Genetic association of lipids and lipid drug targets in abdominal aortic aneurysm

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Abstract

Introduction
Risk factors for abdominal aortic aneurysm (AAA) are largely unknown and this has hampered development of non-surgical treatments to alter the natural history of disease. Here, we use Mendelian randomization (MR) analyses to investigate the association between lipid-associated single nucleotide polymorphisms and AAA.

Methods
Genetic risk scores, composed of lipid trait associated SNPs were constructed and tested for their association with AAA using conventional (inverse-variance weighted) MR in up to 4,914 cases and 47,912 controls across 5 studies. Sensitivity analyses to account for potential genetic pleiotropy included MR-Egger and median weighted MR, and to test the independent effect of lipids and risk of AAA we used multivariable MR. Finally, we assessed the association between AAA and SNPs in loci that can act as proxies for drug targets.

Results.
A 1 standard deviation (SD) genetic elevation of low-density lipoprotein cholesterol (LDL-C) was associated with increased risk of AAA (odds ratio [OR]
1.70, 95% confidence interval [CI] 1.70 – 2.00, P = 4.9 x 10^{-10}). For high density lipoprotein cholesterol (HDL-C), a 1 SD increase was associated with a reduced risk of AAA (OR 0.66, 95% CI 0.54 – 0.80, P = 2.6 x 10^{-5}) while a 1 SD increase in triglycerides (TG) was associated with increased risk of AAA (OR 1.66, 95% CI 1.35 – 2.04, P = 1.4 x 10^{-6}). In multivariable analysis (which can inform on potential independent effects of lipids) and both MR-Egger and weighted median MR (which can account for genetic pleiotropy), the association of each lipid fraction with AAA risk remained largely unchanged. The LDL-C reducing allele of rs12916 in HMGCR (a proxy for statin treatment) was associated with AAA (OR 0.94, 95% CI 0.88 – 0.98, P = 0.009). The HDL-C raising allele of rs3764261 in CETP (a proxy for CETP-inhibition) associated with AAA (OR 0.89, 95% CI 0.85 – 0.93, P = 1 x 10^{-7}). Finally, the LDL-C lowering allele of rs11206510 in PCSK9 (a proxy for PCSK9 inhibition) was weakly associated with a lower risk of AAA (OR 0.94, 95% CI 0.88 – 1.00, P = 0.05) but a second independent LDL-C lowering variant in PCSK9 (rs2479409) was not associated with AAA (OR 0.97, 95% CI 0.84 – 1.02, P = 0.28).

**Conclusion**

Our Mendelian randomization analyses lend support to the hypothesis that lipids play an important role in the etiology of AAA. Our analyses of individual genetic variants used as proxies for drug targets support LDL-C lowering (particularly through statin treatment) as a potential effective treatment strategy for the prevention and treatment of AAA. Furthermore, CETP inhibitors and novel LDL-C lowering treatments may warrant further evaluation in patients with AAA.
Introduction

Abdominal aortic aneurysm (AAA) is an important cardiovascular disease resulting in approximately 4,500 deaths from rupture per year in the United States (US)(1). Around 45,000 operations are carried out each year to prevent rupture, resulting in a further 1,400 deaths(1). AAA screening reduces the burden of AAA rupture in men(2) and therefore many countries now offer such screening to at risk groups(3,4). The US Preventative Services Task Force recommends screening males aged 65-75 with a history of smoking and AHA guidelines suggest surgical repair when the AAA reaches 5.5 cm in diameter.

Recognized non-modifiable risk factors for development of AAA include male sex, increasing age and a family history of the disease. The major modifiable risk factor is cigarette smoking(5-7) and there is evidence that public health policies aimed at reducing smoking have reduced the incidence of AAA(8). The role of other modifiable risk factors in AAA has been poorly defined and in many cases remains controversial(5,9,10).

AAA shares risk factors with occlusive atherosclerotic disease but the magnitude and direction of association is not always consistent. There is a growing body of evidence suggesting considerable heterogeneity of risk factor associations amongst different forms of cardiovascular diseases (CVD) (11-13). For example, the risk of smoking for AAA is at least twofold greater than that for coronary heart disease (CHD)(13), while type 2 diabetes (T2D) appears to be protective for AAA but T2D is a major risk factor for occlusive vascular disease(12). This suggests that AAA may have some distinct causal pathways and understanding...
these will be important for setting public health policies aimed at reducing the risk posed by AAA and its complications.

Genome wide association studies (GWAS) of AAA have identified robust associations of loci that have previously been found for CHD (9p21(14), DAB2IP(15), LDLR(16), SORT1(17), and IL6R(18)) and also a number of variants that do not appear to be associated with other cardiovascular diseases (LRP1(19), SMYD2, ERG, MMP9, and LINC00540(20)). While this again lends support to the hypothesis that AAA and CHD have overlapping pathophysiology, the association of SNPs with AAA and not other CVD suggests that there may well be discrete etiological pathways between these vascular diseases.

Dyslipidemia has long been recognized as a major risk factor for CHD. The role of low-density lipoprotein cholesterol (LDL-C) in CHD is well defined and LDL-C lowering therapies are of clear benefit in reducing CHD risk(21). Genetic studies appear to support a causal role for hypertriglyceridemia in CHD(22-24) but genetic and clinical studies have cast doubt on the status of high density lipoprotein cholesterol (HDL-C) as a causal factor in CHD(22,25-27), despite strong and consistent associations in large scale observational studies(28). In AAA there are a lack of high quality epidemiological data reporting the association between lipid levels and disease risk and/or progression. Previous meta-analysis of observational studies did show a consistent inverse association of HDL-C with risk of AAA but the association with LDL-C was less clear(29,30). It is important, however, to recognize that the studies included in these meta-
analyses were small case-control studies that have not in many cases adjusted for statin use. Furthermore, reverse causality is a major limitation of such case-control studies, meaning that lipid levels may be altered not only by the disease process, but also by concurrent treatment by statins and lifestyle alterations in AAA cases. There is a paucity of any data reporting an association between triglycerides (TG) and AAA risk/progression. Small animal models suggest a potential therapeutic role for both LDL-C(31) and HDL-C(32) but data in humans are lacking. From a clinical point of view it is important to understand the role of lipids in AAA especially considering the excess cardiovascular risks in patients with AAA(6) and recent publications showing low prevalence of LDL-C lowering in AAA patients(33,34).

The co-existence of CHD (symptomatic or asymptomatic) and AAA in many individuals also poses a considerable challenge to deciphering causal pathways(35). Previous genetic association studies have pointed to a potential role of lipids in AAA pathology(16,17,36), but the current study uses a larger panel of SNPs and a considerably larger sample. Mendelian randomization (MR) is an approach that utilizes the unique properties of genotype to investigate causal relationships(37). Specifically, genotype is randomly allocated at conception, owing to Mendel’s second law (a feature that is exploited to minimize confounding), and genotype is not affected by reverse causation. Although MR has traditionally been used to explore causal relationships between circulating biomarkers and disease phenotypes, an
extension of the technique uses genotype to validate drug targets. In this approach, variants in genes encoding potential drug targets are used as instruments to explore the utility of targeting this pathway in specific disease states\textsuperscript{(38,39)}. A major challenge in MR studies of complex traits such as lipid fractions is genetic pleiotropy, whereby SNPs influence circulating concentrations of multiple lipids fractions, and this so-called pleiotropy may reflect an association of a SNP (or multiple SNPs in combination) with multiple discrete pathways that may have differing relationships with AAA, leading to a biased estimate from MR. Recent developments in the technique using both multivariable MR\textsuperscript{(22)}, median weighted MR\textsuperscript{(40)} and MR-Egger\textsuperscript{(41)} have been used to address these issues, but pleiotropy still poses a challenge.

In this study we use conventional MR, multivariate MR, median weighted MR and MR-Egger approaches to investigate the role of lipids in the etiology of AAA.
Methods

The association of genetic risk scores (GRS) for lipid traits with AAA was investigated in five international AAA genome-wide association studies (GWAS)(20). The GRS were composed of SNPs that are robustly associated with serum lipids in the Global Lipids Genetics Consortium (GLGC) meta-GWAS of circulating lipid levels(42).

Study Populations

We used summary SNP – AAA association statistics from previously published GWAS of AAA. This included five genome-wide association studies from the UK(19,20), New Zealand(19,20), the US(20), the Netherlands and Iceland(15). Detailed descriptions of the GWAS analysis are provided in the supplementary data and previous publications. In addition, for the study of single variants in genes encoding lipid drug targets, we supplemented with additional data derived from the Secondary Manifestations of Arterial Diseases (SMART) study. Table 1 includes the number of cases and controls from each study. Descriptions of study cohorts and demographic details are presented in the supplementary data and previous publications. In all studies, the case definition of AAA was an infrarenal aortic diameter ≥3 cm by ultrasound or computed tomography imaging, or previous AAA rupture/repair. Details of the association tests and quality control used in each study are included in the supplementary data and a previously published meta-GWAS.(20). All studies received approval from their respective Institutional Review Boards and Ethics Committees.
Selection of SNPs

SNPs associated with lipids in the Global Lipid Genetics Consortium were identified(42). We used the SNP selection criteria by Do et al(5,22). Briefly, SNPs in association with at least one of the three lipid traits (LDL-C, HDL-C or TG concentrations) at a genome-wide significance level ($P < 5 \times 10^{-8}$) were selected. In Do et al(22) at loci with multiple associated SNPs, single SNPs with the strongest effect estimates were selected and more than one SNP was selected only if there was evidence of minimal linkage disequilibrium ($r^2 < 0.05$). There were data available for 180 of 185 SNPs (Supplementary Table S1) described in Do et al(22).

Data analysis

We first harmonized SNPs across the datasets (GLGC and AAA consortia) by merging SNPs on the rs number. We then ensured that effect alleles were denoted to be the same in both datasets. This was double-checked by investigating effect allele frequencies. We then orientated all variants so that the effect allele was positively associated with each lipid trait (i.e. so that for example, in the MR of LDL-C, all beta coefficients for LDL-C were >0). This resulted in a dataset where each individual SNP was a unique row and we had separate columns for beta and standard errors for each lipid trait and the log odds ratio and corresponding standard error for AAA (see Table S1).
Conventional Mendelian randomization

We conducted a conventional two-sample MR analysis to determine the association between a 1 standard deviation (SD) genetically elevated lipid concentration and risk of AAA. This was conducted using the ‘inverse variance weighted’ method in which the SNP association estimates for the outcome ($\beta_{AAA}$) are regressed on the SNP association estimates ($\beta_{LDL-C}$, $\beta_{HDL-C}$ and $\beta_{TG}$) for each lipid (LDL-C, HDL-C and TG, respectively) individually in turn. The regression was weighted by the inverse variances of the estimated associations of the SNPs with the outcome, and was forced to pass through the origin.

Multivariable Mendelian Randomization

To gauge some insight into potential ‘independent’ effects of the lipids on AAA risk, we used multivariable Mendelian randomization. In this approach, a single regression model with outcome variable $\beta_{AAA}$ was fitted for the predictor variables $\beta_{LDL-C}$, $\beta_{HDL-C}$ and $\beta_{TG}$. The model was implemented as described previously (43) as a multi-linear regression of SNP association estimates weighted by the inverse variances of the estimated associations of SNPs on the outcome, and forced to pass through the origin.

MR-Egger

We used the recently developed MR-Egger method (41) that corrects for unmeasured net pleiotropy. The method comprises an unconstrained linear regression of the SNP association estimates for the outcome on the SNP association estimates for the exposure weighted by the inverse variance of the estimated effect of SNP on outcome. In MR-Egger, any net pleiotropy manifests in
the intercept and (under the assumption that pleiotropic effects are independent
of the associations of the SNPs with the exposure) the regression slope
coefficient is an unbiased MR effect estimate.

**Weighted Median MR**

As a further sensitivity analysis, we also performed the weighted median
method.(40) Whereas the inverse-variance method calculates a weighted mean
of the SNP-specific causal effect estimates, the weighted median method
calculates a weighted version of the median of the SNP-specific causal effect
estimates. As the median of a distribution is not affected by extreme values, the
weighted median method is less sensitive to individual pleiotropic SNPs. The
weighted median estimate is unbiased in large samples if at least 50% of the
weights from SNPs are valid (e.g. not pleiotropic). All non-drug target lipid-
related MR analyses used the “MendelianRandomization” command in R.(44)

**SNPs in drug target analysis**

There have been no large-scale randomized trials of lipid lowering treatments in
patients with AAA, and observational studies have often been small and
retrospective and yielded heterogeneous results. We examined the association of
rs12916 in *HMGCR* (a proxy for statins), rs3764261 in *CETP* (a proxy for CETP
inhibitors) and rs2479409 and rs11206510 in *PCSK9* (a proxy for PCSK9
inhibitors) with AAA to identify the potential utility of pharmacological
modification of these drug targets in AAA.
Results

The numbers of cases and controls for each of the AAA studies is included in Table 1. The complete list of SNPs included in this analysis, together with information on the association statistics for AAA, LDL-C, HDL-C and TG is included in Table S1.

Conventional Mendelian randomization: association of GRS with AAA

Summary statistics for 180 lipid-associated SNPs were available for analysis. Individual SNP-AAA association results are shown in Table S1. As previously reported(17,20), the LDL-lowering alleles of rs6511720 in LDLR (OR 0.75, 95% CI 0.67-0.83, P=5.2x10^{-12}) and rs646776 in SORT1 (OR 0.88, 95%CI 0.82-0.94, P=3.9x10^{-8}) were strongly associated with AAA. None other SNPs out of the 180 were individually associated with AAA at conventional levels of genome-wide significance (P <5.0 x 10^{-8}). Twenty-five out of 180 SNPs were nominally associated with AAA (P <0.05; Table S1); 9 such associations (95% CI: 4 – 15) would be expected by chance alone.

Conventional inverse variance weighted MR analyses using GRS for LDL-C (consisting of 75 SNPs), HDL-C (84 SNPs) and TG (50 SNPs) were then conducted to assess the associations with AAA (Figure 1). The LDL-GRS was strongly associated with AAA risk (OR per SD higher LDL-C 1.70, 95% CI 1.44 – 2.00, P=1.7 x 10^{-10}). One SD higher HDL-C instrumented through the HDL-C GRS was associated with a reduced risk of AAA (OR 0.66, 95% CI 0.54 – 0.8, P = 2.6 x 10^{-5}). Finally, the TG-GRS was also associated with higher risk of AAA (OR per 1-SD higher TG 1.66, 95% CI 1.35 – 2.04, P = 1.4 x 10^{-6}).
Multivariable Mendelian randomization, MR-Egger and weighted median approaches

While it is possible to remove SNPs with pleiotropic effects from the GRS, this diminishes the strength of the instrumental variable(45) and can introduce bias(46). We therefore adopted the multivariable Mendelian randomization approach described by Do et al(22) and modified by Burgess(43) to gain insight into the potential independent effects of these lipid GRS with risk of AAA. To account for any net unbalanced pleiotropy, we used MR-Egger. To reduce the influence of outlying (possibly pleiotropic) variants on the analysis, we used the weighted median method. None of these sensitivity MR analyses resulted in a material change to either the magnitude or significance of the estimates (Figure 1). The point estimates for LDL-C and HDL-C remained largely unaltered whereas for TG the point estimate diminished for multivariate MR, however on MR-Egger and weighted median MR, it remained convincingly associated to AAA.

Association of SNPs in lipid drug targets

We selected rs12916 in HMGCR, rs3764261 in CETP as well as rs2479409 and rs11206510 in PCSK9 as there are drugs that are licensed and/or in development that target pathways related to these genes.

The LDL-C lowering allele of rs12916 (to proxy statin use) was found to be associated with a lower risk of AAA in meta-analysis (OR per LDL-C lowering allele 0.93, 95% CI 0.89 – 0.98, P = 0.009).
PCSK9 inhibitors are a novel class of drugs used to target LDL-C. To date, in CHD, genetic and clinical studies have had concordant results\(^{(42,47)}\). We examined two independent SNPs in \textit{PCSK9} (rs2479409 and rs11206510; linkage disequilibrium \(r^2=0.07\)) that have previously been used as proxies for PCSK9 inhibition in large scale MR analysis\(^{(48)}\) and have strong, independent associations with both LDL-C levels and CHD. The LDL-C lowering allele of rs2479409 was not associated with risk of AAA (OR 0.97, 95% CI 0.84 – 1.02, \(P = 0.28\)). The LDL-C lowering allele of rs11206510 in \textit{PCSK9} was weakly associated with risk of AAA (OR 0.94, 95% CI 0.88 – 1.00, \(P = 0.04\)).

rs3764261 was used as a proxy for CETP inhibition. Although the allele increases HDL-C, it is also associated with lower circulating concentrations of TG and LDL-C, so rs3764261 cannot be considered as an instrument for HDL-C in isolation, but can be used to gauge insight into the potential effects of CETP inhibition\(^{(39)}\). This HDL-raising \textit{CETP} SNP was associated with lowering of AAA risk (OR per HDL-C raising allele 0.89, 95% CI 0.86-0.93, \(P=3.7 \times 10^{-7}\)).

**Discussion**

Understanding the relevance of lipid fractions in the development of AAA has important implications from both etiological and translational standpoints. In this study, we used MR to provide robust evidence that the major lipid fractions, LDL-C, HDL-C and TG, are likely to play important roles in the etiology of AAA. While a similar genetic approach has been used previously\(^{(36)}\), the present study has expanded upon it by including many more individuals and more SNPs.
and using more recent developments in MR, which collectively increase
statistical power and strengthen the validity of the effect estimates we
report(36).

Disentangling the roles of correlated biomarkers in disease etiology continues to
be an analytical challenge, and to this end we used recently developed
techniques for multivariable MR(22). Interestingly, we have shown that there
appear to be independent associations between genetically instrumented levels
of LDL-C, HDL-C and TG and risk of AAA. This is in contrast to studies of CHD
where a similar approach found no association between HDL-C genetic variants
and CHD (once shared pathways with LDL-C and TG and pleiotropy had been
taken into account)(22,24,25,45), or aortic stenosis where only LDL-C appears to
play a causal role(49). This highlights the complexity of lipid pathways across
the diverse biology of CVD, and suggests that results from studies focused solely
upon CHD (which can be defined variably) cannot always be extrapolated to
other vascular diseases such as AAA.

While it has been possible to try and account for pleiotropic effects of genetic
variants used collectively in the lipid GRS employed in the MR analyses that we
present, it is not so straight-forward to disentangle the phenotypic overlap
whereby many patients with AAA also harbor atherosclerotic disease in other
vascular beds. Therefore, while it is tempting to suggest a causal role for lipids
specifically in AAA pathogenesis, these genetic analyses do not provide definitive
evidence. The data do, however, suggest that the burden of genetically influenced
dyslipidemia in AAA patients is considerable, and by extrapolation, these MR
analyses lend support to the lipids playing an important role in AAA etiology and thus targeting lipids through pharmacological modification in patients with small AAA may well be justified. This point is particularly pertinent given recent reports of low prevalence of LDL-C control in AAA patients in both the USA and UK(33,34). This group of patients should also be considered in trials evaluating novel treatments of lipid lowering medications such as CETP or PCSK9 inhibitors.

The use of genetic data to inform drug-trials and/or drug repurposing may be an important translational facet of data derived by large genome-wide consortia(50,51). In addition to the GRS for LDL-C, HDL-C and TG, we looked at four loci that serve as proxies for cardiovascular drug targets that have not been subjected to clinical trials in patients with AAA. Both the LDL-C GRS and a genetic proxy for statin therapy (SNPs in \textit{HMGCR}) were associated with AAA. Prior investigations on the associations of LDL-C with AAA have used cross-sectional datasets with varying findings, and results have been hampered by concurrent LDL-C lowering therapies(9). Indeed, there has been a suggestion that statin use may increase the risk of AAA(5). The collective results from this study suggest that LDL-C plays an important role in the etiology of AAA, which may explain the excess burden of CVD in patients with AAA(6). These data also support a view that patients found to have AAA by screening should be prescribed statins to reduce their CVD risk, though whether or not this will affect the progression of AAA cannot be answered in this study.
PCSK9 inhibitors have recently shown beneficial effects on CVD outcomes in a phase III clinical trial(47). Although the association we found between PCSK9 variants and AAA was weak, if PSCK9 inhibitors do prove to be a safe and cost-effective means of LDL-C lowering, then consideration should be given to evaluation of these drugs in patients with AAA.

As we note above, a genetically-instrumented higher HDL-C was identified to be associated with a reduction in risk of AAA. Variants in CETP have a range of effects similar to pharmacological inhibition of CETP(39) including lowering of LDL-C and raising HDL-C. While trials of CETP inhibition have thus far failed to show benefit in patients following myocardial infarction(27) there are data to support beneficial effects on vascular remodeling(52) that could have more relevance in AAA management. Evaluation of CETP inhibition in patients with AAA may therefore be warranted. Although we cannot specifically determine whether the association between CETP polymorphisms and AAA is via HDL-C, LDL-C or TG (or indeed all, as suggested by our GRS of lipid traits), the results suggest that CETP inhibition could play a role in AAA disease.

The findings related to TG variants also have potential clinical implications with regard to development of novel treatments aimed at TG levels. They suggest that patients with AAA may benefit from TG lowering and if novel therapies such as APOC3 inhibitors progress from phase 2 studies to larger scale phase 3 studies of CVD prevention, then AAA patients could be an important CVD sub-phenotype in whom treatment effect should be evaluated.
Our study used MR, a genetic approach that has important assumptions. The SNPs used in the genetic instruments for each lipid trait were identified from recent GWAS studies that place stringent thresholds on SNP discovery. As such, the genetic instruments are very unlikely to suffer from weak instrument bias, and in any case, since the MR analyses used non-overlapping datasets, such bias would tend to dilute the estimates derived from MR analyses. We also make the assumption that the genetic instruments are not influenced by confounding and that they only associate with AAA through the exposure of interest (i.e. that the genetic instruments do not show evidence of unbalanced horizontal pleiotropy, as pictorially illustrated in Figure 1 of White et al and expanded further in Holmes et al. These assumptions cannot be tested with complete certainty. However, causal estimates obtained from a range of sensitivity analyses, each making different and weaker assumptions, all gave similar results. Nonetheless, it is possible that residual pleiotropy could still influence our findings.

The limitations of this study should be considered. First, we did not have datasets to evaluate AAA progression. Second, due to limited availability of covariate data, we were unable to perform subgroup analyses (e.g. based on age, sex or concurrent lipid lowering therapy). Third, the analyses presented in this manuscript use summary level data as described elsewhere. Fourth, although we attempted to control for pleiotropy in the analyses, this still represents a major challenge to deciphering the roles of specific lipid-based pathways.
Conclusion

Using contemporary MR approaches, these data lend support to the hypothesis that the major lipid fractions are involved in the etiology of AAA. Consideration should be given to public health measures aimed at targeting lipids in patients with AAA, using established and emerging therapies.

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