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Genetic variability within the cholesterol lowering pathway and the effectiveness of statins in reducing the risk of MI

Bas J.M. Peters[1], **Helmi Pett**[1], **Olaf H. Klungel**[1], **Bruno H. Ch. Stricker**[2,3,4], **Bruce M. Psaty**[5], **Nicole L. Glazer**[5], **Kerri L. Wiggins**[5], **Josh C. Bis**[5], **Anthonius de Boer**[1], and **Anke-Hilse Maitland-van der Zee**[1]

[1]Department of Pharmacoepidemiology and Pharmacotherapy, Utrecht Institute for Pharmaceutical Sciences (UIPS), University of Utrecht, Utrecht, The Netherlands [2]Department of Epidemiology, Erasmus MC, Rotterdam, the Netherlands [3]Department of Internal Medicine, Erasmus MC, Rotterdam, the Netherlands [4]Drug Safety Unit, Inspectorate for Health Care, The Hague, the Netherlands [5]Cardiovascular Health Research Unit, Departments of Medicine, Epidemiology, and Health Services, University of Washington, Seattle, WA; Group Health Research Institute, Group Health Cooperative, Seattle, WA

Abstract

Genetic variability has been shown to affect statin responsiveness. Participants from the Utrecht Cardiovascular Pharmacogenetics (UCP) studies were enrolled from a population-based registry of pharmacy records linked to hospital discharge records (PHARMO) to investigate tagging SNPs within candidate genes involved in the cholesterol lowering pathway for modification of the effectiveness of statins in reducing the risk of myocardial infarction (MI). Patients who received a prescription for an antihypertensive drug and/or had hypercholesterolemia were selected from the PHARMO database. We designed a nested case-control study in which cases were hospitalized for MI and controls were not. Patients were contacted through their community pharmacies. For this study, only hypercholesterolemic participants were selected. Logistic regression analysis was used to investigate pharmacogenetic interactions. The Heart and Vascular Health Study (HVH) was used to replicate findings from UCP.

The study population included 668 cases and 1217 controls. We selected 231 SNPs of which 209 SNPs in 27 genes passed quality control. Ten SNPs in eight genes were found to influence the effectiveness of statins in UCP, of which the most significant interaction was found with *SCARB1* rs4765615. Other genes that reached statistical significance ($p<0.05$) included two SNPs in *PCSK9* (rs10888896 and rs505151 (E670G)), two SNPs in *ABCG5* (rs4245786 and rs1864815), *LIPC* rs16940379, *ABCA1* rs4149264, *PPARG* rs2972164, *LRP1* rs715948, and *SOAT1*

Address for correspondence: Anke-Hilse Maitland-van der Zee, Utrecht University, Faculty of Science, Division of Pharmacoepidemiology and Pharmacotherapy, PO box, 80082, 3508 TB Utrecht, The Netherlands, A.H.Maitland@uu.nl.

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rs2493121. None of the total of 5 SNPs that were available for replication in HVH reached statistical significance.

In conclusion, ten SNPs were found to modify the effectiveness of statins in reducing the risk of MI in the UCP study. Five were also tested in the HVH study, but no interactions reached statistical significance.

Keywords

pharmacogenetics; statin; case-control study; cholesterol; myocardial infarction; *PCSK9*; *SCARB1*

Introduction

To reduce the risk of cardiovascular events, statins are among the most prescribed drugs worldwide. Although the efficacy has been well established in clinical trials [1], interindividual differences in response exist [2]. Besides non-genetic factors such as age, concomitant drug use, and co-morbidities, it has been well recognized that variability in statin-related genes contribute to differences in response to statins. These include both genes in the lipid and non-lipid pathways [2].

Statins' foremost pharmacological action is the competitive inhibition of HMG-CoA reductase, the first enzyme and rate-limiting step in the cholesterol biosynthesis cascade. Subsequently, there is an increase in hepatic low-density lipoprotein (LDL) receptors resulting in increased LDL clearance from the blood stream. Although the *HMGCR* gene, encoding HMG-CoA reductase, is an important candidate gene for the pharmacogenomics of statins, a range of other cholesterol pathway related genes may be of importance for statin responsiveness. These include genes that are involved in the hepatic cholesterol metabolism or metabolism and transport of plasma lipoproteins. Well known examples of such genes that have previously been subject of pharmacogenomic research are *LDLR*, encoding LDL receptor, *CETP*, encoding cholesteryl ester transfer protein and *APOE*, encoding apolipoprotein E [2].

Most pharmacogenetic studies have investigated the cholesterol lowering response to statins as opposed to clinically important outcomes such as myocardial infarction (MI). Therefore, the aim of this study was to investigate the genetic influence of tagging SNPs within candidate genes involved in the cholesterol lowering pathway of statins on the effectiveness of statins in reducing the risk of MI.

Methods

Design and Setting

Participants from the Utrecht Cardiovascular Pharmacogenetics (UCP) studies were enrolled from the population-based Pharmaco-Morbidity Record Linkage System (PHARMO, www.pharmo.nl). PHARMO links drug dispensing histories from a representative sample of Dutch community pharmacies to the national registration of hospital discharges (Dutch National Medical Registry).

First, patients who received a prescription for an antihypertensive drug [3], and/or had hypercholesterolemia (prescription for a cholesterol-lowering drug or total cholesterol >5.0 mmol/l) [4], were selected from the PHARMO database for pharmacogenetic studies on antihypertensive drugs [3] and statins [4] respectively. From this cohort, a nested case-

control study was designed using hospital discharge records. Patients hospitalized for MI (International Classification of Diseases (ICD)-9 code 410) were included as cases if they were registered in PHARMO for at least one year and were older than 18 years. The index date was defined as the date of hospitalization for the first MI. Controls met the same eligibility criteria as the cases, but had not developed MI.

Participants were contacted through community pharmacies, where they received a letter in which the purpose of the study was explained. They were asked to return an informed consent form and a filled-out questionnaire. After the participant had consented to participate in the study, he/she was sent material for saliva collection. All participants were explicitly asked to consent for the collection, storage and genotyping of the DNA material. Approval for this study was obtained from the Medical Ethics Committee of the University Medical Center Utrecht, The Netherlands.

For this study, all hypercholesterolemic (prescription for a cholesterol-lowering drug, total cholesterol>5.0 mmol/l, or self-reported hypercholesterolemia) participants were selected. In detail, the case-control ratios for sampling from the nested case-control study on the antihypertensive drugs [3] and statins [4] was one to one and one to three respectively.

Ascertainment of exposure to statins (and other drugs)

Coded pharmacy records were used to ascertain exposure to statins (and other drugs) before the index date. In PHARMO, complete pharmacy records were available as of 1991, including the day of delivery, daily dose, and durations of therapy. To define exposure to statins, we assessed the association of different cumulative defined daily doses (DDD) (cumulative DDD cut-off points of 90, 180, 360, and 720 DDD) with the risk of MI. The DDD is the dose per day for a drug used for its main indication in adults. Our data showed that statins were not effective in reducing the risk of MI in patients exposed to a cumulative dose of 180 DDD or less. The effectiveness of statins in patients exposed for more than 180 days but less than 360 days did not differ from a cumulative exposure for more than 360 or 720 days. Therefore, participants were considered exposed when the cumulative DDD of statin use was more than 180, whereas participants with the cumulative DDD of 180 or less (including 0 DDDs) were considered as the reference group.

To adjust for potential confounding, we identified all prescriptions for concomitant drug use for each patient. The projected end date of a prescription was calculated using information on the daily dose instruction and the quantity dispensed. We considered a patient a current user when the index date was between the start and end date of a prescription. Past users were patients who were not current users, but had used the drug prior to the index date.

Assessment of potential confounding factors and effect modifiers

Questionnaires were used to assess cardiovascular disease (CVD) risk factors such as smoking, hypertension, hypercholesterolemia, diabetes mellitus, use of alcohol, diet, history of CVDs, family history of CVDs, weight and height. Furthermore, information from the general practitioner files and laboratory registrations were available for part of the population. In case of a discrepancy between community pharmacy data and questionnaire data, community pharmacy data was the primary source for defining hypercholesterolemia and diabetes status. Ischemic heart disease (IHD) was defined as “yes” if a participant was hospitalized for an IHD or ever used nitrates.

DNA collection and DNA extraction

Part of patients were send three cotton swabs and tubes containing buffer to collect buccal cell samples as described elsewhere [5]. Other participants were sent an Oragene collection

kit and donor instructions provided by the manufacturer (DNA Genotek, Ottawa, Canada) [4]. DNA was extracted according to the manufacturer's instructions (http://www.dnagenotek.com/techsupport_documents.htm). Samples with a DNA concentration higher than 100 ng/μl were diluted to the Illumina Golden-Gate assay required 50 ng/μl.

Candidate gene selection and SNP selection

We selected a total of 231 SNPs in 27 genes that are involved in the cholesterol lowering pathway of statins. We selected common tagging SNPs within 200 bp (up- and downstream) with a minor-allele frequency (MAF) higher than 0.2 (based on a power calculation with 80% power to detect a SI of 2 or 0.5) and a r^2>0.8 using a web-based tool called QuickSNP version 1.1 (HapMap release 21 [6], U.S. residents with northern and western European ancestry (CEPH individuals)).[7] Additionally, dbSNP [8] nonsynonymous coding SNPs (MAF>0.2) and previously (pharmaco)genetically associated SNPs were included. Illumina SNP designability scores lower than 0.4 (1.1=best validated) or failure codes (http://www.illumina.com/documents/products/technotes/technote_goldengate_design.pdf) were either substituted with a SNP in linkage disequilibrium (LD) or, if unavailable, removed from the SNP list, resulting in a final set of 231 SNPs.

Genotyping

For each individual participating in the study, SNPs were genotyped using the custom GoldenGate assay on an Illumina BeadStation 500 GX (Illumina Inc. San Diego, CA, USA). Genotype calls of all SNPs were individually examined for their resulting quality. SNPs with a low signal, poor clustering, deviation from Hardy–Weinberg equilibrium (HWE) (≤0.01) or a high number of missing genotypes (>10%) were excluded.

Replication study (Heart and Vascular Health Study (HVH))

The setting for the replication study was a large integrated health care system in Washington State, called Group Health Cooperative (GHC). The data were from an ongoing case-control study of incident MI and stroke cases with a shared control group and has been described elsewhere [9, 10]. The study was approved by the human subjects committee at GHC, and all study participants provided an informed consent.

All study participants were GHC members aged 30–79 years. We selected MI cases and controls if they had a prescription for a cholesterol-lowering drug or total cholesterol measurement of >5.0 mmol/l. Cases were hospitalized for a non-fatal incident MI, identified from computerized hospital discharge abstracts and billing records [9, 10]. Controls were a random sample of GHC members frequency matched to MI cases on age, sex, and calendar year of identification. The index date for MI cases was the date of admission for the first acute MI, whereas controls were assigned a computer-generated random date within the calendar year for which they had been selected. Medication use was ascertained using computerised GHC pharmacy records. Definitions of drug exposure matched the definitions from the UCP study. Eligibility and risk factor information were collected by trained medical record abstractors from a review of the GHC medical record using only data available prior to the index date and through a telephone interview.

A venous blood sample was collected from all consenting subjects, and DNA was extracted from white blood cells using standard procedures. Genotype data was available from two sources. Part of the genotype data was available from the Illumina 370CNV BeadChip system. Imputation was performed using BIMBAM with reference to HapMap CEU using release 22, build 36 using one round of imputations and the default expectation-maximization warm-ups and runs. In addition, genotype data was available from a Illumina

(Illumina Inc, San Diego California) GoldenGate custom panel using BeadArray® technology.

SNPs that showed a significant interaction ($p<0.05$) with statin treatment in UCP were identified in the HVH study genotype data. Five of these SNPs had genotype data available from the Illumina 370CNV BeadChip system and/or the Illumina GoldenGate custom panel. For these SNPs, if a subject had genotype results available from both Illumina methods, the GoldenGate panel results were preferentially selected. One SNP had genotype data available only from the Illumina GoldenGate panel (n=865). All remaining SNPs had genotype data available only from the Illumina 370CNV BeadChip system (n=2446). SNPs from the Illumina 370 CNV BeadChip system were chosen with a lower cut-off for the RSQR (or OEvar) score of 0.6. The RSQR denotes the average of the observed-to-expected variance ratio of any SNP, which indicates deviation from Hardy-Weinberg equilibrium and quality of imputation.

Statistical methods

The same analysis was applied to the UCP and HVH study. Logistic regression (LR) analysis was used to study the association between statins and the risk of MI, and to adjust for potential confounders. Matching variables --- age, sex, region, and index date --- were included in our statistical model. The inclusion of potential confounders in the LR model was motivated by the assessment of the influence of each potential confounder on the OR for the association between use of statins and risk of MI. The potential confounding factors that we considered were: Use of different cardiovascular drugs (antihypertensive drugs, platelet aggregation inhibitors, anticoagulants, other cholesterol-lowering drugs, and organic nitrates), use of alcohol, physical activity, family history of CVD, and other factors assessed by the questionnaire. Only covariates IHD and the use of calcium channel blockers showed at least a 5% change in the regression coefficient (beta) for statin use; therefore, they were included in the LR model. We estimated the multiplicative synergy index (SI), which is the ratio of the OR in those with the variant to the OR in those without the variant. For the significant (unadjusted or adjusted) pharmacogenetic associations, ORs were calculated separately in the strata defined by genotype. Heterozygotes and homozygotes for the variant allele of the *PCSK9* E670G (rs505151) polymorhpism were combined because of a low frequency homozygous variant allele carriers. For each SNP, HWE was tested using a X^2 goodness-of-fit test. Analyses were performed using SPSS version 16.0. Subsequently, q-values (the positive false discovery rate (pFDR) analogue of the p-value) were calculated for each gene-treatment interaction that was tested in UCP to account for multiple testing [11].

Results

The data collection procedure for this study has previously been published [4]. Briefly, figure 1 summarizes how the final number of participants was arrived at. For the hypercholesterolemic cohort 9,764 patients could not be approached for various reasons (death of a patient, pharmacy did not participate, amount of controls per case was decreased, or patient was untraceable due to change in the community pharmacy computer information system). For the genotyping assay, out of 1,844 consenting subjects, all cases were selected (n = 315), accompanied by the matched controls and a random sample of unmatched controls, to bring the total population to 1,200 (approximately three controls per case). After exclusion of patients that donated an insufficient amount of DNA, patients of which the genotyping results did not pass quality control (QC), and patients with a self-reported ethnicity other than Caucasian, 307 cases and 831 controls were included from the hypercholesterolemic cohort. From the hypertensive cohort, we selected 429 hypercholesterolemic cases and 429 hypercholesterolemic controls. After excluding patients already included from the hypercholesterolemic cohort study, patients for whom the

genotyping did not pass QC, and patients with a self-reported ethnicity other than Caucasian, we were able to amass 361 cases and 386 controls.

The total UCP study population included 1885 individuals, of which 668 were MI cases and 1217 controls. Table 1 describes the clinical characteristics of the population according to case control status. The well known cardiovascular risk factors smoking (current), a BMI of more than 30 kg/m^2, and the presence of IHD were more frequently seen in cases compared to controls. Current use of other non-statin cholesterol-lowering drugs was associated with a decreased risk of MI. Current use of beta-blockers and calcium channel blockers was more frequently seen in cases than controls, which is due to oversampling of nonantihypertensive users in the control group as described in the methods section [4].

Out of the 231 selected SNPs, 209 passed quality control and were tested for an interaction with statin treatment. The LR analysis revealed ten SNPs in eight genes that significantly ($p<0.05$) interacted with statin treatment (table 2), either with or without adjustment for the additional confounding factors or both. *SCARB1* rs4765615 showed the most significant interaction, with a more beneficial effect of statins for GG and AG carriers (OR 0.30, 95% confidence interval (CI) 0.22–0.42 and OR 0.30, 95%CI 0.18–0.50 respectively) as compared to AA carriers (OR 0.64, 95%CI 0.41–0.98). The *PCSK9*, and *ABCG5* gene were both represented by two SNPs among the significant interactions. The only nonsynonymous SNP that was found to interact with statin treatment, was *PCSK9* rs505151, for which variant allele carriers had no significant benefit from statin treatment (OR 0.63, 95%CI 0.30–1.32) compared to homozygous wildtype carriers who did benefit (OR 0.36, 95%CI 0.28–0.45). The five other SNPs that appeared to be implicated in the pharmacogenetics of statins were found in the *LRP1*, *LIPC*, *ABCA1 SOAT1*, and *PPARG* gene. The q-value for the interaction with *SCARB1* rs4765615 and *PCSK9* rs10888896 was 0.19 and 0.24 respectively, whereas the q-value of the interactions with the other eight SNPs within the significant results was 0.57. The SIs for all of the SNPs can be found in Table II of the Supplementary data.

For the HVH study, 10,860 (2,976 cases and 7,884 controls) subjects were initially eligible. 551 controls were excluded because of a prior MI (2,976 cases and 7,333 controls). Subsequently, 1,097 normocholesterolemic subjects were excluded (2,835 cases and 6,377 controls). An additional 6,766 subjects were exluded because no genotyping results were available resulting in a total study population of 1,182 cases and 1,264 controls. The *PCSK9* SNP was genotyped using the Illumina GoldenGate panel only and the other nine were imputed using the Illumina 370CNV BeadChip system genotype data only. Four of the nine had an RSQR score more than 0.6, and five had an RSQR score less than 0.6 and were therefore not included in the analysis. None of the five interactions tested in HVH showed a significant interaction (table 2). Nonetheless, similarly to the results from the UCP study, *PCSK9* rs505151 variant allele carriers had no significant benefit from statin treatment (OR 1.05, 95%CI 0.18–6.26), whereas homozygous wildtype carriers did (OR 0.61, 95%CI 0.39–0.94). In addition, the point estimates and directionality of the non-significant HVH results for the *LIPC* rs16940379 interaction resemble the UCP results in which homozygous wildtype allele carriers appear to respond better to statin treatment compared to homozygous variant allele carriers.

Discussion

In this population-based retrospective case-control study, we tested 209 SNPs in 27 genes involved in the cholesterol-lowering pathway and found ten SNPs in eight genes to influence the effectiveness of statins in UCP, of which the most significant interaction was found with *SCARB1* rs4765615. Also genetic variability within the *PCSK9*, *LIPC*, *LRP1*, *ABCG5*,

ABCA1, *PPARG*, and *SOAT1* genes were found to affect statin effectiveness. Five out of ten statistically significant SNPs were available in the HVH study for replication but failed to reach statistical significance. In both studies, carriers of the *PCSK9* rs505151 variant allele had no benefit from statin treatment, although the formal test for interaction was not statistically significant in the HVH study.

The highly significant interaction with *SCARB1* rs4765615 showed that homozygous carriers of the A allele did not benefit from statin treatment compared to those carrying one or two G alleles. Scavenger receptor class B member 1, encoded by *SCARB1*, functions as a receptor for high-density lipoprotein (HDL) and plays an important role in the reverse cholesterol transport (RCT). Recently, a variant other than *SCARB1* rs16940379 was shown to affect the LDL cholesterol (LDLc) response to atorvastatin [12]. Despite the unknown underlying mechanism of this gene treatment interaction, also our study indicates a role for *SCARB1* in the response to statins. Genetic variability of *SCARB1* should therefore be investigated in future studies. The imputation score of the HVH study data for *SCARB1* rs16940379 and four other SNPs was too poor and were not used for further analysis. Except for the interaction with *SCARB1* rs16940379 and *PCSK9* rs10888896 (which will be discussed hereafter), these interactions are generally characterized by high q-values, no well defined known functional SNP that is in LD with the interacting tagging SNP, and/or models that lack of gene-dose effect, suggesting that these findings may be false positives.

PCSK9, encoding proprotein convertase subtilisin/kexin type 9, was found to be the third locus involved in autosomal dominant hypercholesterolemia (ADH) [13]. PCSK9 promotes the degradation LDLR and gain-of-function mutations have been shown to lead to higher LDLc levels, whereas loss-of-function mutations have been shown to result in lower LDLc [14] and protection against CHD [15]. The mutations that cause severe hypercholesterolemia are rare, but also common mutations have been shown to affect lipid levels and possibly the response to statins. Such a common nonsynonymous polymorphism is *PCSK9* E670G (included in the current study), which has been shown to be a marker for higher plasma LDLc levels in several [16–19] but not all studies [20–22]. Also an association between *PCSK9* E670G and increased carotid artery intima media thickness was found [18], although others did not show an association with CAD/CHD/vascular disease risk [17, 21, 22]. In turn, it has been suggested that individuals carrying *PCSK9* loss-of-function polymorphisms have an increased lipid response to statins [23], whereas gain-of-function polymorphisms have been shown to result in a decreased lipid response to statin therapy [24, 25].

Three studies investigated the *PCSK9* E670G with respect to statin responsiveness. The PROSPER trial including almost 6000 elderly subjects (mean age 75 years) could not reveal a significant difference in LDLc response or CHD risk reduction between carriers and noncarriers of the variant [21]. Among 49 SNPs in nine candidate genes, the *PCSK9* E670G variant was also included in a pharmacogenetic study in the PROVE IT-TIMI 22 trial. Lipid response of 1378 hypercholesterolemic post ACS subjects randomized to pravastatin or atorvastatin did not differ among *PCSK9* E670G genotype strata [31]. In the Treating to New Targets (TNT) trial, carriers of the 670G allele were found to have a significantly smaller decrease in LDLc levels in response to statin treatment [26]. Similar to the results of the TNT study and the observations that *PCSK9* gain-of-function variants have a deleterious effect on statin responsiveness [24, 25], we show that carriers of the 670G allele have a better response to statin treatment in UCP (significant) and HVH (not significant). Nonetheless, the magnitude of the observation by the TNT study (1.8 mg/dl less LDLc reduction *PCSK9* 670G carriers) does not reflect the large effect of *PCSK9* E670G found in this study, suggesting a partially lipid independent mechanism behind this gene treatment interaction.

Mechanistically, statins decrease the endogenous cholesterol biosynthesis by inhibition of 3-hydroxy-3-methylglutaryl–coenzyme A, which leads to transcriptional activation of both LDLR and PCSK9 [14]. Although PCSK9 counteracts the statin induced increased LDLR activity, the net result of statin treatment is reduction in plasma LDL cholesterol. In the case of *PCSK9* E670G polymorphism, it can be hypothesized that the net result of statin treatment is no longer beneficial due to its gain-of-function nature.

Additionally, our tagging SNP approach revealed a second – more significant – *PCSK9* SNP that affected the response to statins. *PCSK9* rs10888896 resides in the first intron and has not been extensively researched. No effect of this SNP on LDLc reduction was found in the Treating to New Targets (TNT) trial [26]. Possibly, the variant allele of the *PCSK9* is in LD with a (recessive) gain-of-function polymorphism, because our results suggest that homozygous wild-type variant carriers have no benefit from statin treatment. Although this SNP was unavailable in the HVH population, the *PCSK9* gene is of great interest for statin responsiveness, and the effect of *PCSK9* rs10888896 should therefore be assessed in future studies.

None of the results from the UCP study showed a statistically significant interaction in the HVH study (table 2). Besides *PCSK9* E670G, only the interaction with *LIPC* rs16940379 showed a similar trend in the HVH study as was found in UCP study. Hepatic lipase, encoded by *LIPC*, may affect statin responsiveness through its involvement in modulation of LDL size and density, which in turn is has been shown to affect the risk of CHD [27]. Although no data are available on the role of rs16940379 on hepatic lipase activity, our results indicate a role for *LIPC* in the pharmacogenomics of statins.

The present study has several limitations. For statistical power reasons, we assessed only SNPs with a MAF cut-off of 0.2, the consequences of which are that we are likely to miss potentially important SNPs. Nevertheless, our study covers the common genetic variability within the selected candidate genes completely. Furthermore, we considered all statins and all dosage regimes as a homogenous group. Although all statins share the primary working mechanism by which they lower cholesterol, it has been shown that there are differences between the different statins [28, 29]. The sample size of the current study does not allow to study the interaction between individual statins and genetic variability. Also, our replication study has two limitations. First, five out of ten SNPs that were found to interact with statins in UCP were not available in the HVH study, or had a low imputation score. Second, imputation of rs1864815, rs16940379, and rs2972164 gives uncertainty about the true genotype and thereby lowers the statistical power to detect an interaction.

A strength of the current study is the availability of a replication study that used the same study design (case-control, exposure definition, outcome, data analysis) as the UCP study. In addition, a centralized system to define exposure (availability of the community pharmacy records) and outcomes (hospital records) was available. Statin exposure was defined based on pharmacy records, which validity to measure drug exposure has been shown to be good [30]. Testing a large number of variables, the possibility of chance findings (spurious associations) increases. We addressed the issue of multiple testing by calculation of q-values that suggested that a large proportion of our significant interaction were false discoveries. Finally, our study assessed the impact of gene-treatment interactions on the clinically important (endpoint) outcome MI, instead of surrogate parameters.

We show that the *PCSK9* E670G polymorphism may be of great importance for the effectiveness of statins in reducing the risk of MI because carriers of one or two variant alleles do not seem benefit from statin treatment. *PCSK9* gain-of-function variant carriers have been shown to have high untreated and treated cholesterol levels, but a similar

percentage LDLc fall from statin treatment compared to wild-type carriers [24]. Therefore, from a clinical perspective, carriers of *PCSK9* 670G variant allele may benefit from more aggressive lipid-lowering treatment and could especially benefit from novel hypercholesterolemic therapy strategy of PCSK9 inhibition.

In conclusion, variant allele carriers *PCSK9* E670G polymorphism do not seem to benefit from statin treatment and confirmation of the pharmacogenetic associations with *LIPC* rs16940379, *SCARB1*, rs16940379, and *PCSK9* rs10888896 should be subject of future research to pinpoint possible causal variants that affect statin responsiveness.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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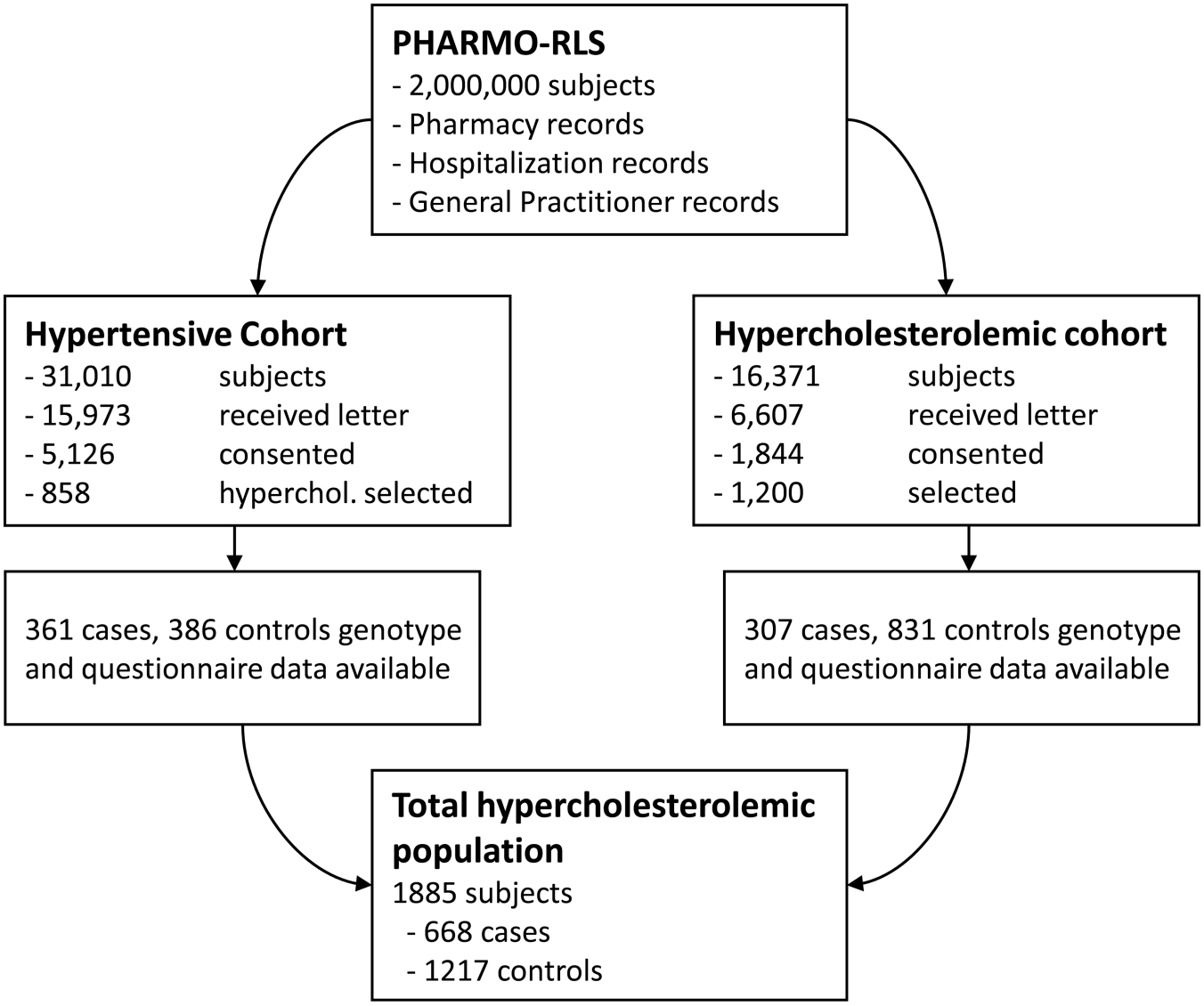

Figure 1.
Flow diagram of study population.

Table 1

Clinical characteristics UCP by case control status.

		Case n=668	%	Control n=1217	%	p
Gender	Female	171	25.6%	285	23.4%	0.290
Age (years)	Mean (sd)	63.0 (9.9)		62.3 (9.4)		0.097
Body Mass Index at ID	>30 kg/m2	140/607	23.1%	193/1104	17.5%	0.005
Familial History CVD	Yes, <60	135/637	21.2%	214/1167	18.3%	0.103
	Yes, >60	266/637	41.8%	464/1167	39.8%	
Diabetes Status	Diabetes, no medication	71/658	10.8%	90/1203	7.5%	0.052
	Diabetes, medication	75/658	11.4%	144/1203	12.0%	
Smoking Status	Current	152/624	24.4%	195/1135	17.2%	0.001
	Past	283/624	45.4%	573/1135	50.5%	
Alcohol Status (consumptions)	No use	118/646	18.3%	162/1188	13.6%	0.062
	<=1	237/646	36.7%	432/1188	36.4%	
	>1 – <2	170/646	26.3%	328/1188	27.6%	
	>2	121/646	18.7%	266/1188	22.4%	
Physical Activity	> 4 hrs a week	491/645	76.1%	939/1191	78.8%	0.180
Cumulative DDD use statins	>180 DDD	218	32.6%	646	53.1%	<0.001
Type of Statin	Atorvastatin	44/218	20.2%	164/646	25.4%	<0.001
	Pravastatin	35/218	16.1%	110/646	17.0%	
	Simvastatin	125/218	57.3%	335/646	51.9%	
	Other	14/218	6.4%	37/646	5.7%	
Ischemic Heart Disease	Yes	211	31.6%	268	22.0%	<0.001
Antihypertensives						
Calcium Channel Blockers	Current use	142	21.3%	193	15.9%	0.003
Diuretics	Current use	82	12.3%	171	14.1%	0.279
Beta Blockers	Current use	275	41.2%	415	34.1%	0.002
Ace Inhibitors	Current use	140	21.0%	273	22.4%	0.459
AT2 Receptor Antagonists	Current use	48	7.2%	110	9.0%	0.165
Other drugs						
Non-statin Cholesterol Lowering drugs	Current use	14	2.1%	46	3.8%	0.046

		Case n=668	%	Control n=1217	%	p
Insulin	Ever use	30	4.5%	54	4.4%	0.957
Oral Antidiabetics	Current Use	52	7.8%	96	7.9%	0.936
Platelet Aggregation Inhibitors	Current Use	219	32.8%	438	36.0%	0.162
Coumarins	Current Use	37	5.5%	76	6.2%	0.537

Abbreviations: ATII = Angiotensin II; DDD = Defined Daily Dosage

Table 2

Significant interactions in UCP and replication results from the HVH study.

UCP									HVH					
Gene	**SNP**	**G**	**#**	**HWE**	**p**	**q**	**OR*(95% CI)**	**OR**(95% CI)**	**RSQR**	**#**	**HWE**	**p**	**OR* (95% CI)**	**OR** (95% CI)**
SCARB1	rs4765615	AA	452				0.80 (0.53–1.20)	0.63 (0.41–0.97)	0.23					
		AG	837	0.54	0.001	0.19	0.33 (0.24–0.46)	0.29 (0.21–0.41)						
		GG	411				0.38 (0.24–0.61)	0.31 (0.19–0.51)						
		--	185											
PCSK9	rs10888896	CC	119				1.44 (0.65–3.18)	1.38 (0.60–3.16)	0.18					
		CG	658	0.12	0.003	0.24	0.37 (0.26–0.54)	0.32 (0.22–0.47)						
		GG	1105				0.40 (0.31–0.53)	0.34 (0.25–0.45)						
		--	3											
ABCG5	rs4245786	AA	1099				0.56 (0.43–0.73)	0.46 (0.35–0.61)	0.08					
		AG	692	0.23	0.016	0.57	0.26 (0.18–0.37)	0.24 (0.16–0.34)						
		GG	93				0.49 (0.19–1.24)	0.38 (0.13–1.12)						
		--	1											
ABCG5	rs1864815	AA	834				0.60 (0.44–0.81)	0.48 (0.35–0.67)	0.68	1071			0.96 (0.70–1.33)	0.80 (0.57–1.13)
		AT	835	0.61	0.022	0.57	0.29 (0.21–0.40)	0.25 (0.18–0.36)		1104	0.646	0.82	0.99 (0.72–1.36)	0.83 (0.60–1.17)
		TT	198				0.38 (0.20–0.74)	0.42 (0.21–0.84)		271			1.23 (0.62–2.44)	0.98 (0.47–2.04)
		--	18							0				
LIPC	rs16940379	GG	986				0.47 (0.35–0.62)	0.38 (0.28–0.51)	0.82	168			0.88 (0.66–1.18)	0.69 (0.51–0.95)
		CG	749	0.68	0.031	0.57	0.32 (0.23–0.45)	0.28 (0.20–0.40)		939	0.637	0.45	1.15 (0.81–1.63)	1.02 (0.70–1.46)
		CC	149				0.83 (0.38–1.82)	0.81 (0.36–1.79)		1339			1.38 (0.54–3.53)	1.43 (0.52–3.93)
		--	1							0				
ABCA1	rs4149264	CC	1192				0.52 (0.40–0.68)	0.44 (0.34–0.58)						
		CG	607	0.67	0.034	0.57	0.27 (0.19–0.40)	0.23 (0.15–0.34)	0.35					
		GG	82				0.54 (0.19–1.51)	0.50 (0.16–1.58)						
		--	4											
PPARG	rs2972164	GG	585				0.42 (0.26–0.65)	0.35 (0.22–0.57)	1.00	507			1.06 (0.73–1.54)	0.96 (0.64–1.43)
		AG	897	0.14	0.034	0.57	0.33 (0.24–0.45)	0.28 (0.20–0.39)		1176	0.48	0.89	1.00 (0.73–1.37)	0.79 (0.57–1.11)
		AA	395				0.60 (0.42–0.85)	0.49 (0.34–0.72)		763			1.02 (0.64–1.64)	0.84 (0.50–1.39)

		UCP							HVH					
Gene	**SNP**	**G**	**#**	**HWE**	**p**	**q**	**OR*(95% CI)**	**OR**(95% CI)**	**RSQR**	**#**	**HWE**	**p**	**OR* (95% CI)**	**OR** (95% CI)**
		--	8							0				
PCSK9	rs505151	AA	1632				0.42 (0.33–0.52)	0.36 (0.28–0.45)	NI†	788			0.69 (0.46–1.04)	0.61 (0.39–0.94)
		G	158	0.23	0.038	0.57	0.91 (0.46–1.79)	0.63 (0.30–1.32)		76	0.621	0.56	1.47 (0.38–5.71)	1.05 (0.18–6.26)
		--	95							1				
LRP1	rs715948	GG	908				0.17 (0.08–0.37)	0.17 (0.08–0.37)	1.05	271			0.95 (0.70–1.29)	0.79 (0.57–1.09)
		AG	794	0.7	0.04	0.57	0.49 (0.36–0.67)	0.44 (0.31–0.60)		1011	0.058	0.93	1.06 (0.76–1.47)	0.90 (0.63–1.28)
		AA	181				0.45 (0.33–0.60)	0.36 (0.26–0.49)		1164			1.12 (0.54–2.31)	0.86 (0.39–1.86)
		--	2							0				
SOAT1	rs2493121	AA	217				0.20 (0.11–0.39)	0.19 (0.10–0.38)						
		AT	832	0.75	0.047	0.57	0.49 (0.36–0.68)	0.43 (0.31–0.60)	0.51					
		TT	824				0.44 (0.32–0.61)	0.36 (0.25–0.50)						
		--	12											

For each gene and SNP, the number of subjects for each genotype are given, together with the HWE. The SI was used to test for the interaction between the genotype and statin treatment. The p value for each interaction denotes whether there is an overall difference in the effectiveness of statins between the three genotype groups (with two degrees of freedom). The q value gives an estimate of the proportion of false discoveries among the statistically significant results. For each genotype stratum, the OR reflects the effectiveness of statins within the specific genotype group. The RSQR is a measure for the quality of an imputed SNP in the HVH (cut-off=0.6).

-- = missing genotype

OR = odds ratio

SI = synergy index

* = adjusted for age, sex, region, index date

** = adjusted for age, sex, region, index date, use of calcium channel blockers, and ischemic heart disease

G = genotype

= number of participants

HWE = Hardy-Weinberg equilibrium

lower = lower limit of the 95% confidence interval

upper = upper limit of the 95% confidence interval

p = p-value for the interaction

q = q-value for the interaction

NI = not imputed

RSQR = the average of the observed by expected variance ratio

† = HVH study data only available from the Illumina GoldenGate pan