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Original Article

Associations of Hydroxysteroid 17-beta Dehydrogenase 13 Variants with Liver Histology in Chinese Patients with Metabolic-associated Fatty Liver Disease

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Abstract

Background and Aims: In Europeans, variants in the *hydroxysteroid 17-beta dehydrogenase 13* (*HSD17B13*) gene impact liver histology in metabolic-associated fatty liver disease (MAFLD). The impact of these variants in ethnic Chinese is unknown. The aim of this study was to investigate the potential associations in Chinese patients. ***Methods:*** In total, 427 Han Chinese with biopsy-confirmed MAFLD were enrolled. Two single nucleotide polymorphisms in *HSD17B13* were genotyped: rs72613567 and rs6531975. Logistic regression was used to test the association between the single nucleotide polymorphisms and liver histology. ***Results:*** In our cohort, the minor allele TA of the rs72613567 variant was related to an increased risk of fibrosis [odds ratio (OR): 2.93 (1.20–7.17), p=0.019 for the additive model; OR: 3.32 (1.39–7.91), p=0.007 for the recessive model], representing an inverse association as compared to the results from European cohorts. In contrast, we observed a protective effect on fibrosis for the minor A allele carriers of the *HSD17B13* rs6531975 variant [OR: 0.48 (0.24–0.98), p=0.043 for the additive model; OR: 0.62 (0.40–0.94), p=0.025 for the dominant model]. *HSD17B13* variants were only associated with fibrosis but no other histological features. Furthermore, *HSD17B13* rs6531975 modulated the effect of *PNPLA3* rs738409 on hepatic steatosis. ***Conclusions:*** *HSD17B13* rs72613567 is a risk variant for fibrosis in a Han Chinese MAFLD population but with a different direction for allelic association to that seen in Europeans. These data exemplify the need for studying diverse populations in genetic studies in order to fine map genome-wide association studies signals.

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Keywords: Metabolic-associated fatty liver disease (MAFLD); Nonalcoholic fatty liver disease (NAFLD); Hydroxysteroid 17-beta dehydrogenase 13 (HSD17B13); Single nucleotide polymorphism (SNP).

Abbreviations: BMI, body mass index; CI, confidence interval; GWAS, genome-wide association studies; HOMA, homoeostasis model assessment; *HSD17B13*, *hydroxysteroid 17-beta dehydrogenase 13*; IFNL3, interferon lambda-3; IR, insulin resistance; MAF, minor allele frequency; MAFL, metabolic-associated fatty liver; MAFLD, metabolic-associated fatty liver disease; *MICA*, *MHC class I polypeptide-related chain A*; *NCAN*, *neurocan*; OR, odds ratio; *PNPLA3*, *patatin-like phospholipase domain containing protein 3*; SNP, single nucleotide polymorphism; TLL1, tolloid-like 1; TLR3, toll-like receptor 3.

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Introduction

Metabolic-associated fatty liver disease (MAFLD) is recognized as a leading cause of liver-related morbidity and mortality.[1,2] In China, the MAFLD burden is increasing, with prevalence rising from 18% to 29% in the last decade.[3] MAFLD comprises a spectrum of disease, ranging from simple steatosis or metabolic-associated fatty liver (MAFL) to the presence of steatohepatitis with varying degrees of

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fibrosis and cirrhosis.[4] MAFLD arises from "multiple hits", with genes acting as important modifiers of the clinical phenotype.[5] Our understanding of the underpinnings of MAFLD has been enhanced by numerous genetic association studies, and all of the polymorphisms identified to date explain only 10–20% of disease heritability.[6,7]

It is broadly acknowledged that there is overrepresentation of subjects of European ancestry in human genetics research, with ~79% of all genome-wide association studies (GWAS) participants being of European descent. This overrepresentation hinders a complete understanding of the human genetic architecture. Moreover, it can also have a negative impact, including prediction accuracies between 1.6-4.9-fold lower for other ethnicities than Europeans.[8] Hence, increasing the representation of diverse populations and studying other ethnicities has become a research priority.

Several variants in the *hydroxysteroid 17-beta dehydrogenase 13* (*HSD17B13*) gene encoding a hepatic lipid droplet protein have been identified to impact the histological features of MAFLD. However, the impact of *HSD17B13* gene variants on MAFLD histology among those of Chinese ancestry is unknown. Notably, allele frequencies, haplotype patterns and the effect size of polymorphisms vary considerably across populations and ethnicities.[6] As *HSD17B13* has been proposed as a therapeutic target for MAFLD, it is pivotal to explore whether the effect of this variant observed in Caucasian populations extends to other populations, as also to the effect size.

It is known that the genetic association of variants in *HSD17B13* with the histological features of MAFLD is complex, with different potentially causative single nucleotide polymorphisms (SNPs) and various SNPs associated with different phenotypic patterns. For example, alleles of rs6834314 and rs72613567 associate with decreased injury and with increased hepatic fat.[9] However, there are other studies that show no association of rs72613567 with steatosis.[10,11] Non-coding SNPs (e.g., rs6531975) not in linkage disequilibrium with rs72613567 have also been associated with decreased hepatic fat.[9] Adding to this complexity, a recent study of 487 patients suggested that those harboring the 'protective' TA-allele of rs72613567 have a numerically increased risk for mortality, liver-related death and hepatic decompensation.[12] Likewise, while some reports have suggested that there is a potential interaction between *HSD17B13* and variants in the *patatin-like phospholipase domain containing protein 3* (*PNPLA3*) gene in MAFLD, subsequent reports have cited a failure to discern an association.[13,14]

Given these controversies, the aims of this study were 1) to explore the role of variants in the *HSD17B13* gene in a cohort of Han Chinese with biopsy-confirmed MAFLD, 2) to clarify the role of the variants on the various morphological features of MAFLD, and 3) to discern if there is any interaction between the variants and variants in *PNPLA3*.

Methods

Study population

We recruited 427 consecutive Han Chinese patients with biopsy-confirmed MAFLD from the PERSONS cohort (2017.01–2019.05). The definition of MAFLD was based on the criteria proposed by an international expert panel.[15] The study cohort included patients from a previously published study as well as additional subjects.[16] To ascertain the effects of the *HSD17B13* variant on liver disease solely due to MAFLD, patients with other causes of liver disease (including alcohol use disorder or viral hepatitis) were excluded. Briefly, all consecutive patients, aged ≥18, with biopsy-proven MAFLD, and without alternative causes of liver disease were recruited to the study.

The study protocol was approved by the ethics committee of the First Affiliated Hospital of Wenzhou Medical University (2016-246, 1 December 2016) and registered in the Chinese Clinical Trial Registry (ChiCTR-EOC-17013562). Written informed consent was obtained from each subject before their participation in the study. Patient identifiers were anonymized and replaced by the health examination number.

Clinical and biochemical data

Clinical and biochemical data were collected from all patients within 24 hours of liver biopsy. Body mass index (BMI) was calculated as weight (kg) divided by the square of height (m). Insulin resistance (IR) was estimated according by the homoeostasis model assessment (commonly referred to as HOMA).[17] Diagnosis of diabetes was based on criteria of the American Diabetes Association.[18]

Assessment of liver histology

Liver biopsies were performed using a 16-gauge needle under ultrasound guidance. The histology was reviewed by a single liver pathologist (X.D. Wang) who was blinded to the clinical and biochemical data. Histologic scoring was based on the Activity Score.[19] Steatohepatitis was diagnosed as a score ≥4 and a score of at least one for each feature of steatosis, ballooning, and lobular inflammation. Severe steatosis, severe ballooning and severe lobular inflammation were defined if their scores were ≥2.

Genetic analysis

Genotyping for the *HSD17B13* (rs72613567 and rs6531975) and *PNPLA3* (rs738409) variants were performed using the MassARRAY (Agena Biosciences, San Diego, CA, USA) or *Taq*Man assay (Bio-Rad, Hercules, CA, USA) platforms, according to the manufacturer's protocol. For the purpose of genotyping, each sample used approximately 20 ng of genomic DNA. Locus-specific PCR and detection primers were designed using Assay Design Suite v3.1.

Statistical analysis

Statistical analyses were performed using R software (v3.5.2; R Foundation for Statistical Computing, Vienna, Austria) and SPSS 19.0 (SPSS Inc., Armonk, NY, USA). Continuous variables were expressed as mean±standard deviation and compared using the one-way analysis of variance test. Categorical variables were expressed as frequency (%) and compared using the chi-square test. The Hardy-Weinberg equilibrium was assessed using the chi-square test. Multivariate logistic regression models were undertaken to test the association between the aforementioned SNPs and liver histology features. A p-value <0.05 was considered to be statistically significant.

Results

Patient characteristics

The study comprised 427 consecutive biopsy-confirmed MAFLD patients; their clinical, biochemical, and histological features are depicted in Supplementary Table 1. The average age was 41 years, with 73.8% being male. About 287

Table 1. Baseline characteristics of biopsy-confirmed MAFLD patients according to rs72613567 genotypes

	T/T (*n*=198)	T/TA (*n*=176)	TA/TA (*n*=45)	*p*-value
Age in years	40.2±11.9	41.4±11.5	43.1±14.8	0.299
Male sex, %	150 (75.8%)	126 (71.6%)	33 (73.3%)	0.657
Diabetes, %	63 (31.8%)	54 (30.7%)	18 (40.0%)	0.484
Hypertension, %	74 (37.4%)	59 (33.5%)	22 (48.9%)	0.161
Waist circumference in cm	92.2±9.0	90.6±8.7	91.7±6.8	0.212
BMI in kg/m^2	27.0±3.5	26.5±3.3	26.3±2.9	0.255
HOMA-IR score	5.3±8.4	5.1±6.6	6.5±7.5	0.541
Platelet count as 10^9/L	242.2±61.0	246.7±56.2	253.1±84.6	0.520
Hemoglobin A1c, %	6.0±1.3	6.2±1.5	6.3±1.5	0.427
Fasting glucose in mmol/L	5.7±1.5	5.5±1.2	6.2±2.4	0.012
Total cholesterol in mmol/L	5.2±1.3	4.9±1.1	5.0±1.0	0.100
Triglycerides in mmol/L	2.4±1.7	2.0±1.1	2.3±1.3	0.044
HDL-cholesterol in mmol/L	1.0±0.2	1.0±0.2	1.1±0.4	0.019
LDL-cholesterol in mmol/L	3.1±1.0	3.0±0.9	2.9±0.8	0.331
Albumin in g/L	46.4±4.2	46.4±3.4	46.2±3.6	0.957
ALT in U/L	83.4±79.9	67.9±56.9	70.6±46.6	0.079
AST in U/L	50.4±35.7	45.2±35.0	40.8±20.6	0.139
GGT in U/L	75.8±83.7	68.7±108.9	84.6±98.2	0.567
Creatinine in μmol/L	67.1±14.3	66.1±12.9	70.6±17.4	0.159
Uric acid in μmol/L	395.7±102.9	385.8±108.1	398.2±120.3	0.615
PNPLA3 rs738409				0.256
C/C	56 (28.7%)	51 (29.7%)	16 (35.6%)	
C/G	101 (51.8%)	73 (42.4%)	19 (42.2%)	
G/G	38 (19.5%)	48 (27.9%)	10 (22.2%)	

Categorical values are shown as *n* (%). Continuous variables are shown as mean±standard deviation.
Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

(67.2%) had fibrosis (≥F1), 226 (52.9%) had severe steatosis (S2-S3), 157 (36.8%) had severe ballooning (B2) and 84 (19.7%) had severe inflammation (A2-A3).

Genotype distribution, Hardy-Weinberg equilibrium calculations

Two SNPs in *HSD17B13* were genotyped: rs72613567 and rs6531975. The genotype distributions of rs72613567 and rs6531975 in *HSD17B13* were in Hardy-Weinberg equilibrium (all, $p>0.05$). The minor allele frequency (MAF) for rs72613567 and rs6531975 was 0.32 and 0.30 in our cohort, respectively. Each of these MAFs is close to the MAF in general East Asian population in the 1000 Genomes Project.[20] The overall genotype distribution of rs72613567 T/T, T/TA and TA/TA was 47.3%, 42.0% and 10.7%, while the distribution of rs6531975 G/G, G/A and A/A was 49.8%, 40.5% and 9.8%, respectively.

Clinical and laboratory characteristics stratified by HSD17B13 variants

The baseline characteristics of study participants according to rs72613567 genotypes is presented in Table 1. There were significant differences in levels of fasting glucose, triglycerides and high-density lipoprotein cholesterol among rs72613567 genotypes (all, $p<0.05$). Table 2 shows the baseline characteristics of study participants according to rs6531975 genotypes. No significant differences were observed among the rs6531975 genotypes.

HSD17B13 variants and hepatic steatosis

The proportion of severe steatosis in rs72613567 T/T, T/TA and TA/TA was 103 (52.0%), 91 (51.7%)and 27 (60.0%) respectively, while the proportion of severe steatosis in rs6531975 G/G, G/A and A/A was 113 (54.1%), 84 (49.4%) and 24 (58.5%) respectively (Table 3). No association between *HSD17B13* variants and severe steatosis was observed in multivariate logistic regression model (Table 4).

HSD17B13 variants and hepatocyte ballooning and lobular inflammation

The proportion of severe ballooning in rs72613567 T/T, T/TA and TA/TA was 73 (36.9%), 58 (33.0%)and 21 (46.7%)

Table 2. Baseline characteristics of biopsy-confirmed MAFLD patients according to rs6531975 genotypes

	G/G (*n*=209)	G/A (*n*=170)	A/A (*n*=41)	*p*-value
Age in years	41.8±12.3	40.6±11.2	38.9±13.8	0.300
Male sex, %	160 (76.6%)	122 (71.8%)	27 (65.9%)	0.287
Diabetes, %	61 (29.2%)	60 (35.3%)	12 (29.3%)	0.420
Hypertension, %	74 (35.4%)	67 (39.4%)	14 (34.1%)	0.672
Waist circumference in cm	91.6±7.9	91.2±9.3	90.8±9.8	0.824
BMI in kg/m^2	26.5±3.1	26.8±3.6	26.7±3.5	0.690
HOMA-IR score	5.8±8.0	5.2±8.8	4.3±3.5	0.472
Platelet count as 10^9/L	246.0±62.3	243.9±60.9	257.4±65.1	0.457
Hemoglobin A1c, %	6.1±1.4	6.1±1.4	5.9±1.3	0.537
Fasting glucose in mmol/L	5.7±1.6	5.7±1.5	5.4±1.1	0.440
Total cholesterol in mmol/L	5.0±1.1	5.1±1.1	5.3±1.6	0.324
Triglycerides in mmol/L	2.2±1.4	2.4±1.6	2.1±1.0	0.284
HDL-cholesterol in mmol/L	1.0±0.2	1.0±0.2	1.0±0.2	0.665
LDL-cholesterol in mmol/L	3.0±0.9	3.0±0.9	3.4±1.2	0.061
Albumin in g/L	46.1±3.6	46.5±4.3	46.7±3.1	0.412
ALT in U/L	70.3±53.4	81.2±93.1	84.3±73.5	0.275
AST in U/L	44.1±30.1	50.2±40.8	51.0±35.7	0.193
GGT in U/L	72.6±103.3	76.7±96.9	60.9±41.7	0.636
Creatinine in μmol/L	68.0±13.0	66.4±15.2	63.5±13.7	0.137
Uric acid in μmol/L	390.8±100.9	391.6±112.9	412.2±115.7	0.489
PNPLA3 rs738409				0.684
C/C	62 (30.1%)	48 (29.1%)	14 (34.1%)	
C/G	93 (45.1%)	83 (50.3%)	16 (39.0%)	
G/G	51 (24.8%)	34 (20.6%)	11 (26.8%)	

Categorical values are shown as *n* (%). Continuous variables are shown as mean±standard deviation.

respectively, while the proportion of severe ballooning in rs6531975 G/G, G/A and A/A was 79 (37.8%), 63 (37.1%) and 11 (26.8%) respectively. The proportion of severe inflammation in rs72613567 T/T, T/TA and TA/TA was 35 (17.7%), 35 (19.9%) and 12 (26.7%) respectively, while the proportion of severe inflammation in rs6531975 G/G, G/A and A/A was 40 (19.1%), 35 (20.6%) and 8 (19.5%) respectively (Table 3). Both severe ballooning and inflammation were unrelated to *HSD17B13* variants in multivariate analysis (Table 4).

HSD17B13 variants and fibrosis

The prevalence of having fibrosis in rs72613567 T/T, T/TA and TA/TA was 135 (68.2%), 111 (63.1%) and 38 (84.4%) respectively. A higher prevalence of fibrosis was observed in patients with the TA/TA genotype in rs72613567 ($p<0.05$) (Table 3). In rs6531975 genotypes, the prevalence of having fibrosis in G/G, G/A and A/A was 150 (71.8%), 109 (64.1%) and 23 (56.1%) respectively. The A allele carriers of rs6531975 showed a nonsignificant trend for a reduced prevalence of having fibrosis ($p=0.082$) (Table 3).

To further understand the association between *HSD17B13* variants and liver histology in Chinese patients with MAFLD, multivariate logistic regression modeling was undertaken. As shown in Table 4, rs72613567 TA/TA increased the risk of fibrosis with an odds ratio (OR) of 2.93 [TA/TA vs. T/T, 95% confidence interval (CI): 1.20–7.17, $p=0.019$] for the additive model and an OR of 3.32 (TA/TA vs. T/T+T/TA, 95% CI: 1.39–7.91, $p=0.007$) for the recessive model after adjusting for age, sex, BMI, presence of diabetes, fasting glucose, triglycerides and high-density lipoprotein cholesterol. In contrast, the rs6531975 A allele appeared to have a protective impact on fibrosis, with an OR of 0.48 (A/A vs. G/G, 95% CI: 0.24–0.98, $p=0.043$) for the additive model and an OR of 0.62 (G/A+A/A vs. G/G, 95% CI: 0.40–0.94, $p=0.025$) for the dominant model after adjusting for age, sex, BMI and presence of diabetes.

Interaction of PNPLA3 and HSD17B13 variants

Next, we conducted interaction analysis for *HSD17B13* (rs72613567 and rs6531975) and *PNPLA3* (rs738409) variants for their impact on liver histology. For fibrosis, no interaction effects were observed between the two genes. In contrast, there was an interaction between rs6531975 and rs738409 with regard to hepatic steatosis (Fig. 1). For the rs738409 risk allele carriers (CG+GG), the proportion of severe steatosis was lower in patients with the rs6531975 A allele (G/A+A/A) compared to those with rs6531975 G/G (Fig. 1A). Using the latter as reference, the rs6531975 A allele (G/A+A/A) attenuated the risk effect of the rs738409

Table 3. Liver histology features of biopsy-confirmed MAFLD patients according to *HSD17B13* genotypes

	***HSD17B13* rs72613567**					***HSD17B13* rs6531975**		
	T/T (*n*=198)	**T/TA (*n*=176)**	**TA/TA (*n*=45)**	***p*-value**	**G/G (*n*=209)**	**G/A (*n*=170)**	**A/A (*n*=41)**	***p*-value**
Steatosis, *n* (%)				0.586				0.484
Mild steatosis: <2	95 (48.0%)	85 (48.3%)	18 (40.0%)		96 (45.9%)	86 (50.6%)	17 (41.5%)	
Severe steatosis: ≥2	103 (52.0%)	91 (51.7%)	27 (60.0%)		113 (54.1%)	84 (49.4%)	24 (58.5%)	
Hepatocyte ballooning, *n* (%)				0.226				0.401
Mild ballooning: <2	125 (63.1%)	118 (67.0%)	24 (53.3%)		130 (62.2%)	107 (62.9%)	30 (73.2%)	
Severe ballooning: =2	73 (36.9%)	58 (33.0%)	21 (46.7%)		79 (37.8%)	63 (37.1%)	11 (26.8%)	
Lobular inflammation, *n* (%)				0.386				0.939
Mild inflammation: <2	163 (82.3%)	141 (80.1%)	33 (73.3%)		169 (80.9%)	135 (79.4%)	33 (80.5%)	
Severe inflammation: ≥2	35 (17.7%)	35 (19.9%)	12 (26.7%)		40 (19.1%)	35 (20.6%)	8 (19.5%)	
Presence of fibrosis, *n* (%)	135 (68.2%)	111 (63.1%)	38 (84.4%)	0.023	150 (71.8%)	109 (64.1%)	23 (56.1%)	0.082

Table 4. Association between *HSD17B13* variants and liver histology features in Chinese MAFLD patients

SNP	**Severe steatosis**			**Severe ballooning**			**Severe inflammation**			**Presence of fibrosis**		
	OR	**95% CI**	***p***	**OR**	**95% CI**	***p***	**OR**	**95% CI**	***p***	**OR**	**95% CI**	***p***
HSD17B13 rs72613567[†]												
Additive model												
T/T	ref.	–	–	ref.	–	–	ref.	–	–	ref.	–	–
T/TA	1.24	0.78–1.96	0.368	0.93	0.60–1.44	0.737	1.24	0.72–2.16	0.437	0.77	0.49–1.20	0.252
TA/TA	1.62	0.77–3.42	0.203	1.37	0.69–2.72	0.368	1.99	0.89–4.43	0.092	2.93	1.20–7.17	0.019
Dominant model												
T/T	ref.	–	–	ref.	–	–	ref.	–	–	ref.	–	–
T/TA+TA/TA	1.30	0.84–2.02	0.234	1.01	0.67–1.52	0.973	1.38	0.83–2.31	0.216	0.96	0.63–1.48	0.867
Recessive model												
T/T+T/TA	ref.	–	–	ref.	–	–	ref.	–	–	ref.	–	–
TA/TA	1.46	0.72–2.98	0.292	1.42	0.74–2.73	0.295	1.80	0.85–3.83	0.127	3.32	1.39–7.91	0.007
HSD17B13 rs6531975[‡]												
Additive model												
G/G	ref.	–	–	ref.	–	–	ref.	–	–	ref.	–	–
G/A	0.69	0.44–1.08	0.104	0.95	0.62–1.45	0.802	0.94	0.56–1.60	0.830	0.65	0.42–1.02	0.063
A/A	0.91	0.43–1.94	0.809	0.59	0.28–1.24	0.164	0.84	0.35–2.00	0.690	0.48	0.24–0.98	0.043
Dominant model												
G/G	ref.	–	–	ref.	–	–	ref.	–	–	ref.	–	–
G/A+A/A	0.73	0.48–1.11	0.138	0.87	0.58–1.30	0.496	0.92	0.56–1.52	0.751	0.62	0.40–0.94	0.025
Recessive model												
G/G+G/A	ref.	–	–	ref.	–	–	ref.	–	–	ref.	–	–
A/A	1.08	0.52–2.23	0.833	0.60	0.29–1.24	0.170	0.86	0.37–1.98	0.726	0.59	0.30–1.16	0.123

[†]OR and 95% CI obtained by binary logistic regression analysis adjusted for age, sex, BMI, presence of diabetes, fasting glucose, triglycerides and HDL-cholesterol.
[‡]OR and 95% CI obtained by binary logistic regression analysis adjusted for age, sex, BMI, presence of diabetes. ref., reference.

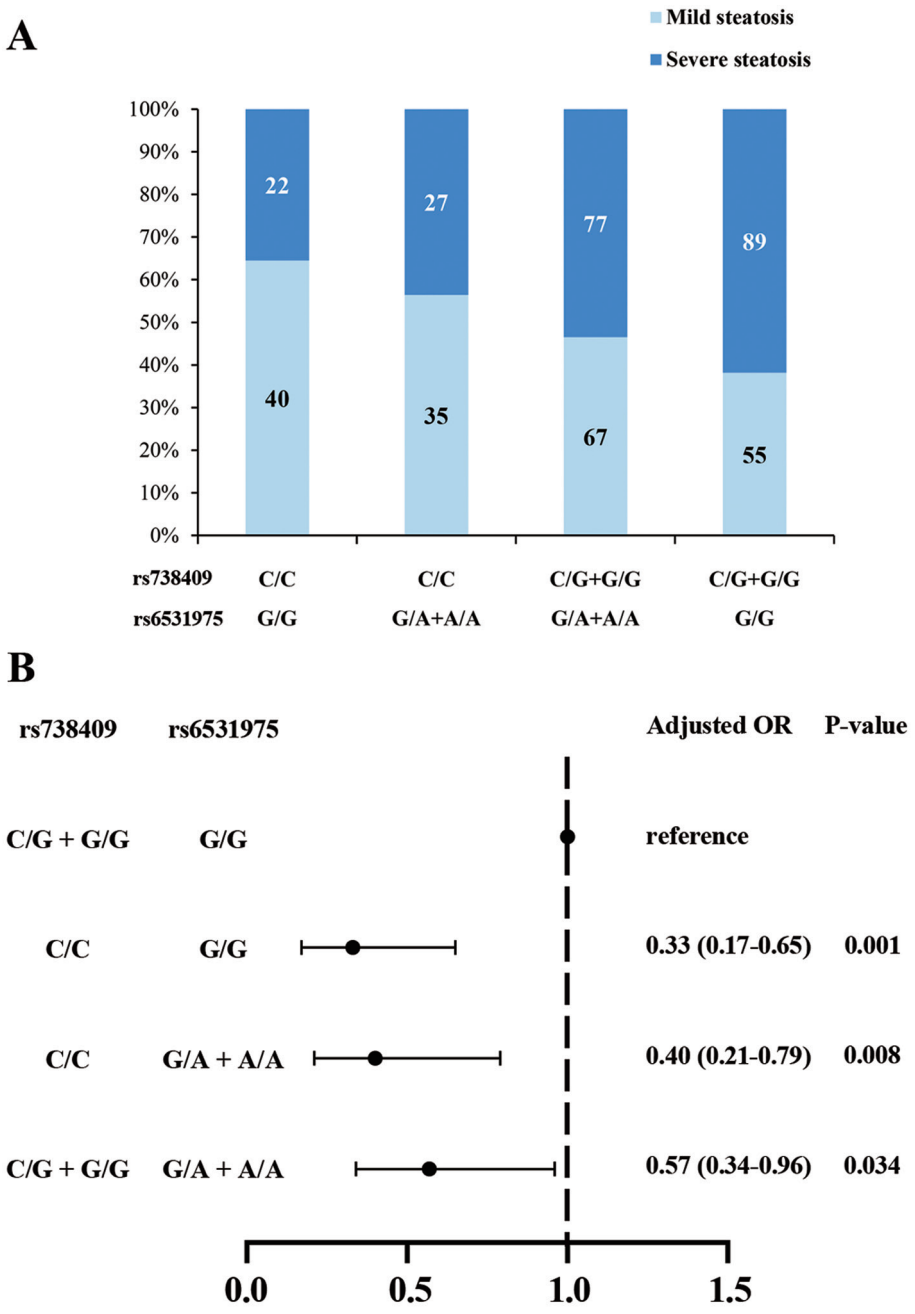

Fig. 1. Interaction of *HSD17B13* rs6531975 and *PNPLA3* rs738409 on liver steatosis. (A) Prevalence of mild steatosis and severe steatosis according to rs6531975 and rs738409 genotypes. (B) Interaction effect of rs6531975 and rs738409 on steatosis after adjusting for age, sex, BMI and presence of diabetes. Patients with the rs6531975 A allele (G/A+A/A) attenuated the risk effect of the rs738409 G allele (C/G+G/G) on steatosis, with an OR of 0.57 (95% CI: 0.34-0.96, p=0.034).

G allele (C/G+G/G) on steatosis, with an OR of 0.57 (95% CI: 0.34–0.96, p=0.034) after adjusting for age, sex, BMI and presence of diabetes (Fig. 1B). The interaction between rs72613567 and rs738409 on liver steatosis was also performed (Fig. 2); however, no effect was observed.

Discussion

We characterized the impact of *HSD17B13* gene variants on histological features in a cohort of Han Chinese with MAFLD. This study has three key findings. First, we confirmed the *HSD17B13* region as a susceptibility locus for MAFLD-related fibrosis but extended these findings toward the identification of an inverse allelic direction of association as compared to that reported in Europeans. Second, the *HSD17B13* variants are only associated with fibrosis and not any other histological feature. Third, the *HSD17B13* variants modulate the effect of *PNPLA3* rs738409 on hepatic steatosis but no other histological features.

The association between *HSD17B13* variants and liver histological features seems to be complex, with multiple

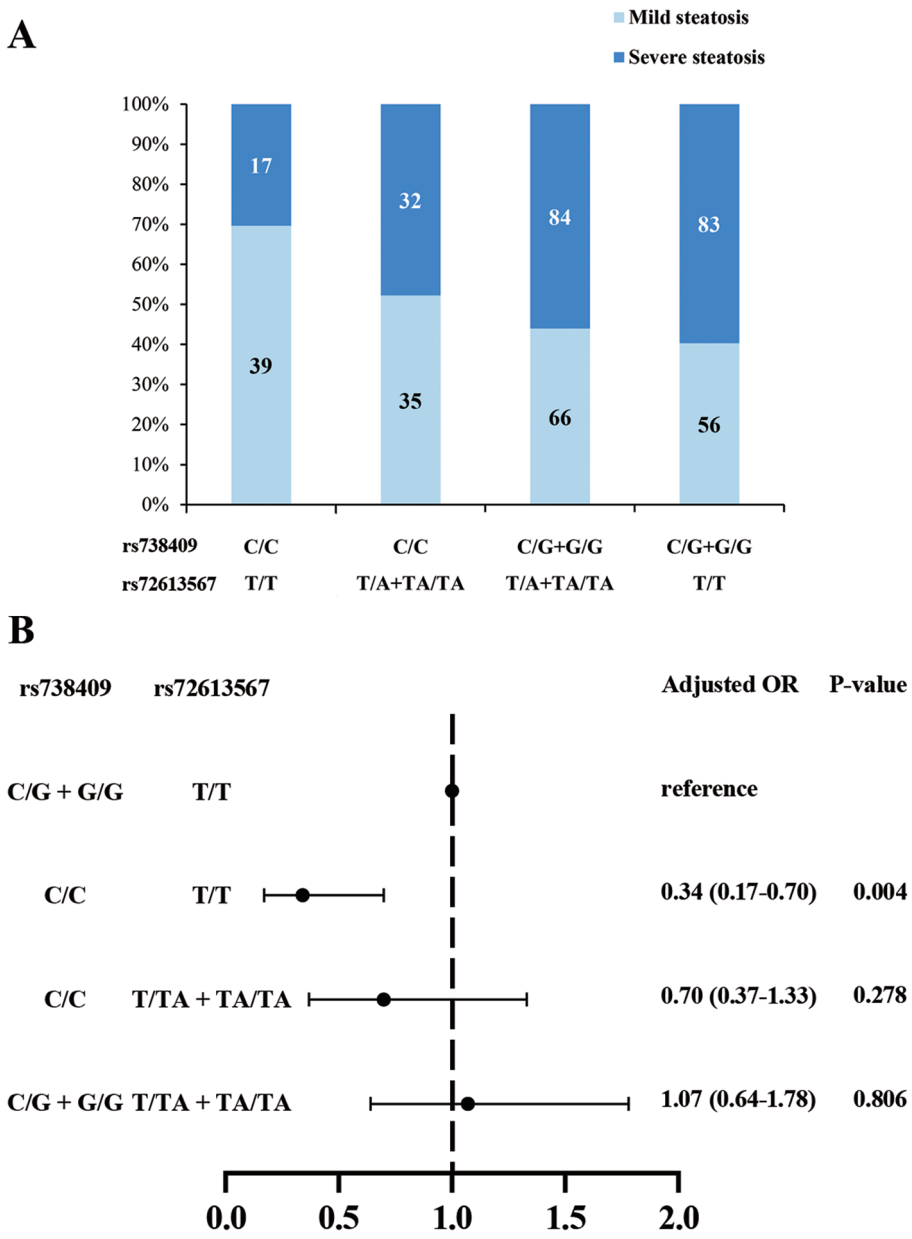

Fig. 2. Interaction of *HSD17B13* rs72613567 and *PNPLA3* rs738409 on liver steatosis. (A) Prevalence of mild steatosis and severe steatosis according to rs72613567 and rs738409 genotypes. (B) Interaction effect of rs72613567 and rs738409 on steatosis after adjusting for age, sex, BMI and presence of diabetes. No interaction effect was observed between rs72613567 and rs738409.

suggested functional variants. Notably, in our cohort, the minor allele TA of the rs72613567 variant was related to an increased risk of fibrosis, representing an inverse association as compared to the results in European cohorts. Hence, if there is a shared causal variant across European and Chinese populations, it is unlikely to be rs72613567. In this regard, we observed a protective effect in the minor A allele carriers of the *HSD17B13* rs6531975 variant, but this is not in strong linkage disequilibrium with rs72613567. Thus, further fine-mapping studies in Han Chinese populations and comparison to other populations would be helpful to identify shared causal variants across different ethnicities.

The differential effect size and allele direction of variants discovered by GWAS between ethnicities is not uncommon. In one Chinese MAFLD cohort, researchers found that the *neurocan* (known as *NCAN*) rs2228603 T variant associated with a higher level of high-density lipoprotein,[21] while it was positively related to liver steatosis in the USA population.[22] Similarly, toll-like receptor 3 (known as *TLR3*) rs3775290[23,24] and interferon lambda-3 (known as *IFNL3*) rs12979860[25,26] variants in Chinese hepatocellular carcinoma populations showed opposite effects to those in non-Asian populations. Inconsistent results have also been observed in other Asian populations, such as among Japanese. For example, tolloid-like 1 (known as *TLL1*) rs17047200[27] and *MHC class I polypeptide-related chain A* (known as *MICA*) rs2596542[28] variants were suggested to have protective impacts on fibrosis and hepatocellular carcinoma in

Caucasians. The associations were inverse to those of a Japanese cohort.[29,30] Besides, there are several MAFLD-related SNPs in Europeans for which there has been no association in Chinese populations.[31–33] Along the same line, lower genetic prediction accuracies (between 1.6-4.9-fold lower) were observed in other ethnicities compared to Europeans.[8] Hence, increasing the representation of diverse populations and studying other ethnicities has recently become a research priority to enhance understanding of the human genetic architecture and its translational implications.

The ethnic differences in the characteristics of patients with MAFLD might also contribute to the observed differences in the genetic findings. There is growing evidence, for example, that the MAFLD disease course in Asian populations is different to that in Caucasians. As an example, for the same BMI, there is a higher prevalence of MAFLD in Asians. Published reports also indicate that lean MAFLD accounts for 36.9% of cases in China,[3] but only 17.3% of the total disease burden in the USA.[34] Differences in metabolic adaptation have been reported between lean and non-lean MAFLD patients, suggesting that lean fatty liver disease likely has a distinct pathophysiology.[35]

Another intriguing aspect of this study is the lack of association found between *HSD17B13* variants and other histological features. To date, the nature of the association between the rs72613567 allelic variant and the histological features of MAFLD, particularly steatosis, is unclear. Abul-Husn and colleagues[10] suggested a lack of association between the rs72613567 TA variant and steatosis in human liver, consistent with the study of Pirola *et al*.[11] However, a study by Ma *et al*.[9] found a significant association with hepatic steatosis. Similarly, in animal and *in vitro* studies, inconsistent results have been reported for an effect of *HSD17B13* on hepatic lipid accumulation. Abul-Husn *et al*.[10] showed no differences in lipid accumulation according *HSD17B13* isoforms. Similarly Ma *et al*.[9] reported that *HSD17B13* overexpression or knockout in HepG2 cells did not affect lipid content. On the other hand, Marion *et al*.[36] noted hepatic steatosis in *HSD17B13* knockout mice, whilst Su *et al*.[37] observed steatosis in mice that overexpressed *HSD17B13.* Collectively, these results imply that *HSD17B13* variants could have a direct impact on fibrosis rather than effects on steatosis. These findings may be associated with retinol metabolism, since retinol plays a crucial role in the activation and transformation of hepatic stellate cells to matrix secreting myofibroblasts and the development of hepatic fibrosis.[38] Since *HSD17B13* participates in the rate limiting step of retinol metabolism,[9] the mutant in *HSD17B13* might conceivably influence the process of fibrosis.

The interaction between *HSD17B13* and *PNPLA3* variants in MAFLD is also a subject of controversy.[14,39] In this work, we noted an interaction between these variants with regard to steatosis, but not with other histological features. As *HSD17B13* has been suggested as a potential therapeutic target for MAFLD and considering the growing concerns about the failure of phase 2 and 3 clinical trials in this disease[40,41] that was at least partially attributed to clinical heterogeneity, our study highlights the importance of first understanding the functional basis of the various proposed genomic and other targets before therapeutic development.[40,42] Collectively, our data support such an approach. The data from *HSD17B13*-knockout mice, in fact, suggest that HSD17B13 triggers steatosis and inflammation,[36] which is opposite to what has been reported in humans.

The present study has limitations. First, the sample size is modest. In case the observed opposite finding is due to the sample size, we performed a post-hoc power analysis. The power calculated for the model was 72%. It is close to but less than 80%. Considering the low proportion of the rs72613567 TA variant in the general population, we think it is acceptable. In addition, lack of a validation cohort from populations in other parts of China or those of Chinese ancestry living outside mainland China is another limitation.

In conclusion, the *HSD17B13* rs72613567 variant appears to be a risk variant for hepatic fibrosis in a Han Chinese MAFLD population, with a different direction for allelic association to that seen in Europeans.

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Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Study concept and design (WYL, ME, JG, MHZ), acquisition of data (HLM, LJT, GL, PWZ), pathology analysis (XDW), drafting of the manuscript (WYL, ME, JG, KIZ, RSR), critical revision of the manuscript (ME, JG, GT, CDB), statistical analysis (WYL, ME, MZL), study supervision (JG, MHZ), guarantor of the article (MHZ).

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