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Eight genetic loci associated with variation in lipoprotein-associated phospholipase A2 mass and activity and coronary heart disease: meta-analysis of genome-wide association studies from five community-based studies

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Aims Lipoprotein-associated phospholipase A2 (Lp-PLA2) generates proinflammatory and proatherogenic compounds in the arterial vascular wall and is a potential therapeutic target in coronary heart disease (CHD). We searched for genetic loci related to Lp-PLA2 mass or activity by a genome-wide association study as part of the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium.

Methods and results In meta-analyses of findings from five population-based studies, comprising 13 664 subjects, variants at two loci (*PLA2G7*, *CETP*) were associated with Lp-PLA2 mass. The strongest signal was at rs1805017 in *PLA2G7* [$P = 2.4 \times 10^{-23}$, log Lp-PLA2 difference per allele (beta): 0.043]. Variants at six loci were associated with Lp-PLA2 activity (*PLA2G7, APOC1, CELSR2, LDL, ZNF259, SCARB1*), among which the strongest signals were at rs4420638, near the *APOE–APOC1–APOC4–APOC2* cluster [$P = 4.9 \times 10^{-30}$; log Lp-PLA2 difference per allele (beta): −0.054]. There were no significant gene–environment interactions between these eight polymorphisms associated with Lp-PLA2 mass or activity and age, sex, body mass index, or smoking status. Four of the polymorphisms (in *APOC1*, *CELSR2*, *SCARB1*, *ZNF259*), but not *PLA2G7,* were significantly associated with CHD in a second study.

Conclusion Levels of Lp-PLA2 mass and activity were associated with *PLA2G7,* the gene coding for this protein. Lipoprotein-associated phospholipase A2 activity was also strongly associated with genetic variants related to low-density lipoprotein cholesterol levels.

Keywords Genome-wide association • Inflammation • Lipoprotein-associated phospholipase A2

Introduction

Lipoprotein-associated phospholipase A2 (Lp-PLA2) is a 45.4-kDa calcium-independent member of the phospholipase A2 family, which is secreted by leucocytes[1] and has been detected in rabbit and human atherosclerotic lesions.[2] In the bloodstream, two-thirds of Lp-PLA2 circulates primarily bound to LDL; the remaining third is distributed between HDL and VLDL. Circulating Lp-PLA2 can be measured by different assays ascertaining mass or activity of Lp-PLA2. However, there is only moderate correlation between mass-based and activity-based measurements of Lp-PLA2 ($r = 0.51$),[3] and the independent role of these two measures in cardiovascular disease (CVD) is unclear.

Several lines of evidence suggest that Lp-PLA2 is associated with the development of atherosclerotic disease. Lipoprotein-associated phospholipase A2 generates pro-inflammatory and pro-atherogenic compounds in the arterial vascular wall. A large meta-analysis with almost 80 000 participants in 32 prospective studies showed that high levels of Lp-PLA2 mass and activity were associated with the risk for coronary heart disease (CHD), stroke, and cardiovascular mortality.[3] Therefore, Lp-PLA2 might represent an emerging biomarker for improved cardiovascular risk assessment in clinical practice and a potential therapeutic target for primary or secondary prevention of CVD.

Although familial factors explain about one-half and one-quarter of the variance in Lp-PLA2 activity and mass, respectively,[4,5] few genetic determinants of Lp-PLA2 have been identified so far.[6] Therefore, to better understand genetic control of Lp-PLA2, we investigated genetic loci related to Lp-PLA2 mass or activity by conducting genome-wide association (GWA) analyses in five population-based studies, as part of the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium.[7]

Methods

For more detailed information on the Methods, see Supplementary material online.

Study data for meta-analysis

We used data from five community-based cohorts in the USA and Europe: Atherosclerosis Risk in Communities (ARIC) Study, Cardiovascular Health Study (CHS), Framingham Heart Study (FHS), Rotterdam Study (RS), and Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA)/Cooperative Health Research in the Region of Augsburg Study (KORA). Baseline clinical and demographic characteristics were assessed at the time of cohort entry for CHS, KORA, and RS and at the time of biomarker collection for ARIC and FHS. All participants gave written informed consent, including consent for genetic studies. All studies received approval from local ethical oversight committees.

Associations between significant single nucleotide polymorphisms (SNPs) and CHD/coronary artery disease (CAD) were assessed in the Coronary Artery Disease Genome-Wide Replication and Meta-Analysis (CARDIoGRAM) consortium data. CARDIoGRAM includes over 22 000 cases with CAD, MI, or both and over 60 000 controls from all published and several unpublished genome-wide association studies (GWAS) in individuals of European descent.[8]

Lipoprotein-associated phospholipase A2 mass concentrations were measured in ARIC, CHS, FHS, and KORA using a commercially available sandwich enzyme immunoassay (PLAC® test; diaDexus, Inc., San Francisco, CA, USA). Lipoprotein-associated phospholipase A2 activity was measured in CHS, FHS, and RS on microtiter plates by colourimetric or radioactive substrate methods (diaDexus CAM Kit, diaDexus, Inc., San Francisco, CA, USA or Perkin Elmer Life Sciences, Inc., Waltham, MA, USA).

Genotyping was performed in each cohort using high-density SNP marker platforms. Genotypes were imputed to ~2.5 million

HapMap SNPs (platform and genetic analysis details provided in Supplementary material online).

Statistical analyses

Associations between genotype and Lp-PLA2 mass and activity were analysed separately within each cohort, using linear regression of natural log-transformed phenotype on number (or imputed dose) of reference alleles, i.e. an additive model (for more details, see Supplementary material online). All analyses were adjusted for age, sex, and if applicable, recruitment site. To assess whether classical cardiovascular risk factors confounded the associations, analyses were additionally adjusted for diabetes, lipid-lowering medication, antihypertensive treatment, aspirin intake $\geq$3 per week, current smoking, hormone replacement therapy (HRT; coded men, women without HRT, women with HRT), body mass index (BMI), systolic blood pressure, diastolic blood pressure, triglycerides, waist circumference, HDL-C, LDL-C, and prevalent CVD. For the most significant SNPs, we also analysed interactions between genetic variant and age, sex, BMI, or smoking status.

To combine results across cohorts, we performed an inverse variance–weighted meta-analysis using the software METAL,[9] which was specifically developed for meta-analysing GWAS results. A meta-analysis is a statistical method that combines analyses from different independent studies to give an overall effect measure. In the case of meta-analysing different GWAS, this approach increases the power to detect significant genetic variants compared with analysing each GWAS alone.[10] Cohort-specific standard errors were adjusted using genomic control.[11] In GWAS, we chose $P = 5 \times 10^{-8}$ as the threshold for significance[12] (see Supplementary material online). To determine whether the multiple SNPs associated in the respective region are due to linkage disequilibrium (LD) with the top SNP or if there are multiple independent signals, we performed a meta-analysis based on models adjusted additionally for the SNPs with the smallest *P*-values.

Results

Sample size, demographics, and laboratory characteristics of each cohort are presented in *Table 1*. Median values for Lp-PLA2 mass (241.8 ng/mL in FHS to 413.0 ng/mL in ARIC) and activity (38.2 nmol/min/mL in CHS to 153.9 nmol/min/mL in FHS) varied substantially across cohorts, but much of these differences may be explained by the different assays used.

Genome-wide association of lipoprotein-associated phospholipase A2 mass and activity

The meta-analysis included 2 661 766 SNPs from one or more studies. Genomic control (λ_{gc}) parameters were small (all $\lambda_{gc} \leq$ 1.058), suggesting negligible inflation in the type-I error rate.

Figure 1A and *B* illustrates the primary findings from the meta-analysis; details are in *Table 2*. In total, 49 SNPs were significantly associated with Lp-PLA2 mass and 59 with Lp-PLA2 activity. For Lp-PLA2 mass, these signals clustered at two loci on chromosomes 6 and 16. For activity, the SNPs clustered at loci on

Table 1 Characteristics of study participants (n = 13 664)

	Study sample				
	ARIC	CHS	FHS	RS	KORA
Number	798	3217	6909	1538	1202
Age (years)	58.6 ± 5.5	72.3 ± 5.3	49.3 ± 13.8	69.1 ± 9.0	53.5 ± 8.9
Male (%)	62	38.5	46.9	39.5	52.6
Current smoking (%)	24.2	11.3	15.7	23.5	17.7
BMI (kg/m^2)	28.0 ± 4.8	26.3 ± 4.5	27.4 ± 5.4	26.2 ± 3.6	27.4 ± 4.1
Waist (cm)	99.8 ± 13.4	93.1 ± 12.9	96.0 ± 15.0	90.2 ± 11.0	91.0 ± 12.7
Systolic blood pressure (mmHg)	125 ± 19	135 ± 21	121 ± 17	138 ± 22	134 ± 19
Diastolic blood pressure (mmHg)	73 ± 10	70 ± 11	75 ± 10	73 ± 11	82 ± 11
Hypertension treatment (%)	34.2	30.2	19.3	31.1	17.5
Lipid-lowering medication (%)	9.4	4.4	13.3	2.2	4.6
Aspirin $\geq$3 per week (%)	—	28.1	18.5	—	5.1
Total cholesterol (mg/dL)	214 ± 42	213 ± 39	194 ± 37	256 ± 50	238 ± 44
HDL-C (mg/dL)	44 ± 15	55 ± 16	54 ± 17	52 ± 15	54 ± 16
LDL-C (mg/dL)	138 ± 36	130 ± 35	115 ± 32	—	147 ± 41
Total/HDL-C ratio	5.3 (2.2)	4.1 ± 1.2	3.9 ± 1.4	5.3 ± 1.6	4.8 ± 1.8
Triglycerides (mg/dL)	159 ± 130	—	125 ± 90	—	187 ± 150
Prevalent diabetes (%)	18.6	11.7	7.5	9.9	4.7
Prevalent CVD[a] (%)	0	0	6.2	7.4	1.9
Hormone-replacement therapy (in women) (%)	29.6	8.6	16.4	21.3	28.6
Lp-PLA2 mass (ng/mL)	413.0 (328.5/513.5)	329.9 (261.4/407.4)	241.8 (208.0/299.3)	—	269.0 (214.0/330.5)
Lp-PLA2 activity (nmol/mL/min)	—	38.2 (30.9/47.0)	153.9 (129.2/181.2)	44.5 (36.3/51.3)	—

Mean ± SD for continuous, per cent for categorical variables, median (25th/75th percentile) for Lp-PLA2 concentrations.
[a]History of myocardial infarction, angina, coronary revascularization, stroke, or transient ischemic attack.

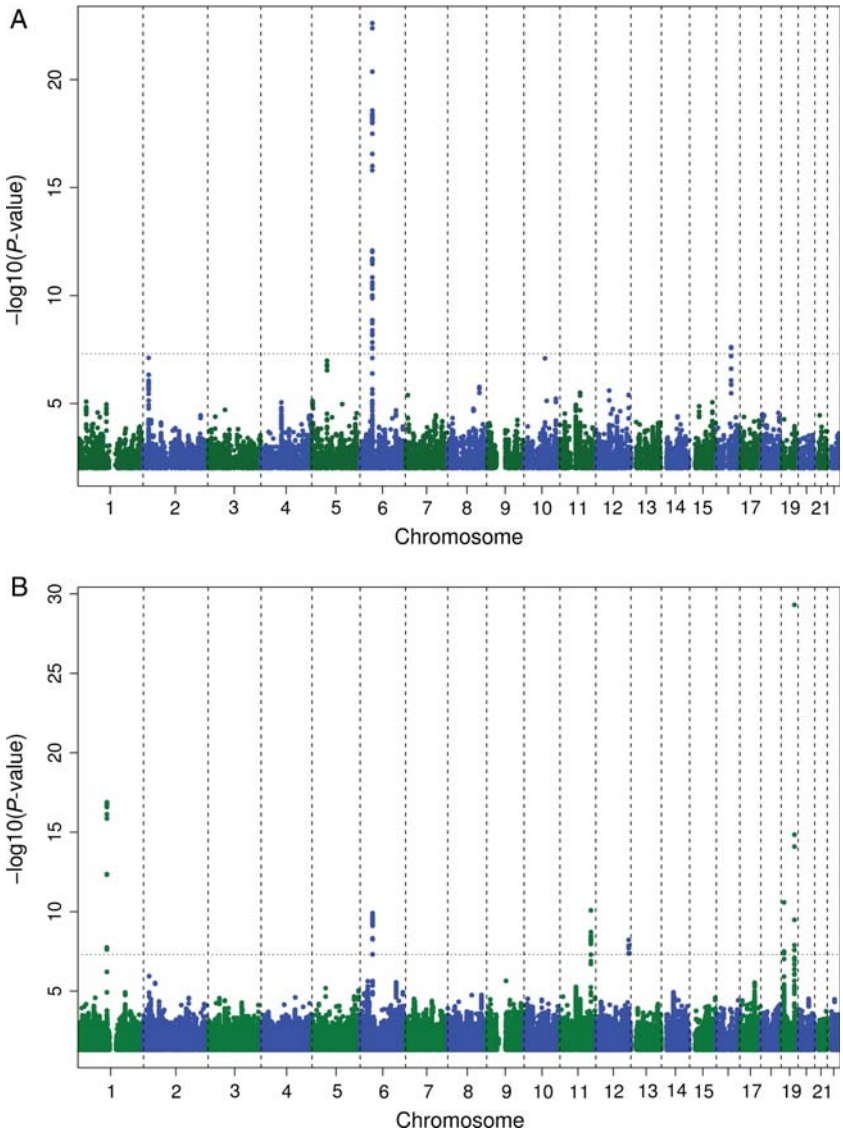

Figure 1 Association of log-transformed lipoprotein-associated phospholipase A2 mass (*A*) and lipoprotein-associated phospholipase A2 activity (*B*) concentrations and 2 661 766 single nucleotide polymorphisms displayed per chromosome and region. The dashed line indicates the significance threshold of $P < 5 \times 10^{-8}$.

chromosomes 1, 6, 11, 12, and (two clusters) 19 (Supplementary material online, *Tables S1* and *S2*).

Lipoprotein-associated phospholipase A2 mass meta-analysis

Of the 49 SNPs significantly associated with Lp-PLA2 mass, 47 were within the Lp-PLA2 gene (*PLA2G7*), at chromosome 6p21.2-p12. The strongest association [$P = 2.4 \times 10^{-23}$; log Lp-PLA2 difference per allele (beta): 0.043] was for rs1805017 (*Figure 2A*), which is located within the coding region of *PLA2G7* in exon 4 and leads to an arginine-to-histidine substitution at position 92 (Arg92His). Supplementary material online, *Table S3* shows unadjusted median values of Lp-PLA2 mass by rs1805017 genotype for each cohort.

Based on pairwise LD (based on HapMap CEU) coefficients, 27 of the 47 signals with *PLA2G7* lie within the same highly preserved haplotype block (Lewontin's $D' > 0.99$). The remaining 21 associated SNPs in this region were correlated with rs1805017 ($r^2 < 0.83$).

Lp-PLA2 mass was also associated with SNPs on chromosome 16 within the cholesteryl ester transfer protein (CETP) gene (*CETP; Figure 2F*): rs247616 ($P = 2.5 \times 10^{-8}$; beta: 0.023).

There were no significant interactions between age, sex, BMI, or smoking and the two most significant SNPs in each region (rs1805017 and rs247616) in relation to Lp-PLA2 mass (Supplementary material online, *Table S4*). The association with Lp-PLA2 remained significant for these two SNPs after multivariable adjustment with similar effect sizes and *P*-values (Supplementary material online, *Table S5*).

Table 2 Association of the top single nucleotide polymorphisms in eight loci with log-transformed lipoprotein-associated phospholipase A2, adjusted for age, sex, and site (if necessary)

SNP		Mass					Activity			
		ARIC	CHS	FHS	KORA	Meta-analysis	CHS	FHS	RS	Meta-analysis
rs4420638 (G/A)	Sample size	798	3217	6899	1202	12 116	**3217**	**6899**	**1538**	**11 654**
Chromosome: 19	*P*-value	4.3E−04	1.9E−01	7.9E−01	5.1E−02	8.6E−02	**5.4E−06**	**1.6E−19**	**3.9E−10**	**4.9E−30**
Location: 50114786	Beta	−0.075	−0.023	0.002	−0.034	−0.009	**−0.071**	**−0.048**	**−0.099**	**−0.054**
Gene: *APOC1*	Standard error	0.021	0.017	0.006	0.017	0.005	**0.016**	**0.005**	**0.016**	**0.005**
Effect allele: A	r^2	0.0140	0.0005	0.0009	0.0032	—	**0.0053**	**0.0162**	**0.0240**	**—**
	Effect allele frequency	0.82	0.83	0.84	0.82	—	**0.83**	**0.84**	**0.80**	**—**
	I^2					0.803				**0.816**
rs1805017 (C/T)	Sample size	**798**	**3217**	**6896**	**1202**	**12 113**	3217	6896	1538	11 651
Chromosome: 6	*P*-value	**2.6E−02**	**9.5E−08**	**5.0E−14**	**1.4E−03**	**2.4E−23**	1.8E−02	1.8E−04	7.1E−02	2.4E−06
Location: 46792181	Beta	**0.043**	**0.051**	**0.040**	**0.052**	**0.043**	−0.021	−0.017	−0.019	−0.018
Gene: *PLA2G7*	Standard error	**0.019**	**0.010**	**0.005**	**0.016**	**0.004**	0.009	0.004	0.010	0.004
Effect allele: T	r^2	**0.0056**	**0.0085**	**0.0097**	**0.0085**	**—**	0.0016	0.0062	0.0014	—
	Effect allele frequency	**0.25**	**0.27**	**0.26**	**0.23**	**—**	0.27	0.26	0.26	—
	I^2					0				**0**
rs7528419 (G/A)	Sample size	NA	3217	6907	NA	10 124	**3217**	**6907**	**1538**	**11 662**
Chromosome: 1	*P*-value	NA	2.6E−03	4.9E−03	NA	7.1E−05	**1.2E−05**	**9.4E−12**	**2.6E−03**	**1.3E−17**
Location: 109618715	Beta	NA	0.029	0.017	NA	0.020	**0.041**	**0.034**	**0.033**	**0.035**
Gene: *CELSR2*	Standard error	NA	0.010	0.006	NA	0.005	**0.009**	**0.005**	**0.011**	**0.004**
Effect allele: A	r^2	NA	0.0026	0.0022	NA	—	**0.0054**	**0.0107**	**0.0051**	**—**
	Effect allele frequency	NA	0.78	0.79	NA	—	**0.78**	**0.79**	**0.76**	**—**
	I^2					0.096				**0**
rs6511720 (G/T)	Sample size	798	3217	6907	1202	12 124	**3217**	**6907**	**1538**	**11 662**
Chromosome: 19	*P*-value	2.4E−01	1.6E−03	3.1E−02	2.8E−01	5.5E−05	**9.9E−10**	**3.1E−03**	**5.2E−03**	**2.6E−11**
Location: 11063306	Beta	−0.043	−0.039	−0.025	−0.035	−0.032	**−0.071**	**−0.029**	**−0.040**	**−0.045**
Gene: *LDLR*	Standard error	0.036	0.012	0.012	0.033	0.008	**0.012**	**0.010**	**0.014**	**0.007**
Effect allele: T	r^2	0.0016	0.0029	0.0016	0.0010	—	**0.0101**	**0.0045**	**0.0043**	**—**
	Effect allele frequency	0.10	0.13	0.10	0.08	—	**0.13**	**0.10**	**0.12**	**—**
	I^2					0				**0.739**
rs964184 (G/C)	Sample size	798	3217	6907	1202	12 124	**3217**	**6907**	**1538**	**11 662**

Chromosome: 11	*P*-value	2.7E−01	1.6E−02	1.3E−01	2.8E−01	5.3E−03	**1.4E−03**	**6.3E−08**	**4.1E−02**	**8.4E-11**
Location: 116154127	Beta	−0.026	−0.034	−0.010	−0.020	−0.016	**−0.044**	**−0.030**	**−0.029**	**−0.032**
Gene: *ZNF259*	Standard error	0.023	0.014	0.007	0.019	0.006	**0.014**	**0.006**	**0.014**	**0.005**
Effect allele: C	r^2	0.0014	0.0017	0.0013	0.0010	—	**0.0030**	**0.0088**	**0.0020**	**—**
	Effect allele frequency	0.85	0.87	0.86	0.87	—	**0.87**	**0.86**	**0.87**	—
	I^2					0				**0**
rs7756935 (C/A)	Sample size	798	3217	6907	1202	12 124	**3217**	**6907**	**1538**	**11 662**
Chromosome: 6	*P*-value	2.8E−01	9.2E−02	1.5E−01	3.3E−01	7.2E−01	**1.5E−03**	**4.9E−06**	**2.1E−04**	**1.3E−10**
Location: 46782984	Beta	0.021	0.017	−0.009	0.016	−0.002	**−0.030**	**−0.023**	**−0.043**	**−0.027**
Gene: *PLA2G7*	Standard error	0.020	0.010	0.006	0.016	0.005	**0.010**	**0.005**	**0.012**	**0.004**
Effect allele: A	r^2	0.0013	0.0009	0.0012	0.0008	—	**0.0030**	**0.0072**	**0.0081**	**—**
	Effect allele frequency	0.78	0.79	0.81	0.79	—	**0.79**	**0.81**	**0.8**	**—**
	I^2					0.568				**0.255**
rs10846744 (G/C)	Sample size	NA	NA	6904	1202	8106	NA	**6904**	**1538**	**8442**
Chromosome: 12	*P*-value	NA	NA	3.3E−05	7.1E−01	4.9E−05	NA	**2.0E−08**	**9.9E−02**	**6.1E−09**
Location: 123878378	Beta	NA	NA	0.026	0.007	0.025	NA	**0.030**	**0.023**	**0.029**
Gene: *SCARB1*	Standard error	NA	NA	0.006	0.020	0.006	NA	**0.005**	**0.014**	**0.005**
Effect allele: C	r^2	NA	NA	0.0041	0.0001	—	NA	**0.0086**	**0.0011**	**—**
	Effect allele frequency	NA	NA	0.16	0.16	—	NA	**0.16**	**0.14**	**—**
	I^2					0				**0.066**
rs247616 (C/T)	Sample size	**798**	**3217**	**6907**	**1202**	**12 124**	3217	6907	1538	11 662
Chromosome: 16	*P*-value	**3.3E−01**	**3.1E−01**	**6.0E−07**	**6.1E−04**	**2.5E−08**	6.4E−01	8.4E−01	2.1E−01	4.2E−01
Location: 55547091	Beta	**0.022**	**0.008**	**0.026**	**0.059**	**0.023**	−0.004	−0.001	−0.013	−0.003
Gene: *CETP*	Standard error	**0.022**	**0.008**	**0.005**	**0.017**	**0.004**	0.008	0.004	0.010	0.004
Effect allele: T	r^2	**0.0011**	**0.0003**	**0.0043**	**0.0098**	**—**	0.0001	0.0034	0.0004	—
	Effect allele frequency	**0.33**	**0.34**	**0.32**	**0.34**	**—**	0.34	0.32	0.33	—
	I^2					**0.618**				**0**

Significant SNPs in the meta-analysis with respective data from each cohort are marked bold.
r^2: increase in r^2 after adding the SNP to a linear regression model with log Lp-PLA2 as outcome and age and sex (+site, if necessary) in the model.
I^2: measure of heterogeneity.

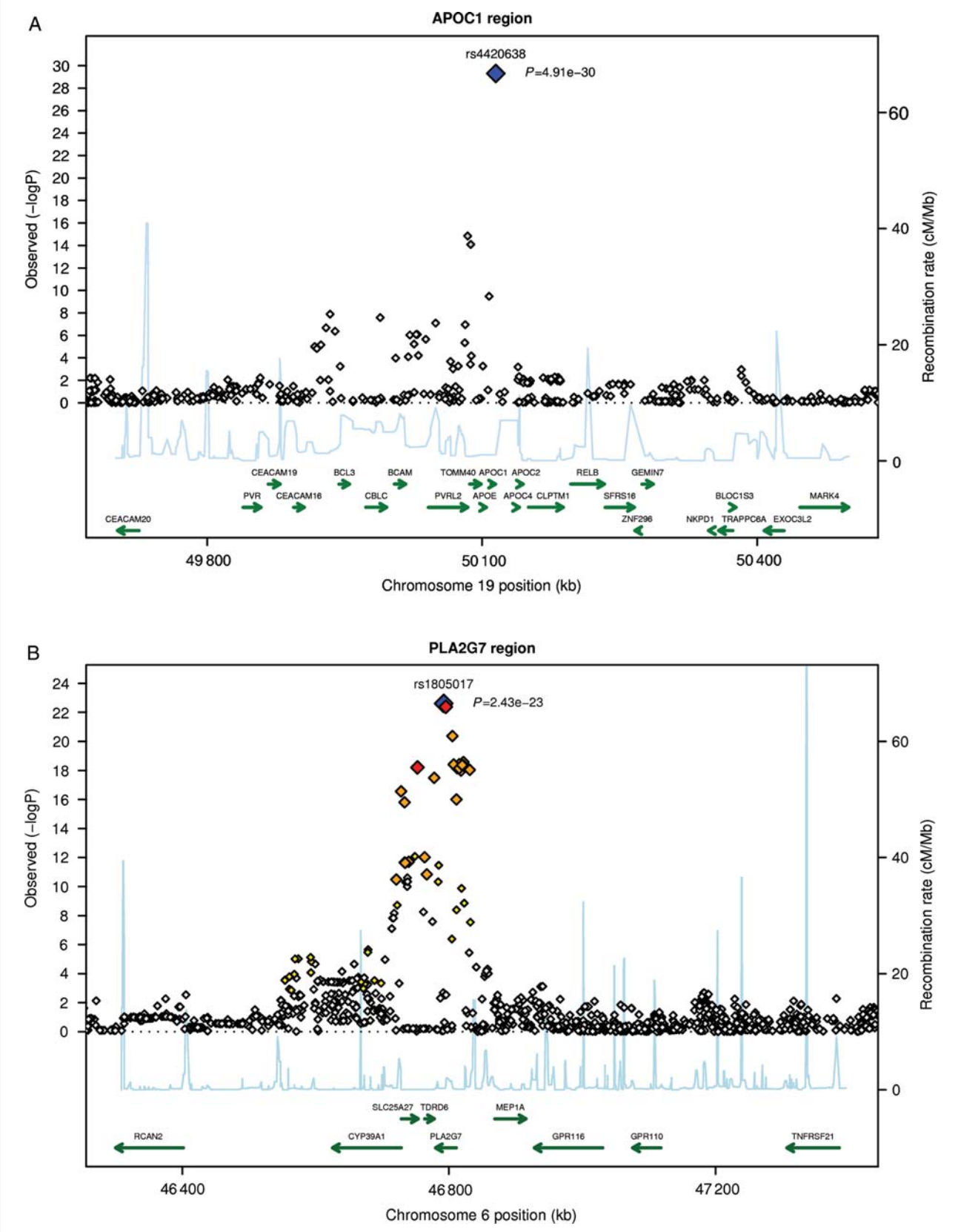

Figure 2 Regional plots for top single nucleotide polymorphisms rs4420638 (*A*), rs1805017 (*B*), rs964184 (*C*), rs7756935 (*D*), rs10846744 (*E*), and rs247616 (*F*). LD: red: r^2 (with primary SNP) >0.8; orange: r^2 between 0.5 and 0.8; yellow: r^2 between 0.2 and 0.5; white: $r^2 < 0.2$.

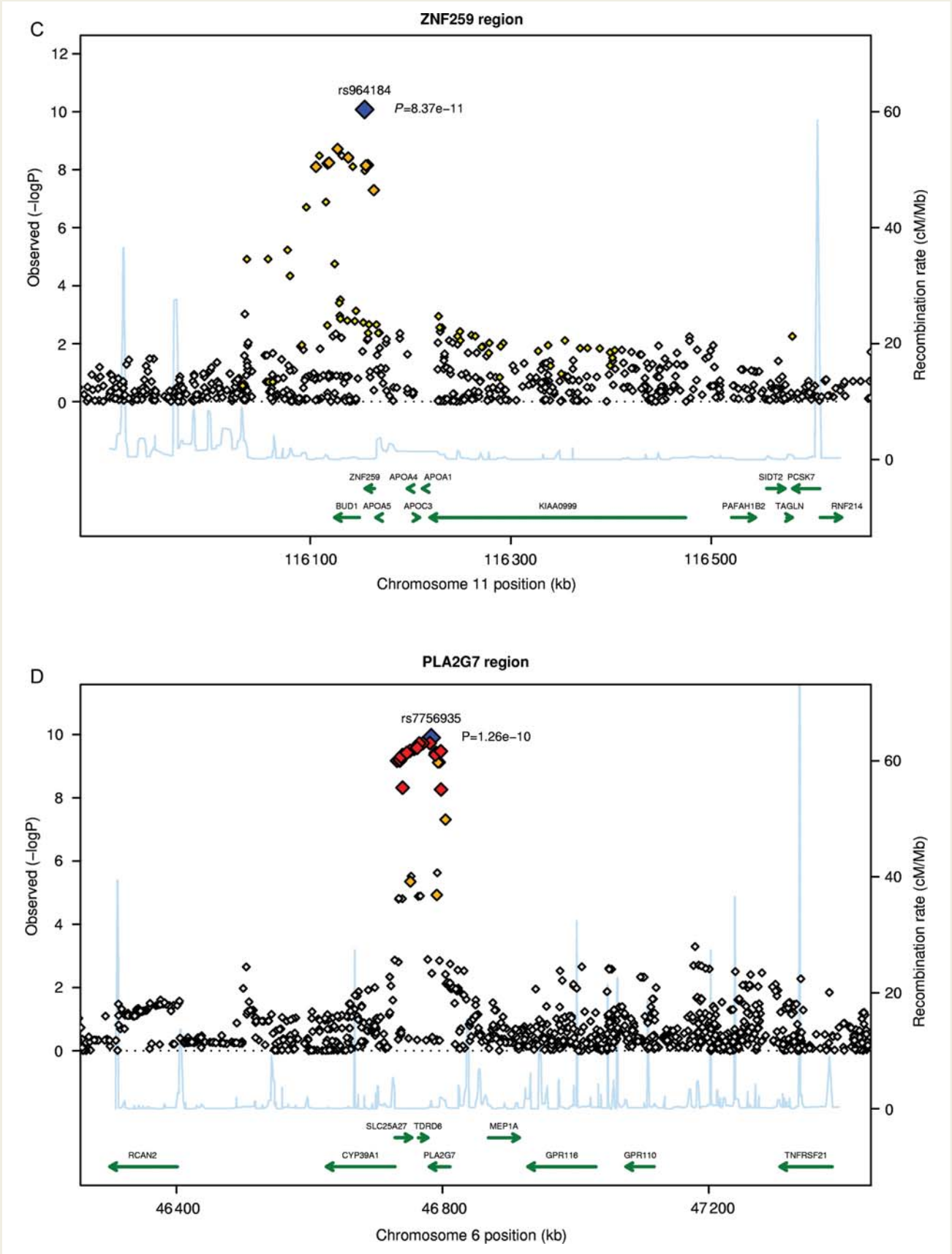

Figure 2 Continued.

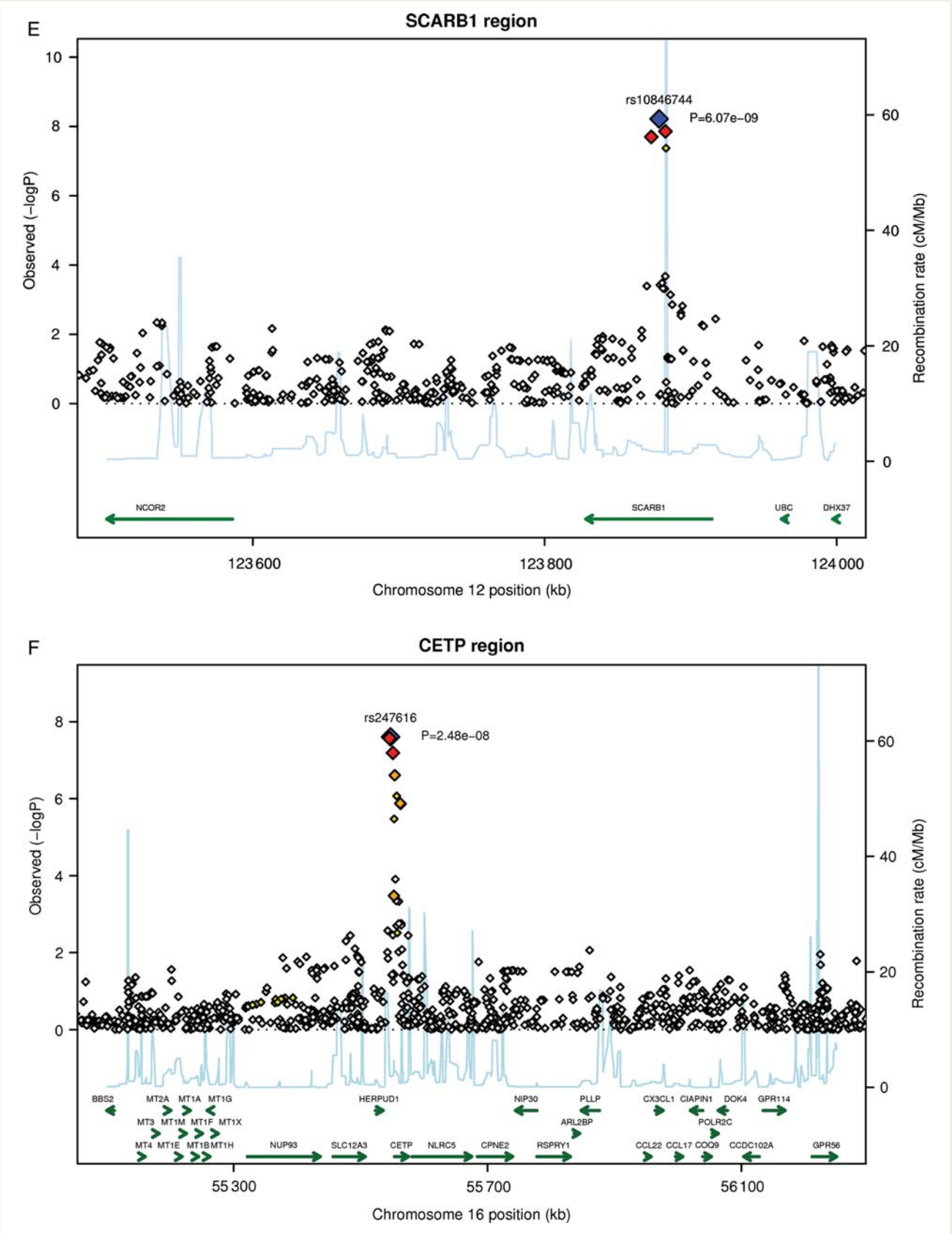

Figure 2 Continued.

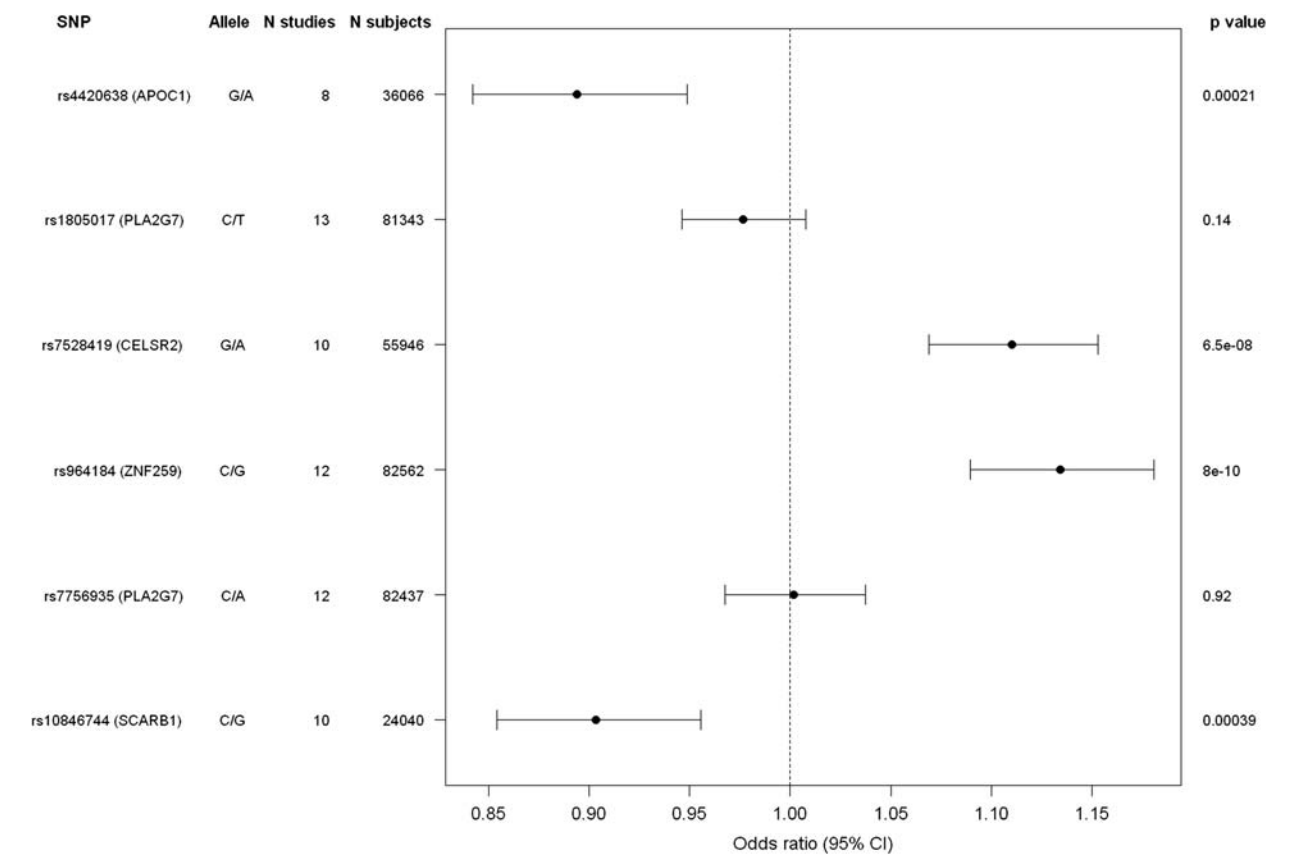

Figure 3 Association of the top single nucleotide polymorphisms in six loci with prevalent CHD/CAD in the CARDIoGRAM consortium.

Lipoprotein-associated phospholipase A2 activity meta-analysis

The smallest *P*-value for association with Lp-PLA2 activity ($P = 4.9 \times 10^{-30}$; beta: −0.054) was for rs4420638 (*Figure 2B*), located on chromosome 19 near the *APOE–APOC1–APOC4–APOC2* cluster and ~11 kb proximal to the *APOE*-ε4 allele. The association was similar in all three studies, with beta coefficients of −0.099 to −0.048 (*Table 2*). Other SNPs with significant associations were rs7528419 (*CELSR2*), rs6511720 (*LDLR*), rs964184 (*ZNF259*), and rs10846744 *(SCARB1)* (*Table 2*). Lipoprotein-associated phospholipase A2 activity was also associated with *PLA2G7*. The strongest association was for rs7756935 ($P = 1.3 \times 10^{-10}$; beta: −0.027), which was in perfect LD with another functional SNP within the *PLA2G7* gene, rs1051931 in exon 11 (Ala379Val).

There were no significant gene–environment interactions of age, sex, BMI, and smoking status with the top SNPs in six loci in relation to log-transformed Lp-PLA2 activity (Supplementary material online, *Table S4*).

Association of top single nucleotide polymorphisms with prevalent coronary heart disease/coronary artery disease

For all but two (rs247616, *CETP;* rs6511720, *LDLR*) of the top SNPs, the association to CHD/CAD could be assessed in the CARDIoGRAM project. Four of the top SNPs related to Lp-PLA2 activity [rs964184 (*ZNF259*), rs4420638 (*APOC1*), rs7528419 (*CELSR2*), rs10846744 (*SCARB1*)] were significantly associated with prevalent CHD/CAD (*Figure 3*). The strongest association was found for rs964184 with a $P = 8.0 \times 10^{-10}$, with an odds ratio of 1.13 per G allele (95% confidence interval 1.09–1.18). The two SNPs in the *PLA2G7* gene most significantly associated with Lp-PLA2 mass or activity were not significantly associated with prevalent CHD/CAD in CARDIoGRAM. The estimated changes in Lp-PLA2 mass or activity per risk allele revealed in our meta-analysis were combined with the CHD risk estimations related to Lp-PLA2 mass or activity drawn from a published meta-analysis[3] to estimate the expected CHD risk changes per allele. For SD values (necessary to calculate the expected risks), we chose the respective data from FHS, as of the five cohorts FHS had the largest number of participants. This approach revealed CHD risk increases between 0.8 and 2.1% per allele and were found to be mostly smaller than that found in the CARDIoGRAM data. The CARDIoGRAM study had ≥70% power to detect CHD risk changes of 3.5% or more (Supplementary material online, *Table S6*).

Discussion

Meta-analysis of GWAS from five population-based studies comprising 13 664 subjects suggests that genes influencing Lp-PLA2

mass and activity differ. While enzyme concentration was regulated mainly by the gene coding for Lp-PLA2 (*PLA2G7*), genetic variants involved in lipid metabolism were strongly associated with Lp-PLA2 activity.

Lipoprotein-associated phospholipase A2 mass

Lipoprotein-associated phospholipase A2 mass was dependent on variants within *PLA2G7*. Previous studies identified numerous SNPs within *PLA2G7:* variants in exon 9 (Val279Phe; rs45619133), exon 11 (Val379Ala; rs1051931), exon 7 (Ile198Thr, Iso195Thr; rs1805018), and exon 4 (Arg92His; rs1805017).[13] A prior GWA of Framingham data accessed through dbGaP also identified multiple SNPs clustered in or near *PLA2G7* that were associated with Lp-PLA2 mass.[6]

Lipoprotein-associated phospholipase A2 mass was also influenced by rs247616 (*CETP*).[14] The T allele of rs247616 was associated with higher HDL-C concentration[14] and higher mean Lp-PLA2 mass. This finding is in contrast to most epidemiological studies, including ARIC[15]and Framingham,[6] which showed an inverse relation between HDL-C level and Lp-PLA2 mass.[3] The mechanism(s) by which reduced CETP activity leading to increased HDL-C would also increase Lp-PLA2 mass is unclear.

Lipoprotein-associated phospholipase A2 activity

All the SNPs associated with Lp-PLA2 activity in our meta-analysis also determine plasma lipoprotein concentrations except for those in *PLA2G7*. The strongest association for Lp-PLA2 activity was for rs4420638 (*APOC1*), previously associated with LDL-C concentrations.[14,16] rs964184 (*ZNF259*), near the *APOA5–APOA4–APOC3–APOA1* cluster, was associated with increased Lp-PLA2 activity and previously with increased triglycerides;[14] elevated triglycerides are associated with increased small dense LDLs, which contain increased Lp-PLA2 mass.[17]

rs7528419 (*CELSR2*) located at 1q13.3, which also contains the genes *PSRC1* and *SORT1,* has strong associations with LDL-C levels and CHD.[14,16,18] Based on HapMap data, rs7528419 was highly correlated with rs599839 (*PSRC1*) and rs646776 (*CELSR2*); minor alleles of rs599839 and rs646776 have been associated with lower LDL-C[14,16,18] and lower CHD risk.[19] The minor allele of rs7528419 also was associated with lower Lp-PLA2 activity, consistent with the reduction in LDL-C in other studies.[14,16,18] rs7528419, or a SNP in LD, might have functional effects due to its location in 3′ UTR of the *CELSR2* gene (encoding for cadherin, EGF LAG seven-pass G-type receptor 2), which includes binding sites for transcriptional factors (e.g. Oct-1).

rs6511720 (*LDLR*) was also associated with lower Lp-PLA2 activity and lower LDL-C.[18] It was highly correlated with rs2228671 (*LDLR*) ($r^2 = 0.734$; $D' = 0.899$), which was related to lower LDL-C and reduced CAD risk.[20]

rs10846744, an intronic SNP within the *SCARB1* gene, which encodes for scavenger receptor type B class 1 (SRB1), a major receptor for HDL, was associated with Lp-PLA2 activity. Although this SNP was not associated with lipids in this study, other SNPs in *SCARB1* have been shown to be associated with levels of HDL-C.[21,22] *SCARB1* may also modulate Lp-PLA2 activity through its role as a scavenger receptor for oxidized LDL, which has been shown to increase Lp-PLA2 secretion by human macrophages.[23] SCARB1 is expressed in macrophages and has been shown to bind avidly to oxidized LDL.[24]

Finally, rs7756935 (*PLA2G7*), associated with increased Lp-PLA2 activity, was in complete LD with rs1051931 *(PLA2G7*; Ala379Val).[24] The V^{379} allele resulted in two-fold lower Lp-PLA2 activity in *in vitro* studies[25] but increased plasma Lp-PLA2 activity in epidemiological studies.[26,27]

Association of top single nucleotide polymorphisms with prevalent coronary heart disease/coronary artery disease

Of the top polymorphisms associated with Lp-PLA2 activity, four (in *ZNF259, APOC1*, *CELSR2, SCARB1*) were significantly associated with CHD or CAD, as also reported in previous studies.[14,16,18,20,28] However, the SNPs in the *PLA2G7* gene that were most significantly associated with Lp-PLA2 mass (rs1805017) or activity (rs7756935) were not associated with prevalent CHD or CAD. These findings are consistent with Casas *et al.*,[27] who found a significant association between Lp-PLA2 activity and CHD but did not find any significant association with *PLA2G7* genotype (12 tagged SNPs including rs1805017). We had >97% power to detect a 5% increase in risk with these two SNPs, but only 35–40% power to detect a 2.5% increase in risk (Supplementary material online, *Table S6*); therefore, if there is an increased risk, it is most likely in the range of a few percent or less. However, considering the modest effect of these common alleles of the *PLA2G7* locus on Lp-PLA2 mass and activity (Supplementary material online, *Table S5*) and the modest strength of the association of Lp-PLA2 mass and activity with CHD,[3] we may have had insufficient power to detect a genetic association that would still be compatible with a causal role for Lp-PLA2 in CHD, despite our large sample size. In contrast to our findings and those of Casas *et al.*, Sutton *et al.*[29] reported significant associations between SNPs in *PLA2G7,* including rs1805017, with CAD in case–control and family data sets. This suggests that the nature of the population, or in fact having familial data, may influence the strength and likelihood of observing a significant association.

The Val279Phe substitution is located within the catalytical domain of the encoded enzyme and therefore leads to reduction (heterozygotes) or complete loss (homozygotes) of enzyme activity. In Japan, this mutation occurs in 30% of the population (27% heterozygous, 4% homozygous and therefore completely lacking plasma Lp-PLA2 activity and mass).[30] Although Val279Phe-mediated loss of activity was an independent CHD risk factor in Japanese men with hypercholesterolaemia, myocardial infarction, stroke, or non-familial dilated and hypertrophic cardiomyopathies and protective from CAD in South Korean men,[31] no association with CHD was observed in a Chinese population.[32]

Clinical implications

In the present meta-analysis, we identified eight SNPs associated with Lp-PLA2 mass or activity which might contribute to the

regulation of plasma levels and give insight as to whether Lp-PLA2 is a risk factor or risk marker for CHD. Most of these genetic loci were in regions that have already been associated with levels of lipoproteins. Of the six top SNPs that could be tested in the CARDIoGRAM consortium, four located in *ZNF259, APOC1, CELSR2,* and *SCARB1* were significantly associated with CHD, whereas the two SNPs in *PLA2G7,* the gene that encodes for Lp-PLA2, did not have any significant association with CHD. This supports the hypothesis that the genetic regions which are known to affect lipoprotein levels also influence both the level of Lp-PLA2 and the development of CHD and is consistent with the hypothesis that Lp-PLA2 level may be a marker of atherogenic lipoproteins; levels of Lp-PLA2 have been shown to be increased in subpopulations of lipoproteins such as electronegative LDL. Although the lack of association does not provide any evidence in support of the hypothesis that Lp-PLA2 is a risk factor for CHD, our data cannot rule out the possibility that Lp-PLA2 is a risk factor. First, despite the large number of CHD cases in CARDIoGRAM, we lacked sufficient power to rule out a small effect. The SNPs identified in *PLA2G7* had modest effects on the levels of Lp-PLA2 mass or activity and would be expected to have even smaller effects on risk for development of CHD. Furthermore, we measured total Lp-PLA2 mass and activity in plasma, not the mass or activity associated with LDL or HDL. It is possible that genes which alter lipoprotein metabolism may also alter the binding of Lp-PLA2 to subspecies of lipoproteins,[17] and Lp-PLA2 may only be atherogenic when it is associated with atherogenic lipoproteins such as LDL, not with HDL. Our observation that a SNP in the *CETP* locus, which is associated with lower CETP activity and higher levels of Lp-PLA2 mass, may have implications for clinical programmes that are developing drugs that inhibit CETP.[33]

Levels of Lp-PLA2 mass and activity were strongly associated with CHD in a large meta-analysis, supporting the view that circulating Lp-PLA2 indeed represents a biomarker of CHD. Experimental data in the pig model indicate that Lp-PLA2 has important proinflammatory effects in the vessel wall.[34] Further, clinical trial data using virtual histology (an intravascular ultrasound–derived modality) as a secondary endpoint suggest that elevated level or increased activity of Lp-PLA2 is related to the progression of atherosclerotic disease.[35] With regard to a potential causal role in the pathophysiology of atherosclerosis and its complications, only large randomized clinical trials assessing the effect of Lp-PLA2 inhibition on cardiovascular endpoints (STABILITY[36] and SOLID–TIMI 52[37]) can provide a definitive answer.

Strengths and limitations

The routine ascertainment of Lp-PLA2 covariates and GWA data in five community-based cohorts based on >13 000 European ancestry subjects are strengths of our study. However, caution should be taken when generalizing these findings to populations with non-European ancestry. In addition, even the most significant SNPs may be in LD with as yet unknown causal variants, and the functional basis of the relation of the identified SNPs with variations in Lp-PLA2 concentrations requires study. Also, it is possible that missense genetic changes may cause a difference in assay immunoreactivity, warranting caution in interpreting genetic effects on levels of Lp-PLA2. Lastly, because many of the cohorts were community based, CHD was largely symptomatic CAD; we acknowledge that asymptomatic CAD may have been misclassified.

Conclusions

We extended the previously reported Framingham findings[6] by conducting a meta-analysis with four additional cohorts. Whereas levels of Lp-PLA2 mass were primarily associated with the gene coding for the protein itself, Lp-PLA2 activity was associated with genetic variants related to LDL-C levels, which were also associated with CHD/CAD. Larger association studies and clinical trials will be needed to determine whether Lp-PLA2 is a causal risk factor for CHD.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

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