# SUPPLEMENTAL MATERIAL

## Protection conferred by *HSD17B13* rs72613567 on hepatic fibrosis is likely mediated by lowering ballooning and portal inflammation

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<u>Selection of the study participants</u>

This cross-sectional analysis includes individuals enrolled in the NASH Clinical Research Network (NASH CRN) studies, including NAFLD Adult Databases 1 and 2 (NCT01030484) along with PIVENS (NCT00063622) and FLINT (NCT01265498) trials. NASH CRN is a multiethnic prospective cohort recruiting patients with biopsy-proven NAFLD from nine clinical centers across the US. Appropriate institutional review boards approved the NASH CRN study, and all participants provided written informed consent. Participants were ≥ 18 years and had a histological diagnosis of NAFLD (≥5% of hepatocytes containing macrovesicular fat) in absence of a significant history of alcohol intake (>7 and >14 standard drinks per week for women and men) in the prior two years of the study enrollment, other causes of CLD, history of total parenteral nutrition, biliopancreatic diversion or bariatric surgery, short bowel syndrome, suspected or confirmed hepatocellular carcinoma and positivity for HIV.

A total of 1697 adults with histologically confirmed NAFLD were enrolled through December 2019. Of them, 1153 (68%) non-Hispanic Whites with detailed information for *HSD17B13* rs72613567, other genetic variants, alcohol intake, centrally scored liver histology severity and clinical phenotypes were included. All laboratory and clinical data analyzed in this study were obtained within 6 months of the liver biopsy.

As per protocol, all liver biopsies are reviewed in a blinded manner by the NASH CRN Pathology Committee and scored according to the NASH CRN Scoring System as follows: steatosis (0-3), lobular inflammation (0-2), ballooning degeneration (0-2) and overall fibrosis (0-4).

<u>*HSD17B13* rs72613567 genotyping</u>

DNA samples were received at Indiana University from the NASH CRN consortium. The *HSD17B13* rs72613567 is an insertion variant (-/A insertion) near the donor splice site of exon 6, predicted to result in a splice donor variant with altered function. The genotyping process included a TaqMan genotyping assay (Applied Biosystems, Foster City, CA USA) according to the manufacturer's instructions. Because genotyping is determined by endpoint reading, the PCR was carried out in standard Applied Biosystems thermocyclers (AB2720, SimpliAmp, Veriti, ThermoFisher). The PCR products were analyzed in an ABI PRISM® 7300 Sequence Detection System (SDS) instrument (Thermofisher). SDS Software 1.3.1 was used to convert the raw data to pure dye components and to plot the results of the allelic discrimination on a scatter plot of different alleles; each genotype appears on the graph as a cluster of points. All DNA samples were blindly duplicated to assess the reproducibility of genotypes. An average reproducibility of 100% was obtained.

**Suppl. Table 1.** Baseline features according to *HSD17B13* rs72613567 genotypes.

| Variables | Entire cohort N=1153 | (-/-) N=737 | (-/A) N=348 | (A/A) N=68 | P value [a] |
|---|---|---|---|---|---|
| Age (years) (mean ± SD) | 50.73 ± 11.46 | 51.13 ± 11.32 | 50.02 ± 11.74 | 50.01 ± 11.57 | .283 |
| Gender (female), n (%) | 727 (63) | 468 (64) | 213 (61) | 46 (68) | .986 |
| Type 2 diabetes mellitus, n (%) | 408 (35) | 265 (36) | 114 (33) | 29 (43) | .940 |
| Hypertension, n (%) | 685 (59) | 446 (61) | 198 (57) | 41 (60) | .452 |
| Body mass index (kg/m$^2$) (mean ± SD) | 34.87 ± 6.41 | 34.77 ± 6.45 | 34.87 ± 6.19 | 35.88 ± 7.14 | .399 |
| Non-heavy drinkers, n (%) | 433 (38) | 277 (38) | 134 (38) | 22 (32) | .704 |
| *PNPLA3* rs738409 (GC) + (GG), n (%) | 776 (67) | 505 (69) | 232 (67) | 39 (57) | .100 |
| **Lab reports** (mean ± SD) | | | | | |
| Triglycerides (mg/dl) | 185.66 ± 178.13 | 183.02 ± 180.98 | 191.65 ± 184.53 | 181.65 ± 94.46 | .745 |
| Total cholesterol (mg/dl) | 192.38 ± 44.30 | 192.56 ± 43.77 | 192.55 ± 44.18 | 189.53 ± 50.84 | .861 |
| HDL cholesterol (mg/dl) | 43.34 ± 11.59 | 43.64 ± 11.53 | 42.90 ± 11.96 | 42.32 ± 10.33 | .471 |
| LDL cholesterol (mg/dl) | 115.74 ± 37.29 | 115.82 ± 36.53 | 115.93 ± 38.51 | 113.80 ± 39.61 | .911 |
| HOMA-IR | 7.02 ± 9.50 | 7.08 ± 9.81 | 6.83 ± 9.26 | 7.33 ± 7.28 | .884 |
| Glycosylated hemoglobin (%) | 6.17 ± 1.13 | 6.20 ± 1.20 | 6.07 ± 1.00 | 6.14 ± 1.09 | .202 |
| Alanine aminotransferase (U/L) | 69.99 ± 47.23 | 74.27 ± 49.33 | 65.00 ± 43.05 | 49.19 ± 35.33 | <.001 |
| Aspartate aminotransferase (U/L) | 51.35 ± 32.30 | 53.93 ± 33.64 | 48.38 ± 30.91 | 38.65 ± 16.86 | <.001 |
| **Histology scores** | | | | | |
| Steatosis (mean ± SD) | 1.86 ± 0.82 | 1.80 ± 0.82 | 1.97 ± 0.82 | 2.00 ± 0.81 | <.01 |
| Categories, n (%) | | | | | <.01 |
| <5% | 21 (1.8) | 19 (2.6) | 2 (0.6) | 0 (0) | |
| 5-33% | 420 (36.4) | 281 (38.1) | 117 (33.6) | 22 (32.4) | |
| 33-66% | 413 (35.8) | 269 (36.5) | 120 (34.5) | 24 (35.2) | |
| >66% | 299 (26) | 168 (22.8) | 109 (31.3) | 22 (32.4) | |
| Lobular inflammation (mean ± SD) | 1.58 ± 0.68 | 1.63 ± 0.68 | 1.50 ± 0.67 | 1.44 ± 0.69 | <.01 |
| Categories, n (%) | | | | | <.01 |

| | | | | | |
|---|---|---|---|---|---|
| No foci | 2 (0.2) | 1 (0.1) | 1 (0.3) | 0 (0) | |
| <2 foci/200x | 606 (52.6) | 356 (48.3) | 204 (58.6) | 46 (67.6) | |
| 2-4 foci/200x | 419 (36.3) | 295 (40) | 110 (31.6) | 14 (20.6) | |
| >4 foci/200x | 126 (10.9) | 85 (11.5) | 33 (9.5) | 8 (11.8) | |
| Ballooning (mean ± SD) | 1.06 ± 0.84 | 1.10 ± 0.84 | 1.00 ± 0.84 | 0.94 ± 0.87 | .110 |
| Categories, n (%) | | | | | .037 |
| None | 383 (33.2) | 231 (31.3) | 124 (35.6) | 28 (41.2) | |
| Few | 320 (27.8) | 204 (27.7) | 100 (28.8) | 16 (23.5) | |
| Many | 450 (39) | 302 (41) | 124 (35.6) | 24 (35.3) | |
| Fibrosis stages (mean ± SD) | 1.68 ± 1.29 | 1.75 ± 1.29 | 1.55 ± 1.25 | 1.60 ± 1.35 | .049 |
| Categories, n (%) | | | | | .032 |
| 0 | 258 (22.5) | 154 (20.9) | 86 (24.7) | 18 (26.5) | |
| 1 | 313 (27.1) | 193 (26.2) | 102 (29.3) | 18 (26.5) | |
| 2 | 238 (20.6) | 154 (20.9) | 71 (20.5) | 13 (19) | |
| 3 | 227 (19.7) | 155 (21) | 61 (17.5) | 11 (16.2) | |
| 4 | 117 (10.1) | 81 (11) | 28 (8) | 8 (11.8) | |
| Portal inflammation (mean ± SD) | 1.14 ± 0.58 | 1.17 ± 0.58 | 1.08 ± 0.57 | 1.15 ± 0.60 | .094 |
| Categories, n (%) | | | | | .118 |
| None | 126 (10.9) | 74 (10) | 44 (12.6) | 8 (11.8) | |
| Mild | 740 (64.2) | 467 (63.4) | 231 (66.4) | 42 (61.8) | |
| > Mild | 287 (24.9) | 196 (26.6) | 73 (21) | 18 (26.4) | |

Abbreviations: PNPLA3, patatin-like phospholipase domain-containing protein 3; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance.

[a] To compute statistical difference among the three *HSD17B13* rs72613567, we used the chi-square statistic test for binary, Jonckheere-Terpstra test for ordered alternatives, and One-Way ANOVA for continuous variables.

**Suppl. Table 2.** Role of intermediate histology traits as mediators between *HSD17B13* rs72613567 and risk of fibrosis (0-4). Results based on covariate-adjusted causal mediation analyses including multiple mediators in a sequential manner.

| | Bootstrapped estimates (n=10,000) | | |
|---|---|---|---|
| | Estimates * | 95% confidence intervals | SEs |
| The total effect of rs72613567 † | -0.12 | -0.24 to -0.006 | 0.007 |
| The direct effect of rs72613567 ‡ | -0.02 | -0.11 to 0.08 | 0.05 |
| The indirect effect through all mediators § | -0.10 | -0.18 to -0.03 | 0.04 |
| The indirect effect of rs72613567 through each sequential combination of mediators | | | |
| *Sequential combinations* | | | |
| Steatosis → Lobular inflammation | 0.002 | -0.001 to 0.004 | 0.001 |
| Steatosis → Ballooning | -0.001 | -0.005 to 0.003 | 0.002 |
| Steatosis → Portal inflammation | -0.009 | -0.017 to 0.001 | 0.004 |
| Lobular inflammation → Ballooning | -0.037** | -0.056 to -0.019 | 0.009 |
| Lobular inflammation → Portal inflammation | -0.009** | -0.018 to -0.003 | 0.004 |
| Ballooning → Lobular inflammation | -0.002 | -0.007 to 0.001 | 0.002 |
| Ballooning → Portal inflammation | -0.007** | -0.015 to -0.001 | 0.004 |
| Portal inflammation → Lobular inflammation | 0.000 | -0.001 to 0.000 | 0.000 |
| Portal inflammation → ballooning | -0.005** | -0.014 to -0.001 | 0.004 |

Abbreviations: SE, standard error.

\* Bootstrapped β coefficients.

Analysis adjusted for age, gender, BMI, type 2 diabetes mellitus, non-heavy alcohol intake, *PNPLA3* rs738409, *HSD17B13* rs72613567, *TM6SF2* rs58542926, and *MBOAT7* rs641738.

† Effect of rs72613567 on the risk of fibrosis including the mediator (intermediate histology features) effects.

‡ Effect of rs72613567 on the risk of fibrosis excluding the mediator (intermediate histology features) effects.

§ Effect of rs72613567 on the risk of fibrosis through the mediator (intermediate histology features). If the 95% bias-corrected CIs do not contain zero, the associations are considered significant. The double star symbol (\*\*) represents statistically significant effects.