Rooted in risk; genetic predisposition for LDL-c level associates with diminished LDL-c response to statin treatment

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Abstract

Aim: To utilize previously reported lead SNPs for LDL-c levels to find additional loci of importance to statin response, and examine whether genetic predisposition to LDL-c levels associates with differential statin response.

Patients/methods: We investigated effects on statin response of 59 LDL-c SNPs, by combining summary level statistics from the Global Lipids Genetics and Genomic Investigation of Statin Therapy consortia.

Results: Lead SNPs for APOE, SORT1, and NPC1L1 were associated with a decreased LDL-c response to statin treatment, as was overall genetic predisposition for increased LDL-c levels as quantified with 59 SNPs, with a 5.4% smaller statin response per standard deviation increase in genetically raised LDL-c levels.

Conclusion: Genetic predisposition for increased LDL-c level may decrease efficacy of statin therapy.

Word count (abstract): 120

Word count (body of article, including future perspective): 2,793

Number of tables: 4 (including 2 supplemental tables)

Number of figures: 1

Number of references: 26

Keywords: statin therapy, pharmacogenetics, MR-Egger

Introduction

HMG-CoA reductase inhibitors, also known as statins, have proven themselves as a highly effective treatment option in the management and prevention of cardiovascular disease, both in research and clinical settings [1, 2]. Their effect is thought to primarily result from reducing low-density lipoprotein cholesterol (LDL-c) levels by up to 50% [3], thereby achieving a 20-30% reduction of cardiovascular events. However, substantial interindividual variability exists in the LDL-c response to statins, in part due to genetic factors, which influences their efficacy in reducing the occurrence of major adverse events.

Recently, through the largest pharmacogenomic meta-analysis for differential LDL-c response to statin therapy to date, the Genomic Investigation of Statin Therapy (GIST) consortium identified four loci (APOE, LPA, SORT1/CELSR2/PSRC1, and SLCO1B1) at a genome-wide significant level, whose effect on statin response was independent of off-treatment LDL-c levels [4]. With the exception of SLCO1B1, these loci have previously been independently reported to associate with LDL-c levels by the Global Lipids Genetics consortium (GLGC) [5]. As loci associated with LDL-c homeostasis are strong mechanistic candidates for differential LDL-c response to statin therapy, we performed a look-up of the previously reported lead SNPs for loci associated with LDL-c levels by the GLGC in the GIST consortium, to examine whether additional loci of importance to differential LDL-c statin response could be identified. Furthermore, we examined whether overall genetic predisposition to higher LDL-c levels (i.e. having more alleles associated with higher LDL-c levels) is associated with differential LDL-c response to statins, by combining summary level statistics from our GIST consortium with publicly available data from the GLGC for all lead SNPs through an inverse-variance weighted approach.

Methods

Selection of Single Nucleotide Polymorphisms (SNPs) associated with LDL-c levels

In the most recent and largest genome-wide association study (GWAS) for blood lipid levels, which examined up to 188,577 European-ancestry individuals, 157 nearly independent loci ($r^2 < 0.10$) were found to associate with lipid levels at p-values lower than 5×10^{-8} [5]. Of the reported 157 lead SNPs, 60 were associated with LDL-c levels (**Supplementary Table 1**). Summary level data of the associations of these 60 lead SNPs with LDL-c levels was downloaded from the University of Michigan GLGC webpage (http://csg.sph.umich.edu//abecasis/public/lipids2013/). Effects on lipid levels were reported in standard deviations. We excluded rs9411489 (ABO) from our analyses, as the genotype could not be imputed in our populations, and therefore included the remaining 59 lead SNPs in our analyses. To further isolate the effects on LDL-c levels from those of other lipids, we repeated all analyses with a restricted SNP list, excluding the 17 variants which also associated with either high-density lipoprotein cholesterol (HDL-c) or triglycerides (TG) levels at a genome-wide significant level. Of these, 5 associated solely with HDL-c, 4 solely with TG, and 8 with both lipid traits. As LDL-c is closely linked to total cholesterol (TC), we did not exclude variants which also associated with TC at a genome-wide significant level. The restricted list therefore included the remaining 42 LDL-c specific SNPs.

Description of pharmacogenetic meta-analysis

The GIST consortium included 6 randomized controlled statin trials (ASCOT, CARDS, CAP, PRINCE, PROSPER, and TNT) and 10 prospective, population-based studies (AGES, ARIC, BioVU, CHS, FHS, GoDARTS I, GoDARTS II, Health ABC, HVH, MESA) for the first stage, comprised of up to 18,596 statin recipients. In addition, 246 SNPS with $p<5x10^{-4}$ were further investigated in three additional studies (HPS, JUPITER, Rotterdam Study), contributing up to 22,318 additional statin-treated subjects to the meta-analysis. Of the 59 lead SNPs for LDL-c levels reported by the GLGC, only one (rs4420638, APOE) was included amongst these 246 SNPs. The GWAS was performed on the difference between natural log-transformed on- and off-treatment LDL-c levels, adjusting for the natural log-transformed off-treatment LDL-c level to control for possible mediation through off-treatment genetic effects. The beta of the corresponding regression therefore represents the fraction of differential LDL lowering in carriers versus non-carriers of each SNP. Details on included studies, genotyping and GWAS analyses have been described previously [4].

Look-up of single SNPs

We performed a look-up of all 59 candidate LDL-c markers within the pharmacogenetic meta-analysis performed by the GIST consortium, assessing their effect on differential LDL-c response to statin therapy adjusted for off-treatment LDL-c values. Adjusted unstandardized beta-coefficients are given for the LDL-c-increasing alleles reported by the GLGC. Multiple testing was taken into account by means of a Bonferroni-corrected p-value threshold of 8.5×10^{-4} (i.e. 0.05/59).

Summary data methods for overall effect of LDL-c predisposition

Next, we investigated whether overall genetic predisposition for LDL-c levels was associated with statin response, making use of summary level data from both the GLGC and GIST consortia. All analyses were carried out separately for the full (n=59) and restricted (n=42) SNP lists. Analogous to pooling estimates from different studies in conventional meta-analysis using inverse-variance weighting (IVW), we pooled the causal estimates from the different genetic variants, defined as the ratio of each SNP's per-allele effect on response to statin therapy to its per-allele effect on LDL-c levels. The average of these ratio estimates was weighted by the inverse of the variance of the per-allele effect on response to statin therapy, and can be visualised as a regression line constrained to pass through the origin [6, 7]. As this approach may be biased by the inclusion of genetic variants violating the underlying assumptions of instrumental variable (IV) methods [8], most notably by the presence of unbalanced pleiotropic effects on phenotypes other than LDL-c, we performed two additional analyses which should be considered as sensitivity analyses for Mendelian randomisation (MR) investigations with multiple genetic variants [9].

We first employed the recently published MR-Egger method [10], which provides a formal test of the presence of directional (i.e. unbalanced) pleiotropy from separate genetic variants by introducing an intercept term to the IVW method and determining whether this term deviates significantly from zero. Based on the Egger test [11], which assesses the presence of small study bias in meta-analysis, this intercept term can be interpreted as the average pleiotropic effect across the genetic variants. After taking these effects into account, the Egger-regression slope reflects the strength of any residual dose-response relationship. Under the assumption that the strength of the association of each variant with LDL-c levels is independent of the pleiotropic effects of the variant (i.e. not via LDL-c), MR-Egger regression gives a valid causal effect estimate even when all the genetic variants are invalid instrumental variables [10].

Secondly, we calculated the weighted median estimator, defined as the 50% weighted percentile of the distribution of causal estimates given weights proportional to the inverse of their variance [9]. As the median of any distribution is less susceptible to outliers, this method provides a consistent causal estimate under the assumption that over 50% of the weight in the analysis is due to valid instruments. We also provide the penalized weighted median estimate, which severely limits the contribution of heterogeneous (i.e. outlying) variants, which are more likely to represent invalid instrumental variables. This penalty is based on the heterogeneity between estimates as quantified by Cochran's Q statistic. We considered p-values of 0.05 or smaller statistically significant for these summary data methods. All analyses were performed with R software version 3.1.1. [12], utilizing the R code provided by the corresponding methodology papers on MR-Egger and median-based methods [9, 10].

Results

Look-up of single SNPs

After correction for multiple testing, three SNPs were found to have attained a statistically significant association with LDL-c response to statins (all p-values < 8.5×10^{-4}, **Table 1**). The results indicate that carriers of these SNPs have a smaller LDL-c response to statin therapy when compared with non-carriers. The magnitudes of these per-allele proportional decreases were 2.5% (APOE, 95% CI: 1.8-3.1), 1.5% (SORT1, 95% CI: 0.9-2.1), and 1.8% (NPC1L1, 95% CI: 0.8-2.7) respectively. When restricting the SNP list to those 42 variants primarily associated with LDL-c, which did not include the lead SNPs for APOE and SORT1, NPC1L1 was the sole statistically significant finding (p=2.1×10^{-4}), also after adjusting the Bonferroni-corrected p-value threshold to 1.2×10^{-3} (i.e. 0.05/42).

Summary results for overall effect of LDL-c predisposition

As shown in **Figure 1** and **Table 2**, the conventional inverse-variance weighted method revealed strong evidence that overall genetic predisposition for higher LDL-c levels associates with a decreased LDL-c response to statin therapy. For the full list (all LDL-c associated variants), this amounted to a 5.4% (95% CI: 4.2-6.7, $p=8.4 \times 10^{-12}$) smaller response per standard deviation increase in genetically raised LDL-c levels. Despite the effect being slightly reduced, the direction of the association was similar for the restricted list (excluding HDL-c and TG-associated variants), showing a 3.2% (95% CI: 1.2-5.1, $p=2.1 \times 10^{-3}$) decreased response per standard deviation increase in genetically raised LDL-c levels.

Results from both sensitivity analyses were largely consistent with those seen for the IVW approach, with regard to magnitude and direction of the association, especially for the restricted SNP-list (**Table 2**). The MR-Egger results indicated the presence of unbalanced pleiotropy for the full list of variants ($p=7.6 \times 10^{-5}$), which was not present when analyses were restricted to those variants primarily associated with LDL-c (p=0.40). Though inconclusive, further attempts to disentangle the influence of HDL-c and TG-associated variants suggested that the variants associated with HDL-c were especially influential with regard to possible unbalanced pleiotropic effects on statin response, as their exclusion led to the greatest decrease in the MR-Egger intercept term (**Supplemental table 2**). Of the median-based methods, the penalized estimator was the most consistent with the IVW-estimate, for both SNP lists.

Discussion

Within the present study, we aimed to examine whether additional loci of importance to LDL-c response to statin therapy could be identified by focusing our efforts on previously reported lead SNPs explaining variation in LDL-c levels. In addition to reconfirming the previously described associations of APOE and SORT1 with LDL-c response to statin therapy, we found suggestive evidence that NPC1L1 is of importance to statin pharmacogenetics. Of note, our previously reported association of LPA with statin response was not among these results, reflecting the different lead SNP reported by the GLGC, which also explains why the association with statin response was not genome-wide significant for SORT1. Consistent with the results for the individual lead SNPs, we found strong evidence that overall genetic predisposition for higher LDL-c levels is associated with a decreased LDL-c statin response, and robustly quantified this association using summary level data from the largest and most recent GWA studies on lipid levels and LDL-c response to statin therapy.

Localized to gastrointestinal tract epithelial cells as well as hepatocytes, the Niemann-pick C1-like 1 (NPC1L1) protein is a key regulator of cholesterol absorption [13], and is the drug target of ezetimibe [14]. Shown to associate with interindividual variation in response to ezetimibe treatment [15, 16], genetic variation in NPC1L1 has also been previously linked to LDL-c response to statin therapy in smaller studies. In 37 men with central obesity, Chan and colleagues found that subjects with the NPC1L1 2/2 haplotype had a greater reduction in LDL-c levels than non-2/2 haplotype subjects, independent of their higher baseline LDL-c levels [17]. Moreover, in the PROSPER trial, the NPC1L1 -133A>G variant was found to associate with greater 6-month change in lipid levels in pravastatin-treated individuals, but also with higher baseline LDL-c levels, which were not adjusted for in the analyses [18].

In contrast, our findings are unlikely to be explained by differences in off-treatment LDL-c levels, as these were statistically accounted for in the GIST meta-analysis. Rather, the genetic associations with LDL-c levels reflect lifelong effects on lipid metabolism, which we now show may influence the efficacy of clinical interventions later in life. Unfortunately, our use of summary level data precludes providing more detailed mechanistic insights, though there exists some evidence that statin therapy efficacy interacts with cholesterol synthesis and absorption, possibly in part through changes in intestinal expression of NPC1L1 [19, 20].

While the MR-Egger test did not show evidence for directional pleiotropy after excluding variants associated with HDL-c or TG at a genome-wide significant level, it is possible that the remaining variants are not solely of importance to LDL-c homeostasis, as meaningful sub-threshold associations may exists for HDL-c or TG. Similarly, we cannot be certain that the associations with HDL-c and TG of the excluded genetic variants reflected true biological pleiotropy, or merely down-stream effects of LDL-c on other phenotypic traits (lipid or otherwise), which are specifically the effects of interest in MR investigations [21]. However, by creating a restricted list we attempted to isolate variants more specific to LDL-c levels, as has previously been done when constructing genetic risk scores consisting of large numbers of genetic variants [22]. In line with this, the consistency of the different methods for the restricted score indicates that this score is less likely to contain invalid instruments. Furthermore, the relatively large difference in mean estimates between the MR-Egger and median-weighted methods for the full list of variants possibly reflects violation of MR-Egger's underlying assumptions, as variants associated with LDL-c levels might be proportionally associated with HDL-c and TG levels.

As we included summary level data from partially overlapping data sources, our findings may have been influenced by weak IV bias [23]. More specifically, of the 10 prospective, population-based studies which contributed to the first-stage meta-analysis of GIST, 6 (AGES, ARIC, CHS, FHS, Go-DARTS I, Go-DARTS II) also contributed to the GLGC meta-analysis. With the exception of rs4420638 (APOE), which was validated in additional populations in the second-stage meta-analysis of GIST, this means that up to 43% of GIST participants included in the first-stage meta-analysis were possibly also included in the GLGC analyses. However, the median F-statistic of our instruments for LDL-c levels was 58.35 (IQR 42.51-118.59), making it unlikely to have substantially influenced our results, as instruments with F-statistics over 10 are generally considered sufficiently strong [24].

In summary, we investigated whether 59 lead SNPs known to associate with LDL-c levels also associate with differential LDL-c response to statin therapy. After taking multiple testing into account, we found that three lead SNPs (for APOE, SORT1, and NPC1L1) were associated with smaller LDL-c response to statin treatment, thereby identifying one new locus of importance to statin response, namely NPC1L1. In addition, our findings indicate that individuals with overall genetic predisposition for high LDL-c levels are less likely to respond well to statins.

Future perspective

To date, pharmacogenetic research on statin therapy has identified genetic variants with only modest effect sizes and therefore limited clinical utility [25]. However, our results suggest that risk stratification based on a LDL-c genetic risk score might identify individuals most likely to benefit from combination therapy of statin and non-statin lipid-lowering medication, as genetic predisposition to higher LDL-c levels may not affect their efficacy to the same degree. If genetic information becomes available, large experimental studies such as the recently completed IMPROVE-IT trial [26] would be most suited to determine possible clinical significance. In addition, pharmacogenetic studies of non-statin LDL-lowering therapies should also consider examining the role of genetic predisposition for higher LDL-c levels.

Executive summary

Background

- There exists substantial interindividual variation in low-density lipoprotein cholesterol (LDL-c) response to statin treatment, in part due to genetic factors. Several genetic loci have been found to associate with differential LDL-c response to statins, independent of off-treatment LDL-c levels.
- The majority of these loci have additionally been found to associate with LDL-c levels. LDL-c level-associated loci may therefore represent strong candidates for pharmacogenetic studies on statin therapy.

Patients & methods

- To identify additional loci of importance to statin response, we performed a look-up of 59 lead SNPs for LDL-c levels in the pharmacogenetic meta-analysis of the GIST consortium.
- We further examined whether overall genetic predisposition for higher LDL-c levels associates with statin response, by combining summary statistics from the GLGC and GIST consortia for 59 lead SNPs for LDL-c levels from the GLGC, through an inverse-variance weighted approach. MR-Egger regression and median-based methods were then performed as sensitivity analyses.

Results: main findings

- Lead SNPs for APOE, SORT1, and NPC1L1 were associated with diminished statin response, as was overall genetic predisposition for increased LDL-c level.

Disclosure/acknowledgments

We would like to thank the GLGC consortium for making available the result files of their meta-analysis. In addition, we wish to express our gratitude to all studies participating in the GIST consortium.

BMP serves on the DSMB of a clinical trial funded by the manufacturer, and on the Steering Committee of the Yale Open Data Access Project funded by Johnson & Johnson. DIC received research support for independent genetic analysis in JUPITER from AstraZeneca. JWJ is an Established Clinical Investigator of the Netherlands Heart Foundation (grant 2001 D 032). RMK serves on the Merck Global Atherosclerosis Advisory Board. The remaining authors declare no competing financial interests.

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Table 1. Candidate markers significantly associated with LDL-c response to statin therapy

			Global Lipids Genetics consortium (GLGC)					Genomic Investigation of Statin Therapy (GIST) consortium		
SNP	Locus	Chr	EA	EA freq. (1000G)	Beta (SE)*	p-value	Other lipids	EA freq.	Beta (SE)†	p-value
rs4420638	APOE	19	G	0.19	0.225 (0.008)	$2x10^{-178}$	HDL-c, triglycerides, TC	0.17	0.025 (0.003)	$3.9x10^{-15}$
rs629301	SORT1	1	T	0.79	0.167 (0.005)	$5x10^{-241}$	HDL-c, TC	0.77	0.015 (0.003)	$9.4x10^{-7}$
rs2072183	NPC1L1	7	C	0.24	0.039 (0.004)	$7 x10^{-16}$	TC	0.24	0.018 (0.005)	$2.1x10^{-4}$

Listed variants are those with p-values smaller than the Bonferroni-corrected threshold of $8.5x10^{-4}$ (i.e. 0.05/59) for the association with statin response.

Chr, chromosome; EA, effect allele for increased LDL-c levels from the GLGC consortium; HDL-c, high-density lipoprotein cholesterol; TC, total cholesterol

* Beta for effect on LDL-c levels, in standard deviations

† Beta for difference between the natural log-transformed on- and off-treatment LDL-c levels adjusted for natural log-transformed off-treatment LDL-c, age-, sex- and study-specific covariates. A negative beta indicates a better statin response, a positive beta a worse statin response.

Table 2. IVW, MR-Egger, and median-based estimators for the association between LDL-c levels and proportional LDL-c response to statin therapy

	Full list of 59 variants		42 variants primarily associated with LDL-c	
Analysis method	Beta (SE)	p-value	Beta (SE)	p-value
Inverse-variance weighted	0.054 (0.006)	**8.4×10^{-12}**	0.032 (0.010)	**2.1×10^{-3}**
MR-Egger: slope	0.089 (0.010)	**1.0×10^{-11}**	0.044 (0.018)	**1.7×10^{-2}**
MR-Egger: intercept	-0.003 (0.001)	**7.6×10^{-5}**	-0.001 (0.001)	0.40
Weighted median	0.070 (0.011)	**4.2×10^{-8}**	0.043 (0.015)	**6.4×10^{-3}**
Penalized weighted median	0.051 (0.011)	**2.8×10^{-5}**	0.043 (0.015)	**6.8×10^{-3}**

Beta's (SE) given as differential LDL-c response to statin therapy per standard deviation increase in LDL-c levels. The MR-Egger intercept term provides a formal test of directional pleiotropy. P-values in bold reflect statistically significant results, using a p-value threshold of 0.05.

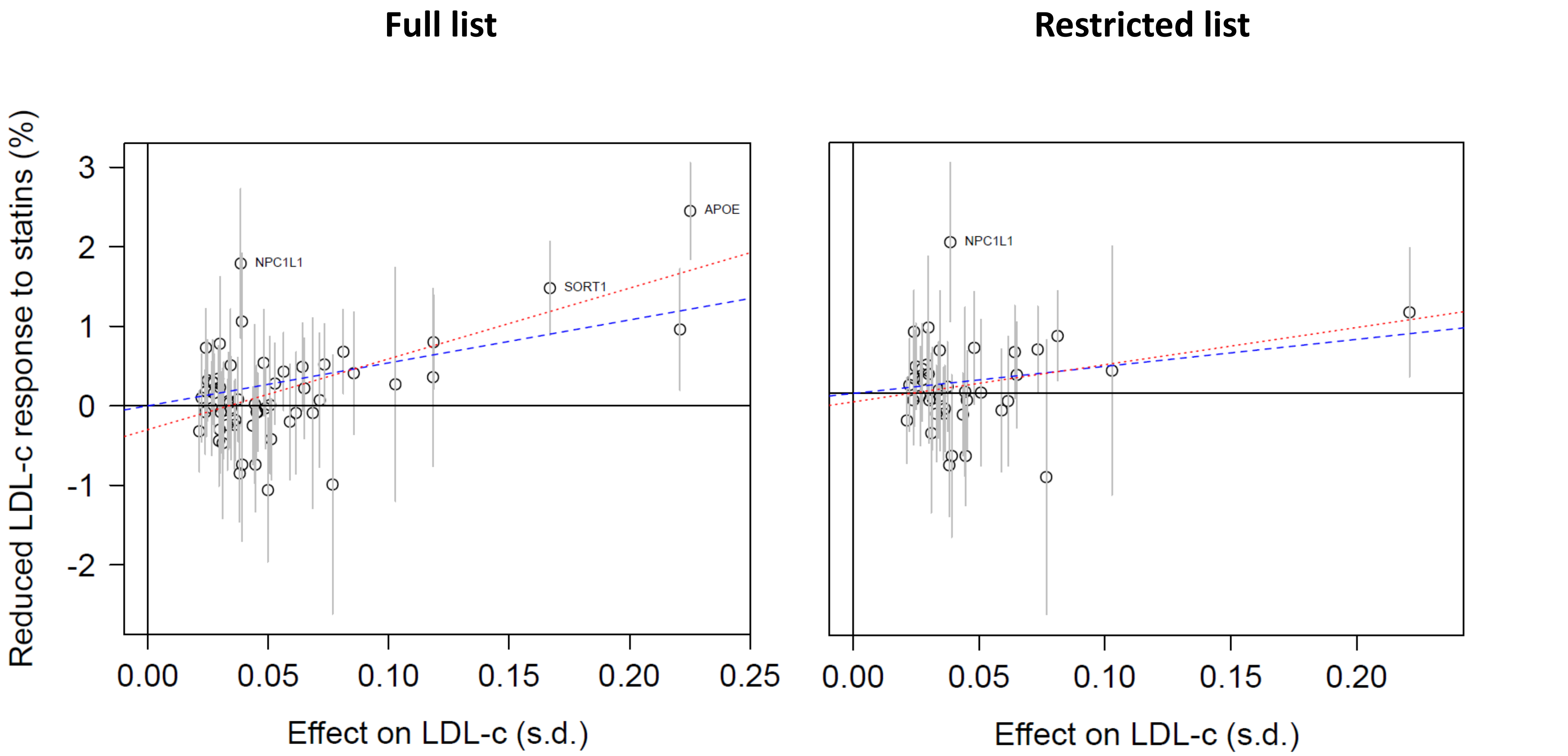

Figure 1. Scatter plots of the genetic associations with LDL-c against genetic associations with differential LDL-c response to statin therapy, both plotted as per-allele effects. In addition, 95% CI's are presented for the genetic associations with statin response. The blue (dashed) and red (dotted) line correspond to the inverse-variance weighted and MR-Egger estimators respectively, and are shown for the full (59 SNPs) and restricted (42 SNPs) lists, with a positive slope reflecting a worse statin response.