



Effect of Loss-of-Function Genetic Variants in *PCSK9* on Glycemic Traits, Neurocognitive Impairment, and Hepatobiliary Function

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OBJECTIVE

To evaluate the association between *PCSK9* predicted loss-of-function (pLoF) variants and glycemic traits, hepatobiliary function, and neurocognitive traits.

RESEARCH DESIGN AND METHODS

We identified carriers of *PCSK9* pLoF variants in UK Biobank exome sequencing data. We assessed the aggregate effects of these variants on lipid and lipoprotein traits, which served as a positive control. Association of *PCSK9* pLoF carrier status and glycemic traits, hepatobiliary function, and neurocognitive traits was then evaluated as a measure for adverse effects.

RESULTS

We identified 374 individuals carrying one of 41 unique *PCSK9* pLoF variants. As expected, we found that *PCSK9* pLoF carriers had significantly lower LDL cholesterol C levels ($P = 7.4 \times 10^{-55}$) and apolipoprotein B levels ($P = 7.6 \times 10^{-50}$) than did noncarriers. However, we found no significant associations between pLoF carrier status and glycemic traits, hepatobiliary function, and neurocognitive traits ($P > 0.05$).

CONCLUSIONS

Our results do not support adverse effects of *PCSK9* pLoF variants on glycemic traits, hepatobiliary function, or neurocognitive traits.

Results from genetic studies and clinical trials have led to concerns regarding the long-term safety of *PCSK9* inhibitors (1–5). Studying predicted loss-of-function (pLoF) variants, predicted to largely or entirely abolish the function of affected gene products, can provide insights into the long-term safety of *PCSK9* inhibition. In the present study, we examined the effect of *PCSK9* pLoF variants on glycemic traits, hepatobiliary function, and neurocognitive traits, using UK Biobank (UKB) exome sequencing data from ~170,000 European individuals.

RESEARCH DESIGN AND METHODS

Variant Annotation

We used exome sequenced data, provided by the UKB, for *PCSK9* from ~170,000 individuals of European ancestry. The definition of ancestry is provided in the Supplementary Material. Detailed information on exome sequencing methodology,

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alignment, variant calling, and annotation has been described previously (6). We filtered variants with a genotype quality of <20 , genotype depth of <10 , missing genotypes >0.1 , and minor allele frequency >0.01 . Variants were annotated using SnpEff (7). Variants in *PCSK9* were identified via positional intersection with Ensembl transcript (ENSG00000169174). We defined pLoF variants as follows: single nucleotide variations (SNVs) leading to loss of a start codon, loss of a stop codon, or to a premature stop codon; open-reading-frame shifting insertions and deletions leading to the formation of a premature stop codon; and SNVs or insertions and deletions disrupting canonical splice acceptor or donor sites. We prioritized Sequence Ontology standardized terms (<https://www.sequenceontology.org/>) with putative high impact. Furthermore, we evaluated splice-site region variants with dbcsSNV (8). AdaBoost and Random Forest scores >0.9 were set as splice-altering effects and classified as pLoF variants. We calculated the mean coverage depth for each exon in *PCSK9* using a random subset of 2,000 individuals, described in detail in Supplementary Material.

Outcome Definitions

Our outcomes of interest were lipids (namely, LDL cholesterol [LDL-C], HDL cholesterol, triglycerides) and lipoprotein traits (i.e., apolipoprotein A, apolipoprotein B, and lipoprotein(a)); glycemic traits (i.e., hemoglobin A_{1c} and blood glucose) and type 2 diabetes (T2D); hepatobiliary function (namely, ALT, γ -glutamyltransferase, bilirubin); and neurocognitive tests (specifically, a trail-making test, numeric memory, and fluid intelligence score) and brain structure (i.e., gray matter volume and white matter volume, obtained from brain MRI data). Details on UKB data fields that were used to define outcomes are provided in Supplementary Table 1.

Statistical Analysis

Association tests aggregating rare variants in *PCSK9*, using the Set Mixed Model Association Test, were performed for each trait separately to assess the aggregate effect of rare pLoF variants within *PCSK9* and associated regulatory regions (9). First, we tested the

association between *PCSK9* pLoF carrier status and lipid and lipoprotein traits, which served as a positive control. We then evaluated the association of *PCSK9* pLoF-variant carrier status and hepatobiliary function, glycemic traits, and neurocognitive traits as measures for adverse effects. Non-normally distributed variables were log-transformed. All models were adjusted for age at enrollment, sex, assessment center, and genetic ancestry (as quantified by the first five principal components). We also assessed the association between the five most abundant variants (≥ 20 carriers) and the listed outcomes to test whether results were concordant across variants.

Sensitivity Analysis

First, because individuals enrolled in the *PCSK9* inhibitor landmark trials were patients with established atherosclerotic cardiovascular disease and LDL-C levels outside clinically recommended targets, we investigated whether the adverse effects of lifelong *PCSK9* inhibition through pLoF variants differed across tiers of genetically determined LDL-C and coronary artery disease (CAD) risk by using polygenic risk score (PRS) analysis (described in more detail in the Supplementary Material). Second, in the study by Ference et al. (3), the risk of T2D was reported to be confined to patients at clinically high risk for developing T2D; therefore, we analogously tested whether the association between pLoF variants and glycemic traits differed across PRS quintiles.

RESULTS

We identified 374 individuals carrying one of 41 unique *PCSK9* pLoF variants (Supplementary Table 2), equating to a carrier frequency of 0.2%. We found that *PCSK9* pLoF carriers had significantly lower LDL-C ($P = 7.4 \times 10^{-55}$) and apolipoprotein B ($P = 7.6 \times 10^{-50}$) levels compared with noncarriers (Table 1). Also, *PCSK9* pLoF-variant carriers had significantly higher apolipoprotein A levels compared with noncarriers ($P = 0.016$).

Next, we evaluated the relationship of *PCSK9* pLoF-variant carriers and potential adverse effects. We found no significant difference between carrier status, glycemic traits, hepatobiliary function, neurocognitive function, or

brain structure (all $P > 0.05$). Nor did we find any significant association with T2D (odds ratio 1.15; 95% CI 0.79–1.68; $P = 0.467$). Results are summarized in Table 1. Individually, we also found concordant effects across the five most abundant pLoF variants (Supplementary Table 3). In addition, the effect of pLoF variants did not differ across PRS quintiles for LDL-C, CAD, or T2D (for all, P for interaction > 0.05 ; Supplementary Results and Supplementary Tables 4–6).

CONCLUSIONS

For this work, we leveraged large-scale exome sequencing data from $\sim 170,000$ European individuals and used *PCSK9* pLoF variants as genetic proxies for the effect of anti-*PCSK9* antibodies on lipid and lipoprotein traits, glucose traits, hepatobiliary function, and neurocognitive function. We found that *PCSK9* pLoF-variant carriers exhibit lipid changes that mirror those observed in randomized controlled trials, a finding that supports the validity of our approach. However, as opposed to some previous reports, we found that lifelong *PCSK9* inhibition was not associated with any detrimental effects on glucose metabolism or neurocognitive or hepatobiliary functions, which is in line with shorter-term safety data from clinical trials on *PCSK9* monoclonal antibodies.

Conflicting evidence exists about the role of *PCSK9* inhibition in disruption of pancreatic islet function. Safety concerns were mainly spurred by the results of three genetic studies that showed selected common genetic variants of *PCSK9* were associated with increased fasting glucose levels (1) and development of overt T2D (1–3). On the basis of these findings, subsequent landmark trials evaluated the effects of *PCSK9* inhibitors on glycemic markers and incidence of T2D (10,11). In none of these trials was an increased risk of new-onset T2D with *PCSK9* inhibitors shown (10,11). Nor did they report significant differences in levels of HbA_{1c} or fasting glucose levels between individuals who received *PCSK9* inhibitors compared with those who received placebo (10). Findings from the present study, in which we looked at pLoF variants with larger functional effects compared with prior genetic studies, are in line with results from these shorter-term clinical trials.

Table 1—Association between PCSK9 predicted loss-of-function variants and lipid and lipoprotein traits, glycemic traits, hepatobiliary, and neurocognitive function

Trait	Noncarriers		Carriers of PCSK9 loss-of-function variants		P
	No. of participants	Median level (IQR)	No. of participants	Median level (IQR)	
Lipid and lipoprotein trait					
LDL cholesterol, mmol/L	165,794	3.53 (2.96–4.13)	347	2.85 (2.36–3.35)	7.4×10^{-55}
HDL cholesterol, mmol/L	152,694	1.41 (1.18–1.69)	324	1.42 (1.17–1.74)	0.279
Triglyceride, mmol/L	166,261	1.49 (1.05–2.14)	355	1.38 (0.95–2.17)	0.159
Apolipoprotein A, g/L	151,775	1.52 (1.36–1.71)	324	1.55 (1.41–1.75)	0.016
Apolipoprotein B, g/L	166,267	1.02 (0.87–1.18)	345	0.83 (0.69–0.98)	7.6×10^{-50}
Lipoprotein A, nmol/L	132,424	20.0 (9.3–59.7)	262	18.2 (9.7–68.4)	0.984
Glycemic trait					
Blood glucose, mmol/L	152,876	4.94 (4.62–5.31)	326	4.90 (4.57–5.24)	0.585
HbA _{1c} , mmol/mol	166,742	35.1 (32.7–37.6)	354	35.1 (32.7–37.8)	0.443
HbA _{1c} , %	166,742	5.4 (5.1–5.6)	354	5.4 (5.1–5.6)	0.443
Hepatobiliary trait					
ALT, units/L	166,352	20.1 (15.4–27.3)	355	19.6 (15.0–28.2)	0.648
γ -glutamyltransferase, units/L	166,321	26.0 (18.4–40.3)	355	25.8 (18.0–38.2)	0.847
Bilirubin, μ mol/L	165,742	8.06 (6.44–10.40)	354	7.80 (6.51–9.96)	0.608
Neurocognitive function					
Trail-making test	39,952	35.6 (29.2–45.1)	94	35.5 (29.7–43.5)	0.847
Fluid intelligence score	47,314	7.00 (5.00–8.00)	109	6.00 (5.00–8.00)	0.585
Numeric memory	42,687	7.00 (6.00–8.00)	100	7.00 (6.00–8.00)	0.893
Gray matter volume, mL	19,565	793.1 (760.6–826.5)	40	805.6 (784.4–838.9)	0.533
White matter volume, mL	19,565	703.7 (676.7–731.5)	40	706.9 (687.5–731.3)	0.622

P values were obtained using the Efficient Variant-Set Mixed Model Association Test, after adjustment for age, sex, assessment center, and the first five principal components of ancestry. A genetic-relatedness matrix was included as a random-effects covariate. Values for triglycerides, lipoprotein A, ALT, γ -glutamyltransferase, bilirubin, HbA_{1c}, glucose levels, and trail-making test were log-transformed before association testing to obtain normality of residuals. IQR, interquartile range.

Concerns regarding adverse neurocognitive events also were raised in prior trials (4,5). However, these were refuted by the subsequent EBBINGHAUS trial, in which researchers found no difference in cognitive function between participants randomized to receive evolocumab versus placebo (12). Also, in the REGARDs study, in which Black participants with PCSK9 pLoF variants were studied, researchers did not report any association between carrier status and neurocognitive adverse effects (13). Our results complement the results from the EBBINGHAUS trial and extend the lack of association between PCSK9 pLoF variants and neurocognitive function to a European population.

Last, on the basis of findings from preclinical studies in which knockouts of PCSK9 in mice exhibited liver steatosis (14), there are concerns regarding the long-term hepatobiliary safety of PCSK9 inhibition in humans. Our long-term results are in line with safety data from the shorter-term trials (4,5) that indicate that a partial to complete reduction of the PCSK9 protein in humans is unlikely

to result in the severe phenotypes observed in model organisms.

Our study must be interpreted within the context of its limitations. It should be taken into consideration that the analyses were mainly restricted to biomarkers as proxies for drug efficacy and toxicity, and evaluation of harder end points requires even larger data sets. Biomarkers were measured in the non-fasting state, which potentially adds residual error to the models, thereby reducing statistical power. Additional studies are needed to determine the effect of PCSK9 on biomarkers in fasted participants. Participants recruited for this study were between the ages of 40 and 69 years, which increased the risk of survival bias. The low response rate (5.5%) to the UKB recruitment may have introduced a healthy responder bias to the analyses. However, none of our sensitivity analyses indicated that PCSK9 pLoF variants conferred any differential safety risk in people at genetically predicted high risk of T2D, CAD, or high LDL-C levels. A lifelong pLoF of PCSK9 may not be equivalent to

therapeutic inactivation later in life, if bio-

logical compensation occurs. Therefore, more clinical trials with longer follow-up may be needed to definitely confirm these findings.

In conclusion, PCSK9 pLoF variants were not associated with impaired glucose metabolism, neurocognitive function, or hepatobiliary function.

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Author Contributions. J.G. is the guarantor of this study and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. J.G. and G.A. researched data and wrote the manuscript. H.B. and M.S.O. contributed to the discussion and reviewed and edited the manuscript.

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