Deciphering the Causal Role of sPLA2s and Lp-PLA2 in Coronary Heart Disease

Philippa J. Talmud, Michael V. Holmes

Abstract—Over the last 10 to 15 years, animal and human observational studies have identified elevated levels of both proinflammatory secretory phospholipase A2-IIA and lipoprotein-associated phospholipase A2 as potential risk factors for coronary heart disease. However, Mendelian randomization, a genetic tool to test causality of a biomarker, and phase III randomized controlled trials of inhibitors of these enzymes (varespladib and darapladib) converged to indicate that elevated levels are unlikely to be themselves causal of coronary heart disease and that inhibition had little or no clinical utility. The concordance of findings from Mendelian randomization and clinical trials suggests that for these 2 drugs, and for other novel biomarkers in future, validation of potential therapeutic targets by genetic studies (such as Mendelian randomization) before embarking on costly phase III randomized controlled trials could increase efficiency and offset the high risk of drug development, thereby facilitating discovery of new therapeutics and mitigating against the exuberant costs of drug development. (Arterioscler Thromb Vasc Biol. 2015;35:2281-2289. DOI: 10.1161/ATVBAHA.115.305234.)

Key Words: darapladib ■ lipoprotein-associated phospholipase A2 ■ Mendelian randomization ■ secretory phospholipase-IIA ■ varespladib

Despite the rising burden of coronary heart disease (CHD) worldwide, the rate of novel drug development to treat at-risk individuals has remained stagnant over the past 20 years.1 In conjunction with an overall ≈90% failure rate of drugs,2 which impacts directly the rising cost of drug development, this is creating a perfect storm that is threatening to make the high risk of drug development, thereby facilitating discovery of new therapeutics and mitigating against the exuberant costs of drug development.

PLA2 Super Family

Phospholipids are the major component of cell membranes, as well as the outer bilayer of lipoprotein particles, and comprise ≈40% of the lipidome.4 The fatty acid variation and different phospholipid head groups, phosphatidylcholine (PC), phosphatidylserine, or phosphatidyl ethanolamine, make them heterogeneous. The phospholipases, enzymes which hydrolyse phospholipids, are defined by their molecular weight, their catalytic residues, and their (in)dependence on calcium. It is outside the scope of this review to consider all phospholipases, and the reader is directed to several excellent reviews which detail the characterization of these enzymes.5-10

The A2 group of phospholipases (PLA2s) specifically hydrolyse the fatty acid at the sn-2, or second carbon positions, on the glycerol backbone of the phospholipids and, in doing so, release both a fatty acid, which can act as a second messenger signaling molecule in downstream processes, and lysophospholipid (Figure 2A). An early indication that PLA2s may be implicated in atherogenesis was the identification of a depletion of phospholipid and the concomitant increase of lysophospholipids in the arterial intima of atherosclerotic plaques.11,12 We have focussed on 2 subgroups of the PLA2 family (Table 1), which have recently been targeted for therapeutic intervention: 3 of the secretory PLA2s (sPLA2-IIA, sPLA2-V, and sPLA2-X) and the lipoprotein-associated PLA2s...
(Lp-PLA2), all of which have been reported to be associated with atherothrombotic disease from animal and human observational studies. The predicted efficacy of their inhibition on the development of atherosclerosis and CHD has been tested using the genetic approach of MR (see Figure 1 for a description of MR and comparison to conventional RCT), and this, together with the outcome of the drug trials, is reviewed.

**Secretory PLA2s and Atherogenesis**

The sPLA2 enzymes are the largest group of this family of enzymes, representing around 30% of all known phospholipases. They are small calcium-dependent, disulfide-rich enzymes with molecular weights ranging between ≈14 and 16 kDa, with the exception of sPLA2-III, which has a molecular weight of ≈55 kDa. The reason for the biological requirement for so many sPLA2s may arise because of their specificity for different phospholipid substrates, as well as their cellular and tissue-specific diversity.

The purported impact of sPLA2s on inflammatory diseases, such as atherosclerosis and rheumatoid arthritis, stems from their enzymatic action, generating proinflammatory biolipid mediators (Figure 2B). Of the sPLA2s, sPLA2-IIA, sPLA2-IIA-V, and sPLA2-X have been identified in atherosclerotic plaques by immunohistochemistry. The ability of sPLA2s to promote atherosclerosis is suggested to reflect their action on the phospholipid bilayer of low-density lipoprotein (LDL) particles, generating proatherogenic, small dense LDL with 50% less surface phospholipids. This results in a conformational change in the apolipoprotein B on the LDL particle, exposing proteoglycan-binding sites. This leads to the aggregation and retention of the modified lipoprotein by proteoglycans in the intima, where they are prone to oxidation and subsequent uptake by macrophages, generating foam cells.

In the intima of the vessel wall, phospholipase hydrolysis continues, producing more bioactive nonesterified fatty acids and lysophospholipids. sPLA2-V, with high affinity for cell membrane PC, has the potential to act extracellularly, whereas sPLA2-IIA has low affinity for native phospholipids and higher affinity for oxidised phospholipids and is active within the intima and macrophages. By contrast, sPLA2-X, under physiological conditions, is inactive and absent from healthy arteries, but is activated in proinflammatory conditions and also has high affinity for PC. Thus, the histological and in vitro studies suggest that high levels of these sPLA2s are likely to influence both the initial and the later stages of the development of the atherosclerotic plaque, suggesting that lowering levels of these enzymes would be beneficial.

**Overexpression and Knockout Animal Models of sPLA2 Support Their Atherogenic Role**

Knockout and transgenic animal models of these sPLA2s present with different phenotypes, reinforcing the notion that the sPLA2 isoenzymes have unique specification. The C57BL/6 mouse is a natural knockout model for sPLA2-IIA because of a frame shift mutation in exon 3. The PL2G2A human transgenic mouse had 8-fold elevated expression of sPLA2-IIA and developed atherosclerosis even on standard chow. Mice carrying the Pla2g5 transgene by retroviral transfer on an Ldlr−/− background had a 2.7-fold increase in aortic arch atherosclerosis, whereas the double Ldlr−/− Pla2g5−/− mice had 36% reduced atherosclerosis. By contrast, although sPLA2-X is present in atherosclerotic lesions, animal models

![Figure 1. Comparison of conventional randomized controlled trial (RCT) to Mendelian randomization. A, A conventional RCT where individuals are randomized to an intervention or comparator group. The randomization process means that individuals in the 2 groups should be similar to one another in all regards other than one group that receives the intervention and the other the placebo; this randomization obviates confounding. That the intervention precedes disease mitigates reverse causality. For these reasons, appropriately conducted RCTs abolish 2 fundamental sources of bias that affect observational studies: confounding and reverse causality. B, Mendelian randomization. If a genetic variant modifies a biomarker, and certain other assumptions are met (see Lawlor et al), grouping individuals in the population by their genotype should mean that individuals in the 2 groups are similar in all respects apart from the difference in the biomarker encoded by the genetic variant—that is, exactly the same process that abolishes confounding in a conventional RCT. This arises because of Mendel’s second law, where genotype is allocated independent of potential confounders. Because genotype cannot be modified by disease status, reverse causality is also removed. Thus, just like RCTs, appropriately conducted Mendelian randomization studies can provide causal estimates that are free from confounding and reverse causality. RCTs typically use interventions with large effects on biomarkers over short periods of time (months-years). In contrast, Mendelian randomization studies use genetic variants with typically small effects, but because randomization occurs at conception, it encodes lifetime differences in biomarkers or exposures. CHD indicates coronary heart disease; Lp-PLA, lipoprotein-associated PLA2; PLA2, phospholipase A2; and sPLA, secretory phospholipase A2.

**Figure 1.** Comparison of conventional randomized controlled trial (RCT) to Mendelian randomization. A, A conventional RCT where individuals are randomized to an intervention or comparator group. The randomization process means that individuals in the 2 groups should be similar to one another in all regards other than one group that receives the intervention and the other the placebo; this randomization obviates confounding. That the intervention precedes disease mitigates reverse causality. For these reasons, appropriately conducted RCTs abolish 2 fundamental sources of bias that affect observational studies: confounding and reverse causality. B, Mendelian randomization. If a genetic variant modifies a biomarker, and certain other assumptions are met (see Lawlor et al), grouping individuals in the population by their genotype should mean that individuals in the 2 groups are similar in all respects apart from the difference in the biomarker encoded by the genetic variant—that is, exactly the same process that abolishes confounding in a conventional RCT. This arises because of Mendel’s second law, where genotype is allocated independent of potential confounders. Because genotype cannot be modified by disease status, reverse causality is also removed. Thus, just like RCTs, appropriately conducted Mendelian randomization studies can provide causal estimates that are free from confounding and reverse causality. RCTs typically use interventions with large effects on biomarkers over short periods of time (months-years). In contrast, Mendelian randomization studies use genetic variants with typically small effects, but because randomization occurs at conception, it encodes lifetime differences in biomarkers or exposures. CHD indicates coronary heart disease; Lp-PLA, lipoprotein-associated PLA2; PLA2, phospholipase A2; and sPLA, secretory phospholipase A2.
suggest that it might be anti-inflammatory. Although transgenic Pla2g10 mice die neonatally from lung pathologies, macrophage-specific expression of Pla2g10 inhibits macrophage activation and inflammatory responses. Bone marrow transplantation into Ldlr−/− mice from Pla2g10 mice showed significant reduction of macrophage response and lesion size. Overall, these mouse studies support the view that high levels of these sPLA2 enzymes are proatherogenic, with the caveat that these are mouse models and they may not be directly extrapolatable to the human situation.

### Measuring sPLA2s

For a biomarker to be of use in the clinical setting, a reliable assay must be available. Of these 3 sPLA2 enzymes, only

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**Table 1. Chromosomal Location, Tissue-Specific Expression, Substrate Specificity, and Inhibitory Drugs for sPLA2-IIA-V, -X, and Lp-PLA2**

<table>
<thead>
<tr>
<th>Major PLA2 Subgroups</th>
<th>Isoforms</th>
<th>Gene</th>
<th>Chromosome Location</th>
<th>Variant Used in MR</th>
<th>Enzymatic Active Site and Protein Size</th>
<th>Tissue-Specific Expression</th>
<th>Phospholipid Substrate Specificity</th>
<th>Inhibitory Drug and IC50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Secretory phospholipase A2</td>
<td>sPLA2-IIA</td>
<td>PLA2G2A</td>
<td>Chr 1p34-36</td>
<td>rs11573156C&gt;G; MAF 0.23</td>
<td>His-Asp; Ca2+-dependent; 13–15 kDa</td>
<td>SMC in intima and media, adventitia macrophage,</td>
<td>PE, PS, and oxidised lipoproteins</td>
<td>Varespladib 9–14 nM</td>
</tr>
<tr>
<td></td>
<td>sPLA2-V</td>
<td>PLA2G5</td>
<td>Chr 1p34-36</td>
<td>rs525380; C&gt;A; MAF 0.44</td>
<td>His-Asp; Ca2+-dependent; 13–15 kDa</td>
<td>SMC in media but not adventitia</td>
<td>PC on HDL and LDL. Prefers oleic acid species</td>
<td>Varespladib 77 nM</td>
</tr>
<tr>
<td></td>
<td>sPLA2-X</td>
<td>PLA2G10</td>
<td>Chr 16p12-13</td>
<td>rs4003228; C&gt;T; R38C; MAF 0.02</td>
<td>His-Asp; Ca2+-dependent; 13–15 kDa</td>
<td>Not present in normal arteries</td>
<td>PC on LDL and HDL. Prefers AA species</td>
<td>Varespladib 15 nM</td>
</tr>
</tbody>
</table>

AA indicates arachidonic acid; HDL, high-density lipoprotein; IC50, half maximum inhibitory concentration; LDL, low-density lipoprotein; Lp-PLA2, lipoprotein-associated PLA2; MAF, minor allele frequency; MR, Mendelian randomization; PAF, platelet activating factor; PC, phosphatidyl choline; PE, phosphatidyl ethanolamine; PLA2, phospholipase A2; PS, phosphatidylserine; and SMC, smooth muscle cells.
sPLA2-IIA levels can be measured with a commercially available ELISA. No assay is available, as yet, to measure sPLA2-V or sPLA2-X levels. Serum sPLA2 activity, measured by a selective fluorometric assay, shows only a weak correlation with sPLA2 type IIA levels ($r=0.20$), supporting the idea that sPLA2 activity measures are a composite of several sPLA2, including possibly sPLA2-IIA, sPLA2-V, and sPLA2-X.

sPLA2-IIA and Cardiovascular Disease

Observational studies have indicated that higher circulating sPLA2-IIA levels and sPLA2 activity are associated with increased risk of incident and recurrent cardiovascular events (cardiovascular death, myocardial infarction [MI], and stroke), but these do not provide evidence of causation. Prospective cohorts reported that individuals with higher sPLA2-IIA levels had an elevated risk of incident disease, with effect estimates ranging from odds ratio (OR) 1.34 in the general population (comparing top quartile of sPLA2-IIA to bottom quartile) to OR 1.49 to 5.08 for individuals with preexisting vascular disease (although the exposure categories were slightly different). In a meta-analysis of individual participant data, we identified a 1-log increase in sPLA2-IIA to associate with an OR of 1.45 for incident fatal/nonfatal MI, but for patients with preexisting vascular disease, the corresponding estimate for recurrent MI was OR 1.09.

Lipoprotein-Associated Phospholipase, Lp-PLA2 (PLA2-GVII), and In Vitro and In Vivo Effects

Lp-PLA2 is able to bind to 2 distinct domains of apolipoprotein B, and it circulates in the blood associated with both LDL37 and Lp(a),38 as well as binding to apolipoprotein AI on high-density lipoprotein.39 It is secreted by monocytes and macrophages, mast cells and T-lymphocytes, with a favored substrate of oxidized PC produced during the oxidation of LDL and Lp(a), generating the soluble proinflammatory lipid mediators, lyso-PC and oxidized nonesterified fatty acids. Lp-PLA2 can also hydrolyze platelet activating factor, and, therefore, is also known as platelet activating factor-acetylhydrolase (Figure 2B). Since platelet activating factor is proinflammatory, this suggests that lowering it might have anti-inflammatory and antithrombotic activity.40 These opposing pro- and antiatherogenic properties of Lp-PLA2 have been demonstrated both in human and animal models. Lp-PLA2 is expressed by macrophages in human and rabbit lesions.41 In vitro, specific inhibition of Lp-PLA2 reduced the cytotoxic effects of oxidised LDL on monocyte–macrophages.42 In contrast, adenoviral overexpression of Lp-PLA2 in Apoe$^{-/-}$ mice led to reduced endothelial damage and spontaneous atherosclerosis,43 along with a protection of plasma lipoproteins from oxidation,44 promoting an antiatherogenic role.

Measuring Lp-PLA2 and Risk of Cardiovascular Disease

Both commercial radiometric and colorimetric assays are available to measure Lp-PLA2 activity, which correlates moderately well with Lp-PLA2 levels ($r=0.5$), measured by ELISA or commercial immunoassay.45 The first study to identify Lp-PLA2 as an independent risk marker for CHD was the West of Scotland Study.46 The Lp-PLA2 Studies Collaboration that analyzed 32 prospective studies with over 79,000 participants and confirmed the association of higher Lp-PLA2 measures with risk of CHD.45 A 1 SD increment in Lp-PLA2 mass and activity was associated with a relative risk (95% confidence interval) of 1.10 (1.05–1.16) and 1.11 (1.07–1.16) for CHD, respectively.45

Varespladib and Darapladib: Inhibitors of sPLA2-IIA and Lp-PLA2

Varespladib, a pan-sPLA2 inhibitor, targets the catalytic dyad that is unique to sPLA2s, providing a pharmacodynamic target specific to sPLA2s.47 Although initially designed to inhibit just the IIA isofrom of sPLA, the drug was subsequently found to have inhibitory effects on sPLA2-V and sPLA2-X.47 The IC$_{50}$ (half maximum inhibitory concentration) value for

![Figure 3.](http://journals.ahajournals.org/doi/abs/10.1161/atvbaha.115.303022)
sPLA2-IIA is around 10 nM, whereas the values are 77 and 15 nM for sPLA2-V and sPLA2-X, respectively (Figure 3).54 Phase II dose-finding trial of varespladib showed a positive dose–response relationship,36,49 with a 500 mg/d dose of varespladib reducing sPLA2-IIA by ≈80%,46 and this did not report any adverse effects.50

Darapladib, a substituted pyrimidine, is a freely reversible, noncovalently bound inhibitor of Lp-PLA2 with an IC50 of 5 nM (Figure 3). Darapladib is relatively specific for Lp-PLA2, with no inhibitory action identified for sPLA2-IIA and with only weak effects on sPLA2-V.31 Phase II RCTs showed that 40 and 80 mg/d of darapladib was efficacious at reducing plasma Lp-PLA2 activity by 57% and 82%, respectively, and was well tolerated.52

Use of MR to Test Causality of sPLA2 and Lp-PLA2
A large LR study of sPLA2-IIA, including over 93,000 individuals of European descent from 29 studies, used a functional genetic variant (rs11573156C>G in PLA2G2A)55 that explained 21% of circulating sPLA2-IIA levels (see Table 2).56 Individuals carrying one copy of the C allele had 38% lower circulating levels of sPLA2-IIA, and those carrying 2 copies had ≈60% lower sPLA2-IIA values, similar to values obtained from 500 mg/d of varespladib, the dose used in clinical trials. However, despite use of a functional genetic variant with a strong association with sPLA2-IIA levels, and specific to the -IIA isoform, meta-analysis did not show evidence of a causal effect of sPLA2-IIA on risk of CHD.36 In pooled analysis from 13 studies with 8021 incident and 7513 prevalent CHD events, the OR (95% confidence interval) per C allele of rs11573156 was 1.02 (0.98–1.06) and 0.99 (0.95–1.03) for incident and prevalent major vascular events. In instrumental variable analysis, the OR per 1-log reduction in sPLA2-IIA was 1.04 (0.96–1.13), which was statistically different to the observational estimate of 0.69 (0.61–0.79) for the same reduction in sPLA2-IIA, showing strong divergence of findings from the observational and genetic (ie, causal) analyses.56 These important findings, interrogating whether sPLA2-IIA was causal in CHD pathogenesis, were published while the VISTA-16 RCT was ongoing and cast serious doubt on whether the trial would show efficacy of varespladib.

For sPLA2-V and sPLA2-X, 2 further MR studies were conducted.59,60 The sPLA2-V MR used a novel approach given the lack of available assays to directly quantify sPLA2-V levels. In contrast, we used expression data to select the single nucleotide polymorphism most strongly associated with PLA2G5 gene expression.59 This approach, although making certain assumptions, was validated by our prior sPLA2-IIA

### Table 2. Mendelian Randomization and Randomized Controlled Trials of sPLA2s and LpPLAs

<table>
<thead>
<tr>
<th>Study name/ID</th>
<th>Mendelian Randomization Studies</th>
<th>Randomized Controlled Trials</th>
<th>Mendelian Randomization Studies</th>
<th>Randomized Controlled Trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population</td>
<td>Europeans; healthy or those with recent ACS</td>
<td>VISTA-1664</td>
<td>Mixed ethnicity (88% White, 9% Asian, 2% Black, 1% other); recent ACS</td>
<td>Casas et al45</td>
</tr>
<tr>
<td>Events/total sample size</td>
<td>18054/109179</td>
<td>5145</td>
<td>Up to 10494 events in 26118 individuals</td>
<td>1533 CHD cases in 8564 Europeans and 2386 black</td>
</tr>
<tr>
<td>Intervention and dose (SNP or drug)</td>
<td>PLA2G2A rs11573156; 38% lower sPLA2-IIA per effect allele</td>
<td>Varespladib 500 mg/d; 78% lower sPLA2-IIA*</td>
<td>PLA2G7 rs1051931; 3% difference in Lp-PLA2 per effect allele</td>
<td>PLA2G7 rs14002965; carriers have 2.8-fold difference in Lp-PLA2 in Europeans and 1.8-fold difference in black</td>
</tr>
<tr>
<td>Primary outcome</td>
<td>Fatal/nonfatal MI and fatal/nonfatal stroke (+all-cause mortality for studies in established CAD)</td>
<td>Cardiovascular mortality, nonfatal MI, nonfatal stroke, or unstable angina with evidence of ischemia requiring hospitalization</td>
<td>CHD and angiographic coronary artery disease</td>
<td>Incident CHD</td>
</tr>
<tr>
<td>Result</td>
<td>OR 1.00 (95% CI, 0.07, 1.03) per C-allele</td>
<td>HR 1.25; 95% CI, 0.97–1.61 varespladib vs placebo</td>
<td>OR 0.96; 95% CI, 0.87–1.06 per effect allele</td>
<td>HR 1.06; 95% CI, 0.33–2.45 for Europeans and HR 0.92; 95% CI, 0.35–1.49 for black</td>
</tr>
<tr>
<td>Concordance?</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

ACS indicates acute coronary syndrome; CAD, coronary artery disease; CHD, coronary heart disease; CI, confidence interval; HR, hazard ratio; Lp-PLA2, lipoprotein-associated PLA2; MI, myocardial infarction; OR, odds ratio; sPLA2, secretory phospholipase A2; SNP, single nucleotide polymorphism; SOLID-TIMI, Stabilization of plaque using Darapladib-Thrombosis in Myocardial Infarct; sPLA2, secretory phospholipase A2; STABILITY, Stabilization of atherosclerotic plaque by initiation of Darapladib therapy; and VISTA-16, Vascular Inflammation suppression to treat acute coronary syndrome in 16 weeks.

MR study because the same genetic variant that showed strongest association with plasma levels of sPLA2-IIA showed the strongest association with PLA2G2A expression. For sPLA2-V, we investigated the association of the genetic variant in a pooled analysis of 27,230 CHD events and 70,500 controls. Our data did not find evidence in support of sPLA2-V being causally implicated in CHD. Furthermore, a smaller-scale genetic analysis of sPLA2-X also suggested no strong evidence of association of the -X isoform with risk of CHD. Taken together, these MR studies put into question whether sPLA2s play anything other than a bystander (or reverse causality) effect. This is supported by the wide range of cardiovascular traits with which sPLA2-IIA levels and sPLA2 activity associate, making confounding a likely scenario.

For Lp-PLA2, an MR study that used 7 genetic variants in PLA2G7 with a total of 10,494 CHD cases and 15,624 controls failed to identify evidence of a causal relationship of Lp-PLA2 on risk of CHD (Table 2). Although the genetic variant with the strongest association on Lp-PLA2 (rs1051931) only reduced Lp-PLA2 by 3% per minor allele, the study was adequately powered to detect a per-allele OR of 1.2 at \( P = 0.001 \), and variants in PLA2G7 were not related to risk of CHD or CHD events (OR 0.98 [0.82–1.17]).

In contrast, a subsequent study using a loss of function variant (PLA2G7 279F) in Asian males found some evidence of a protective effect; however, inconsistent findings were observed in women, where the variant was not associated with CHD.

A more recent MR study used sequencing to identify a loss-of-function variant (rs140020965; Q287X) that associated with Lp-PLA2 at \( P < 2.64 \times 10^{-6} \). Carriers of the loss-of-function variant had almost 3-fold lower levels of Lp-PLA2 activity than the noncarriers, but no difference in risk of incident CHD was found in whites (hazard ratio [HR] 1.06 [0.33–2.45]; \( P = 0.93 \)). Similar findings were seen in blacks where the HR was 0.92 (0.35–1.49; \( P = 0.78 \)).

## Drug Trial Outcomes of Varespladib and Darapladib

To test whether inhibition of sPLA2-IIA reduced CHD, vascular inflammation suppression to treat acute coronary syndrome for 16 weeks (VISTA-16), a phase III RCT, was initiated. VISTA-16 randomized 5189 patients with acute coronary syndrome to either varespladib (500 mg/d) or placebo with follow-up for 16 weeks. The primary outcome was a composite of cardiovascular death, nonfatal MI, nonfatal stroke, or documented unstable angina with objective evidence of ischemia requiring hospitalization.

At the prespecified interim analysis, the independent data and safety monitoring board reviewed evidence, including 245 primary events, in 5012 randomized patients. Analyses showed no effect of varespladib on the primary outcome (HR 1.25 [0.97–1.61; \( P = 0.08 \)], comparing varespladib to placebo).

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### Figure 4

**A**, Meta-analysis of randomized studies investigating the causality of secretory phospholipase A2 (sPLA2)-IIA in coronary heart disease (CHD). Fixed-effects meta-analysis. **B**, Meta-analysis of SOLID-TIMI 52 and STABILITY trials. Outcome is a composite of cardiovascular death, MI, or stroke. Fixed-effects meta-analysis. CI indicates confidence interval; HR, hazard ratio; MI, myocardial infarction; MR, Mendelian randomization; RR, relative risk; SOLID-TIMI 52; Stabilization of plaque using Darapladib—Thrombosis in Myocardial Infarction; STABILITY, Stabilization of atherosclerotic plaque by initiation of Darapladib therapy; and VISTA-16, vascular inflammation suppression to treat acute coronary syndrome for 16 weeks.
and a statistically significant increase in risk of the secondary outcome MI (HR 1.66 [1.16–2.39; P=0.005]). Because of these findings, the trial was terminated and further assessments of varespladib for CHD prevention were abandoned.

It is opportune to compare and contrast estimates from the MR analysis that used the extreme genotype comparison (PLA2G2A rs11573156 CC versus GG), resulting in 60% lower sPLA2-IIA levels, to those of the VISTA-16 trial, which used 500 mg/d varespladib and resulted in ≈78% lower sPLA2-IIA (Table 2). As can be seen in Figure 4A, the genetic subset had severalfold more events than VISTA-16 (10 346 in the extreme genotype comparison versus 245 in the phase III RCT) with a similar effect on sPLA2-IIA levels. When we meta-analyze both sources of evidence (permissible because both are randomized designs [see Figure 1], with roughly similar effects on the intermediate phenotype, sPLA2-IIA), the summary estimate is a relative risk of 1.00 (0.93, 1.07; Figure 4A). Although this relies on prior data showing varespladib to predominantly inhibit the -IIA isoform of sPLA2 and makes certain assumptions, such as no off-target effects of varespladib, these data provide compelling evidence that sPLA2-IIA is unlikely to play a causal role in development of CHD and, therefore, is not a suitable therapeutic target for CHD prevention.

To assess therapeutic modification of Lp-PLA2, there were 2 phase III RCTs, SOLID-TIMI 5257 and STABILITY.58 SOLID-TIMI 52 randomized 13 026 patients with recent acute coronary syndrome to once-daily darapladib (160 mg) or placebo with follow-up for 2.5 years. The incidence of the primary end point, a composite of CHD death, MI, or urgent revascularization for myocardial ischemia, did not differ according to treatment allocation (HR, 1.00 [0.91–1.09; Figure 4A]). Although this relies on prior data showing varespladib to predominantly inhibit the -IIA isoform of sPLA2 and makes certain assumptions, such as no off-target effects of varespladib, these data provide compelling evidence that sPLA2-IIA is unlikely to play a causal role in development of CHD and, therefore, is not a suitable therapeutic target for CHD prevention.

In STABILITY, 15 828 patients with stable CHD received 160 mg/d darapladib or placebo and were followed-up for a median of 3.7 years. The primary outcome (cardiovascular death, MI, or stroke) was similar between the 2 groups (HR in the darapladib group, 0.94 [0.85–1.03]; P=0.20).58 Pooling results from the 2 trials together, with >3250 major vascular events (comprising CV death, MI or stroke), showed that darapladib had no convincing effect on risk of CHD (HR 0.96, [0.90–1.03]; Figure 4B). These are compared with the PLA2G7 MRs in Table 2.

Conclusions
We have reviewed the evidence base for sPLA2s and Lp-PLA2 in CHD. Despite strong biological plausibility and compelling evidence from multiple observational studies, MR studies failed to show evidence of causation, which was borne out in the RCTs. Where possible, use of MR before embarking on expensive, high-risk phase III RCTs could help reduce late-stage attrition caused by clinical futility and separate on- from off-target adverse effects. This could offset the high risk of drug development for CHD and mitigate costs. Ultimately, this should facilitate the identification of which novel biomarkers are most likely to be causally implicated in the pathogenesis of CHD and shed light on valid therapeutic targets to reduce residual cardiovascular risk.


Apart from the example of low-density lipoprotein cholesterol and blood pressure, selecting from conventional coronary heart disease risk factors as being causal mediators of coronary heart disease, and thus useful targets for the development of new drugs, has not been hugely successful. Although animal studies and human observational studies identify the proinflammatory phospholipase A2s as potential risk factors for coronary heart disease, this has not been supported by the genetic tool Mendelian randomization or the phase III randomized controlled trials of the phospholipase A2 inhibitors, varespladib and darapladib. The convergence of Mendelian randomization with randomized controlled trials strongly supports the use of Mendelian randomization before embarking on phase III controlled trials, thereby making it possible for the pharmaceutical industry to pre-empt the outcome of pharmacological modification of a therapeutic target, offset risk, and save billions of dollars.
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