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17-Beta Hydroxysteroid Dehydrogenase 13 Is a Hepatic Retinol Dehydrogenase Associated With Histological Features of Nonalcoholic Fatty Liver Disease

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Nonalcoholic fatty liver disease (NAFLD) is a common cause of chronic liver disease. A single-nucleotide polymorphism (SNP), rs6834314, was associated with serum liver enzymes in the general population, presumably reflecting liver fat or injury. We studied rs6834314 and its nearest gene, 17-beta hydroxysteroid dehydrogenase 13 (*HSD17B13*), to identify associations with histological features of NAFLD and to characterize the functional role of HSD17B13 in NAFLD pathogenesis. The minor allele of rs6834314 was significantly associated with increased steatosis but decreased inflammation, ballooning, Mallory-Denk bodies, and liver enzyme levels in 768 adult Caucasians with biopsy-proven NAFLD and with cirrhosis in the general population. We found two plausible causative variants in the *HSD17B13* gene. rs72613567, a splice-site SNP in high linkage with rs6834314 ($r^2 = 0.94$) generates splice variants and shows a similar pattern of association with NAFLD histology. Its minor allele generates simultaneous expression of exon 6-skipping and G-nucleotide insertion variants. Another SNP, rs62305723 (encoding a P260S mutation), is significantly associated with decreased ballooning and inflammation. Hepatic expression of *HSD17B13* is 5.9-fold higher ($P = 0.003$) in patients with NAFLD. HSD17B13 is targeted to lipid droplets, requiring the conserved amino acid 22-28 sequence and amino acid 71-106 region. The protein has retinol dehydrogenase (RDH) activity, with enzymatic activity dependent on lipid droplet targeting and cofactor binding site. The exon 6 deletion, G insertion, and naturally occurring P260S mutation all confer loss of enzymatic activity. *Conclusion:* We demonstrate the association of variants in *HSD17B13* with specific features of NAFLD histology and identify the enzyme as a lipid droplet–associated RDH; our data suggest that HSD17B13 plays a role in NAFLD through its enzymatic activity. (Hepatology 2019;69:1504-1519).

The rise of nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH) has sparked considerable interest in pathogenic mechanisms of the disease. Racial and ethnic variability and a known heritable component[1,2] hinted at a genetic contribution. Genome-wide association studies (GWAS) identified loci associated with NAFLD, most predominantly rs738409, a single-nucleotide polymorphism (SNP) in the patatin-like phospholipase domain-containing protein 3 (*PNPLA3*) gene.[3-5] Subsequently, we and others[6-8] demonstrated that rs738409 is strongly

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CI, confidence interval; GFP, green fluorescent protein; GWAS, genome-wide association study; HSD17B13, 17-beta hydroxysteroid dehydrogenase 13; indel, insertion/deletion; LD, linkage disequilibrium; MGI, Michigan Genomics Initiative; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NASH CRN, NASH Clinical Research Network; OR, odds ratio; PNPLA3, patatin-like phospholipase domain-containing protein 3; RA, retinoic acid; RDH, retinol dehydrogenase; SDR16C, short-chain dehydrogenase/reductase 16C; SNP, single-nucleotide polymorphism; TM6SF2, transmembrane 6 superfamily member 2.

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associated with histological features and severity of NAFLD. Although the association is highly significant statistically, the effect size with regard to liver fat accumulation[9] is quite weak. Similarly, the major allele of the transmembrane 6 superfamily member 2 (*TM6SF2*) SNP rs58542926 is associated with decreased liver fat[10] but, conversely, with higher serum lipid levels and increased risk of atherosclerosis and cardiovascular disease.[11] *In vivo* and *in vitro* studies provided evidence that TM6SF2 regulates very low-density lipoprotein secretion and acts as a determinant of liver damage or cardiovascular disease under metabolic stress.[10-14] Of other genes associated with NAFLD by GWAS, only a few (such as membrane-bound O-acyltransferase domain containing 7 [*MBOAT7*][15-17]) were studied in large histologically characterized NAFLD cohorts, and in even fewer has their mechanism been elucidated.

A recent large-scale GWAS identified loci associated with plasma liver enzyme levels.[18] In unselected population surveys, elevated transaminases occur concomitantly with metabolic syndrome risk factors[19,20] and are presumed to reflect hepatic fat accumulation and related injury. However, a direct association between these SNPs and NAFLD has not yet been shown. We hypothesized that plasma transaminase activity in the GWAS reflected the presence of fatty liver and associated injury and that genetic associations with transaminases could identify genes involved in NAFLD pathogenesis. Our goals were to establish which of these described SNPs is associated with histological features of NAFLD, identify the genes associated with these SNPs, and elucidate the mechanism by which these genes affect NAFLD.

In this work we demonstrate that SNPs in or near 17-beta hydroxysteroid dehydrogenase 13 (*HSD17B13*)

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are associated with histological features of NAFLD, identify HSD17B13 as a retinol dehydrogenase (RDH), and characterize the impact of genetic variants on its enzymatic activity. Our study provides a link between genetic variants, retinoid metabolism, and NAFLD pathogenesis and identifies HSD17B13 as a potentially druggable target in NAFLD.

Patients and Methods

STUDY DESIGN

We initially genotyped in a cohort of subjects with biopsy-proven NAFLD 38 SNPs described by Chambers et al.[18] to identify loci associated with histological features. After identifying a locus of interest, we genotyped additional SNPs in the candidate gene locus in the same cohort and confirmed associations in additional cohorts. Finally, we performed functional analyses to characterize the gene product and identify the functional impact of gene variants (Supporting Fig. S1).

STUDY POPULATIONS

The NAFLD cohort has been described in detail.[6] Briefly, we included patients from the NASH Clinical Research Network (NASH CRN) observational database[21] and PIVENS[22] studies, as well as patients with biopsy-proven NAFLD seen at the National Institutes of Health Clinical Center. Analysis was limited to Caucasians aged ≥18 years at the time of liver biopsy. All subjects had histological evidence of NAFLD or NASH on liver biopsy. Liver histology was scored semiquantitatively using the NASH CRN scoring system.[23]

Confirmatory analyses were performed in a cohort of patients with chronic hepatitis C and available liver histology and in two population-based cohorts—the Michigan Genomics Initiative (MGI) and the UK Biobank.

HEPATIC GENE EXPRESSION

Deidentified normal and NASH human liver samples were obtained from the Liver Tissue Cell Distribution System and from clinical trials at the National Institutes of Health Clinical Center. Gene expression was quantified by real-time quantitative PCR.

INTRACELLULAR LOCALIZATION

Cells were stained with primary antibodies against organelle markers, LipidTox Red (Life Technologies, Carlsbad, CA) for lipid droplets, and Hoechst 33342 for nuclei. Staining was visualized using a confocal microscope.

RDH ACTIVITY

RDH activity was tested in a cell-based assay as described.[24] Briefly, HEK293 cells were transfected with protein expression vectors and treated with all-trans-retinol for 8 hours, followed by retinaldehyde and retinoic acid (RA) quantification by high-pressure liquid chromatography (HPLC) and protein quantification by western blot.

STATISTICAL ANALYSIS

Associations between genotypes and histology were determined by a two-step method. Univariate analysis was performed using the Jonkheere-Terpstra test for ordered differences. SNPs with a univariate significance level <0.1 were selected for multivariate ordinal regression, with age, body mass index (BMI), gender, and type 2 diabetes as covariates. We used the Benjamini-Hochberg[25] false discovery rate to correct for multiple comparisons, using the calculator by Pike,[26] with $q < 0.05$ considered significant. *P* values are reported from the multivariate ordinal regression. Odds ratios (ORs) of associations with histology were calculated using binary logistic regression. An additive inheritance model was used. The Student *t* test was used for two-group differences, and one-way analysis of variance followed by Dunnett's multiple comparison test were used for more than two groups in the *in vitro* enzymatic assays. All significance testing was two-sided. Statistical analyses were performed using SPSS Statistics V.19 (IBM) and Prism V.7 (GraphPad).

ETHICS

Human subject data were obtained from studies conducted according to the Declaration of Helsinki principles that were reviewed by institutional review boards. All subjects gave written informed consent prior to inclusion.

Additional details can be found in the Supporting Information.

Results

We obtained DNA from 768 adult Caucasians with biopsy-proven NAFLD (mean age, 49; mean BMI, 34.6 kg/m^2; 28.5% diabetic). Histologically, 59.5% had NASH and 12.1% had cirrhosis (Supporting Table S1). We genotyped in these subjects 38 SNPs described by Chambers et al.[18] (Supporting Table S2). The associations between SNPs and histological features of NAFLD are detailed in Supporting Table S3. Multivariate ordinal logistic regression adjusting for age, gender, diabetes, and BMI found nine SNPs to be significantly associated with steatosis grade, four SNPs associated with degree of ballooning degeneration, and two SNPs associated with Mallory-Denk bodies. No SNP was associated with severity of inflammation or fibrosis. Associations were independent of the *PNPLA3* rs738409 genotype (Supporting Table S3). Of the SNPs associated with NAFLD histology, only rs6834314 was also associated with serum transaminases in patients with NAFLD (Supporting Table S4) and thus was the focus of subsequent study.

rs6834314 IS ASSOCIATED WITH NAFLD HISTOLOGY

rs6834314 is located on chromosome 4, 11 kb downstream of the *HSD17B13* gene. Each copy of the minor G allele increased the risk of having liver fat content ≥33% (OR, 1.32; confidence interval [CI], 1.02-1.70, Fig. 1A; Supporting Fig. S2A). The mean steatosis grade in subjects with rs6834314G/G genotype was 0.2 units higher than in those with A/A (1.88 ± 0.87 versus 1.68 ± 0.89), an effect similar to the impact of the *PNPLA3* rs738409 in the same subjects (0.21 units; 1.59 ± 0.84 for rs738409C/C versus 1.80 ± 0.89 for G/G).[6] In contrast to its association with increased liver fat, the minor G allele was significantly associated with lower risks for inflammation (OR, 0.77 for total inflammatory score ≥3, CI 0.60-0.99), significant ballooning (OR, 0.67 for ballooning score >1, CI 0.51-0.87), and the presence of Mallory-Denk bodies (OR, 0.68; CI, 0.51-0.91) and showed a nonsignificant trend for reduced fibrosis (OR, 0.79; CI, 0.60-1.05) on binary logistic regression. The associations with inflammation, ballooning, and Mallory-Denk bodies were independent of the association with steatosis and, in fact, were stronger when steatosis grade was included in the regression model (Supporting Table S5). The minor allele of rs6834314 was also associated with lower serum transaminases and gamma-glutamyltransferase (Fig. 1B). NAFLD has been associated with genetic variants in *PNPLA3* (rs738409), *TM6SF2* (rs58542926), *MBOAT7* (rs626283), and glucokinase regulator (rs1260326). After adjusting for these variants, rs6834314 remained significantly associated with steatosis ($P = 0.013$), ballooning ($P = 0.031$), and Mallory-Denk bodies ($P = 0.030$) and trended for association with inflammation ($P = 0.067$; Supporting Table S6). rs6834314 genotype did not differ between patients with NASH and those with steatosis only.

To validate the association of rs6834314 with steatosis, we genotyped it in an independent sample of 317 Caucasian patients with chronic hepatitis C and available liver histology (Supporting Table S7). Liver fat was present in 179 (57.2%) subjects. As with the NAFLD cohort, rs6834314 genotype was significantly associated with the degree of steatosis in univariate ($P = 0.032$, Jonkheere-Terpstra test) and multivariate ($P = 0.028$, ordinal regression adjusted for BMI, age, and gender) analyses. Each copy of the minor G allele was significantly associated with the presence of liver fat (OR, 1.67; CI, 1.11-2.51; $P = 0.015$; Fig. 1C; Supporting Fig. S2B). As expected, rs6834314 genotype was not associated with hepatitis C virus–driven inflammation or fibrosis.

As further confirmation, we determined the association of rs6834314 with liver enzymes and diagnoses in the MGI cohort (Supporting Table S8). rs6834314-G was associated with lower alanine aminotransferase (ALT) ($P = 3.3 \times 10^{-4}$) and aspartate aminotransferase (AST) ($P = 5.1 \times 10^{-5}$) and with a lower risk of cirrhosis diagnosis (OR, 0.79; $P = 7.5 \times 10^{-4}$; Supporting Fig. S2C). The MGI cohort is population-based, and histological data are not available for detailed analyses.

OTHER VARIANTS IN *HSD17B13* ARE ASSOCIATED WITH NAFLD

rs6834314 is downstream of *HSD17B13* and in high linkage disequilibrium (LD) with multiple adjacent SNPs, overlapping parts of the *HSD17B13* gene. To

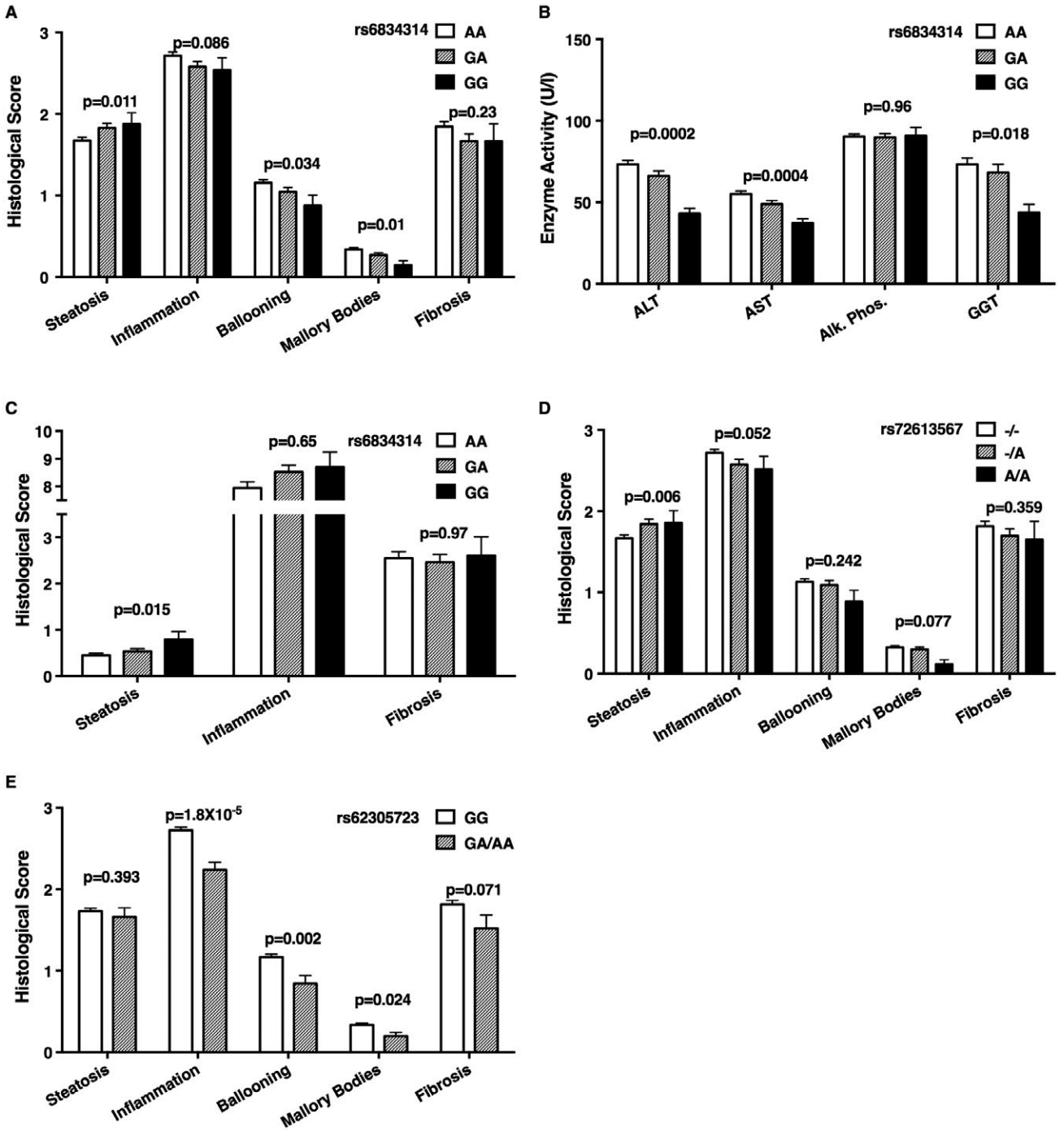

FIG. 1. *HSD17B13*-related SNPs are associated with liver disease. Association of rs6834314 genotype with (A) liver histology scores and (B) blood levels of liver enzymes in subjects with NAFLD (AA, n = 485; GA, n = 226; GG, n = 41). (C) Association of rs6834314 genotype with liver histology scores in subjects with chronic hepatitis C (AA, n = 165; GA, n = 125; GG, n = 23). (D) Association of rs72613567 genotype with liver histology scores in subjects with NAFLD. (–/–, n = 503; –/A, n = 220; A/A, n = 35). (E) Association of rs62305723 genotype with liver histology scores in subjects with NAFLD (GG, n = 615; GA/AA, n = 76). Mean ± SEM. *P* values for histology from multivariate ordinal regression adjusted for age, gender, and BMI. *P* values for enzymes from linear regression of log-transformed enzyme activity, adjusted for age, gender, and BMI. Abbreviations: Alk. Phos., alkaline phosphatase; GGT, gamma-glutamyltransferase.

determine whether the associations of rs6834314 reflect a biological effect of *HSD17B13*, we selectively genotyped additional variants in the gene region, including two nonsynonymous coding SNPs, an insertion/deletion (indel) polymorphism in a splice site, and a frameshift coding SNP, as well as seven SNPs that capture most of the common variants in the gene region (Table 1). The tag SNP, rs6834314, is in strong LD ($D' = 0.995$, $r^2 = 0.93$) with the indel rs72613567, located immediately adjacent to the 3′ end of exon 6 (Supporting Fig. S3). As expected, this indel had a similar pattern of association with NAFLD histology and liver enzymes as rs6834314 (Fig. 1D). The minor allele, rs72613567-A, was associated with increased steatosis (OR, 1.36; CI, 1.04-1.77) but with decreased inflammation (OR, 0.74; CI, 0.57-0.96) and trended toward decreased ballooning (OR, 0.78; CI, 0.60-1.03), Mallory-Denk bodies (OR, 0.77; CI, 0.57-1.03), and fibrosis (OR, 0.77; CI, 0.58-1.03; Supporting Fig. S4A).

rs72613567 was not genotyped in the MGI cohort but was genotyped in the UK Biobank cohort (Supporting Table S9). The minor allele A insertion was significantly less common in the NAFLD cohort (19.1%) compared to the population-based UK Biobank (27.9%, $P < 0.001$) and was associated with a reduction in the risk of fibrosis and cirrhosis (OR, 0.83; $P = 9 \times 10^{-4}$; Supporting Fig. S4B). rs72613567-A was also associated with lower risk of diagnoses of liver disease (OR, 0.93; $P = 8 \times 10^{-4}$), other inflammatory liver diseases (a diagnostic code that includes NASH; OR, 0.82; $P = 0.0012$), and alcoholic liver disease (OR, 0.87; $P = 0.015$). Histological data or liver enzymes are not available for this cohort.

Another SNP, the nonsynonymous coding rs62305723G>A in exon 6, encodes a P260S substitution and is not in LD with rs6834314 ($r^2 = 0.02$). The rs62305723 minor A allele is significantly associated with decreased inflammation (OR, 0.46; CI, 0.28-0.74), ballooning (OR, 0.48; CI, 0.30-0.76), and Mallory-Denk bodies (OR, 0.51; CI, 0.28-0.91) but, in contrast to rs72613567/rs6834314, is not associated with steatosis (Fig. 1E; Supporting Fig. S5A). The minor A allele was associated with a lower risk of NASH compared to steatosis (OR, 0.39; CI, 0.23-0.68; $P = 0.001$). In the UK Biobank cohort, rs62305723 was not associated with liver-related diagnoses. Interestingly, the minor A allele was associated with a diagnosis of blindness or low vision (OR, 1.3; $P = 0.007$; Supporting Fig. S5B).

TABLE 1. *P* Values for Association of SNPs in the *HSD17B13* Gene With Histological Features and ALT in the NAFLD Cohort: Arrows Denote Direction of Effect for the Minor Allele

SNP	SNP Type	Alleles	Minor Allele Frequency*	Genotype Frequency*	R^2 versus rs6834314 Genotype†	Fat‡	Inflammation‡	Ballooning‡	Mallory-Denk Bodies‡	Fibrosis‡	ALT§
rs72613567	Splice site	–/A	0.194	0.66/0.29/0.05	0.938	0.008¶↑	0.063↓	0.202	0.062↓	0.373	4.2×10^{4}¶↓
rs62305723	Nonsynonymous coding	G/A	0.056	0.89/0.11/0.003	0.022	0.326	5×10^{4}¶↓	0.002¶↓	0.025↓	0.065↓	0.217
rs10433937	Noncoding	T/G	0.199	0.65/0.29/0.05	0.979	0.019¶↑	0.062↓	0.042↓	0.013↓	0.323	2.3×10^{4}¶↓
rs6531975	Noncoding	G/A	0.202	0.64/0.32/0.05	0.019	0.016¶↓	0.098	0.728	0.641	0.392	0.744
rs10022237	Noncoding	C/T	0.428	0.33/0.49/0.18	0.403	0.004¶↑	0.021↓	0.05↓	0.100	0.090	0.033↓
rs6531973	Noncoding	C/A	0.238	0.57/0.39/0.05	0.091	0.535	0.621	0.853	0.321	0.402	0.207
rs6531972	Noncoding	A/C	0.404	0.35/0.49/0.16	0.171	0.067	0.648	0.532	0.993	0.603	0.333
rs7692397	Noncoding	C/A	0.241	0.58/0.36/0.06	0.104	0.074	0.068	0.257	0.231	0.227	0.215
rs9647458‖	Noncoding	A/G	0.096	0.82/0.18/0.008	0.035	0.646	0.696	0.137	0.472	0.661	0.579

*Allele and genotype frequencies from the NAFLD cohort.
†R^2 for linkage between SNPs and rs6834314 from LDLink, limited to populations of European ancestry.
‡Multivariate ordinal logistic regression adjusted for age, BMI, gender, and diabetes.
§Linear regression of log-transformed enzyme activity adjusted for age, BMI, gender, and diabetes.
‖rs76926692 (nonsynonymous coding) and rs75049937 (frameshift) were genotyped but omitted for minor allele frequency ≤0.3%.
¶False discovery rate q value < 0.05.

Conditional analysis found rs62305723 and rs72613567 to associate with NAFLD histology independently of each other (Supporting Table S10), suggesting that both are causal variants. Including *PNPLA3* rs738409 in the model did not impact significance (Supporting Table S10), and the association of HSD17B13 SNPs with histology and ALT was seen for all rs738409 genotypes (Supporting Fig. S6). We found no significant interaction between rs738409 and *HSD17B13* SNPs, although our study was not powered to detect such an effect. Two additional noncoding SNPs (rs6531975, rs10022237), neither in LD with rs6834314, were associated with histological features; and rs10022237 was also associated with ALT. Detailed mapping supports a functional role for *HSD17B13* in the pathogenesis of NAFLD.

HEPATIC *HSD17B13* EXPRESSION IS ELEVATED IN NASH PATIENTS

To determine whether *HSD17B13* is expressed differentially in NAFLD, we quantified its expression in human NASH (n = 43) or normal (n = 14) liver samples. Hepatic expression of *HSD17B13* was 5.9-fold higher in NASH patients compared with controls ($P = 0.003$; Fig. 2A).

In the GTEx database, rs6834314 genotype did not affect hepatic *HSD17B13* expression (Supporting Fig. S7A; $P = 0.6$) and neither did rs72613567 and rs62305723 (data not shown). We confirmed this in a pooled analysis of our liver tissue samples (Fig. 2B). Because *HSD17B13* expression is up-regulated in NAFLD, we examined whether rs6834314 modulates gene expression only in these subjects but found no significant impact of genotype on hepatic *HSD17B13* expression in either NASH (Supporting Fig. S7B) or normal (Supporting Fig. S7C) liver, although nearly all high-expression samples had A/A genotype. To confirm that rs6834314 does not affect *HSD17B13* expression in hepatocytes, as liver tissue samples contain other cell types, we quantified *HSD17B13* mRNA in primary human hepatocytes from 22 donors and genotyped them for rs6834314. No donor was homozygous for the minor G allele, and there was no difference in *HSD17B13* expression between A/A and A/G genotypes (Supporting Fig. S7D). Thus, hepatic *HSD17B13* expression is up-regulated in NAFLD, but the association between rs6834314 genotype and NAFLD phenotype is not due to changes in expression levels and more likely reflects a change in protein function.

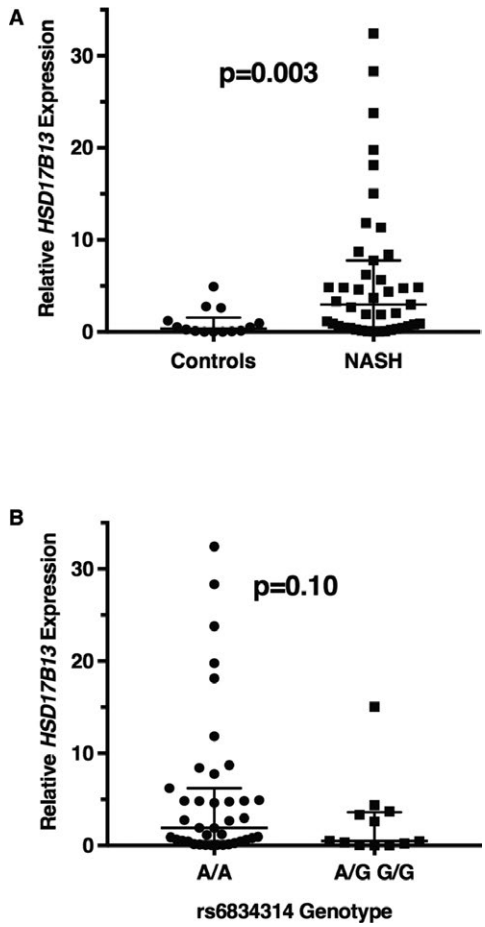

FIG. 2. Hepatic expression of *HSD17B13*. (A) Expression levels of *HSD17B13* by real-time quantitative PCR in liver samples from healthy controls (n = 14) and NASH patients (n = 43). (B) Expression levels of *HSD17B13* by real-time quantitative PCR according to rs6834314 genotype in liver samples from control and NASH patients (A/A, n = 45; A/G or G/G, n = 12). rs6834314A/G and G/G were pooled together due to lower frequency. Lines denote median and interquartile range and are normalized to controls. The Mann-Whitney test was used for significance testing.

HSD17B13 LOCALIZES TO LIPID DROPLETS

To better understand the function of HSD17B13, we determined its subcellular localization by transfecting *HSD17B13*–green fluorescent protein (GFP) into HepG2 cells and staining for cellular organelle markers. HSD17B13-GFP colocalizes with the lipid

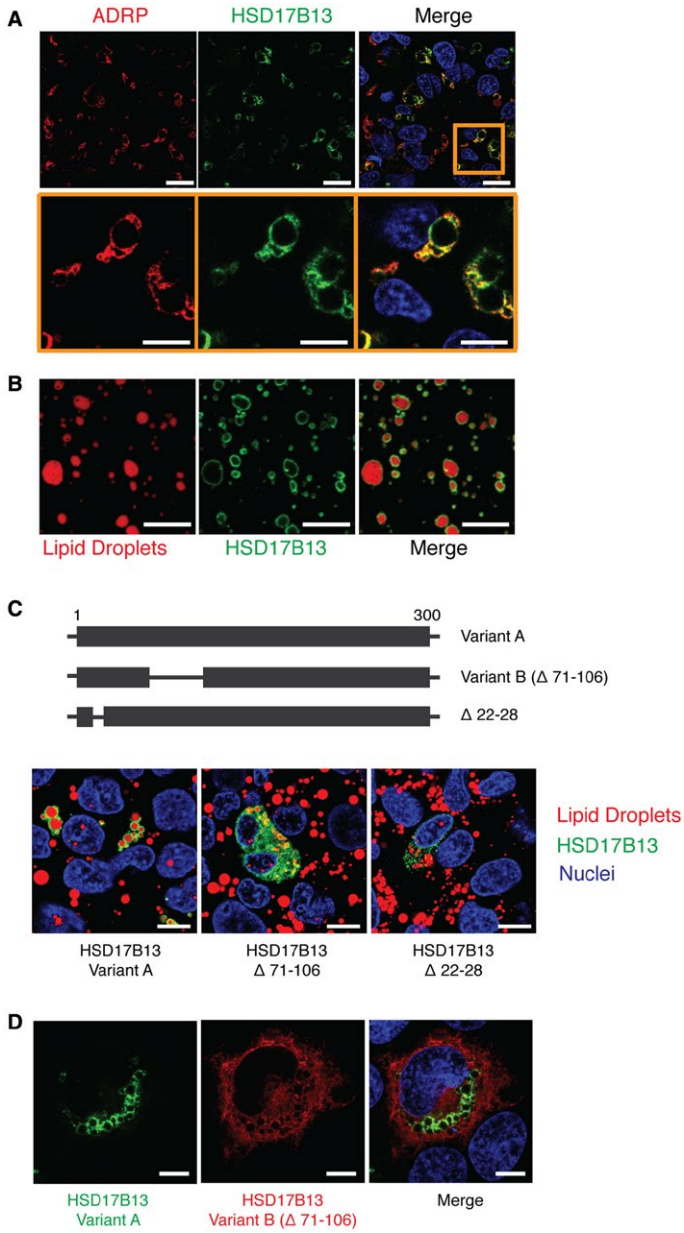

FIG. 3. HSD17B13 localizes with lipid droplets. (A) HepG2 cells were transfected with HSD17B13(A)-GFP and stained for the lipid droplet marker protein ADRP (perilipin-2). (B) Lipid droplets were isolated from HSD17B13(A)-GFP-transfected cells and stained with the lipid droplet–specific dye LipidTox. (C) Mutations were generated and tested for cellular localization with LipidTox, which was used to stain lipid droplets. (D) HSD17B13(A)-GFP and HSD17B13(B)-Flag were cotransfected into HEK293 cells. Immunofluorescence staining was used to visualize HSD17B13(B)-Flag. Nuclei were counterstained with Hoechst 33342. One representative image from repeated experiments is shown. Bars, 10 μm.

droplets (Fig. 3A) and is detected on the surface of purified droplets from transfected cells (Fig. 3B) but does not colocalize with other organelles (Supporting Fig. S8A).

There are two described transcript variants for the human *HSD17B13* gene, formed by alternative splicing: variant A contains all seven exons, while variant B is missing exon 2 (Supporting Fig. S3), resulting in deletion of amino acids 71-106 (Fig. 3C). Transfected HSD17B13(B)-GFP localized to the cytoplasm but not to lipid droplets (Fig. 3C,D). By site-directed mutagenesis, we identified the N-terminal amino acids

22-28 of HSD17B13 as essential for lipid droplet targeting (Fig. 3C). This seven–amino acid sequence is conserved within adipose differentiation–related protein (ADRP) and tail-interacting protein 47 kDa (perilipin-3), which are also lipid droplet–associated proteins[27] (Supporting Fig. S8B), and appears to be crucial for protein stability as its deletion results in low protein expression.

HSD17B13 DOES NOT AFFECT HEPATOCYTE LIPID CONTENT

To assess the functional role of HSD17B13 in the liver, we investigated whether its expression level affects fat content of HepG2 cells incubated with a high concentration of fatty acids. Expression of HSD17B13 protein was dose-dependently induced by doxycycline using the Tet-on inducible expression system (Supporting Fig. S9A) with no effect on lipid content (Supporting Fig. S9B,C). Similarly, stable HSD17B13 overexpression or knockout in HepG2 cells did not affect their lipid content (Supporting Fig. S9D,E), suggesting that HSD17B13 does not regulate hepatocyte lipid content in a direct manner.

HSD17B13 HAS RDH ACTIVITY *IN VITRO*

HSD17B13 is a member of the 17β hydroxysteroid dehydrogenase (HSD) family, 14 structurally related enzymes implicated in steroid and fatty acid metabolism[28]; the substrate of HSD17B13 is currently unknown. Given the association of *HSD17B13* with NAFLD, we hypothesized that its functional role is due to its enzymatic activity and that SNPs modulate that activity. Beyond its similarity to the 17β-HSD family, HSD17B13 is structurally similar to other members of the short-chain dehydrogenase/reductase 16C family (SDR16C),[29] which are known to play a critical role in retinoid metabolism through their RDH enzymatic activity, catalyzing the oxidation of retinol to retinaldehyde, the rate-limiting step in all-trans-RA biosynthesis (Fig. 4A). Cells transfected with HSD17B13(A) (variant A) demonstrated RDH activity comparable to that of the positive control RDH10[30] (Fig. 4B-D). In contrast, HSD17B13(B) had no RDH activity (Fig. 4E-G). HSD17B13-Δ22-28, which does not target lipid droplets, also lacked RDH activity, although its expression was markedly lower than that of variants A and B, possibly because of low protein stability (Supporting Fig. S10). Thus, HSD17B13 demonstrates *in vitro* RDH activity that requires lipid droplet targeting for its stability and enzymatic function.

HSD17B13 contains the conserved TGXXXGXG NAD(P)(H) cofactor binding site[31] common to SDRs.[32] We generated a mutation within this motif (HSD17B13-G47/G49A), which resulted in loss of RDH activity (Fig. 4H-J), confirming the need for cofactor binding for enzymatic activity.

THE IMPACT OF GENETIC VARIANTS IN *HSD17B13* ON ENZYMATIC ACTIVITY

The nonsynonymous coding SNP rs62305723G>A encodes a P260S substitution. P260 is highly conserved in vertebrate HSD17B13 orthologs and in other SDR16C family members (Fig. 5A), even though it is outside the predicted catalytic or cofactor binding sites, suggesting its importance for protein function. We generated and tested this mutant form for enzymatic activity. Although the mutant protein HSD17B13(A)-P260S retains lipid droplet localization (Fig. 5B) similarly to wild-type protein, it lacks RDH activity (Fig. 5C-E). Thus, the minor allele of rs62305723 is generating a loss-of-function mutation that is associated with decreased hepatic inflammation and injury in patients with NAFLD.

The indel rs72613567 was associated with histological and serological features of hepatic fat and injury (Table 1). The indel is located in the 5′ end of the intron between exons 6 and 7, with the minor allele (A insertion) modifying a conserved splice donor sequence (GTAAGT to GTAAAGT). To determine whether this SNP affects splicing, we amplified and sequenced the surrounding regions from DNA and RNA of 36 human liver samples. We identified two *HSD17B13* splice variants—one with a single G-nucleotide insertion (G insertion) between exons 6 and 7, causing a frameshift and premature stop codon, and another with exon 6 skipping (delta-6) (Fig. 6A). Both the delta-6 and the G-insertion variants retain localization to lipid droplets (Fig. 6B) but have no RDH activity (Fig. 6C) and were only found

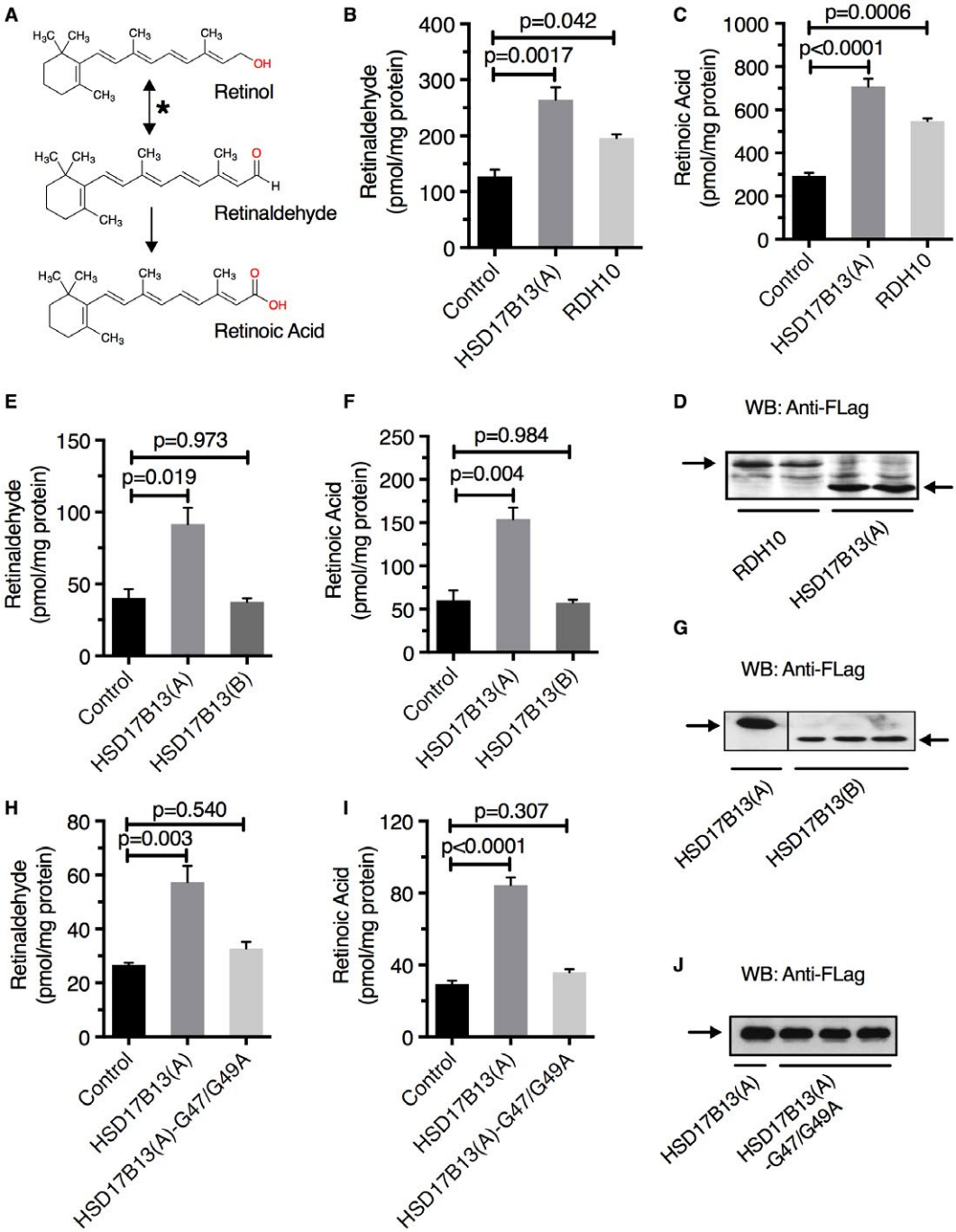

FIG. 4. HSD17B13 is an RDH. (A) Oxidation of retinol to retinaldehyde by RDHs (asterisk) is the rate-limiting step of RA synthesis. HEK293 cells transfected with HSD17B13(A), empty vector, or RDH10 as positive control were treated with retinol (5 μM), and levels of synthesized retinal (B) and RA (C) were measured by HPLC. Transfection of HSD17B13(B) variant does not lead to increased retinaldehyde (E) or RA **(F)** synthesis. Cofactor binding site mutation protein HSD17B13(A)-G47/49A has no RDH activity (H,I) in HEK293 cells treated with 2 μM retinol. Anti-Flag western blot used to determine protein transfection efficiency in each experiment (D,G,J). Data are presented as mean ± SEM. Abbreviation: WB, western blot.

in subjects with the minor rs72613567A allele (Fig. 6D,E), who, as expected, also carried the rs6834314 minor G allele (Supporting Fig. S11A,B). Delta-6 was expressed simultaneously with exon 6–containing variants at a relatively fixed ratio (Supporting Fig. S11C,D).

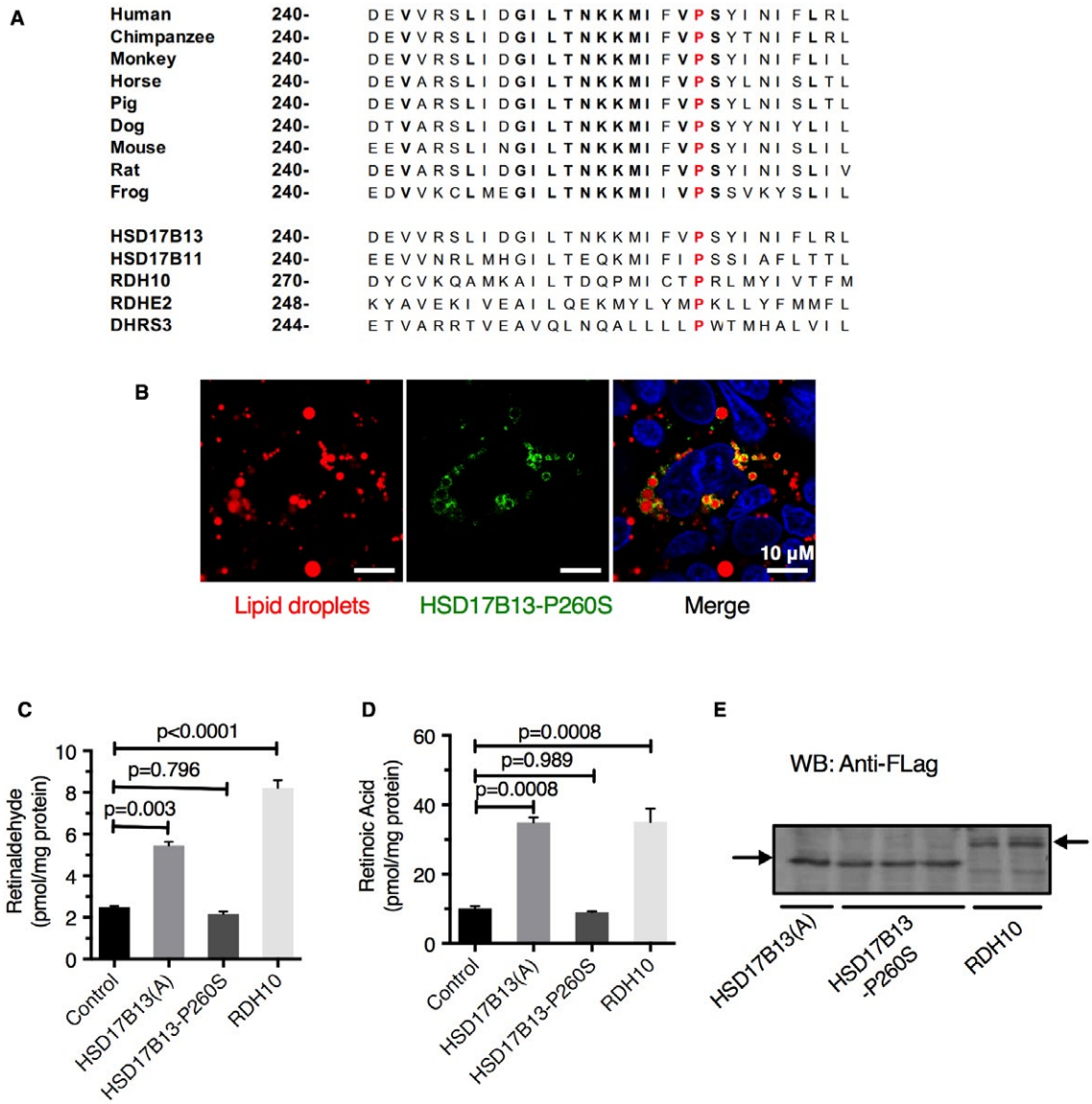

FIG. 5. The P260S mutation in HSD17B13 (rs62305723G>A) is associated with loss of RDH activity despite lipid droplet targeting. (A) The P260S substitution is located within a highly conserved region of HSD17B13 orthologs and human homologs. (B) In HepG2 cells transfected with HSD17B13-P260S-GFP, the mutant protein, similar to the wild-type protein, can be detected in lipid droplets. Transfection of HEK293 cells with HSD17B13-P260S-Flag does not lead to increased retinaldehyde (C) or RA (D) synthesis in the presence of 2 μM retinol despite adequate transfection efficiency (E). Data are presented as mean ± SEM. Abbreviation: WB, western blot.

Discussion

In otherwise normal subjects, elevated plasma liver enzymes are often considered a surrogate for the presence of liver fat but can also reflect other liver processes, derive from nonhepatic sources, or reflect variability in the genes for the enzymes themselves. Chambers et al.[18] found at the population level several genetic variants that associate with liver enzyme levels. We aimed to identify which of these associations actually reflects fatty liver or injury by assessing the association of the variants with histological features of NAFLD. We identified several SNPs that associated with NAFLD features and by focusing on one of them, rs6834314, identified HSD17B13 as a lipid droplet enzyme associated with the disease.

Other 17β-HSDs act as molecular switches, modulating the activation of steroid hormone receptors

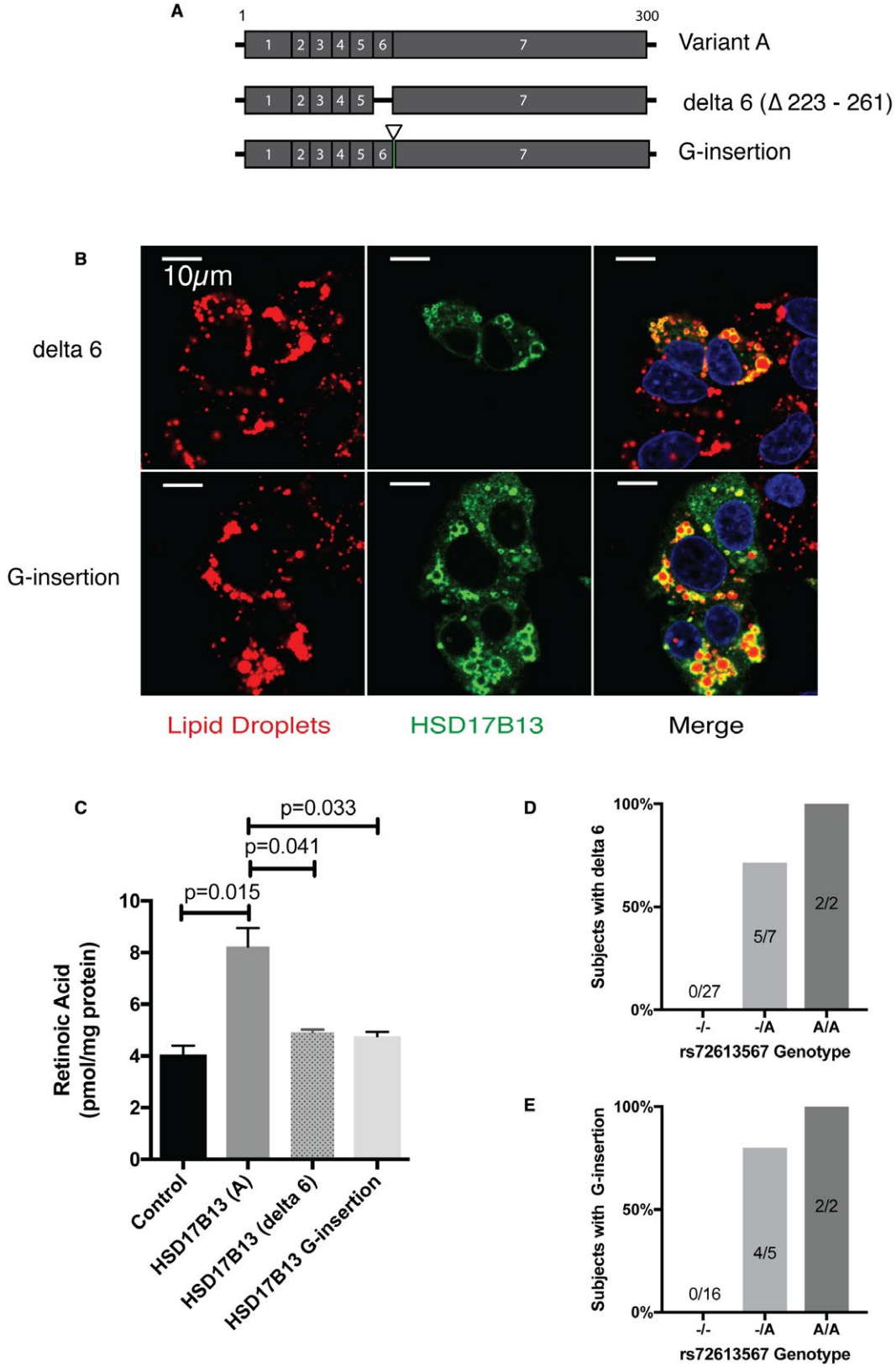
A
1
300
1 2 3 4 5 6 7
Variant A
1 2 3 4 5 7
delta 6 (Δ 223 - 261)
1 2 3 4 5 6 7
G-insertion
B
10μm
delta 6
G-insertion
Lipid Droplets
HSD17B13
Merge
C
p=0.033
p=0.041
p=0.015
Retinoic Acid (pmol/mg protein)
10
8
6
4
2
0
Control
HSD17B13 (A)
HSD17B13 (delta 6)
HSD17B13 G-insertion
D
Subjects with delta 6
100%
50%
0%
0/27
5/7
2/2
-/-
-/A
A/A
rs72613567 Genotype
E
Subjects with G-insertion
100%
50%
0%
0/16
4/5
2/2
-/-
-/A
A/A
rs72613567 Genotype

FIG. 6. HSD17B13 delta 6 and G-insertion variants localize with lipid droplets, lose RDH activity, and are associated with rs72613567. (A) Schema of HSD17B13 variants A, delta 6, and G insertion. (B) In HepG2 cells transfected with HSD17B13-delta 6-Flag or HSD17B13-G insertion-Flag, the mutant proteins can be detected in lipid droplets stained by LipidTox. (C) Transfection of HEK293 cells with HSD17B13-delta 6 or HSD17B13-G insertion does not lead to increased RA levels. The delta 6 (D) and G-insertion (E) variants are only detected in subjects who carry the rs72613567 minor A allele.

in target tissues.[28] *In vivo*, some 17β-HSDs may have targets other than steroid hormones, such as prostaglandins, bile acids, and cholesterol; and some have been implicated in fatty acid metabolism.[33] Compared to other family members that are restricted to gonads or ubiquitously expressed, HSD17B13 is predominantly expressed in the liver, and the protein is targeted from the endoplasmic reticulum to lipid droplets by its N-terminal region.[27,31,34] We confirmed HSD17B13's lipid droplet localization and identified a conserved seven–amino acid sequence (amino acids 22-28) that is crucial for lipid droplet targeting. Interestingly, a naturally occurring splice variant, *HSD17B13(B)*, that lacks exon 2 is not targeted to lipid droplets, despite the presence of amino acids 22-28. In overexpression experiments using equal amounts of plasmid, the Δ22-28 variant and *HSD17B13(B)* demonstrate lower protein levels than wild type, suggesting that proper targeting is crucial for protein stability and protection from degradation; a similar phenotype was described for TM6SF2 variants.[10]

We found *HSD17B13* to be highly expressed in NAFLD patients, consistent with a recent report.[34] However, its specific target and physiological function have not been defined, and neither has its role in NAFLD pathogenesis. We establish that HSD17B13 has RDH activity and that its enzymatic activity depends on lipid droplet targeting and cofactor binding. HSD17B13 clearly has RDH activity *in vitro*, and this assay can be used to assess the impact of genetic variations on enzymatic function. Although *in vivo* the enzyme may have other substrates in addition to retinol, retinol is still a plausible *in vivo* substrate. Retinoids are stored as retinyl esters in the lipid droplets of hepatic stellate cells that, when activated, play critical roles in hepatic fibrogenesis. There is significant evidence that vitamin A metabolites (retinaldehyde and RA) and retinol binding protein (RBP4) are associated with the pathogenesis of hepatic steatosis, fibrosis, adipogenesis, and insulin resistance.[35-40] Enzymes involved in retinol metabolism are upregulated in hepatic lipid droplets in NAFLD.[34] Furthermore, PNPLA3, strongly associated with NAFLD,[3] acts as a retinyl-palmitate lipase to control serum retinol and the RBP4 level in NAFLD patients and to modulate stellate cell fibrogenesis.[41-44] This supports the critical role retinoid metabolism plays in NAFLD progression. The association of the P260S substitution with a diagnosis of blindness or low vision further supports a role for HSD17B13 in retinoid metabolism. In addition, the putative substrate binding sites (L153/L156, K208, L199/E202), homodimer interaction sites, and the P260 residue found in this study are all crucial for enzymatic activity and are conserved within RDH10, DHRS3, and HSD17B11 but not with other 17β-HSDs (Supporting Fig. S12). In addition, a recent study in mice heterozygous for Rdh10 knockout found increased steatosis on a high-fat diet despite only a modest decrease in hepatic RA.[45] This suggests unique substrate specificity and is consistent with retinoids being the substrate for HSD17B13. Ongoing *in vivo* experiments are aimed at confirming these findings. Hepatic HSD17B13 expression appears limited to hepatocytes, with no detectable expression in quiescent or activated stellate cells (data not shown). Whether HSD17B13 acts directly on the hepatocyte through modulation of hepatocyte RA synthesis or indirectly through a paracrine or autocrine effect remains to be shown. Our data suggest that HSD17B13 is a hitherto undescribed hepatic RDH and that its effect on NAFLD pathogenesis relates to its enzymatic activity and possibly to its impact on retinoid homeostasis. Further characterization of the enzymatic activity of HSD17B13 *in vitro* and *in vivo* can help clarify its role. The increased levels of HSD17B13 in NAFLD patients may reflect a separate pathological process in the multifactorial pathogenesis of NAFLD.

The genetic association of variants in *HSD17B13* with features of NAFLD is complex, with different SNPs associated with different phenotypic patterns. We identified two different and independent SNPs that result in loss-of-function variants and reduction in NAFLD-associated injury and inflammation and are likely causal variants. The rs62305723 SNP

encodes a P260S mutation that abolishes the RDH activity *in vitro*. Conservation of P260 among vertebrates in the *HSD17B13* gene and in the SDR16C family indicates that this site is essential for protein function. A second SNP, rs72613567, generates two simultaneously expressed splice variants that are also devoid of enzymatic function and associated with decreased injury. This SNP is in high LD with the tag SNP, rs6834314, and likely explains the associations found with it, as well as the association with ALT reported by Chambers et al.[18] and Xu et al.,[46] especially because we demonstrate that rs6834314 is not an expression quantitative trait locus. Association with fibrosis stage, the major determinant of prognosis in NASH, was weaker; but rs6834314 and rs72613567 are associated with cirrhosis in the MGI and UK Biobank cohorts, respectively, consistent with their impact on liver injury. Interestingly, both rs62305723 and rs72613567 are located in or near exon 6 of the gene, away from the conserved catalytic and cofactor binding motifs of the 17β-HSD family.[28] Overall, our data support a role for HSD17B13 in mediating NAFLD-associated injury through its enzymatic activity.

Some variants in *HSD17B13* were associated with histological degree of steatosis, including rs6834314, its associated splice SNP rs72613567, and other independent SNPs. The association of rs6834314 with steatosis was confirmed in an independent cohort of patients with chronic hepatitis C. The alleles that associate with increased hepatic fat are those that associate with decreased injury, and the association with injury was independent of the association with steatosis. However, the loss-of-function P260S variant is not associated with steatosis. This suggests that the association with hepatic fat may be unrelated to the enzymatic activity and could be driven by another mechanism. This is also supported by our *in vitro* data, where knocking out or overexpressing *HSD17B13* in hepatoma cells does not affect their fat-storage capacity. Consistent with our data, Abul-Husn et al.[47] reported that HSD17B13 overexpression does not change cellular lipid content. In contrast, Adam et al.[48] recently found that Hsd17b13 knockout mice showed hepatic steatosis only at an older age, indicating that HSD17B13 might affect hepatic fat metabolism *in vivo*; the underlying mechanism is yet to be explored.

In a proteomic analysis of hepatic lipid droplets, Su et al.[34] recently identified up-regulation of HSD17B13 in subjects with NAFLD, consistent with our results. They demonstrated an increase in lipid droplet size in cells overexpressing *HSD17B13* tagged with GFP; we obtained similar results but were not able to replicate them when transfecting with *HSD17B13* with a smaller tag (Flag epitope), suggesting that the large GFP tag on lipid droplet–associated HSD17B13 causes nonspecific lipid aggregation (data not shown). *HSD17B13* expression appears to be regulated by liver X receptor and sterol regulatory element binding protein 1c,[49] which can explain our finding of its up-regulation in patients with NASH and is consistent with a functional role in NAFLD pathogenesis. Additional evidence supporting a role for *HSD17B13* in lipid metabolism comes from a recent GWAS[50] in which rare variants in *HSD17B13* were found to impact the effect of fenofibrate on plasma triglycerides and high-density lipoprotein.

Recently, Abul-Husn et al.[47] also reported that the splice-site SNP rs72613567 is associated with levels of ALT and AST and with the risk of chronic liver disease. Their findings, including the presence of loss-of-function splice variants associated with rs72613567, are consistent with ours and together highlight the role of HSD17B13 in liver injury. Whether the association with injury is specific to fatty liver disease is unclear. A major strength of our study compared to that of Abul-Husn et al.[47] is our focus on genetic associations with the separate specific histological components of NAFLD, allowing us to highlight the different impact on hepatic fat and injury. Our findings suggest that HSD17B13 affects injury and inflammation differently in NAFLD compared to viral hepatitis and that the association with steatosis may be due to a different mechanism. Furthermore, beyond the data on the impact of truncated or unstable forms generated by the splicing SNP, also demonstrated by Abul-Husn et al.,[47] we describe a single–amino acid mutation of HSD17B13 that confers loss of enzymatic activity despite stable protein expression and proper cellular localization and associate this mutation with histological severity of NAFLD. This confirms the link between enzymatic function and phenotype.

In conclusion, we confirmed that the association of rs6834314 with ALT reflects its association with NAFLD and that *HSD17B13* represents the affected gene of interest. Our data establish HSD17B13 as a lipid droplet–associated protein and point to its RDH

activity as a key aspect of function, with a potential pathogenic link to NAFLD. Genetic variations in HSD17B13 have complex consequences that vary by type and locus, and further investigation into the genotype–phenotype association is warranted.

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REFERENCES

1) Schwimmer JB, Celedon MA, Lavine JE, Salem R, Campbell N, Schork NJ, et al. Heritability of nonalcoholic fatty liver disease. Gastroenterology 2009;136:1585-1592.
2) Browning JD, Szczepaniak LS, Dobbins R, Nuremberg P, Horton JD, Cohen JC, et al. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. Hepatology 2004;40:1387-1395.
3) **Romeo S**, **Kozlitina J**, Xing C, Pertsemlidis A, Cox D, Pennacchio LA, et al. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. Nat Genet 2008;40:1461-1465.
4) **Speliotes EK**, **Yerges-Armstrong LM**, **Wu J**, **Hernaez R**, **Kim LJ**, Palmer CD, et al. Genome-wide association analysis identifies variants associated with nonalcoholic fatty liver disease that have distinct effects on metabolic traits. PLoS Genet 2011;7:e1001324.
5) Yuan X, Waterworth D, Perry JR, Lim N, Song K, Chambers JC, et al. Population-based genome-wide association studies reveal six loci influencing plasma levels of liver enzymes. Am J Hum Genet 2008;83:520-528.
6) **Rotman Y**, **Koh C**, Zmuda JM, Kleiner DE, Liang TJ. The association of genetic variability in patatin-like phospholipase domain-containing protein 3 (PNPLA3) with histological severity of nonalcoholic fatty liver disease. Hepatology 2010;52: 894-903.
7) Sookoian S, Castano GO, Burgueno AL, Gianotti TF, Rosselli MS, Pirola CJ. A nonsynonymous gene variant in the adiponutrin gene is associated with nonalcoholic fatty liver disease severity. J Lipid Res 2009;50:2111-2116.
8) Speliotes EK, Butler JL, Palmer CD, Voight BF, Hirschhorn JN. PNPLA3 variants specifically confer increased risk for histologic nonalcoholic fatty liver disease but not metabolic disease. Hepatology 2010;52:904-912.
9) Kotronen A, Peltonen M, Hakkarainen A, Sevastianova K, Bergholm R, Johansson LM, et al. Prediction of non-alcoholic fatty liver disease and liver fat using metabolic and genetic factors. Gastroenterology 2009;137:865-872.
10) **Kozlitina J**, **Smagris E**, Stender S, Nordestgaard BG, Zhou HH, Tybjaerg-Hansen A, et al. Exome-wide association study identifies a TM6SF2 variant that confers susceptibility to nonalcoholic fatty liver disease. Nat Genet 2014;46:352-356.
11) **Holmen OL**, **Zhang H**, **Fan Y**, Hovelson DH, Schmidt EM, Zhou W, et al. Systematic evaluation of coding variation identifies a candidate causal variant in TM6SF2 influencing total cholesterol and myocardial infarction risk. Nat Genet 2014;46:345-351.
12) Mahdessian H, Taxiarchis A, Popov S, Silveira A, Franco-Cereceda A, Hamsten A, et al. TM6SF2 is a regulator of liver fat metabolism influencing triglyceride secretion and hepatic lipid droplet content. Proc Natl Acad Sci USA 2014;111:8913-8918.
13) Anstee QM, Day CP. The genetics of nonalcoholic fatty liver disease: spotlight on PNPLA3 and TM6SF2. Semin Liver Dis 2015;35:270-290.
14) Kahali B, Liu YL, Daly AK, Day CP, Anstee QM, Speliotes EK. TM6SF2: catch-22 in the fight against nonalcoholic fatty liver disease and cardiovascular disease? Gastroenterology 2015;148:679-684.
15) **Buch S**, **Stickel F**, **Trepo E**, Way M, Herrmann A, Nischalke HD, et al. A genome-wide association study confirms PNPLA3 and identifies TM6SF2 and MBOAT7 as risk loci for alcohol-related cirrhosis. Nat Genet 2015;47:1443-1448.
16) Simons N, Isaacs A, Koek GH, Kuc S, Schaper NC, Brouwers M. PNPLA3, TM6SF2, and MBOAT7 genotypes and coronary artery disease. Gastroenterology 2017;152:912-913.
17) **Mancina RM**, **Dongiovanni P**, Petta S, Pingitore P, Meroni M, Rametta R, et al. The MBOAT7-TMC4 variant rs641738 increases risk of nonalcoholic fatty liver disease in individuals of European descent. Gastroenterology 2016;150:1219-1230.
18) **Chambers JC**, **Zhang W**, **Sehmi J**, **Li X**, **Wass MN**, **Van der Harst P**, et al. Genome-wide association study identifies loci influencing concentrations of liver enzymes in plasma. Nat Genet 2011;43:1131-1138.
19) Clark JM, Brancati FL, Diehl AM. The prevalence and etiology of elevated aminotransferase levels in the United States. Am J Gastroenterol 2003;98:960-967.
20) Ioannou GN, Boyko EJ, Lee SP. The prevalence and predictors of elevated serum aminotransferase activity in the United States in 1999–2002. Am J Gastroenterol 2006;101:76-82.
21) Neuschwander-Tetri BA, Clark JM, Bass NM, Van Natta ML, Unalp-Arida A, Tonascia J, et al. Clinical, laboratory and histological associations in adults with nonalcoholic fatty liver disease. Hepatology 2010;52:913-924.
22) Sanyal AJ, Chalasani N, Kowdley KV, McCullough A, Diehl AM, Bass NM, et al. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. N Engl J Med 2010;362: 1675-1685.
23) Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 2005;41:1313-1321.
24) Belyaeva OV, Korkina OV, Stetsenko AV, Kim T, Nelson PS, Kedishvili NY. Biochemical properties of purified human retinol dehydrogenase 12 (RDH12): catalytic efficiency toward retinoids and C9 aldehydes and effects of cellular retinol-binding protein type I (CRBPI) and cellular retinaldehyde-binding protein (CRALBP) on the oxidation and reduction of retinoids. Biochemistry 2005;44:7035-7047.
25) Benjamini Y, Hochberg Y. Controlling the false discovery rate—a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society Series B Methodological 1995;57:289-300.
26) Pike N. Using false discovery rates for multiple comparisons in ecology and evolution. Methods Ecol Evol 2011;2:278-282.

27) Horiguchi Y, Araki M, Motojima K. Identification and characterization of the ER/lipid droplet-targeting sequence in 17beta-hydroxysteroid dehydrogenase type 11. Arch Biochem Biophys 2008;479:121-130.
28) Marchais-Oberwinkler S, Henn C, Moller G, Klein T, Negri M, Oster A, et al. 17Beta-hydroxysteroid dehydrogenases (17beta-HSDs) as therapeutic targets: protein structures, functions, and recent progress in inhibitor development. J Steroid Biochem Mol Biol 2011;125:66-82.
29) Belyaeva OV, Chang C, Berlett MC, Kedishvili NY. Evolutionary origins of retinoid active short-chain dehydrogenases/reductases of SDR16C family. Chem Biol Interact 2015;234:135-143.
30) Belyaeva OV, Johnson MP, Kedishvili NY. Kinetic analysis of human enzyme RDH10 defines the characteristics of a physiologically relevant retinol dehydrogenase. J Biol Chem 2008;283:20299-20308.
31) Liu S, Huang C, Li D, Ren W, Zhang H, Qi M, et al. Molecular cloning and expression analysis of a new gene for short-chain dehydrogenase/reductase 9. Acta Biochim Pol 2007;54:213-218.
32) Duax WL, Pletnev V, Addlagatta A, Bruenn J, Weeks CM. Rational proteomics I. Fingerprint identification and cofactor specificity in the short-chain oxidoreductase (SCOR) enzyme family. Proteins 2003;53:931-943.
33) Shafqat N, Marschall HU, Filling C, Nordling E, Wu XQ, Bjork L, et al. Expanded substrate screenings of human and *Drosophila* type 10 17beta-hydroxysteroid dehydrogenases (HSDs) reveal multiple specificities in bile acid and steroid hormone metabolism: characterization of multifunctional 3alpha/7alpha/7beta/17beta/20beta/21-HSD. Biochem J 2003;376:49-60.
34) **Su W**, **Wang Y**, **Jia X**, Wu W, Li L, Tian X, et al. Comparative proteomic study reveals 17beta-HSD13 as a pathogenic protein in nonalcoholic fatty liver disease. Proc Natl Acad Sci USA 2014;111:11437-11442.
35) Chen G. The link between hepatic vitamin A metabolism and nonalcoholic fatty liver disease. Curr Drug Targets 2015;16:1281-1292.
36) Tsuchiya H, Ikeda Y, Ebata Y, Kojima C, Katsuma R, Tsuruyama T, et al. Retinoids ameliorate insulin resistance in a leptin-dependent manner in mice. Hepatology 2012;56:1319-1330.
37) Ziouzenkova O, Orasanu G, Sharlach M, Akiyama TE, Berger JP, Viereck J, et al. Retinaldehyde represses adipogenesis and diet-induced obesity. Nat Med 2007;13:695-702.
38) Kim SC, Kim CK, Axe D, Cook A, Lee M, Li T, et al. All-trans-retinoic acid ameliorates hepatic steatosis in mice by a novel transcriptional cascade. Hepatology 2014;59:1750-1760.
39) **Yang Q**, **Graham TE**, Mody N, Preitner F, Peroni OD, Zabolotny JM, et al. Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. Nature 2005;436:356-362.
40) **Graham TE**, **Yang Q**, Bluher M, Hammarstedt A, Ciaraldi TP, Henry RR, et al. Retinol-binding protein 4 and insulin resistance in lean, obese, and diabetic subjects. N Engl J Med 2006;354:2552-2563.
41) **Mondul A**, **Mancina RM**, **Merlo A**, Dongiovanni P, Rametta R, Montalcini T, et al. PNPLA3 I148M variant influences circulating retinol in adults with nonalcoholic fatty liver disease or obesity. J Nutr 2015;145:1687-1691.
42) Bruschi FV, Claudel T, Tardelli M, Caligiuri A, Stulnig TM, Marra F, et al. The PNPLA3 I148M variant modulates the fibrogenic phenotype of human hepatic stellate cells. Hepatology 2017;65:1875-1890.
43) **Pirazzi C**, **Valenti L**, **Motta BM**, Pingitore P, Hedfalk K, Mancina RM, et al. PNPLA3 has retinyl-palmitate lipase activity in human hepatic stellate cells. Hum Mol Genet 2014;23:4077-4085.
44) Valenti L, Romeo S. Destined to develop NAFLD? The predictors of fatty liver from birth to adulthood. J Hepatol 2016;65:668-670.
45) Yang D, Vuckovic MG, Smullin CP, Kim M, Lo CP, Devericks E, et al. Modest decreases in endogenous all-trans-retinoic acid produced by a mouse Rdh10 heterozygote provoke major abnormalities in adipogenesis and lipid metabolism. Diabetes 2018;67:662-673.
46) Xu L, Jiang CQ, Lam TH, Zhang WS, Zhu F, Jin YL, et al. Mendelian randomization estimates of alanine aminotransferase with cardiovascular disease: Guangzhou Biobank Cohort study. Hum Mol Genet 2017;26:430-437.
47) Abul-Husn NS, Cheng X, Li AH, Xin Y, Schurmann C, Stevis P, et al. A protein-truncating HSD17B13 variant and protection from chronic liver disease. N Engl J Med 2018;378:1096-1106.
48) Adam M, Heikela H, Sobolewski C, Portius D, Maki-Jouppila J, Mehmood A, et al. Hydroxysteroid (17) dehydrogenase 13 deficiency triggers hepatic steatosis and inflammation in mice. FASEB J 2018;32:3434-3447.
49) **Su W**, **Peng J**, Li S, Dai YB, Wang CJ, Xu H, et al. Liver X receptor alpha induces 17beta-hydroxysteroid dehydrogenase-13 expression through SREBP-1c. Am J Physiol Endocrinol Metab 2017;312:E357-E367.
50) Rotroff DM, Pijut SS, Marvel SW, Jack JR, Havener TM, Pujol A, et al. Genetic variants in HSD17B3, SMAD3, and IPO11 impact circulating lipids in response to fenofibrate in individuals with type 2 diabetes. Clin Pharmacol Ther 2018;103:712-721.

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Supporting Information

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