SUPPLEMENTAL MATERIAL:

PLA2G7 genotype, Lp-PLA2 activity and coronary heart disease risk in 10,494 cases and 15,624 controls of European ancestry

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Supplemental methods

Lp-PLA2 activity, cardiovascular traits and risk of coronary heart disease

To evaluate the associations of Lp-PLA2 activity with cardiovascular traits, quartiles of log-Lp-PLA2 activity were generated within NPHS-II and EPIC-Norfolk, and the unadjusted mean with its respective standard deviations were obtained for the following cardiovascular traits: age (years), body mass index (kg/m2), systolic blood pressure (mm Hg), total-cholesterol (mmol/L), LDL cholesterol (mmol/L), HDL-cholesterol (mmol/L), log-triglycerides (mmol/L), log-C-reactive protein (mg/dL) and alcohol consumption (units/week). Then using the bottom quartile (Q1) as the reference category, mean difference for each quartile was obtained and results were pooled across studies using random model effects. For these associations, only individuals without known coronary heart disease at the time of measuring the Lp-PLA2 activity were included, to reduce possible reverse causation.

Association between Lp-PLA2 activity and incident coronary heart disease was assessed using Cox proportional hazards regression. Hazard ratios and 95% confidence intervals (CI) were obtained for each quartile, using the bottom quartile as the reference group within the NPHS-II and EPIC-Norfolk studies. Then hazard ratios (and ORs for EPIC-Norfolk) and their 95%CI for each quartile were pooled across the two studies to obtain a summary effect for each quartile. A progressive adjustment for potential confounders were conducted as follows: model-1 includes age (continuous), gender and practice (for NPHS-II) and enrolment date (for EPIC); model-2: adjusted for variables in previous model-1 plus body mass index (continuous), smoking (current-ex vs. never), diabetes (yes vs. no), systolic blood pressure (continuous), C-reactive protein (continuous), fibrinogen (continuous), alcohol (continuous as: units/week). Model 3: Adjusted for variables in model-2 plus: total-cholesterol (continuous), triglycerides (continuous) Apo-A1 (continuous), Apo-B

(continuous). If Apo-A1 was not available HDL-cholesterol (continuous) was used. Similarly, if Apo-B was not available LDL-cholesterol (continuous) was used.

PLA2G7 gene variants and Lp-PLA2 activity levels

To evaluate the effect of the different *PLA2G7* variants on levels of Lp-PLA2 activity, 6094 individuals from 7 studies with available DNA and Lp-PLA2 activity levels were studied; See Supplementary Table 1. The unadjusted mean values of the log-LP-PLA2 activity with its respective standard deviation were obtained for each genotype from the seven PLA2G7 variants. Mean differences in log-Lp-PLA2 (log-ratio) for each genotype were obtained and results were pooled across studies using random model effects.

PLA2G7 gene variants and cardiovascular traits

From the previous analyses, the gene-variant exhibiting the largest genotype effect on the Lp-PLA2 activity was then used as proxy of the likely effect on cardiovascular traits to be observed when using an Lp-PLA2 inhibitor in a clinical trial (i.e. darapladib). Such effect is expected to be proportional to the magnitude of the effect that the gene-variant exhibit on the Lp-PLA2 activity levels. For this, unadjusted mean values of cardiovascular traits with their respective standard deviations were obtained for each genotype. Mean differences in the cardiovascular traits for each genotype were calculated and then data were pooled across studies using random effect models. For cohort studies with repeated measures of some continuous traits, the baseline or first available measure (in the case of missing data) was utilized. Cardiovascular traits included here are those evaluated in the observational component, in order to allow a more like-with-like comparison.

PLA2G7 gene variants and risk of coronary heart disease

To evaluate the effect of the PLA2G7 variants on the risk of coronary heart disease, a total of 9 studies including up to 8506 cases were used. For each gene-variant, using individuals homozygous for the common allele as the reference group a measure of association was obtained for each genotype comparison. Specifically, log-odds ratio and its standard error for case-control studies and log-hazard ratio with its standard error for cohort studies were obtained for each genotype comparison from each gene-variant analysed. All measures of effect were adjusted by age (continuous), and gender. Enrolment date or practice centre were also included if relevant to each study. Assuming equivalence of log-odds ratio with log-hazard ratios, these measures of effect were then pooled across studies using random effect models, from now on this will be referred to as odds ratios. Initially, pooled odds ratios were obtained separately according to the outcome reported; coronary artery disease (determined by coronary angiography) vs. coronary heart disease. If no evidence of heterogeneity was observed between the groups a summary odds ratio was then calculated.

Supplemental Table 1. Details of the 12 studies used in the current analysis

Study Name	Study Design (location)	Sampling frame	Main selection criteria	Primary analysis		
				Genotype& LpPLA2 level (No subjects)	**Genotype & CV Traits** (No subjects)	**Genotype & CHD** (No subjects)
NPHS-II	Population-based Prospective Cohort (United Kingdom)	All men aged between 50 to 63 years registered with 9 primary care practices.	Individuals were excluded if had: Pre-existing cardiovascular disease (CHD or stroke), Coronary surgery or malignant disease, or were taking Aspirin or anticoagulant .	Yes (2099)	Yes (2386)	Yes (2706)
EPIC-Norfolk	population-based Prospective cohort: a nested case-control was used for this analysis (United Kingdom)	All men and women aged between 40 and 79 years registered in primary care practices.	Participants with prevalent CHD or stroke were excluded. CHD is defined as ICD9 410-414 or ICD10 I20-I25. Controls were study participants who remained free of any cardiovascular disease during follow-up and were matched to each case by sex, age (within 5 years), and time of enrolment (within 3 months).	Yes (1485 controls)	Yes (1969 controls)	Yes (4350)
CYPRUS	Prospective cohort (Cyprus)	All men and women > 40 years identified through the population list at the Mayor's office	To be included subjects had to be inhabitants from Pedoulas or Nissou villages in Cyprus or their relatives living in major cities.	Yes (633)	Yes (638)	Yes (731)
HIFMECH	Case-control (UK, Sweden, France, Italy)	Cases: male MI survivors. Controls: men matched by age and regional areas.	Subjects with Familial hypercholesterolaemia, or Insulin-dependent diabetes mellitus were excluded.	No	Yes (565 controls)	Yes (1093)
THROMBO	Prospective cohort (United States of America)	Consecutive series of post-infarction subjects	Individuals with diabetes were excluded.	Yes (524)	Yes (530)	No

Study Name	Study Design (Location)	Sampling frame	Main selection criteria	Primary analysis		
				Genotype & LpPLA2 level (No subjects)	**Genotype & CV Traits** (No subjects)	**Genotype & CHD** (No subjects)
Athero*Gene*	Case-control (Germany)	Cases: patients with stable angina attending for diagnostic coronary angiography Controls : men and women recruited either from GP's offices (routine check-up) or by newspaper announcement	Cases were excluded if there was evidence of significant co-morbidities: valvular heart disease, cardiomyopathy, malignant disease or febrile condition. Controls: individuals with evidence, from the interview, on atherosclerosis or with pathological ECG were excluded	Yes (468 controls)	Yes (484 controls)	Yes (1304)
Whitehall-II	Prospective cohort (United Kingdom)	All civil servants in 20 departments at Whitehall	To be included individuals had to have a job-contract in a London-based civil service department	No	Yes (5154)	Yes (5611)
UDACS	Case-control (United Kingdom)	Consecutive subjects recruited to diabetic clinic Cases: men and women with diabetes and cardiovascular disease. Controls: men and women with diabetes but without cardiovascular disease.	To be included individuals had to have T2DM according to WHO criteria	Yes (235 controls)	Yes (194 controls)	No
Southampton Atherosclerosis Study	Collection of cases with coronary stenosis (United Kingdom)	Consecutive patients undertaking diagnostic and interventional coronary angiography	N/A	No	No	Yes (1091)
LURIC	Case-control (Germany)	Men and women hospitalized for coronary angiography.	N/A	Yes (728 controls)	Yes (730 controls)	Yes (2581)
Edinburgh Artery Study	Population-based prospective cohort (United Kingdom)	A random sample stratified by age and selected from 10 general practises throughout the city of Edinburgh.	Subjects were excluded if they were unfit to participate (e.g. due to mental illness)	No	Yes (894)	Yes (895)
WTCCC1 CHD Cases and joint National Blood Service and 1958 Birth Cohort Controls	Case Control (United Kngdom)	Recruitment of cases on a national basis by direct approach to public via media or mailing family physicians with	CAD cases with validated history of either MI or coronary revascularisation (CABG or PCA) under the age of 66, verified through hospital or primary care physician records	No	No	Yes (4992)

Supplemental Table 2. List of tagging-SNPs within the *PLA2G7* gene evaluated in the NPHS-II study. 11= Homozygous for the common allele. 12= Heterozygous and 22 = Homozygous for the rare allele. Minor and major alleles are described in supplementary methods.

	genotype	N	Mean Lp-PLA2	Standard deviation	N	P value (additive)
	11	637	48.06	15.73	637	
rs974670	12	862	48.66	16.03	862	
	22	309	49.32	15.31	309	0.237619
	11	1195	48.1	15.07	1195	
rs1051931	12	550	49.16	16.72	550	
	22	64	51.83	20.61	64	0.044157
	11	806	49.32	16.73	806	
rs2216465	12	800	48.48	15.24	800	
	22	201	45.89	13.77	201	0.009986
	11	1592	48.72	15.95	1592	
rs1805018	12	82	46.61	13.53	82	
	22	1	60.37		1	0.313898
rs17288905	11	1569	48.43	15.48	1569	
	12	229	49.36	17.81	229	
rs16874962	11	1593	48.56	15.78	1593	
	12	210	48.53	16.07	210	
	22	6	46.63	16.46	6	0.906231
rs12195701	11	1117	48.84	16.13	1117	
	12	610	48.28	15.52	610	
	22	82	46.67	13.31	82	0.220458
rs10948300	11	1097	48.92	16.16	1097	
	12	624	48.27	15.39	624	
	22	87	46.33	13.72	87	0.138449
rs12528807	11	1524	48.61	16.09	1524	
	12	274	48.31	14.31	274	
	22	11	46.8	12.24	11	0.683216
rs1421368	11	1466	48.48	15.64	1466	
	12	329	48.89	16.52	329	
	22	13	50.9	13.36	13	0.557218
rs1421378	11	647	49.68	16.3	647	
	12	873	48.32	15.5	873	
	22	287	46.86	15.38	287	0.008984

Supplementary Table 3. Numbers of individuals included from each study for the gene-traits associations.

rs_number	NPHS-II	UDACS	EPIC-Norfolk	THROMBO	Cyprus	Atherogene	LURIC	WH-II	HIFMECH	EAS
rs974670										
LP-PLA2 activity	2099	231		522	633	458				
rs1805017										
LP-PLA2 activity	2085	235	1479	471	628	468	728			
rs9381475										
LP-PLA2 activity	2080	230	1477	474	625	464				
rs10948300										
LP-PLA2 activity	1808	188	1477							
rs2216465										
LP-PLA2 activity	1807	188	1476							
rs1421378										
LP-PLA2 activity	1807	231	1485	520	631	467				
rs1051931										
Age	2386	194	1969	530	638	484	730	5154	565	894
LP-PLA2 activity	1809	188	1453	524	632	468	727			
BMI	2383	191	1969	530	638	484	730	5146	566	894
Systolic BP	2384	193	1969		623			5147	564	893
Total-cholesterol	2371	192	1969		638	484		5129	547	893
LDL	1533	186	1969		638	484		4693		888
Apo-B	2072		1272	511	638	244			547	
HDL	1619	192	1969		638	484		4765		888
Apo-A	2072		1200	522	638	244				
Log-TGL	2373	192	1969		638			4782	547	893
Log-CRP	2000	194	1348	491	609	327		4529	544	603
Fibrinogen	2377		1880	529	614	244		4484	527	875
Alcohol	2386		1969		637				555	

Supplementary Table 4. Summary hazard ratio (HR) of coronary heart disease risk by quartiles of Lp-PLA2 activity in NPHS-II and EPIC-Norfolk, according to different levels of adjustment.

	Model-1	**Model-2**	**Model-3**
Comparison	**Hazard ratio (95% CI)**	**Hazard ratio (95% CI)**	**Hazard ratio (95% CI)**
Q1	Reference	Reference	Reference
Q2	1.31 (1.06, 1.63)	1.33 (1.05, 1.67)	1.23 (0.97, 1.57)
Q3	1.46 (1.18, 1.80)	1.44 (1.14, 1.81)	1.18 (0.92, 1.50)
Q4	1.61 (1.31, 1.99)	1.56 (1.24, 1.96)	1.17 (0.91, 1.51)

Variables included in the different models were as follows. Model-1 includes age, sex, enrolment date & practice. Model-2 includes variables from Model-1 plus BMI, smoking, diabetes, systolic BP, CRP, fibrinogen & alcohol. Model-3 includes variable from model-2 plus total-cholesterol, triglycerides, Apo-A & Apo-B.

Supplemental Figure 1

Haploview map of PLA2G7 and the tSNPs used to genotype NPHS-II. Those boxed were included in the list of SNPs used to genotype the other studies.