Novel Genetic Approach to Investigate the Role of Plasma Secretory Phospholipase A2 (sPLA<sub>2</sub>)-V Isoenzyme in Coronary Heart Disease

Modified Mendelian Randomization Analysis Using PLA2G5 Expression Levels

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Background—Secretory phospholipase A<sub>2</sub> (sPLA<sub>2</sub>) enzymes are considered to play a role in atherosclerosis. sPLA<sub>2</sub> activity encompasses several sPLA<sub>2</sub> isoenzymes, including sPLA<sub>2</sub>-V. Although observational studies show a strong association between elevated sPLA<sub>2</sub> activity and CHD, no assay to measure sPLA<sub>2</sub>-V levels exists, and the only evidence linking the sPLA<sub>2</sub>-V isoform to atherosclerosis progression comes from animal studies. In the absence of an assay that directly quantifies sPLA<sub>2</sub>-V levels, we used PLA2G5 mRNA levels in a novel, modified Mendelian randomization approach to investigate the hypothesized causal role of sPLA<sub>2</sub>-V in coronary heart disease (CHD) pathogenesis.

Methods and Results—Using data from the Advanced Study of Aortic Pathology, we identified the single-nucleotide polymorphism in PLA2G5 showing the strongest association with PLA2G5 mRNA expression levels as a proxy for sPLA<sub>2</sub>-V levels. We tested the association of this SNP with sPLA<sub>2</sub> activity and CHD events in 4 prospective and 14 case–control studies with 27,230 events and 70,500 controls. rs525380C>A showed the strongest association with PLA2G5 mRNA expression (P=5.1×10<sup>-6</sup>). There was no association of rs525380C>A with plasma sPLA<sub>2</sub> activity (difference in geometric mean of sPLA<sub>2</sub> activity per rs525380 A-allele 0.4% [95% confidence intervals [−0.9%, 1.6%]; P=0.56). In meta-analyses, the odds ratio for CHD per A-allele was 1.02 (95% confidence intervals [0.99, 1.04]; P=0.20).

Conclusions—This novel approach for single-nucleotide polymorphism selection for this modified Mendelian randomization analysis showed no association between rs525380 (the lead single-nucleotide polymorphism for PLA2G5 expression, a surrogate for sPLA<sub>2</sub>-V levels) and CHD events. The evidence does not support a causal role for sPLA<sub>2</sub>-V in CHD. (Circ Cardiovasc Genet. 2014;7:144-150.)

Key Word: Mendelian randomization analysis

The secretory phospholipases (sPLA<sub>2</sub>s) are a family of enzymes that hydrolyse phospholipids on lipoprotein particles, initially in the plasma, leading to the modification of low-density lipoproteins (LDLs) to small, dense, proatherogenic LDL particles that can transcytose the endothelial layer of the arterial wall. Further modification of these intimal apolipoprotein B–containing lipoproteins, by sPLA<sub>2</sub>s in the arterial wall, leads to their accumulation and retention on the proteoglycans within the intima, a proatherosclerotic process. Additionally, by hydrolyzing lipoprotein phospholipids, sPLA<sub>2</sub>s generate lysophospholipid and nonesterified free fatty acids, such as arachidonic acid, a precursor of eicosanoids and leukotrienes that are proinflammatory cytokines.

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Three sPLA₂ isoenzymes have been identified in human atherosclerotic lesions: sPLA₂-IIa, sPLA₂-V, and sPLA₂-X. It is thought that sPLA₂-V may contribute to the quantitative trait sPLA activity, a composite measure of sPLA₂-IIa, -V, and -X, although no direct biological proof exists. There is converging evidence from both the European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk study and Global Registry of Acute Coronary Events (GRACE), a study of patients with acute coronary syndrome, that sPLA₂ activity shows stronger association with cardiovascular risk than sPLA₂-IIa levels alone. This provides some indirect evidence that sPLA₂ activity might encompass more than just sPLA₂-IIa, and this identifies sPLA₂-V as a potential contributor to CHD risk in humans. Although a large body of observational studies support the relationship between higher sPLA₂-IIa levels and risk of CHD in humans, no such studies exist for sPLA₂-V. A specific ELISA assay exists that enables the quantification of sPLA₂-IIa levels, but there is currently no assay to specifically measure sPLA₂-V levels. Despite the lack of observational studies in humans for sPLA₂-V, animal studies report a proatherogenic role for sPLA₂-V as well as -IIa, showing increased susceptibility to atherosclerosis in sPLA₂-V (Pla2g5) and sPLA₂-IIa (Pla2g2a) transgenic mice. Studies of human tissue also indicate that sPLA₂-V is expressed in human endothelial cells, macrophages, and lipid-loaded macrophages. Suggested mechanisms by which sPLA₂-V is thought to increase risk of CHD include increasing the entrapment of LDLs in the atherosclerotic plaque and modification of LDLs to encourage generation of foam cells.

The specific catalytic dyad found in sPLA₂ enzymes makes them suitable drug targets, and indeed an sPLA₂ inhibitor has been developed. The varespladib, with a primary target of sPLA₂-IIa, also inhibits sPLA₂-V. However, because the exact contribution of sPLA₂-V to plasma sPLA₂ activity is unknown, it is challenging to infer the nature of the relationship between sPLA₂-V and CHD events.

Genetics provides a powerful tool to examine whether a relationship between a biomarker and a disease outcome is likely to be causal. This process, called Mendelian randomization (MR), makes use of a genetic variant that associates with the biomarker of interest as a means to investigate whether the biomarker is causally related to disease. There are 3 steps in traditional MR analysis, often referred to as MR triangulation. The first side of the triangle usually is the starting point of the analysis and arises from observational studies, which report the association of the biomarker with CHD, in this case it would be sPLA₂-V levels. However, in the absence of measures of plasma sPLA₂-V levels, observational studies report the association of elevated levels of the composite measure of sPLA₂ activity with CHD risk. The second side of the MR triangle validates the association of the genetic variant with the biomarker of interest. In this modified MR study, the absence of a specific assay to quantify sPLA₂-V levels motivated us to pursue a novel approach that exploited the availability of vascular tissue mRNA expression of PLA2G5 (the gene encoding sPLA₂-V) as a proxy for sPLA₂-V levels, and for this we identified a common PLA2G5 gene variant most strongly associated with PLA2G5 mRNA expression. We feel this novel approach is justified because a recent study we performed for sPLA₂-IIa found that the single-nucleotide polymorphism (SNP) showing strongest association with PLA2G2A mRNA was in strong linkage disequilibrium with the SNP that showed strongest association with sPLA₂-IIa (a specific assay for sPLA₂-IIa).

Finally, to validate whether the biomarker is causal, the MR triangle is completed by examining the association of the PLA2G5 variant with CHD risk and comparing this value to the observational estimate for a similar difference in biomarker.

Methods

SNP Selection for Mendelian Randomization Using mRNA Expression

We searched publicly available eQTL data sets to identify SNPs in PLA2G5 associated with eQTL effects at genome-wide significance in circulating cells in blood. This did not identify any associations, and we therefore focused on mRNA expression in tissue samples in our own data set. We used the Advanced Study of Aortic Pathology (ASAP) (n=272) as a source of PLA2G5 mRNA expression. Individuals undergoing valve surgery had tissue biobanked from liver (n=212), mammary artery intima-media (n=89), ascending aorta intima-media (n=138), aorta adventitia (n=133), and heart (n=127), and subsequently mRNA levels extracted. mRNA levels were quantified using Affymetrix Gene Chip Human Exon 1.0 ST expression arrays, and DNA was genotyped using Illumina Human 610 W-Quad Bead array. We investigated the association between SNPs in and within 200 kb of the PLA2G5 gene with mRNA expression of PLA2G5 and selected the SNP that showed the strongest differential association with PLA2G5 expression levels. SNPs with a call rate <80% or Hardy–Weinberg chi square statistic >3.84 were excluded. The overall call rate per SNP was 99.84%. Twelve samples were genotyped in duplicate, and the concordance was 99.99%. The rs525380 SNP was in Hardy–Weinberg equilibrium (P=0.54) and had a call rate of 100%.

Association of the Gene Variant With Nonindex mRNA Expression and sPLA₂ Activity

To investigate the specificity of our genetic variant, we examined the relationship between the SNPs with mRNA levels of PLA2G2A and PLA2G10. To gauge insight into the relative contribution of sPLA₂-V to sPLA₂ activity, we investigated the per-allele association of the SNPs with sPLA₂ activity in EPIC-Norfolk (measured by a selective fluorometric assay).

Genotyping of rs525380

The lead SNP in the analysis, rs525380, was present on various genome-wide association studies platforms used by the CARDioGRAM studies. For EPIC-Netherlands, Whitehall II, and Women’s Health Initiative, genotyping was performed using the IBC CardioChip array (Illumina HumanCVD). For the remaining study (EPIC-Norfolk), the rs525380 SNP was genotyped using TaqMan technology (Applied Biosciences, ABI, Warrington, UK; Table I in the Data Supplement). In each study, rs525380 was in Hardy–Weinberg equilibrium with call rates >97%.

Association of the Gene Variant With LDL-C Levels

We previously reported an association of PLA2G5 SNPs with LDL-cholesterol (LDL-C) levels in a small study of patients with type 2 diabetes mellitus. To investigate whether LDL-C may represent a mediator between sPLA₂-V and CHD, we looked up the association of rs525380 in a recent large gene-centric analysis of 32 studies including 66,240 individuals of European ancestry.

Association of the Gene Variant With CHD Events

Data from 18 studies were used in the analysis of the association between the PLA2G5 lead SNP and CHD risk, comprising 3 nested case–control studies (Women’s Health Initiative, EPIC-Norfolk, and EPIC-Netherlands). 1 prospective cohort (Whitehall II), and
14 case–control studies (participants in the CARDIoGRAM GWA meta-analysis of coronary artery disease [CAD]). All studies were approved by their institutional review committees, and subjects gave informed consent.

These studies are described in Table I in the Data Supplement and the details of the CARDIoGRAM consortium in Table II in the Data Supplement.

Statistical Analysis
All gene expression values were log transformed before analysis as part of the microarray preprocessing algorithm. Association strength between genotype and gene expression levels were calculated using a linear regression model with the gene expression as response variable and the genotype recorded numerically (as 0, 1, and 2) as the explanatory variable. A Bonferroni-adjusted P value threshold of \( P < 8.4 \times 10^{-4} \) was taken as the level of significance for the association of SNPs with mRNA expression. The mRNA analysis was performed using R 2.13.0 and Bioconductor.

sPLA\(_2\), activity was log(e) transformed before analysis because of a skewed distribution. We used an additive model for the genetic association analysis of rs525380 with sPLA\(_2\), activity and CHD events. The univariate per-minor A-allele estimates otherwise stated, were performed using Stata 12.1 (StataCorp, College Station, TX).

Results

Identification of the SNP Showing the Strongest Association With \( \text{PLA2G5 Expression} \)

The SNP showing the strongest association with \( \text{PLA2G5 mRNA expression} \) was rs525380 at \( P = 5.1 \times 10^{-6} \) (n=272, Figure 1), which surpassed our Bonferroni-adjusted \( P \) value threshold. \( \text{PLA2G5} \) was most highly expressed in the heart (Figure 2), where it was among the top 11% most highly expressed genes and in the top 50% of expression in other investigated tissues (mammary artery, liver, aorta media, and adventitia). The rs525380C>A was associated with the strongest differential mRNA expression of \( \text{PLA2G5} \) in the aortic adventitia explaining 14.5% of the \( \text{PLA2G5 mRNA variance} \) (n=133, Figure 2; Figure I in the Data Supplement); the rare A-allele was associated with 37.6% higher mRNA levels than the common C allele. Associations of rs525380 with \( \text{PLA2G5 mRNA expression} \) were also identified in the aortic media and mammary artery (\( P < 0.001 \); Figure 2). The regional plot for rs525380, showing the linkage disequilibrium (LD) with SNPs in the vicinity, is presented in Figure I in the Data Supplement. This plot shows that the LD falls off around the lead SNP, rs525380, in \( \text{PLA2G5} \) and shows little LD with \( \text{PLA2G2A SNPs} \), with \( R^2 \leq 0.2 \).

Bioinformatic Analysis of rs525380
rs525380 is located \( 12.5 \) kb downstream of the \( \text{PLA2G5 transcription start site} \) within a potential enhancer motif, experimentally determined by DNase1-seq and FAIRE-seq open chromatin marks (liver and vascular cells), and by ChIP-seq for the transcription factor GATA-2 (UCSC Genome Browser GRCh37/hg19\(^3\); Figure II in the Data Supplement), suggesting rs525380 may be functional, potentially playing a distal regulatory role and altering \( \text{PLA2G5 expression} \).

Association of \( \text{PLA2G5 SNPs With PLA2G2A and PLA2G10 mRNA Expression Levels and sPLA2 Activity} \)

We next examined the association of \( \text{PLA2G5} \) rs525380 with \( \text{PLA2G2A} \) (lying head to tail with \( \text{PLA2G5} \) on chr1) and \( \text{PLA2G10} \) (chr10) mRNA expression levels. We did not observe an association of rs525380 and \( \text{PLA2G2A mRNA expression} \) in vascular tissues, but we did identify an association of rs525380 with liver \( \text{PLA2G2A mRNA expression} \) (n=212, \( P = 0.001 \), Figure III in the Data Supplement). rs525380 showed no association with \( \text{PLA2G10 mRNA expression} \) in any tissue (\( P > 0.05 \) for all associations).

There was no association between the A-allele of rs525380 and plasma sPLA\(_2\) activity (n=3095, 0.4% difference in geometric mean per A-allele of rs525380; 95% confidence interval [CI] [−0.9%, 1.6%]; \( P = 0.56 \)).

Association of rs525380 With LDL-C Levels
A look-up in a large meta-analysis across 32 studies\(^9\) yielded a pooled per A-allele estimate of 0.002 mmol/l (95% CI [−0.008, 0.012]) difference in LDL-C in 66 240 individuals (\( P = 0.71 \)), thus showing no association between rs525380 and LDL-C levels.

Association of rs525380 With CHD Events
The pooled estimate of the association of rs525380 with CHD events in meta-analysis of 18 studies with 27 230 CHD events in 97 730 individuals did not identify any evidence of association. The per-A-allele estimate was OR 1.02 (95% CI [0.99, 1.04]; 146 cases and 131 691 controls; \( P = 0.12 \)).

Figure 1. Manhattan plot of the association between single-nucleotide polymorphisms in the \( \text{PLA2G5 region and PLA2G5 mRNA expression by tissue type} \). rs525380 A>C showed the strongest association with \( \text{PLA2G5 mRNA expression} \) in the aorta adventitia (\( P = 5.05 \times 10^{-6} \)). The black horizontal line above the scale represents the position of \( \text{PLA2G5} \). Total number of individuals providing tissue samples for analysis=272 (samples available for each tissue: mammary artery 89, liver 212, aorta med 138, aorta adventitia 133, heart 127).
1.04), and the heterogeneity was low ($I^2=0\%$; 95\% CI [0\%, 48\%]; Figure 3). When we restricted the analysis to only large studies with >1000 CHD events (11 studies with 22,757 cases in 85,494 individuals), the estimate remained unchanged (OR 1.01; 95\% CI [0.99, 1.04]).

Discussion

We performed a modified MR analysis to evaluate whether the relationship between sPLA$_2$-V and CHD events is likely to be causal. In the absence of a suitable assay to directly quantify sPLA$_2$-V levels, we took the novel approach of using vascular mRNA expression levels of the gene encoding sPLA$_2$-V, $PLA2G5$, as a proxy measure. We found $PLA2G5$ to be highly expressed in all available tissues, being among the top 11\% genes expressed in the heart and in the top 50\% of expression in other investigated tissues. We identified an SNP that surpassed the predefined Bonferroni-adjusted $P$ value threshold for association with $PLA2G2A$ mRNA expression, explaining 14\% of the variance in $PLA2G5$ mRNA levels. We took this genetic variant forward to investigate the association with CHD in a large collection of studies. In analysis of 27,230 cases, we found the association of $PLA2G5$ rs525380 (per A-allele) with CHD in 27,230 cases in a total of 97,730 individuals. When limited to studies with <1000 CHD events, the odds ratio (OR) was 1.04 (95\% CI [0.97, 1.11]) with an $I^2$ of 31\% (95\% CI [0\% to 71\%]). For studies with >1000 CHD events, the OR was 1.01 (95\% CI [0.99, 1.04]) with an $I^2$ of 0\% (95\% CI [0\% to 43\%]).

![Figure 2. Overall expression of all probe-sets and the differential expression of $PLA2G5$ rs525380 C>A with $PLA2G5$ mRNA in the 5 tissue types. AMed indicates dilated and nondilated ascending aorta intima-media; MMed, mammary artery intima-media; and aorta ADV, aorta adventitia. The sample sizes for samples from individuals with the genotypes CC, CA, and AA are as follows: heart 43/68/16, MMed 21/51/17, AMed 44/70/24, aorta ADV 38/73/22, and liver 59/120/32.]

![Figure 3. Forest plot of the association of $PLA2G5$ rs525380 (per A-allele) with CHD in 27,230 cases in a total of 97,730 individuals. When limited to studies with <1000 CHD events, the odds ratio (OR) was 1.04 (95\% CI [0.97, 1.11]) with an $I^2$ of 31\% (95\% confidence intervals [CI] [0\% to 71\%]). For studies with >1000 CHD events, the OR was 1.01 (95\% CI [0.99, 1.04]) with an $I^2$ of 0\% (95\% CI [0\% to 43\%]).]
CHD events across 97,730 total individuals, the SNP was not associated with CHD, suggesting that sPLA$_2$-V may not be causally involved in CHD pathogenesis.

We recently used a similar technique for SNP selection when we investigated the role of sPLA$_2$-IIa in CHD. In the case of sPLA$_2$-IIa, we did have access to a trait that directly quantified circulating levels of sPLA$_2$-IIa. We showed that the SNP showing the strongest association with circulating sPLA$_2$-IIa levels was in high linkage disequilibrium with the SNP showing the strongest association with PL2G2A mRNA expression. This serves to justify the method we used here: that is, we assume that if we could quantify circulating sPLA$_2$-V levels, we would find that the SNP that showed the strongest association with sPLA$_2$-V levels would also show the strongest association with PL2G5 mRNA expression.

mRNA expression is considered a good proxy for its encoded protein, although it might only reflect a proportion of protein expression because post-transcriptional and post-translational modifications may further influence protein levels. Thus, the association of rs525380 with PL2G5 mRNA in vascular tissue may be a good marker of sPLA$_2$-V expression. This is supported by immunohistochemistry and in situ hybridization of sPLA$_2$-V in human atherosclerotic aortas showing that sPLA$_2$-V protein expression was limited to smooth muscle cells, and this correlated well with PL2G5 mRNA expression.

One of the limitations of our study is the lack of observational data on the association of sPLA$_2$-V levels and risk of CHD, limited by the absence of an available sPLA$_2$-V ELISA. PL2G5 mRNA expression measures are limited by availability of data sets with tissue mRNA expression in individuals with and without CHD. The lack of a quantitative trait also means that a formal Mendelian triangulation analysis is not possible. However, the genetic analysis that we present is a form of MR as the SNP (rs525380) will, according to Mendel’s second law, be randomized at conception, meaning that individuals grouped by rs525380 genotype should be equal in all respects apart from exposure to differing PL2G5 mRNA expression levels. Several animal studies support an atherosclerotic role of sPLA$_2$-V, although we accept that positive findings from animal studies do not always translate into meaningful advances in combatting human disease. Even in the absence of availability of an observational quantification of the association of sPLA$_2$-V (or for that matter PL2G5 mRNA) with CHD events in humans, our genetic findings show that if such an association were to exist, it would most likely be attributable to confounding and reverse causality rather than a causal relationship. We have made the assumption that vascular expression of sPLA$_2$-V is a likely proatherogenic mediator, and therefore we have considered vascular PL2G5 mRNA as a good proxy for circulating levels of sPLA$_2$-V levels. Our findings do not support those from animal studies and suggest sPLA$_2$-V is not an important cause of CHD in humans. The outcome of this study is in part validated by the phase III Vista 16 trial of varespladib (a drug that inhibits sPLA$_2$-IIa, sPLA$_2$-V, and sPLA$_2$-X), prematurely terminated because of lack of efficacy.

Because we had no measure of sPLA$_2$-V levels, we were unable to estimate the effect of the PL2G5 SNP on sPLA$_2$-V levels, and without an estimate of the observed association between sPLA$_2$-V and CHD to obtain the expected effect size, we were unable to perform a power calculation. However, for comparison, in our MR analysis of sPLA$_2$-IIA, in studies set in the general population, we had a total of 15,534 incident and prevalent cardiovascular events out of a total of 74,683 individuals. In this current study, we almost doubled the number of events with 27,230 events in 97,730 individuals. With an OR of 1.02 (95% CI [0.99, 1.04]) between rs525380 and CHD, we are able to exclude a large effect of the SNP on CHD. Furthermore, the $F$ value of 0%, indicating low heterogeneity, means that the values reported in the individual studies included in this meta-analysis were similar (ie, low between-study heterogeneity), adding further confidence to a true negative finding.

Plasma sPLA$_2$ activity is suggested to represent a composite of the activities of the -IIa, -V, and -X isoenzymes. However, currently no experimental evidence supports this. In a recent MR investigation of sPLA$_2$-IIa, we reported that the SNP showing the strongest association with sPLA$_2$-IIa levels explained 31% of PL2G2A mRNA expression and 21% of sPLA$_2$-IIa variance, yet accounted for only 0.5% of the variance of sPLA$_2$ activity. In a similar fashion, the rs525380 SNP, which explained 15% of the variance of PL2G5 mRNA, may only explain a small variance of sPLA$_2$ activity (for which we may be underpowered to detect with precision in the current analysis). Thus, sPLA$_2$-V may only make a minor contribution to plasma sPLA$_2$ activity. An alternative explanation is that, despite rs525380 showing the strongest association with PL2G5 mRNA in the aortic adventitia of the vasculature, rs525380 may not represent a suitable proxy for circulating sPLA$_2$-V. The high level of expression of PL2G5 mRNA in several relevant atherosclerosis-prone tissues, such as heart, mammary artery intima-media, ascending aorta intima-media, and aorta adventitia that we identified, suggest that sPLA$_2$-V may have its greatest biological effect in these tissues and not in the plasma. Thus, circulating plasma sPLA$_2$ activity may not reflect tissue levels of sPLA$_2$-V.

PL2G5 rs525380 showed a weak association with PL2G2A expression in the liver but not in the other tissues we examined. This association could be a spurious finding because it did not exceed the Bonferroni adjusted $P$ value threshold. Alternatively, it could represent a real association. Our Bioinformatic analysis suggests that rs525380, 12.5 kb downstream of the PL2G5, disrupts the binding site of the transcription factor GATA-2. GATA-2 is a transcription factor implicated in endothelial inflammatory responses.

This might be particularly relevant to PL2G5 given that Pla2g5 knockout mice show 50% reduction in eicosanoid generation in response to zymosan stimulus, thus suggesting that sPLA$_2$-V plays an important role in innate immunity. The association of the PL2G3 SNP rs525380 with PL2G2A expression in the liver suggests that GATA-2 might also act as a transcription factor for the control of expression of PL2G2A. However, although the rare A-allele of rs525380 is associated with higher PL2G5 expression levels, the common C allele is associated with higher PL2G2A expression levels. We previously showed that of the 5 tissues available in ASAP, PL2G2A was most highly expressed in the liver, a tissue that showed the lowest level
of \textit{PLA2G5} expression in this current study. This suggests a potential complementarity expression of these 2 transcripts in the liver, possibly under the control of GATA-2.

In conclusion, we identified no association between an SNP strongly linked to \textit{PLA2G5} mRNA tissue levels in atherosclerosis-prone tissues and risk of CHD. Although the findings we report are by no means definitive, they do not support the hypothesis that sPLA\textsubscript{2}-V plays an important role in CHD. The methods we present demonstrate that in the absence of a specific plasma biomarker measure, it may be possible to use mRNA expression levels of the coding gene as a surrogate to examine the potential causal relationship of a biomarker.

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**Disclosures**

None.

**Appendix**

From the Division of Transplant Surgery, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA (M.V.H., B.J.K.); Genetic Epidemiology Group (M.V.H., M. Kivimaki, M. Kumar, A.D.H.), Centre of Cardiovascular Genetics (H.J.E., M.G., J.A.C., K.-W.L., A.J.P.S., S.E.H., P.J.T.), Faculty of Population Health Sciences, Institute of Cardiovascular Genetics (H.J.E., M.G., J.A.C., K.-W.L., A.J.P.S., S.E.H., P.J.T.), School of Biomedical Sciences, University of Leeds, Leeds, UK; Department of Molecular Genetics, University of Cambridge, Cambridge, UK (K.-T.-K.); Atherosclerosis Research Unit, Department of Medicine Solna (F.V.H., P.E.), Cardiothoracic Surgery Unit, Department of Molecular Medicine and Surgery, Karolinska Institutet (A.F.-C.), Center for Molecular Medicine, Karolinska University Hospital Solna, Stockholm, Sweden (F.v.H., P.E.); Department of Cardiology, Division Heart and Lungs (F.W.A.), Department of Medical Genetics, University Medical Centre Utrecht (N.C.O.M.), Durrer Center for Cardiogenetic Research, ICIN-Netherlands Heart Institute, Utrecht, The Netherlands (F.W.A., N.C.O.M.); National Institute for Public Health and the Environment (RIVM), Bilthoven (J.M.A.B.), University of Groningen, University Medical Center Groningen, Department of Molecular Genetics, Groningen, the Netherlands (M.H.); Universität zu Lübeck, Medizinische Klinik II, Lübeck, Germany (J.E.); Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA (A.P.R.); Center for Applied Genomics, Children’s Hospital of Philadelphia, Philadelphia, PA (B.J.K.); Division of Cardiovascular Medicine, University of Cambridge, Addenbrooke’s Hospital, Cambridge, UK (Z.M.).

**Investigation Into the Role of sPLA\textsubscript{2}-V in CHD**


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CLINICAL PERSPECTIVE

Secretory phospholipase (sPLA₂) enzymes are thought to be involved in the development of coronary heart disease (CHD) through the synthesis of proinflammatory bioactive lipids and proatherogenic modification of low-density lipoprotein cholesterol. Animal studies support a causal role of sPLA₂-V in CHD; however, findings from animal studies do not always translate into humans. In an attempt to answer whether sPLA₂-V is causally related to CHD in humans, we performed a modified Mendelian randomization study. Using a hypothesis-free approach, we identified the single-nucleotide polymorphism that associated most strongly with PLA2G5 mRNA expression (rs525380). We then investigated the association of this rs525380 single-nucleotide polymorphism with CHD in a pooled data set of >27,000 CHD cases and 70,000 controls and found no association with CHD (odds ratio per A-allele of rs525380 was 1.02; 95% confidence interval [0.99, 1.04]; P = 0.20). This suggests that sPLA₂-V is not causally related to CHD. The findings are of importance because a recent phase III randomized clinical trial of a drug that inhibits sPLA₂-II, V, and X (varespladib) was terminated because of futility. Our previous Mendelian randomization analysis identified no causal effect of sPLA₂-IIA on CHD, and this present study adds to it by suggesting that sPLA₂-V is also noncausal for CHD. Taken together, the current evidence does not support a causal role for sPLA₂ enzymes in CHD.
**Supplementary Table 1.** Characteristics of the studies used in the analyses.

<table>
<thead>
<tr>
<th>Study</th>
<th>Study Design</th>
<th>Geographic Location</th>
<th>Sampling Frame</th>
<th>Number of individuals</th>
<th>CHD definition</th>
<th>Baseline Year(s)</th>
<th>% Female</th>
<th>Age (Mean/SD)</th>
<th>Genotype platform</th>
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<td>ADVANCE</td>
<td>C-C</td>
<td>USA</td>
<td>Electronic records</td>
<td>590</td>
<td>Clinical non fatal CAD (men ≤45 yrs, women ≤55 yrs) including AMI (enzymes), typical angina with ≥1 artery with &gt;50% stenosis, positive non invasive test, or PCI or CABG</td>
<td>2000</td>
<td>58.5</td>
<td>45.3 (5.7)</td>
<td>Illumina 550k v3</td>
</tr>
<tr>
<td>ASAP</td>
<td>Cohort</td>
<td>Sweden</td>
<td>Single centre biobank</td>
<td>272</td>
<td>Not CHD. Consecutive biobanking study of all individuals undergoing valve surgery at one clinic</td>
<td>2007</td>
<td>28.7</td>
<td>63.6 (12.4)</td>
<td>Illumina Human 610W-Quad Bead array</td>
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<td>CHARGE</td>
<td>C-C</td>
<td>Europe/USA</td>
<td>5 Prospective cohorts</td>
<td>24311</td>
<td>CHD: definite or probable MI, PTCA or CABG, or ECG MI</td>
<td>2007-2008</td>
<td>46.5</td>
<td>63.1 (8.0)</td>
<td>Affymetrix 6.0, Affymetrix 500K, Illumina Infinium HumanHap 550K</td>
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<tr>
<td>deCODE</td>
<td>C-C</td>
<td>Iceland</td>
<td>Icelandic</td>
<td>34251</td>
<td>MI: MONICA criteria</td>
<td>1998</td>
<td>49.1</td>
<td>53.7 (21.5)</td>
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</tr>
<tr>
<td>Study Name</td>
<td>Study Type</td>
<td>Country</td>
<td>Recruitment Criteria</td>
<td>Population Size</td>
<td>Year Range</td>
<td>Disease Distribution</td>
<td>Technology Used</td>
<td>Platform/Array Set</td>
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<td>EPIC-Netherlands</td>
<td>Nested C-C</td>
<td>Netherlands</td>
<td>Existing cohorts, (aged &lt;75 yrs) or discharge diagnosis of MI; CAD: PCI or participation in CVD genetics program with self report of CABG or PCI, or discharge diagnosis of angina pectoris, MI or chronic ischaemic heart disease</td>
<td>5194</td>
<td>1993-1997</td>
<td>78.11</td>
<td>HH300/HHCNV370</td>
<td>IBC CardioChip</td>
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<tr>
<td>EPIC-Norfolk</td>
<td>Nested C-C</td>
<td>UK</td>
<td>GPs, ICD9 codes 410-414</td>
<td>3039</td>
<td>1993-1997</td>
<td>34.65</td>
<td>TaqMan</td>
<td>IBC CardioChip</td>
<td></td>
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<tr>
<td>GERMIFIS I</td>
<td>C-C</td>
<td>Germany</td>
<td>Multi-centre, MI (&lt;65 yrs) with &gt;1 1st degree sibling with severe CAD (PTCA; MI; CABG)</td>
<td>2488</td>
<td>1997-2002</td>
<td>50</td>
<td>Affymetrix</td>
<td>Mapping 500K Array Set</td>
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<tr>
<td>GERMIFIS II</td>
<td>C-C</td>
<td>Germany</td>
<td>Multi-centre, MI (&lt;60 yrs); 59.4% with family history of CAD</td>
<td>2509</td>
<td>2002</td>
<td>40.7</td>
<td>Affymetrix</td>
<td>Genome-Wide Human SNP Array 6.0</td>
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<tr>
<td>GERMIFIS III (KORA)</td>
<td>C-C</td>
<td>Germany</td>
<td>Multi-centre, MI (&lt;60 yrs); MONICA criteria</td>
<td>2905</td>
<td>1999-2001</td>
<td>34.5</td>
<td>Affymetrix</td>
<td>Genome-Wide Human SNP Array 5.0 / 6.0</td>
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<tr>
<td>CADomics</td>
<td>C-C</td>
<td>Germany</td>
<td>Single centre, CAD: &gt;50% stenosis in 1 major coronary artery and/or MI</td>
<td>5030</td>
<td>1996-1997</td>
<td>38.7</td>
<td>Affymetrix</td>
<td>Genome-Wide Human SNP</td>
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<tr>
<td>Study</td>
<td>Design</td>
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<td>Type of Centre</td>
<td>Sample Size</td>
<td>Inclusion Criteria</td>
<td>Start Year</td>
<td>End Year</td>
<td>p-Value (SE)</td>
<td>Array Type</td>
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<tr>
<td>LURIC/AtheroRemo 1</td>
<td>C-C</td>
<td>Germany</td>
<td>Single centre</td>
<td>865</td>
<td>Symptoms of angina pectoris, NSTEMI, STEMI, or &gt;50% coronary stenosis</td>
<td>1997-2002</td>
<td>33.2</td>
<td>58.3 (12.1)</td>
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<td>LURIC/AtheroRemo 2</td>
<td>C-C</td>
<td>Germany</td>
<td>Single Centre</td>
<td>782</td>
<td>Symptoms of angina pectoris, NSTEMI, STEMI, or &gt;50% coronary stenosis</td>
<td>2002</td>
<td>36</td>
<td>56.4 (12.7)</td>
<td>Affymetrix Mapping 500K Array Set</td>
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<tr>
<td>MedStar</td>
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<td>USA</td>
<td>Single-centre hospital</td>
<td>1322</td>
<td>Angiography (≥1 coronary vessel with &gt;50% stenosis); &lt;65yrs</td>
<td>2004-2007</td>
<td>54.59</td>
<td>59.7 (8.9)</td>
<td>Affymetrix Genome-Wide Human SNP Array 6.0</td>
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<td>MIGEN</td>
<td>C-C</td>
<td>Europe/USA</td>
<td>Multi-centre</td>
<td>2681</td>
<td>MI (men &lt;50 yrs / women &lt;60 yrs)</td>
<td>1987-1991</td>
<td>38.6</td>
<td>43.0 (7.8)</td>
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<td>OHGS1</td>
<td>C-C</td>
<td>Canada</td>
<td>Single centre</td>
<td>2997</td>
<td>Angiographic (&gt;50% stenosis)</td>
<td>2010</td>
<td>36.1</td>
<td>75.0 (5.0)</td>
<td>Affymetrix Mapping 500K Array Set / Genome-Wide Human SNP Array 6.0 platform</td>
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<td>PennCath</td>
<td>C-C</td>
<td>USA</td>
<td>Single-centre hospital</td>
<td>1516</td>
<td>Angiography (≥1 coronary vessel with &gt;50% stenosis);</td>
<td>1998-2003</td>
<td>51.92</td>
<td>61.7 (9.6)</td>
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<td>Country</td>
<td>Setting</td>
<td>Cases</td>
<td>Cases</td>
<td>&lt;65 yrs</td>
<td>Year Range</td>
<td>Number</td>
<td>Mean (SD)</td>
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<tr>
<td>Whitehall II</td>
<td>Cohort</td>
<td>UK</td>
<td>Workplace</td>
<td>5018</td>
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<td>1985-1988</td>
<td>26.44</td>
<td>43.9 (5.9)</td>
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<td>Women’s Health Initiative</td>
<td>Nested C-C</td>
<td>USA</td>
<td>Community</td>
<td>5729</td>
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<td></td>
<td>1993-1998</td>
<td>100</td>
<td>68.0 (3.3)</td>
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<td>WTCCC</td>
<td>C-C</td>
<td>UK</td>
<td>1958 Birth cohort and National blood donor register</td>
<td>4864</td>
<td></td>
<td></td>
<td>2005</td>
<td>35.4</td>
<td>49.8 (7.7)</td>
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</tbody>
</table>

**Footnotes:** CABG: coronary artery bypass graft; CAD: coronary artery disease; C-C: case control; ICD: International Classification of Disease; MI: myocardial infarction; NSTEMI: non-ST-segment elevation myocardial infarction; PTCA: percutaneous transluminal coronary angioplasty, RCT: randomized clinical trial; STEMI: ST-segment elevation myocardial infarction
<table>
<thead>
<tr>
<th>Supplementary Table 2</th>
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</thead>
<tbody>
<tr>
<td><strong>The CARDioGRAM Consortium</strong></td>
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<tr>
<td><strong>Executive Committee:</strong> Sekar Kathiresan, Muredach P. Reilly, Nilesh J. Samani, Heribert Schunkert, Jeanette Erdmann</td>
</tr>
<tr>
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</tr>
<tr>
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</tr>
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<tr>
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</tr>
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<td><strong>deCODE:</strong> Solveig Gretarsdottir, Jeffrey R. Gulcher, Hilma Holm, Augustine Kong, Kari Stefansson, Gudmundur Thorleifsson, Karl Andersen, Gudmar Thorleifsson, Unnur Thorsteinsdottir</td>
</tr>
<tr>
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</tr>
<tr>
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</tr>
<tr>
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</tr>
</tbody>
</table>
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Supplementary Figures

Supplementary Figure 1 Regional haploblock plot of *PLA2G5* rs525380 showing low linkage disequilibrium with closely linked *PLA2G2A* SNPs.

**Footnote:** Representing Caucasian (CEU) data
Supplementary Figure 2 Bioinformatic details of rs525380 in intron 1, identifying position relative to GATA-2 and a DNAse hypersensitive region. There is no evidence that this SNP affects splicing. Taken from www.genome.ucsc.edu
**Supplementary Figure 3.** Association of *PLA2G5* rs525380 with *PLA2G2A* mRNA expression in the liver.

Footnote: $P=0.001$ for differential expression by rs525380, using an additive model.
Novel Genetic Approach to Investigate the Role of Plasma Secretory Phospholipase A2 (sPLA₂)-V Isoenzyme in Coronary Heart Disease: Modified Mendelian Randomization Analysis Using PLA2G5 Expression Levels


on behalf of the CARDIoGRAM Consortium*

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