Supplementary material

Association Between Coronary Artery Disease Genetic Variants and Subclinical Atherosclerosis: an Association Study and Meta-analysis

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Table 1, Supplementary Material.

Age- and Sex-adjusted Mean and Standard Deviation of the Mean and Maximum Carotid Bulb and Internal Carotid Artery Intima Media Thickness Across the Groups Defined by the Genotypes Studied

Genetic variant	Mean IMTbul		Maximum IMTbul		Mean IMTica		Maximum IMTica	
Single Nucleotide Polymorphisms								
rs17465637								
AA	N=210	0.733±0.015	N=179	1.024±0.029	N=202	0.581±0.011	N=163	0.800±0.020
AC	N=1020	0.765±0.007	N=904	1.052±0.013	N=1000	0.593±0.005	N=853	0.796±0.009
CC	N=1248	0.766±0.006	N=1105	1.051±0.012	N=1222	0.593±0.005	N=1036	0.799±0.008
P Value	.122		.657		.579		.948	
rs6725887								
CC	N=45	0.770±0.032	N=41	1.118±0.060	N=44	0.624±0.024	N=38	0.845±0.041
CT	N=611	0.765±0.009	N=548	1.047±0.016	N=602	0.600±0.007	N=498	0.812±0.011
TT	N=1779	0.759±0.005	N=1561	1.045±0.010	N=1737	0.589±0.004	N=1483	0.792±0.007
P Value	.808		.488		.163		.179	
rs9818870								
CC	N=1843	0.762±0.005	N=1645	1.047±0.009	N=1802	0.592±0.004	N=1531	0.796±0.007
CT	N=578	0.764±0.009	N=497	1.051±0.017	N=567	0.593±0.007	N=472	0.804±0.012
TT	N=34	0.720±0.037	N=28	1.016±0.073	N=33	0.612±0.028	N=31	0.822±0.046
P Value	.525		.895		.786		.706	
rs12526453								
CC	N=1018	0.764±0.007	N=909	1.053±0.013	N=995	0.593±0.005	N=835	0.806±0.009
CG	N=1124	0.758±0.006	N=990	1.037±0.012	N=1103	0.590±0.005	N=949	0.787±0.008
GG	N=315	0.765±0.012	N=273	1.067±0.023	N=306	0.599±0.009	N=254	0.813±0.01

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P Value	.824		.433		.720		.169	
rs1333049								
CC	N=650	0.768±0.009	N=572	1.060±0.016	N=636	0.592±0.006	N=542	0.799±0.011
GC	N=1233	0.769±0.006	N=1095	1.060±0.012	N=1207	0.595±0.005	N=1013	0.802±0.008
GG	N=595	0.744±0.009	N=521	1.013±0.017	N=581	0.586±0.007	N=498	0.787±0.011
P Value	.067		.048		.536		.564	
rs1746048								
CC	N=1863	0.761±0.005	N=1637	1.051±0.010	N=1822	0.589±0.004	N=1558	0.796±0.006
TC	N=560	0.772±0.009	N=500	1.045±0.017	N=548	0.603±0.007	N=446	0.806±0.012
TT	N=50	0.725±0.031	N=46	0.979±0.057	N=49	0.576±0.023	N=43	0.767±0.039
P Value	.277		.444		.173		.573	
rs9982601								
CC	N=1874	0.762±0.005	N=1662	1.052±0.009	N=1835	0.594±0.004	N=1535	0.798±0.007
CT	N=548	0.763±0.009	N=478	1.042±0.018	N=538	0.592±0.007	N=474	0.805±0.012
TT	N=32	0.730±0.038	N=29	0.936±0.071	N=30	0.548±0.029	N=26	0.719±0.050
P Value	.699		.248		.310		.245	
rs10455872								
AA	N=2116	0.758±0.005	N=1872	1.041±0.009	N=2070	0.590±0.004	N=1747	0.793±0.006
GA	N=371	0.791±0.011	N=324	1.094±0.021	N=364	0.604±0.009	N=303	0.821±0.015
GG	N=13	0.767±0.061	N=11	1.114±0.116	N=12	0.615±0.047	N=10	0.781±0.081

P Value	.027		.061		.271		.207	
ALOX5AP Haplotype B								
0*	N=1433	0.763±0.006	N=1262	1.049±0.011	N=1401	0.593±0.004	N=1195	0.798±0.007
1*	N=899	0.764±0.007	N=804	1.051±0.014	N=880	0.589±0.005	N=735	0.799±0.009
2*	N=157	0.754±0.018	N=131	1.041±0.034	N=154	0.598±0.013	N=130	0.786±0.022
P Value	.875		.966		.726		.871	

IMTbul, carotid bulb intima-media thickness; IMTica, internal carotid intima-media thickness

*Number of risk alleles (AGA)

Table 2, Supplementary Material.

Summary of Results of the Process of Selecting Manuscripts for Inclusion in the Meta-analysis According to the PRISMA Statement

	Number of Manuscripts Including Each SNP				
	Identified Through the PubMed Search	**Initially Selected After Title-abstract Screening**	**Excluded After Review of Full Manuscript**	**Identified Through Other Sources**	**Total Included in Meta-analysis**
rs17465637	2	1	1[1]	2	2[2,3]
rs6725887	0	0	0	2	2[2,3]
rs9818870	29	0	0	2	2[2,3]
rs12526453	1	0	0	2	2[2,3]
rs1746048*	78	2	1[4]	2	3[2,3,5]
rs9982601	0	0	0	2	2[2,3]
rs1333049†	124	7	2[6,7]	2	7[2,3,8-12]
rs10455872	2	1	0	0	1[13]

SNP, single nucleotide polymorphisms.

*Some studies analyzed the SNP rs501120 in linkage disequilibrium (LD) with rs1746048

†Some studies analyzed the SNP rs4977574 in LD with rs1333049

Table 3, Supplementary Material.

Results of the Association Between the Different Genetic Variants Included in Our Study and Mean Common Carotid Intima Media Thickness in the Individual Studies Included in the Meta-analysis.

SNP	N	Beta	SE
rs17465637			
CAPS[2]	*993*	0.004	0.007
KORA[2]	*1552*	-0.002	0.005
YFS[3] *(rs17011666)*	*2015*	0.001	0.004
REGICOR	*2116*	0.010	0.004
rs6725887			
CAPS[2]	*993*	-0.027	0.009
KORA[2]	*1552*	-0.024	0.006
YFS[2]	*2425*	-0.002	0.004
Bogalusa[3]	*755*	-0.011	0.011
REGICOR	*2076*	-0.007	0.006
rs9818870			
CAPS[2]	*993*	0.008	0.008
KORA[2]	*1552*	0.003	0.006
YFS[2]	*2425*	0.004	0.005
REGICOR	*2096*	-0.007	0.006
rs12526453			
CAPS[2]	*993*	-0.002	0.007
KORA[2]	*1552*	0.004	0.004
YFS[2]	*2425*	-0.003	0.003
Bogalusa[3]	*755*	-0.020	0.008
REGICOR	*2096*	0.001	0.004
rs1333049			
CAPS (rs4977574)[2]	*993*	-0.002	0.006
KORA (rs4977574)[2]	*1552*	-0.000	0.004
Bogalusa (rs4977574)[3]	*755*	-0.004	0.007
YFS[8]	*2277*	-0.001	0.005
Health 2000[8]	*1295*	0.011	0.011

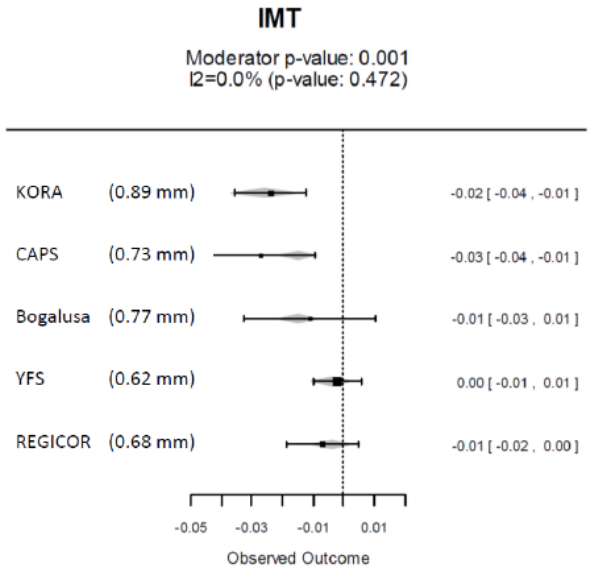
PAGE[9]			
European Americans	*8418*	0.000	0.002
African Americans	*3299*	-0.001	0.006
American Indians	*5411*	-0.007	0.004
Three City + EVA studies[10]	*4097*	0.003	0.002
Han Chinese population [11]			
Men	*490*	0.022	0.012
Women	*584*	0.001	0.002
Bruneck[12]	*769*	0	0.010
REGICOR	*2116*	-0.010	0.004
rs1746048			
CAPS[2]	*993*	0.007	0.009
KORA[2]	*1552*	0.003	0.006
YFS[2]	*2425*	0.002	0.004
Bogalusa[3]	*755*	0.012	0.011
Bruneck[5] *(rs501120)*	*738*	0.042	0.013
Health 2000[5] *(rs501120)*	*1237*	0.017	0.010
HTO[5] *(rs501120)*	*770*	0.011	0.016
REGICOR	*2111*	0.001	0.006
rs9982601			
CAPS[2]	*993*	-0.001	0.009
KORA[2]	*1552*	0.002	0.006
YFS[2]	*2425*	-0.004	0.004
Bogalusa[3]	*755*	-0.020	0.012
REGICOR	*2092*	0.003	0.006
rs10455872			
IMPROVE[13]*	*2984*	-0.003	0.002
KORA[13]*	*1552*	-0.000	0.011
REGICOR	*2140*	0.002	0.007

*Results are presented as an association between carotid intima-media thickness and a genetic risk score composed by two SNPs in the *LPA* gene. It is assumed that the effect of the SNP of interest represents half of the magnitude shown in the original publication.

SE, standard error; SNP, single nucleotide polymorphisms.

Figure, Supplementary Material.

Results of the meta-regression analyses showing that the heterogeneity between studies assessing the association between rs6725887 and carotid intima-media thickness was explained by the population mean intima-media thickness.



IMT, intima-media thickness

REFERENCES

1. García-Bermúdez M, López-Mejías R, González-Juanatey C, Corrales A, Castañeda S, Miranda-Filloy JA, et al. Association study of MIA3 rs17465637 polymorphism with cardiovascular disease in rheumatoid arthritis patients. DNA Cell Biol. 2012;31:1412-7.
2. Conde L, Bevan S, Sitzer M, Klopp N, Illig T, Thiery J, et al. Novel associations for coronary artery disease derived from genome wide association studies are not associated with increased carotid intima-media thickness, suggesting they do not act via early atherosclerosis or vessel remodelling. Atherosclerosis. 2011;219:684-9.
3. Hernesniemi JA, Seppälä I, Lyytikäinen LP, Mononen N, Oksala N, Hutri-Kähönen N, et al. Genetic Profiling Using Genome-Wide Significant Coronary Artery Disease Risk Variants Does Not Improve the Prediction of Subclinical Atherosclerosis: The Cardiovascular Risk in Young Finns Study, the Bogalusa Heart Study and the Health 2000 Survey – A Meta-Analysis of Three Independent Studies. PLoS One. 2012;7:e28931.
4. López-Mejías R, García-Bermúdez M, González-Juanatey C, Castañeda S, Miranda-Filloy JA, Gómez-Vaquero C, et al. Lack of association between the CXCL12 rs501120 polymorphism and cardiovascular disease in Spanish patients with rheumatoid arthritis. Hum Immunol. 2012;73:543-6.
5. Kiechl S, Laxton RC, Xiao Q, Hernesniemi JA, Raitakari OT, Kähönen M, et al. Coronary artery disease-related genetic variant on chromosome 10q11 is associated with carotid intima-medica thickness and atherosclerosis. Arterioscler Thromb Vasc Biol. 2010;30:2678-83.
6.-Nambi V, Boerwinkle E, Lawson K, Brautbar A, Chambless L, Franeschini N, et al. The 9p21 genetic variant is additive to carotid intima media thickness and plaque in improving coronary heart disease risk prediction in white participants of the Atherosclerosis Risk in Communities (ARIC) Study. Atherosclerosis. 2012;222:135-7.
7. Cunnington MS, Mayosi BM, Hall DH, Avery PJ, Farrall M, Vickers MA, et al. Novel genetic variants linked to coronary artery disease by genome-wide association are not associated with carotid artery intima-media thickness or intermediate risk phenotypes. Atherosclerosis. 2009;203:41-4.
8. Samani NJ, Taitakari OT, Sipilä K, Tobin MD, Schunkert H, Juonala M, et al. Coronary artery disease-associated locus on chromosome 9p21 and early markers of atherosclerosis. Arterioscler Thromb Vasc Biol. 2008;28:1679-83.
9. Zhang L, Buzkova P, Wassel CL, Roman MJ, North KE, Crawford DC, et al. Lack of associations of ten candidate coronary heart disease risk genetic variants and subclinical

atherosclerosis in four U.S. populations: The Population Architecture using Genomics and Epidemiology (PAGE) study. Atherosclerosis. 2013;228:390-9.
10. Plichart M, Empana JP, Lambert JC, Amouyel P, Tiret L, Letenneur L, et al. Single polymorphism nucleotide rs1333049 on chromosome 9p21 is associated with carotid plaques but not with common carotid intima-media thickness in older adults. A combined analysis of the Three-City and the EVA studies. Atherosclerosis. 2012;222:187-90.
11. Lin HF, Tsai PC, Lin RT, Khor GT, Sheu SH, Juo SH. Sex differential genetic effect of chromosome 9p21 on subclinical atherosclerosis. PLoS One. 2010;5:e15124.
12. Ye S, Willeit J, Kronenberg F, Xu Q, Kiechl S. Association of genetic variation on chromosome 9p21 with susceptibility and progression of atherosclerosis: a population-based, prospective study. J Am Coll Cardiol. 2008;52:378-84.
13. Helgadottir A, Gretarsdottir S, Thorleifsson G, Holm H, Patel RS, Gudnason T, et al. Apolipoprotein(a) genetic sequence variants associated with systemic atherosclerosis and coronary athersoclerotic burden but not with venous thromboembolism. J Am Coll Cardiol. 2012;60:722-9.