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A genetic risk score is associated with statin-induced LDL-cholesterol lowering

Short title: Genetic risk score for LDL-cholesterol lowering

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Keywords

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Abstract

Aims

To find new genetic loci associated with statin response, and to investigate the association of a genetic risk score (GRS) with this outcome.

Patients & Methods

In a discovery meta-analysis (five studies, 1,991 individuals), we investigated the effects of approximately 50,000 SNPs on statin response, following up associations with $p < 1e-4$ (three independent studies, 5,314 individuals). We further assessed the effect of a GRS based on SNPs in ABCG2, LPA, and APOE.

Results

No new SNPs were found associated with statin response. The GRS was associated with reduced statin response: 0.0394 mmol/L per allele (95%CI 0.0171;0.0617, $p=5.37e-4$).

Conclusions

The GRS was associated with statin response, but the small effect size (~2% of the average LDLc reduction) limits applicability.

Introduction

The primary goal of statin therapy is to reduce plasma low-density lipoprotein cholesterol (LDLc) levels, thereby lowering the risk of cardiovascular events. Statin treatment has proven effective in reducing risk for major coronary events and all-cause mortality (average risk reductions of 27% and 15%, respectively) [1,2]. However, the response to statin therapy shows a degree of interindividual variability influenced by genetic variation and environmental factors [3,4].

A better understanding of the biological pathways and determinants involved in this variation in statin response could lead to improved treatment. Genome-wide association studies (GWAS) have identified many (95+) loci associated with plasma lipid and lipoprotein traits, including several not previously known to be related to lipoprotein metabolism [5]. In contrast, studies searching for single nucleotide polymorphisms (SNPs) related to statin response have yielded a relatively low number of robust results. Evidence from several studies shows that the $\epsilon 4$ allele of *APOE* is associated with decreased LDLc lowering by statin therapy [3,6–9]. Additionally, SNPs at *ABCG2* and *LPA* were associated with LDLc response at the level of genome-wide significance [8,9]. These GWAS have generally been performed in data from randomized controlled trials (RCTs), and it is not certain if these results also apply to statin users in the general population. In addition, other approaches than hypothesis-free GWAS genotyping across the genome may be a useful complement to uncover further associated genetic variants.

Using a gene-centric approach, we therefore aimed to identify additional genetic associations underlying interindividual variation in statin response. To this end, we performed a meta-analysis of five studies comprising 2,159 individuals of European ancestry by using the candidate-gene ITMAT-Broad-CARe (IBC) array, also known as the CardioChip or the Human CVD BeadArray [10]. This array aims to capture genetic diversity related to cardiovascular, inflammatory, and metabolic phenotypes in greater depth than classic hypothesis-free GWAS using approximately 50,000 SNPs selected across ~2,000 candidate

loci. Compared to normal GWAS arrays, the equal or greater marker density might permit a better identification of multiple functional polymorphisms, or their proxies, at each locus.

Compared with single SNPs, genetic risk scores consisting of multiple SNPs previously identified by GWAS increase statistical power to find associations, and summarize the total genetic effect; therefore these risk scores might be of more clinical value than single SNPs. However, as the single SNPs robustly associated with statin response were found in GWA studies with RCT participants, the value of a genetic risk score in the general population is at present unclear. We therefore investigated whether a genetic risk score is associated with statin response in the lowering of LDLc response in observational studies as well as in RCTs.

Methods

Study selection

In our focus on a gene-centric discovery approach, we selected studies that used the 50k Cardiochip array [10] for genotyping, having more than one measurement of LDLc, information on statin use, and participants of European ancestry. We included five studies, the Atherosclerosis Risk in Communities (ARIC) study, the Framingham Heart Study (FHS), the Multi-Ethnic Study of Atherosclerosis (MESA), Whitehall II (WHII), and AMC-PAS (Academic Medical Centre Amsterdam Premature Atherosclerosis Study). All these studies were observational. Replication of the results was sought in other studies, detailed below. The baseline measurement was defined in these studies as the last measurement of LDLc before reporting use of statins. The baseline value is therefore not necessarily measured on the same visit for all participants. The on-statin measurement was defined as the first measurement of LDLc after the first report of statin use. We then calculated LDLc change (delta LDLc) by subtracting the baseline value from the on-statin value. Details of the set-up and analysis for each study can be found in

the Supplementary Material. Informed consent for DNA analysis was received from each respective local institutional and/or national ethical review board.

Genotyping and Quality Control

Genotyping was performed using the gene-centric IBC array [10]. Several studies have been published using this array and have confirmed previously established associations and identified new associations of SNPs with several phenotypes and disease outcomes, including blood pressure [11], coronary artery disease [12,13], plasma lipids [14], and type 2 diabetes (T2D) [15]. Array data were clustered into genotypes with Illumina BeadStudio software. Quality-control filters were applied within each cohort at the sample and SNP levels and are described in the Supplementary Methods. SNPs with a call rate below 95% or Hardy-Weinberg p -value $< 1e-6$ were excluded from analysis. Only SNPs with a minor allele frequency $> 1\%$ were included in this analysis.

Association analysis

We used linear regression to test the association of a SNP with LDLc change. Association testing was performed using an additive genetic model, adjusting for age, sex, baseline LDLc, and other relevant study covariates (see Supplementary Material). Samples were excluded based on ancestry outliers by PCA, removals of duplicates and first and second degree relatives. Each study was further adjusted for population stratification using the principal component approach in EIGENSTRAT [16]. Adjustment for baseline LDLc was necessary because many of the SNPs previously associated with statin response are also associated with cholesterol levels before treatment with statins. Meta-analysis was performed with METAL [17], using the inverse variance method and a fixed-effects model and I^2 as a measure of between-study heterogeneity [18]. To maintain the conventional 5% false-positive rate, the appropriate multiple-testing-corrected threshold for statistical significance was set at $p < 2.4e-6$, taking into account the ~20,500 independent tests generated for individuals of European descent [19]. We selected all SNPs

reaching $p < 1e-4$ for subsequent replication. Power calculations for the chip-wide association analysis were done using QUANTO [20], assuming a mean LDLc-reduction of 1.3 mmol/L with a SD of 0.6, and a minor allele frequency of 20%.

Replication

Independent replication was attempted for associations with $p < 1e-4$. Look-ups were performed in three additional studies, the UK and Irish subjects from the observational and the randomized arm of the Anglo Scandinavian Cardiac Outcomes Trial (ASCOT-UK RCT and ASCOT-UK OBS) and the RCT JUPITER (Justification for the Use of Statins in Primary Prevention: An Intervention Trial Evaluating Rosuvastatin), with data from a total of 5,314 individuals in the three studies. Details of characteristics and methodological details for cohorts can be found in the Supplementary Material. The association of the genetic risk score was also investigated in these replication studies.

Risk score composition

We calculated a genetic risk score (genetic risk score) on the basis of previous findings related to statin response, selecting risk alleles achieving genome-wide significance ($p < 5e-8$) in GWAS of LDLc change, replicated in at least one other study. In total, we selected 3 SNPs reported by Chasman et al [9], independently found by Deshmukh *et al.* [8] and Tomlinson *et al.* [21]. These SNPs were rs2231142 (a proxy SNP with $r^2 = 0.92$ for rs2199936 in *ABCG2*), rs10455872 in *LPA*, and rs2075650 (a proxy SNP with $r^2 = 0.95$ for rs71352238 in *APOE*). For all three loci, the lead SNP or a good proxy SNP ($r^2 > 0.8$) was available. One of the studies, AMC-PAS, was not included in this analysis due to the unavailability of one of the SNPs in the risk score.

The approach used to calculate the genetic risk score was a simple count of the alleles decreasing statin response, i.e. lowering the efficacy and bringing the response closer to zero. The simple count approach, rather than weighted scores, was chosen as SNP effects found in GWAS were similar.

Association of the risk score with delta LDLc was investigated using linear regression, using the risk score as a continuous variable. Models were adjusted for age, sex, baseline LDLc, and relevant study covariates (e.g. correction for population stratification with principal components) as specified in the Supplementary Material.

Study-specific estimates of the risk score effect (beta) were subsequently combined using random effects meta-analysis with the inverse variance weighting method, using R [22] version 3.0.2 and the 'meta' package [23] 3.5-0. A p-value < 0.05 was considered to constitute a statistically significant effect. We used I^2 to quantify between-study heterogeneity [18].

Results

Characteristics of studies

For the discovery data set, we included five studies with a total of 1,991 participants (Table 1). This gave us 80% statistical power to detect effects of 0.125 mmol/L per allele, which is close to the effect sizes found by other studies [9]. LDLc levels were consistently lower on statin use compared with before statin use, with an average reduction of 1.18 mmol/L. In the replication phase, a total of 5,314 participants in three studies were included with an average LDLc reduction of 1.25 mmol/L. For the risk score analysis, one study was excluded due to unavailable SNPs, and the total sample size was 7,121.

Association analysis

A total of 37,465 SNPs in five cohort studies were tested in the association analysis. No SNPs reached the significance threshold of $p < 2.4e-6$ (Figure S1: QQ-plot, Figure S2: Manhattan plot). One SNP reached $p < 1e-4$ (Table 2), rs17171676 on chromosome 7, and was taken to the replication phase. This SNP was not statistically significantly associated in this replication phase (replication $p > 0.05$), nor in a joint analysis of the discovery and replication phase (Table 2).

Association of genetic risk score

The distribution of the risk score (Table 3) shows that only 4% of the individuals of European ancestry have 4 or more risk alleles, with the majority (more than 95%) having 1-3 alleles. Although statins show a consistent average reduction in LDLc, a higher genetic risk score is associated with a smaller change in LDLc due to statin treatment. The risk score decreased the effect of statins on delta LDLc by 0.0394 mmol/L per allele (95%CI 0.0171; 0.0617), with a p-value of 5.37e-4 (Figure 1). Heterogeneity was low in this analysis ($I^2 = 18\%$). When adjustment for baseline LDLc was not included, the effect size was similar, 0.0354 mmol/L per allele but with a larger 95% confidence interval (0.0054; 0.0654), which is still statistically significant ($p = 0.00206$). The effect size was larger in the observational studies ($\beta = 0.0509$ mmol/L per allele, 95%CI: 0.0172; 0.0847, $p = 0.0031$) than in the RCTs ($\beta = 0.0391$ mmol/L per allele, 95%CI: -0.0097; 0.0880, $p = 0.117$).

Discussion

Using data from six population-based cohort studies and two RCTs, with a total of 7,305 participants, we investigated the effect of common genetic variation on the LDLc response to statins using a cardiovascular gene-centric SNP array. The LDLc lowering effect of statins we found in the whole population, -1.30 mmol/L, is in the range of the values reported in a large meta-analysis of clinical trials (from -1.08 to -1.8 mmol/L)[1,24].

No new loci were found to be associated to LDLc response to statins. Although one locus reached a p-value of $p < 1e-4$, it could be replicated. These results are consistent with a genetic model involving multiple genes, each with modest effects, on LDLc change. The observation that GWAS have revealed robust associations with statin-induced response suggests that there are genuine genetic factors but that much larger samples will be needed to find them with unequivocal evidence.

We also investigated the association of a genetic risk score, consisting of SNPs previously associated with statin response at the genome-wide level, with statin-induced LDLc lowering. The direction of effect of the risk score agreed with what was previously found. However, the effect was small, 0.0313 mmol/L per extra allele, which is about 2% of the total lowering of LDLc we found in the studies included in our analysis. Furthermore, more than 95% of individuals with European ancestry only have 1-3 risk alleles, resulting in a maximum of 0.09 mmol/L difference in statin response. The small effect of the risk score, the modest effective range of a risk score based on 3 SNPs, and the fact that LDLc was substantially reduced in most participants starting statin therapy, suggest that the possible role of genetics in the prediction of statin efficacy is small. The same conclusion was also reached by investigators studying the effects of single SNPs [8,9,25]. This study adds to the existing knowledge that taking the genetic background of a patient into account when prescribing statins, based on the known SNPs that affect statin-efficacy, does not seem justified. Although larger sample sizes in future studies may well result in the identification of additional loci that influence LDLc response, it seems unlikely that a revised genetic risk score would explain a sufficiently large fraction of the observed interindividual variation in LDLc response for it to be clinically useful. These limitations notwithstanding, genetic research into adverse events such as myopathy [26] and rhabdomyolysis might be used to predict and prevent these side effects, as has been done for other drugs and side effects, as effect sizes for adverse drug reactions may be much larger [27,28].

It is important to distinguish between direct genetic effects on (baseline) LDLc and genetic effects on statin-induced LDLc reduction. These can be difficult to separate when there are also associations with baseline LDLc, as is the case for the SNPs used in the risk score [5,9]. Adjusting for baseline LDLc values slightly increased the effect of the risk score, and narrowed the 95% CI. This indicates that associations with LDLc change can be obscured by effects of SNPs on baseline LDLc, and underscores the importance of this adjustment.

A strong point of this study is that it includes evidence from both observational cohort studies and clinical trials, demonstrating that the genetic risk score has a similar effect in both types of studies. A limitation of this study, however, is that we were not able to investigate the genetic effects for the different types of statins separately. This is partly a result of lack of data: the studies in the discovery phase have incomplete dosing and statin type data or complete absence of it. This is different from the data of clinical trials ASCOT and JUPITER (10 mg/d atorvastatin and 20mg/d rosuvastatin, respectively). However, when we would restrict our analyses to those subjects with information on dose and type of statin, the power reduction due to a lower participant count would negatively impact the possible identification of SNPs affecting statin effectiveness. Further, the study is small compared to meta-analyses of main SNP effects, which now typically include more than 100,000 participants [5]. This is a result of the fact that only a subset of the participants in observational studies (and also of placebo-controlled statin trials) is prescribed statin medication. Additionally, several population-based studies only have cholesterol measures at only one time point, excluding the possibility of investigating longitudinal changes in LDLc, while other studies do not have (reliable) information on drug use. For pharmacogenetic research, which is hampered by relatively small sample sizes [29], it would be valuable if drug use data and longitudinal follow-up data were routinely collected in large samples.

In summary, we investigated whether new individual SNPs associated with statin-induced change in LDLc levels could be identified from a gene-centric discovery approach; we further investigated whether a genetic risk score, composed of SNPs previously associated with statin response, was associated with statin-induced change in LDLc levels. We did not find new SNPs associated with the magnitude of statin-induced LDLc reduction, but we found that there is a small but statistically significant association of the risk score with statin-induced LDLc reduction. However, use of statins lowers LDLc substantially regardless of genotype, and each additional allele in the genetic risk score decreases the total LDLc change only by 2%. Therefore, genotype-based clinical decision making for statin therapy is unlikely to

improve efficacy, and at this moment there is no role for genetic testing in clinical practice to guide statin treatment.

Future perspective

To date, several polymorphisms have been found to affect response to statin therapy based on the achieved lowering of LDL cholesterol (LDLc). A further increase of the number of loci associated with LDLc response can probably be achieved with larger studies using GWAS methodology, although large numbers of statin users are required to effectively perform these. Because of the small effect sizes identified, there does not seem to be a genetically identifiable subgroup in which statins do not lower LDLc. The utility of genetic research into statin response might lie more in a better understanding of pharmacokinetics and pharmacodynamics, and ultimately the development of new drugs, than in direct clinical application (e.g. choosing a drug type or dose). Alternatively, research into the (prediction of) adverse drug reactions side effects of statin therapy might have a faster impact on clinical decisions.

Summary points

Background

- The response to statin therapy shows a degree of interindividual variability influenced by genetic variation and environmental factors.
- Several studies have found genetic variants that have an effect on the statin-induced LDLc lowering.

Patients & methods

- We investigated the association of statin response on a cardiovascular gene-centric SNP array in a discovery data set consisting of five studies and 1,991 individuals, aiming to find novel associations with this outcome.
- We further assessed the effect of a composite genetic risk score on statin response, based on previously associated SNPs in ABCG2, LPA, and APOE.

Results: main findings

- A genetic risk score was significantly associated with LDLc-response to statins. We found no novel SNPs to be significantly associated with LDLc-response to statins

- The small effect size of this association suggests that the applicability of this score in predicting statin response in clinical practice will be limited.

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Conflict of Interest/Disclosure

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mean age of the offspring cohort was 37 years; 52 percent were women. A third generation cohort of 4,095 children of offspring cohort participants (mean age 40 years; 53 percent women) was enrolled beginning in 2002. At each clinic visit, a medical history was obtained with a focus on cardiovascular content, and participants underwent a physical examination including measurement of height and weight from which BMI was calculated; **MESA**: The Multi-Ethnic Study of Atherosclerosis Study (MESA) is a multicenter prospective cohort study initiated to study the development of subclinical cardiovascular disease. A total of 6814 women and men between the age of 45 and 84 year were recruited for the first examination between 2000 and 2002. Participants were recruited in six US cities (Baltimore, MD; Chicago, IL; Forsyth County, NC; Los Angeles County, CA; Northern Manhattan, NY; and St. Paul, MN). This study was approved by the institutional review boards of each study site, and written informed consent was obtained from all participants. This cohort was genotyped as part of the National Heart Lung and Blood Institute's (NHLBI) Candidate Gene Association Resource (CARE) (Musunuru, K., Lettre, G., Young, T., Farlow, D.N., Pirruccello, J.P., Ejebe, K.G., Keating, B.J., Yang, Q., Chen, M.H., Lapchyk, N. et al. Candidate gene association resource (CARE): design, methods, and proof of concept. *Circ. Cardiovasc. Genet*, 3, 267-275.); **JUPITER**: The Jupiter study and genotyping of the study participants was funded by AstraZeneca.

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Figure legends

Figure 1 Forest plot for the association between the genetic risk score and LDLc reduction. Analyses were adjusted for baseline LDLc values.