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Genetic variation in the mitochondrial glycerol-3-phosphate acyltransferase is associated with liver injury

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Abstract

Most of the genetic basis of chronic liver disease remains undiscovered. To identify novel genetic loci that modulate the risk of liver injury, we performed genome-wide association studies (GWAS) on circulating levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and total bilirubin across 312,671 White British participants in

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the UK Biobank. We focused on variants associated with elevations in all four liver biochemistries at genome-wide significance ($P<5x10^{-8}$) and that replicated using Mass General Brigham Biobank in 19,323 European ancestry individuals. We identified a genetic locus in mitochondrial glycerol-3-phosphate acyltransferase (*GPAM* rs10787429) associated with increased levels of ALT ($P=1.4x10^{-30}$), AST ($P=3.6x10^{-10}$), ALP ($P=9.5x10^{-30}$) and total bilirubin ($P=2.9x10^{-12}$). This common genetic variant was also associated with an allele dose-dependent risk of alcoholic liver disease (OR 1.34, $P=2.6x10^{-5}$) and fatty liver disease (OR 1.18, $P=5.8x10^{-4}$) by ICD-10 codes. We identified significant interactions between *GPAM* rs10787429 and elevated body mass index in association with ALT and AST (P-interaction=$7.1x10^{-9}$ and $3.95x10^{-8}$, respectively), as well as between *GPAM* rs10787429 and weekly alcohol consumption in association with ALT, AST, and alcoholic liver disease (P-interaction=$4.0x10^{-2}$, $1.6x10^{-2}$ and $1.3x10^{-2}$, respectively). Unlike previously described genetic variants that are associated with an increased risk of liver injury but confer a protective effect on circulating lipids, *GPAM* rs10787429 was associated with an increase in total cholesterol ($P=2.0x10^{-17}$), LDL cholesterol ($P=2.0x10^{-10}$), and HDL cholesterol ($P=6.6x10^{-37}$). Single-cell RNA sequencing data demonstrated hepatocyte-predominant expression of *GPAM* in cells that co-express genes related to VLDL production ($P=9.4x10^{-103}$). In conclusion, genetic variation in *GPAM* is associated with susceptibility to liver injury. *GPAM* may represent a new therapeutic target in chronic liver disease.

Keywords

glycerol-3-phosphate acyltransferase; genomic analysis; precision medicine

Chronic liver disease leads to over 40,000 deaths annually in the United States and represents an area of substantial unmet medical need (1). While environmental factors play a role in the development of chronic liver disease, prospective twin studies have demonstrated that up to half of the observed variation in hepatic fibrosis and steatosis is attributable to genetic factors (2). Elucidation of the genetic underpinnings of chronic liver disease may reveal new targets for therapeutic intervention; drug development targets with human genetic evidence of disease association are more likely to lead to FDA-approved drugs (3).

Hepatocellular injury is characterized by elevations in serum alanine (ALT) and aspartate (AST) aminotransferases, while cholestatic liver injury is associated with elevated serum alkaline phosphatase (ALP) and total bilirubin levels. A powerful approach for understanding the molecular basis of liver disease has been to perform genome-wide association studies (GWAS) of levels of circulating liver enzymes across large population samples (4-13). Combined GWAS of ALT and AST have previously revealed sequence variations associated with liver disease, such as *PNPLA3* I148M (11) and *HSD17B13* rs72613567 (13); this has catalyzed the development of new therapeutics targeting these genes for the treatment of chronic liver disease (14). A more recent large trans-ethnic GWAS of ALT and AST identified a missense variant in the gene encoding manganese 28 efflux transporter SLC30A10 (rs188273166) associated with liver disease and extrahepatic cholangiocarcinoma (15).

A limitation of combined GWAS of liver transaminases is that elevations in ALT or AST may be caused by kidney, heart or muscle damage (16), and therefore only a subset of identified loci are likely to be causally implicated in liver damage. In addition, in contrast to other metabolic disorders, such as obesity or type two diabetes, where hundreds of genetic risk loci have been identified (17), few genetic associations have been identified to date for chronic liver disease. This highlights the need for studies with increased sample size and the exploration of novel liver-related endophenotypes.

To further study genetic variants that may confer susceptibility to liver injury, we performed GWAS on circulating levels of ALT, AST, ALP, and total bilirubin across 312,671 unrelated White British participants in UK Biobank, a large population-based study. We focused on variants associated with elevations in all four liver biochemistries at genome-wide significance ($P<5x10^{-8}$), hypothesizing that genetic variants associated with both elevated liver aminotransferases (ALT, AST) and elevated markers of cholestasis (ALP, total bilirubin) would be more likely to play a role in liver health. We replicated our findings in 19,323 unrelated European ancestry subjects from the Mass General Brigham Biobank, a hospital-based cohort. For variants that associated with elevated risk of liver injury across both cohorts, we also sought to determine whether the variants were associated with clinical liver disease phenotypes by ICD-10 codes, whether the variants demonstrated differential effect by other risk factors for liver disease (gene by environment interaction), and whether the variants were associated with circulating lipids. Finally, we analyzed single-cell RNA sequencing data from healthy human livers to determine the relative contribution of cell sub-populations to the expression of identified target genes.

EXPERIMENTAL PROCEDURES

Cohort descriptions and quality control

The UK Biobank is a population-based cohort consisting of 502,682 individuals (18). To minimize confounding due to population structure in our dataset, we restricted our analysis to include only subjects estimated to have British ancestry. We also excluded individuals with more genome-wide heterozygosity than expected, an excess of missing genotype calls, putative sex chromosome aneuploidy, and more than 10 third-degree relatives. We further removed at least one individual from each related pair with kinship coefficient >0.0625, giving preference to inclusion of patients with all-cause cirrhosis by ICD-10 codes. After quality control, 312,671 unrelated White British subjects were included in the analysis. We replicated our findings using 19,323 unrelated (kinship coefficient <0.0885) European ancestry samples from the Mass General Brigham Biobank, a hospital-based cohort of 43,534 individuals (19).

Phenotype data

In UK Biobank, blood biochemistry values were obtained for ALT (field 30620), AST (field 20650), ALP (field 30610), total bilirubin (field 30840), cholesterol (field 30690), LDL direct (field 30780), and HDL cholesterol (field 30760). In Mass General Brigham Biobank, blood biochemistry values for ALT, AST and ALP were obtained. We log-transformed ALT, AST, ALP and total bilirubin, resulting in approximately normal distributions. We defined

a severe alcoholic liver disease phenotype by combining the following ICD-10 codes: K702 (alcoholic fibrosis and sclerosis of liver), K703 (alcoholic cirrhosis of liver), and K704 (alcoholic hepatic failure). For the all-cause cirrhosis phenotype, we combined the severe alcoholic liver disease ICD-10 codes with K740 (hepatic fibrosis), K741 (hepatic sclerosis), K742 (hepatic fibrosis with hepatic sclerosis), K746 (other and unspecified cirrhosis of the liver), K766 (portal hypertension), I850 (bleeding esophageal varices), I859 (esophageal varices), K717 (toxic liver disease with fibrosis and cirrhosis of liver), K721 (chronic hepatic failure), K729 (hepatic failure, unspecified), and K767 (hepatorenal syndrome). We also investigated a phenotype for all-cause fatty liver by combining K700 (alcoholic fatty liver), K701 (alcoholic hepatitis), K709 (alcoholic liver disease, unspecified), K760 (fatty liver, not elsewhere classified), K758 (other specified inflammatory liver diseases), and K759 (inflammatory liver disease, unspecified).

Genotype data

In UK Biobank, genotyping was performed using either the UK BiLEVE Axiom array or the UK Biobank Axiom array, then imputed into the Haplotype Reference Consortium (HRC) and UK10K + 1000 Genomes panels. We used genotype data from the UK Biobank dataset release version 2 and the hg19 human genome reference for all analyses in this study. For Mass General Brigham Biobank, genotyping was performed using the Illumina MEGA array, QC steps were conducted consistent with prior studies (19), and imputation was performed to the HRC using the Michigan Imputation Server. We filtered out variants with minor allele frequency < 0.01 and imputation quality < 0.5. Content overlap between the UK Biobank and Mass General Brigham Biobank genotyping arrays was between 74.3% and 81.3% per chromosome.

Genome-wide association analyses in UK Biobank

We performed GWAS using linear regression for ALT, AST, ALP and total bilirubin, adjusting for age, sex, body mass index (BMI), total number of medications taken by each participant, genotyping batch, and the first 10 principal components of genetic ancestry. Association analyses were performed using linear regression in PLINK 2.0 alpha (20). Linkage disequilibrium score regression (LDSC) was used to estimate heritability and test statistic inflation due to confounding by population substructure (21). GWAS summary statistics were uploaded to the FUnctional Mapping and Annotation of GWAS tool (FUMA; version 1.3.5) (22). We used a panel of 10,000 randomly selected unrelated UK Biobank participants, release 2b, as the reference population for calculating linkage disequilibrium (LD). For each GWAS, we identified independent genomic risk loci defined by a genome-wide significance value of $P<5x10^{-8}$, an r^2 threshold of 0.2, and a maximum distance between LD blocks to merge into a locus of < 250 kb. To define shared association signals across the four phenotypes, we identified SNPs within these blocks with $r^2 > 0.8$ that were genome-wide significant and in common across the ALT, AST, ALP and total bilirubin GWAS. The lead independent SNPs that generated the common proxy SNP were identified from the ALT, AST, ALP and total bilirubin GWAS, and the SNP with the smallest p-value was kept. To correct for potential test statistic inflation, we also performed a sensitivity analysis where we inflated GWAS standard errors by the square root of the estimated intercept from the LD score regression.

Replication of top results from UK Biobank in the Mass General Brigham Biobank

We focused on variants associated with elevations in all four liver biochemistries in UK Biobank at genome-wide significance ($P<5x10^{-8}$) for replication in Mass General Brigham Biobank in 19,323 unrelated European ancestry individuals. We examined the association of these genetic variants with ALT, AST, and ALP using linear regression, assuming an additive model, and adjusting for age, sex, BMI, and first ten principal components of ancestry.

Association analyses for binary liver disease phenotypes and additional quantitative traits

For replicated variants, we investigated the association with binary liver disease phenotypes by ICD-10 codes in UK Biobank. We used R 3.6.3 to perform logistic regression. To account for case-control imbalance for binary liver disease phenotypes and lower allele frequencies, all logistic regression results were analyzed using the Firth penalized likelihood approach. We also examined the association of genetic variants with circulating lipids (total cholesterol, direct LDL, and HDL) using linear regression. We assumed an additive model for all association analyses and adjusted all models for age, sex, BMI, total number of medications taken by each participant, genotyping batch, and first ten principal components of ancestry.

Sensitivity analyses for alcohol use and type 2 diabetes in UK Biobank

Given the known important role of alcohol intake on liver injury, we performed a sensitivity analysis on variants that replicated in Mass General Brigham Biobank, using self-reported number of alcoholic drinks consumed as a covariate. We calculated the average units of alcohol consumed per week for each participant in UK Biobank, assuming 2 units (16g) of pure alcohol in a pint of beer/cider; 1.5 units (12g) in a glass of red wine, champagne, white wine, fortified wine, and “other” alcohol drink; and 1 unit (8g) in a measure of spirits. For participants who reported consuming alcohol monthly rather than weekly, we multiplied by 0.23 to convert monthly alcohol consumption to weekly. Weekly intake values greater than 6 standard deviations from the mean were excluded, reflective of greater than 92 alcoholic drinks per week. We also performed a sensitivity analysis adjusting for the presence of type 2 diabetes, which was defined as self-report of type 2 diabetes, followed by a verbal interview with a trained nurse to confirm the diagnosis, or hospitalization for diabetes (ICD code E11).

Assessing for gene-environment interactions in UK Biobank

For genetic variants associated with elevated risk of liver injury in both UK Biobank and Mass General Brigham Biobank, we investigated the combined effects of the genetic variant and BMI on biomarkers of hepatocellular injury. We performed an interaction analysis by modeling the main effects of the genetic variant and BMI, as well as an interaction term, using an additive model. BMI was entered as a continuous variable in all analyses. To depict the interaction between genotype and BMI visually, participants were divided into four categories of BMI: lean (<25 kg/m^2), overweight (25–30 kg/m^2), obese (30–35 kg/m^2) and very obese (>35 kg/m^2). We performed a similar analysis to assess the interaction between the genetic variant and weekly alcohol consumption on markers of hepatocellular

injury and alcoholic liver disease. We depicted the interaction between genotype and alcohol use by dividing participants into four categories: no alcohol use, low risk alcohol use (<21 units of alcohol/week for men, <14 units of alcohol/week for women), hazardous drinking (22-49 units of alcohol/week for men, 15-35 units of alcohol/week for women), and harmful drinking (>50 units of alcohol/week for men, >36 units of alcohol/week for women) (23).

Single-cell RNA sequencing

Merged single-cell RNA data from non-diseased human livers was acquired (24). We assessed the expression of target genes of interest in parenchymal and non-parenchymal liver cells, and in hepatocytes with or without a gene expression signature of VLDL production. This signature was defined by co-expression of more than three transcripts of *APOB* and *MTTP.* Expression of target genes of interest in VLDL-producing compared to non-VLDL producing hepatocytes was tested for statistical difference using Wilcoxon rank sum test. Data was analyzed using Seurat and R 3.6.3.

RESULTS

Genome-wide association study and replication results

We performed GWAS of 9.9 million genetic variants on circulating levels of ALT, AST, ALP and total bilirubin across 312,671 unrelated White British participants in UK Biobank. SNP-based heritability estimates (standard error) in our sample were 10.8% (0.8%) for ALT, 10.7% (1.0%) for AST, 19% (2.6%) for ALP, and 10.1% (3.2%) for total bilirubin. According to the estimated intercept (standard error) from a linkage disequilibrium (LD) score regression, some of the genomic inflation in test statistics for ALT, AST, and total bilirubin may be due to biases such as residual population stratification: 1.0774 (0.013) for ALT; 1.0688 (0.01) for AST; 1.14 (0.0257) for ALP, and 1.0489 (0.0154) for total bilirubin. We identified 204 distinct genomic risk loci associated with ALT, 201 associated with AST, 274 associated with ALP, and 99 associated with total bilirubin at $P<5x10^{-8}$. We next identified shared association signals across the four liver biomarkers, with traits having either the same index variant or a variant in strong linkage disequilibrium ($r^2 > 0.8$ with the index variant). This identified 58 distinct loci associated with both ALT and AST, 18 distinct loci associated with both ALP and total bilirubin, and 5 distinct loci associated with ALT, AST, ALP and total bilirubin (Figure 1, Figure 2, Table 1). Of these 5 loci, 2 of them, rs10787429 (closest gene, *GPAM*) and rs11601507 (closest gene, *TRIM5*), were associated with elevated levels across all four liver biochemistries. The other 3 genetic variants, such as the well-characterized locus at *SUGP1/TM6SF2* rs200210321, were highly associated with elevated ALT, AST and total bilirubin but were also associated with significantly decreased ALP (Table 1). We focused on the 2 identified variants associated with elevations across all four liver biochemistries, suggestive of a possible common pathway towards liver cholestasis and hepatocellular injury. We attempted to replicate the association of *GPAM* rs10787429 and *TRIM5* rs11601507 with elevated markers of liver injury in Mass General Brigham Biobank, a hospital-based cohort. *GPAM* rs10787429 was associated with log-transformed ALT ($P=2.7x10^{-3}$), AST ($P=3.3x10^{-2}$) and ALP ($P=1.9x10^{-2}$) in Mass General Brigham Biobank (Table 2). *TRIM5* rs11601507 was not significantly associated with liver injury in Mass General Brigham Biobank, perhaps due to insufficient statistical power (minor allele

frequency for *GPAM* rs10787429 0.234 compared to 0.06 for *TRIM5* rs11601507) (Table 2). As *GPAM* rs10787429 was the only risk locus associated with elevated ALT, AST, ALP and total bilirubin in UK Biobank that replicated in Mass General Brigham Biobank, we focused further analysis on this locus (Figure 3). In UK Biobank, the results for *GPAM* rs10787429 remained at genome-wide significance after inflating the standard errors by the square root of the LD score regression intercept for each biomarker of liver injury GWAS (Supplementary Table 1).

Association with ICD-10 based definitions of chronic liver disease in UK Biobank

We investigated the association of *GPAM* rs10787429 with broad categories of liver disease defined by ICD-10 codes in UK Biobank, including severe alcoholic liver disease (N=413), all-cause cirrhosis (N=1,493), and fatty liver disease (N=979). A Bonferroni adjusted significance level of P<0.017 was used for the three clinical liver disease phenotypes. *GPAM* rs10787429 was associated with increased risk of severe alcoholic liver disease (OR 1.34, $P=2.6x10^{-5}$) and fatty liver disease (OR 1.18, $P=5.8x10^{-4}$) in an allele dose-dependent manner; these odds were comparable to those of well-established genetic variants, including *PNPLA3* I148M and *TM6SF2* E167K (Figure 4). A nominally significant increased risk of all-cause cirrhosis was observed for *GPAM* rs10787429 (OR 1.09, $P=2.6x10^{-2}$) (Supplementary Figure 1).

Sensitivity analyses for alcohol use and type 2 diabetes in UK Biobank

Given the role of alcohol intake on liver damage, we performed a sensitivity analysis analyzing the association of *GPAM* rs10787429 with biomarkers of liver damage while accounting for weekly alcohol consumption. The results were consistent and remained at genome-wide significance across all biomarkers of liver injury, suggesting *GPAM* rs10787429 has impact on liver damage independent of alcohol consumption (Supplementary Table 2). The associations also remained significant for alcoholic liver disease and fatty liver disease phenotypes (Supplementary Figure 2, 3). The results adjusted for the presence of type 2 diabetes were similar (Supplementary Table 3; Supplementary Figure 4, 5).

Analysis of gene-environment interactions in UK Biobank

To determine if the effect of the *GPAM* rs10787429 variant on hepatocellular injury is modified by BMI, we analyzed the relationship between *GPAM* rs10787429 and ALT and AST after stratifying UK Biobank participants into four categories based on BMI. We performed linear regression modelling main effects of *GPAM* rs10787429 and BMI as well as an interaction term, assuming an additive model. Significant interactions were identified with elevated BMI and *GPAM* rs10787429 in association with ALT (P-interaction=$7.1x10^{-9}$) and AST (P-interaction=$4.0x10^{-8}$), suggesting a gene-environment interaction (Figure 5). We also performed a similar analysis dividing participants into four categories of alcohol use: no alcohol use, low risk use, hazardous drinking, and harmful drinking, according to UK guidelines (23). We demonstrated a nominally significant interaction between elevated weekly alcohol use and *GPAM* rs10787429 in association with ALT (P-interaction=$4.0x10^{-2}$) and AST (P-interaction=$1.6x10^{-2}$), using self-reported weekly alcohol use as a continuous variable (Supplementary Figure 6). There was also a

nominally significant interaction between alcohol consumption and *GPAM* rs10787429 in association with severe alcoholic liver disease (P-interaction=$1.3x10^{-2}$).

Association with circulating lipids in UK Biobank

The previously described genetic variants *PNPLA3* I148M and *TM6SF2* E167K increase the risk of liver injury but are also associated with decreased circulating lipids and cardiovascular protection (25). This raises the possibility that therapeutic strategies targeting these genes associated with liver disease may adversely impact cardiovascular risk. However, we found that the *GPAM* rs10787429 allele that associated with increased liver biochemistries also associated with increased total cholesterol ($P=2.0x10^{-17}$), LDL cholesterol ($P=2.0x10^{-10}$), and HDL cholesterol ($P=6.6x10^{-37}$) (Supplementary Table 4).

Single-cell RNA sequencing

Single-cell RNA sequencing from 28 healthy human livers (24) demonstrated hepatocyte-predominant expression of *GPAM* (Figure 6). An expression-based analysis revealed that *GPAM* was present in 48.5% of VLDL-producing cells and only 9.5% of VLDL-non-producing cells ($P=9.4x10^{-103}$) (Figure 6).

DISCUSSION

In this study, we performed GWAS on circulating levels of ALT, AST, ALP and total bilirubin in UK Biobank, a large population-based cohort. Our analysis of shared GWAS risk loci for ALT and AST, as well as the results of a recent trans-ethnic study in UK Biobank (15), confirm the key role in liver injury played by *PNPLA3* (11, 26), *TM6SF2* (27, 28), *SERPINA1* (29), *HSD17B13* (13), *SH2B3* (30), *PANX1* (7, 31), *EFNA1* (4, 32), *ERLIN1* (33), *AKNA* (7), *MTTP* (34), *ZNF827* (4, 7), and *EFHD1* (4, 7). Consistent with the hypothesis that loci associated with ALT or AST could be related to kidney or muscle damage, we identified *ANO5* rs7481951, for which homozygosity has been shown to lead to muscular dystrophy (35). Of the 18 shared genome-wide significant loci from our ALP and total bilirubin GWAS, we confirm several loci previously associated with liver injury at *TM6SF2* (27, 28), *HNF1A* (36), *ADH4* (37, 38), *PCCB* (7, 39), *CPS1* (7, 40), and *APOE* (12, 41).

We focused on variants associated with elevations across all four liver biochemistries and that also associated with liver injury in Mass General Brigham Biobank, a hospital-based cohort. This identified a novel genetic locus at the mitochondrial glycerol-3-phosphate acyltransferase (*GPAM* rs10787429). In addition to demonstrating allele dose-dependent effects across four biomarkers of liver injury, *GPAM* rs10787429 also associated with several categories of liver disease based on ICD-10 diagnostic codes, demonstrated an interaction with environmental risk factors for liver disease, and associated with elevated circulating lipids. Our study is the first to perform an analysis across all four biomarkers of liver injury, and complements recent reports that have associated variation in *GPAM* with quantified liver fat by abdominal MRI imaging in UK Biobank (12, 42), and with severity of histologic assessment of liver steatosis in European cohorts (12).

The genetic risk locus at *GPAM* shares several features in common with other variants previously linked to liver disease. First, genetic variants in *PNPLA3, TM6SF2*, and *HSD17B13* are strong genetic determinants of both fatty liver disease and alcohol-related liver cirrhosis (11, 13, 27, 28, 43). The overlap between the genetic determinants of these two diseases suggests that fatty liver, regardless of cause, is harmful and may progress to inflammation and fibrosis. Consistent with these results, we find that genetic variation in *GPAM* is associated with both fatty liver disease and severe alcohol-related liver cirrhosis in UK Biobank. Second, a unique feature of genetic variants associated with liver disease is a strong interaction with the environment. For example, a recent study demonstrated that adiposity amplifies the risk of fatty liver disease conferred by multiple loci: *PNPLA3* I148M, *TM6SF2* E167K and *GCKR* P446L (44). Similar to these results, we found that BMI as well as weekly alcohol use amplified the risk of hepatocellular injury associated with sequence variation at *GPAM* rs10787429. We suggest that certain genetic variants, in combination with an environmental trigger, such as high BMI or excess alcohol intake, act synergistically to uncover liver disease phenotypes.

The *PNPLA3* I148M and *TM6SF2* E167K alleles that increase the risk of cirrhosis have been reported to decrease circulating atherogenic lipid particles and are associated with lower risk of cardiovascular disease (25). This raises the possibility that therapeutic strategies targeting *PNPLA3* or *TM6SF2* may adversely impact cardiovascular risk. Interestingly, *GPAM* rs10787429 was associated with both increased risk of liver injury as well as elevated total cholesterol, LDL cholesterol, and HDL cholesterol. It is therefore plausible that therapeutic targeting of *GPAM* may protect against liver disease without adversely impacting the risk of cardiovascular disease, although further laboratory-based validation of this concept is required.

Glycerol-3-phosphate acyltransferase catalyzes the rate-limiting step in the de novo pathway of glycerolipid synthesis (45). Four isoforms of this enzyme have been identified; the mitochondrial glycerol-3-phosphate acyltransferase, encoded by a nuclear gene, is located in the outer mitochondrial membrane and is responsible for 30-50% of the total glycerol-3-phosphate acyltransferase activity in the liver (45). Consistent with these results, we demonstrate hepatocyte-predominant expression of *GPAM* in subclusters that overlap with cells involved in VLDL production. In addition to catalyzing the committed step in triacylglycerol synthesis, *GPAM* also directs the flux of fatty acids towards glycerolipid synthesis and away from β-oxidation (46). AMP-activated kinase, a sensor of cellular energy stores, has been shown to inhibit *GPAM*, thereby decreasing triacylglycerol synthesis and up-regulating β-oxidation when cellular energy stores are low (46).

Prior studies have linked *GPAM* to liver triglyceride content, body weight, and plasma lipid levels in murine overexpression and knockout experiments (46-48). Several small molecule inhibitors of glycerol-3-phosphate acyltransferase have been reported, including compounds with lower half maximal inhibitory concentration to *GPAM* (49). In diet-induced obese mice, pharmacologic inhibition of glycerol-3-phosphate acyltransferase reduced food intake, decreased body weight and adiposity, enhanced fatty acid oxidation, enhanced insulin sensitivity, and reversed hepatic steatosis (50). Together, these observations support further investigation of the role of *GPAM* as a therapeutic target in liver disease.

Our results should be interpreted in the context of several important limitations. Further research will be needed to confirm these results across multiple ethnicities. Among liver disease cases analyzed in this study, the presence of hepatitis B or hepatitis C was not systematically assessed. ICD-10 diagnostic codes are known to be imprecise in the context of clinical care; additional studies on the impact of the *GPAM* rs10787429 locus and other loci on biopsy-confirmed liver disease are warranted. We did not confirm associations with binary liver disease phenotypes or gene environment interactions in Mass General Brigham Biobank; lack of associations could be due to false positives or insufficient power, and validation in additional cohorts is warranted. Finally, there are multiple single nucleotide polymorphisms at the *GPAM* locus in high linkage disequilibrium; further studies will be necessary to uncover the causative variant and its functional significance.

In conclusion, we identified a common genetic variant in *GPAM* that is associated with susceptibility to liver injury and also associated with increased circulating lipid particles. The risk of liver injury may increase synergistically in the setting of higher BMI and increasing alcohol use. Our findings provide human genetic support for the role of *GPAM* in liver injury; therapeutic targeting of this enzyme should be explored as a potential approach for the treatment of liver disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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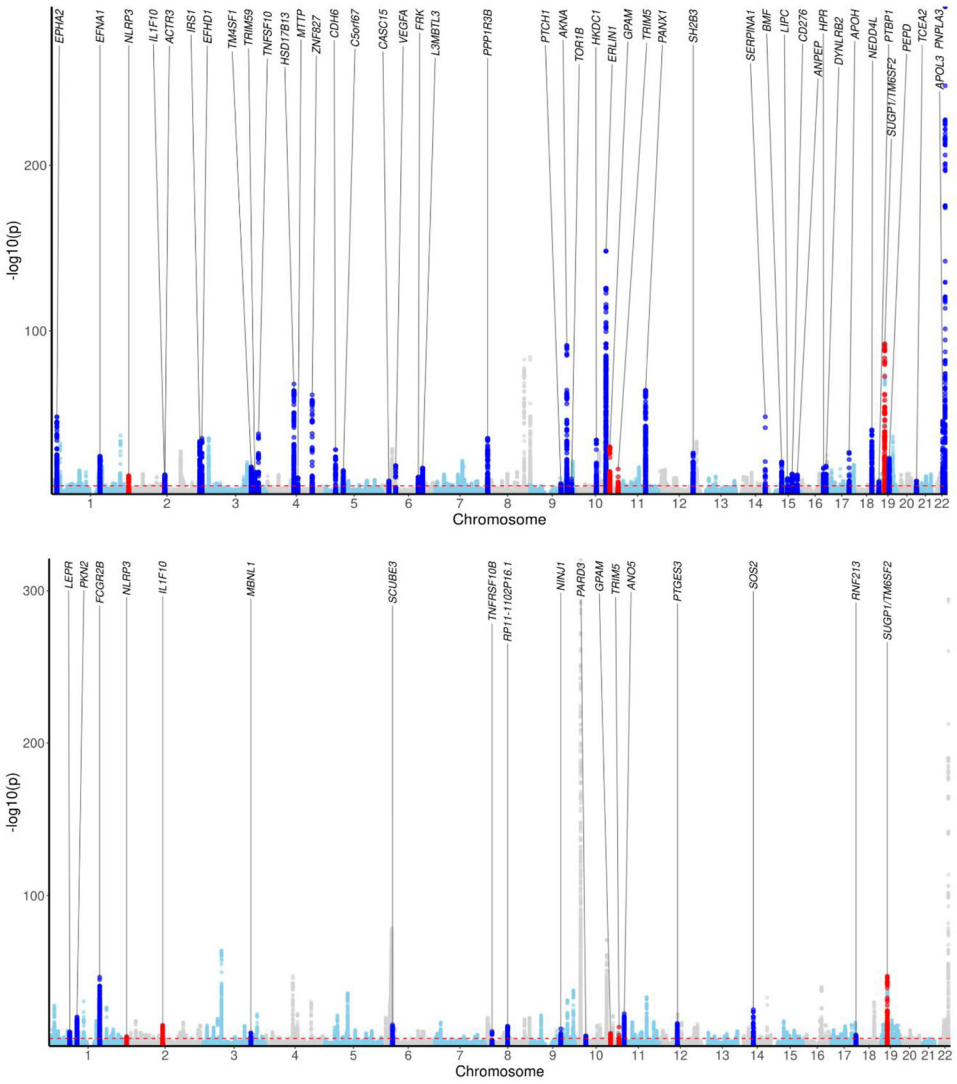

Figure 1:

Manhattan plots of GWAS results in 312,671 unrelated White British participants in UK Biobank for (A) ALT, (B) AST. Red dots indicate lead SNPs for shared signals across all four liver biochemistry GWAS. Dark blue dots (A, B) indicate lead SNPs for shared loci between ALT and AST GWAS; the shared signals are marked only once, on the plot for which the p-value for the association is smaller. Dashed line indicates $P<5x10^{-8}$.

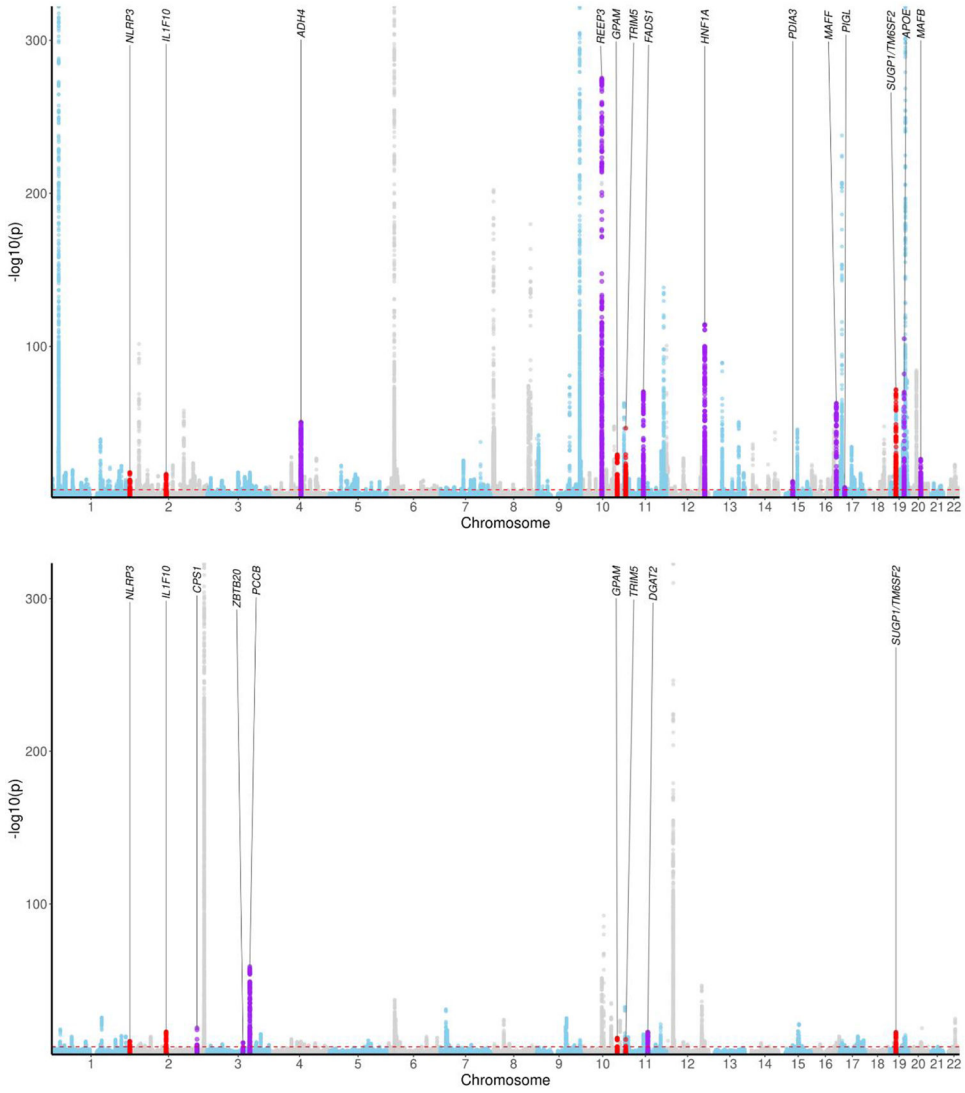

Figure 2:

Manhattan plots of GWAS results in 312,671 unrelated White British participants in UK Biobank for (A) ALP, (B) total bilirubin. Red dots indicate lead SNPs for shared signals across all four liver biochemistry GWAS. Purple dots (A, B) indicate lead SNPs for shared loci between ALP and total bilirubin GWAS; the shared signals are marked only once, on the plot for which the p-value for the association is smaller. Dashed line indicates $P<5x10^{-8}$.

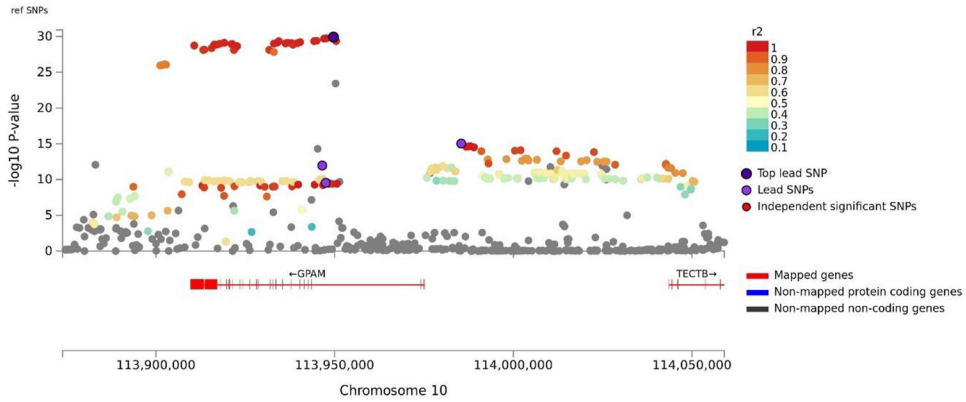

Figure 3:

Representative regional plot of GWAS p-values with genes and functional annotations of genomic risk loci on chromosome 10 for GWAS of ALT. Genes prioritized by FUMA are highlighted in red; SNPs are colored based on r^2.

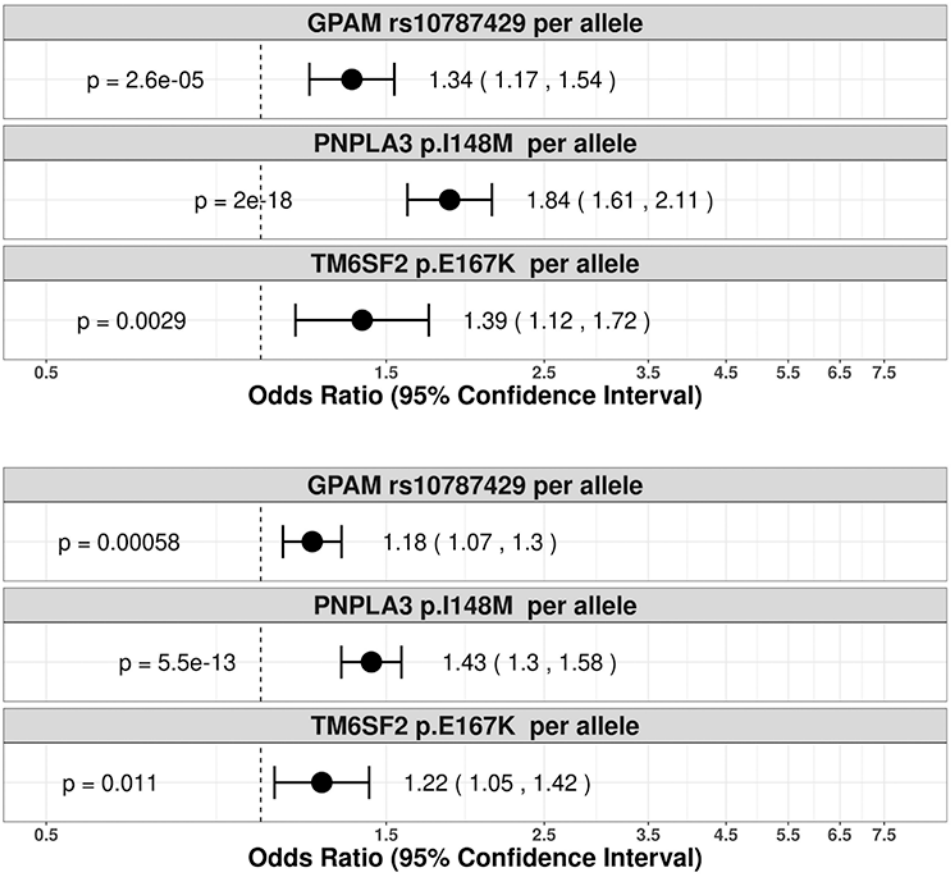

Figure 4:

Association of *GPAM* rs10787429, *PNPLA3* I148M, and *TM6SF2* E167K with (A) severe alcoholic liver disease and (B) all-cause fatty liver disease, by ICD-10 diagnostic codes in UK Biobank, using the Firth penalized likelihood approach. Odds ratios were calculated with the use of logistic regression, with adjustment for age, sex, BMI, total number of medications, genotyping batch, and first ten principal components of ancestry.

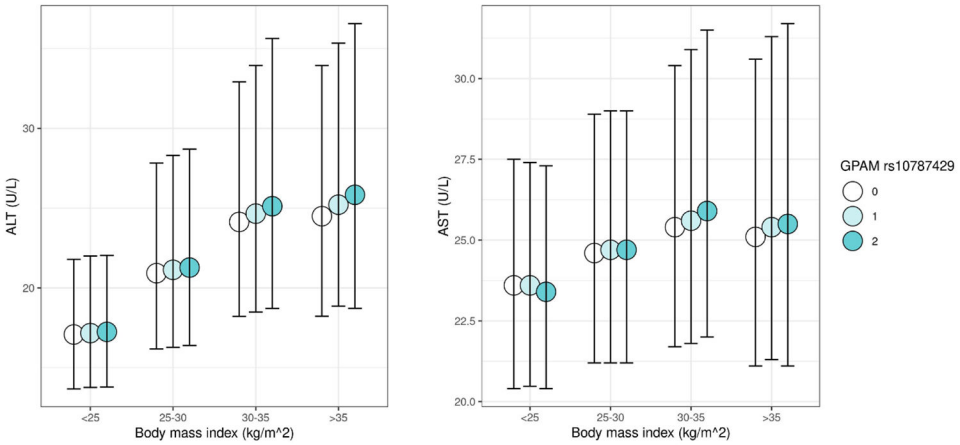

Figure 5:

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) by BMI and *GPAM* rs10787429 status in UK Biobank. Circles and bars depict medians the interquartile ranges, respectively. The ALT- and AST- increasing effect of the *GPAM* rs10787429 was amplified by increasing BMI (p for interaction rs10787429 x BMI on ALT = 7.1×10^{-9}; p for interaction rs10787429 x BMI on AST = 4.0×10^{-8}).

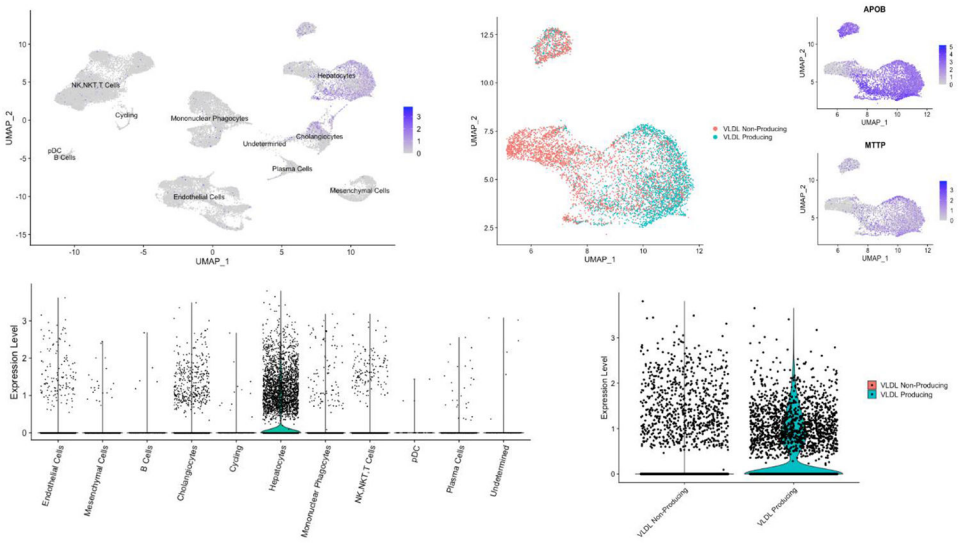

Figure 6:

Single-cell RNA sequencing reveals that *GPAM* is predominately expressed in hepatocytes and co-expresses with VLDL production-related genes. (A) *GPAM* expression in parenchymal and non-parenchymal liver cells shown in purple, (B) violin plot of *GPAM* expression by cell identity, (C) VLDL-producing hepatocytes are defined by co-expression of more than three transcripts of *APOB* and *MTTP*, (D) *GPAM* expression in VLDL-producing compared to non-VLDL producing hepatocytes. *GPAM* was present in 48.5% of VLDL-producing cells and only 9.5% of VLDL non-producing cells ($P=9.4x10^{-103}$).

Table 1:

Association signals shared across ALT, AST, ALP and total bilirubin GWAS studies, defined by having a lead SNP from each GWAS (ALT, AST, ALP, total bilirubin) either identical or with shared proxies with $r^2 > 0.8$. The lead SNP with the smallest p-value was kept. Linear regression was performed adjusting for age, sex, BMI, number of medications, genotyping batch, and the first ten principal components of ancestry. Biomarkers of liver injury are log-transformed.

Variant	Measure (U/L)	Estimate	Std. Error	P-value
GPAM rs10787429	ALT	$1.37e^{-02}$	$1.19e^{-03}$	$1.37e^{-30}$
[10:113949664:C:T]	AST	$4.84e^{-03}$	$7.73e^{-04}$	$3.59e^{-10}$
	ALP	$8.90e^{-03}$	$7.85e^{-04}$	$9.51e^{-30}$
	total bilirubin	$7.56e^{-03}$	$1.08e^{-03}$	$2.93e^{-12}$
Variant	**Measure (U/L)**	**Estimate**	**Std. Error**	**P-value**
TRIM5 rs11601507	ALT	$1.75e^{-02}$	$2.08e^{-03}$	$4.09e^{-17}$
[11:5701074:A:C]	AST	$1.03e^{-02}$	$1.35e^{-03}$	$1.96e^{-14}$
	ALP	$1.98e^{-02}$	$1.37e^{-03}$	$3.23e^{-47}$
	total bilirubin	$1.29e^{-02}$	$1.89e^{-03}$	$7.58e^{-12}$
Variant	**Measure (U/L)**	**Estimate**	**Std. Error**	**P-value**
NLRP3 rs56015600	ALT	$7.92e^{-03}$	$1.10e^{-03}$	$5.51e^{-13}$
[1:247602968:A:G]	AST	$3.55e^{-03}$	$7.12e^{-04}$	$6.33e^{-07}$
	ALP	$-6.29e^{-03}$	$7.24e^{-04}$	$3.75e^{-18}$
	total bilirubin	$6.37e^{-03}$	$9.98e^{-04}$	$1.68e^{-10}$
Variant	**Measure (U/L)**	**Estimate**	**Std. Error**	**P-value**
IL1F10 rs6743171	ALT	$-7.23e^{-03}$	$1.09e^{-03}$	$2.90e^{-11}$
[2:113840058:C:G]	AST	$-5.33e^{-03}$	$7.04e^{-04}$	$3.99e^{-14}$
	ALP	$6.01e^{-03}$	$7.16e^{-04}$	$4.74e^{-17}$
	total bilirubin	$-8.18e^{-03}$	$9.87e^{-04}$	$1.12e^{-16}$
Variant	**Measure (U/L)**	**Estimate**	**Std. Error**	**P-value**
SUGP1/TM6SF2 rs200210321	ALT	$4.11e^{-02}$	$2.04e^{-03}$	$2.31e^{-90}$
[19:19393890:A:AG]	AST	$1.89e^{-02}$	$1.32e^{-03}$	$1.93e^{-46}$
	ALP	$-2.38e^{-02}$	$1.34e^{-03}$	$1.11e^{-70}$
	total bilirubin	$1.47e^{-02}$	$1.85e^{-03}$	$2.12e^{-15}$

Table 2:

Association of genetic variants of interest with log-transformed biomarkers of liver injury in Mass General Brigham Biobank (n = 19,323). Variants were selected based on their association associated with elevated ALT, AST, ALP and total bilirubin in UK Biobank. Linear regression was performed adjusting for age, sex, BMI, batch, and the first ten principal components of ancestry.

Variant	Measure (U/L)	Estimate	Std. Error	P-value
GPAM rs10787429	ALT	$2.28e^{-02}$	$7.60e^{-03}$	$2.73e^{-03}$
[10:113949664:C:T]	AST	$1.19e^{-02}$	$5.59e^{-03}$	$3.31e^{-02}$
	ALP	$1.32e^{-02}$	$5.61e^{-03}$	$1.87e^{-02}$
Variant	**Measure (U/L)**	**Estimate**	**Std. Error**	**P-value**
TRIM5 rs11601507	ALT	$1.64e^{-02}$	$1.31e^{-02}$	$2.10e^{-01}$
[11:5701074:A:C]	AST	$7.54e^{-03}$	$9.63e^{-03}$	$4.33e^{-01}$
	ALP	$1.85e^{-02}$	$9.66e^{-03}$	$5.57e^{-02}$