# *PLA2G10* Gene Variants, sPLA2 Activity and Coronary Heart Disease Risk

Running title: *Guardiola et al.; Investigation into the role of sPLA2-X in CHD*

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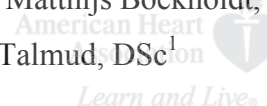
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Abstract:

Background - Observational studies report that secretory phospholipase A2 (sPLA2) activity is a marker for CHD risk, and activity measures are thought to represent the composite activity of sPLA2-IIA, -V and -X. The aim of this study was to use genetic variants of *PLA2G10,* encoding sPLA2-X, to investigate the contribution of sPLA2-X to the measure of sPLA2 activity, and coronary heart disease (CHD) risk traits and outcome.

Methods and Results - Three *PLA2G10* tagging SNPs (rs72546339, rs72546340, rs4003232) and a previously studied *PLA2G10* cSNP rs4003228, R38C, were genotyped in a nested case: control cohort drawn from the prospective EPIC-Norfolk Study (2175 cases and 2175 controls). Meta-analysis of rs4003228 (R38C) and CHD was carried out using data from the Northwick Park Heart Study II and two published cohorts AtheroGene and SIPLAC, providing in total an additional 1884 cases and 3119 controls. EPIC-Norfolk subjects in the highest tertile of sPLA2 activity were older and had higher inflammatory markers compared to those in the lowest tertile for sPLA2 activity. None of the *PLA2G10* tSNPs nor R38C, a functional variant, were significantly associated with sPLA2 activity, intermediate CHD risk traits or CHD risk. In meta-analysis the summary OR for R38C was OR=0.97 (95%CI 0.77-1.22).

Conclusions - *PLA2G10* variants are not significantly associated with plasma sPLA2 activity or with CHD risk.

Key words: coronary heart disease risk, genetic polymorphism, sPLA2 activity, PLA2G10

Introduction

Observational studies have identified secretory phospholipase A2 (sPLA2)-IIA mass/levels, measured by a specific ELISA, as a marker for CHD risk [1], with elevated levels associated with an increased risk of CHD events [2-4]. In the prospective EPIC-Norfolk study, comparing the prognostic ability of sPLA2-IIA levels with sPLA2 activity, sPLA2 activity appeared to be the better risk predictor, independent of circulating sPLA2-IIA levels [5-6]. This raised the possibility that sPLA2 activity was a composite measure of all secreted sPLA2s, including sPLA2-IIA, -V and -X and possibly -III [7] providing additional association over and above that of sPLA2-IIA levels alone. However, there is now supporting evidence that it is only sPLA2-IIA that is strongly induced under pathologic conditions associated with inflammation, tissue injury or infection, and there seems to be little evidence that other sPLA2 isoforms are present in the circulation [8,9].

The proatherogenic role of sPLA2-IIA (*PLA2G2A*) and sPLA2-V (*PLA2G5*) is supported by animal studies showing increased susceptibility to atherosclerosis in sPLA2-IIA (*Pla2g2a*) [10,11] and sPLA2-V (*Pla2g5*) transgenic mice [12]. Thus by extrapolation sPLA2-X, as the most potent of the three sPLA2s in hydrolysing the most abundant phospholipid phosphotidylcholine (PC), [13] could also be considered to be pro-atherogenic and indeed it has been shown that *PLA2G10* enhances foam cell formation *in vitro* by promoting atherogenic LDL formation [14]. Transgenic mice over-expressing *Pla2g10* have been shown to die neonatally due to severe lung pathology, suggesting a role for sPLA2-X over-expression in inflammatory airway diseases [15].

The ELISA assay to measure circulating sPLA2-IIA mass/levels is very specific to –IIA, but no such assays exist to measure sPLA2-V or -X levels. At present, it is not possible to measure the individual contributions of these sPLA2s to plasma sPLA2 activity and the aim of

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this study was to use genetics to investigate whether sPLA2-X does contribute to the measure of sPLA2 activity.

Gora et al [16] identified 8 SNPs in *PLA2G10,* all in strong linkage disequilibrium with each other, amongst them a non-synonymous variant R38C (rs4003228). The substitution of cysteine for arginine at position 38 lies near an arginine doublet where the propeptide is cleaved. *In vitro* protein expression studies led to the conclusion that R38C causes misfolding of sPLA2-X resulting in a catalytically inactive and unstable enzyme [16]. Without sPLA2 activity measures the study was unable to examine the relationship of R38C with sPLA2 activity. They also reported that R38C was not associated with CHD risk in their relatively small cohorts.

In the current paper we have taken this work forward and examined the association of the functional SNP, R38C and a number of *PLA2G10* tagging (t) SNPs with sPLA2 activity, and CHD risk traits in the EPIC-Norfolk study, and with CHD events in meta-analysis of 3667 CHD cases and 4945 controls.

Methods

Study populations

The EPIC (European Prospective Investigation into Cancer and Nutrition)-Norfolk study is a prospective study of 25,663 men and women aged 45-79 years, resident in Norfolk, UK, recruited from age-sex registers of general practices in Norfolk between 1993 and 1997 and followed-up for around 6 years, excluding those reporting a history of heart attack or stroke. The design and methods of the study have been described in detail [17]. The study was approved by the Norwich District Health Authority ethics committee and all participants gave signed informed consent. A nested case: control cohort was drawn from this larger study. Cases were those identified as having CHD on follow-up if they had a hospital admission and/or died with CHD as

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the underlying cause. Controls were free of disease and matched to cases by sex, age and enrolment time. A total of 2175 cases were matched to 2175 controls for the genotype analysis. sPLA2 activity measures were available for 1200 cases and 1468 controls.

Additional studies included in the meta-analysis of R38C and CHD risk

The Northwick Park Heart Study II (NPHS-II) is a prospective study of 3012 healthy middle-aged men aged 50–64 years at recruitment, sampled from nine UK general practices between 1989 and 1994 [18]. Men were free from disease at the time of recruitment, and information on lifestyle habits, height, weight, blood pressure were recorded at baseline and on subsequent prospective follow-up. A DNA repository was established using samples from 2775 men obtained at the time of recruitment. Full details of recruitment, measurements, follow-up and definitions of incident disease have been reported elsewhere [18]. We included published data from AtheroGene and SIPLAC [16] in the meta-analysis of R38C on CHD.

Biochemical analysis for EPIC-Norfolk Study

Blood samples were stored at -80°C at the Department of Clinical Biochemistry, University of Cambridge. Serum levels of total cholesterol, high-density lipoprotein cholesterol, and triglycerides were measured on fresh samples with the RA 1000 (Bayer Diagnostics, Basingstoke, UK), and low-density lipoprotein cholesterol (LDL-C) levels were calculated with the Friedewald formula. Serum sPLA2 activity was measured by a selective fluorometric assay [5] by using fluorescent substrate 1-hexadecanoyl-2-(1-pyrenedecanoyl)-sn-glycero-3 phosphomethanol, sodium salt (Interchim, Montluçon, France), as previously described [6]. One hundred percent hydrolysis of the fluorescent substrate was measured using 0.1 U sPLA2 from bee venom (Sigma Chemical Co). The hydrolysis of substrate in the absence of plasma was used as negative control and deduced from sPLA2 activity. All samples were tested in duplicate and

plasma activity was expressed as nmol/min per mL. The minimum detectable activity was 0.10

nmol/min per mL. Plasma concentrations of CRP were measured with a sandwich-type enzyme-

linked immunosorbent assay as previously described [19]. Results were related to a standard

consisting of commercially available CRP (Behringwerke AG, Marburg, Germany). The lower

detection limit was 0.1 mg/L.

Genotyping

We identified 10 tagging SNPs in *PLA2G10* using the STRAM algorithm [20]. Seven of these

tSNPs occurred at very low frequencies and were not taken forward. We genotyped EPIC-

Norfolk for three tSNPs, rs72546339, rs72546340 and rs4003232 and also included in our study

the previously reported cSNP rs4003228 (R38C) [16]. Rs72546339 and rs72546340 were

genotyped using TaqMan technology (Applied Biosciences, ABI, Warrington UK). Reactions

were performed on 384-well microplates and analysed using ABI TaqMan 7900HT software.

Rs4003228 and rs4003232 were determined by nested PCR amplified from a single large

fragment encompassing both variant sites, with primers Forward: 5'-

GCGTGACCTGCCACACCTATG-3' and Reverse: 5'-TTCACTGGCCATGTTATCC-3'.

Rs4003228 was determined using the following internal primers (Forward: 5'-

GAGGAAACCAAGGCCCAGAGAGG-3' and Reverse: 5'-TGCCACTTCCAGGATCCCACG-

3'), and genotype was assessed by *Bsa AI* digestion, yielding fragments of 220, 186 and 34 bp;

common allele generating 186 and 34 base pairs in length, and rare allele generating the large

fragment. rs4003232 genotype was determined using internal primers (Forward: 5'-

CCCAGCCGGATTATAATA-3' and Reverse: 5'-GCATGAGCCTGAGAAGAGCCA-3')

followed by *Alu I* digestion yielding fragment sizes of 216, 150 and 66 bp; common allele

generating 150 and 66 base pairs in length, and rare allele generating the large fragment.

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Fragments were resolved using MADGE (Microtitre Array Diagonal Gel Electrophoresis) gels [21]. NPHSII was genotyped for rs4003228 (R38C) alone.

PLA2G10 mRNA expression in the ASAP Study and BiKE

Data from the Advance Study of Aortic Pathology (ASAP) was used to determine the mRNA expression levels for *PLA2G10* in mammary artery, aortic media, aortic adventitia, liver and heart tissues. ASAP recruited 223 patients undergoing aortic valve surgery at the Karolinska University Hospital, Stockholm Sweden [22]. Tissue biopsies were taken from liver, mammary arteries and dilated and non-dilated ascending aorta and heart during surgery. The medial and adventitial layers of the vascular specimen were isolated by adventectomy, and incubated with RNAlater (Ambion, Austin, Texas, USA) and homogenised for mRNA extraction as previously detailed [22]. Affymetrix Gene Chip Human Exon 1.0 ST expression arrays were used. mRNA expression was evaluated using RMA pre-processing as previously described [22]. Briefly, the paper proposes to apply a quantile normalization which is an approach to make the hybridization intensity distribution of the probes in all arrays the same. The essence is that each gene is assigned a value that indicates its expression relative to all other genes. Participants were genotyped using the Illumina Human 610W-Quad Bead array [22].

Human carotid endarterectomy samples from 127 patients undergoing surgery for asymptomatic or symptomatic carotid stenosis were part of Biobank of Karolinska Endarterectomies (BiKE) study [23].

All samples were collected with consent from patients, organ donors or organ donors' guardians. The Ethical Committee of Northern Stockholm approved the study. RNA, extracted from endarterectomy and control specimens, was analysed by Affymetrix HG-U133 plus 2.0 Genechip arrays. Robust multi-array average (RMA) normalization was performed and

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processed gene expression data was returned in a $\log_2$-scale.

Statistical analysis

The analysis was performed using Stata version 13.1 (StataCorp, Texas).

Summary statistics of the baseline characteristics were presented by tertile of sPLA2 activity and p values for trend obtained from linear regression models. Variables were log-transformed where necessary to give a normal distribution. For these variables summary statistics were back-transformed from the log scale to give the geometric mean and approximate standard deviation (SD). Geometric means and approximate SDs were also presented for sPLA2 activity by genotype. Linear regression was used to obtain beta coefficients and standard errors for the additive genetic model (both unadjusted and adjusted for age and gender) along with p values for trend. Adjustment was made for age and gender by including them as covariates in the model. Linear regression was also used to assess the effect of genotype on intermediate traits. Association with CHD risk was determined by unconditional logistic regression analysis to obtain results consistent with those from the published studies. An additive genetic model was used. Results were adjusted for age and gender. Results from EPIC-Norfolk were then combined with those from NPHSII and the published studies SIPLAC and AtheroGene [16] using fixed effects meta-analysis to examine the association of rs4003228 (R38C) with CHD risk. [16]. For eQTL studies, association was calculated using an additive linear model in which the genotypes were numerically encoded as 0, 1, and 2.

Results

The basic characteristics of the EPIC-Norfolk case-control cohort, by tertiles of sPLA2 activity are presented in Table 1. Compared to the lowest tertile of sPLA2 activity, those in the highest tertile were older, and had higher body mass index (BMI), blood pressure (both systolic and

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diastolic), circulating cholesterol, LDL-cholesterol, triglycerides, CRP and sPLA2 mass levels and lower HDL-cholesterol levels.

PLA2G10 gene variants and sPLA2 activity and intermediate traits

We genotyped the EPIC-Norfolk cohort for four *PLA2G10* SNPs (rs4003232, rs4003228, rs72546339 and rs72546340). All genotypes were in Hardy-Weinberg equilibrium and their minor allele frequencies are given in Table 2. In EPIC-Norfolk, none of the variants, including the functional rs4003228 (R38C) which was postulated to affect sPLA2-X activity [16], showed an association with sPLA2 activity (additive genetic model using linear regression), but rs4003232 alone showed association with HDL-cholesterol and CRP levels (Supplementary data).

PLA2G10 R38C and CHD risk

We then concentrated on the cSNP rs4003232 (R38C). Meta-analysis was carried out on data from EPIC- Norfolk, NPHSII, SIPLAC and AtheroGene studies using fixed effects meta-analysis. The summary odds ratio was 0.97 (95%CI 0.77-1.22) (Figure 1).

PLA2G10 mRNA expression in ASAP and BiKE

We tested the potential allele-specific expression of *PLA2G10* in the tissues available in the ASAP study, namely liver, mammary arteries, dilated and non-dilated ascending aorta and heart, from 223 patients undergoing aortic valve surgery. Overall mRNA expression of *PLA2G10* was very low when compared to that of *PLA2G2A* and *PLA2G5*, as illustrated by expression in the aortic adventitia, as an example (Figure 2). In addition, compared to *PLA2G2A* [24] and *PLA2G5* [25] where differential mRNA expression of lead SNP reached p=8.71 x 10^{-19} and 5.1 X 10^{-6}, respectively, differential expression of PLA2G10 was ~1x$10^{-1.5}$ (Figure 3). None of our selected SNPs of interest were present on the ASAP arrays and a limitation of these results is that only a small number of *PLA2G10* specific SNPs were available on the array [22].

The BiKE study, examining RNA expression in carotid endarterectomy samples from 127 individuals, enabled us to examine expression levels of *PLAG10* in diseased tissue compared to controls, and *PLA2G10* expression levels were similar between control and carotid endarterectomy samples (data not shown), but as with tissue from ASAP, expression levels of *PLA2G10* were very low, suggesting that *PLA2G10* is weakly expressed in these samples (Figure 3).

Discussion

We have had a long standing interest in the sPLA2 enzymes [26,27] since they were identified as potential risk markers for CHD both from animal studies [10-12] and observational analyses. The aim of the present study was to investigate the contribution of sPLA2-X to the complex measure of sPLA2 activity, a marker of CHD risk [28], by examining the association of *PLA2G10* variants with sPLA2 activity. We also aimed to extend the work of Gora et al [16] examining, through meta-analysis, the association of *PLA2G10* SNPs with CHD risk.

sPLA2 activity is not a widely available measure. For this reason we chose to study a CHD nested case-control set drawn from the EPIC-Norfolk cohort [3], with available measures of sPLA2 activity [5]. sPLA2 is considered to be pro-inflammatory, by generating arachidonic acid which is a precursor of the inflammatory eicosanoid pathway [29]. Indeed it was evident that EPIC-Norfolk participants in the top tertile of sPLA2 activity had a poorer CHD risk profile than those in the lowest tertile; they were older, had a higher BMI, a worse lipid profile and increased levels of inflammatory markers CRP and sPLA2-IIA mass/ levels.

Rs4003228 (R38C) was identified as a functional SNP which could strongly affect sPLA2-X activity [16]. The functional analysis of this SNP suggested that the synonymous arginine to cysteine change at codon 38 had a large impact on sPLA2-X activity *in vitro* with the extra,

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unpaired cysteine residue resulting in ~95% decrease in sPLA2-X protein in the cell supernatant of the fibroblast-like cell line (COS cells) and ~85% decrease in the cell lysate. We were able to examine whether this SNP had an effect on sPLA2 activity *in vivo*. If sPLA2-X was indeed contributing to the overall measure of sPLA2 activity then we anticipated that the functional SNP R38C should show association with sPLA2 activity. Yet we found no association of R38C or the other 3 *PLA2G10* tSNPs with sPLA2 activity. In addition to sPLA2-X, sPLA2 activity has been considered to result from the combined activity of sPLA2-IIA and sPLA2-V. We previously reported the association of *PLA2G2A* functional SNP, rs11573156, with sPLA2 activity [24,30], but we were unable to ratify the contribution of sPLA2-V to the overall activity measure using genetic variants of *PLA2G5* [25]. From this we conclude that sPLA2-V and -X play a very minor role, if any, to the measure of plasma sPLA2 activity.

We analysed the association of the 4 *PLA2G10* gene variants with a range of cardiovascular risk factors in EPIC-Norfolk. We studied anthropometric measurements (BMI, SBP and DBP), circulating cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides, and inflammatory markers (sPLA2, CRP), and we could not find an association with any intermediate CHD trait. This was in agreement with the findings Gora et al [16].

To examine the expression of *PLA2G10* we made use of data from the ASAP study. Expression of *PLA2G10* across all available tissues was considerably lower than *PLA2G2A* and *PLA2G5* expression, and no single *PLA2G10* SNP on the array had an association with differential mRNA expression. This fits with results from Kudo et al showing sPLA2-X expression was lower than sPLA2-IIA and -V in tissues such as the heart and liver [31]. Our results do not reflect the expression of *PLA2G10* in tissues associated with inflammation such as thymus, spleen, leukocytes, and airways as reported elsewhere [32,33]. Although sPLA2-X has also

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been identified in atherosclerotic plaques [34] mRNA expression levels of sPLA2-X in the carotid endarterectomy samples from the BiKE study were very low, raising doubt about the level of expression of sPLA2-X in plaques.

The meta-analysis of R38C and CHD risk, using data from EPIC-Norfolk, NPHSII and the two studies published by Gora et al, AtheroGene and SIPLAC [16], providing in total 3667 cases and 4945 controls, yielded a summary OR of 0.94 (95%CI 0.75-1.19). Thus, there was no association of this functional variant with CHD. An interesting recent finding has suggested that sPLA2-X may *not* be pro-atherogenic. Ait-Oufella et al [35] reported that, contrary to expectation, *Ldlr-/-* recipients mice receiving bone marrow derived macrophage from *Pla2g10-/-* donor mice displayed *enhanced* atherosclerosis, suggesting that sPLA2-X may in fact behave in an anti-atherogenic manner *in vivo*, raising questions about the difference of *in vitro* and *in vivo* studies [35].

Causality of a biomarker in the development of cardiovascular disease is a prerequisite for the clinical efficacy of a drug therapy targeting that biomarker. Drugs are often developed based on epidemiological observational data, despite the fact that association does not imply causality. Mendelian randomization (MR) makes use of genetics to overcome the two pitfalls of observational data, namely confounding and reverse causation. We have used this approach with both sPLA2-IIa [24] and sPLA2-V [25] and our analyses indicate that neither of these sPLA2 enzymes are causal of CHD. However, the gold standard to test causality of a biomarker is the randomized control trial. Varespladib is a selective inhibitor of sPLA2 reported to have inhibitory effect against sPLA2 isoforms [36] with the aim of reducing cardiovascular events. However, the recent results from the phase III trial of the varespladib, VISTA 16, show that in the setting of acute coronary syndrome, varespladib had no impact on the primary composite

outcome (a composite of cardiovascular mortality, nonfatal myocardial infarction, nonfatal stroke, or unstable angina) and increased the risk of MI [37]. These results validated our *PLA2G2A* MR [24]. One of the suggestions put forward by Nicholls et al [37], concerning the failure of the inhibitor was that it might not only inhibit the pro-atherogenic effects of sPLA2-IIA and –V. Referring to, but taking into account the results from Ait-Oufella et al showing anti-atherogenic effects of sPLA2-X [35], the suggestion is that the inhibitor may at the same time reduce these anti-atherogenic effects of sPLA2-X.

One limitation of our study is that the STRAM method used to select *PLA2G10* tSNPs may now be outdated by more recent algorithms and data from genome wide association studies, and many of the tSNPs we identified had very low MAFs, also in agreement with those identified by Gora et al by sequence analysis [16]. R38C, although a functional SNP, has a MAF of only 0.03. Secondly our meta-analysis is powered to detect an odds ratio of CHD risk of 1.34 with 80% power at the 5% significance level, whereas the result suggests if an effect was present, it would be of smaller magnitude (likely to lie between 0.77 and 1.22). The low minor allele frequency of the R38C suggests that a larger cohort would be needed before firm conclusions can be drawn about the contribution of sPLA2-X to sPLA2 activity. The study is 90% powered to detect a medium effect size (0.5 SDs of sPLA2 activity), but to detect a small effect size (0.2 SDs) would require 5116 participants in order to have 80% power at the 5% significance level. The observed effect size is just 0.01 SDs with a confidence interval of -0.16 to 0.24. Thirdly, there has been the incorrect notion that all or most mammalian sPLA2s are induced during inflammation and can exist in the plasma. However, this is possibly only true for sPLA2-IIA and not the other sPLA2s [9,38], making it unlikely that sPLA2-X is a component of the plasma measure of sPLA2 activity, as supported by our results.

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Taking these results altogether, the rank order of the hydrolytic potency of various human sPLA2s, as evaluated by Electrospray Ionisation Mass Spectrometry is X > V > III > IIF > IIA, IIE for both LDL and HDL. This order appears to roughly correlate with the ability of these sPLA2s to interact with PC-rich vesicles and with PC-rich cellular plasma membranes [8,9]. However the expression level of sPLA2-IIA is considerably higher than those of other sPLA2s and it is the only sPLA2 isoform detected in the circulation of mammals (except mice) [9,38].

In conclusion, we conducted a genetic study to test the hypothesis that sPLA2-X contributes to plasma measures of sPLA2 activity. Our results do not support this, and may reflect the current evidence that sPLA2-X is not active in the plasma. Furthermore our results do not support the hypothesis that sPLA2-X is associated with risk of CHD.

Conflict of Interest Disclosures: None

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Table 1: Baseline characteristics of EPIC-Norfolk by sPLA2 activity tertiles

sPLA2 activity (nmol/min per mL)	Tertile 1 <4.041 N=891	Tertile 2 4.042-4.856 N=890	Tertile 3 >4.856 N=887	P value (trend)
Variable	Mean (sd)	Mean (sd)	Mean (sd)	
Age (years)	64.31 (7.73)	64.38 (7.88)	65.60 (7.53)	4.1×10^{-04}
BMI (kg/m^2)	25.93 (3.36)	26.44 (3.56)	27.10 (3.64)	3.01×10^{-12}
SBP (mm Hg)	138.1 (17.7)	138.6 (18.2)	142.3 (18.0)	1.44×10^{-06}
DBP (mm Hg)	82.9 (11.0)	83.2 (11.1)	85.0 (11.7)	7.70×10^{-05}
Cholesterol (mmol/L)	5.85 (1.01)	6.23 (1.07)	6.72 (1.24)	7.47×10^{-58}
LDL-C (mmol/l)	3.91 (0.93)	4.16 (0.98)	4.39 (1.09)	3.09×10^{-22}
HDL-c (mmol/l)	1.31 (0.36)	1.28 (0.36)	1.22 (0.34)	1.50×10^{-08}
Triglycerides (mmol/l)	1.36 (0.54)	1.72 (0.76)	2.43 (1.21)	9.09×10^{-143}
CRP (mg/L)	1.53 (1.82)	1.71 (1.96)	2.35 (2.67)	6.01×10^{-15}
sPLA2 mass (ng/mL)	7.92 (4.70)	8.75 (5.18)	10.48 (6.32)	8.42×10^{-23}
sPLA2 activity (nmol/min/mL)	3.49 (0.47)	4.43 (0.24)	5.82 (1.05)	-

All results are geometric mean (approx sd) except for age and LDL where results are presented as mean (SD)

Table 2: Associations of *PLA2G10* gene variations with sPLA2 activity

SNP		EPIC		
		N	sPLA2 activity geometric mean, (nmol/min per mL) (approximate SD)	MAF
rs72546340	GG	2353	4.49 (1.12)	0.03
	GC	148	4.59 (1.05)	
	CC	2	3.97 (.09)	
	B (se) [*]		0.018 (0.020)	
	P value [*]		0.39	
	B (se) [†]		0.017 (0.020)	
	P value [†]		0.40	
	Genotype success rate (%)		94.5	
rs72546339	CC	2315	4.49 (1.13)	0.05
	CA	251	4.43 (0.96)	
	AA	9	4.25 (1.05)	
	B (se) [*]		-0.016 (0.015)	
	P value [*]		0.31	
	B (se) [†]		-0.014 (0.015)	
	P value [†]		0.35	
	Genotype success rate (%)		97.2	
rs4003228 (R38C)	CC	2113	4.47 (1.12)	0.02
	CT	87	4.41 (0.96)	
	TT	1	5.95 (0)	
	B (se) [*]		-0.007 (0.027)	
	P value [*]		0.80	
	B (se) [†]		-0.010 (0.026)	
	P value [†]		0.71	
	Genotype success rate (%)		94	
rs4003232	TT	1435	4.47 (1.06)	0.21
	TC	748	4.49 (1.19)	
	CC	106	4.54 (1.22)	
	B (se) [*]		0.005 (0.009)	
	P value [*]		0.55	
	B (se) [†]		0.005 (0.009)	
	P value [†]		0.57	
	Genotype success rate (%)		97.3	

All results are represented as geometric mean (approximate SD).

[*] unadjusted b coefficient and standard error ($\log_e$ scale) from additive model with p values for trend.

[†] age and gender adjusted b coefficient and standard error ($\log_e$ scale) from additive model with p value for trend.

MAF: mean allele frequency

Figure Legends:

Figure 1: Meta-analysis of *PLA2G10* R38C (rs4003232) and risk of CHD

Figure 2: Expression value of all genes in the aortic adventitia, results from the ASAP study. *PLA2G10* expression in aortic adventitia compared to *PLA2G2A* and *PLA2G5*. Each dot shows one gene, sorted by expression-rank on X-axis and log10-expression on Y-axis. The red-crosses indicate the expression of Y-chromosome genes in female samples and serves as basis for approximate absence cutoff, i.e. the horizontal line.
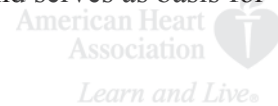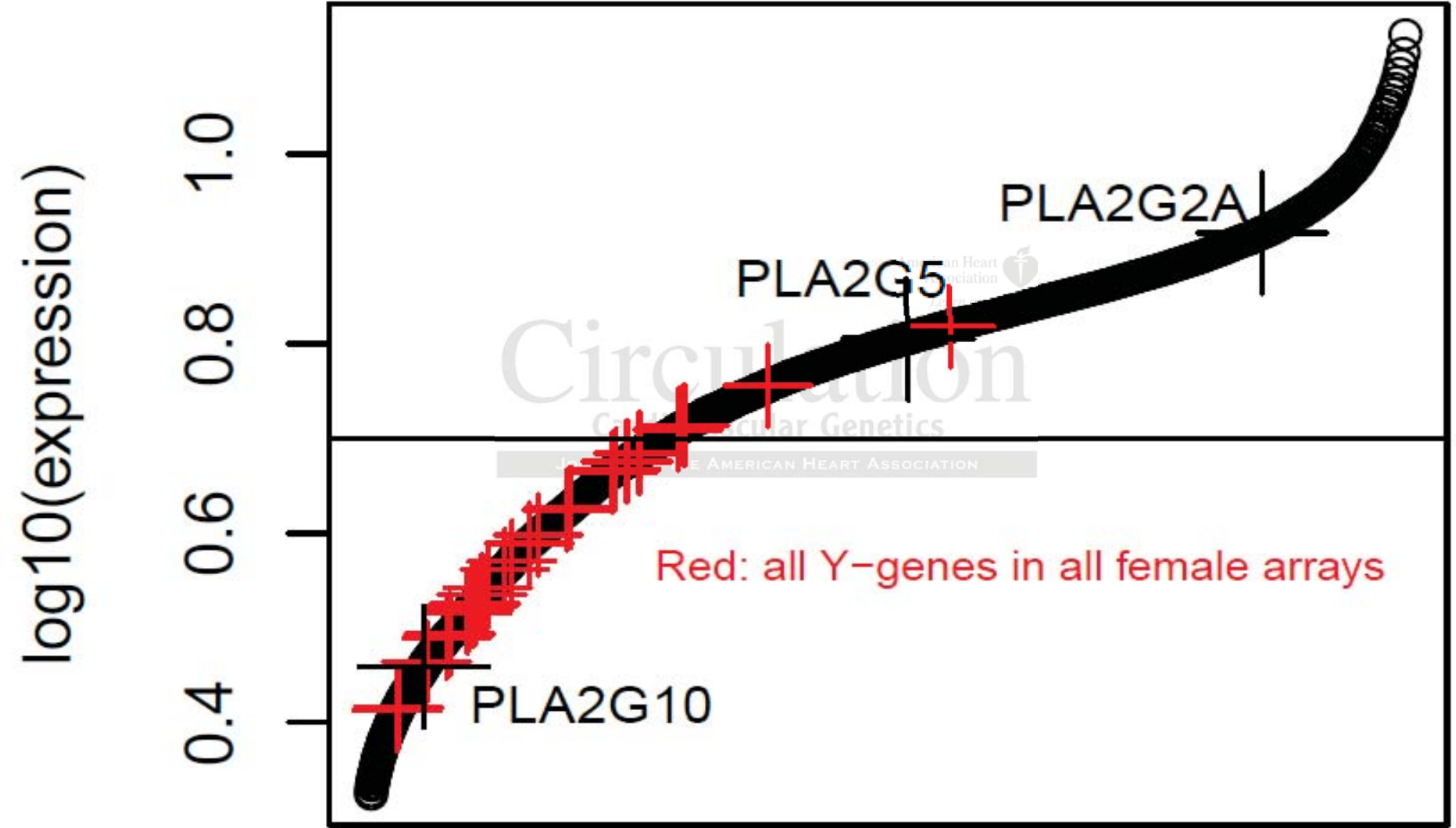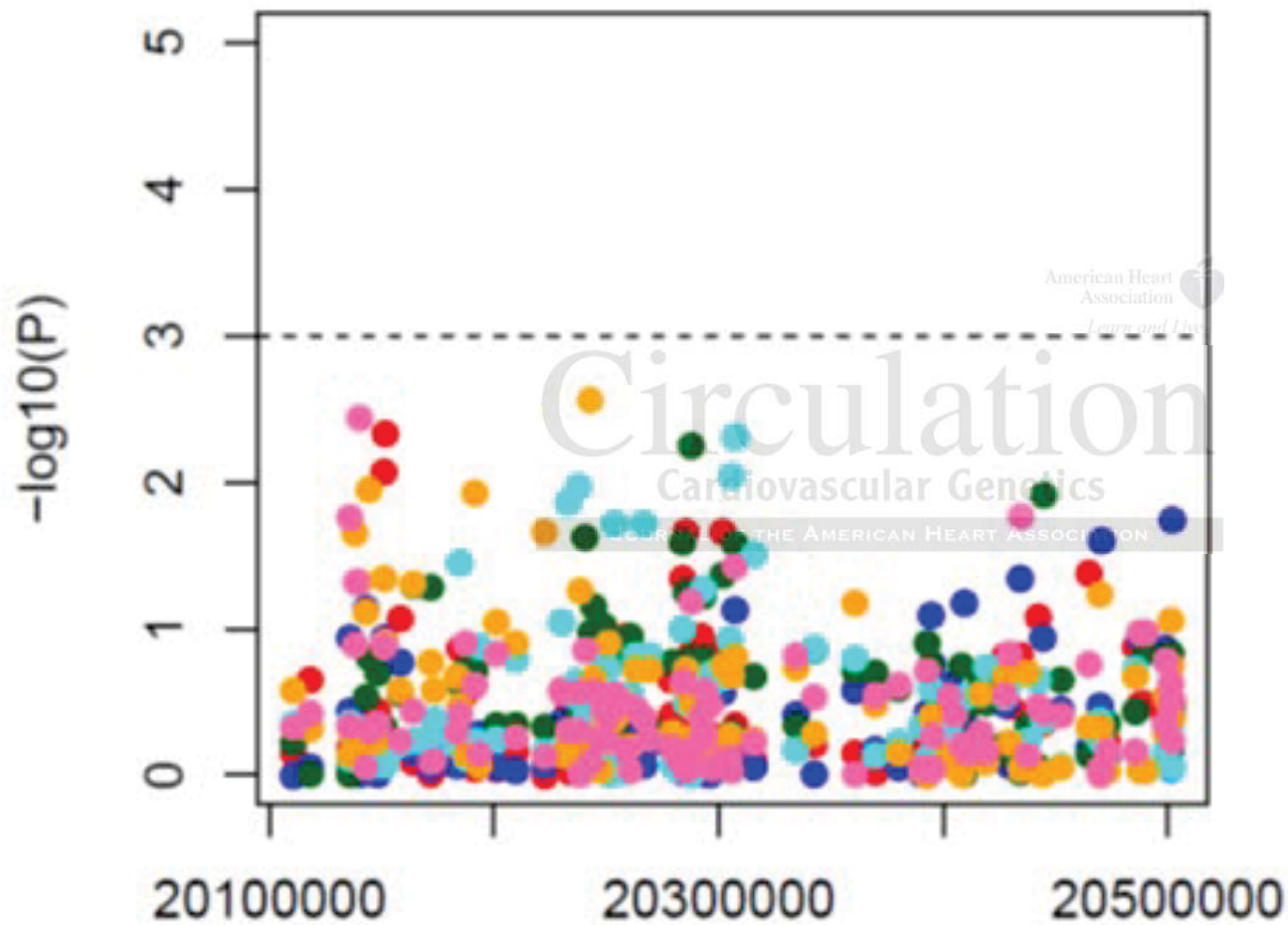
Figure 3: Results from the ASAP study eQTL effect of all SNPs within 200 kb of *PLA2G10* in 6 different tissues. Each dot indicates the association of one SNP to *PLA2G10*, in tissue type as indicated by colour-code. X-axis show genomic location and Y-axis shows -log10(P) value of eQTL effect. Horizontal dashed line indicates P=0.001. No SNPs were significant at a false-discovery rate of 0.05.

Study	N controls/N cases		OR (95% CI)
NPHSII	2318/272		1.25 (0.77, 2.03)
EPIC	1826/1783		1.01 (0.73, 1.40)
SIPLAC	317/312		0.60 (0.26, 1.38)
Atherogene	484/1299		0.79 (0.48, 1.32)
Overall (I-squared = 0.5%, p = 0.389)			0.97 (0.77, 1.22)
			p=0.78

.5   1   2
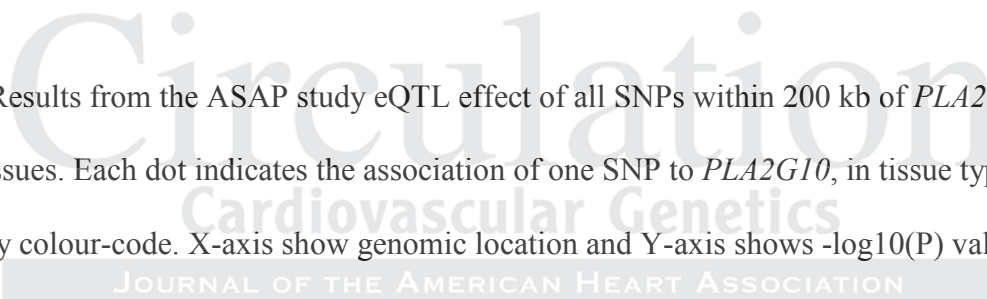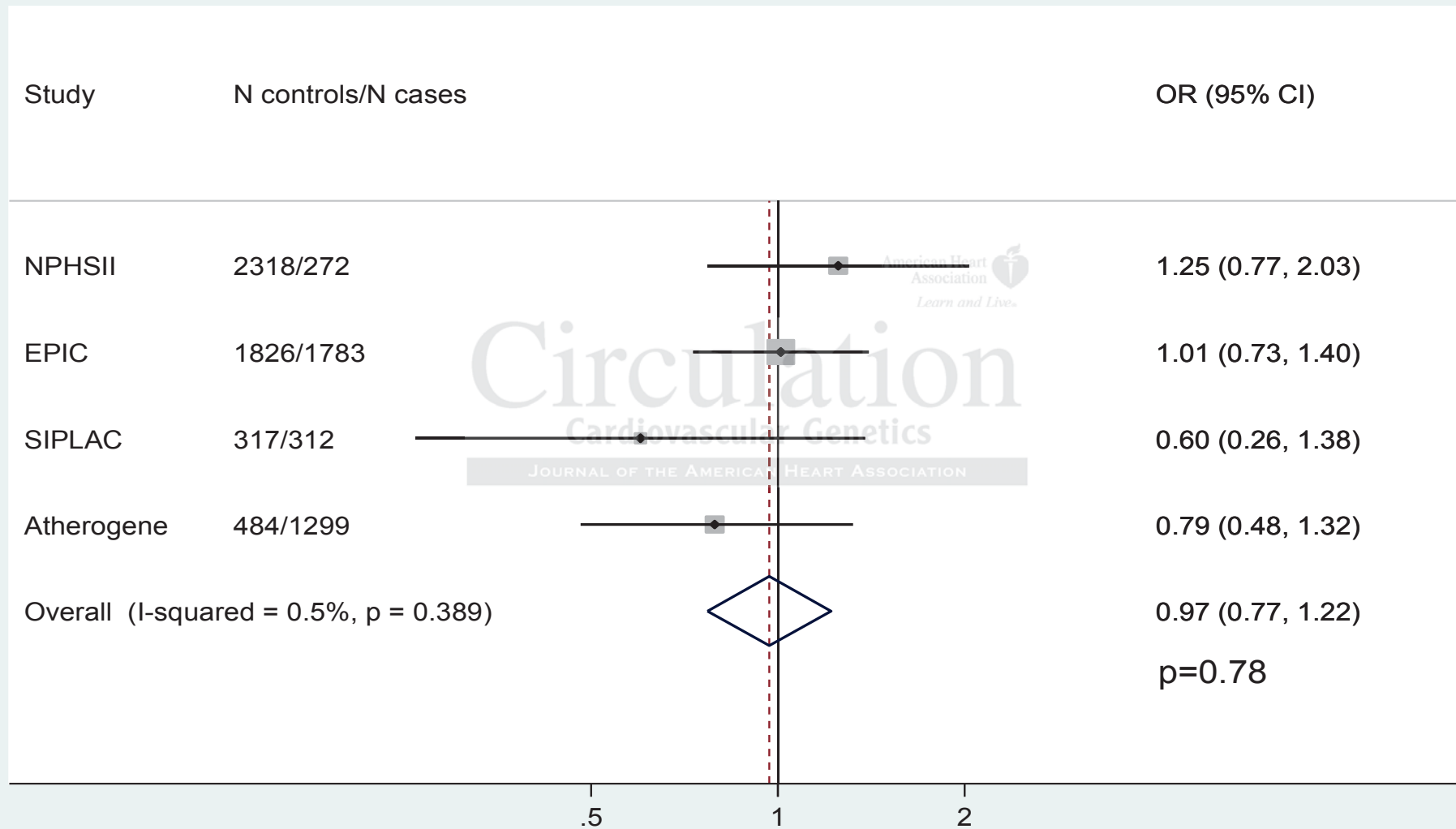
Supplemental Material.

Supplemental table. Intermediate traits according to *PLA2G10* SNPs in the EPIC-Norfolk nested case control cohort.

rs72546340	N	Age (years)	BMI (kg/m^2)	SBP (mm Hg)	DBP (mm Hg)	CHOL (mmol/L)	CRP (mg/L)	sPLA2 mass (ng/mL)
GG	2394	64.7 (7.7)	26.5 (3.5)	139.5 (17.9)	83.7 (11.2)	6.26 (1.16)	1.81 (2.11)	8.93 (5.32)
GC	150	65.2 (7.9)	26.6 (3.7)	139.2 (19.2)	82.6 (11.7)	6.23 (1.16)	1.75 (2.15)	10.22 (6.76)
CC	2	65.5 (3.5)	23.4 (3.8)	163.5 (0)	98.5 (0)	4.54 (1.68)	0.59 (0.14)	4.20 (0.28)
P value [*]		0.646	0.91	1.00	0.35	0.42	0.52	0.03
rs72546339								
CC	2353	64.8 (7.7)	26.5 (3.5)	139.6 (18.0)	83.7 (11.3)	6.25 (1.15)	1.84 (2.16)	8.98 (5.43)
CA	256	64.4 (8.1)	26.3 (3.6)	139.4 (17.9)	83.2 (11.3)	6.23 (1.20)	1.62(1.93)	9.09 (5.43)
AA	9	61 (9.6)	27.1 (3.6)	144.9 (23.0)	86.5(14.1)	6.16 (1.50)	1.68 (1.45)	9.80 (6.98)
P value [*]		0.25	0.59	0.87	0.76	0.71	0.12	0.66
rs4003228								
CC	3466	64.5 (7.9)	26.6 (3.6)	139.7 (18.0)	83.7 (11.2)	6.26 (1.16)	1.82 (2.15)	8.91 (5.39)
CT	141	64.3 (7.8)	26.6 (3.5)	140.1 (18.3)	83.8 (11.7)	6.22 (1.02)	1.82 (2.27)	8.62 (5.88)
TT	2	64.5 (0.7)	27.9 (2.4)	124.6 (0)	72.8 (7.8)	7.98 (0.71)	3.90 (0)	12.49 (0)
P value [*]		0.86	0.77	P=0.96	0.86	0.91	0.91	0.71
rs4003232								
TT	2324	64.4 (8.1)	26.6 (3.7)	139.5 (17.8)	83.7 (11.2)	6.27 (1.18)	1.93 (2.25)	9.10 (5.58)
TC	1229	64.4 (7.7)	26.5 (3.6)	140.1 (18.3)	83.8 (11.2)	6.25 (1.14)	1.70 (2.01)	8.76 (5.37)
CC	192	65.9 (7.3)	26.3 (3.7)	140.1 (17.7)	83.4 (10.9)	6.38 (0.98)	1.70 (1.88)	8.17 (4.35)
P value [*]		0.09	0.15	0.35	0.93	0.66	0.02	0.04

rs72546340	N	HDL (mmol/L)	TG (mmol/L)
GG	2394	1.27 (0.36)	1.78 (0.90)
GC	150	1.23 (0.37)	1.87 (0.94)
CC	2	1.35 (0.07)	0.99 (0.49)
P value [*]		0.17	0.43
rs72546339			
CC	2353	1.26 (0.36)	1.78 (0.90)
CA	256	1.30 (0.36)	1.78 (0.92)
AA	9	1.24 (0.28)	2.03 (0.61)
P value [*]		0.19	0.81
rs4003228			
CC	3466	1.28 (0.36)	1.77 (0.89)
CT	141	1.26 (0.32)	1.84 (0.96)
TT	2	1.15 (0.07)	2.90 (0.14)
P value [*]		0.40	0.23
rs4003232			
TT	2324	1.28 (0.36)	1.77 (0.90)
TC	1229	1.29 (0.36)	1.76 (0.90)
CC	192	1.35 (0.37)	1.76 (0.92)
P value [*]		0.02	0.74

[*] unadjusted p value for trend

PLA2G10 Gene Variants, sPLA2 Activity and Coronary Heart Disease Risk

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