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**Variation in serum PCSK9, cardiovascular disease risk and an investigation of potential unanticipated effects of PCSK9 inhibition: A GWAS and Mendelian randomization study in the Nord-Trøndelag Health Study, Norway**

**Short title:** Brumpton et al. Effects of variation in serum PCSK9

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Proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors reduce serum low-density lipoprotein cholesterol (LDL-C) by increasing up-take in the liver<sup>1</sup>. While some long-term trials have evaluated their safety, broad investigations of outcomes over the life-time, leveraging genetic variation in serum PCSK9, have seldomly been conducted. We investigated effects of these variants on a range of outcomes to explore unanticipated effects of long-term PCSK9 inhibition.

We linked genotype and phenotype data from 69,422 participants in the population-based Nord-Trøndelag Health Study (HUNT) with hospital records. Coronary heart disease, myocardial infarction, heart failure, venous thrombosis, diabetes, asthma, chronic obstructive pulmonary disease, allergic rhinitis and mood disorders were indicated by ICD-9 or ICD-10 codes recorded between September 1987 and March 2016 or self-report in HUNT2 (1995-1997) or HUNT3 (2006-2008). Using self-report in HUNT, we assessed anxiety, depression, musculoskeletal pain, headaches, pain or stiffness in muscles or joints in at least 3 consecutive months during the last year, gastrointestinal pain, pain in either leg while walking and pain in the legs while at rest.

Blood pressure, height, weight and lung function were measured in HUNT by trained nurses or technicians. Non-fasting serum levels of total cholesterol, high-density lipoprotein cholesterol (HDL-C), triglycerides, glucose and C-reactive protein were measured, and LDL-C was calculated. All-cause mortality was updated from the National Population Registry.

Serum concentrations of PCSK9 were measured in a sub-sample by enzyme immune-assay with antibodies obtained from R&D Systems (Minneapolis, Minnesota) as described elsewhere<sup>2</sup>.

Because of the sensitive nature of the data, requests to access the dataset from qualified researchers may be sent to HUNT ([www.ntnu.edu/hunt](http://www.ntnu.edu/hunt)). The Regional Committee for Ethics in Medical Research approved the study and participants gave informed consent.

We performed a genome-wide association study (GWAS) of serum PCSK9 in 3,697 adults using BOLT-LMM adjusted for sex, age, genotyping batch and four principal components. Serum PCSK9 measurements were rank-transformed to normality. The replication cohort included 5,304 adults from the TwinGene Study recruited between 2004 and 2008<sup>3</sup>. We defined a new genetic risk score for serum PCSK9, and regressed this and an existing score (rs11206510, rs2479409, rs2149041, rs2479394, rs10888897, rs7552841, rs562556) on a range of outcomes<sup>1</sup>. For comparison, we used LDL-C-associated variants on *HMGCR* (rs12916, rs17238484, rs5909, rs2303152, rs10066707, rs2006760), the target of statins<sup>1</sup>. The associations of PCSK9 and *HMGCR* genetic risk scores with outcomes in HUNT were assessed using linear regression for continuous and logistic regression for binary outcomes, adjusted for age and sex. We performed two-sample Mendelian randomization on 48 outcomes using large biobanks available in MR-Base<sup>4</sup>.

We assessed 11.67 million single nucleotide and indel variants imputed from a combined reference panel including 2,202 low-pass sequenced HUNT genomes and the Haplotype Reference Consortium panel. We identified genome-wide significant associations at two loci – *PCSK9* and *PCSK2*. Three independent genome-wide significant variants were identified, the most strongly associated variant was rs11591147 in *PCSK9* (p.R46L), which was associated with a 1.30 unit decrease in rank-transformed serum PCSK9 ( $P=2.9 \times 10^{-31}$ ) per effect allele (standard

deviation of original PCSK9 measurements=47.5 ng/ml, interquartile range=100.6-154.9), followed by the intronic rs499883 in *PCSK9* ( $\beta=0.19$ ,  $P=2.3 \times 10^{-15}$ ) and rs192265866 in *PCSK2* ( $\beta=-1.05$ ,  $P=2.8 \times 10^{-8}$ ) of which the two variants in *PCSK9* (but not the variant in *PCSK2*) replicated in TwinGene and were used to construct a new genetic risk score for PCSK9.

Our results demonstrated probable causal associations between lower serum PCSK9 and both lower serum LDL-C ( $P=2.8 \times 10^{-43}$ ) and lower risk of coronary heart disease ( $P=8.9 \times 10^{-4}$ ) (Figure A). As analyses within HUNT suggested associations between PCSK9 and HMGCR genetic risk scores and risk of venous thrombosis and asthma, respectively, we investigated associations using summary level data from the INVENT Consortium and GABRIEL<sup>4,5</sup>. Neither associations replicated (venous thrombosis, inverse variance weighted, OR=1.38, 95% CI=0.88, 2.20; asthma, inverse variance weighted, OR=1.13, 95% CI=0.64, 1.98). We did not observe any other convincing associations across a range of cardiovascular, respiratory, mental, diabetes, or pain related outcomes.

Utilising GWAS summary statistics and two-sample MR, the PCSK9 genetic risk score was associated with LDL-C, HDL-C and total cholesterol as expected (Figure B). Moreover, genetically decreased serum PCSK9 was associated with higher father's age at death ( $\beta=0.04$ , 95% CI=0.01, 0.08), parents age at death ( $\beta=0.05$ , 95% CI=0.01, 0.10) and waist-to-hip ratio ( $\beta=0.03$ , 95% CI=0.01, 0.06) (Figure B). Additionally, genetically decreased serum PCSK9 was associated with decreased risk of coronary heart disease (odds ratio [OR]=0.84, 95% CI=0.78, 0.89) and myocardial infarction (OR=0.81, 95% CI=0.70, 0.93), and an increased risk of Alzheimer's disease (OR=1.27, 95% CI=1.04, 1.55) (Figure B).

In summary, we confirmed two independent genetic variants associated with serum PCSK9, and causal associations of lower serum PCSK9 with lower LDL-C and reduced risk of coronary heart disease. Additionally, we did not observe any other consistent unexpected associations when investigating a range of outcomes. The lack of association with a range of potential adverse outcomes is reassuring for long-term use of PCSK9 inhibitors.



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**Disclosure**

None

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**Figure. A**, The association between PCSK9 and HMGCR genetic risk scores and a range of continuous traits (**left**) and diseases or traits (**right**) in the Nord-Trøndelag Health Study, scaled to a genetic risk score-determined 0.259 mmol/L (10 mg/dL) decrease in LDL cholesterol. The PCSK9 genetic risk score is defined from the discovery GWAS in this study. The existing PCSK9 score and HMGCR score are defined and weighted using results for LDL cholesterol associated variants from the Global Lipids Genetics Consortium. **B**, The association between genetically determined serum PCSK9 and a range of continuous traits (Amygdala volume, Birth weight, Body fat, Body mass index, Caudate volume, Chronotype, Depressive symptoms, Father's age at death, HDL cholesterol, Hip circumference, Hippocampus volume, Intracranial volume, LDL cholesterol, Mean platelet volume, Mother's age at death, Neuroticism, Nucleus accumbens volume, Pallidum volume, Parents' age at death, Platelet count, Putamen volume, Sitting height ratio, Sleep duration, Thalamus volume, Top 1 % survival, Total cholesterol, Triglycerides, Waist circumference, Waist-to-hip ratio, Years of schooling) (**left**) and diseases or traits (Alcohol dependence, Alzheimer's disease, Amyotrophic lateral sclerosis, Autism, Bulimia nervosa, Coronary heart disease, Crohn's disease, Eczema, Inflammatory bowel disease, Lung adenocarcinoma, Lung cancer, Multiple sclerosis, Myocardial infarction, Rheumatoid arthritis, Schizophrenia, Squamous cell lung cancer, Type 2 diabetes, Ulcerative colitis) (**right**) in MR-Base. The new PCSK9 genetic risk score had a  $r^2$  and F-statistics of 2.1% and 80.55 for serum PCSK9.

CI, confidence interval; COPD, chronic obstructive pulmonary disease; BMI, body mass index; FEV1, forced expiratory volume in one second; FVC, forced vital capacity; HADS, Hospital Anxiety and Depression Scale; HDL, high-density lipoprotein; HMGCR, 3-hydroxy-3-methylglutaryl-Coenzyme A reductase; LDL, low-density lipoprotein; OR, odds ratio; PCSK9: Proprotein convertase subtilisin/kexin type 9.