

SUPPLEMENTAL MATERIAL

Plasma phospholipid transfer protein (PLTP) activity inversely correlates with carotid artery disease: Effects of paraoxonase 1 enzyme activity and genetic variants on PLTP activity

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Supplemental Table S1. Demographic and clinical characteristics of the studied CLEAR subset stratified by CAAD case status.

Variable	N	CAAD Controls (n=622)	CAAD Cases (n=493)	Total (n=1115)	P-Value
Censored age, y	1115	66.7 ± 8.0	66.5 ± 9.1	66.6 ± 8.5	0.98 ^a
Female, %	1115	106 (17%)	60 (12%)	166 (15%)	0.031 ^b
Diabetic, %	1115	61 (10%)	115 (23%)	176 (16%)	< 0.001 ^b
BMI, kg/m ²	1115	28.4 ± 5.0	27.9 ± 4.7	28.2 ± 4.9	0.14 ^a
Statin use, %	1115	109 (18%)	353 (72%)	462 (41%)	< 0.001 ^b
Current smoking status, %	1115	35 (6%)	94 (19%)	129 (11%)	< 0.001 ^b
Total cholesterol, mg/dl	1100	197 ± 40	176 ± 40	187 ± 41	< 0.001 ^a
apoA1, mg/dl	1110	148 ± 28	138 ± 27	144 ± 28	< 0.001 ^a
HDL-C, mg/dl	1100	53 ± 17	47 ± 15	50 ± 17	< 0.001 ^a
HDL-2, mg/dl	1098	10 ± 6.5	8.6 ± 5.8	9.6 ± 6.3	< 0.001 ^a
HDL-3, mg/dl	1099	43 ± 12	38 ± 11	41 ± 11	< 0.001 ^a
Ln(Triglycerides)	1100	4.7 ± 0.56	4.8 ± 0.55	4.8 ± 0.56	< 0.001 ^a
Ln(VLDL)	1100	3.1 ± 0.55	3.3 ± 0.54	3.2 ± 0.55	< 0.001 ^a
PON1 AREase activity, IU	1077	148 ± 50	128 ± 50	139 ± 50	< 0.001 ^a
PLTPa, μM/hr	1115	13.8 ± 4.2	13.2 ± 4.7	13.5 ± 4.4	0.008 ^a

Abbreviations: apoA1 = apolipoprotein A1; BMI = body mass index; CAAD = carotid artery disease; censored age = age at CAAD diagnosis for cases, age at enrollment for controls; CLEAR = Carotid Lesion Epidemiology and Risk cohort; HDL = high-density lipoprotein; PON1 AREase activity = Paraoxonase 1 arylester hydrolysis activity; PLTPa = phospholipid transfer protein activity; VLDL = very-low-density lipoprotein.

Tests used for p-value calculations for differences between subsets: ^a = Wilcoxon rank sum test; ^b = Pearson chi-square test.

Supplemental Table S2. Sensitivity analysis of PLTPa predictors from stepwise linear regression in controls (n=601).*

Variable	Coefficient ± SE	% PLTPa	P-Value
<i>Intercept</i>	11.79 ± 2.16	-	-
Current age	0.00014 ± 0.025	0.12%	0.99
Female gender	-0.13 ± 0.52	0.066%	0.79
Ln(Triglycerides)	0.92 ± 0.35	1.31%	0.0097
Diabetes status	1.73 ± 0.61	1.69%	0.0045
PON1 AREase activity	0.0056 ± 0.0042	0.53%	0.19
Current smoking status	1.07 ± 0.77	0.37%	0.17
apoA1, mg/dl	0.0078 ± 0.0079	0.19%	0.32
Statin use	-1.02 ± 0.48	0.54%	0.037

Abbreviations: apoA1 = apolipoprotein A1; PON1 AREase activity = paraoxonase 1 arylester hydrolysis enzymatic activity; PLTPa = phospholipid transfer protein activity.

These analyses relate to **Table 2 in the main manuscript. They show the association of each individual covariate identified as predictive of PLTP activity stratified by CAAD status (associations for CAAD controls reported in this table).*

Supplemental Table S3. Sensitivity analysis of PLTPa predictors from stepwise linear regression in CAAD cases (n=455).*

Variable	Coefficient ± SE	% PLTPa	P-Value
<i>Intercept</i>	11.28 ± 2.67	-	-
Current age	-0.031 ± 0.028	1.12%	0.48
Female gender	-1.04 ± 0.72	0.19%	0.15
Ln(Triglycerides)	0.98 ± 0.46	1.59%	0.034
Diabetes status	0.93 ± 0.51	0.52%	0.074
PON1 AREase activity	0.011 ± 0.0048	1.31%	0.023
Current smoking status	0.18 ± 0.59	0.016%	0.75
apoA1, mg/dl	0.0043 ± 0.0091	0.048%	0.64
Statin use	0.13 ± 0.53	0.13%	0.80

Abbreviations: apoA1 = apolipoprotein A1; PON1 AREase activity = paraoxonase 1 arylester hydrolysis enzymatic activity; PLTPa = phospholipid transfer protein activity.

These analyses relate to **Table 2 in the main manuscript. They show the association of each individual covariate identified as predictive of PLTP activity stratified by CAAD status (associations for CAAD cases reported in this table).*

Supplemental Table S4. PLTPa predicts CAAD status adjusting for confounders through multivariate logistic regression (n=1056)*.

Variable	OR (95% CI)	% CAAD Prediction	P-Value
Censored Age	1.00 (0.99-1.02)	0.054%	0.58
Female Gender	1.07 (0.69-1.64)	0.65%	0.79
Ln(Triglycerides) ^a	1.27 (1.06-1.53)	2.34%	0.005
Diabetes Status	3.25 (2.24-4.74)	3.59%	6.31x10 ⁻⁹
PON1 AREase activity ^b	0.94 (0.92-0.98)	2.69%	0.001
Current Smoking Status	4.55 (2.92-7.21)	4.34%	1.38x10 ⁻¹²
apoA1 ^b	0.96 (0.90-1.02)	0.30%	0.12
PLTPa ^c	0.91 (0.88-0.94)	2.92%	7.90x10 ⁻⁹

Abbreviations: apoA1 = apolipoprotein A1; CAAD = carotid artery disease; PON1 AREase activity = paraoxonase 1 arylester hydrolysis enzymatic activity; PLTPa = phospholipid transfer protein activity.

^a Reported odds ratio for ln(triglyceride) association with CAAD odds is for a 2-fold increase in triglycerides.

^b Reported odds ratios for PON1 AREase activity and apoA1 associations with CAAD odds are for a 10 unit increase in each respective covariate.

^c PLTPa adjusted for statin use by the average decrease seen in controls using statins (-1.02).

*1056 participants had complete data on all parameters considered in this stepwise linear regression model. All included covariates were previously associated with PLTPa (see **Table 2**).

Supplemental Table S5. *PLTP* and local region SNP associations ($P \leq 0.05$) with covariate-adjusted odds of CAAD (logistic regression) and PLTPa (linear regression).

SNP	Position ^a	Gene ^b	Function	MAF	N	CAAD OR (95% CI) ^c	CAAD P ^c	PLTP Beta ^d	PLTP P ^d
rs4810479	20:44545048	(<i>PLTP</i>)	5'	0.253	1025	0.84 (0.67-1.04)	0.097	-1.183	2.63E-07
rs4812975	20:44545460	(<i>PLTP</i>)	5'	0.253	1022	0.84 (0.68-1.05)	0.12	-1.043	2.28E-06
rs2868346	20:44547970	(<i>PLTP</i>)	5'	0.2486	1015	0.84 (0.67-1.04)	0.11	-1.012	4.60E-06
rs6065904	20:44534651	<i>PLTP</i>	Intron 8 ^e	0.2212	1025	0.74 (0.59-0.94)	0.012	-1.054	1.35E-05
chr20:44546198:D	20:44546198	(<i>PLTP</i>)	TA>T Del	0.1867	1001	0.77 (0.60-0.99)	0.040	-1.056	1.91E-05
rs73307905	20:44545773	(<i>PLTP</i>)	5'	0.1863	1021	0.77 (0.59-0.98)	0.034	-1.058	1.94E-05
rs7679	20:44576502	<i>PCIF1</i>	3'UTR	0.1772	987	0.76 (0.59-0.98)	0.035	-1.102	2.84E-05
rs58847685	20:44544947	(<i>PLTP</i>)	5'	0.1863	1018	0.78 (0.61-0.99)	0.044	-1.03	3.28E-05
rs111602331	20:44557474	(<i>PLTP</i>)	5'	0.1753	986	0.73 (0.57-0.95)	0.017	-1.048	4.35E-05
rs139953093	20:44559748	(<i>PLTP</i>)	5'	0.1767	982	0.76 (0.59-0.99)	0.038	-1.03	4.73E-05
rs6073957	20:44549476	(<i>PLTP</i>)	5'	0.1841	1019	0.77 (0.60-0.98)	0.034	-0.9936	5.98E-05
rs118024629	20:44546197	(<i>PLTP</i>)	5'	0.1852	1019	0.76 (0.60-0.98)	0.031	-0.9902	6.33E-05
rs12185764	20:44550020	(<i>PLTP</i>)	5'	0.1847	1019	0.76 (0.60-0.98)	0.031	-0.9902	6.33E-05
rs59329875	20:44547672	(<i>PLTP</i>)	5'	0.1852	1019	0.76 (0.60-0.98)	0.031	-0.9902	6.33E-05
rs73307913	20:44548301	(<i>PLTP</i>)	5'	0.1852	1019	0.76 (0.60-0.98)	0.031	-0.9902	6.33E-05
rs6065906	20:44554015	(<i>PLTP</i>)	5'	0.1849	1011	0.75 (0.59-0.96)	0.025	-0.986	6.80E-05
rs12185776	20:44551600	(<i>PLTP</i>)	5'	0.1841	1017	0.77 (0.60-0.98)	0.035	-0.9682	9.18E-05
rs6073958	20:44551855	(<i>PLTP</i>)	5'	0.1937	998	0.82 (0.64-1.04)	0.099	-0.9515	0.0001
rs6073952	20:44536932	<i>PLTP</i>	Intron 4 ^e	0.1974	1025	0.80 (0.63-1.01)	0.069	-0.8945	0.0003
rs6073966	20:44570192	<i>PCIF1</i>	Intron 7 ^f	0.1644	986	0.75 (0.58-0.98)	0.031	-0.9076	0.0004
rs6065905	20:44537837	<i>PLTP</i>	Intron 4 ^e	0.198	1018	0.81 (0.64-1.03)	0.080	-0.8009	0.0009
rs378114	20:44538427	<i>PLTP</i>	Intron 3 ^e	0.2656	1025	0.95 (0.77-1.17)	0.61	0.5145	0.024
rs435306	20:44538484	<i>PLTP</i>	Intron 3 ^e	0.2656	1025	0.95 (0.77-1.17)	0.61	0.5145	0.024
rs4810478	20:44544732	(<i>PLTP</i>)	5'	0.05424	1004	1.13 (0.75-1.71)	0.55	-0.8958	0.036
rs6073955	20:44544571	(<i>PLTP</i>)	5'	0.05424	1004	1.13 (0.75-1.71)	0.55	-0.8958	0.036
rs2903809	20:44552895	(<i>PLTP</i>)	5'	0.2705	998	0.93 (0.75-1.15)	0.49	0.447	0.037
rs3843763	20:44548193	(<i>PLTP</i>)	5'	0.2721	1004	0.93 (0.76-1.15)	0.53	0.4247	0.048

rs41305805	20:44587926	<i>ZNF335</i>	Phe>Val	0.01749	995	1.18 (0.61-2.28)	0.63	1.335	0.049
rs118013520	20:44563133	(<i>PCIF1</i>)	5'	0.0175	995	1.18 (0.61-2.27)	0.62	1.329	0.04933
rs181914932	20:44561279	(<i>PCIF1</i>)	5'	0.0175	995	1.18 (0.61-2.27)	0.62	1.329	0.04933
rs79629788	20:44562148	(<i>PCIF1</i>)	5'	0.0175	995	1.18 (0.61-2.27)	0.62	1.329	0.04933

Abbreviations: Beta = beta coefficient from linear regression; CI = confidence interval; MAF = minor allele frequency; OR = odds ratio.

^a = Position based on hg19/GRCh37.

^b = Noncoding SNPs are represented in parentheses naming the nearest gene, e.g. (*PLTP*).

^c = Logistic regression analyses adjusted by censored age, sex, diabetes status, ln(triglyceride levels), current smoking status, and PON1 AREase activity.

^d = Linear regression analyses adjusted by age, sex, diabetes status, statin use, ln(triglyceride levels), current smoking status and PON1 AREase activity.

^e = *PLTP* gene has 14 total introns.

^f = *PCIF1* gene has 15 total introns.

Supplemental Figure S1. Linkage disequilibrium (LD) of *PTLP* and surrounding genomic region SNPs with minor allele frequency greater than 5%. This plot demonstrates the local correlation (R^2), or LD, of *PLTP* region SNPs that were studied. Red denotes strong correlation, indicating that these SNPs' genotypes (which create "haplotypes") are inherited together frequently, while white indicates no correlation. Note the lack of solid "blocks" of SNPs in LD (continuous red blocks) that would indicate large regions or haplotypes are inherited together.

Pairwise LD for SNPs in *PLTP* locus and surrounding region

