Original Investigation

Association of Low-Density Lipoprotein Cholesterol–Related Genetic Variants With Aortic Valve Calcium and Incident Aortic Stenosis

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IMPORTANCE Plasma low-density lipoprotein cholesterol (LDL-C) has been associated with aortic stenosis in observational studies; however, randomized trials with cholesterol-lowering therapies in individuals with established valve disease have failed to demonstrate reduced disease progression.

OBJECTIVE To evaluate whether genetic data are consistent with an association between LDL-C, high-density lipoprotein cholesterol (HDL-C), or triglycerides (TG) and aortic valve disease.

DESIGN, SETTING, AND PARTICIPANTS Using a Mendelian randomization study design, we evaluated whether weighted genetic risk scores (GRSs), a measure of the genetic predisposition to elevations in plasma lipids, constructed using single-nucleotide polymorphisms identified in genome-wide association studies for plasma lipids, were associated with aortic valve disease. We included community-based cohorts participating in the CHARGE consortium (n = 6942), including the Framingham Heart Study (cohort inception to last follow-up: 1971-2013; n = 1295), Multi-Ethnic Study of Atherosclerosis (2000-2012; n = 2527), Age Gene/Environment Study–Reykjavik (2000-2012; n = 3120), and the Malmö Diet and Cancer Study (MDCS, 1991-2010; n = 28 461).

MAIN OUTCOMES AND MEASURES Aortic valve calcium quantified by computed tomography in CHARGE and incident aortic stenosis in the MDCS.

RESULTS The prevalence of aortic valve calcium across the 3 CHARGE cohorts was 32% (n = 2245). In the MDCS, over a median follow-up time of 16.1 years, aortic stenosis developed in 17 per 1000 participants (n = 473) and aortic valve replacement for aortic stenosis occurred in 7 per 1000 (n = 205). Plasma LDL-C, but not HDL-C or TG, was significantly associated with incident aortic stenosis (hazard ratio [HR] per mmol/L, 1.28; 95% CI, 1.04-1.57; *P* = .02; aortic stenosis incidence: 1.3% and 2.4% in lowest and highest LDL-C quartiles, respectively). The LDL-C GRS, but not HDL-C or TG GRS, was significantly associated with presence of aortic valve calcium in CHARGE (odds ratio [OR] per GRS increment, 1.38; 95% CI, 1.09-1.74; *P* = .007) and with incident aortic stenosis in MDCS (HR per GRS increment, 2.78; 95% CI, 1.22-6.37; *P* = .02; aortic stenosis incidence: 1.9% and 2.6% in lowest and highest GRS quartiles, respectively). In sensitivity analyses excluding variants weakly associated with HDL-C or TG, the LDL-C GRS remained associated with aortic valve calcium (*P* = .03) and aortic stenosis (*P* = .009). In instrumental variable analysis, LDL-C was associated with an increase in the risk of incident aortic stenosis (HR per mmol/L, 1.51; 95% CI, 1.07-2.14; *P* = .02).

CONCLUSIONS AND RELEVANCE Genetic predisposition to elevated LDL-C was associated with presence of aortic valve calcium and incidence of aortic stenosis, providing evidence supportive of a causal association between LDL-C and aortic valve disease. Whether earlier intervention to reduce LDL-C could prevent aortic valve disease merits further investigation.

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Aortic valve disease remains the most common form of heart valve disease in Europe and North America and is the most common cause of valve replacement.[1,2] Despite the heavy disease burden, no medical treatments are known to stop or retard disease progression. Although aortic valve disease shares several risk factors with vascular disease,[3] it remains largely unknown which factors are causal and should be targeted to reduce valve disease. Our group recently described evidence for a causal association between a common variant in the *LPA* gene, via elevated plasma lipoprotein(a) (Lp[a]), and aortic valve disease.[4] Whether other plasma lipids are causally associated with the development of aortic valve disease remains unclear.

GRS *genetic risk score*

GWAS *genome-wide association study*

HDL-C *high-density lipoprotein cholesterol*

LDL-C *low-density lipoprotein cholesterol*

SNPs *single-nucleotide polymorphisms*

TG *triglycerides*

Low-density lipoprotein cholesterol (LDL-C) is an important risk factor for aortic valve disease in epidemiologic studies[3]; however, large randomized trials of LDL-C–lowering therapy in patients with advanced aortic stenosis have failed to demonstrate effectiveness in reducing disease progression.[5-7] Nonetheless, if LDL-C plays a causal role in the earlier stages of aortic valve disease, this could have important implications for prevention.

Because of the random allocation of genetic information that occurs at conception, genetic variation can be used as an effective tool to distinguish potentially causal from noncausal biomarkers. Termed "Mendelian randomization," this approach has been successfully applied to assess for causality of several biomarkers with various clinical end points.[4,8] Genetic risk scores (GRSs) for lipids, incorporating multiple genetic variants, have been shown to be strongly associated with their corresponding lipid levels in both children[9] and adults,[10] providing strong support for the contention that a higher GRS confers life-long exposure to higher lipid levels. Here, we used a Mendelian randomization approach to determine whether genetic contributions to elevations in LDL-C and other lipids were associated with early subclinical aortic valve disease and incident clinical aortic stenosis.

Methods

Associations of GRSs with aortic valve calcium were evaluated in the 3 CHARGE cohorts where data from computed tomographic (CT) imaging were available; associations with incident aortic stenosis were estimated in the population-based Malmö Diet and Cancer Study (MDCS).

CHARGE Cohorts

The Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium is an ongoing collaboration among several large, well-phenotyped, prospective, longitudinal cohort studies from the United States and Europe.[11] This analysis included white European participants with aortic valve calcium data and genome-wide association study (GWAS) data from the Multi-Ethnic Study of Atherosclerosis (MESA, n = 2527; cohort inception to last follow-up: 2000-2012), the Age, Gene/Environment Susceptibility–Reykjavik Study (AGES-RS, n = 3120; from 2000-2012), and the Framingham Heart Study (FHS, n = 1295, from 1971-2013). Details of the CHARGE consortium and each of these cohorts have been previously described.[4,11] The studies were approved by relevant regulatory bodies and participants provided informed consent.

Malmö Diet and Cancer Study

The MDCS is a prospective, population-based cohort study from the city of Malmö in southern Sweden. Data collection, sample characteristics, and clinical definitions for MDCS have been described previously.[12] Briefly, 30 447 randomly selected men born between 1923 and 1945 and women born between 1923 and 1950 attended a baseline examination between 1991 and 1996. Follow-up for events was completed on December 31, 2010. For the present study, DNA was available for 28 461 participants. Participants underwent sampling of peripheral venous blood and measurement of blood pressure and anthropometric measures and filled out a questionnaire. Measurements of cholesterol (high-density lipoprotein cholesterol [HDL-C], triglycerides [TG], total cholesterol) were performed in a random subset of 5269 participants, on fresh blood samples according to standard procedures at the Department of Clinical Chemistry, Skåne University Hospital, Malmö.[12] Levels of LDL-C were calculated according to the Friedewald formula, with exclusion of individuals where TG exceeded 4.52 mmol/L (to convert TG to mg/dL, divide by 0.0113). Informed consent was obtained from all participants and the study was approved by the ethics committee of Lund University, Sweden.

Genotyping, Imputation, and Construction of Lipid Genetic Scores

CHARGE Cohorts

Details of the genotyping and HapMap imputation methods used in the FHS, AGES-RS, and MESA have previously been described.[4,11] To construct each lipid GRS, we included previously reported, linkage disequilibrium-pruned (ie, uncorrelated) single-nucleotide polymorphisms (SNPs) from large-scale lipid GWAS[13,14] that were associated with the primary lipid traits (LDL-C, HDL-C, and TG) at P value $<5.0 \times 10^{-8}$ and were available in the aortic valve calcium GWAS data set. We constructed separate weighted genetic scores for LDL-C (57 SNPs), HDL-C (70 SNPs), and TG (39 SNPs) weighted by the β coefficients of each individual SNP from large-scale lipid GWASs.[13,14] The possible range of the GRS was from 0 to 5.17, 0 to 6.94, and 0 to 3.66 for LDL-C, HDL-C, and TG, respectively. Because of the known association of aortic valve calcium and aortic stenosis with Lp(a), we specifically excluded the rs10455872 SNP, which is strongly associated with Lp(a).[4] We also calculated secondary SNP scores that were specific for each single lipid trait by excluding SNPs that were associated with a secondary lipid trait (P value $<5.0 \times 10^{-8}$). The SNPs included in each score are summarized in eTables 1 through 3 in the Supplement.

Malmö Diet and Cancer Study

Genotyping and genotype quality control for the 116 SNPs associated with plasma concentrations of LDL-C, HDL-C, or TG in the Global Lipid Genetics consortium (GLGC) study[13] in the MDCS are described in detail in the eMethods in the Supplement. The final SNPs included in the genetic scores for LDL-C (31 SNPs), HDL-C (41 SNPs), and TG (26 SNPs) are summarized in eTables 4-7 in the Supplement. Weighted GRSs were calculated using PLINK version 1.07[15] as the sum of the number of alleles associated with increased LDL-C or TG or decreased HDL-C weighted by the β coefficients (expressed in mmol/L) of each individual SNP from the GLGC study. The possible range of the GRS was from 0 to 3.61, 0 to 1.70, and 0 to 2.84 for LDL-C, HDL-C, and TG, respectively. The secondary SNP scores specific for each lipid trait are described in eTables 4-6 in the Supplement.

Outcomes

Aortic Valve Calcium in CHARGE Cohorts

To assess aortic valve calcium, standard CT scanning was performed on all participants and images were analyzed for the presence of aortic valve calcium using offline digital software. Using standard Agatston methods, the presence of calcium was defined as 3 or more contiguous pixels with a brightness of at least 130 Hounsfield units.[4] Aortic valve calcium was defined as calcium residing within the aortic-valve leaflets or commissures, excluding aortic annulus, proximal aorta, and coronary arteries.

Aortic Stenosis in MDCS

Prevalent and incident diagnoses of aortic stenosis and aortic valve replacement were identified by record linkage with nationwide registers on hospitalizations and causes of death as described previously.[4] Incident aortic stenosis includes all cases of aortic stenosis identified in the MDCS whereas the aortic valve replacement outcome includes all aortic stenosis cases that underwent aortic valve replacement in the MDCS. High diagnostic validity of aortic stenosis in national Swedish registers has previously been confirmed, and most patients with an aortic stenosis diagnosis in national registers had moderate to severe aortic stenosis.[4] A summary of case ascertainment procedures is provided in the eMethods in the Supplement.

Statistical Analysis

CHARGE Cohorts

To test the hypothesis of a possible causal association between each lipid trait and aortic valve calcium, we used GWAS summary-level data from the 3 CHARGE participating cohorts with aortic valve calcium data.[4] The CHARGE aortic valve calcium GWAS has been previously described[4] and was performed using an age- and sex-adjusted additive model. To estimate the association for each lipid GRS (LDL-C, HDL-C, and TG) with aortic valve calcium presence, we extracted summary-level SNP data from the CHARGE aortic valve calcium GWAS for all lipid SNPs in each GRS and used the Genetics ToolboX (gtx) R package version 0.08[16] to generate GRS effect sizes (β_{GRS}) expressed as odds ratios ($OR_{GRS} = e^{\beta GRS}$) with 95% confidence intervals for presence of aortic valve calcium. This method has been shown to provide GRS estimates that are equivalent to using individual participant data for GRS association studies.[17,18] Details of the statistical methods can be found in the eMethods in the Supplement.

We also performed 3 sensitivity analyses to address the issue of genetic pleiotropy, in which a given lipid SNP may have several biological effects other than their known effects on plasma lipid levels. First, using the same approach outlined above, we reanalyzed the association between each lipid GRS and aortic valve calcium using a secondary "specific" GRS in which SNPs associated with more than 1 lipid were excluded. Second, we also addressed pleiotropy using a recently developed regression method called multivariable Mendelian randomization,[10] which uses a linear regression model to adjust for any possible known pleiotropic effects of the included SNPs by other lipids traits. The details of this method are available in the eMethods in the Supplement. Third, as a final sensitivity analysis, we simulated 1000 LDL-C GRS randomly excluding 30% of the available LDL-C SNPs in each set. Each LDL-C GRS was then tested against aortic valve calcium and the β coefficient for the GRS association was plotted against the LDL-C effect for each of these LDL-C GRS. We also report the mean GRS association and the upper and lower 95% confidence intervals for the effect estimate across all 1000 simulated GRS.

Malmö Diet and Cancer Study

In contrast to the summary-level data used in the CHARGE cohorts, direct genotype-phenotype data were used in the MDCS. The association of each linear GRS with incident clinical aortic stenosis was examined using Cox proportional hazards regression with censoring at death from other causes, emigration, or aortic valve replacement from other causes, adjusting for age, sex, height, weight, diabetes, hypertension, and current smoking at baseline. Age was used as the time scale, and both prevalent and incident diagnoses at the first study examination were included. Wald tests were used for significance testing of the hazard ratios (HRs). A sensitivity analysis was also performed, restricted to participants who underwent aortic valve replacement due to aortic stenosis. Cumulative incidence by GRS quartiles was calculated using cumulative incidence functions (CIF macro in SAS).

To estimate the possible causal association of LDL-C on aortic stenosis risk, an instrumental variable analysis was performed in the MDCS that considered both the GRS association with aortic stenosis and the GRS association with LDL-C and provided an estimate for the increased risk of aortic stenosis per unit change in LDL-C. We used a 2-stage regression approach in which (1) the association between the LDL GRS and LDL-C was estimated in the subset of MDCS with cholesterol measurements (n = 5269) and (2) the association between the predicted LDL-C levels (from the first level regression) and incident aortic stenosis was estimated in the entire MDCS cohort (n = 28 461). Analyses were adjusted for age and sex. To account for error terms across both stages, we used the delta method to estimate standard errors of the instrumental variable ratio estimates.[19] Analyses in the MDCS were performed

Table 1. Characteristics of CHARGE Participants

	Cohorts		
	FHS	AGES-RS	MESA
Country of origin	United States	Iceland	United States
Population	White European	White European	White European
No. of participants	1295	3120	2527
Age, mean (SD), y	60 (9)	76 (5)	63 (10)
Female, No. (%)	616 (47)	1811 (58)	1321 (52)
Presence of aortic valve calcium, No. (%)	510 (39)	1338 (43)	397 (16)
Lipids, mean (SD), mg/dL			
LDL-C	131.3 (30.9)	135.1 (61.8)	115.8 (30.9)
HDL-C	50.2 (15.4)	61.8 (19.3)	54.1 (15.4)
Triglycerides	185.8 (132.7)	106.2 (53.1)	132.7 (88.5)

Abbreviations: AGES-RS, Age, Gene/Environment Susceptibility–Reykjavik Study; CHARGE, Cohorts for Heart and Aging Research in Genomic Epidemiology; FHS, Framingham Heart Study; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MESA, Multi-Ethnic Study of Atherosclerosis.

SI conversion factors: To convert HDL and LDL cholesterol to mmol/L, multiply by 0.0259; triglycerides to mmol/L, multiply by 0.0113.

using IBM SPSS version 21.0 (IBM Corporation), Stata version 12.0 (StataCorp), and SAS version 9.4 (SAS Institute). For all analyses, we considered a 2-tailed *P* value of <.05 to be statistically significant.

Results

Baseline characteristics of CHARGE and MDCS participants are detailed in **Table 1** and **Table 2**. The numbers of individuals who qualified for analysis were as follows: FHS, n = 1295; AGES-RS, n = 3120; MESA, n = 2527; MDCS, n = 28 461. The prevalence of aortic valve calcium across the 3 CHARGE cohorts was 32% (n = 2245). In the MDCS, over a median follow-up time of 16.1 years (interquartile range, 14.8-17.7 years), aortic stenosis developed in 17 per 1000 participants (n = 473) and aortic valve replacement for aortic stenosis occurred in 7 per 1000 (n = 205). In the MDCS, we confirmed that each lipid GRS was associated with the corresponding lipid level as shown in eTable 8 in the Supplement. Each GRS explained 7.1%, 5.7%, and 4.3% of the variance of LDL-C, HDL-C, and TG, respectively. Pleiotropic associations with other lipid traits were also noted for all scores. However, the specific LDL-C GRS that excluded variants with associations with lipid fractions in the GLGC study (at $P < 5.0 \times 10^{-8}$) other than LDL-C was only significantly associated with LDL-C and total cholesterol (eTable 8 in the Supplement). The LDL-C GRS was also not associated with Lp(a) levels in the CHARGE cohorts with available Lp(a) data. As expected, Lp(a) levels were appropriately randomized across LDL-C GRS tertiles (eTables 9-10 in the Supplement).

Association Between Plasma Cholesterol Levels and Aortic Stenosis

In the subcohort of the MDCS where lipid fractions were measured (n = 5269), baseline LDL-C was significantly associated with incident aortic stenosis (HR per mmol/L, 1.28; 95% CI, 1.04-1.57; *P* = .02; incidence of aortic stenosis: 1.3% and 2.4% in lowest and highest LDL-C quartiles, respectively). Baseline HDL-C or TG levels were not significantly associated with incident aortic stenosis (**Table 3**).

Table 2. Baseline Characteristics for Participants of the Malmö Diet and Cancer Study

Characteristic	MDCS
Country of origin	Sweden
Population	White European
No. of participants	28 461
Age, mean (SD), y	58.0 (7.7)
Female sex, No. (%)	17 166 (60.3)
Diabetes, No. (%)	1220 (4.3)
Hypertension, No. (%)	17 444 (61.3)
Smoking, current, No. (%)	8121 (28.5)
Lipids, mean (SD), mg/dL[a]	
LDL-C	162.2 (38.6)
HDL-C	54.1 (15.4)
Triglycerides	123.9 (70.8)

Abbreviations: HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MDCS, Malmö Diet and Cancer Study.

SI conversion factors: To convert HDL and LDL cholesterol to mmol/L, multiply by 0.0259; triglycerides to mmol/L, multiply by 0.0113.

[a] Lipids were measured in a subset of the cohort (n = 5269).

Lipid GRSs and Aortic Valve Calcium in CHARGE Participants

In CHARGE participants, the LDL-C GRS, but not the HDL-C or the TG GRS, was significantly associated with presence of aortic valve calcium (OR per GRS increment, 1.38; 95% CI, 1.09-1.74; *P* = .007) (**Table 4**). After exclusion of pleiotropic SNPs that were associated with more than 1 lipid trait, a "specific" LDL-C GRS remained associated with presence of aortic valve calcium (OR per GRS increment, 1.39; 95% CI, 1.04-1.86; *P* = .03).

Lipid GRSs, LDL-C, and Aortic Stenosis in MDCS Participants

In the MDCS, the LDL-C GRS was strongly associated with LDL-C (+2.4 mmol/L per GRS increment; $P = 4 \times 10^{-93}$). Only the LDL-C GRS, but not the HDL-C or TG GRS, was significantly associated with increased aortic stenosis incidence (HR per GRS increment in LDL-C, 2.78; 95% CI, 1.22-6.37; *P* = .02; aortic stenosis incidence: 1.9% and 2.6% in lowest and highest GRS quartiles, respectively) (Table 4). For aortic stenosis requiring aortic valve replacement, the increased risk was not statistically significant (HR per GRS increment, 3.15; 95% CI, 0.90-11.03; *P* = .06; aortic valve replacement incidence: 0.8%

Table 3. Baseline Lipid Levels by Incident Aortic Stenosis Status and Associated Relative Risks Among MDCS Participants With Available Lipid Values

	Lipid, Mean (SD), mg/dL		Relative Risk for Aortic Stenosis per mmol/L of Lipid	
	No Aortic Stenosis (n = 5184)	Aortic Stenosis (n = 85)	Adjusted HR (95% CI)[a]	*P* Value
LDL-C	161.0 (38.1)	173.3 (37.6)	1.28 (1.04-1.57)	.02
HDL-C	53.4 (14.4)	49.8 (12.7)	0.67 (0.33-1.33)	.25
Triglycerides	121.8 (71.1)	128.4 (64.4)	0.89 (0.67-1.18)	.41

Abbreviations: HDL-C, high-density lipoprotein cholesterol; HR, hazard ratio; LDL-C, low-density lipoprotein cholesterol; MDCS, Malmö Diet and Cancer Study.

SI conversion factors: To convert HDL and LDL cholesterol to mmol/L, multiply by 0.0259; triglycerides to mmol/L, multiply by 0.0113.

[a] Cox proportional hazards model adjusting for age, sex, height, weight, diabetes, hypertension, and current smoking at baseline.

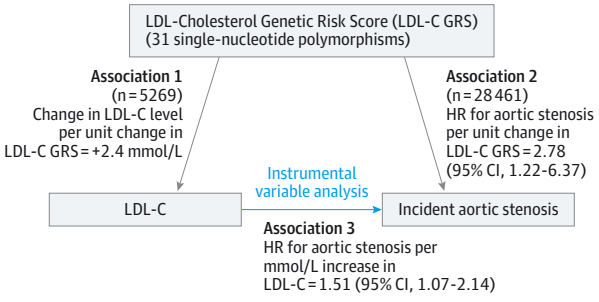
Table 4. Association of Genetic Risk Scores With Aortic Valve Calcium, Aortic Stenosis, and Aortic Valve Replacement[a]

	CHARGE Consortium (n = 6942)		MDCS Cohort (n = 28 461)			
	Prevalent Aortic Valve Calcium, OR (95% CI)	*P* Value	Incident Aortic Stenosis, HR (95% CI)	*P* Value	Incident Aortic Valve Replacement, HR (95% CI)	*P* Value
LDL-C GRS	1.38 (1.09-1.74)	.007	2.78 (1.22-6.37)	.02	3.15 (0.90-11.03)	.06
Specific LDL-C GRS[a]	1.39 (1.04-1.86)	.03	3.85 (1.37-10.79)	.009	4.95 (1.03-23.78)	.05
HDL-C GRS	1.07 (0.60-1.91)	.81	0.21 (0.03-1.50)	.12	0.08 (0.004-1.58)	.10
Triglyceride GRS	1.24 (0.95-1.62)	.11	2.03 (0.74-5.58)	.17	2.29 (0.49-10.60)	.29

Abbreviations: GRS, genetic risk score; HDL-C, high-density lipoprotein cholesterol; HR, hazard ratio; LDL-C, low-density lipoprotein cholesterol; MDCS, Malmö Diet and Cancer Study; OR, odds ratio.

[a] A specific LDL-C GRS excluded single-nucleotide polymorphisms also associated with HDL-C or triglycerides in the Global Lipids Genetics Consortium study.[13] Results are presented per unit increment in the GRS with 95% CIs and *P* value.

Figure 1. Mendelian Randomization of LDL-C and Risk of Aortic Stenosis in the Malmö Diet and Cancer Study



The aim of Mendelian randomization is to provide a robust test of the association between low-density lipoprotein cholesterol (LDL-C) and aortic stenosis (association 3). Association 3 can be tested simply using standard epidemiologic methods, but these methods may be biased (eg, confounding, reverse causality, etc). To overcome this bias, Mendelian randomization indirectly tests association 3 by first establishing via linear regression that LDL-C–related single-nucleotide polymorphisms (SNPs) increase LDL-C (association 1). These LDL-C SNPs are then tested for an association with aortic stenosis (association 2). Under the assumption that the entire effect of the LDL-C SNPs on aortic stenosis (association 2) is mediated by their effect on increasing LDL-C (association 1), an unconfounded assessment of association 3 can be obtained (ie, instrumental variable estimate).

and 1.4% in lowest and highest GRS quartiles, respectively). After exclusion of pleiotropic SNPs that were associated with more than 1 lipid trait, a "specific" LDL-C GRS remained associated with incident aortic stenosis (HR per GRS increment, 3.85; 95% CI, 1.37-10.79; *P* = .009; aortic stenosis incidence: 1.6% and 2.7% in lowest and highest GRS quartiles, respectively).

In formal instrumental variable analysis, which may provide a more robust causal association estimate between change in LDL-C and risk of aortic stenosis, genetic increases in LDL-C were significantly associated with an increased risk of aortic stenosis (HR per mmol/L, 1.51; 95% CI, 1.07-2.14; *P* = .02) (**Figure 1**).

Sensitivity Analyses

In CHARGE participants, we also used multivariable Mendelian randomization, a regression-based method, to assess the association between β_{LDL} and $\beta_{aortic\ valve\ calcium}$ and observed a strong linear association (*P* for linear trend 7×10^{-13}) indicating a significant relation between the magnitude of change in LDL-C and the increase in aortic valve calcium presence (**Figure 2**). Adjustments for β_{HDL} and β_{TG} did not materially change the observed relation between β_{LDL} and $\beta_{aortic\ valve\ calcium}$ (*P* for linear trend 3×10^{-13}) (eTable 11 in the Supplement). Finally, the results after simulating 1000 LDL-C GRS by random SNP exclusion were largely in keeping with the main analysis, demonstrating no major dependencies of the LDL-C GRS association with aortic valve calcium on any set of specific SNPs (eFigure 1 in the Supplement).

Discussion

In this study of 6942 participants with subclinical data on aortic valve calcium and more than 28 000 participants with more than 15 years of follow-up for aortic stenosis, we demonstrate that genetic elevation in LDL-C, as determined by a GRS, is associated with both presence of aortic valve calcium

and incident aortic stenosis. We also found no evidence of association between genetically elevated HDL-C or TG with presence of valve calcium or aortic stenosis, indicating that a mechanism specifically attributable to LDL-C is likely responsible for the association with aortic valve disease. Our findings link a genetically mediated increase in plasma LDL-C with early subclinical valve disease, as measured by aortic valve calcium,[20,21] and incident clinical aortic stenosis, providing supportive evidence for a causal role of LDL-C in the development of aortic stenosis. Our results are in keeping with prior observations of a higher risk of aortic stenosis in patients with familial hypercholesterolemia,[22] due to marked elevations in LDL-C, caused by rare mutations in the LDL receptor gene (and other related genes), and extend these findings to individuals with common variants that modestly increase LDL-C in the general population. These data suggest that, in addition to the established risks for myocardial infarction and other vascular diseases, increases in LDL-C are also associated with increased risk for aortic stenosis.

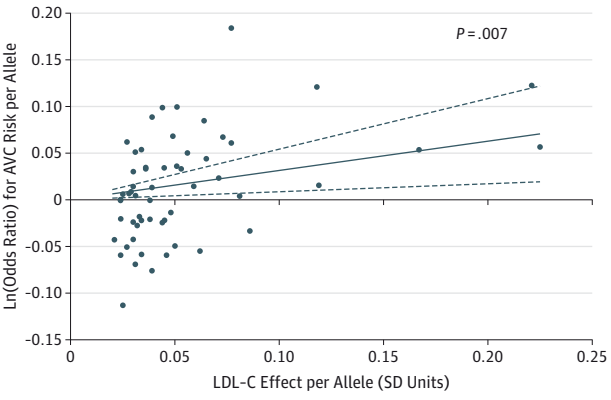
Epidemiological studies have previously demonstrated that higher LDL-C is a risk factor for aortic valve calcium and aortic stenosis.[3,23,24] Although observational studies have also reported a reduction in the incidence and progression of aortic stenosis in statin users,[25] this was not observed in subsequent large randomized trials.[5-7] The disappointing results of these trials have cast doubt on the LDL hypothesis in aortic valve disease and dampened enthusiasm for LDL-C lowering in the prevention of valve disease. So how do we reconcile our Mendelian randomization data supporting an association between LDL-C and the development of aortic valve disease with the failure of LDL-C lowering in randomized trials of aortic stenosis?

All trials of LDL-C lowering for aortic stenosis enrolled older participants with established valve disease (in most cases, moderate or severe aortic stenosis),[5-7] in which LDL-C may no longer be an important mediator of progressive disease. Once valve calcification and remodeling are well established, factors other than LDL-C, including hemodynamic forces and other procalcifying mediators, may become more important for progression. We have previously shown that higher total cholesterol in early adulthood, prior to any valve disease, was strongly associated with presence of valve calcification 25 years later.[23] Similarly, in MESA, the association between LDL-C and aortic valve calcium was strongest among younger, rather than older, participants.[24] It also remains to be seen whether part of the failure of prior randomized clinical trials may be related to the use of statins for lipid lowering, which may promote calcification in advanced valve disease.[26]

Lipoprotein deposition is a seminal process in both valve disease and vascular atherosclerosis. In early valve lesions, apolipoprotein B (the carrier molecule of LDL-C) has been demonstrated to co-localize to regions of early leaflet remodeling and calcification.[27] Although LDL-C may play a common role in both vascular and valvular disease, LDL-C may play a more important role in the early calcification and mineralization phase that is strongly characteristic of early valve lesions, as opposed to vascular lesions.[28] Low-density lipoprotein cholesterol may promote calcification in these early lesions, via the formation of cholesterol microcrystals that act as nidi for initial calcification,[29] and also via oxidized LDL, a potent proinflammatory and pro-oxidant mediator that is known to strongly induce an osteogenic phenotype in valvular cells.[30] The importance of LDL-C in the early stages of aortic valve disease is also well supported by transgenic animal data demonstrating that the genetic induction of hypercholesterolemia leads to an early procalcific phenotype at the aortic valve that can be arrested, but not reversed, with reversion to normal cholesterol levels.[31] Based on the known biology of valve calcification and the failure of prior randomized trials targeting advanced disease, our results suggest that early lipid lowering, prior to the development of even mild forms of aortic stenosis, may be required to prevent aortic valve disease; this hypothesis remains to be tested in a randomized trial.

Our study has limitations that deserve mention. First, the use of summary-level data from the CHARGE consortium precluded adjustment for additional covariates. However, large Mendelian randomization studies are unlikely to be confounded because of the inherent randomization in these analyses. Indeed, adjusting for several potential confounders in the MDCS did not materially change our findings. Second, although we performed several sensitivity analyses to exclude potential pleiotropic effects of the included SNPs, a non–LDL-C–related mechanism for the observed associations cannot be entirely excluded: an important caveat of all Mendelian randomization studies. In addition, our estimates from instrumental variable analysis may be biased due to time-varying LDL-C and other considerations; therefore, the magnitude of these estimates must be interpreted cautiously.[32] Third, we used diagnostic codes to identify incident aortic stenosis in the MDCS. However, these codes

Figure 2. Magnitude of Genetic Increase in LDL-C and Odds of Aortic Valve Calcium Across All LDL-C SNPs in CHARGE Participants



Each dot represents a single low-density lipoprotein cholesterol (LDL-C) single-nucleotide polymorphism (SNP). Across all 57 LDL-C–associated SNPs, a given genetic increase in LDL-C is correlated with a concomitant increase in the odds of aortic valve calcium (AVC). The solid line represents the best line of fit, and the dashed lines represent the 95% CI for this relationship. *P* value reported is for the linear association.

have been validated and shown to have a predictive value greater than 90% when compared with echocardiography.[4] Fourth, the LDL-C GRS in the CHARGE and MDCS analyses differed because of the lack of GWAS data and fewer SNPs available in the MDCS; however, results in CHARGE were not materially different when we used the same LDL-C GRS used in the MDCS. Finally, our study was performed only in individuals of European descent; whether our results apply to other ethnicities will require further study.

Conclusions

Genetic predisposition to elevated LDL-C was associated with presence of aortic valve calcium and incidence of aortic stenosis, providing evidence supportive of a causal association between LDL-C and aortic valve disease. Whether earlier intervention to reduce LDL-C could prevent aortic valve disease merits further investigation.

ARTICLE INFORMATION

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