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Association of Triglyceride-Related Genetic Variants With Mitral Annular Calcification

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ABSTRACT

BACKGROUND Mitral annular calcium (MAC), commonly identified by cardiac imaging, is associated with cardiovascular events and predisposes to the development of clinically important mitral valve regurgitation and mitral valve stenosis. However, its biological determinants remain largely unknown.

OBJECTIVES The authors sought to evaluate whether a genetic predisposition to elevations in plasma lipids is associated with the presence of MAC.

METHODS The authors used 3 separate Mendelian randomization techniques to evaluate the associations of lipid genetic risk scores (GRS) with MAC in 3 large patient cohorts: the Framingham Health Study, MESA (Multiethnic European Study of Atherosclerosis), and the AGE-RS (Age, Gene/Environment Susceptibility-Reykjavik Study). The authors provided cross-ethnicity replication in the MESA Hispanic-American participants.

RESULTS MAC was present in 1,149 participants (20.4%). In pooled analyses across all 3 cohorts, a triglyceride GRS was significantly associated with the presence of MAC (odds ratio [OR] per triglyceride GRS unit: 1.73; 95% confidence interval [CI]: 1.24 to 2.41; $p = 0.0013$). Neither low- nor high-density lipoprotein cholesterol GRS was significantly associated with MAC. Results were consistent in cross-ethnicity analyses among the MESA Hispanic-Americans cohort (OR per triglyceride GRS unit: 2.04; 95% CI: 1.03 to 4.03; $p = 0.04$). In joint meta-analysis across all included cohorts, the triglyceride GRS was associated with MAC (OR per triglyceride GRS unit: 1.79; 95% CI: 1.32 to 2.41; $p = 0.0001$). The results were robust to several sensitivity analyses that limit both known and unknown forms of genetic pleiotropy.

CONCLUSIONS Genetic predisposition to elevated triglyceride levels was associated with the presence of MAC, a risk factor for clinically significant mitral valve disease, suggesting a causal association. Whether reducing triglyceride levels can lower the incidence of clinically significant mitral valve disease requires further study. (J Am Coll Cardiol 2017;69:2941–8) © 2017 by the American College of Cardiology Foundation. Published by Elsevier. All rights reserved.

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ABBREVIATIONS AND ACRONYMS

CI = confidence interval

CT = computed tomography

GRS = genetic risk score

GWAS = genome-wide association study

HDL-C = high-density lipoprotein cholesterol

LDL-C = low-density lipoprotein cholesterol

MAC = mitral annular calcium

MR = Mendelian randomization

MV = mitral valve

OR = odds ratio

SNP = single nucleotide polymorphism

Mitral annular calcium (MAC) is commonly identified by cardiac imaging and has been shown to be independently associated with cardiovascular events (1-5). MAC also is associated with the development of clinically significant mitral valve (MV) regurgitation and MV stenosis (6-8). Although MAC is common among older individuals and has been associated with significant morbidity and mortality (9), its biological determinants have not been well established. We have previously shown that MAC was associated with genetic variants near the *IL1F9* gene, a finding that was replicated among Hispanic Americans (10), but additional genetic contributions to MAC remain largely unknown. A better understanding of the biological determinants of MAC could provide novel therapeutic targets for the prevention of MV disease.

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In observational studies, MAC has been associated with obesity and several metabolic traits, including dyslipidemia (11-14). However, whether elevated lipid levels cause MAC and mediate these associations remains unknown. Accordingly, as we have performed previously for aortic valve calcium (15), we used a Mendelian randomization (MR) approach to evaluate whether a lifelong genetic predisposition to elevations in plasma lipids was associated with the presence of MAC in participants of the CHARGE (Cohorts for Heart and Aging Research in Genetic Epidemiology) consortium. We also utilized several novel MR methodologies to address some of the potential limitations of MR.

METHODS

The CHARGE consortium is an ongoing collaboration among several large, well-phenotyped, prospective, longitudinal cohort studies from the United States and Europe (16). We evaluated the association of lipid genetic risk score (GRS) with the presence of MAC in CHARGE participants; specifically, white European participants with MAC data by cardiac computed tomography (CT) imaging and genome-wide association study (GWAS) data from MESA (Multi-Ethnic Study of Atherosclerosis) and the FHS (Framingham Heart Study), with additional data later added from AGES-RS (Age, Gene/Environment Susceptibility-Reykjavik Study). We also performed cross-ethnicity analyses using MESA Hispanic Americans, the only ethnicity in the MESA study with adequate statistical power for GRS analyses, as there were only 37 cases of MAC among MESA Chinese Americans; the triglyceride GRS, which has not been validated across ethnicities, was also poorly predictive of triglycerides in MESA African Americans (data not shown). Details of each of these cohorts, as well as the CHARGE extracoronary calcium consortium, have been previously described (17-20). High-density lipoprotein cholesterol (HDL-C) and triglycerides were measured using standard techniques as previously described (16). Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald equation. The studies were approved by relevant regulatory bodies, and participants provided informed consent.

To construct each lipid GRS, we included linkage disequilibrium-pruned single nucleotide polymorphisms (SNPs) that were reported in the 2013 Global Lipid Genetics consortium study (21) to be associated with LDL-C, HDL-C, or triglyceride levels and for which summary results were available in the previously reported GWAS for MAC (10). As performed previously, we constructed separate weighted GRS for LDL-C (57 SNPs), HDL-C (70 SNPs), and triglycerides (39 SNPs) (i.e., weighted by the β coefficients of each individual SNP from large-scale

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lipid GWASs) (21). The SNPs included in each GRS are summarized in Online Tables 1 to 3.

OUTCOMES. MAC in CHARGE cohorts was examined using standard CT scanning that was performed on all participants; images were analyzed for the presence of MAC using offline digital software. The presence of MAC was then defined as ≥3 contiguous pixels with a brightness of 130 Hounsfield units or more to indicate the presence of calcium, as defined by Agatston et al. (22). MAC was identified as lesions along the mitral annulus circumferentially, exclusive of the MV leaflets (except in the FHS cohort, where leaflet calcium was not specifically excluded) (10).

STATISTICAL ANALYSIS. To estimate the association between each lipid GRS and the presence of MAC, we extracted age- and sex-adjusted summary-level data from the CHARGE MAC GWAS (which included the FHS plus MESA cohorts) for all SNPs included in each GRS. We used the Genetics Toolbox R package version 0.08 (23) to generate association estimates for each lipid GRS with the presence of MAC, which were expressed as odds ratios (ORs) with 95% confidence intervals (CIs). At the time of the initial CHARGE GWAS, data from the AGES-RS cohort were not included. Therefore, we estimated the association between each lipid GRS and MAC in AGES-RS participants at the participant level by logistic regression adjusted for age and sex. Each GRS in the AGES-RS cohort included the same SNPs as used in the summary-level analyses from the available CHARGE cohorts. Each GRS was quantified as the weighted sum of the number of risk alleles for each individual; weights were determined on the basis of the effect size for each lipid parameter for a given SNP based on large-scale lipid GWAS (24,25). Results from the AGES-RS cohort were then pooled with CHARGE summary-level estimates to provide the final estimate for the triglyceride GRS association with MAC in participating CHARGE cohorts. In MESA Hispanic Americans, analyses were performed using participant-level data, as performed for AGES-RS. For MESA Hispanic Americans, models were adjusted for age, sex, field center, and principal components. In a joint meta-analysis, estimates for the triglyceride GRS with MAC were pooled across all the cohorts (CHARGE, AGES-RS, and MESA Hispanics) using a fixed-effects meta-analysis. In sensitivity analyses, we demonstrated that the results were robust to the meta-analytic model used (i.e., fixed vs. random effects; data not shown).

We performed additional sensitivity analyses using the CHARGE summary-level statistics to address the issue of genetic pleiotropy that could explain the association between triglyceride SNPs and MAC, in which a given SNP might have several biological effects other than its known effects on triglyceride levels. First, we used multivariable MR, a technique that addresses known pleiotropy of included SNPs (26). Second, we generated 1,000 triglyceride GRS sets randomly excluding 30% of the available lipid-associated SNPs in each set (for LDL-C, HDL-C, and triglycerides). Each lipid risk score was then tested against MAC, and we reported the mean GRS association and the upper and lower 95% CIs for the effect estimate across all 1,000 simulated GRS (15). Third, we also utilized MR-Egger regression to ensure our results were robust to unknown pleiotropy (27).

TABLE 1 Baseline Characteristics

	CHARGE Cohorts		
	FHS (n = 1,298)	AGES-RS (n = 1,826)	MESA (n = 2,527)
Country of origin	United States	Iceland	United States
Ethnicity	Caucasian	Caucasian	Caucasian
Age, yrs	60 ± 9	75 ± 5	63 ± 10
Female	616 (47)	1,068 (58)	1,321 (52)
Presence of MAC	259 (20)	581 (32)	309 (12)
LDL-C, mg/dl	131.3 ± 30.9	136.1 ± 39.7	115.8 ± 30.9
HDL-C, mg/dl	50.2 ± 15.4	61.5 ± 17.1	54.1 ± 15.4
Triglycerides, mg/dl	185.8 ± 132.7	105.0 ± 54.8	132.7 ± 88.5

Values are mean ± SD or n (%).

AGES-RS = Age, Gene/Environment Susceptibility-Reykjavik Study; CHARGE = Cohorts for Heart and Aging Research in Genetic Epidemiology; FHS = Framingham Heart Study; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; MAC = mitral annulus calcium; MESA = Multiethnic European Study of Atherosclerosis.

RESULTS

Table 1 summarizes the baseline characteristics of CHARGE participants (N = 5,651). Data on MAC were available for all participants in the 3 cohorts: the FHS and MESA (n = 3,795) and AGES-RS (n = 1,826) cohorts. The prevalence of MAC across the CHARGE cohorts was 20.4% (n = 1,149). To confirm our findings, we also included data from MESA Hispanic Americans (n = 2,025; prevalence of MAC 9.7%).

The ORs per GRS increment for the triglyceride GRS for MAC in the FHS plus MESA cohorts (using combined GWAS summary data) were 2.55 (95% CI: 1.62 to 4.02) and, using individual-level data, the AGES-RS cohort showed an OR of 1.10 (95% CI: 0.67 to 1.79). In pooled analyses of these CHARGE cohorts, the triglyceride GRS was significantly associated with the presence of MAC (OR per GRS unit: 1.73; 95% CI: 1.21 to 2.41). The LDL-C and HDL-C GRS were not significantly associated with MAC (LDL-C OR per GRS unit:

TABLE 2 GRS of All Lipid Fractions and MAC Association

Lipid Fraction	Phenotype	OR (per Unit Change)	95% CI	p Value	m
Triglycerides					
	GTX	2.55	1.62–4.02	**5.26E-05**	39
	Multivariable	3.21	2.09–4.93	**2.70E-07**	39
	Random SNP exclusion	2.58	1.47–4.54	**0.0046**	27
	Egger	2.34	1.14–4.80	**0.021**	39
LDL-C					
	GTX	1.47	0.96–2.27	0.079	55
	Multivariable	1.03	0.73–1.45	0.89	55
	Random SNP exclusion	1.49	0.88–2.52	0.20	38
	Egger	1.30	0.58–2.90	0.52	55
HDL-C					
	GTX	1.00	0.68–1.48	0.99	68
	Multivariable	1.40	0.99–1.99	0.061	68
	Random SNP exclusion	1.01	0.62–1.62	0.66	48
	Egger	0.70	0.39–1.26	0.29	68

Values in **bold** are statistically significant.

CI = confidence interval; GRS = genetic risk score; GTX = Genetic ToolBox package in R; m = number of single nucleotide polymorphisms used in the analysis; OR = odds ratio; SNP = single nucleotide polymorphisms; other abbreviations as in Table 1.

1.47; 95% CI: 0.96 to 2.27; HDL-C OR per GRS unit: 1.00; 95% CI: 0.68 to 1.48) (Table 2).

In cross-ethnicity analyses of the MESA Hispanic-American cohort, triglyceride GRS was strongly predictive of triglyceride levels and significantly associated with MAC (adjusted OR: 2.04; 95% CI: 1.03 to 4.03; p = 0.04) (Table 3). In a joint meta-analysis of the FHS plus MESA cohorts, the AGES-RS cohort, and MESA Hispanic Americans, triglyceride GRS was significantly associated with presence of MAC (OR: 1.79; 95% CI: 1.32 to 2.41; p = 0.0001) (Central Illustration).

SENSITIVITY ANALYSES. First, we conducted a multivariable MR analysis to address any pleiotropy from other lipid fractions, such as LDL-C and HDL-C (Table 2). Across the 181 lipid GWAS SNPs, β_{MAC} was significantly associated with $\beta_{triglyceride}$ in unadjusted and adjusted models for $\beta_{LDL\text{-}C}$ and $\beta_{HDL\text{-}C}$ (adjusted OR: 2.23; 95% CI: 1.55 to 3.20; $p = 2.28 \times 10^{-5}$) (Online Table 4). We also used a multiple linear regression model using all the predictors in a single model. The results were similar with only triglycerides showing a strong and significant association with MAC (adjusted OR: 3.21; 95% CI: 2.09 to 4.93; $p = 2.70 \times 10^{-7}$) (Table 2). We found no significant association between β_{MAC} and $\beta_{LDL\text{-}C}$ or $\beta_{HDL\text{-}C}$. Second, after computing 1,000 triglyceride GRS sets by random SNP exclusions, the association with MAC was largely in keeping with the main analysis, demonstrating no major dependencies of the triglyceride GRS association with any specific SNP or any set of specific SNPs, as seen in Online Figure 1. Third, MR-Egger estimates, which account for unknown pleiotropy of included SNPs, were also consistent with our main analysis (Table 2).

TABLE 3 Triglyceride GRS Associations With Triglycerides and MAC in MESA Hispanic Americans

	Subjects (n)	Cases (n)	Adjusted Beta for Triglycerides (95% CI)	p Value	OR for MAC (95% CI)	p Value
Hispanic	2,105	197	103.2 mg/dl (82.8–123.5)	3.1×10^{-23}	2.04 (1.03–4.03)	0.04

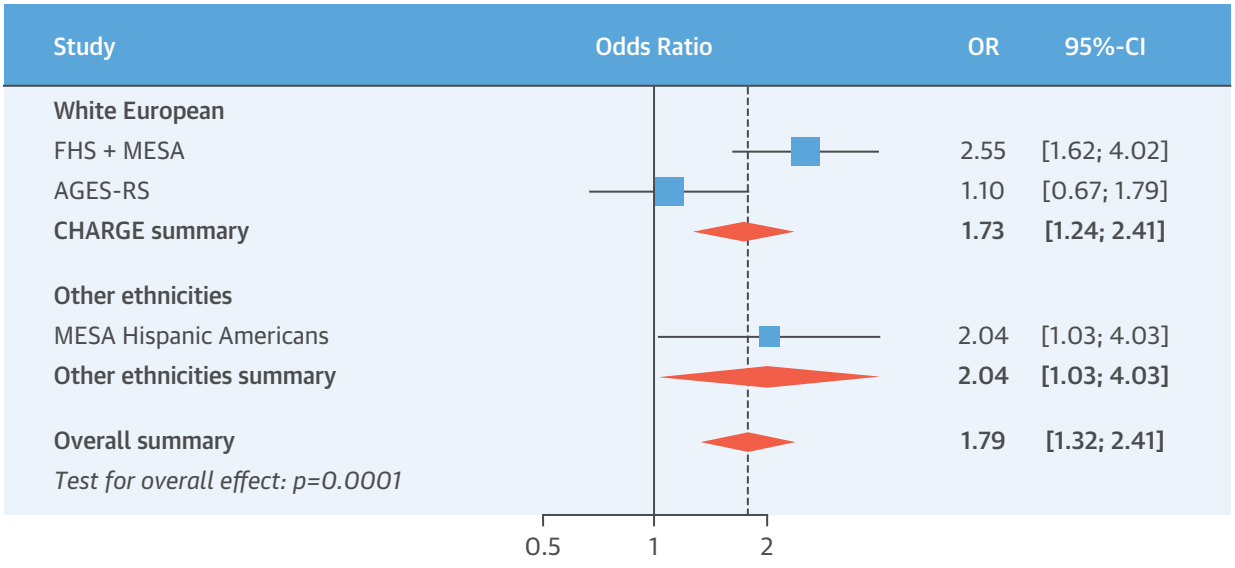
Abbreviations as in Tables 1 and 2.

DISCUSSION

In this analysis of data from 5,651 individuals with MAC quantified by CT imaging, we demonstrated that a triglyceride GRS, which has been shown to be highly correlated with circulating triglyceride levels (10,21), is strongly associated with the presence of MAC (Central Illustration). Although the triglyceride GRS is known to be highly pleiotropic and associated with several other lipid traits, we used 3 separate MR methods, including multivariable MR, a random SNP exclusion approach, and Egger-regression, to limit the effects of other possible pleiotropic pathways and improve causal inference. First, we demonstrated that after adjustment for the known associations between each triglyceride SNP with other non-triglyceride lipid measures, the triglyceride GRS remained significantly associated with MAC. Second, by simulating 1,000 triglyceride GRS sets by random exclusion of triglyceride SNPs, we showed that the association between the triglyceride GRS and MAC was highly consistent across all simulated triglyceride GRS sets. This demonstrates that the triglyceride GRS association with MAC is not dependent on any specific SNP or set of SNPs, limiting potential pleiotropic mechanisms and further supporting our finding that this association is mediated primarily by circulating triglycerides. Finally, Egger-regression estimates, which can correct estimates for unknown pleiotropy, were also supportive of an association between triglycerides and MAC. In cross-ethnicity analyses in MESA Hispanic Americans, where the triglyceride GRS strongly correlated with triglyceride levels (and thus was a valid genetic instrument for MR), we confirmed the association between the triglyceride GRS and MAC.

In contrast to our prior work examining the genetic determinants of aortic valve calcium, where we identified strong associations with the LDL-C GRS

CENTRAL ILLUSTRATION **Association Between Triglyceride GRS and MAC**



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In Hispanic Americans in the MESA study, the triglyceride genetic risk score (GRS) was strongly predictive of triglyceride levels and also significantly associated with mitral annular calcium (MAC). This association could not be evaluated in African Americans or Chinese Americans, given that the triglyceride GRS was poorly predictive of triglyceride levels in these cohorts. AGES-RS = Age, Gene/Environment Susceptibility-Reykjavik Study; CHARGE = Cohorts for Heart and Aging Research in Genetic Epidemiology; CI = confidence interval; FHS = Framingham Heart Study; MESA = Multiethnic European Study of Atherosclerosis; OR = odds ratio.

(15), we found no similar association with MAC. Both an LDL-C and an HDL-C GRS were not associated with presence of MAC suggesting that lifelong elevations of these lipoproteins is unlikely causal in the genesis of MAC. Although the LDL-C GRS was nearly significant for an association with MAC in our main analysis, this association was not robust to sensitivity analyses addressing pleiotropy. Our results therefore suggested possible differences in the calcification of the mitral annulus and the aortic leaflets and might indicate unique biological pathways for calcification of each valve. Whether differences in the lipid composition of the relevant lipoprotein or their propensity to enter each tissue explains these disparate associations will require further study.

The development and progression of MAC is now believed to be a tightly regulated process, with marked similarities to bone formation (28,29). MAC has been associated with several cardiovascular risk factors including diabetes mellitus, hypertension, and dyslipidemia (11-14). In a small case-control study with 107 MAC cases and 107 controls, Nair et al. (11) demonstrated that patients with MAC had a significantly higher prevalence of diabetes mellitus and hypertension. Aronow et al. (12) identified 559 patients, 55% of whom had MAC based on echocardiography. Patients with MAC were more likely to have hypertension, diabetes, and high total cholesterol levels (12). A study by Boon et al. (13) examined more than 8,000 patients from a prospective echocardiographic database and identified 8% with MAC. Using multivariable regression analysis, they identified hypertension, diabetes mellitus, and dyslipidemia to be associated with MAC (13). In a longitudinal analysis of incident MAC, Elmariah et al. (14) have shown that age, diabetes mellitus, hypertension, dyslipidemia, and smoking were associated with incident MAC. We have also previously shown that MAC was associated with age, mean total cholesterol, smoking, and, interestingly, C-reactive protein among 1,300 participants in the Framingham Offspring Study (30). We have now extended these observational associations to provide new evidence that triglycerides, but not other lipid fractions, are causally associated with MAC.

MAC might be caused by ectopic direct deposition of triglycerides in the mitral annulus similar to their deposition within cardiac myocytes (31), as well as by the formation of calcium-phosphate crystals in plasma that might become insoluble, resulting in

metastatic calcification (28,32). This process has been observed in secondary hyperparathyroidism (33), which has been associated with obesity and metabolic syndromes, independent of hypovitaminosis D and renal failure (34–36). Triglycerides have also been shown to be correlated with calcium levels (37), which have been attributed to decreased triglyceride clearance in hyperparathyroid states, (38,39). However, it remains unknown whether hypertriglyceridemia could also predispose to bone resorption, increased calcium phosphate, or hyperparathyroidism directly. Although understanding the biological mechanisms for this association will require further study, our results of a causal role for triglycerides in MAC provided a possible explanation for the longstanding association observed between MAC and dysmetabolic states, such as obesity and the metabolic syndrome, that are characterized by increased triglyceride levels. In addition, our results also suggested that the observed associations between MAC and cardiovascular events (1–5), which are unlikely due to MAC itself, might be mediated by the presence of high triglycerides, which predispose to increased cardiovascular risk. Indeed, genetic evidence now strongly supports a causal link between circulating triglycerides and myocardial infarction (40).

Although MAC represents a common subclinical phenotype that has little clinical impact when mild, it is now recognized that the presence of MAC may predispose to the development of clinically significant valve disease by several mechanisms (6,7). Calcification of the mitral annulus has been shown to impede the annular contraction that occurs in systole and that ensures proper apposition of the mitral leaflets (6). Furthermore, MAC is also suggested to cause tethering of leaflets with resultant mitral regurgitation (7). More severe MAC also may predispose to mitral stenosis due to calcium encroachment in the mitral annulus (6,8). Currently, the only effective treatment for MV disease is valve repair or replacement when significant regurgitation and/or stenosis become evident, a procedure that is associated with significant morbidity and mortality (41,42). Whether prevention of progressive MAC by reduction of circulating triglycerides, by lifestyle management (e.g., weight loss), or through novel pharmacological agents, including recently developed apolipoprotein CIII antisense molecules (43–47), could provide novel strategies to prevent progressive MV disease and the need for MV intervention merits further investigation.

STUDY STRENGTHS AND LIMITATIONS. Our study had several strengths, including a large sample size with CT imaging of the mitral annulus providing excellent resolution of MAC. In addition, the use of Mendelian randomization, as well as several sensitivity analyses, provided strong support for the association between MAC and genetically elevated triglyceride levels. Nonetheless, several limitations deserve mention. First, by using summary-level data from the cohorts in the CHARGE consortium, we were unable to adjust for additional covariates. This limitation, however, is unlikely to have affected our findings, given that large MR studies limit both confounding and reverse causality. Second, all MR studies have the potential to be limited by pleiotropy of the included SNPs. For this reason, we performed 3 separate sensitivity analyses to limit the effects of other possible pleiotropic pathways, but a non-triglyceride-related mechanism for the observed association cannot be completely ruled out. Third, we noted significant heterogeneity with the effect of the triglyceride GRS in the AGES-RS cohort, as compared with the other cohorts. Whether this represents random variation or a true biological difference due to the older age, lower triglyceride levels, or other clinical differences in the AGES-RS cohort as compared with the other cohorts will require further study. However, even considering this heterogeneity, our joint meta-analysis (using either fixed or random effects) remained significant. Lastly, the association could not be evaluated in African Americans or Chinese Americans from the MESA cohort because the triglyceride GRS was poorly predictive of triglyceride levels (it was not a valid instrument for circulating triglycerides), and statistical power was very low due to the small sample size available. Nonetheless, in Hispanic Americans, where the sample size was adequate and the triglyceride GRS successfully predicted triglyceride levels, we were able to confirm this association.

CONCLUSIONS

Genetic predisposition to elevated triglyceride levels was associated with the presence of MAC, a risk factor for mitral regurgitation and stenosis, suggesting a causal association between triglycerides and MV disease. Future studies are required to assess whether reducing circulating triglyceride levels could lower the incidence of clinically significant MV disease.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: A genetic predisposition to hypertriglyceridemia is associated with the development of mitral annular calcification (MAC) and may partly explain the link between MAC and cardiovascular events.

TRANSLATIONAL OUTLOOK: Whether lowering blood levels of triglycerides can prevent progressive MAC and valve dysfunction and improve clinical outcomes requires further investigation.

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APPENDIX For supplemental tables and a figure, please see the online version of this article.