Genetic variants associated with low-density lipoprotein cholesterol and systolic blood pressure and the risk of recurrent cardiovascular disease in patients with established vascular disease

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Abstract

Background and aims: Polygenic risk scores (PRS) can be used to quantify the effect of genetic contribution to LDL-cholesterol (LDL-C) and systolic blood pressure (SBP). Several PRS for LDL-C and SBP have been shown to be associated with cardiovascular disease (CVD) in the general population. This study aimed to evaluate the effect of an LDL-C PRS and an SBP PRS on the risk of recurrent CVD in patients with CVD.

Methods: Genotyping was performed in 4,416 patients included in the UCC-SMART study. A weighted LDL-C PRS (279 LDL-C related SNPs) and SBP PRS (425 SBP related SNPs) were calculated. Linear regression models were used to evaluate the relation between both PRSs and LDL-C and SBP. The effects of the LDL-C PRS and SBP PRS, and its combination on the risk of recurrent CVD (stroke, myocardial infarction, and vascular death) were analyzed with Cox proportional-hazard models.

Results: Per SD increase in LDL-C PRS, LDL-C increased by 0.18 mmol/L; 95%CI 0.15–0.21. Per SD increase in SBP PRS, SBP increased by 3.19 mmHg; 95%CI; 2.60–3.78. During a follow-up of 11.7 years (IQR 9.2–15.0) 1,198 recurrent events occurred. Neither the LDL-C nor the SBP PRS were associated with recurrent CVD (HR 1.05 per SD increase in LDL-C PRS; 95%CI; 0.99–1.11 and HR 1.04 per SD increase in SBP PRS; 95%CI 0.98–1.10). The combination of both scores was neither associated with recurrent CVD (HR 1.09; 95%CI 0.93–1.28).

Conclusions: In patients with vascular disease, an LDL-C PRS and SBP PRS, both separately and in combination, were not associated with recurrent CVD.

Keywords: Polygenic risk score, low-density lipoprotein cholesterol, systolic blood pressure, cardiovascular events, secondary prevention

Introduction

Increased low-density lipoprotein cholesterol (LDL-C) and systolic blood pressure (SBP) are among the most important risk factors for the development and progression of cardiovascular disease (1). SBP and LDL-C are highly heritable traits, involving a large set of genes contributing to disease (2). Hundreds of single nucleotide polymorphisms (SNPs) associated with plasma LDL-C and SBP, have been identified through genome-wide association studies (GWAS) and this is still increasing (3-5). These genetic variants represent lifelong exposure to LDL-C or SBP in which the small individual effects of each SNP are assumed to be cumulative. Polygenic risk scores (PRS) aggregate the modest effects of multiple SNPs into a single score as a proxy for lifelong exposure to a given trait (6). As demonstrated earlier, including genetic information in risk models could potentially contribute to the improvement of personalized cardiovascular risk prediction or to the identification of high-risk patients who might benefit from stricter treatment goals through treatments (7-9). Previous studies in the general population showed that a PRS for LDL-C and SBP is associated with an increased risk of incident cardiovascular events (8, 10-12). However, very few studies have reported on the association between such PRSs and recurrent cardiovascular events. One study evaluated the effect of an LDL-C PRS in a selected study population that underwent carotid endarterectomy (13). Treatment with lipid-lowering and antihypertensive medications could modulate the effects of genetic variants on LDL-C and SBP in patients with stable vascular disease. In addition, the effects of these genetic variants on recurrent vascular events may be different compared to first events, because patients with few risk alleles may have other risk factors that caused the first event that also increase the risk of recurrent vascular events (14). The aim of the present study is therefore twofold. First, to replicate the effect of PRSs for known genetic variants associated with LDL-C or SBP on these risk factors within a cohort of patients with

established vascular disease. Second, to evaluate the effect of these PRSs for LDL-C and SBP on the risk of recurrent cardiovascular events in this high-risk patient population.

Methods

Study population

Data from patients enrolled in the Utrecht Cardiovascular Cohort – Second Manifestations of Arterial Disease (UCC-SMART) study were used. The UCC-SMART study is an ongoing, single-center, prospective cohort at the tertiary referral center University Medical Center Utrecht (UMCU) in the Netherlands. Patients aged 18-80 years referred to the UMCU with established cardiovascular disease (coronary artery disease (CAD), cerebrovascular disease (CeVD), peripheral arterial disease (PAD) or abdominal arterial aneurysm (AAA) underwent vascular screening. A description of the study rationale has been published previously (15). The UCC-SMART study was approved by the Medical Ethics Committee of the UMCU, and all patients provided written informed consent prior to inclusion. For the current study, data of patients that were included between September 1996 and August 2010 were used, as these patients were genotyped (n=6,971).

Baseline measurements

At baseline, all patients underwent a standardized vascular screening protocol including a health questionnaire, physical examination, laboratory testing, ankle-branchial index, and an abdominal, aortic and carotid ultrasound. Office blood pressure measurements were performed with automated blood pressure monitors (Iso-Stabil 5; Speidel & Keller, Jungingen, Germany) on the arm with the highest blood pressure. The mean of 3 measurements on that arm was recorded. Smoking, alcohol use, and medication use were self-reported. Lipid-lowering

medication included use of statins, fibrates, bile acid sequestrants or nicotinic acid. Prescription of high intensity statins was defined as atorvastatin ≥40 mg or rosuvastatin ≥20 mg. Antihypertensive medications were grouped based on drug class (angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, beta-blockers, alpha-blockers, calcium antagonists, diuretics, aldosterone antagonists, central acting antihypertensives, direct vasodilators). Type 2 diabetes mellitus (T2DM) was defined as either a referral or self-reported diagnosis of T2DM, or a fasting plasma glucose ≥7 mmol/L at study inclusion with initiation of glucose-lowering treatment within 1 year, or baseline use of hypoglycemic agents or insulin.

Laboratory measurements

Laboratory blood testing was performed in the fasting state. Total cholesterol (TC) and triglycerides (TG) were measured with a commercial enzymatic dry chemistry kit (Johnson & Johnson, New Brunswick, USA). HDL-cholesterol (HDL-C) was measured with a commercial enzymatic kit (Boehringer, Mannheim, Germany) and LDL-cholesterol (LDL-C) was calculated using the Friedewald formula up to triglyceride levels of 9 mmol/L to reduce missing values in this analysis (16, 17). The estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula (18).

Genotyping and quality control

Genotyping of the cohort was performed using the Illumina GSA array. All SNPs went through a thorough quality control (QC) check using PLINK v. 1.9 (19). Genotype imputation has been performed using IMPUTE2 v2.3.0. After imputation 91.3 million SNPs were available. SNPs with an imputation quality (R^2) <0.3 (n=36.8 million), a minor allele frequency below 0.1% (n=71.2 million) and SNPs with a Hardy-Weinberg equilibrium *p*-value $<1 \times 10^{-6}$ (n=90) were

also excluded, resulting in 19.9 million imputed SNPs available. Patients of non-European ancestry (n=543), with low quality genotyping (n=212) or those who were related to each other (n=203) were excluded. In case of the latter, the patient with the latest (most recent) date of inclusion was excluded. Other reasons for exclusion during quality control were samples with likely sample contamination based on high degree of relatedness with other samples (n=37), or when samples were >5 standard deviations from median for inbreeding coefficient (n=32), with a sex mismatch between genotype and phenotype (n=18), and samples without phenotype data available (n=43). Finally, 4,416 patients were available for further analysis.

SNP selection and calculation of the polygenic risk scores

To identify SNPs for both PRSs we first retrieved the most recent (at the time of conducting the analysis) meta-analyses of GWAS describing genetic variants associated with either LDL-C (5) or SBP (3, 4) at genome-wide level of significance ($p < 5x10^{-8}$). From these meta-analyses, a total of 444 SNPs and 616 SNPs were identified as potentially relevant for the construction of each PRS. To remove highly correlated variants, we performed LD pruning on the summary data of these SNPs extracted from the Pan-ancestry genetic analysis of the UK biobank (21) using PLINK v.1.9 (22). To this end we used the '--indep-pairwise 1000 10 0.2' flag in PLINK, meaning we used a window of 1000 SNPs, calculated LD between each pair of SNPs in the window, removed one of a pair of SNPs if the LD was greater than $r^2 = 0.2$, and shifted the window 10 SNPs forward and repeat the procedure. This resulted in a final selection of 279 and 425 SNPs associated with LDL-C and SBP, respectively.

For each patient, two weighted PRSs were calculated by summing the dosages of alternate alleles (labeled as the alternate alleles; ranging from 0 to 2) of an individual patient at each SNP multiplied by the β-coefficient of the respective alternate allele. Because the UCC-SMART

study population is from European descent, we used the β-coefficients from European ancestry sub-analysis of the Pan-UKB. These β-coefficients were adjusted for use of medication (row 4,491 for LDL-C and row 4,519 for SBP) (23). A list of genetic variants and their β-coefficients used to derive both PRSs is provided in **Supplemental table 1a and 1b.**

Follow-up

Follow-up duration was defined as time from inclusion in the cohort until development of first cardiovascular event, death, loss to follow-up or the preselected date of 1 July 2019. From 1996 till 1 July 2019, 360 patients were lost to follow-up (8%). During follow-up patients received questionnaires on hospital admissions and outpatient clinic visits twice a year. If an event was reported, all relevant hospital documents, and laboratory and radiologic findings were collected. All events were audited independently by three physicians of the UCC-SMART endpoint committee. The primary outcome for this study was the combination of non-fatal and fatal vascular events, consisting of non-fatal myocardial infarction (MI), non-fatal stroke and vascular death. Secondary outcomes were the separate components of the composite outcome (non-fatal MI, non-fatal stroke and vascular death). For detailed description of the outcomes see **Supplemental table 2.**

Data analyses

Baseline characteristics are presented in four groups, according to the median of both polygenic risk scores (the distributions of both PRSs are displayed in **Supplementary figure 1**); one reference group with genetically lower LDL-C and SBP (LDL-C PRS $\leq$ median and SBP PRS $\leq$ median), one group with genetically higher SBP (LDL-C PRS $\leq$ median, SBP PRS > median), one group with genetically higher LDL-C (LDL-C PRS > median, SBP PRS $\leq$ median), and

one group with both genetically higher SBP and LDL-C (LDL-C PRS > median, SBP PRS > median). The organization of patients according to both PRSs is provided in **Supplemental figure 2).**

Baseline data are presented as number and percentage for categorical variables, mean ± standard deviation (SD) for normally distributed variables or median with interquartile range (IQR) in case of a skewed distribution. For the association between the LDL-C PRS and LDL-C and the SBP PRS and SBP values, respectively, linear regression models were fitted. Three models were built. The first model was adjusted for age, sex, and the first five principal components. The second model was additionally adjusted for BMI, T2DM, smoking, alcohol use, eGFR, and triglycerides. The third model was additionally adjusted for use of lipid-lowering- or antihypertensive medication. For these analyses the LDL-C - and SBP PRS were standardized. Hence, the beta coefficient corresponds to the change per SD increase in the PRS. In addition, the beta-coefficients derived from the linear regression models were plotted according to quartiles of the LDL-C and SBP PRS.

Cox proportional hazard models were used to determine the relationship between the (standardized) LDL-C PRS and SBP PRS and recurrent events. Linearity of the relationships between LDL-C PRS and SBP PRS with recurrent vascular events was assessed with restricted cubic splines. The Cox proportional hazard assumption was visually checked and confirmed by plotting Schoenfeld residuals against time. Two models were built. The first model was adjusted for age, sex, and the first five principal components. The second model was additionally adjusted for BMI, T2DM, smoking, alcohol use, eGFR, triglycerides, and systolic blood pressure and lipid lowering medication (in model for LDL PRS), or LDL-C and antihypertensive medication (in model for SBP PRS). Additionally, to evaluate potential effect modification between the LDL-C and SBP PRS Cox models were fitted between the combined

LDL-C and SBP PRS groups and recurrent cardiovascular events. To evaluate whether several key characteristics (T2DM, sex, age, type of vascular disease at baseline, and use of lipid-lowering and antihypertensive medication) might modify the association between both PRSs and recurrent vascular events, we included interaction terms into the models.

Several sensitivity analyses were performed. To assess whether a different distribution of patient groups will influence the results, we classified patients according to the highest quintile and decile of both PRSs and compared the hazard of recurrent MACE in those with genetically higher LDL-C and SBP (top quintiles and top deciles of both PRSs) versus all others. Also, to evaluate whether the results were influenced by pleiotropy, we performed a sensitivity analysis by excluding SNPs that were significantly associated with either SBP or LDL-C PRS (*p*-value adjusted for multiple testing = 0.018 for LDL-C and *p*-value adjusted for multiple testing = 0.012 for SBP, Supplemental Tables 7 and 8).

To improve statistical accuracy, missing values of variables of interest [BMI (n=9; 0.2%), smoking status (n=17, 0.4%), eGFR (n=19, 0.4%), triglycerides (n=28, 0.6%), systolic blood pressure (n=9, 0.2%), LDL-C (n=38, 0.9%)] were completed by single regression imputation using predictive mean matching (24). There were no missing values for age, sex, T2DM, lipid-lowering- and antihypertensive medication. All analyses were performed with R statistical software (Version 3.5.1; R foundation for Statistical Computing, Vienna, Austria).

Results

Baseline characteristics

Baseline characteristics of the patients stratified according to the medians of both PRSs are shown in **Table 1**. The mean age was 61 ± 10 years and 75% of the patients were male, 61%

had a history of CAD, 27% of CeVD, 21% of PAD, and 9% of AAA. Compared to the reference group (genetically lower LDL-C and SBP), the group with genetically higher LDL-C and SBP had a higher mean SBP (143 ± 21 mmHg versus 139 ± 20 mmHg) and a higher mean LDL-C (3.02 ± 1.07 mmol/L versus 2.87 ± 1.04 mmol/L). This group also had a higher prescription rate for lipid-lowering (68% versus 59%) and antihypertensive medications (75% versus 70%) compared to the reference group. There were no clinically relevant differences with respect to the other variables at baseline between the four groups.

Relation between polygenic risk scores and traits

LDL-C polygenic risk score and LDL-C

Supplemental table 3 shows that the LDL-C PRS was significantly associated with LDL-C (per SD increase in PRS, LDL-C increased by 0.11 mmol/L; 95% CI 0.08 – 0.14). Additional adjustment for the use of lipid-lowering medication further strengthened this relation (β-coefficient per SD 0.18 mmol/L; 95% CI 0.15 – 0.21). To evaluate whether the effect of the PRS was different in patients with or without lipid-lowering, we added use of lipid-lowering as an interaction term in the model. (p=0.08). **Figure 1** shows mean LDL-C levels according to LDL-C PRS quartiles stratified for use of lipid-lowering medication after adjustment for age, sex, BMI, SBP, smoking, alcohol use, T2DM, eGFR, triglycerides, and the first 5 principal components. Mean LDL-C levels were higher in patients without lipid-lowering medication in all quartiles.

SBP polygenic risk score and SBP

The SBP PRS was significantly associated with SBP, as shown in **Supplemental table 4.** One SD increase in the SBP PRS corresponded to an increment of 3.15 mmHg (95% CI 2.56 – 3.74) in SBP. Additional adjustment for use of antihypertensive medication did not change the results meaningfully (β 3.19; 95% CI 2.60 – 3.78). **Figure 2** shows mean SBP according to SBP PRS quartiles, stratified for use of antihypertensive mediation after adjustment for age, sex, BMI, LDL-C, smoking, alcohol use, T2DM, eGFR, triglycerides, and the first 5 principal components. SBP levels were similar in patients with and without antihypertensive medication indicating that the effect of the SBP does not depend on the use of antihypertensive drugs, which was confirmed by the non-significant interaction between SBP PRS and use of antihypertensive drugs (p = 0.17).

Relation between polygenic risk scores and recurrent cardiovascular events

During a median follow-up of 11.7 years IQR: 9.2 – 15.0 years; 51.991 person-years), the composite outcome (consisting of non-fatal myocardial infarction, non-fatal stroke, and vascular death) occurred in 1,198 patients.

LDL-C polygenic risk score and recurrent cardiovascular events

After adjustment for traditional cardiovascular risk factors including age, sex, BMI, T2DM, smoking, alcohol use, eGFR, triglycerides, SBP, and lipid-lowering medication, the LDL-C PRS was not associated with the risk of recurrent cardiovascular events (hazard ratio (HR) per one SD increase in PRS; 1.05; 95% CI 0.99 – 1.11) **(Table 2)**. There was no interaction with use of lipid-lowering medication (p for interaction=0.39). Also, there was no effect

modification by age, sex, T2DM and type of vascular disease at baseline in the relation between LDL-C PRS and recurrent cardiovascular events (p for all interactions >0.05). Exploratory analyses examining secondary outcomes showed similar results (non-fatal MI (HR 1.05; 95% CI 0.96 - 1.16), non-fatal stroke (HR 1.00; 95% CI 0.90 – 1.12), and vascular death (HR 1.05; 95% CI 0.98 – 1.13) (**Supplemental table 5**).

SBP polygenic risk score and recurrent cardiovascular events

The SBP PRS was not associated with recurrent cardiovascular events (HR 1.04 per one SD increase in PRS; 95% CI; 0.98 – 1.10) **(Table 2)**. The effects were similar in patients with or without antihypertensive mediation (p for interaction=0.79). No interaction was observed with age, sex, T2DM and type of vascular disease at baseline (p for all interactions >0.05). Analyses examining secondary outcomes also found no statistically significant association between the SBP PRS and non-fatal MI (HR 1.03; 95% CI 0.94 – 1.13) and non-fatal stroke (HR 0.99; 95% CI 0.89 – 1.10), but did find a significant association with vascular death (HR 1.11; 95% CI 1.03 – 1.19) (**Supplemental table 5)**.

Combined polygenic risk scores and recurrent cardiovascular events

Patients with a genetically higher LDL-C and SBP experienced 303 recurrent cardiovascular events during follow-up (incidence rate 25.2 per 1,000 person-years). Patients with a genetically lower LDL-C and SBP experienced 295 recurrent cardiovascular events (incidence rate 24.8 per 1,000 person-years). Compared to patients with a genetically lower LDL-C and SBP, there was no statistically significant difference in the risk of recurrent cardiovascular events in patients with a genetically higher LDL-C and SBP (HR 1.09; 95% CI 0.93 – 1.28) **(Table 3)**.

Also, there was no significant difference in the risk of the separate cardiovascular outcomes (non-fatal MI (1.10; 95% CI 0.84 – 1.44), non-fatal stroke (1.02; 95% CI 0.75 – 1.39) and vascular death (1.14; 95% CI 0.93 – 1.40)) when comparing both groups (**Supplemental table 6).**

Sensitivity analyses

Repeating the analyses after classification of patients according to the highest quintile and decile of both PRSs showed comparable results (**Supplemental tables 9-10**). Furthermore, to determine whether the results were influenced by pleiotropy we performed a sensitivity analysis in which we excluded SNPs that were significantly associated with both LDL-C and SBP. For the LDL-C PRS, a total of 81 SNPs were excluded, and for the SBP PRS, a total of 77 SNPs. Exclusion of these SNPs from both PRSs did not change the estimates meaningfully (**Supplemental tables 11 - 14**).

Discussion

In this prospective cohort study of patients with vascular disease, we replicated the association of a PRS for LDL-C and a PRS for SBP with these risk factors, constructed by SNPs identified through the latest large-scale genome-wide association studies. However, no statistically significant association was observed between these PRSs and recurrent cardiovascular events.

Results of the current study are in line with the results from a study that investigated an LDL-C PRS in patients that underwent carotid endarterectomy. This study also found no

association between an LDL-C PRS and recurrent cardiovascular events within a follow-up of 3 years (HR (per one SD increase) 1.03 (95%CI; 0.92 – 1.15)) (13).

To our knowledge, the combined effect of a PRS for LDL-C and a PRS for SBP on cardiovascular events only has been evaluated in apparently healthy individuals enrolled in the UK biobank (10). In contrast to our study, this study found that relatively small absolute differences in combined exposure to genetically lower LDL-C and SBP translated into a large difference in the risk for major coronary events (odds ratio (OR) 0.61 (95% CI 0.59 – 0.64)) (10). Although a direct comparison of PRS effect sizes may be challenging due to use of varying (number of) SNPs and outcomes it remains somewhat notable that the present study found no effect of either PRSs on the risk of recurrent cardiovascular events, also given the abundant evidence on LDL-C and SBP as causal contributors to cardiovascular risk. Several mechanisms may explain why no association was observed in this study.

First, the present study was conducted in a relatively small cohort compared to previous studies evaluating a PRS (10, 11). This may have resulted in limited power to demonstrate a genuine lack of associations, especially when the magnitude of the effect is small. This is supported by the ambivalent results we obtained: both PRSs did not associated with the primary outcome, but we did observe a nominally significant association between the PRS for SBP and the secondary outcome vascular death. Hence, before drawing any definitive conclusions, replication in larger cohorts of patients with vascular disease is needed. Second, index-event bias has been proposed as an explanation for differences in associations of PRS in patients with cardiovascular events compared to patients without prior cardiovascular disease (25). This can be understood by considering the onset of vascular events as the sum of the effect of multiple causal factors. If one important causal risk factor (such as a high genetically determined LDL-C or SBP (reflected in a high LDL-C or SBP PRS)) is already present, less effect of other factors

is required for disease onset. Subsequently, comparing patients with a genetically unfavorable LDL-C and/or SBP profile to patients with a genetically favorable LDL-C and/or SBP profile who already have developed vascular disease, leads to a relatively healthy risk profile in the former compared to the latter and hence a bias of the results towards null. This type of bias is recently investigated in a study using data from the UK biobank (26). The authors demonstrated that associations of a CAD PRS with incident cardiovascular outcomes were greatly attenuated among those with established CAD compared to those without CAD. Nonetheless, the estimates did not change after adjustment for most known risk factors for vascular disease, making index event bias a less likely explanation.

Finally, use of lipid-lowering- or antihypertensive medication and healthy lifestyle may have contributed to the lack of an association between both PRSs and recurrent vascular events. As demonstrated in the baseline table, patients with both the LDL-C PRS and SBP PRS above the median had a higher prescription rate for lipid-lowering and antihypertensive medications compared to patients with both scores below median. Moreover, patients with a genetically higher LDL-C and SBP may be more likely to be treated more intensively with these type of medications and potentially adopt a more healthy lifestyle during follow-up, which eventually compensates for the higher genetically determined LDL-C and SBP levels. Moreover, these types of medication and the change to a healthy lifestyle may be more effective in patients with genetically higher LDL-C and SBP. This concept is supported by previous studies showing that both statins, Proprotein Convertase Subtilisin-Kexin type 9 (PCSK9) monoclonal antibodies, and a healthy lifestyle are able to modify the risk of (recurrent) cardiovascular events associated with a high PRS (27-30).

This study shows that genetically determined LDL-C and SBP do not explain differences in residual cardiovascular risk in patients with established vascular disease. Although this is an

etiologic study, these results support the recommendations in international guidelines not to routinely collect genetic information for CVD risk stratification. In general, the position of genetic risk scores in clinical practice is under debate. Currently, PRSs are considered of limited use for the prediction of CVD events (31). Moreover, in the scenario that PRSs will play an important role in clinical practice in the future, it is likely that its greatest value lies in the first decades of life, prior to clinical events and even prior to definable plaque burden by imaging.

Strengths of the present study include the prospective cohort study design reflecting clinical practice of patients with vascular disease being treated according to national guidelines, the substantial follow-up duration and the large number of validated clinically relevant outcomes. Also, genotyping and quality control were performed according to a highly standardized protocol by experts in the field. Lastly, elaborate sensitivity analyses were performed to further investigate the main findings of this study.

Some limitations need to be considered. In the present study two PRSs were used based on 704 different SNPs related to either LDL-C or SBP identified through GWAS in the general population. Some have argued that such PRSs are of limited value in populations with established vascular disease and advocate the design and use of dedicated GWAS of disease progression (26, 32, 33). However, this study demonstrated a robust effect of the selected SNPs on plasma LDL-C and SBP levels in patients with vascular disease, independent of the use of lipid-lowering or antihypertensive medication. Moreover, differences in LDL-C and SBP levels when stratified for LDL-C or SBP PRS, were comparable with the differences observed in the general population (7, 8). In addition, the allele frequencies of the selected SNPs in the current study population were comparable to the allele frequencies found in the general European population **(Supplemental table 1).** Another important limitation is that use of medication such as lipid-lowering and antihypertensive medications was only recorded at baseline. Although the

use of these types of medication probably increased during follow-up, since treatment advice was part of the screening for this study, we were not able to account for these changes in the analyses. Lastly, the PRSs used in this study are only applicable to populations of European descent, which may limit the generalizability of the results and poses an ethical dilemma (34, 35).

In conclusion, in patients with established cardiovascular disease, we replicated the known association of PRSs for LDL-C and SBP with these risk factors. We found no statistically significant association between an LDL-C PRS and an SBP PRS, nor in combination, and recurrent cardiovascular events. These results suggests that genetically determined LDL-C and SBP do not explain the differences in residual cardiovascular risk in patients with established vascular disease.

Conflicts of interest

None

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Author contributions

All authors contributed to either the acquisition, analysis, or interpretation of the data for the work.

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Data and code availability

Github respository with R scripts: https://github.com/CirculatoryHealth/UKB_Lipids_SBP

SMART dataset in DataverseNL: doi:10.34894/TCAZ6T

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522

Tables

Table 1 – Baseline characteristics according to combined LDL-C and SBP polygenic risk score

	Reference group	**LDL-C PRS ≤ median, SBP PRS > median**	**LDL-C PRS > median, SBP PRS ≤ median**	**LDL-C PRS and SBP PRS > median**	**Total**	***p*-value**
	n = 1123	n = 1085	n = 1085	n = 1123	n = 4416	
Male sex	840 (75%)	808 (74%)	815 (75%)	831 (74%)	3294 (75%)	0.94
Age (years)	61 ± 10	61 ± 10	60 ± 10	60 ± 10	61 ± 10	<0.05
Current smoker	402 (36%)	348 (32%)	372 (34%)	354 (32%)	1476 (33%)	0.12
Current alcohol use	550 (49%)	536 (49%)	548 (51%)	577 (51%)	2211 (50%)	0.66
Body mass index (kg/m2)	26.8 ± 3.8	26.9 ± 3.9	26.7 ± 4.0	26.7 ± 3.9	26.7 ± 3.9	0.35
Systolic blood pressure (mmHg)	139 ± 20	144 ± 22	138 ± 21	143 ± 21	141 ± 21	0.07
Diastolic blood pressure (mmHg)	81± 11	82 ± 11	80 ± 12	83 ± 11	81 ± 11	<0.05
History of vascular disease						
Diabetes mellitus type 2	173 (15%)	199 (18%)	156 (14%)	177 (16%)	705 (16%)	0.08
Coronary artery disease	651 (58%)	632 (58%)	702 (65%)	720 (64%)	2705 (61%)	<0.05
Peripheral artery disease	231 (21%)	251 (23%)	217 (20%)	237 (21%)	936 (21%)	0.30
Cerebrovascular disease	338 (30%)	305 (28%)	260 (24%)	300 (27%)	1203 (27%)	<0.05
Abdominal aortic aneurysm	107 (10%)	90 (8%)	95 (9%)	101 (9%)	393 (9%)	0.78
Laboratory values						
Total cholesterol (mmol/l)	4.82 ± 1.19	4.84 ± 1.21	5.03 ± 1.23	5.04 ± 1.31	4.93 ± 1.24	<0.05
HDL-cholesterol (mmol/l)	1.23 ± 0.36	1.21 ± 0.38	1.20 ± 0.35	1.21 ± 0.37	1.21 ± 0.36	0.08
LDL-cholesterol (mmol/l)	2.87 ± 1.04	2.89 ± 1.08	3.08 ± 1.08	3.02 ± 1.07	2.97 ± 1.07	<0.05
Triglycerides (mmol/l)	1.3 (0.9 - 1.9)	1.4 (1.0 - 2.0)	1.4 (1.0 - 2.0)	1.5 (1.1 - 2.2)	1.4 (1.0 - 2.0)	<0.05

Estimated GFR (ml/min/1.73m2)	75 ± 17	74 ± 18	76 ± 17	76 ± 18	75 ± 18	<0.05
hsCRP (mg/L)	2.2 (1.0 - 4.6)	2.3 (1.0 - 4.9)	1.9 (0.9 - 4.3)	2.0 (1.0 - 4.4)	2.1 (1.0 - 4.5)	<0.05
Medication use						
Lipid lowering medication	660 (59%)	641 (59%)	770 (71%)	764 (68%)	2835 (64%)	<0.05
High intensity statins	54 (5%)	61 (6%)	85 (8%)	79 (7%)	279 (6%)	<0.05
Antihypertensive medication	789 (70%)	819 (75%)	783 (72%)	845 (75%)	3236 (73%)	<0.05
Number of antihypertensive drugs (mean, range)	1.2 (0 – 5)	1.4 (0 – 7)	1.3 (0 – 5)	1.4 (0 – 6)	1.3 (0 – 7)	<0.05
Platelet inhibitors	819 (73%)	796 (73%)	813 (75%)	864 (77%)	3292 (75%)	0.12

Abbreviations: HDL; high-density lipoprotein, LDL; low-density lipoprotein, SBP; systolic blood pressure, GFR; glomerular filtration rate, hsCRP; high sensitivity C-reactive protein

Table 2 – LDL-C and SBP polygenic risk score and recurrent cardiovascular events (non-fatal MI, non-fatal stroke and vascular death)

			LDL-C PRS	**SBP PRS**
			N = 4416	N = 4416
		Model	HR per SD increase in PRS (95% CI)	HR per SD increase in PRS (95% CI)
Recurrent cardiovascular events	#events		1198	1198
		I	1.02 (0.96 - 1.08)	1.04 (0.99 - 1.10)
		II	1.05 (0.99 - 1.11)	1.04 (0.98 - 1.10)

Model I: adjusted for age and sex, and the first five principal components.
Model II:
LDL-C PRS:
Model I + additional adjustment for BMI, type 2 diabetes mellitus, smoking, alcohol use, eGFR, triglycerides, first 5 principal components, SBP, and lipid-lowering medication
SBP PRS:
Model I + additional adjustment for BMI, type 2 diabetes mellitus, smoking, alcohol use, eGFR, triglycerides, first 5 principal components, LDL-C, and antihypertensive medication

Table 3 – Combined LDL-C and SBP polygenic risk score and recurrent cardiovascular events (non-fatal MI, non-fatal stroke and vascular death)

			LDL-C PRS ≤ median, SBP PRS < median (Reference group) n=1123	**LDL-C PRS ≤ median, SBP PRS > median n= 1085**	**LDL-C PRS > median, SBP PRS ≤ median n= 1085**	**LDL-C PRS > median, SBP PRS > median n= 1123**
	Model		HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)
Recurrent cardiovascular events	# events		295	320	280	303
		I	Reference	1.08 (0.92 - 1.26)	0.98 (0.83 - 1.15)	1.06 (0.91 - 1.25)
		II	Reference	1.06 (0.90 - 1.24)	1.03 (0.87 - 1.22)	1.09 (0.93 - 1.28)

Model I: adjusted for age, sex, and the first 5 principal components
Model II: Model I + additionally adjusted for BMI, type 2 diabetes mellitus, smoking, alcohol use, eGFR, triglycerides, lipid-lowering medication, antihypertensive medication

Figures

Figure 1 – Relation LDL-C polygenic risk score and LDL-C values in quartiles in patients with and without use of lipid-lowering medication

Linear regression analyses describing the association between mean LDL-C level and use of lipid-lowering-specific quartile of LDL-C PRS. Models were adjusted for age, sex, BMI, SBP, smoking, alcohol use, T2DM, eGFR, triglycerides, and the first 5 principal components.

Figure 2 – Relation SBP polygenic risk score and SBP values in quartiles in patients with and without use of antihypertensive medication

Linear regression analyses describing the association between mean SBP and use of antihypertensives-specific quartile of SBP PRS. Models were adjusted for age, sex, BMI, LDL-C, smoking, alcohol use, T2DM, eGFR, triglycerides, and the first 5 principal components