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Phenome-wide association analysis of LDL-cholesterol lowering genetic variants in PCSK9

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Abstract

Background: We characterised the phenotypic consequence of genetic variation at the PCSK9 locus and compared findings with recent trials of pharmacological inhibitors of PCSK9.

Methods: Published and individual participant level data (300,000+ participants) were combined to construct a weighted PCSK9 gene-centric score (GS). Seventeen randomized placebo controlled PCSK9 inhibitor trials were included, providing data on 79,578 participants. Results were scaled to a one mmol/L lower LDL-C concentration.

Results: The PCSK9 GS (comprising 4 SNPs) associations with plasma lipid and apolipoprotein levels were consistent in direction with treatment effects. The GS odds ratio (OR) for myocardial infarction (MI) was 0.53 (95% CI 0.42; 0.68), compared to a PCSK9 inhibitor effect of 0.90 (95% CI 0.86; 0.93). For ischemic stroke ORs were 0.84 (95% CI 0.57; 1.22) for the GS, compared to 0.85 (95% CI 0.78; 0.93) in the drug trials. ORs with type 2 diabetes mellitus (T2DM) were 1.29 (95% CI 1.11; 1.50) for the GS, as compared to 1.00 (95% CI 0.96; 1.04) for incident T2DM in PCSK9 inhibitor trials. No genetic associations were observed for cancer, heart failure, atrial fibrillation, chronic obstructive pulmonary disease, or Alzheimer’s disease – outcomes for which large-scale trial data were unavailable.

Conclusions: Genetic variation at the PCSK9 locus recapitulates the effects of therapeutic inhibition of PCSK9 on major blood lipid fractions and MI. While indicating an increased risk of T2DM, no other possible safety concerns were shown; although precision was moderate.

Keywords: Genetic association studies, Mendelian randomisation, LDL-cholesterol, Phenome-wide association scan

Background

Statins and ezetimibe reduce the risk of major coronary events and ischemic stroke via lowering of low density lipoprotein-cholesterol (LDL-C) [1–3]. Loss-of-function mutations in PCSK9 are associated with lower LDL-C and a reduced risk of coronary heart disease (CHD) [4, 5]. Antibodies (mAbs) inhibiting PCSK9, reduce LDL-C in patients with hypercholesterolemia, and received market access in 2015. The FOURIER and ODYSSEY OUTCOMES trials tested the efficacy of PCSK9-inhibition versus placebo on the background of statin treatment and both found that PCSK9 inhibition led to a 15% relative risk reduction of major vascular events in patients with established CVD and recent acute coronary syndrome over a median follow up of 2.2 to 2.8 years [6, 7].

Evidence is limited on the effect of PCSK9 inhibition on clinical outcomes, and on safety outcomes that might only become apparent with prolonged use. Nor is evidence available on the efficacy and safety of PCSK9 inhibitors in subjects other than the high-risk patients studied in trials. Mendelian randomisation for target validation uses naturally-occurring variation in a gene encoding a drug target to identify mechanism-based consequences of pharmacological modification of the same target [8]. For example, previous studies showed that variants in HMGCR, encoding the target for statins, were associated with lower concentrations of LDL-C and lower risk of coronary heart disease (CHD), while confirming the on-target nature of the effect of statins on higher body weight and higher risk of type 2 diabetes (T2DM) [9].

We characterised the phenotypic consequences of genetic variation at PCSK9 in a large, general population sample focussing on therapeutically relevant biomarkers, cardiovascular disease (CVD), individual CVD components and non-CVD outcomes such as cancer, Alzheimer’s disease, and chronic obstructive pulmonary disease (COPD). Effect estimates from the genetic analysis were compared to those from intervention trials where the outcomes under evaluation overlapped.

Methods

We summarise methods briefly here as they have been previously described in detail [14].

Genetic variant selection

SNPs rs11583680 (minor allele frequency [MAF] = 0.14), rs11591147 (MAF = 0.01), rs2479409 (MAF = 0.36) and rs11206510 (MAF = 0.17) were selected as genetic instruments at the PCSK9 locus based on the following criteria: (1) an LDL-C association as reported by the Global Lipids Genetics Consortium (GLGC) [15]; (2) low pairwise linkage
Individual participant-level and summary-level data

Participating studies (Additional file 1: Table S2) provided analyses of individual participant-level data (IPD) based on a common analysis script (available from AFS), submitting summary estimates to the UCL analysis centre. These data were supplemented with public domain data from relevant genetic consortia (Additional file 1: Table S1). Studies contributing summary estimates to genetic consortia were excluded from the IPD component of the analysis to avoid duplication.

Biomarker data were collected on the major routinely measured blood lipids (LDL-C, HDL-C, triglycerides [TG], total cholesterol [TC]); apolipoproteins A1 [ApoA1] and B [ApoB], and nominal lipoprotein (Lp) (a); systolic (SBP) and diastolic (DBP) blood pressure; inflammation markers C-reactive protein (CRP), interleukin-6 (IL-6), and fibrinogen; haemoglobin; glycated haemoglobin (HbA1c); liver enzymes gamma-glutamyltransferase (GGT), alanine aminotransferase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP); serum creatinine, and cognitive function (standardized to mean 0, and standard deviation 1, see Additional file 1: Table S5).

We focussed on individual clinical endpoints, rather than composites, which have been assessed in outcome trials, as well as disease end-points commonly seen in patients likely to be eligible for PCSK9 inhibitor treatment. Ischemic CVD endpoints studied were myocardial infarction (MI), ischemic stroke, revascularization, and angina. The following non-ischemic CVD events were considered: haemorrhagic stroke, heart failure, and atrial fibrillation. Non-CVD outcome data was collected on common chronic diseases: COPD, any cancer (including those of the breast, prostate, colon and lung), Alzheimer’s disease, and T2DM. Study endpoints and biomarker were chosen based on a combination of 1) available sample size, 2) clinical relevance, and 3) evaluation in RCTs of PCSK9 inhibitors, as per Schmidt et al. 2017 [6]. Briefly, systematic searches were performed using the Cochrane Central Register of Controlled Trials (CENTRAL), MEDLINE, Embase, Web of Science registries, Clinicaltrials.gov and the International Clinical Trials Registry Platform databases. Data from placebo controlled trials were extracted and combined using the inverse variance weighted method for fixed effect meta-analysis. Study-specific associations were excluded if the SNP was not in Hardy-Weinberg equilibrium (see Additional file 1: Table S4, based on a Holm-Bonferroni alpha criterion), with no variants failing this test. We estimated the effect at the PCSK9 locus by combining all four SNPs in a gene centric score (GS) as the inverse variance weighted effect of the 4 variants, that were subsequently scaled by the inverse variance weighted effect on LDL-C.

We did not a priori hypothesize on the likelihood of PCSK9 being associated with any of the available phenotypes. Specific cancer sites evaluated here: chronic lymphocytic leukaemia, multiple myeloma, Hodgkin, meningioma, glioma, melanoma, colorectal cancer, prostate cancer, breast cancer, lung adenocarcinoma, and small-cell lung cancer.

Finally, aggregated trial data on the effect of monoclonal PCSK9 (13 alirocumab trials, and 4 evolocumab trials) inhibitors were compared to placebo for MI, revascularization, ischemic or haemorrhagic stroke, cancer, and T2DM abstracted from the Cochrane systematic review [6, 17], with the addition of the OUTCOMES alirocumab trial published afterwards [18]. We compared effects on biomarkers and clinical endpoints common to both the genetic analysis and trials.

Statistical analyses

In all analyses, we assumed an additive allelic effect with genotypes coded as 0, 1 and 2, corresponding to the number of LDL-C lowering alleles; model comparison tests did not show signs of non-additivity [14]. Continuous biomarkers were analysed using linear regression and binary endpoints using logistic regression. Study-specific associations were pooled for each SNP using the inverse variance weighted method for fixed effect meta-analysis. Study-specific associations were excluded if the SNP was not in Hardy-Weinberg equilibrium (see Additional file 1: Table S4, based on a Holm-Bonferroni alpha criterion), with no variants failing this test. We estimated the effect at the PCSK9 locus by combining all four SNPs in a gene centric score (GS) as the inverse variance weighted effect of the 4 variants, that were subsequently scaled by the inverse variance weighted effect on LDL-C.

Trial data were assembled as per Schmidt et al. 2017 [6]. Results are presented as mean differences (MD) or odds ratios (OR) with 95% confidence intervals (CI). Analyses were conducted using the statistical programme R version 3.4.1 [19]. For study specific estimates please contact AFS.

Results

Participant level data were available from up to 246,355 individuals, and were supplemented by summary effect estimates from data repositories, resulting in a sample size of 320,170 individuals, including 95,865 cases of MI, 16,437 stroke, 11,920 ischemic stroke, 51,623 T2DM, 54,702 cancer, 25,630 Alzheimer’s disease and 12,412 of COPD.
Lipid and apolipoprotein associations

As reported previously [14], the four PCSK9 SNPs were associated with lower LDL-C blood concentrations ranging from $-0.02 \text{ mmol/L}$ (95% CI $-0.03$, $-0.02$) per allele for rs11583680 to $-0.34 \text{ mmol/L}$ (95% CI $-0.36$, $-0.32$) for rs11591147 (See Additional file 2: Figure S1). PCSK9 SNPs associated with a lower LDL-C concentration were also associated with lower concentrations of apolipoprotein B proportionate to the LDL-C association.

Associations of the GS with the other lipids or apolipoproteins, scaled to a 1 mmol/L lower LDL-C were (Table 1): $0.05 \text{ mmol/L}$ (95% CI $0.02$, $0.07$) for HDL-C, $-0.07 \text{ mmol/L}$ (95% CI $-0.12$, $-0.01$) for TG, $-0.20 \text{ g/L}$ (95% CI $-0.25$, $-0.18$) for ApoB, $0.02 \text{ g/L}$ (95% CI $-0.01$, $0.06$) for ApoA1, and $-4.12 \text{ mg/dL}$ (95% CI $-8.62$, $0.38$) for Lp(a).

The associations of the PCSK9 GS with blood-based lipid markers were directionally concordant with effects from treatment trials of therapeutic inhibition of PCSK9 (Fig. 1).

Table 1 Biomarker associations of a PCSK9 gene centric score, effect presented as mean difference (MD) with 95% confidence interval in brackets with the effects scaled to a 1 mmol/L decrease in LDL-C

| Biomarker               | Total sample size | MD (95% CI)     |
|-------------------------|-------------------|-----------------|
| Lipids related biomarkers |                   |                 |
| HDL-C in mmol/L         | 314,078           | 0.05 (0.02; 0.07) |
| TG in mmol/L            | 298,069           | $-0.07 (-0.12; -0.01)$ |
| TC in mmol/L            | 320,170           | $-1.06 (-1.12; -1.00)$ |
| ApoA1 in g/L            | 55,477            | 0.02 $(-0.01; 0.06)$ |
| ApoB in g/L             | 54,643            | $-0.20 (-0.25; -0.18)$ |
| LP [a] in mg/dL         | 21,181            | $-4.12 (-8.62; 0.38)$ |
| Safety related biomarkers |                   |                 |
| SBP in mmHg             | 182,487           | 0.03 $(-0.05; 0.10)$ |
| DBP in mmHg             | 182,497           | 0.08 $(-0.001; 0.15)$ |
| CRP in log (mg/L)       | 91,990            | 0.03 $(-0.07; 0.14)$ |
| IL-6 in log (pg/mL)     | 22,370            | $-0.08 (-0.21; 0.04)$ |
| GGT in log (IU/L)       | 69,488            | 0.03 $(-0.04; 0.10)$ |
| Fibrinogen in log(g/dL) | 63,288            | 0.02 $(-0.01; 0.04)$ |
| Hemoglobin in g/L       | 52,109            | 1.16 $(-0.38; 2.70)$ |
| ALT in log (IU/L)       | 83,223            | 0.03 $(-0.02; 0.08)$ |
| AST in log (IU/L)       | 49,556            | 0.01 $(-0.03; 0.05)$ |
| ALP in log (IU/L)       | 60,222            | $-0.06 (-0.09; -0.02)$ |
| Creatinine in umol/L    | 100,206           | 0.06 $(-1.43; 1.55)$ |

Nota bene, TG triglycerides, TC Total cholesterol, ApoA1 Apolipoprotein A1, ApoB Apolipoprotein B, LP Lipoprotein a, SBP Systolic blood pressure, DBP Diastolic blood pressure, CRP C-reactive protein, IL-6 Interleukin-6, GGT Gamma-glutamyltransferase, ALT Alanine transaminase, AST Aspartate transaminase, ALP Alkaline phosphatase

Genetic associations with other biochemical and physiological measures

The GS estimates with SBP and DBP were $0.03 \text{ mmHg}$ (95% CI $-0.05$, $0.10$) and $0.08 \text{ mmHg}$ (95% CI $0.0001$, $0.15$), respectively, per 1 mmol/L lower LDL-C. The PCSK9 GS was associated with nominally lower ALP (IU/L) $-0.06$ (95% CI $-0.09$, $-0.02$), but not with other liver enzymes (Table 1).

Genetic associations with ischemic cardiovascular events

The PCSK9 GS was associated with a lower risk of MI (OR 0.53; 95% CI 0.42; 0.68; 95,865 cases), which was directionally consistent with results from placebo-controlled PCSK9 inhibition trials: OR 0.90 (95% CI 0.86, 0.93), with both estimates scaled to a 1 mmol/L lower LDL-C (mmol/L). Results are pooled using a fixed effect model. Trial estimates are based on the systematic review by Schmidt et al 2017 [6, 17].

The PCSK9 GS association with coronary revascularization (OR 0.75 95% CI 0.44; 1.27) was directionally consistent with the PCSK9 inhibitor trials (OR 0.90; 95% CI 0.86, 0.93) (Fig. 3).
Genetic associations with non-ischemic cardiovascular disease

The point estimate for the GS association with hemorrhagic stroke (Fig. 2), OR 1.29 (95% CI 0.76, 2.19), was discordant to the estimate from PCSK9 inhibitor trials (OR 0.96 95% CI 0.75; 1.23) (Fig. 3), although the confidence intervals overlapped. Comparing the association of PCSK9 GS with hemorrhagic and ischemic stroke indicated the GS had a differential effect (p-value = 0.02). No PCSK9 GS association was observed with atrial fibrillation (OR 0.92 95% CI 0.72; 1.18; 41,485 cases), or heart failure (OR 1.06 95% CI 0.48; 2.32; 1803 cases) (Fig. 2).

Associations with non-cardiovascular disease and related biomarkers

The PCSK9 GS was not associated with the risk of any cancer (OR 0.97: 95%CI 0.81; 1.17; 54,702 cases, see Fig. 4), nor with any of 12 specific types of cancer (Additional file 2: Figure S2). We did not observe an association with either Alzheimer’s disease or cognitive performance: for Alzheimer’s the OR was 0.91 (95% CI 0.55, 1.51) and for cognition (per standard deviation) -0.03 (95% CI -0.22, 0.16). As reported before [14] the GS was associated with T2DM (OR 1.29 95% CI 1.11; 1.50) (Fig. 4), higher body weight (1.03 kg, 95% CI 0.24, 1.82), waist to hip ratio 0.006 (95% CI 0.003, 0.011) and fasting glucose 0.09 mmol/L (95% CI 0.02, 0.15). The OR for COPD was 0.89 (95% CI 0.67, 1.18).

Discussion

The genetic findings presented here show that variation in PCSK9 is associated with lower circulating LDL-C and apoB concentrations, lower risk of MI and, with lesser confidence, the risk of ischemic stroke and coronary revascularization. These effects are consistent in direction to effects observed in PCSK9 inhibitor trial’s [20].

A recent systematic review of trial data [21] indicated PCSK9 inhibition was associated with increased fasting glucose (0.17 as standardized mean difference [SMD] 95% CI 0.14; 0.19) and glycosylated haemoglobin (0.10 SMD 95% CI 0.07, 0.12, 21), although these associations were dependent on the inclusion of the terminated bococizumab trials. Recently we, and others, showed natural genetic variation PCSK9 was associated with elevated fasting glucose and T2DM [14, 22, 23] and that variation at other LDL-C-associated loci also influence risk of T2DM [24, 25]. However, the FOURIER and ODYSSEY OUTCOMES trials, the largest treatment trials of PCSK9 inhibitors to date, did not find an association with risk of incident T2DM, at a median follow up of 2.2 and 2.8 years respectively. It is possible this reflects a genuine discordance between the findings from trials and genetic analyses. Alternatively, the exposure durations in the two largest trials may simply have been too short for subjects to develop T2DM. The risk increasing effect of statins on T2DM was only apparent after conducting a
The available trial data showed PCSK9 inhibitors had a similar effect on MI (OR 0.90, 95% CI 0.86; 0.93) and ischemic stroke (OR 0.85 95% CI 0.78; 0.93). By contrast, the genetic analysis indicated a directionally concordant, but larger effect on MI (OR 0.53; 95% CI 0.42; 0.68) than ischemic stroke, (OR 0.84 95% CI 0.57; 1.22). The genetic analysis was, however, based on only 11,920 stroke cases, about one-fifth of the number of cases available for the genetic analysis of MI and as such confidence interval overlapped. We did observe a differential association between PCSK9 SNPs and ischemic and hemorrhagic stroke (interaction p-value = 0.02). Findings from statin trials previously suggested LDL-C lowering through inhibition of HMG-coA reductase is associated with a reduced risk of ischemic but potentially increased risk of hemorrhagic stroke [30–32]. Our findings suggest that a different effect on ischemic and hemorrhagic stroke subtypes may be eventually identified for PCSK9 inhibitors.

Despite previous concerns about a potential effect of this class of drugs on cognition [33], the genetic analysis did not reveal a significant association of the PCSK9 variants with cognitive function or Alzheimer’s disease, nor with COPD or cancer, though this does not preclude an effect on such outcomes from drug treatment given in later life. While we explored the associations with any cancer (54,702 events) as well as individual cancer sites (Additional file 2: Figure S2), we did not have data on some clinically relevant cancer types such as endometrial cancer.

This neutral effect on cognition has been recently reported by the EBBINGHAUS study, nested within the FOURIER trial, which reported a non-significant PCSK9 inhibitor effect on multiple measures of cognition confirming (using a non-inferiority design) an absence of effect [33]; it should be noted that similar to the FOURIER, the EBBINGHAUS follow-up time was limited. The absence of an effect on cognition during PCSK9 inhibitor treatment was also observed in the ODYSSEY OUTCOMES trial, which had a median follow-up [7] of 2.8 years.

Drugs (even apparently specific monoclonal antibodies) can exert actions on more than one protein if such targets belong to a family of structurally similar proteins. PCSK9, for example, is one of nine related proprotein convertases [34]. Such ‘off-target’ actions, whether beneficial or deleterious, would not be shared by variants in the gene encoding the target of interest. In addition, monoclonal antibodies prevent interaction between circulating PCSK9 and LDL-receptor and should not, in theory, influence any intracellular action of the protein [35].

Genetic association studies of the type conducted here tend to examine the risk of a first clinical event, whereas
clinical trials such as ODYSSEY OUTCOMES focus on patients with established disease, where mechanisms may be modified. Proteins influencing the risk of a first event may also influence the risk of subsequent events, as observed in the case of the target of statin drugs that are effective in both primary and secondary prevention [1]. For this and other reasons [36–38], examination of the effects of PCSK9 variants on the risk of subsequent CHD events in patients with established coronary atherosclerosis is the subject of a separate analysis led by the GENIUS-CHD consortium [38].

Conclusions

PCSK9 SNPs associated with lower LDL-C predict a substantial reduction in the risk of MI and concordant associations with a reduction in risk of ischemic stroke, but with a modestly increased risk of T2DM. In this preliminary analysis we did not observe associations with other non-cardiovascular safety outcomes such as cancer, COPD, Alzheimer’s disease or atrial fibrillation.

Additional files

| Additional file 1: | Supplemental tables. (XLSX 62 kb) |
|--------------------|-----------------------------------|
| Additional file 2:  | Supplemental figures and study acknowledgments. (PDF 154 kb) |

Abbreviations

ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; ApoA1: Apolipoproteins A1; ApoB: Apolipoproteins B; AST: Aspartate transaminase; CADD: Combined annotation dependent depletion; CHD: Coronary heart disease; CI: Confidence interval; COPD: Chronic obstructive pulmonary disease; CRP: C-reactive protein; CVD: Cardiovascular disease; DBP: Diastolic blood pressure; GGT: Gamma-glutamyltransferase; GLGC: Global lipids genetics Consortium; GSN: Gene-centric score; HbA1c: Glycated haemoglobin; IL-6: Interleukin-6; IPD: Individual participant-level data; LD: Linkage disequilibrium; LDL-C: Low density lipoprotein-cholesterol; LPA: Lipoprotein a; mAbs: Monoclonal antibodies; MAF: Minor allele frequency; MD: Mean difference; MI: Myocardial infarction; OR: Odds ratio; SBP: Systolic blood pressure; SMD: Standardized mean difference; T2DM: Type 2 diabetes mellitus; TC: Total cholesterol; TG: Triglycerides

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Not applicable

Authors’ contributions

AFS, DIS, MWH, RSP, FWA, JPC, BJK, ADH, DP, NS contributed to the idea and design of the study. AFS, DIS, MWH, designed the analysis scripts shared with individual centres. AFS performed the meta-analysis and had access to all the data. The authors jointly drafted the manuscript, and contributed to subsequent critical revisions. All authors have approved the submitted manuscript, and take responsibility for the integrity and the accuracy of the data and presented results.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.
Ethics approval and consent to participate
Local ethics committees for studies contributing data to these analyses granted approval for the work.

Consent for publication
Not applicable

Competing interests
Dr. Holmes has collaborated with Boehminger Ingelheim in research, and in accordance with the policy of the Translational Research Unit and Epidemiological Studies Unit (University of Oxford), did not accept any personal payment. David Preiss consulted for Amgen on a single occasion but, in accordance with the policy of the Clinical Research Unit at University Oxford (University of Oxford), did not accept any personal payment. He is an investigator on a clinical trial of PCSK9 inhibition funded by Amgen. Naveed Sattar consulted for AstraZeneca, Sanofi, and NovoNordisk, and was an investigator on clinical trials of PCSK9 inhibition funded by Amgen. Naveed Sattar has also consulted for Boehminger Ingelheim, Janssen, Eli-Lilly and NovoNordisk. Daniel Swedlow has consulted to Pfizer for work unrelated to this paper. Folkert W. Asselbergs is supported by UCL Hospitals NIHR Biomedical Research Centre. Dr. Patel has received honoraria and speaker fees from Sanofi, Amgen and Bayer. Kees Hovingh or his institution (AMC) received honoraria for consultancy, ad boards, and/or conduct of clinical trials from: AMGEN, Aegerion, Pfizer, AstraZeneca, Sanofi, Regeneron, Kowa, Ionis pharmaceuticals and Cerenis. Bertrand Cariou has received research funding from Pfizer and Sanofi, received honoraria from AstraZeneca, Pierre Fabre, Janssen, Eli-Lilly, MSD Merck Co. and NovoNordisk, Sanofi, and Takeda, and has acted as a consultant/advisory panel member for Amgen, Eli Lilly, Novo-Nordisk, Sanofi, and Regeneron. Andzej Pajak acted as a consultant/advisory panel member for Amgen. Erik Ingeisson is a scientific advisor for Precision Welling and Olink Proteomics for work unrelated to this paper. JCH is a scientific advisor to a clinical trial of PCSK9 inhibition. AE Honoraria: Takeda, BMS, Amgen, Consulting: Takeda, BMS. Amgen, SEH acknowledges BHF funding (PG008/08) and support from the UCL BRC. All other authors declare no competing interests.

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