glucose, insulin, serum lipids, and lipoprotein lipids were determined, as described.⁽¹⁵⁾

BILE ACID MEASUREMENTS

Bile acids were measured from fasting serum samples and from samples obtained from transhepatic gallbladder punctation during elective laparoscopic Roux-en-Y

gastric bypass (LRYGB). Measurements were carried out using a Nexera X2 ultra high-performance liquid chromatography system (Shimadzu, Kyoto, Japan) coupled to a 5500 Qtrap mass spectrometer interfaced with an electrospray ion source (ABSciex, Toronto, Canada), as described.⁽¹⁶⁾ Total, primary, secondary, tertiary, primary conjugated, and secondary conjugated bile acids were calculated based on individual bile acids. Secondary conjugated bile acids in plasma were calculated without taurothiocholate acid and secondary conjugated bile acids in bile were calculated without glycolithocholic acid because these were not measured.

Cholesterol and phospholipids from bile samples were measured enzymatically using a Clariostar analyzer (BMG Labtech, Ortenberg, Germany), as described.⁽¹⁷⁾ Total lipid content and cholesterol

TABLE 1. CHARACTERISTICS OF THE LIVER mRNA COHORT (N = 242) BASED ON THE *PPP1R3B* rs4240624 GENOTYPE

	AA n = 170	AG n = 67	GG n = 5	PValue (K-W)
Sex (male/female)	48/122	22/45	3/2	0.267*
Age (years)	47.8 ± 9.1	46.2 ± 8.7	52.7 ± 8.1	0.205
BMI (kg/m ²)	43.5 ± 5.7	42.5 ± 4.7	44.1 ± 3.2	0.430
Total cholesterol (mmol/L)	4.2 ± 0.9	4.1 ± 0.9	3.9 ± 0.5	0.067
LDL cholesterol (mmol/L)	2.3 ± 0.8	2.3 ± 0.8	1.8 ± 0.4	0.327
HDL cholesterol (mmol/L)	1.1 ± 0.3	1.1 ± 0.3	1.0 ± 0.2	0.697
Triglycerides (mmol/L)	1.6 ± 0.3	1.1 ± 0.3	1.0 ± 0.4	0.093
Type 2 diabetes (%)	36	43	0	0.133*
Fasting glucose (mmol/L)	6.5 ± 1.8	6.7 ± 2.0	5.7 ± 0.5	0.524
Fasting insulin (mU/L)	18.5 ± 11.8	21.4 ± 22.1	14.8 ± 8.0	0.439
ALT (U/L)	46 ± 31	39 ± 20	35 ± 16	0.312
Histology (n = 239)				
Steatosis grade (n)				0.403*
<5%	59	32	3	
5%-33%	65	19	1	
33%-66%	24	6	1	
>66%	21	8	0	
Lobular inflammation grade (n)				0.503*
None	109	49	10	
<2 foci per 200× field	49	12	4	
2-4 foci per 200× field	11	4	0	
>4 foci per 200× field	0	0	0	
Fibrosis stage (n)				0.906*
0	90	40	2	
1	68	21	3	
2	7	2	0	
3	3	2	0	
4	1	0	0	

*chi-square test.

Abbreviations: ALT, alanine aminotransferase; HDL, high-density lipoprotein; K-W, Kruskal-Wallis; LDL, low-density lipoprotein.

saturation index (CSI) were calculated based on the critical tables by Carey.⁽¹⁸⁾

LIVER BIOPSIES AND HISTOLOGY

Liver biopsies were obtained with ultrasonic scissors during the elective LRYGB operation. Overall histologic assessment of liver biopsy samples was performed by one pathologist according to standard criteria.^(19,20)

GENOTYPING

PPP1R3B rs4240624 was genotyped with TaqMan single-nucleotide polymorphism genotyping assays (Applied Biosystems) according to the manufacturer's protocol.

LIVER RNA-Seq DATA

RNA samples were isolated using the miR-Neasy (Qiagen) kit, and sequencing libraries were prepared using Ribo-Zero Gold (Illumina). Reads were aligned against the human reference genome GRCh38 (release 29) using two-pass STAR RNA-aligner.⁽²¹⁾ On average, 37.5 million (77.4%) read-pairs mapped to a unique site of the human genome and were used for further analysis. The Rsubread R package⁽²²⁾ was used to count all reads mapping within exon features. To reduce random variability resulting from low counts, we required at least 80% of samples to have at least 10 reads, resulting in 15,243 genes in the final analysis. The gene level count values were then normalized using a trimmed-mean of M values converted to count per million using edgeR⁽²³⁾ and inverse normal transformed. Technical factors were obtained from STAR RNA-aligner (summary of mapping statistics) and from Picard⁽²⁴⁾ (quality metrics). Principal component analysis (PCA) was used to identify effects of confounding factors on the transcriptome. Expression data were corrected for uniquely aligned reads percentage and 3' bias, body mass index (BMI), sex, and age for the subsequent statistical analyses.

STATISTICAL ANALYSIS

Data are presented as mean \pm SD. Differences in categorical variables were evaluated by the χ^2 test. Differences between the study groups were compared using Kruskal-Wallis and Mann-Whitney

independent samples test. Spearman's rank correlation was used for correlation analyses. Analyses were conducted with SPSS version 25 (IBM Inc., Armonk, NY). $P < 0.05$ was considered statistically significant.

Pairwise gene correlations were measured with Pearson correlation coefficients. The Benjamini-Hochberg (false discovery rate [FDR]) method was applied to adjust for multiple testing. An FDR value < 0.05 was considered statistically significant.

EXPRESSION QUANTITATIVE TRAIT LOCI ANALYSIS

Expression quantitative trait loci (eQTL) analysis was used to investigate the effect of the rs4240624 genotype on gene expression levels in the liver. To improve power to map eQTL and remove the impact of cofactors, which can mask the genetic signal, we determined the hidden covariates with PCA. Matrix eQTL⁽²⁵⁾ was run in the R environment (www.r-project.org) using the additive linear model with 23 principal components that were selected based on optimization on chromosome 21 as covariates to account for confounding variation. The rs4240624 variant was defined as *cis*-eQTL if it were within 500 kb of either end of the gene and as *trans*-eQTL if it were beyond that point. eGenes were defined as genes associated with eQTL (*cis*-eGenes for local and *trans*-eGenes for distant transcripts).

To reveal the functional relationship among key genes, gene ontology (GO) pathway analysis was performed on *trans*-eGenes with nominal $P < 0.01$ as a cutoff, using the web-based gene set analysis toolkit.⁽²⁶⁾

Results

PPP1R3B rs4240624 GENOTYPE DID NOT CORRELATE WITH LIVER HISTOLOGY

From the initial 242 subjects (73 men, 169 women, age 47.6 ± 9.0 years, BMI 43.2 ± 5.4 kg/m²), 170 subjects had *PPP1R3B* rs4240624 genotype AA, 67 had genotype AG, and 5 had genotype GG. Clinical characteristics did not differ based on the genotype (Table 1). There were no significant associations

between *PPP1R3B* rs4240624 genotype and liver histology (available from 239 subjects) in this cohort.

BILE ACIDS IN FASTING PLASMA DID NOT DIFFER BASED ON THE rs4240624 GENOTYPE

From the whole study cohort, bile acids were measured from 50 subjects (16 men, 34 women, age 46.8 ± 10.0 years, BMI 41.7 ± 4.5 kg/m²); from those, 33 had *PPP1R3B* rs4240624 genotype AA and 17 had genotype AG. Clinical characteristics did not differ based on the genotype (Supporting Table S1).

Total bile acids were not associated with the rs4240624 genotype (Fig. 1). However, glycodeoxycholic acid (GDCA) was higher in those with the AG genotype compared to those with the AA genotype (0.14 ± 0.11 μ M vs. 0.31 ± 0.26 μ M; $P = 0.034$). Importantly, serum 7 α -hydroxy-4-cholesten-3-one (C4), a marker of bile acid synthesis,⁽²⁷⁾ did not associate with the genotype (0.07 ± 0.08 μ M vs. 0.05 ± 0.06 μ M; $P = 0.241$) (Supporting Table S2).

TOTAL BILE ACID LEVELS IN BILE WERE SIGNIFICANTLY LOWER IN THOSE WITH THE *PPP1R3B* rs4240624 AG GENOTYPE

Next, we analyzed if the *PPP1R3B* rs4240624 genotype had an effect on bile acid concentration in

samples taken from gallbladder. The total concentration of bile acids in bile was much lower in those with the AG genotype compared to the AA genotype (108.7 ± 55.4 vs. 35.1 ± 19.0 mM; $P = 1.0 \times 10^{-5}$) (Fig. 1). Similarly, all individual bile acid concentrations in bile, including C4 (0.001 ± 0.001 vs. 0.0002 ± 0.001 mM; $P = 0.015$), were lower in those with the AG genotype (Table 2).

Next, we calculated ratios of different bile acid to total bile acid concentration in bile to estimate possible changes independent of the change in concentration of total bile acids. The ratio of primary conjugated bile acids to total bile acids was lower in those with the AG genotype. In contrast, the ratio of secondary conjugated bile acids to total bile acids (glycolithocholic acid was not available for our analysis) was higher in those with the AG genotype. In addition, the ratio of GDCA to total bile acids was higher in those with the AG genotype (Supporting Table S3).

TOTAL LIPID CONTENT IN BILE IS DECREASED IN THOSE WITH THE *PPP1R3B* AG GENOTYPE

Because bile acids are known to drive the biliary secretion of phospholipid and cholesterol, we also measured the concentration of these lipids in the bile. Similar to the level of bile acids, cholesterol (25.7 ± 22.3 vs. 13.3 ± 11.1 mM; $P = 0.011$) and phospholipid (74.1 ± 36.3 vs. 36.9 ± 19.9 mM;

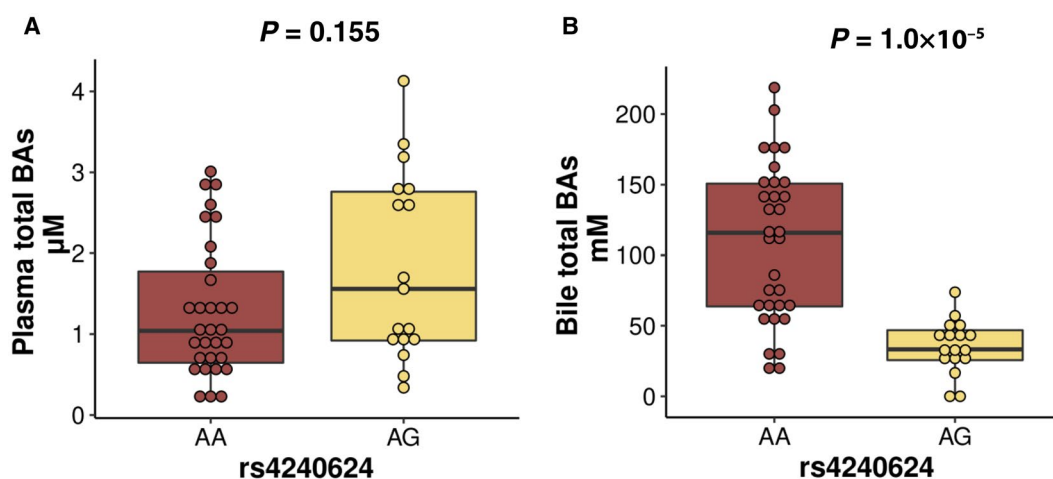


FIG. 1. Total bile acid levels based on the *PPP1R3B* genotypes AA and AG at rs4240624. (A) In plasma. (B) In gallbladder bile. Kruskal-Wallis test used for statistics. Horizontal line indicates the median, whiskers indicate the minimum and maximum values and box borders indicate lower and upper quartiles. Abbreviation: BA, bile acid.

TABLE 2. DIFFERENT BILE ACIDS IN BILE BASED ON THE *PPP1R3B* rs4240624 GENOTYPE

Bile Acids in Bile (mM)	AA (n = 33)	AG (n = 17)	P Value (K-W)
Primary BAs	0.030 ± 0.035	0.009 ± 0.014	3.3 × 10 ⁻⁴
Cholic acid	0.017 ± 0.024	0.006 ± 0.011	0.002
Chenodeoxycholic acid	0.012 ± 0.013	0.003 ± 0.004	3.1 × 10 ⁻⁴
Secondary BAs	0.005 ± 0.004	0.002 ± 0.001	1.6 × 10 ⁻⁴
Deoxycholic acid	0.004 ± 0.004	0.001 ± 0.001	3.8 × 10 ⁻⁴
Lithocholic acid	0.0003 ± 0.0002	0.0001 ± 0.0001	0.006
Tertiary BAs			
Ursodeoxycholic acid	0.001 ± 0.001	0.0003 ± 0.0004	0.041
Primary conjugated BAs	79.3 ± 48.5	22.2 ± 12.2	5.7 × 10 ⁻⁶
Glycocholic acid	30.7 ± 20.0	9.6 ± 5.4	2.8 × 10 ⁻⁴
Taurocholic acid	10.5 ± 7.1	3.3 ± 2.6	3.2 × 10 ⁻⁵
Glycochenodeoxycholic acid	31.4 ± 25.8	7.5 ± 4.6	5.8 × 10 ⁻⁵
Taurochenoxycholic acid	6.7 ± 5.6	1.8 ± 1.6	1.7 × 10 ⁻⁵
Secondary conjugated BAs*	29.4 ± 15.2	12.9 ± 8.6	1.4 × 10 ⁻⁴
Glycodehydrocholic acid	18.6 ± 10.6	9.0 ± 6.5	0.004
Taurodeoxycholic acid	6.9 ± 4.5	2.8 ± 2.7	0.001
Taurolithocholate	0.2 ± 0.2	0.1 ± 0.1	0.037
Glycoursodeoxycholic acid	3.3 ± 2.9	0.9 ± 0.7	1.1 × 10 ⁻⁴
Tauroursodeoxycholic acid	0.4 ± 0.3	0.1 ± 0.1	3.8 × 10 ⁻⁵
Hyodeoxycholic acid	0.0002 ± 0.0003	0.00004 ± 0.0001	0.018
C4	0.001 ± 0.001	0.0002 ± 0.001	0.015

*Missing glycolithocholic acid (GLCA).

Abbreviations: BA, bile acid; K-W, Kruskal-Wallis.

$P = 0.001$) concentrations were lower in subjects with the AG genotype compared to those with the AA genotype. Concordantly, total lipid content in bile was lower in those with the AG genotype (16.6 ± 6.6 vs. 10.1 ± 5.3 g/dL; $P = 0.003$), but the CSI did not differ ($99.8\% \pm 60.2\%$ vs. $101.2\% \pm 46.8\%$; $P = 0.444$) (Fig. 2).

LIVER-SPECIFIC IMPACT OF rs4240624 ON *PPP1R3B*, *TNKS*, *AC022784.1*, *AC021242.1*, AND *AC022784.6* EXPRESSION

Because changes in gene expression are one of the most important determinants of phenotypic changes, we investigated the effect of the rs4240624 genotype on messenger RNA (mRNA) expression by using the liver RNA-seq data from the whole cohort ($n = 242$). The genomic region surrounding rs4240624 (± 500 kb) is comprised of 25 genes (four protein coding genes, five pseudogenes, and 16 RNA genes), seven of which were detected in the liver (Fig. 3). Interestingly, while all four protein coding genes were expressed both in the liver and in subcutaneous adipose tissue collected from the same individuals (data not shown), the expression

of *Homo sapiens* chromosome 8, clone RP11-10A14 (*AC022784.6*), *Homo sapiens* chromosome 8 clone RP11-375N15.1 (*AC021242.1*), and *Homo sapiens* chromosome 8 clone RP11-10A14.5 map 8 (*AC022784.1*) were detected only in the liver. We identified five genes (*cis*-eGenes) regulated by the rs4240624 variant: *PPP1R3B*, *AC022784.6*, *AC021242.1*, *AC022784.1*, and *TNKS* ($\beta = 0.787$, FDR, 7.1×10^{-30} ; $\beta = 0.360$, FDR, 3.4×10^{-4} ; $\beta = -1.037$, FDR, 1.0×10^{-32} ; $\beta = -0.966$, FDR, 4.8×10^{-35} ; and $\beta = -0.769$, FDR, 3.8×10^{-17} , respectively) (Fig. 3). Importantly, rs4240624 did not affect subcutaneous adipose tissue expression levels of any of the detected genes in *cis* (Fig. 3; data not shown), indicating that association of rs4240624 with expression levels of local genes is tissue specific. Additionally, we detected 202 *trans*-eGenes with a nominal $P < 0.01$.

EXPRESSION OF *PPP1R3B*, *AC021242.1*, AND *AC022784.6* CORRELATED WITH TOTAL BILE ACIDS IN BILE

Although correlations were not very strong, mRNA expression of *PPP1R3B* and to a lesser extent

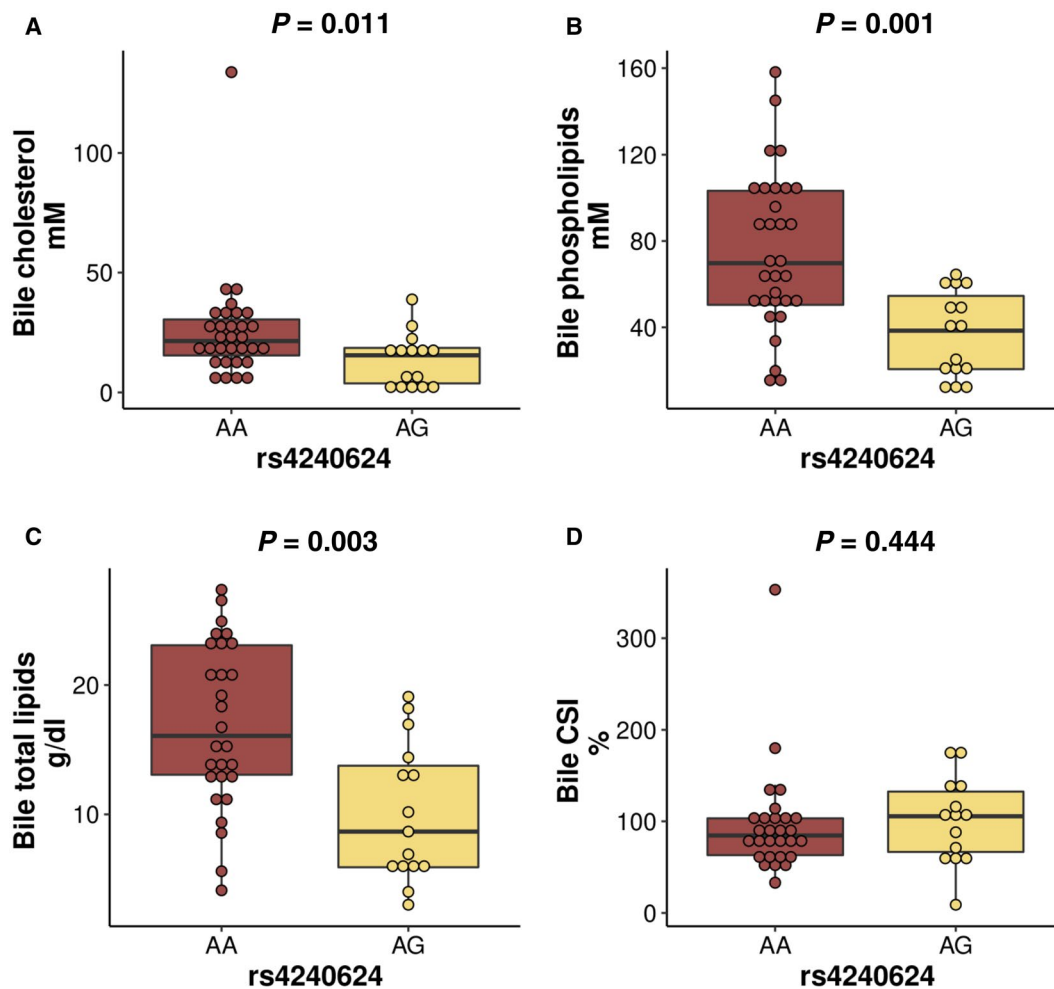


FIG. 2. Lipids in gallbladder bile based on the *PPP1R3B* genotypes AA and AG at rs4240624. (A) Cholesterol, (B) phospholipid, (C) total lipid content, and (D) CSI. Kruskal-Wallis test used for statistics. Horizontal line indicates the median, whiskers indicate the minimum and maximum values and box borders indicate lower and upper quartiles.

AC021242.1 and *AC022784.6* tended to correlate with several bile acids in bile but not in plasma (except between *PPP1R3B* and total bile acids) (Supporting Tables S4 and S5).

PATHWAY ANALYSIS REVEALED CHANGES IN THE TRANSMEMBRANE TRANSPORTER ACTIVITY LIBRARY BASED ON THE *PPP1R3B* GENOTYPE

To further explore the biological functions of rs4240624-mediated regulation of gene expression in

the liver, we performed a GO analysis. Identification of *trans*-eGenes is challenging due to the fact that the effect sizes of *trans*-eQTLs are typically small and underpowered due to a heavy multiple testing correction burden.⁽²⁸⁾ Hence, we performed the GO analysis on nominally significant *trans*-eGenes to get more insight into potentially affected pathways rather than particular genes. The overrepresented groups identified according to GO molecular function were involved in active transmembrane transporter activity (GO:0022804 enrichment ratio, 3.5; FDR, 0.028) and phospholipid binding (GO:0005543 enrichment ratio, 3.2; FDR, 0.028). In addition, we observed a significant overrepresentation of nuclear receptors (Wikipathways

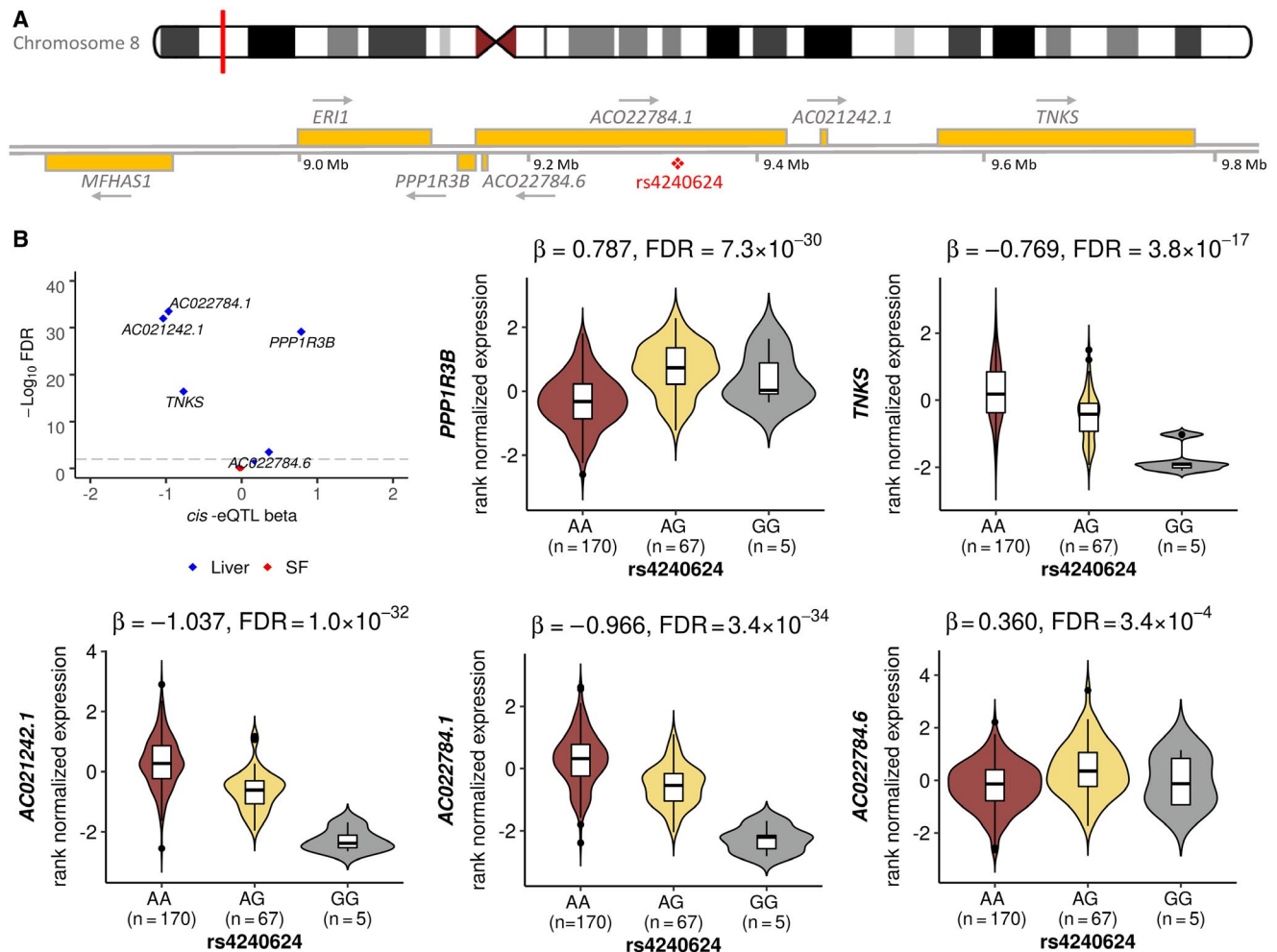


FIG. 3. Genes detected in the liver. (A) Genomic region of rs424062 (yellow boxes) depict genes detected in the liver samples. Genomic coordinates are based on the Human Genome Assembly GRCh38 (hg38). (B) NAFLD-associated variant rs4240624 is a *cis*-eQTL for five genes in the liver (*ACO22784.1*, *ACO21242.1*, *TNKS*, *PPP1R3B*, *ACO22784.6*) but not in the subcutaneous adipose tissue. Violin plots display gene expression levels in liver based on the rs4240624 genotype (AA, AG, and GG); effect sizes (β) and *cis*-eQTL FDR values are presented. Overlaid boxplot represent upper and lower quartiles, with horizontal line depicting the median. Whiskers depict 1.5 times interquartile range. Abbreviations: ERI1, exoribonuclease 1; MFHAS1, malignant fibrous histiocytoma amplified sequence 1.

WP2882 enrichment ratio, 7.82; FDR, 1.1×10^{-4}). Strikingly among the *trans*-eGenes, we found *FXR* (*NR1H4*; nominal $P = 0.007$) (Table 3).

TRANSCRIPTS IN THE *PPP1R3B* LOCI HAVE DIFFERENT CO-EXPRESSION PATTERNS

Finally, to further explore how the changes in the expression of rs4240624-associated *cis*-eGenes might

affect the expression of distant genes, we performed gene expression correlation analysis using Pearson's correlation coefficient as a measure of gene co-expression. Based on our results from bile samples and the role of *PPP1R3B* in glycogen metabolism, we focused on selected genes of bile acid synthesis, regulation, and transport and furthermore on genes taking part in glycogen metabolism and water transport (Fig. 4). We observed significant correlations between expression of *PPP1R3B* and several genes involved

TABLE 3. GO AND WIKIPATHWAYS ENRICHMENT ANALYSIS OF A SET OF *PPP1R3B* rs4240624-ASSOCIATED *trans*-eGenes

Identified eight enriched genes belonging to the molecular function active transmembrane transporter activity (GO:0022804), enrichment ratio, 3.5; FDR, 0.028

ENSG00000118777	<i>ABCG2</i>	ATP binding cassette subfamily G member 2 (junior blood group)
ENSG00000123191	<i>ATP7B</i>	ATPase copper transporting beta
ENSG00000124564	<i>SLC17A3</i>	solute carrier family 17 member 3
ENSG00000127249	<i>ATP13A4</i>	ATPase 13A4
ENSG00000128524	<i>ATP6V1F</i>	ATPase H ⁺ transporting V1 subunit F
ENSG00000139370	<i>SLC15A4</i>	Solute carrier family 15 member 4
ENSG00000171365	<i>CLCN5</i>	Chloride voltage-gated channel 5
ENSG00000173214	<i>MFSD4B</i>	Major facilitator superfamily domain containing 4B

Identified 10 enriched genes belonging to the molecular function phospholipid binding (GO:0005543), enrichment ratio, 3.2; FDR, 0.028

ENSG00000021762	<i>OSBPL5</i>	Oxysterol binding protein-like 5
ENSG00000100003	<i>SEC14L2</i>	SEC14-like lipid binding 2
ENSG00000110243	<i>APOA5</i>	Apolipoprotein A5
ENSG00000118960	<i>HS1BP3</i>	Hematopoietic cell-specific Lyn substrate 1 binding protein 3
ENSG00000135317	<i>SNX14</i>	Sorting nexin 14
ENSG00000141738	<i>GRB7</i>	Growth factor receptor bound protein 7
ENSG00000154217	<i>PITPNC1</i>	Phosphatidylinositol transfer protein cytoplasmic 1
ENSG00000159023	<i>EPB41</i>	Erythrocyte membrane protein band 4.1
ENSG00000197858	<i>GPA1</i>	Glycosylphosphatidylinositol anchor attachment 1
ENSG00000247596	<i>TWF2</i>	Twinfilin actin binding protein 2

Identified seven enriched genes belonging to the nuclear receptors meta-pathway (WP2882), enrichment ratio, 7.8; FDR, 1.1×10^{-4}

ENSG00000110243	<i>APOA5</i>	Apolipoprotein A5
ENSG00000114315	<i>HES1</i>	Hes family bHLH transcription factor 1
ENSG00000012504	<i>NR1H4</i>	Nuclear receptor subfamily 1 group H member 4
ENSG00000186350	<i>RXRA</i>	Retinoid X receptor alpha
ENSG00000072310	<i>SREBF1</i>	Sterol regulatory element binding transcription factor 1
ENSG00000118503	<i>TNFAIP3</i>	TNF alpha-induced protein 3
ENSG00000136810	<i>TXN</i>	Thioredoxin

Identified four enriched genes belonging to the interleukin-18 signaling pathway (WP4754), enrichment ratio, 5.4; FDR, 0.022

ENSG00000125817	<i>CENPB</i>	Centromere protein B
ENSG00000159023	<i>EPB41</i>	Erythrocyte membrane protein band 4
ENSG00000106397	<i>PLOD3</i>	Procollagen-lysine,2-oxoglutarate 5-dioxygenase 3
ENSG00000118503	<i>TNFAIP3</i>	TNF alpha-induced protein 3

Identified five enriched genes belonging to the VEGFA-VEGFR2 signaling pathway (WP3888), enrichment ratio, 3.46; FDR, 0.031

ENSG00000175183	<i>CSRP2</i>	Cysteine and glycine rich protein 2
ENSG00000159023	<i>EPB41</i>	Erythrocyte membrane protein band 4
ENSG00000133961	<i>NUMB</i>	NUMB, endocytic adaptor protein
ENSG00000106397	<i>PLOD3</i>	Procollagen-lysine,2-oxoglutarate 5-dioxygenase 3
ENSG00000136810	<i>TXN</i>	Thioredoxin

Abbreviations: ATP, adenosine triphosphate; ATPase, adenosine triphosphatase; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; WP, WikiPathways.

in glycogen metabolism, confirming its reported role in glycogenesis. Additionally, we observed correlations between *PPP1R3B* and genes involved in bile acid biosynthesis (cytochrome P450 family 7 subfamily B member 1 [*CYP7B1*], alpha-methylacyl-coenzyme A [CoA] racemase [*AMACR*], solute carrier family 27 member 5 [*SLC27A5*], bile acid-CoA:amino acid N-acyltransferase [*BAAT*], D-box

binding PAR bZIP transcription factor [*DBP*], and hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 7 [*HSD3B7*]) and regulation (*NR1H4* [i.e., *FXR*], ; i.e., *NR1H3*], and nuclear receptor subfamily 1 group I member 3 [*NR1I3*]). Furthermore, *PPP1R3B* expression correlated with canalicular multispecific organic anion transporter 2 (encoded by the adenosine triphosphate

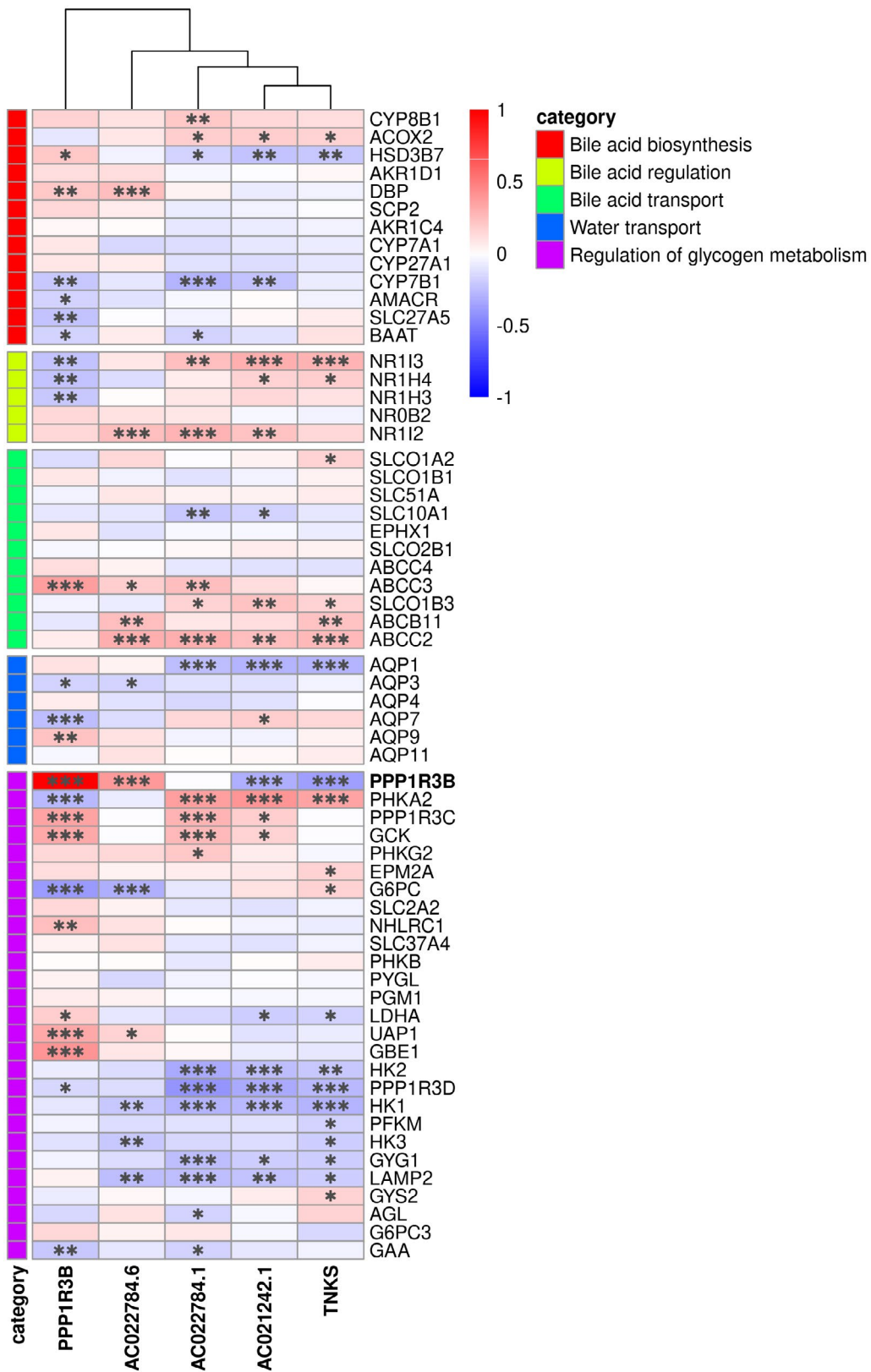


FIG. 4. Pearson's correlation between identified *cis*-eQTL genes and selected genes taking part in bile acid and glycogen metabolism and water transport. Pearson's correlation coefficients are shown with continuous gradient colors, with red representing positive and blue representing negative correlation. Asterisk symbols correspond to FDR-adjusted * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Abbreviations: ABCB11, ATP binding cassette subfamily B member 11 aka BSEP, bile salt export pump; ABCC2, ATP binding cassette subfamily C member 2; ABCC3, ATP binding cassette subfamily C member 3; ABCC4, ATP binding cassette subfamily C member 4; ACOX2, acyl-CoA oxidase 2; AGL, amylo- α -1, 6-glucosidase, 4- α -glucanotransferase; AKR1C4, 3 α -steroid dehydrogenase; AKR1D1, delta-5-3-oxysterol 5 β -reductase; AMACR, alpha-methylacyl-CoA racemase; AQP1, aquaporin 1; AQP11, aquaporin 11; AQP3, aquaporin 3; AQP4, aquaporin 4; AQP7, aquaporin 7; AQP9, aquaporin 9; CYP27A1, sterol 27-hydroxylase; CYP7A1, cholesterol 7 α -hydroxylase; CYP7B1, oxysterol 7 α -hydroxylase; CYP8B1, sterol 12 α -hydroxylase; DBP, D-box binding PAR bZIP transcription factor; EPHX1, epoxide hydrolase 1; EPM2A, glucan phosphatase, laforin; G6PC, glucose 6-phosphatase catalytic subunit; G6PC3, glucose 6-phosphatase catalytic 3; GAA, glucosidase alpha, acid; GBE1, 1,4- α -glucan branching enzyme 1; GCK, glucokinase; GYG1, glycogenin 1; GYS2, glycogen synthase 2; HK1, hexokinase 1; HK2, hexokinase 2; HK3, hexokinase 3; HSD3B7, 3 β -hydroxy-delta5-C27-steroid oxoreductase; LAMP2, lysosome-associated membrane protein 2; LDHA, lactate dehydrogenase A; NHLRC1, NHL repeat containing E3 ubiquitin protein ligase 1; NR1H3, nuclear receptor subfamily 1 group H member 3; NR1H4, nuclear receptor subfamily 1 group H member 4 aka FXR, farnesoid X-activated receptor; NR1I2, nuclear receptor subfamily 1 group I member 2; NR1I3, nuclear receptor subfamily 1 group I member 3; NROB2, nuclear receptor subfamily 0 group B member 2; PFKM, phosphofructokinase, muscle; PGM1, phosphoglycomutase 1; PHKA2, glycogen phosphorylase kinase regulatory subunit alpha 2; PHKB, phosphorylase kinase regulatory subunit beta; PHKG2, glycogen phosphorylase kinase catalytic subunit gamma 2; PPP1R3C, protein phosphatase 1 regulatory subunit 3C; PPP1R3D, protein phosphatase 1 regulatory subunit 3D; PYGL, glycogen phosphorylase L; SCP2, sterol carrier protein 2; SLC10A1, solute carrier family 10 member 1; SLC27A5, solute carrier family 27 member 5; SLC2A2, solute carrier family 2 member 2; SLC37A4, glucose 6-phosphatase translocase; SLC51A, solute carrier family 51 alpha subunit; SLCO1A2, solute carrier organic anion transporter family member 1A2; SLCO1B1, solute carrier organic anion transporter family member 1B1; SLCO1B3, solute carrier organic anion transporter family member 1B3; SLCO2B1, solute carrier organic anion transporter family member 2B1; UAP1, UDP-N-acetylglucosamine pyrophosphorylase 1.

binding cassette subfamily C member 3 [*ABCC*] gene, more often called MRP3) involved in bile acid transport.⁽²⁹⁾ Moreover, we observed a correlation between *PPP1R3B* and aquaporin (*AQP*)3, *AQP*7, and *AQP*9. Finally, we found correlations between remaining *cis*-eGenes (*AC022784.6*, *AC021242.1*, *AC022784.1*, and *TNKS*) and bile acid metabolism and transport-related genes (Fig. 4).

Interestingly, transcripts in the *PPP1R3B* loci showed different co-expression patterns. *PPP1R3B* expression correlated positively with *AC022784.6* ($r = 0.38$; FDR, 2.1×10^{-7}) and negatively with *TNKS* and *AC021242.1* ($r = -0.37$; FDR, 3.2×10^{-7} ; and $r = -0.32$; FDR, 1.6×10^{-5} , respectively). On the other hand, *TNKS* showed a very strong positive correlation with *AC022784.1* and *AC021242.1* ($r = 0.56$; FDR, 2.0×10^{-17} ; and $r = 0.80$; FDR, 4.4×10^{-52} , respectively) (Supporting Fig. S1). In addition, hexokinase 1 (*HK1*), *HK2*, *HK3*, phosphofructokinase, muscle (*PFKM*), glycogenin 1 (*GYG1*), glycogen synthase 2 (*GYS2*), and lysosomal associated membrane protein 2 (*LAMP2*) did not correlate with *PPP1R3B* but showed association with *TNKS*. Finally, we observed that *TNKS* correlated with *ABCB11* (also known as bile salt export pump [*BSEP*]), the transporter responsible for canalicular transport of the major bile acid species, and other transporters, such as *ABCC2*, solute carrier organic anion transporter family member 1A2

(*SLCO1A2*), and *SLCO1B3*, whereas *PPP1R3B* did not.

Discussion

The major finding of this study is that *PPP1R3B* rs4240624 associated with bile acid concentration in gallbladder bile. In addition, liver mRNA expression of *PPP1R3B* showed positive correlation with total bile acids in plasma and negative correlation with total bile acids in gallbladder bile (Supporting Tables S4 and S5). Interestingly, the genotype had only minimal effect on bile acid concentrations in plasma or bile acid synthesis monitored by C4. Furthermore, cholesterol, phospholipid, and total lipid concentrations in gallbladder bile were lower in subjects with the AG genotype. However, the CSI did not associate with the genotype, explained by the fact that the decrease in concentrations of the biliary lipids was proportional.

Our study is the first to report the association of *PPP1R3B* rs4240624 genotype with altered biliary bile acid secretion. While variants of *PPP1R3B* at rs4240624 and rs4841132 have been linked with NAFLD and nonalcoholic steatohepatitis (NASH) in some studies,^(6,30) the totality of evidence in the literature does not support an association

between this variant of *PPP1R3B* and NAFLD and NASH.^(7,31) Accordingly, we did not observe association between NAFLD and rs4240624 in this cohort. The link between *PPP1R3B* and bile acid metabolism has been suggested based on the finding that *PPP1R3B* was a target of *FXR* (*NR1A4*) in slices of human liver and in mouse liver samples.⁽¹⁰⁾ It is also known that bile acids can increase glycogenesis and decrease gluconeogenesis through activation of *FXR*.⁽³²⁾ Thus, we analyzed *FXR* and several other genes involved in bile acid metabolism to reveal more information about the function of the *PPP1R3B* gene. Our gene expression analyses based on liver mRNA-seq data showed that those with the G allele had higher *PPP1R3B* mRNA expression in the liver, which is in agreement with our previous report linking the rs4841132 variant (in complete linkage disequilibrium with rs4240624) with a lower risk of steatosis and fibrosis in a cohort of 1,388 individuals⁽³⁰⁾ but contrary to some other findings.⁽⁶⁾ Moreover, liver mRNA expression of *FXR* correlated with *PPP1R3B*, strengthening the finding of the association of these two genes.⁽¹⁰⁾ Notably, we found that rs4240624 is a *trans*-eQTL for *FXR* (nominal $P = 0.007$). A direct interaction of *PPP1R3B* with *FXR* should have influenced bile acid synthesis but was not observed in this study, indicating more complex and indirect mechanisms. *FXR* might be an important mediator between *PPP1R3B* and bile acids, but we conclude that it did not explain the effect of the *PPP1R3B* genotype on bile acids.

On the other hand, gene expression quantitative trait locus analysis of ~16,000 liver genes followed by GO analysis revealed significant enrichment in the GO category involved in transport (active transmembrane transporter activity) (Table 3). Interestingly, this pathway included two genes from the solute carrier family, which are membrane-bound transporters, that have substrates that include, for example, glucose, fatty acids, lipids, and bile salts; for example, *SLC17A3* belongs to the vesicular glutamate transporter family.⁽³³⁾ It has been suggested that bile acids could link to glutamate metabolism through *FXR*.⁽³⁴⁾

Importantly, we observed that four other transcripts located in close proximity to rs4240624 were significantly associated with the *PPP1R3B* rs4240624 genotype. Three of these (*TNKS*, *AC022784.1*, and *AC021242.1*) were down-regulated in subjects

with the G allele and *AC022784.6* was moderately up-regulated (Fig. 3). Based on these transcriptomics results, we suggest that another possible link between *PPP1R3B* and altered bile composition in gallbladder bile could be *TNKS*. We found that *TNKS* mRNA expression in the liver was associated with the *PPP1R3B* genotype and liver mRNA expression of *PPP1R3B* (Figs. 3 and 4). *TNKS* regulates WNT/ β -catenin signaling,⁽³⁵⁾ which has been shown to play a role in bile acid synthesis and transport through regulation of *FXR* activation.⁽³⁶⁾ Importantly, we observed a correlation between *TNKS* and bile salt export pump (*ABCB11*), a critical player involved in the secretion of bile salts into bile.⁽²⁹⁾

We also found that the *PPP1R3B* genotype associated with *AC022784.1*, *AC022784.6*, and *AC021242.1* mRNA expression in the liver (Fig. 3). Interestingly, *LOC157273*, one of the transcript variants of the *AC022784.1* gene (*AC022784.1-248*), is a long noncoding RNA that has been reported to down-regulate *PPP1R3B* expression.⁽³⁷⁾ While we could not link *AC022784.1* with *PPP1R3B* expression or with bile acid levels, we cannot exclude it because our analysis did not focus on the splice variant composition. On the other hand, we observed a correlation between expression of *PPP1R3B* and another long noncoding RNA, *AC022784.6* (Fig. 4; Supporting Fig. S1). Furthermore, *AC021242.1* had positive correlation with total bile acids in bile and negative correlation with the CSI (Supporting Tables S4 and S5). Therefore, our results suggest that *AC021242.1* might function as a liver-specific regulator of bile acid metabolism together with *PPP1R3B*.

Our results also provide further insight into earlier findings suggesting alterations in bile acid metabolism have been associated with NAFLD.⁽¹²⁾ In our study, the secondary bile acid GDCA was increased in plasma and decreased in bile in subjects with the AG genotype compared to those with the AA genotype. However, the ratio of GDCA to total bile acids in bile was increased in those with the AG genotype. This bile acid has been shown to be decreased in the liver in patients with NASH.⁽³⁸⁾ In contrast, high serum concentrations of GDCA have been associated with liver injury.⁽³⁹⁾ We also found that the ratio of primary conjugated bile acids to total bile acids in bile was lower in AG while that to secondary conjugated bile acids to total bile acids was higher in AG than in AA phenotypes. Lower bile acid concentration

probably influences the composition of the microbiota and hence may increase bile acid dehydroxylation.⁽⁴⁰⁾

Variants of *PPP1R3B* have not been associated with gallstones.⁽⁴¹⁾ Intriguingly, our preliminary data with limited number of subjects with gallstone disease data available showed that 2 out of 25 (8%) subjects with genotype AA had gallstones compared to 5 out of 15 (33%) with genotype AG ($P = 0.021$). This observation will require further studies. Importantly, our findings that bile acids from bile associated with the polymorphism remained essentially the same when excluding those with gallstones, indicating the observed changes in bile acid levels are primarily associated with genotype not gallstones.

A major strength of this study is that we had fasting bile acid samples taken from gallbladder punctation during the LRYGB, which in conjunction with plasma samples provided new insights into bile acid homeostasis. To our knowledge, there are only few human studies in more recent literature with bile acids measured from bile. The presence of liver mRNA sequencing data allowed us to investigate the molecular pathways regulated by the *PPP1R3B* genotype. A limitation of this study is the relatively small sample size, which might limit the statistical power, especially with RNA-seq data. It is probably the reason why we could not link the genotype with liver histology, although we have done it previously in a larger cohort (rs4841132 with complete linkage disequilibrium with rs4240624).⁽³⁰⁾

In conclusion, we found that the *PPP1R3B* genotype at rs4240624 associates strongly with total bile acid concentration in bile but not in plasma. Because the genotype did not associate with phospholipid and cholesterol content in bile, our results strongly suggest that factors involved in biliary bile acid transport are primarily affected. The role of altered expression of *TNKS* and three noncoding genes associated with the rs4240624 variant in the liver needs to be investigated further.

Acknowledgment: We acknowledge CSC–IT Center for Science, Finland, for computational resources.

REFERENCES

- 1) Mehta MB, Shewale SV, Sequeira RN, Millar JS, Hand NJ, Rader DJ. Hepatic protein phosphatase 1 regulatory subunit 3B (Ppp1r3b) promotes hepatic glycogen synthesis and thereby regulates fasting energy homeostasis. *J Biol Chem* 2017;292:10444–10454.

- 2) Niazi RK, Sun J, Have CT, Hollensted M, Linneberg A, Pedersen O, et al. Increased frequency of rare missense PPP1R3B variants among Danish patients with type 2 diabetes. *PLoS One* 2019;14:e0210114.
- 3) Seidelin AS, Nordestgaard BG, Tybjaerg-Hansen A, Stender S. Genetic variation at PPP1R3B increases hepatic CT attenuation and interacts with prandial status on plasma glucose. *J Clin Endocrinol Metab* 2020;105:dga151.
- 4) Li WJ, Yin RX, Huang JH, Bin Y, Chen WX, Cao XL. Association between the PPP1R3B polymorphisms and serum lipid traits, the risk of coronary artery disease and ischemic stroke in a southern Chinese Han population. *Nutr Metab (Lond)* 2018;15:27.
- 5) Tin A, Balakrishnan P, Beaty TH, Boerwinkle E, Hoogveen RC, Young JH, et al. GCKR and PPP1R3B identified as genome-wide significant loci for plasma lactate: the atherosclerosis risk in communities (ARIC) study. *Diabet Med* 2016;33:968–975.
- 6) Speliotes EK, Yerges-Armstrong LM, Wu J, Hernaez R, Kim LJ, Palmer CD, et al.; NASH CRN; GIANT Consortium; MAGIC Investigators. Genome-wide association analysis identifies variants associated with nonalcoholic fatty liver disease that have distinct effects on metabolic traits. *PLoS Genet* 2011;7:e1001324.
- 7) Stender S, Smagris E, Lauridsen BK, Kofoed KF, Nordestgaard BG, Tybjaerg-Hansen A, et al. Relationship between genetic variation at PPP1R3B and levels of liver glycogen and triglyceride. *Hepatology* 2018;67:2182–2195.
- 8) Doherty MJ, Cadefau J, Stalmans W, Bollen M, Cohen PT. Loss of the hepatic glycogen-binding subunit (GL) of protein phosphatase 1 underlies deficient glycogen synthesis in insulin-dependent diabetic rats and in adrenalectomized starved rats. *Biochem J* 1998;333(Pt. 2):253–257.
- 9) Liu Y, Bastly N, Whitcher B, Bell JD, van Bruggen N, Thomas EL, et al. Systematic quantification of health parameters from UK Biobank abdominal MRI using deep learning. *bioRxiv* 2020. <https://doi.org/10.1101/2020.07.14.187070>.
- 10) Ijssennagger N, Janssen AWF, Milona A, Ramos Pittol JM, Hollman DAA, Mokry M, et al. Gene expression profiling in human precision cut liver slices in response to the FXR agonist obeticholic acid. *J Hepatol* 2016;64:1158–1166.
- 11) Chiang JY. Bile acid metabolism and signaling. *Compr Physiol* 2013;3:1191–1212.
- 12) Jiao N, Baker SS, Chapa-Rodriguez A, Liu W, Nugent CA, Tsompana M, et al. Suppressed hepatic bile acid signalling despite elevated production of primary and secondary bile acids in NAFLD. *Gut* 2018;67:1881–1891.
- 13) Haeusler RA, Camastra S, Nannipieri M, Astiarraga B, Castro-Perez J, Xie D, et al. Increased bile acid synthesis and impaired bile acid transport in human obesity. *J Clin Endocrinol Metab* 2016;101:1935–1944.
- 14) Männistö VT, Simonen M, Soininen P, Tiainen M, Kangas AJ, Kaminska D, et al. Lipoprotein subclass metabolism in non-alcoholic steatohepatitis. *J Lipid Res* 2014;55:2676–2684.
- 15) Pihlajamäki J, Grönlund S, Simonen M, Käkälä P, Moilanen L, Pääkkönen M, et al. Cholesterol absorption decreases after Roux-en-Y gastric bypass but not after gastric banding. *Metabolism* 2010;59:866–872.
- 16) Xiang X, Han Y, Neuvonen M, Laitila J, Neuvonen PJ, Niemi M. High performance liquid chromatography–tandem mass spectrometry for the determination of bile acid concentrations in human plasma. *J Chromatogr B Analyt Technol Biomed Life Sci* 2010;878:51–60.
- 17) Aronson SJ, Bakker RS, Shi X, Duijst S, Ten Bloemendaal L, de Waart DR, et al. Liver-directed gene therapy results in long-term correction of progressive familial intrahepatic cholestasis type 3 in mice. *J Hepatol* 2019;71:153–162.

- 18) Carey MC. Critical tables for calculating the cholesterol saturation of native bile. *J Lipid Res* 1978;19:945-955.
- 19) Brunt EM, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. *Am J Gastroenterol* 1999;94:2467-2474.
- 20) Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, et al.; Nonalcoholic Steatohepatitis Clinical Research Network. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 2005;41:1313-1321.
- 21) Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, et al. STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* 2013;29:15-21.
- 22) Liao Y, Smyth GK, Shi W. The R package rsubread is easier, faster, cheaper and better for alignment and quantification of RNA sequencing reads. *Nucleic Acids Res* 2019;47:e47.
- 23) McCarthy DJ, Chen Y, Smyth GK. Differential expression analysis of multifactor RNA-seq experiments with respect to biological variation. *Nucleic Acids Res* 2012;40:4288-4297.
- 24) Broad Institute. Picard. <http://Broadinstitute.github.io/picard/>. Accessed December 2019.
- 25) Shabalin AA. Matrix eQTL: ultra fast eQTL analysis via large matrix operations. *Bioinformatics* 2012;28:1353-1358.
- 26) Liao Y, Wang J, Jaehnig EJ, Shi Z, Zhang B. WebGestalt 2019: gene set analysis toolkit with revamped UIs and APIs. *Nucleic Acids Res* 2019;47:W199-W205.
- 27) Galman C, Arvidsson I, Angelin B, Rudling M. Monitoring hepatic cholesterol 7 α -hydroxylase activity by assay of the stable bile acid intermediate 7 α -hydroxy-4-cholesten-3-one in peripheral blood. *J Lipid Res* 2003;44:859-866.
- 28) Grundberg E, Small KS, Hedman AK, Nica AC, Buil A, Keildson S, et al.; Multiple Tissue Human Expression Resource (MuTHER) Consortium. Mapping cis- and trans-regulatory effects across multiple tissues in twins. *Nat Genet* 2012;44:1084-1089.
- 29) Kullak-Ublick GA, Stieger B, Meier PJ. Enterohepatic bile salt transporters in normal physiology and liver disease. *Gastroenterology* 2004;126:322-342.
- 30) Dongiovanni P, Meroni M, Mancina RM, Baselli G, Rametta R, Pelusi S, et al. Protein phosphatase 1 regulatory subunit 3B gene variation protects against hepatic fat accumulation and fibrosis in individuals at high risk of nonalcoholic fatty liver disease. *Hepatology* 2018;67:666-675.
- 31) Anstee QM, Darlay R, Cockell S, Meroni M, Govaere O, Tiniakos D, et al.; EPoS Consortium Investigators. Genome-wide association study of non-alcoholic fatty liver and steatohepatitis in a histologically characterised cohort. *J Hepatol* 2020;73:505-515.
- 32) **Shapiro H, Kolodziejczyk AA, Halstuch D**, Elinav E. Bile acids in glucose metabolism in health and disease. *J Exp Med* 2018;215:383-396.
- 33) He L, Vasiliou K, Nebert DW. Analysis and update of the human solute carrier (SLC) gene superfamily. *Hum Genomics* 2009;3:195-206.
- 34) Renga B, Mencarelli A, Cipriani S, D'Amore C, Zampella A, Monti MC, et al. The nuclear receptor FXR regulates hepatic transport and metabolism of glutamine and glutamate. *Biochim Biophys Acta* 2011;1812:1522-1531.
- 35) Kim MK. Novel insight into the function of tankyrase. *Oncol Lett* 2018;16:6895-6902.
- 36) Thompson MD, Moghe A, Cornuet P, Marino R, Tian J, Wang P, et al. Beta-catenin regulation of farnesoid X receptor signaling and bile acid metabolism during murine cholestasis. *Hepatology* 2018;67:955-971.
- 37) Manning AK, Goustin AS, Kleinbrink EL, Thepsuwan P, Cai J, Ju D, et al. A long non-coding RNA, LOC157273, is an effector transcript at the chromosome 8p23.1-PPP1R3B metabolic traits and type 2 diabetes risk locus. *Front Genet* 2020;11:615.
- 38) Lake AD, Novak P, Shipkova P, Aranibar N, Robertson D, Reily MD, et al. Decreased hepatotoxic bile acid composition and altered synthesis in progressive human nonalcoholic fatty liver disease. *Toxicol Appl Pharmacol* 2013;268:132-140.
- 39) Woolbright BL, McGill MR, Staggs VS, Winefield RD, Gholami P, Olyae M, et al.; Acute Liver Failure Study Group. Glycodeloxycholic acid levels as prognostic biomarker in acetaminophen-induced acute liver failure patients. *Toxicol Sci* 2014;142:436-444.
- 40) Funabashi M, Grove TL, Wang M, Varma Y, McFadden ME, Brown LC, et al. A metabolic pathway for bile acid dehydroxylation by the gut microbiome. *Nature* 2020;582:566-570.
- 41) Ferkingstad E, Oddsson A, Gretarsdottir S, Benonisdottir S, Thorleifsson G, Deaton AM, et al. Genome-wide association meta-analysis yields 20 loci associated with gallstone disease. *Nat Commun* 2018;9:5101.

Author names in bold designate shared co-first authorship.

Supporting Information

Additional Supporting Information may be found at onlinelibrary.wiley.com/doi/10.1002/hep4.1630/supinfo.