The relationship between blood lipids and risk of atrial fibrillation:
Univariable and multivariable Mendelian randomization analysis

ABSTRACT

Aims: We performed univariable and multivariable Mendelian randomization analysis to evaluate the association between blood lipid including low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglyceride (TG), Apolipoprotein A1, Apolipoprotein B and AF risk.

Methods: Single nucleotide polymorphisms (SNPs) related to blood lipids, AF were selected from the genome-wide association (GWAS) of UK Biobank and latest meta-analysis of GWASs with six independent cohorts, respectively. The univariable MR analysis aimed to investigate the association of individual lipid-related traits with AF and multivariable MR analysis with three models aimed to compare the independent effects of correlated lipid-related traits.

Results: The IVW estimate showed that genetically predicted LDL cholesterol (OR: 1.016, 95% CI: 0.962-1.073, P=0.560), HDL cholesterol (OR: 0.951, 95% CI: 0.895-1.010, P=0.102), triglycerides (OR: 0.961, 95% CI: 0.889-1.038, P=0.313), Apolipoprotein A1 (OR: 0.978, 95% CI: 0.933-1.025, P=0.356) and Apolipoprotein B (OR: 1.008, 95% CI: 0.959-1.070, P=0.794) were not causally associated with risk of AF. Sample mode (OR: 0.852, 95% CI: 0.731-0.993, P=0.043) and weighted mode (OR: 0.907, 95% CI: 0.841-0.979, P=0.013) both showed that triglycerides was associated with lower risk for AF. In the multivariable MR analysis with mutual adjustment for model 1, model 2 and model 3, the association was consistent. The multivariable MR analysis showed that triglycerides, LDL cholesterol and HDL cholesterol were associated with lower risk for AF, while Apolipoprotein A1 and Apolipoprotein B were inversed but still non-significant.

Conclusions: Our MVMR analyses provide genetic evidence that none of the genetically determined lipid traits was significantly associated with AF. Furthermore, more work is needed to confirm the potential association between lipid traits and AF.

Keywords: Blood lipids; Atrial fibrillation; Mendelian randomization; Causal effect

Introduction

Atrial fibrillation (AF) is a common arrhythmia with significant health and socioeconomic impact[1]. The prevalence of AF is increasing, estimated to rise to 12.1 million in 2030 in the United States and 17.9 million in 2060 in the European Union[2]. AF is associated with high healthcare system utilization, low quality of life, and increased risk for hospitalization, heart failure, stroke, and death[3].

Significant effort has been made to define the underlying mechanisms of AF, such as basic electrophysiological and structural changes within the left atrium, and the links to comorbidities and wider systemic and metabolic perturbations[4]. Of note, patients with hyperlipidemia have increased arterial inflammation[5]. Since evidence from human genetics supports a causal role of LDL cholesterol, triglycerides, and apolipoprotein B in CHD[6-9], it seems likely that hyperlipidemia would also be a risk factor for AF.
However, a few published studies exploring the link between blood lipids and AF have yielded inconsistent and paradoxical results. High levels of total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C), were unexpectedly found to be inversely associated with the risk of AF in several observational studies\[10-14\]. However, the Multi-Ethnic Study of Atherosclerosis and the Framingham Heart Study found HDL-C and triglycerides (TG) but not LDL-C or TC were associated with the risk of AF\[15\]. Meanwhile, low levels of both apolipoprotein A1 and B were associated with incident AF\[16\]. A systematic review of large cohort studies found an inverse relationship between serum TC, LDL-C, and high-density lipoprotein cholesterol (HDL-C) levels and AF risk, although there was no significant association between TG levels and incident AF\[17\]. Another meta-analysis reported a nonlinear (U-shaped) association between TC and new-onset AF and a nonlinear (reverse spoon-shaped) association between concentrations of LDL-C and the risk of new-onset AF\[18\]. Notably, these studies included limited sample sizes and confounders. Confirmation of a causal association is a challenging as the reverse causation and confounding between bilirubin and the risk of AF. Mendelian randomization (MR) has emerged as a powerful method for identifying the causation between risk factors and diseases using genetic variants as instrument variables (IVs)\[19\]. MR analysis can largely overcome the confounders with random assignment of an individual’s genetic variants at conception. Furthermore, the risk of reverse causation is also minimized, since the presence of a disease cannot affect individuals’ genotypes\[20\]. Meanwhile, it is plausible to assume that each lipid-related entity played an individual causal role or that one trait, such as apoB, predominated and accounted for the associations of related lipoprotein particle entities\[21\]. In our study, we performed a univariable MR analysis aimed to investigate the association of individual lipid-related traits with AF and multivariable MR analysis with three models aimed to compare the independent effects of correlated lipid-related traits on AF in which we used the summary statistics from the public GWAS data.

**Methods**

**Data resources and study design**

Genome-wide association studies (GWAS) were searched to extract leading single-nucleotide polymorphisms (SNPs) as genetic instrumental variables. Summary statistic data for LDL and HDL cholesterol, triglycerides, and Apolipoprotein A1 and Apolipoprotein B was from a meta-analyzed GWAS for 35 lab biomarkers from the UK Biobank (UKB) in 318,340, 291,830, 318,674, 290,198, 317,412 participants of White British European ancestry, respectively\[22\]. UK Biobank is a prospective cohort that recruited more than 500,000 men and women aged 40–96 years between 2006 and 2010 and their health is being followed long term\[23\]. Data for AF was obtained from the latest meta-analysis of GWASs for AF with six independent cohorts (The Nord-Trøndelag Health Study, Michigan Genomics Initiative, DECODE, UK Biobank, DiscovEHR Collaboration Cohort, and AF Gen Consortium) with more than 1,000,000 subjects of European ancestry, including 60,620 cases with AF and 970,216 controls\[24\].
This study identified 142 independent risk variants at 111 loci and prioritized 151 functional candidate genes likely to be involved in atrial fibrillation\cite{24}. The details were presented in Table 1.

We designed univariable and multivariable Mendelian randomization analysis to estimate the causal effects of lipid traits on AF using genetic predictors of lipid levels as instrumental variables (IVs). In order for a genetic variant to qualify as a valid instrument for causal inference in a MR study, it must satisfy three core assumptions\cite{25}:

a. The genetic variant must be truly associated with the exposure;
b. The genetic variant should not be associated with confounders of the exposure-outcome relationship;
c. The genetic variant should only be related to the outcome of interest through the exposure under study.

The univariable MR analysis aimed to investigate the association of individual lipid-related traits with AF and the multivariable MR analysis aimed to compare the independent effects of correlated lipid-related traits on AF. In the model 1 of multivariable MR, we adjusted for pleiotropic effects across the included lipid traits including Apolipoprotein B, LDL cholesterol and triglycerides for the causal associations with AF. In the model 2 of multivariable MR, we adjusted for pleiotropic effects across the included lipid traits including Apolipoprotein A1, HDL cholesterol. Finally, in the model 3, we included GWAS-related SNPs for all circulating lipids traits\cite{21}.

**Selection of genetic instrumental variables**

All genetic variants associated with LDL and HDL cholesterol, triglycerides, and Apolipoprotein A1 and Apolipoprotein B levels reaching genome-wide significance (P < 5 × 10^{-8}) were selected as instruments for the MR analysis. The corresponding linkage disequilibrium was tested to confirm that there were any SNPs in a linkage disequilibrium state and the SNPs were independent by pruning SNPs within a 10,000kb window with an \( r^2 < 0.001 \) threshold\cite{26}. Then, the SNPs were extracted which were associated with any potential confounders of the outcomes. In this study, blood pressure, blood glucose, BMI, chronic nephropathy, coronary artery disease and C-reactive protein were identified as confounding factors when AF was identified as the outcome(\url{http://www.phenoscan.medschl.cam.ac.uk/})\cite{27}. SNP harmonization was conducted to correct the orientation of the alleles. In univariable MR analysis, we used 147 SNPs as instrument variables for LDL cholesterol, 136 SNPs for HDL cholesterol, 139 SNPs for triglycerides, 118 SNPs for Apolipoprotein A1, and 117 SNPs for Apolipoprotein B (Supplementary table 1-5). The SNPs of all lipid traits used in the multivariable MR analysis were acquired by clumping to a linkage disequilibrium threshold of \( r^2 < 0.001 \). Finally, we used 301 SNPs in the multivariable MR analysis of model 1, 173 SNPs in the multivariable MR analysis of model 2 and 437 SNPs in the multivariable MR analysis of model 3. \( F \) statistics for every instrument-exposure association were from 28.422 to 49.559 in our study, demonstrating the small possibility
of weak instrumental variable bias (Table 1).

**Statistical analysis**

The way to obtain an MR estimate was to perform an inverse variance weighted (IVW) meta-analysis of each Wald Ratio\(^\text{28}\). When there was no evidence of directional pleiotropy (p for MR-Egger intercept > 0.05) among the selected IVs, the IVW method was considered the most reliable\(^\text{29}\).

Complementary analyses using the weighted median method\(^\text{30}\), sample mode method\(^\text{31}\), weighted mode method\(^\text{31}\) and MR-egger method\(^\text{30}\) were utilized as supplements to IVW. The weighted median analysis can generate consistent estimates if at least 50% of the weight in the analysis comes from valid instrumental variables\(^\text{32}\).

Cochran’s Q test was applied to assess heterogeneity between individual genetic variants estimates. If the P value of Cochran’s Q test was less than 0.05, the final results of MR referred to a multiplicative random-effects model of IVW; otherwise, a fixed-effects model was used\(^\text{33}\). To examine whether there was violation of the main MR assumptions due to directional pleiotropy, the MR-Egger test for directional pleiotropy was performed\(^\text{30}\). In MR-Egger test, the intercept estimates the average pleiotropic effect across the genetic variants; a value that differs from zero indicates that the IVW estimate is biased\(^\text{34}\). We also examined possible directional pleiotropy via observing asymmetry in funnel plots to gauge the reliability of the current MR analyses. Finally, MR-PRESSO was performed to support the results by IVW method, which detects and corrects the effects from outliers, yielding causal estimates that are robust to heterogeneity\(^\text{35}\). The leave-one-out sensitivity analyses were implemented by removing a single SNP each time to assess if the variant was driving the association between the exposure and the outcome variable.

R-squared were calculated to estimate the proportion of variance in outcomes, and the true value of the F-statistic was calculated to mitigate the bias and predict the strength of IVs.

Given genetic and phenotypic correlations across lipid traits as prior study reported (Pearson’s R ranging from -0.49 to 0.96)\(^\text{21}\), we further used multivariable inverse-variance weighted method with 3 models to disentangle and compare the effects of correlated lipid-traits on AF. The multiple testing was not tailed for multivariable MR analysis due to the mutual adjustment nature of multivariable MR analysis. Furthermore, we performed a linear regression-based approach, which gives estimates for each of the risk factors separately\(^\text{36}\). The intuitive rationale is that these residuals represent any causal effects that are not explained by the alternative risk factors but are potentially explained by the risk factor of interest\(^\text{36}\).

A two-sided P value of < 0.05 was considered suggestive for significance. All analyses were performed using the package “Two-Sample-MR” (version 0.5.6) and “MR-PRESSO” (version 1.0) in R (version 4.0.5).

**Results**

**Univariable MR analysis of lipid traits on AF**
Figure 1 reported the univariable MR estimated of lipid traits on AF. The IVW estimate showed that genetically predicted LDL cholesterol (OR: 1.016, 95% CI: 0.962–1.073, P=0.560), HDL cholesterol (OR: 0.951, 95% CI: 0.895–1.010, P=0.102), triglycerides (OR: 0.961, 95% CI: 0.889–1.038, P=0.313), Apolipoprotein A1 (OR: 0.978, 95% CI: 0.933–1.025, P=0.356) and Apolipoprotein B (OR: 1.008, 95% CI: 0.959–1.070, P=0.794) were not causally associated with risk of AF (Figure 1). The association was consistent in complementary analyses using weighted median methods and weighted mode methods (Figure 1). However, sample mode (OR: 0.852, 95% CI: 0.731–0.993, P=0.043) and weighted mode (OR: 0.907, 95% CI: 0.841–0.979, P=0.013) both showed that triglycerides was associated with lower risk for AF. To ensure the robustness of our results, MR-PRESSO was also conducted which showed the similar results that lipid traits were not associated with the risk of AF (Table 2).

There were potential heterogeneities but no directional pleiotropies for the analysis results (Supplementary table 6). The scatter plots and forest plots were displayed in Supplementary Figure 1A-1E, 2A-2E. The funnel plots were symmetrical (Supplementary Figure 3A-3E) and the leave-one-out approach revealed that no individual SNP was substantially driving the association between lipids traits and AF (Supplementary Figure 4A-4E).

**Multivariable MR Analysis of model 1**

In the multivariable MR analysis with mutual adjustment for LDL cholesterol, triglycerides, and Apolipoprotein B, the estimate for LDL cholesterol was reversed on mutual adjustment to yield an adjusted OR of 0.972 but still non-significant (N=301, 95% CI: 0.505–1.439, P=0.891). The ORs of AF were 0.962 (N= 301 SNPs, 95% CI: 0.737–1.076, P=0.500) and 1.039 (N= 301SNPs, 95% CI: 0.710–1.520, P=0.845), respectively, for one-SD increase of triglycerides and Apolipoprotein B (Figure 2, Supplementary table 7). The association was consistent in complementary analyses using a linear regression-based approach (Supplementary table 8).

**Multivariable MR Analysis of model 2**

In the multivariable MR analysis with mutual adjustment for HDL cholesterol and Apolipoprotein A1, the estimate for Apolipoprotein A1 was reversed on mutual adjustment to yield an adjusted OR of 1.006 but still non-significant (N=173, 95% CI: 0.867–1.145, P=0.950, Figure 2, Supplementary table 7)). The association was consistent in complementary analyses using a linear regression-based approach (Supplementary table 8).

**Multivariable MR Analysis of on model 3**

In the multivariable MR analysis with mutual adjustment for LDL cholesterol, triglycerides, and Apolipoprotein A1, HDL cholesterol and Apolipoprotein A1, the association was consistent with model 1 and model 2. The multivariable MR analysis showed that triglycerides (N=437 SNPs, OR: 0.905, 95% CI: 0.744–1.101, P=0.322), LDL cholesterol (N= 437 SNPs, OR: 0.903, 95% CI: 0.505–1.616, P=0.731) and HDL cholesterol (N=437 SNPs, OR: 0.787, 95% CI: 0.522–1.185, P=0.252) were associated
with lower risk for AF, while Apolipoprotein A1 (N=437 SNPs, OR: 1.271, 95% CI: 0.841–1.922, P=0.257) and Apolipoprotein B (N=437 SNPs, OR: 1.157, 95% CI: 0.635–2.108, P=0.632) were inversed but still non-significant (Figure 2, Supplementary table 7). The association was consistent in complementary analyses using a linear regression-based approach (Supplementary table 8).

Discussion
Using a comprehensive approach, including conventional, multivariable Mendelian randomization, we tested for a causal relationship between lipid traits and AF. In univariable MR analysis, we demonstrated that genetically predicted triglyceride was associated with lower risk for AF with sample mode and weighted mode. However, none of the genetically determined lipid traits was significantly associated with AF in univariable MR analysis with other methods and multivariable analyses with three models.

Observational studies have provided discrepant results concerning the relationship of LDL-C, HDL-C, triglycerides, Apolipoprotein A1 and Apolipoprotein B with AF. For example, in a pool analysis of 2 community-based cohorts, HDL-C and TG but not LDL-C or TC were associated with the risk of AF, accounting for other cardiometabolic risk factors[15]. In the Niigata Preventive Medicine Study, high TC and LDL-C as well as high HDL-C were found to be associated with increased risk of AF[10]. However, a prospective large community-based cohort in China found that lower levels of TC and LDL-C were associated with increased risk of AF, whereas no significant association of incident AF was observed with HDL-C and TG[17]. Lack of adjustment for important confounders including obesity, geographical and ethnic variations and other CV risk factors may partly explain inconsistencies between studies. A meta-analysis of large cohort studies found an inverse relationship between serum TC, LDL-C, and HDL-C levels and AF risk, although there was no significant association between TG levels and incident AF[17].

It seems there is a “cholesterol paradox” in AF[14]. The potential mechanisms of the identified inversed causal effects of LDL-C and TC were as following. Firstly, it is alterations in cardiac ion channel handling by cholesterol[38-41]. Some studies have demonstrated that cholesterol modulates the distribution and function of the Kv1.5 K+ channel, Kir2.1 K+ channel, and Na+ channel, which may be involved in the pathogenesis of AF. Secondly, it may be confounding by hyperthyroidism status as hyperthyroidism has been found to simultaneously decrease LDL-C levels and increase AF risk[42-44]. Another confounding factor may be natriuretic peptides (NT-proBNP or BNP). It has been described an inverse association between LDL-C levels and NT-proBNP[45], and natriuretic peptides are strong predictors of AF risk[15, 46-48]. Thirdly, high levels of TC have an anti-inflammatory effect in certain circumstances, which may also be involved in the pathogenesis of AF[49]. In univariable MR analysis, we demonstrated that genetically predicted triglyceride was associated with lower risk for AF. It may be explained by the confounder factors such as other lipid traits. This was why the association was lost in the multivariable MR analysis with mutual adjustment for LDL cholesterol, triglycerides, and Apolipoprotein A1, HDL cholesterol and
Apolipoprotein A1 in our study. In a word, the potential mechanisms of lipid and AF are still not fully illuminated. Both LDL cholesterol and triglycerides are carried in atherogenic lipoproteins, each containing an apolipoprotein B molecule\cite{50, 51}. Some cross-sectional analyses\cite{52, 53}, prospective observational studies\cite{54}, suggested Apolipoprotein B to be a more accurate marker of cardiovascular risk than total cholesterol or LDL-C. Mendelian randomization has added to the evidence that the number of apolipoprotein B particles within the arterial lumen is the most direct measure of the atherogenic injury that the apolipoprotein B particles will inflict over time on the arterial wall. Given correlations across lipid-related traits, multivariable MR analysis with three models, as an extension to traditional MR method, were aimed to compare the independent effects of each lipid trait. However, our results indicated that high apolipoprotein B levels were not associated with the increased risk of AF with univariable and multivariable MR analysis. Epidemiologic and clinical studies suggest that elevated triglyceride levels are a biomarker of cardiovascular (CV) risk\cite{55}. But we found there was no cause effect between triglyceride and AF.

Our MVMR analyses provide genetic evidence that none of the genetically determined lipid traits was significantly associated with AF. Firstly, data was obtained from different sample of individuals, genetic associations with the exposure/outcomes can be obtained from large consortia, which greatly increases statistical power to detect small effects in complex phenotypes\cite{56}. Secondly, the genetic variants we selected were located in different chromosome, the potential gene-gene interaction may have little effect on the estimated value\cite{57}. There are some limitations in our study. Firstly, there was heterogeneity among our results. Due to the GWAS data, any potential nonlinear relationships or stratification effects which varies by health status, age or sex cannot be examined which may be the resource of heterogeneity. Secondly, The CIs in the multivariable MR analysis were wide which might reveal some degree of compromise of the precision of MR statistical model fitting strongly correlated exposures\cite{21}. Thirdly, despite the lack of indication of directional pleiotropy in the analysis, we could not exclude the association, which is almost completely mediated through other causal pathways. Fourthly, we did not explore the association between blood lipids and different AF subtypes. Finally, our datasets included the majority of the European populations which limited applicability of results to non-European populations.

**Conclusion**
In conclusion, Our MVMR analyses provide genetic evidence that none of the genetically determined lipid traits was significantly associated with AF. Furthermore, more work is needed to confirm the potential association between lipid traits and AF.

**Conflict of interest**
The Author(s) declare(s) that there is no conflict of interest.
References


Table 1. Details of studies included and predictive strength of IVs in Mendelian randomization analyses (two-sided α = 0.05)

<table>
<thead>
<tr>
<th>Exposures/Outcomes</th>
<th>Consortium</th>
<th>Ethnicity</th>
<th>Sample sizes</th>
<th>R-squared % (of variance in AF)</th>
<th>F-statistic (total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL-cholesterol</td>
<td>UKB</td>
<td>European</td>
<td>291,830</td>
<td>1.582</td>
<td>34.375</td>
</tr>
<tr>
<td>LDL-cholesterol</td>
<td>UKB</td>
<td>European</td>
<td>318,340</td>
<td>1.296</td>
<td>28.422</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>UKB</td>
<td>European</td>
<td>318,674</td>
<td>1.543</td>
<td>38.704</td>
</tr>
<tr>
<td>Apolipoprotein A1</td>
<td>UKB</td>
<td>European</td>
<td>290,198</td>
<td>1.976</td>
<td>49.559</td>
</tr>
<tr>
<td>Apolipoprotein B</td>
<td>UKB</td>
<td>European</td>
<td>317,412</td>
<td>1.354</td>
<td>31.788</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>HUNT, DECODE, DiscovEHR, MGI, UKB, and AF Gen Consortium</td>
<td>European</td>
<td>1,030,836</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

AF, atrial fibrillation; UKB, UK Biobank; HDL, high density lipoprotein; LDL, low density lipoprotein; HUNT, The Nord-Trøndelag Health Study; DECODE, DiscovEHR, Collaborative analysis of Diagnostic criteria in Europe study; MGI, Michigan Genomics Initiative; AF Gen, Atrial Fibrillation Genetics.
Table 2: MR-PRESSO for causal effect between circulation bilirubin levels and AF

<table>
<thead>
<tr>
<th>Exposure</th>
<th>nSNP</th>
<th>Beta</th>
<th>OR (95%CI)</th>
<th>P-value</th>
<th>nSNP</th>
<th>Beta</th>
<th>OR (95%CI)</th>
<th>P-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL cholesterol</td>
<td>138</td>
<td>-0.025</td>
<td>0.975(0.920,1.030)</td>
<td>0.379</td>
<td>137</td>
<td>-0.018</td>
<td>0.982(0.928,1.036)</td>
<td>0.517</td>
<td>0.695</td>
</tr>
<tr>
<td>LDL-cholesterol</td>
<td>147</td>
<td>-0.001</td>
<td>0.999(0.947,1.051)</td>
<td>0.966</td>
<td>145</td>
<td>-0.004</td>
<td>0.996(0.946,1.046)</td>
<td>0.882</td>
<td>0.942</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>129</td>
<td>-0.041</td>
<td>0.960(0.885,1.035)</td>
<td>0.291</td>
<td>125</td>
<td>-0.047</td>
<td>0.954(0.897,1.011)</td>
<td>0.114</td>
<td>0.847</td>
</tr>
<tr>
<td>Apolipoprotein A1</td>
<td>118</td>
<td>-0.002</td>
<td>0.998(0.994,1.002)</td>
<td>0.921</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Apolipoprotein B</td>
<td>137</td>
<td>0.008</td>
<td>1.008(0.959,1.070)</td>
<td>0.786</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
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</table>

HDL, high density lipoprotein; LDL, low density lipoprotein; SNP, single nucleotide polymorphisms; OR, odds ratio.
Figure 1. Associations of HDL cholesterol, LDL cholesterol, triglycerides, apolipoprotein A1, and apolipoprotein B with AF in Univariable Mendelian randomization analysis. HDL, high-density lipoprotein; LDL, low-density lipoprotein; IVW, inverse variance weighted.
Figure 2. Model 1, associations of LDL cholesterol, triglycerides, apolipoprotein B with AF in multivariable Mendelian randomization analysis. Model 2, associations of HDL cholesterol, apolipoprotein A1 with AF in multivariable Mendelian randomization analysis. Model 3, associations of HDL cholesterol, LDL cholesterol, triglycerides, apolipoprotein A1, and apolipoprotein B with AF in multivariable Mendelian randomization analysis. HDL, high-density lipoprotein; LDL, low-density lipoprotein.