Secretory Phospholipase A₂-IIA and Cardiovascular Disease

A Mendelian Randomization Study

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Objectives   This study sought to investigate the role of secretory phospholipase A$_2$ (sPLA$_2$)-IIA in cardiovascular disease.

Background   Higher circulating levels of sPLA$_2$-IIA mass or sPLA$_2$ enzyme activity have been associated with increased risk of cardiovascular events. However, it is not clear if this association is causal. A recent phase III clinical trial of an sPLA$_2$ inhibitor (varespladib) was stopped prematurely for lack of efficacy.

Methods   We conducted a Mendelian randomization meta-analysis of 19 general population studies (8,021 incident, 7,513 prevalent major vascular events [MVE] in 74,683 individuals) and 10 acute coronary syndrome (ACS) cohorts (2,520 recurrent MVE in 18,355 individuals) using rs11573156, a variant in *PLA2G2A* encoding the sPLA$_2$-IIA isoenzyme, as an instrumental variable.

Results   *PLA2G2A* rs11573156 C allele associated with lower circulating sPLA$_2$-IIA mass (38% to 44%) and sPLA$_2$ enzyme activity (3% to 23%) per C allele. The odds ratio (OR) for MVE per rs11573156 C allele was 1.02 (95% confidence interval [CI]: 0.98 to 1.06) in general populations and 0.96 (95% CI: 0.90 to 1.03) in ACS cohorts. In the general population studies, the OR derived from the genetic instrumental variable analysis for MVE for a 1-log unit lower sPLA$_2$-IIA mass was 1.04 (95% CI: 0.96 to 1.13), and differed from the non-genetic observational estimate (OR: 0.69; 95% CI: 0.61 to 0.79). In the ACS cohorts, both the genetic instrumental variable and observational ORs showed a null association with MVE. Instrumental variable analysis failed to show associations between sPLA$_2$ enzyme activity and MVE.

Conclusions   Reducing sPLA$_2$-IIA mass is unlikely to be a useful therapeutic goal for preventing cardiovascular events. (J Am Coll Cardiol 2013;62:1966–76) © 2013 by the American College of Cardiology Foundation

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The secretory phospholipase A$_2$ (sPLA$_2$) enzymes, mostly comprising sPLA$_2$-IIA, sPLA$_2$-III, sPLA$_2$-V, and sPLA$_2$-X, hydrolyze phospholipids from the cell membrane surface and lipoproteins, producing pro-inflammatory lysophospholipids and eicosanoids (1). This activity may also modify low-density lipoprotein cholesterol (LDL-C) particles in the circulation increasing the binding of LDL-C onto blood vessel wall proteoglycans, promoting foam cell formation and the development of atherosclerosis (2). sPLA$_2$-IIA is thought to be the most highly expressed of the

sPLA$_2$ enzymes (2) and its mass can be quantified specifically in plasma by enzyme-linked immunosorbent assay (3). In contrast, the assay for sPLA$_2$ enzyme activity does not distinguish between the secretory isoenzymes IIA, III, V, and X (2).

Observational studies have indicated that higher circulating sPLA$_2$-IIA mass and sPLA$_2$ enzyme activity are associated with an increased risk of incident and recurrent MVE (comprising cardiovascular death, myocardial infarction [MI], and stroke) (3–6), with the evidence being more compelling in primary prevention (4) than in patients with ACS (7). This suggests that sPLA$_2$ isoenzymes, in particular IIA, may represent a novel therapeutic target for cardiovascular disease prevention. This hypothesis is supported by studies in mouse models that show over-expression of sPLA$_2$-IIA associates with increased atherosclerotic lesion size (8).

Despite these encouraging findings, mechanistic studies in animals may not faithfully model the disease process in humans, and observational studies in humans cannot provide

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definitive evidence on causation. This is because higher sPLA$_2$-IIA mass or sPLA$_2$ enzyme activity may be a consequence not a cause of atherosclerosis or reflect an association with causal risk factors. An evaluation of sPLA$_2$ as a therapeutic target is timely to help put into context the recent announcement that a phase III randomized trial (VISTA-16 [Vascular Inflammation Suppression to Treat Acute Coronary Syndrome for 16 Weeks] trial) (10) of varespladib, a first-in-class sPLA$_2$ inhibitor (9) for major vascular event (MVE) prevention in patients with acute coronary syndrome (ACS), was stopped prematurely for lack of efficacy (11). Varespladib was designed to selectively block sPLA$_2$-IIA, however, it also has minor inhibitory effects on other sPLA$_2$ isoenzymes (Online Fig. 1) (12,13).

Methods

A total of 109,179 individuals of European descent from 36 studies were included in the analysis (Online Table 1), comprising 19 in general populations and 10 studies in patients with ACS. In addition, we included 4 case control studies of coronary artery disease, 1 cohort of patients with established arterial vascular disease or risk factors for cardiovascular disease (SMART [Second Manifestations of

ARTerial disease] study), and 1 nested case-control study of coronary artery restenosis in patients with ACS undergoing percutaneous coronary intervention (GENDER [GENetic Determinants of Restenosis] study). These additional studies did not contribute toward the analyses set in general population or ACS studies, and were analyzed and reported separately (Online Appendix). Finally, tissue samples from 1 cohort of patients undergoing aortic valve surgery (ASAP [Advanced Study of Aortic Pathology]) were used to investigate the association of single-nucleotide polymorphisms (SNPs) in *PLA2G2A* with mRNA expression in liver, mammary artery, aorta, and heart with an external data source comprising 206 transplant donor liver samples used for replication (14). Approval from relevant ethical committees was obtained for collaborating studies. All analyses, unless otherwise stated, were performed using Stata 12.1 (StataCorp, College Station, Texas).

Measurement of sPLA₂-IIA mass and sPLA₂ enzyme activity. sPLA₂-IIA mass and sPLA₂ enzyme activity were measured in 7 and 6 of the collaborating studies, respectively (Online Table 2). Assay methods are reported in Online Table 3. Owing to the time of blood sampling being greater than 1 month after the acute coronary event, samples for the KAROLA (Langzeiterfolge der KARdiOLogischen Anschlussheilbehandlung) study were not included in the analysis. The distributions of both sPLA₂-IIA mass and sPLA₂ enzyme activity were skewed, hence the traits were natural log(e) transformed prior to analysis.

Observational analysis. We investigated correlations between log sPLA₂-IIA mass and log sPLA₂ enzyme activity in studies that measured both traits (Online Appendix, Online Fig. 2). To investigate the association between circulating sPLA₂-IIA mass and sPLA₂ enzyme activity with incident major vascular events in general populations, we used the European Prospective Investigation of Cancer (EPIC)-Norfolk study and to investigate the association with recurrent events in patients with ACS, we used 4 ACS cohorts (FAST-MI [French Registry of Acute ST-Elevation or Non–ST-elevation Myocardial Infarction], GRACE [Global Registry of Acute Coronary Events]-France, GRACE-Scotland, and MIRACL [Myocardial Ischemia Reduction with Acute Cholesterol Lowering] trial). For EPIC-Norfolk, the outcome was a composite of fatal and nonfatal MI, whereas for the 4 ACS cohorts, it was a composite of all-cause mortality or MI.

First, in the EPIC-Norfolk study we evaluated the cross-sectional correlates of sPLA₂-IIA and sPLA₂ enzyme activity with established and emerging risk factors using linear or logistic regression as appropriate. Second, we evaluated the shape of the association between sPLA₂-IIA mass and sPLA₂ enzyme activity with MI in the general population study, and with MI/all-cause mortality in the ACS cohorts. Third, we estimated the magnitude of the association per 1 log unit lower sPLA₂-IIA mass and sPLA₂ enzyme activity with MI or MI/all-cause mortality after statistical adjustment for potential confounders using logistic regression. Finally, we explored the independent effect of log sPLA₂-IIA mass and log sPLA₂ enzyme activity with MI or MI/all-cause mortality by fitting a logistic regression model that included both log sPLA₂-IIA mass, and log sPLA₂ enzyme activity in addition to potential confounders. The summary estimates were pooled across studies using fixed-effects meta-analysis. For full details of the observational analyses, please see the Online Appendix.

Genetic analysis. All studies apart from the MIRACL trial contributed toward the genetic analysis (Online Table 2). Genotype coding was arranged to be directionally consistent with the effect of varespladib on sPLA₂-IIA mass and sPLA₂ enzyme activity. Traits that were nonnormally distributed (sPLA₂-IIA mass, sPLA₂ enzyme activity, C-reactive protein, triglycerides, and interleukin-6) were log(e) transformed, and differences between genotype groups were reported as a percentage difference.

Selection of the genetic instrument and evaluation of its specificity and effect size. SNP SELECTION. Six tagging SNPs (15) that captured >90% of genetic variation in *PLA2G2A* in Europeans were evaluated in 3 studies (EPIC-Norfolk, GRACE-France, and UDACS [University College London Diabetes and Cardiovascular Study]). The rs11573156 variant that showed the lowest p value with sPLA₂-IIA mass and sPLA₂ enzyme activity (Online Fig. 3) was chosen for Mendelian randomization analysis. Rs11573156 was directly genotyped in all studies except 2 that imputed it and 1 that used a proxy SNP. Genotype frequencies were consistent across studies (Online Fig. 4) and did not deviate from Hardy-Weinberg equilibrium (at p < 0.001) (Online Table 4).

SPECIFICITY OF GENETIC INSTRUMENT FOR *PLA2G2A*. Affymetrix GeneChip Human Exon 1.0 ST expression arrays were used to quantify mRNA expression in the ASAP study, in which participants were genotyped using Illumina Human 610W-Quad Beadarray (including 101 SNPs in the region 200 kb upstream and downstream from the *PLA2G2A* locus). Please see the Online Appendix for further details of estimation of genotype association with mRNA expression.

STRENGTH OF GENETIC INSTRUMENT (RS11573156 C>G) ON SPLA₂. We estimated the per C allele association between *PLA2G2A* rs11573156 and sPLA₂ measures, as well as the proportion of variance (R^2) of these measures explained by the rs11573156 variant.

ASSOCIATION BETWEEN GENETIC INSTRUMENT AND PUTATIVE AND ESTABLISHED CARDIOVASCULAR RISK FACTORS. Twenty studies of individuals in which blood sampling occurred prior to the cardiovascular event were used to test the association of *PLA2G2A* rs11573156 (per C allele) with cardiovascular risk factors within each study using linear regression. Results were pooled using fixed (default) and random effects meta-analysis.

CARDIOVASCULAR OUTCOMES EXAMINED. For the general population studies, MVE were separated into prevalent and incident, whereas for ACS cohorts, all events after recruitment were included and labeled as recurrent.

Prevalent MVE were a composite of nonfatal MI and nonfatal stroke, and incident MVE were a composite of fatal/nonfatal MI and fatal/nonfatal stroke. For ACS cohorts, recurrent MVE were a composite of nonfatal MI, nonfatal stroke, and all-cause mortality. Individual components of MVE were also reported separately. See the Online Appendix for outcomes definitions per study and Online Table 5 for study contribution to the composite outcome.

ASSOCIATION BETWEEN GENETIC INSTRUMENT AND MVE. We conducted 2 genetic approaches: first, a genetic association analysis of the *PLA2G2A* rs11573156 variant with MVE, and; second, an instrumental variable analysis that quantified a causal effect per 1 log unit lower sPLA₂-IIA mass and sPLA₂ enzyme activity on MVE, under the assumptions of instrumental variable analysis (16). A total of 26 studies contributed to these 2 approaches, comprising 17 in general populations and 9 studies in patients with ACS.

For the genetic association approach, we estimated the within-study odds ratio (OR) per C allele of *PLA2G2A* rs11573156 with MVE using logistic regression and the results were pooled using fixed (default) and random-effects meta-analysis and used I^2 to measure heterogeneity. All meta-analyses were stratified by clinical setting (general population or patients with ACS).

For the instrumental variable analysis, we first applied the pooled estimate of the gene variant on log sPLA₂-IIA mass and log sPLA₂ enzyme activity to each study, including studies that did not have measures of sPLA₂-IIA mass or sPLA₂ enzyme activity (17). An instrumental variable estimate was then generated (taking into account the uncertainty in both the gene-sPLA₂ and gene-outcome associations) (18) for each study. The study-specific instrumental variable estimates were pooled using fixed-effects meta-analysis. Full details of the methodology are provided in the Online Appendix. We compared the instrumental variable estimates to the expected estimates based on the observational association between sPLA₂-IIA mass, sPLA₂ enzyme activity, and cardiovascular events.

Treatment trials of varespladib. In order to contextualize the effect of the genetic instrument with the sPLA₂ inhibitor (varespladib), we conducted a systematic review of RCTs (following PRISMA guidance) (19) to evaluate the effects of varespladib on sPLA₂-IIA mass and other cardiovascular traits. To investigate the dose response between varespladib and sPLA₂-IIA mass, we conducted a meta-regression analysis (for details, see the Online Appendix).

Results

Observational analysis of sPLA₂-IIA mass and sPLA₂ enzyme activity. Lower levels of sPLA₂-IIA mass and lower levels of sPLA₂ enzyme activity each were associated with a reduced risk of cardiovascular events in the general population with an OR for fatal/nonfatal MI of 0.67 (95% confidence interval [CI]: 0.59 to 0.75) and 0.30 (95% CI: 0.22 to 0.41) per 1 log unit lower sPLA₂-IIA mass and sPLA₂ enzyme activity, respectively, after adjustment for age and sex (Fig. 1). For

studies set in ACS, the corresponding summary ORs for all-cause mortality or MI were 0.93 (95% CI: 0.84 to 1.04) and 0.82 (95% CI: 0.69 to 0.98), respectively (Fig. 1). The log-linear model provided the best fit (p ≥ 0.1 for a quadratic model in all comparisons) (Online Table 6), indicating a constant proportional decrease in the relative odds per 1 log unit lower sPLA₂-IIA mass or sPLA₂ enzyme activity.

sPLA₂-IIA mass and sPLA₂ enzyme activity associated with several established and emerging cardiovascular risk factors in the general population (Online Tables 7 and 8). In general, adjustment for cardiovascular risk factors diminished the association between sPLA₂-IIA mass and sPLA₂ enzyme activity with incident MI in the general population, though the association persisted following multivariate adjustment (Fig. 1). Interestingly, both associations (sPLA₂-IIA mass and sPLA₂ enzyme activity with MI) remained after adjustment for one another. For ACS cohorts, only sPLA₂ enzyme activity was associated with recurrent events (Fig. 1).

Selection and validation of the genetic instrument for sPLA₂-IIA. rs11573156 C>G showed the lowest p value with sPLA₂-IIA mass (p = 5.49 × 10⁻¹⁸⁰) and sPLA₂ enzyme activity (p = 3.29 × 10⁻⁵) and was prioritized for analysis in the remaining studies (Online Fig. 3).

To evaluate the specificity of the rs11573156 variant for sPLA₂-IIA, we analyzed the association of SNPs in *PLA2G2A* with mRNA expression of 3 different sPLA₂ isoenzymes, encoded by distinct genes (*PLA2G2A* for sPLA₂-IIA and *PLA2G5* for sPLA₂-V in close proximity on chromosome 1, and *PLA2G10* for sPLA₂-X on chromosome 10). *PLA2G2A* was mainly expressed in the liver, aortic adventitia and heart (Online Fig. 5). The SNP showing strongest association with *PLA2G2A* mRNA expression in liver was rs10732279A>G (p = 8.71 × 10⁻¹⁹) (Fig. 2A), in strong linkage disequilibrium with rs11573156 (R^2 = 0.91 in Europeans, HapMap release 21) and explained 31% of the variance of *PLA2G2A* mRNA. These findings were replicated in an external data source comprising 206 transplant donor liver samples (p = 4.76 × 10⁻⁸) (14). rs10732279 showed no association with either *PLA2G5* or *PLA2G10* mRNA expression confirming the specificity of the genetic instrument for sPLA₂-IIA (Figs. 2B and 2C).

Association of rs11573156 with sPLA₂-IIA mass and sPLA₂ enzyme activity. In 3 studies of 1,400 individuals with ACS and 2 general population studies of 3,533 individuals, an allele dose-dependent association was observed between rs11573156 and sPLA₂-IIA mass and sPLA₂ enzyme activity (Fig. 3). For each additional C allele of rs11573156, sPLA₂-IIA mass was lower by 38% (95% CI: 36% to 40%) in studies of general populations and 44% (95% CI: 37% to 50%) in studies of ACS patients, compared with individuals homozygous for the G allele. The proportion of variance of sPLA₂-IIA mass explained by rs11573156 in general population and ACS studies was 21% (95% CI: 18% to 23%) and 6% (95% CI: 3% to 9%), respectively.

In contrast, the effect of the rs11573156 C allele on sPLA₂ enzyme activity was smaller and varied considerably by study

Figure 1　**Association of a 1 Log Unit Lower sPLA₂-IIA Mass or sPLA₂ Enzyme Activity With Fatal/Nonfatal Myocardial Infarction in General Population Studies and All-Cause Mortality/Myocardial Infarction in Acute Coronary Syndrome Studies**

The general population study was EPIC-Norfolk and the 4 acute coronary syndrome cohorts were FAST-MI (French Registry of Acute ST-Elevation or Non–ST-elevation Myocardial Infarction), GRACE (Global Registry of Acute Coronary Events)-France, GRACE-Scotland, and MIRACL (Myocardial Ischemia Reduction with Acute Cholesterol Lowering). In Model 1, only age and gender were introduced as covariates. We then additionally adjusted for covariates (blood pressure [BP], body mass index [BMI], type 2 diabetes [T2D]) that could confound the association between secretory phospholipase A₂ (sPLA₂) and coronary heart disease (CHD; Model 2). Because lipids may mediate the association between sPLA₂-IIA and CHD, we did not include lipids in Model 2, but included them in Model 3 (only available in the general population cohort). Finally, to investigate whether there was an independent association between sPLA₂-IIA mass **(orange)**, sPLA₂ enzyme activity **(blue)**, and CHD, we additionally included sPLA₂ enzyme activity where sPLA₂-IIA mass was the explanatory variable (and vice-versa; Model 4). CI = confidence interval; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; OR = odds ratio; TG = triglycerides.

setting, with a 3% reduction (95% CI: 1% to 5%) in studies of general populations and 23% reduction (95% CI: 19% to 27%) for studies of ACS patients. The proportion of variance of sPLA₂ enzyme activity explained by rs11573156 was 0.5% (95% CI: 0.0% to 1.0%) and 3% (95% CI: 1% to 5%) in the general population and ACS cohorts, respectively.

We identified no major associations between rs11573156 and blood pressure, lipid fractions, inflammation markers, or carotid intima-media thickness (Online Tables 9 to 11). **Comparison of pharmacological modification of sPLA₂ in randomized clinical trials and carriage of the PLA2G2A variant in populations.** We identified 4 randomized clinical trials (RCTs) of the sPLA₂ inhibitor varespladib in a total of 1,300 individuals (Online Fig. 6,

Online Table 12) (9,20–22). A meta-regression suggested varespladib treatment produced a dose-dependent reduction in sPLA₂-IIA mass (p for meta-regression = 0.06) (Online Fig. 7). The most frequently studied dose of varespladib (500 mg/day) reduced sPLA₂-IIA mass by 78% (95% CI: 62% to 94%). The effect of varespladib on sPLA₂ enzyme activity was not reported in RCTs because activity was reported to be beneath the lower limit of quantification of the assay (20–22). **Association between rs11573156 and MVE.** In a meta-analysis across 13 population studies (8,021 incident events in 56,359 individuals), there was no association between the C allele of rs11573156 and incident MVE (OR: 1.02; 95% CI: 0.98 to 1.06), nor with any of the individual components (Fig. 4, Online Fig. 8). Similarly, in 12 studies with 7,513
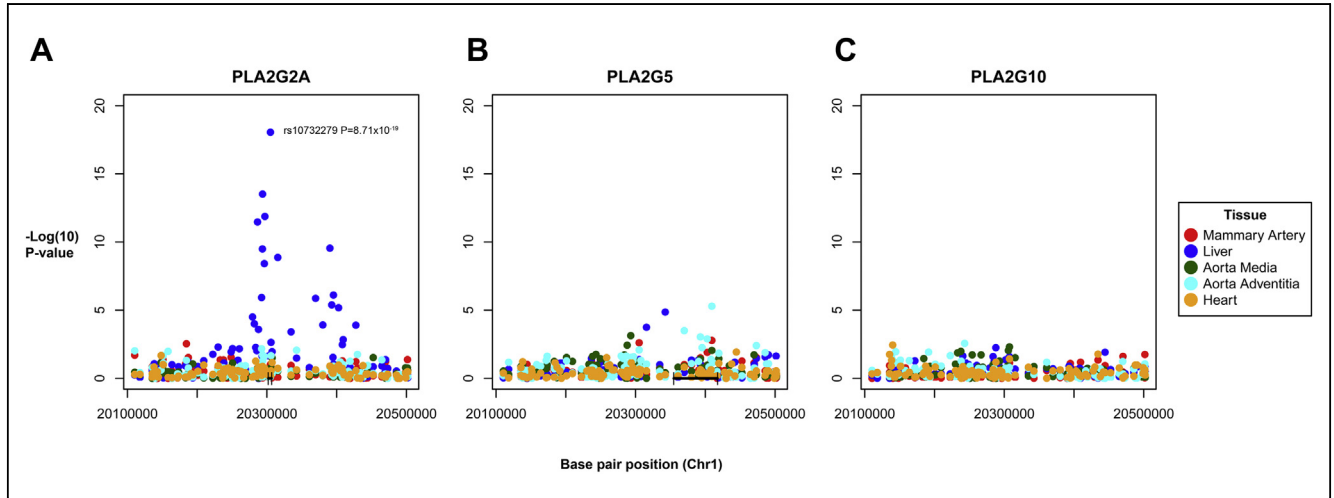
Figure 2 Association of SNPs in the *PLA2G2A* Region With mRNA Expression

Manhattan plots of single-nucleotide polymorphisms (SNPs) in the *PLA2G2A* region and association with mRNA expression of **(A)** *PLA2G2A*, **(B)** *PLA2G5*, and **(C)** *PLA2G10*, color-coded by tissue type. The p values for the association between rs10732279 and mRNA expression of *PLA2G5* or *PLA2G10* were 0.04 and 0.88, respectively.

prevalent events in 55,523 individuals, there was no association between the rs11573156 C allele with prevalent MVE (OR: 0.99; 95% CI: 0.95 to 1.03), or with any of the individual components (Fig. 4, Online Fig. 9). For the 9 ACS studies with 2,520 recurrent events in 15,768 patients, there was also no association between the C allele of rs11573156 and
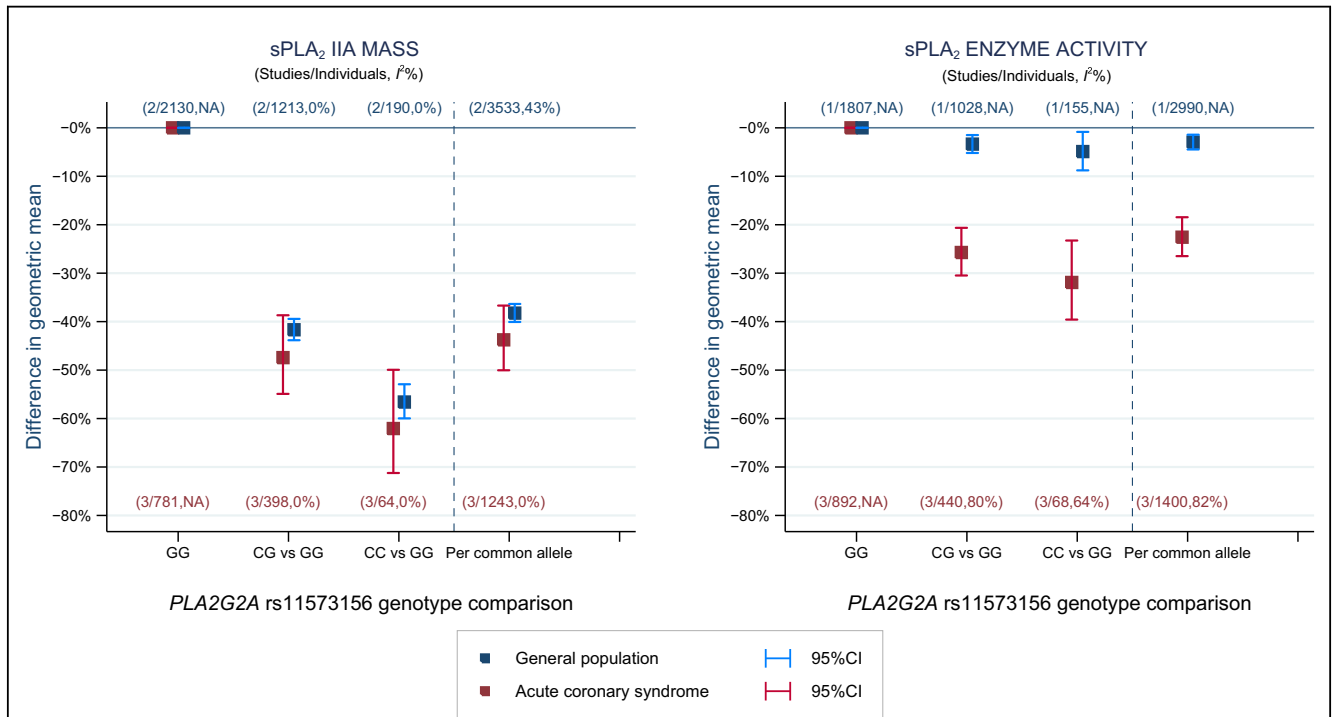
Figure 3 Meta-Analysis Pooled Estimates of the Association Between *PLA2G2A* rs11573156 With sPLA₂-IIA Mass and sPLA₂ Enzyme Activity

The analysis is separated by study setting into general populations (EPIC [European Prospective Investigation of Cancer]-Norfolk, UDACS [University College London Diabetes and Cardiovascular Study]; **blue**) and acute coronary syndrome (FAST-MI [French Registry of Acute ST-Elevation or Non–ST-elevation Myocardial Infarction], GRACE [Global Registry of Acute Coronary Events]-France, GRACE-Scotland; **red**). The percentage estimate was obtained by back-transforming the *PLA2G2A* rs11573156 log sPLA₂ association to obtain the relative difference, which was converted to a percentage by subtracting 1 from the relative difference and multiplying the fraction by 100. NA = not applicable either because there were too few studies (<3 studies) to synthesize an I^2 estimate, or the value could not be calculated for the reference genotype group (GG). sPLA₂ = secretory phospholipase A₂. The 3 genotype groups for the rs11573156 SNP are: 1) GG = reference group; 2) CG: 1 copy of the sPLA₂-lowering (common) C-allele; 3) CC: 2 copies of the sPLA₂-lowering C-allele.

Figure 4   **Meta-Analysis Pooled Estimates of the Association Between *PLA2G2A* rs11573156 and MVE (Including Individual Components) Stratified by Clinical Setting Into General Population and ACS Patients**

Each plot represents the *PLA2G2A* rs11573156 per C allele odds ratio, with genotype grouping arranged to mimic the effects of pharmacological lowering of secretory phospholipase A$_2$ (sPLA$_2$)-IIA (i.e., if lowering of sPLA$_2$-I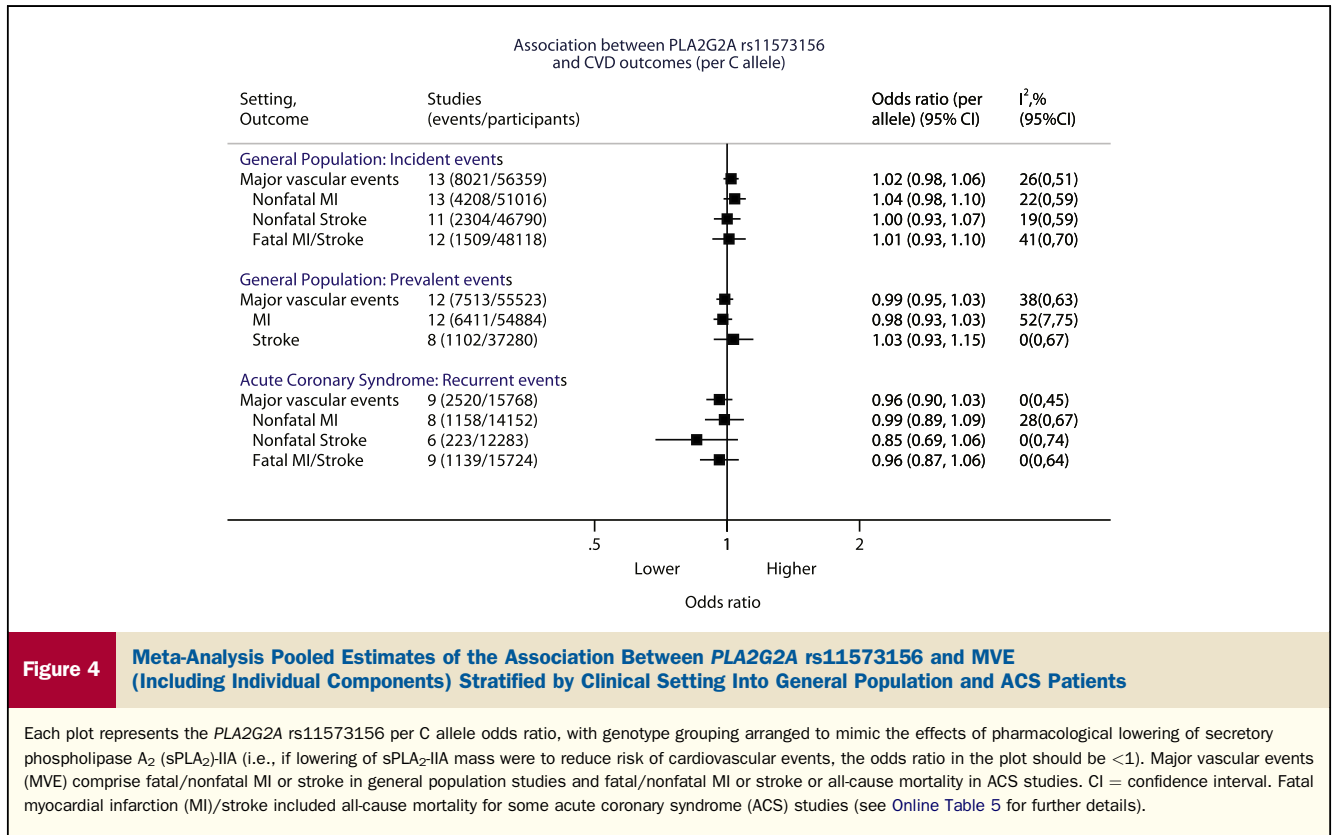IA mass were to reduce risk of cardiovascular events, the odds ratio in the plot should be <1). Major vascular events (MVE) comprise fatal/nonfatal MI or stroke in general population studies and fatal/nonfatal MI or stroke or all-cause mortality in ACS studies. CI = confidence interval. Fatal myocardial infarction (MI)/stroke included all-cause mortality for some acute coronary syndrome (ACS) studies (see Online Table 5 for further details).

recurrent MVE (OR: 0.96; 95% CI: 0.90 to 1.03) (Fig. 4, Online Fig. 10). Similar findings were obtained using a random-effects model for meta-analysis (Online Table 13). **Extreme genotype comparison.** Individuals homozygous for the rs11573156 C allele had a 57% to 62% lower sPLA$_2$-IIA mass compared with those homozygous for the G allele (Fig. 3), which was similar in magnitude to the 78% reduction seen with 500 mg/day varespladib dose used in VISTA-16. Using this genotype comparison, a null effect was again observed for MVE in all clinical settings: incident (5,175 cases; OR: 0.99; 95% CI: 0.89 to 1.10), prevalent (3,545 cases; OR: 1.00; 95% CI: 0.88 to 1.13), and recurrent (1,626 cases; OR 0.89; 95% CI: 0.74 to 1.06). **Instrumental variable analysis of sPLA$_2$ on MVE.** For the general population studies, instrumental variable analysis showed a null effect between sPLA$_2$-IIA mass and incident MVE (OR per 1 log unit lower sPLA$_2$-IIA mass: 1.04; 95% CI: 0.96 to 1.13) that was in contrast to the expected association based on observational analysis (OR: 0.69; 95% CI: 0.61 to 0.79). Similarly, for sPLA$_2$ enzyme activity, observational studies showed an OR of 0.50 (95% CI: 0.35 to 0.71), yet null associations were obtained for the instrumental variable estimates for sPLA$_2$ enzyme activity and incident MVE (OR: 1.87; 95% CI: 0.47 to 7.49), although the CIs were wide due to the weak effect of the rs11573156 variant on sPLA$_2$ enzyme activity in the general population.

For the ACS studies, the instrumental variable estimate for sPLA$_2$-IIA mass and recurrent MVE was also null (OR

per 1 log unit lower sPLA$_2$-IIA mass: 0.93; 95% CI: 0.83 to 1.05) and consistent with the observational estimate (OR: 0.92; 95% CI: 0.81 to 1.03). For sPLA$_2$ enzyme activity, no association was identified for the instrumental variable estimate with MVE (OR: 0.86, 95% CI: 0.66 to 1.12), which was similar to the observational estimate (OR: 0.77, 95% CI: 0.64 to 0.93) (Fig. 5).

Discussion

We used a genetic approach to judge the causal role of sPLA$_2$-IIA on MVE and, by extension, to evaluate if inhibition of sPLA$_2$-IIA represents a valid therapeutic target for cardiovascular prevention. We identified a SNP in *PLA2G2A* (rs11573156) that had a large and specific effect on circulating sPLA$_2$-IIA mass and a small-to-modest effect on sPLA$_2$ enzyme activity, but found no association between rs11573156 and incident, prevalent or recurrent MVE. This study provides evidence that the observational association between sPLA$_2$-IIA mass and MVE is likely due to residual confounding or reverse causality.

Our Mendelian randomization analysis used a single genetic instrument that had a remarkable effect on sPLA$_2$-IIA mass, explaining between 6% and 21% of its variance, a value several times higher than that observed for all genome wide association studies hits on blood pressure (1% for 29 SNPs combined) (23) and similar to that for LDL-C (~12% for 49 SNPs combined) (24). The strength of our
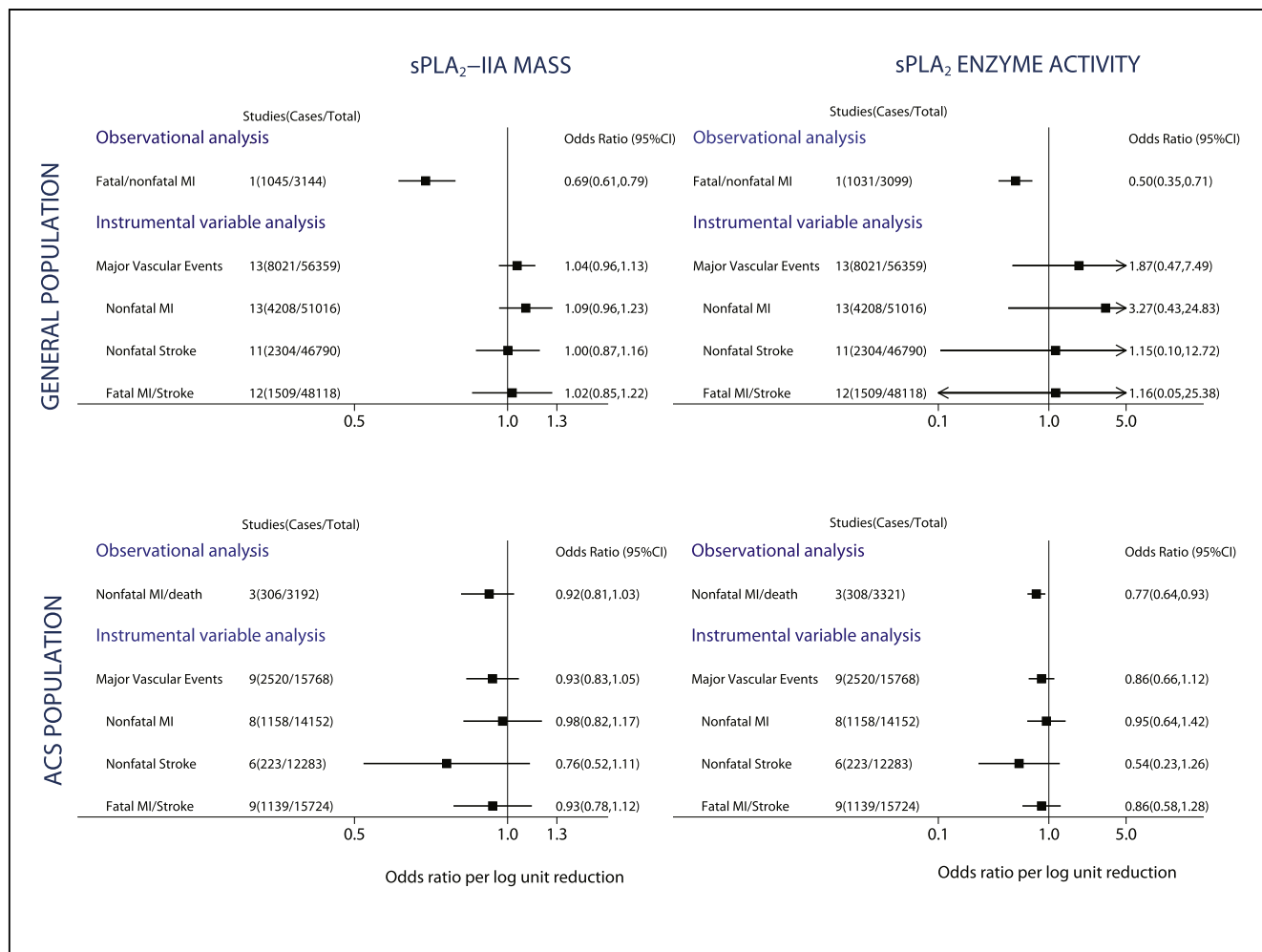
Figure 5   Observational and Instrumental Variable Estimates Per 1 Log Unit Lower sPLA₂-IIA Mass and sPLA₂ Enzyme Activity With Major Vascular Events

The observational analyses were adjusted for age, sex, BMI, BP, T2D, LDL-C, HDL-C, and TG in general population cohorts (corresponding to Model 3 in Fig. 1) and for age, sex, BMI, and BP in ACS cohorts (corresponding to Model 2 in Fig. 1). Abbreviations as in Figure 1.

genetic instrument together with the large sample size analyzed strongly support the instrumental variable estimates that indicate a null effect of sPLA₂-IIA mass with cardiovascular events.

The key SNP in our study (rs11573156) had a smaller impact on sPLA₂ enzyme activity than sPLA₂-IIA mass, in particular for general population studies. Because sPLA₂ enzyme activity is a composite of several sPLA₂ isoenzymes (2), it is not surprising that a *PLA2G2A* SNP (specific for sPLA₂-IIA) explained a smaller proportion of variance of sPLA₂ enzyme activity compared with sPLA₂-IIA mass.

While this manuscript was being prepared, a phase III RCT of varespladib (VISTA-16) (10) was halted for lack of efficacy (11). VISTA-16 was to enroll up to 6,500 patients with ACS and randomize them to 500 mg/day varespladib or placebo for 16 weeks with a primary combined endpoint of cardiovascular death, nonfatal MI, nonfatal stroke, or documented unstable angina. We hypothesize that the null

findings from our Mendelian randomization analysis may provide an eventual explanation for the lack of efficacy of varespladib in VISTA-16.

We did not find an association between lower sPLA₂-IIA mass and lower rates of recurrent MVE in ACS patients, unlike earlier reports (10). With CIs that span ORs from 0.81 to 1.03, we cannot rule out a false negative finding due to a limited number of events. Alternatively, initial studies often overestimate the effect of a biomarker on a health outcome, and as more evidence accrues, the magnitude of the effect may diminish and in some cases disappears, known as the "winner's curse."

Study limitations. First, we did not have data from a common set of participants with all 3 key variables: sPLA₂ measures, genetic information, and cardiovascular events. This is a common scenario with large-scale meta-analyses of Mendelian randomization studies that include novel biomarkers (25), but the instrumental variable approach helps overcome this problem. Second, given the impact of the SNP

on sPLA$_2$ enzyme activity was more modest than its effect on sPLA$_2$-IIA mass, our genetic analyses do not exclude a possible causal role of other isoforms such as sPLA$_2$-III, -V, and -X in cardiovascular disease. However, our genetic data do provide clear evidence against a causal role of sPLA$_2$-IIA mass in incident MVE in the general population. With regard to an ACS population, our analysis includes 2,520 recurrent MVE in patients with ACS, which is 6-fold greater than the 385 primary events that VISTA-16 intended to accrue (10). Furthermore, comparing individuals homozygous for the rs11573156 C allele to those homozygous for the G allele resulted in a reduction in sPLA$_2$-IIA mass similar to the effect of varespladib 500 mg/day and also showed no association with MVE.

Conclusions

Our large-scale Mendelian randomization analysis suggests that sPLA$_2$-IIA is unlikely to be a valid therapeutic target for prevention of cardiovascular events. The concordance of our genetic findings with the lack of efficacy of the phase III varespladib trial supports the wider use of this type of genetic approach earlier in drug development to prioritize which drug targets to take through to RCTs.

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▶ **APPENDIX**

For an expanded methods and results sections and supplemental figures and tables, please see the online version of this article.