Genetic Inhibition of PCSK9 and Liver Function

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RESEARCH LETTER

Genetic Inhibition of *PCSK9* and Liver Function

Inhibition of proprotein convertase subtilisin/kexin type 9 (PCSK9) with anti-PCSK9 monoclonal antibodies (alirocumab and evolocumab) or with small-interfering RNAs (inclisiran) lowers plasma low-density lipoprotein cholesterol (LDL-C) levels, a major risk factor for the development of atherosclerotic cardiovascular disease (ASCVD).[1,2] So far, the pharmacologic inhibition of PCSK9 presents with a favorable safety profile, but longer-term safety remains to be proven. Studies in preclinical models and in humans have suggested a potential link between PCSK9 deficiency and the risk of nonalcoholic fatty liver disease (NAFLD),[3,4] a spectrum of progressive liver diseases ranging from simple steatosis to fibrosis that can lead to cirrhosis and hepatocellular carcinoma. Notably, it has been shown that *PCSK9* knockout mice are more prone to develop severe hepatic steatosis and fibrosis when receiving a high-fat diet.[3]

This study aims to study the associations of the lifelong genetic inhibition of *PCSK9* with plasma liver enzymes and NAFLD by using the loss-of-function variant *PCSK9*-p.Arg46Leu as a genetic instrument. For comparison, we used 2 variants in *PNPLA3* and *TM6SF2,* known to be involved in the pathogenesis of NAFLD.[5]

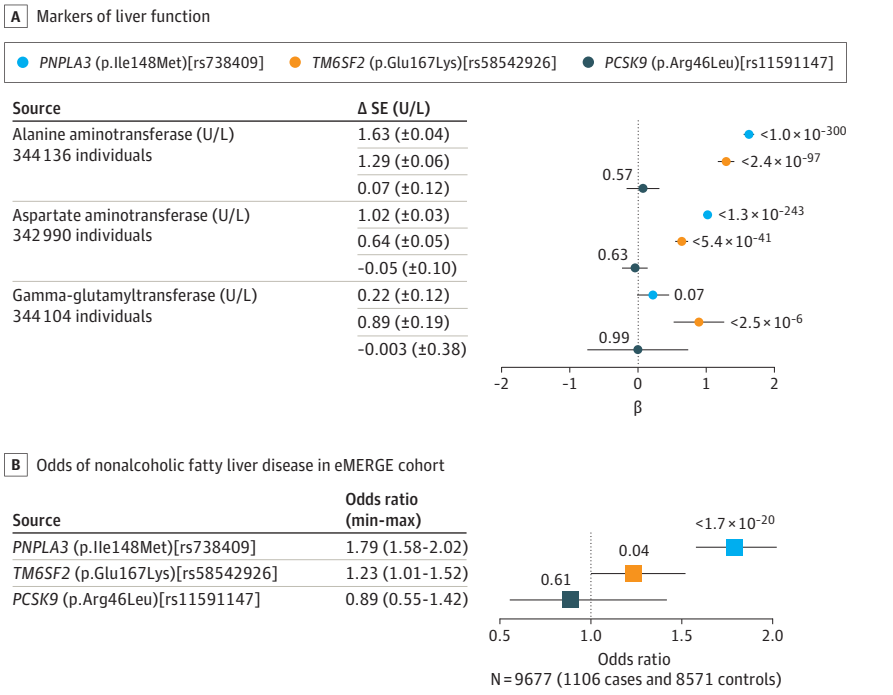
Methods | *Approval*. Both the UK Biobank and the Electronic Medical Record and Genomics (eMERGE) cohort have obtained regulatory approvals, and all participants provided written informed consent. Because the data were publicly available, we did not obtain specific approval for this study.

UK-Biobank Data Sets. Genetic associations with biologic traits (plasma circulating liver enzymes) in the UK-Biobank cohort were extracted from the second-round results (released August 1, 2018) by the Nealelab (see description in http://www.nealelab.is/uk-biobank/). These data were obtained from 361 194 participants (194 174 women and 167 020 men) of the UK-Biobank cohort (https://www.ukbiobank.ac.uk).

The eMERGE Cohort. Genetic association with risk of NAFLD was assessed by using summary data from the study published by Namjou et al[5] in 2019 that studied 9677 individuals (1106 cases and 8571 controls) from the eMERGE cohort (https://emerge-network.org).

Results | Using data from the UK-Biobank, we show that the variant *PCSK9*-p.Arg46Leu is not associated with higher levels in circulating liver enzymes (alanine aminotransferase [ALT], mean [SD] β, 0.071 [0.123] U/L; *P* = .57, aspartate aminotransferase [AST], mean [SD] β, 0.045 [0.095] U/L; *P* = .63, γ-glu-

Figure. Association of *PCSK9*-p.Arg46Leu With Plasma Liver Enzymes and Nonalcoholic Fatty Liver Disease (NAFLD)



A, Markers of liver function (plasma ALT, AST, and GGT) as a function of *PNPLA3*-p.Ile148Met (blue), *TM6SF2*-p.Glu167Lys (orange), and *PCSK9*-p.Arg46Leu variants (dark blue) in more than 300 000 individuals from UK-Biobank as β coefficients per alternative allele compared with noncarriers (horizontal bars show standard error). B, Odds ratio for NAFLD as a function of *PNPLA3*-p.Ile148Met (blue), *TM6SF2*-p.Glu167Lys (orange), and *PCSK9*-p.Arg46Leu variants (dark blue) compared with noncarriers from the eMERGE study (1106 cases vs 8571 controls). The effects are shown as odds ratio per alternative allele. Horizontal bars depict maximum and minimum values.

tamyltransferase [GGT] mean [SD] β, −0.004 [0.379] U/L; P = .99, in more than 340 000 individuals [**Figure**, A]).

In contrast, we confirm that the genetic variants *PNPLA3*-p.Ile148Met and *TM6SF2*-p.Glu167Lys, which have been robustly linked to NAFLD, are significantly associated with higher ALT and AST levels in the same cohort (Figure, A). Of note, statistical associations of *PNPLA3* and *TM6SF2* variants are weaker for GGT plasma levels (mean [SD] β, 0.219 [0.121] U/L; P = .07 and mean [SD] β, 0.893 [0.190] U/L; $P = 2.5 \times 10^{-06}$).

We further demonstrate that *PCSK9*-p.Arg46Leu is not associated with NAFLD in the eMERGE cohort (P = .61; odds ratio, 0.89; 95% CI, 0.55-1.42), whereas *PNPLA3*-p.Ile148Met and *TM6SF2*-p.Glu167Lys are indeed associated with an increased risk for NAFLD (*PNPLA3*-p.Ile148Met, $P = 1.7 \times 10^{-20}$; odds ratio, 1.79; 95% CI, 1.58-2.02; and *TM6SF2*-p.Glu167Lys, P = .04; odds ratio, 1.24; 95% CI, 1.01-1.52).

Discussion | In contrast to data published in 2017 and 2019,[3,4] this study clearly demonstrates that the genetic inhibition of *PCSK9* in humans is not associated with an increased risk of NAFLD in 2 large cohorts. These findings are consistent with the safety data of randomized clinical trials with anti-PCSK9 monoclonal antibodies and inclisiran, which have found no significant changes in liver enzymes.[1,2]

Hepatic adverse effects have been major concerns of other intracellular lipid-lowering therapies, such as mipomersen and lomitapide, which reduce LDL-C by blocking the hepatic secretion of apolipoprotein B (ApoB) and very low-density lipoproteins. In contrast, PCSK9 inhibitors and statins reduce LDL-C by affecting the LDL receptor pathway. In accordance with a neutral effect of PCSK9 inhibition on liver function, statins do not worsen NAFLD and even might exert a beneficial effect on NAFLD progression to NASH.[6] These data suggest that lipid-lowering drugs targeting ApoB secretion present a risk for NAFLD development but that molecules targeting ApoB catabolism do not. As such, the data presented here are reassuring for the hepatic safety of long-term intracellular PCSK9 inhibition and support the potential use of PCSK9 inhibitors in patients with NAFLD who are at increased risk of ASCVD.

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Concept and design: Rimbert, Smati, Le May, Cariou.
Acquisition, analysis, or interpretation of data: Rimbert, Dijk.
Drafting of the manuscript: Rimbert, Dijk, Cariou.
Critical revision of the manuscript for important intellectual content: Rimbert, Smati, Dijk, Le May.
Statistical analysis: Rimbert.
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1. Guedeney P, Giustino G, Sorrentino S, et al. Efficacy and safety of alirocumab and evolocumab: a systematic review and meta-analysis of randomized controlled trials. *Eur Heart J*. 2019;ehz430. doi:10.1093/eurheartj/ehz430

2. Ray KK, Wright RS, Kallend D, et al; ORION-10 and ORION-11 Investigators. Two phase 3 trials of inclisiran in patients with elevated LDL cholesterol. *N Engl J Med*. 2020;382(16):1507-1519. doi:10.1056/NEJMoa1912387

3. Lebeau PF, Byun JH, Platko K, et al. *Pcsk9* knockout exacerbates diet-induced non-alcoholic steatohepatitis, fibrosis and liver injury in mice. *JHEP Rep*. 2019;1(6):418-429. doi:10.1016/j.jhepr.2019.10.009

4. Baragetti A, Balzarotti G, Grigore L, et al. PCSK9 deficiency results in increased ectopic fat accumulation in experimental models and in humans. *Eur J Prev Cardiol*. 2017;24(17):1870-1877. doi:10.1177/2047487317724342

5. Namjou B, Lingren T, Huang Y, et al. GWAS and enrichment analyses of non-alcoholic fatty liver disease identify new trait-associated genes and pathways across eMERGE Network. *BMC Med*. 2019 17;17(1):135.

6. Dongiovanni P, Petta S, Mannisto V, et al. Statin use and non-alcoholic steatohepatitis in at risk individuals. *J Hepatol*. 2015;63(3):705-712. doi:10.1016/j.jhep.2015.05.006