SUPPLEMENTAL MATERIAL

ANGPTL3 Deficiency and Risk of Hepatic Steatosis

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Table of Contents

Supplemental Methods

Magnetic Resonance Imaging data analysis

All imaging datasets were reviewed by one radiologist (M.D.M.) with more than ten years of experience in liver imaging who was blinded to the participant's genotype. Hepatic fat fraction (HFF), estimated as percentage, was measured as previously described and validated[30,44,45]. Briefly, regions of interest (ROI) were drawn in all liver segments on the HFF map dataset, and the final value corresponded to the mean of these measurements. According to results of previous investigations, patients showing HFF ≥ 5% were classified as having NAFLD, and its severity was estimated as previously described[38,46,47]. Steatosis according to MR-S technique was rated as follows: absent (< 5% of hepatocytes were visibly steatotic); mild (5–33% steatotic hepatocytes); moderate (34–66% steatotic hepatocytes); or severe (> 66% steatotic hepatocytes). In addition, the degree of hepatic steatosis as estimated by MR-PDFF was classified as absent (<5% of hepatocytes were visibly steatotic); mild (5-9.9%); moderate (10-32.9%); or severe (≥33%)[38].

MR spectra were reconstructed on a dedicated workstation with SAGE Dev2 0017.1 software (General Electric Healthcare, Milwaukee, WI). Raw data were zero-filled once, and no filter was used. The data were phase-corrected, Fourier-transformed, baseline corrected and averaged. A Marquardt curve-fitting procedure was performed using a Lorentzian function to calculate the area under the fat and water peaks. Spectra were referenced to residual water and the dominant methylene lipid (-CH3 and -CH2) peak at δ 4.8-5.2 and δ 0.9-1.1 and 1.3-1.6 and 2.1-2.3, respectively. The fat fraction percentage (FF) was defined as follows: FF = FA/(FA + WA) x 100, where FA is the area under the fat peak and WA is the area under the water peak.

Fat-only datasets from 3D GRE T1-weighted sequence were transferred to a personal computer and analyzed using a commercially available software package (Slice-O-Matic; Tomovision Inc.; Montreal, Canada) according to a procedure that has been previously described[38]. VAT and SAT were calculated at L4-L5 by drawing a free-form ROI and utilizing manual thresholding values. Intermuscular adipose tissue (IMAT) was assessed as described by Boettcher et al. [48].

Table S1. Hepatic fat fraction for participants with ANGPTL3 levels ≤ 48 µg/L or ≤ 59 µg/L compared to participants without ANGPTL3 LoF mutations.

	ANGPTL3 ≤ 48 µg/L (N=10)	ANGPTL3 ≤ 59 µg/L (N=14)	Controls without ANGPTL3 LoF mutations (N=83)	Comparison between 48 µg/L or 59 µg/L groups with WT, respectively
HFF-MRS, mean (standard deviation)	4.8% (6.9%)	4.7% (6.0%)	10.8% (15.9%)	P=0.85, P=0.66
HFF-PDFF, mean (standard deviation)	6.9% (7.6%)	6.8% (6.7%)	6.8% (5.9%)	P=0.99, P=0.89

Two ANGPTL3 cutoffs were chosen to approximate the levels observed with vupanorsen dosing of 120mg Q4W or 160mg Q2W which significantly associated with a 24% and 76% increase in hepatic steatosis, respectively. Vupanorsen 120mg Q4W resulted in a mean ANGPTL3 level of 34.2 µg/L with standard deviation of 24.3 µg/L while vupanorsen 160mg Q2W resulted in a mean ANGPTL3 level of 22.2 µg/L with standard deviation of 26.0 µg/L. Assuming normal distributions, approximately 85% of participants (i.e., the cumulative distribution below the mean + one standard deviation) receiving 120mg Q4W vupanorsen would have had ANGPTL3 levels less than ~59 µg/L while approximately 85% of participants receiving the highest dose of vupanorsen (160mg Q2W) would have had ANGPTL3 levels less than ~48 µg/L. We compared these groups to those without ANGPTL3 LoF mutations to reflect individuals on placebo. Comparison was performed using Mann-Whitney test for non-parametric variables.

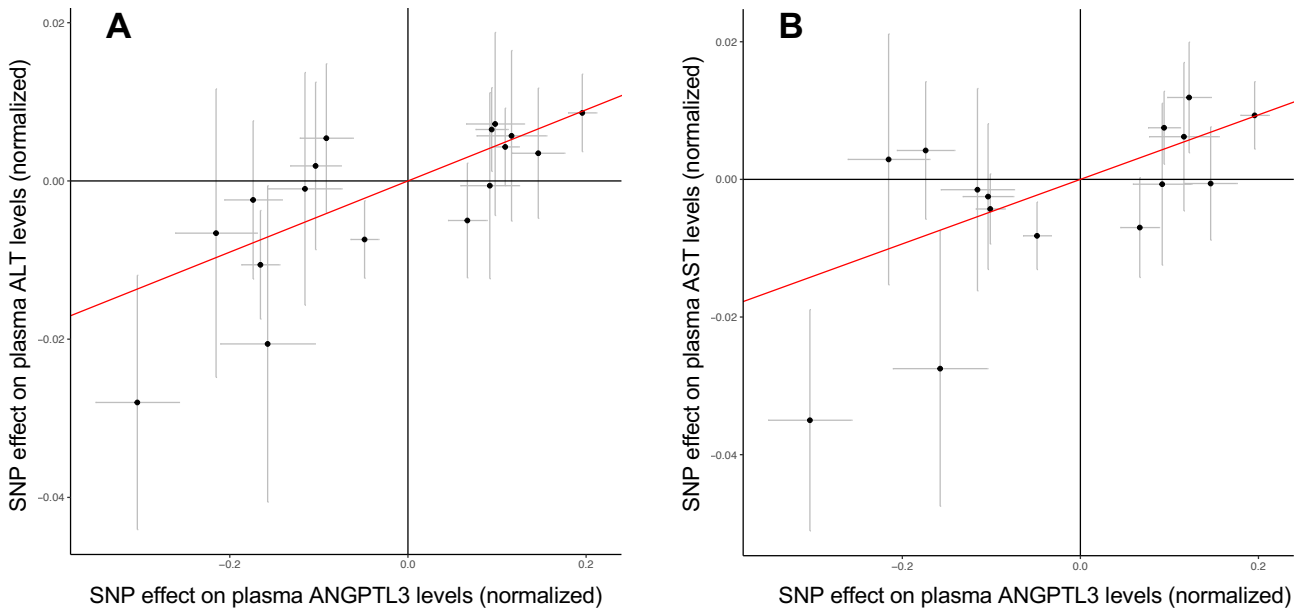

Figure S1. Mendelian randomization of genetically determined plasma ANGPTL3 with ALT and AST in the UK Biobank study. Estimated effect (with 95% confidence intervals) of each variant included in the Mendelian Randomization analysis on ANGPTL3 levels for (**A**) plasma ALT (P=4.7x10^{-7}) and (**B**) plasma AST (P=2.6x10^{-4}). Red lines indicate the causal effect estimates. ALT, alanine aminotransferase; AST, aspartate aminotransferase.

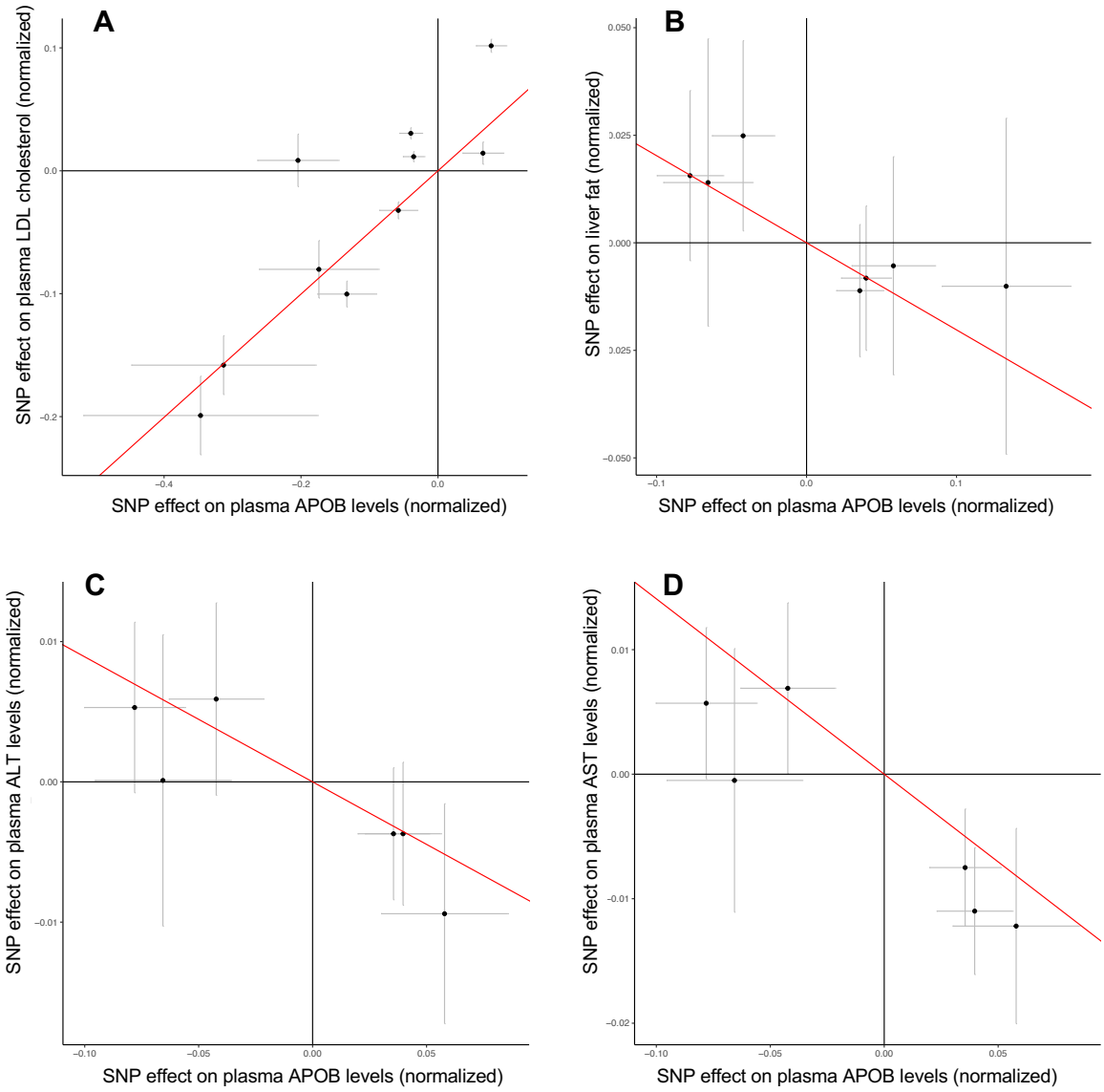

Figure S2. Mendelian randomization of genetically determined plasma ApoB in the UK Biobank study. Estimated effect (with 95% confidence intervals) of each variant included in the Mendelian Randomization analysis on ApoB levels for (**A**) plasma LDL cholesterol (P=0.009), (**B**) hepatic fat (P=0.005), (**C**) plasma ALT (P=3.7x10^{-4}), and (**D**) plasma AST (P=4.2x10^{-4}). Red lines indicate the causal effect estimates. AST, aspartate aminotransferase; ALT, alanine aminotransferase.