Title: Cross-sectional gene-smoking interaction analysis in relation to subclinical atherosclerosis -Results from the IMPROVE study

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Running Title (49/50): gene-smoking interactions and C-IMT

Words in abstract: 225
Count words: 2069
Lines: 484
Tables: 3
Figure: 1
References: 86
Supplemental Material: Materials and Methods, 6 Tables and Members of the IMPROVE

Abstract (225/300)

Background: Smoking is associated with carotid intima-media thickness (C-IMT). However, knowledge about how genetics may influence this association is limited. We aimed to perform non-hypothesis driven gene-smoking interaction analyses to identify potential genetic variants, among those included in immune and metabolic platforms, that may modify the effect of smoking on C-IMT.

Materials: We used baseline data from 1,551 men and 1,700 women, aged 55-79, included in a European multi-center study. C-IMT_{max}, the maximum of values measured at different locations of the carotid tree, was dichotomized with cut-point values >=75^{th}, respectively. Genetic data were retrieved through use of the Illumina Cardio-Metabo- and Immuno- Chips. Gene-smoking interactions were evaluated through calculations of Synergy index (S). After adjustments for multiple testing, p-values of <$2.4x10^{-7}$ for S were considered significant. The models were adjusted for age, sex, education, physical activity, type of diet and population stratification.

Results: Our screening of 207,586 SNPs available for analysis, resulted in the identification of 47 significant gene-smoking synergistic interactions in relation to C-IMT_{max}. Among the significant SNPs, 28 were in protein coding genes, 2 in non-coding RNA and the remaining 17 in intergenic regions.

Conclusions: Through non-hypothesis-driven analyses of gene-smoking interactions, several significant results were observed. These may stimulate further research on the role of specific genes in the process that determines the effect of smoking habits on the development of carotid atherosclerosis.

Keywords: carotid intima-media thickness, single nucleotide polymorphism, epidemiological studies, smoking

Non-standard Abbreviations and Acronyms

IMPROVE: Carotid Intima Media Thickness [IMT] and IMT-Progression as Predictors of Vascular Events in a High Risk European Population

C-IMT: Carotid intima media thickness

C-IMT$_{max}$: The maximum of C-IMT values measured at different locations of the carotid tree

Introduction

Subclinical atherosclerosis is an asymptomatic, chronic condition that is easily undiagnosed until a clinical event occurs, such as myocardial infarction or stroke [1]. Carotid intima media thickness (C-IMT), assessed with B-mode ultrasound, a non-invasive method, has been shown to be a valid surrogate marker for subclinical atherosclerosis [2], and a predictor for future cardiovascular disease [3,4]

Previous studies indicate that genetic susceptibility plays an important role in the pathogenesis of atherosclerosis [5-8]. The reported proportions of heritability of carotid atherosclerosis vary between 2% to 78% [8]. Part of this heritability is likely to be explained by gene-environment interactions [8]. There are hopes from the scientific community and healthcare that personalized medicine, such as knowledge of how genetic background can interact with modifiable factors and thereby influence cardiovascular risk, will be able to contribute to improved prevention of cardiovascular disease.

Among the risk factors for premature atherosclerosis, smoking has been identified as a major determinant of atherosclerotic development [9-11]. Studies have shown that smoking exposure and duration of smoking cessation can affect carotid artery structure in all phases of atherosclerosis [12,13]. In an earlier investigation based on data from a European multi-center study (IMPROVE), smoking was found to be a major determinant of C-IMT [14].

Previous studies that have investigated gene-smoking interactions behind carotid atherosclerosis were generally performed with a candidate gene approach and the results are inconclusive [15-33]. Only two studies evaluated gene-smoking interactions with an explorative approach, using the whole genome, one based on 669 Hispanics, mainly women, residing in N.Y. [34], and the other based on 1,776 men from West Africa [35]. These studies are insufficient to detect all important gene-smoking interactions due to their limited sample size. In addition, it is doubtful whether the results can be generalized to populations of other ancestries.

Hence, we aimed to explore gene-smoking interactions behind carotid subclinical

atherosclerosis in a multi-center study including men and women of European ancestry. We limited the search for interactions to include genetic variants available via platforms for genetic studies of cardiovascular, metabolic and immune traits.

Materials and methods

The data that support the findings of this study are available from the corresponding author upon reasonable request.

The Institutional review board (IRB) at each recruitment center (Karolinska Institutet, Stockholm, Sweden; University of Milan, Milan, Italy; University of Kuopio and Kuopio Research Institute of Exercise Medicine, Kuopio, Finland; University Hospital Groningen, Groningen, The Netherlands; University of Perugia, Perugia, Italy; Groupe Hôpital Pitie-Salpetriere, Paris, France) approved the study. Written informed consents for general participation and for the genotyping were provided by all participants. The study was carried out in accordance with the Helsinki Declaration.

Full materials and methods are available in Supplemental Materials.

Results

Baseline characteristics of all study participants and by their smoking status are presented in Table 1. The current smokers were younger, less physically active and educated than non-smokers. Smokers had also higher levels of total cholesterol, triglycerides, low-density lipoprotein cholesterol (LDL-C), blood glucose and Hs-C-reactive protein. However, their level of uric acid and creatinine were lower than in non-smokers.

In total, 207,586 genetic variants were available for analyses. Results from the main analysis investigating gene-smoking interaction in relation to C-IMT_{max} cut-off at the 75^{th} percentile are shown in Table 2. We found 47 SNPs significant (p for Synergy index $<2.4x10^{-7}$) after Bonferroni correction. All the aforementioned interaction results were synergistic, with Synergy index point estimates in the range between 3.3 and 5.8 (*Supplemental Table I*). Compared to the reference group of non-smokers without the risk variant, the odds for having C-IMT $>75^{th}$ percentile associated with smoking and having the risk variant were approximately 3-4-fold higher (Table 2). Of the 47 significant SNPs, 28 were in protein coding genes, 2 in non-coding RNA and the remaining 17 in intergenic regions (Table 3). None of the 47 SNPs involved in the interactions identified in our study were among the published quantitative trait locus (QTL) data included in the Genotype-Tissue Expression (GTEX) (accessed March 25th, 2022).

Additional analysis that used C-IMT_{max} cut-off at the 50^{th} percentile resulted in the identification of 146 SNPs for which a significant synergistic interaction with smoking was observed (*Supplemental Table II*). Among those SNPs, 75 were in protein coding genes, 21 in non-coding RNA and the remaining 50 in intergenic regions (*Supplemental Table III*). Two of these significant SNPs (rs6032180 in *LOC105372631* and rs3744761 in *PLCD3*) were found both when using the 75th and the 50th percentile C-IMT_{max} cut-off values.

Analyses of gene-smoking interactions that also considered data where the number of observations for each of the possible combinations of the exposures considered are less than 10 resulted in the identification of additional significant results for the C-

180 IMT_{max}, cut-off 75th percentile (*Supplemental Table IV*), and for C-IMT_{max}, cut-off 50th
181 percentile (*Supplemental Table V*). All the observed interactions were synergistic. Of
182 the SNPs that appeared in these results, 130 are located in protein coding genes, 43
183 in long non-coding RNA and 84 in intergenic regions (*Supplemental Table VI*).
184
185 We observed no significant results of interaction on the multiplicative scale.

Discussion

In this population of European descent at high risk of CVD but free of clinical manifestations of CVD, our non-hypothesis-based analyses of gene-smoking interactions resulted in the identification of several genetic variants that may have a role in the process behind the effects of smoking on the development of carotid atherosclerosis. Among the 47 SNPs identified in the main analyses, 8 SNPs (Figure 1) are located in any of 7 coding genes that in previous research have been linked to atherosclerosis development: rs72676073 in the interleukin 23 receptor (*IL23R*), rs9877192 in the LIM domain containing preferred translocation partner in lipoma (*LPP*), rs2278392 in the 5-hydroxytryptamine receptor 4 (*HTR4*), rs10810371 in the tetratricopeptide repeat domain 39B (*TTC39B*), rs7068194 and rs12251673 in the 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase (*PFKFB3*), rs2511241 in the purinergic receptor P2Y2 (*P2RY2*), and rs915064 in the potassium voltage-gated channel subfamily H member 5 (*KCNH5*) [36-42]. None of these coding genes were identified in two previous studies that evaluated gene-smoking interactions with an explorative approach in relation to carotid atherosclerosis [34,35]. These two studies were based on the whole genome and assessed interaction on the multiplicative scale only; significant findings of interaction with smoking were observed for a few genetic variants (rs112017404; rs144170770; rs4941649; rs1192824; rs77461169, rs3751383) [34,35] that were not available in the Cardio-Metabo- and Immuno- Chips.

Scientific support for relevance of the *IL23R* gene seems to be emerging; it encodes for a protein, interleukin 23 receptor, involved in the cascade of pro-inflammatory mediators which may in turn play a role in the development of atherosclerosis [36]. Further, the *IL23R* gene has been previously related to autoimmune disease [43,44] and smoking behavior [45]. It has been found to synergically interact with smoking in relation to sarcoidosis, an autoimmune disease, in a Swedish population-based case-control study [43].The *HTR4* and *P2RY2* genes may also possibly be of particular interest. These proteins belong to the family of serotonin and purinergic receptors, respectively. The activation of extracellular nucleotide purinergic receptors, such as adenosine triphosphate (ATP), has been suggested to stimulate inflammatory mediators [46] and regulate the expression of vascular cell adhesion molecule (VCAM), which is thought to be important for the pathogenesis of atherosclerosis [39]. The *HTR4*

gene has been noted to associate to C-IMT in a previous study based on the IMPROVE study material using a candidate gene approach [41].

Among the 47 SNPs identified in our main analysis of interaction as well as in our additional analyses that used the 50th percentile cut-off, there is a SNP (rs3744761), located in a protein coding gene, the phospholipase C delta 3 (*PLCD3*) gene, which may be of particular interest due to its link to hypertension. This gene has been identified in the Global Blood Pressure Genetics (BPGEN) Consortium genome-wide association study (GWAS) including >34,000 study participants, as one of eight genes linked to hypertension [47]. Hypertension, in turn, has been consistently associated with increased C-IMT in several studies including the IMPROVE [14,48]. The identification of the PLCD3 gene in the BPGEN was not confirmed in a later larger GWAS: the International Consortium for Blood Pressure (approximately 200,000 study participants including also BPGEN participants) [49]. A possible explanation for this lack of replication may relate to underlying gene-smoking interaction.

Among the 146 significant interaction results generated from analyses that used the 50th percentile C-IMT$_{max}$ cut-off, 75 are in protein coding genes. Among those, perhaps the most interesting finding involves the *APOB* gene (rs550619 and rs570877). The *APOB* gene encodes for the well-known apolipoprotein B (APOB) protein involved in the transportation and metabolism of lipids such as Low-Density-Lipoprotein (LDL), which in turn seems to play a fundamental role in CVD pathophysiology [50]. Findings from recent Mendelian randomization studies suggest APOB as the predominant lipoprotein trait that accounts for a causal mechanism that links LDL to CVD [51,52]. Also, levels of APOB have been noted to increase in relation to smoking tobacco [53], however, not consistently [54].

The remaining significant results (not discussed above) from analyses based on the C-IMT$_{max}$ 75th or 50th percentile cut-offs, involve SNPs located in genes previously discussed in relation to: 1) regulation of cardiometabolic factors and related diseases such as obesity, hypertension and diabetes (e.g. *COBLL1; HFM1, CXCR1; COL21A1, DOCK3; DGKB, BMP1; IDE; KCNQ1 and KCNQ1-AS1, ZC3H10*) [55-64], 2) endothelial inflammation and dysfunction (e.g. *TNFAIP8L1*, *CCNY*, *GSE1*) [65-67], 3)

vascular smooth muscle cell proliferation (e.g. *VEGFA*) [68], 4) inflammatory diseases (e.g *PSORS1C1*) [69], 5) risk of CVD hard endpoint such as atrial fibrillation and venous thromboembolism (e.g. *ZFPM2; LMO7*) [70,71] and 6) addiction behavior including nicotine dependence (e.g. *SP140L, THSD7B*) [72,73].

From the results of our analyses restricted to cell counts of 10 or below, the identification of a SNP located in the PIN2/TERF1 interacting, telomerase inhibitor 1 (*PINX1)* gene is potentially interesting, because this gene was previously identified in GWAS of subclinical atherosclerosis [6] and carotid plaque [7]. However, it was not found to interact with smoking in a previous study on C-IMT using a candidate gene approach [33]. The study addressed multiplicative interactions only.

An important advantage of our study is that we did not limit the gene-smoking interaction analyzes to involve SNPs identified in previous GWAS of C-IMT. It is possible that a gene itself is not associated with C-IMT but becomes important only when smoke exposure occurs. Interestingly, none of the SNPs we have identified as significantly involved in smoking interaction are among the significant findings reported in previous GWAS in relation to C-IMT or smoking behavior [5,7].

Limitations

Our study, just like other exploratory studies, cannot determine which findings are truly positive, and as to whether there are other true effects we did not detect. However, we used the most conservative approach available to adjust for multiple testing, which increases the likelihood that reported findings are true positive. Further, to our knowledge, our study is the largest to date investigating gene-smoking interaction in relation to subclinical atherosclerosis with an explorative approach. Interactions we may have failed to identify should be of a smaller magnitude than those we have identified. Concerning our positive findings, replication analyses using an external study material would have been a good complement. However, no suitable material for replication analyses was available. Another study limitation is that our genetic data were extracted from genetic chips which do not encompass the whole genome; our results are thus limited to genes related to cardiovascular and immunological traits which means that some of the relevant SNPs related to smoking

predisposition may not have been included. An additional limitation is that our results may not be generalized to populations other than those with European ancestry and at high risk of CVD. Finally, there is also a limitation linked to the fact that our chosen method for interaction analyses requires dichotomization of exposure variables; the results may have been diluted because we included former smokers in the same category as the current smokers. However, smoking cessation is considered a risk factor for CVD [74]. Further, studies on the relation between smoking cessation and C-IMT have not shown any clear decreased risk of C-IMT progression [75].

Conclusions

In this European population at high risk of CVD, we identified several significant gene-smoking interactions in relation to C-IMT. Further research in this field is urged to build strong scientific evidence that may open new possibilities for improving cardiovascular prevention through personalized recommendations or drug development.

Acknowledgments

a) Acknowledgments: The authors thank all the study participants of the IMPROVE study. We also thank Max Vikström and Paolo Frumento for their statistical support.

b) Sources of funding: The IMPROVE study was supported by the European Commission [Contract number: QLG1- CT- 2002- 00896] (to ET, DB, PG, SK, MP); Ministero della Salute Ricerca Corrente, Italy [RC2017 BIO30 ID:2631169; RC2018 MMP4.9 ID:2634520; RC2019 MPP 4D ID:2755475] (to DB). The IMPROVE was also funded by the Swedish Research Council (8691 and 0593), European Commission (Contract number: QLG1- CT- 2002- 00896), the Swedish Heart-Lung Foundation, the Swedish Foundation for Strategic Research, the Stockholm County Council (project 562183), and the British Heart Foundation (RG2008/008). The present study was supported by the Swedish Research Council (project 2016-02815 to KL). RJS is supported by a UKRI Innovation-HDR-UK Fellowship (MR/S003061/1).

c) Conflict of interest: None

Supplemental Materials:

Supplemental Methods

Supplemental Tables I-VI

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Table 1: Characteristics of the IMPROVE study participants by smoking status

	Entire sample (N = 3,251)	**Current smokers (n = 520)**	**Non-smokers (n = 2,731)**
Male; n (%)	1,551 (47.7)	273 (52.5)	1,278 (46.8)
Age, years; mean±SD	64.4 ± 5.4	61.5 ± 5.2	64.9 ± 5.7
Geographical gradient; n (%)			
Kuopio	928 (28.5)	166 (31.9)	762 (27.9)
Stockholm	488 (15.0)	63 (12.1)	425 (15.6)
Groningen	400 (12.3)	77 (14.8)	323 (11.8)
Paris	434 (13.3)	48 (9.2)	386 (14.1)
Milan	517 (15.9)	93 (17.9)	424 (15.5)
Perugia	484 (14.9)	73 (14.0)	411 (15.0)
Anthropometric variables; mean±SD			
BMI, kg/m^2	26.7 ± 4.3	26.3 ± 4.2	26.7 ± 4.6
Waist/hip ratio	0.92 ± 0.09	0.92 ± 0.08	0.92 ± 0.09
Blood pressure; mean±SD			
Diastolic blood pressure, mmHg	81 ± 9	81 ± 10	81 ± 10
Systolic blood pressure, mmHg	140 ± 19	140 ± 18	140 ± 18
Physical activity level; n (%)			
Low	613 (18.8)	114 (22.0)	499 (18.3)
Medium	1,453 (44.8)	234 (45.1)	1,219 (44.7)
High	1,180 (36.4)	171 (32.9)	1,009 (37.0)
Education; n (%)			
≤9 years	1,467 (45.6)	238 (46.6)	1,229 (45.4)
9-12 years	803 (25.0)	133 (26.0)	670 (24.7)
≥12 years	944 (29.3)	140 (27.4)	804 (29.7)
Mediterranean diet score*; n (%)			
0	316 (9.7)	68 (13.1)	248 (9.1)
1	758 (23.3)	142 (27.3)	616 (22.6)
2	930 (28.6)	159 (30.6)	771 (28.2)
3	731 (22.5)	102 (19.6)	629 (23.0)
4	427 (13.1)	42 (8.1)	385 (14.1)
5	80 (2.5)	7 (1.3)	73 (2.7)
6	9 (0.3)	0 (0.0)	9 (0.3)
Biochemical markers; mean ± SD			
Total cholesterol, mmol/l	5.44 ± 1.11	5.53 ± 1.12	5.43 ± 1.25
HDL cholesterol, mmol/l	1.21 ± 0.36	1.15 ± 0.36	1.22 ± 0.30

Triglycerides, mmol/l	1.29 ± 1.01	1.41 ±1.13	1.27 ±1.17
LDL cholesterol, mmol/l	3.51 ± 1.01	3.57 ± 1.01	3.49 ± 0.94
Uric acid, µmol/l	313.8 ± 71.6	309.0 ± 72.0	314.7 ± 71.5
Blood glucose, mmol/l	5.50 ± 1.54	5.60 ± 1.45	5.50 ± 1.42
Creatinine, µmol/l	80.7 ± 17.7	80.1 ± 17.3	80.8 ± 17.7
C-reactive protein	2.79 ± 4.30	2.98 ± 3.60	2.76 ± 4.42
Medical history and drug use; n (%)			
Hypercholesterolemia[†]	2,299 (70.8)	337 (64.9)	1,962 (71.9)
Hypertriglyceridemia[‡]	827 (25.5)	151 (29.0)	676 (24.8)
Hypertension[‖]	2,327 (71.6)	331 (63.6)	1,996 (73.1)
Diabetes[#]	775 (24.2)	125 (24.3)	650 (24.2)
Statin use	1,290 (39.7)	166 (31.9)	1,124 (41.2)

Results are expressed as mean and standard deviation (SD) for continuous variables and as count and proportion (%) for categorical variables;
***** The score indicates level of adherence; zero corresponds to the lowest level
[†] Serum total cholesterol>5.17 mmol/L
[‡] Serum triglycerides>1.7 mmol/L
[‖] Self-reported and/or use of antihypertensive drugs
[#] Self-reported and/or use of antidiabetic drugs

Table 2: Significant gene-smoking interaction results* after Bonferroni adjustment for multiple testing in relation to C-IMT$_{max}$ with cut-off at the 75th percentile. A dominant genetic model was assumed†.

	Number of observations										Odds Ratio (95% Confidence Interval) ‡			
	Non-smokers without the risk variant		Non-smokers with the risk variant		Smokers without the risk variant		Smokers with the risk variant				Reference group: Non-smokers without the risk variant			
	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Risk allele	MAF (%)	Non-smokers with the risk variant	Smokers without the risk variant	Smokers with the risk variant	p Synergy index
Chr 1														
rs12134420	1874	597	65	22	324	144	11	13	C	20	1.11(0.67;1.83)	1.62(1.29;2.03)	4.92(2.09;11.57)	$4.43x10^{-14}$
rs2446622	1702	538	230	75	306	131	26	25	G	6	1.02(0.77;1.36)	1.56(1.23;1.98)	3.86(2.14;6.97)	$9.17x10^{-18}$
rs72676073	1683	532	256	87	290	119	45	38	G	7	1.19(0.91;1.55)	1.50(1.17;1.92)	3.53(2.22;5.62)	$8.51x10^{-15}$
rs73009101	1742	545	197	74	315	137	20	20	G	5	1.18(0.88;1.58)	1.60(1.27;2.02)	4.03(2.12;7.68)	$5.96x10^{-13}$
Chr 2														
rs6758414	1746	558	191	61	309	134	26	23	A	5	1.00(0.73;1.37)	1.56(1.23;1.97)	3.60(2.00;6.50)	$6.51x10^{-15}$
rs9789490	1386	443	553	176	253	93	82	64	G	16	1.01(0.82;1.24)	1.33(1.01;1.74)	2.96(2.06;4.24)	$4.34x10^{-17}$
Chr 3														
rs9877192	1801	572	138	47	317	139	18	18	A	26	1.12(0.79;1.6)	1.6(1.27;2.02)	4.21(2.07;8.54)	$1.48x10^{-13}$
Chr 4														
rs11736632	1620	503	319	116	281	110	54	47	A	9	1.13(0.89;1.44)	1.48(1.15;1.9)	3.13(2.05;4.78)	$9.51x10^{-13}$
Chr 5														
rs13176964	1587	490	352	128	285	116	50	41	G	10	1.16(0.92;1.46)	1.52(1.18;1.94)	3.24(2.08;5.05)	$9.03x10^{-13}$
rs2278392	1553	495	386	124	270	108	65	49	T	11	1.01(0.80;1.28)	1.44(1.12;1.87)	2.86(1.92;4.27)	$7.74x10^{-13}$
rs4867490	1622	517	316	102	291	121	44	36	G	40	1.06(0.82;1.36)	1.50(1.17;1.91)	3.27(2.05;5.21)	$8.27x10^{-15}$
rs7722352	1684	533	252	86	306	132	29	25	G	7	1.04(0.79;1.36)	1.56(1.23;1.98)	3.45(1.95;6.10)	$3.08x10^{-13}$
Chr 7														
rs28695838	1580	504	353	115	290	116	44	41	G	10	1.03(0.81;1.31)	1.43(1.12;1.84)	3.62(2.31;5.69)	$2.21x10^{-23}$
Chr 8														
rs12545167	1302	412	637	207	246	97	89	60	A	18	1.03(0.84;1.25)	1.37(1.05;1.80)	2.81(1.96;4.03)	$8.11x10-^{14}$
rs4301463	1678	530	261	89	304	127	31	30	A	7	1.14(0.87;1.49)	1.54(1.21;1.96)	3.77(2.2;6.44)	$9.53x10^{-16}$
rs6997802	1679	530	260	89	304	127	31	30	T	7	1.14(0.88;1.49)	1.54(1.21;1.96)	3.77(2.20;6.44)	$1.13x10^{-15}$
rs752039	1266	404	673	215	242	93	93	64	A	19	1.01(0.83;1.23)	1.33(1.01;1.76)	2.82(1.98;4.02)	$5.92x10^{-15}$
Chr 9														
rs10810371	1414	430	509	185	258	97	72	58	G	15	1.17(0.95;1.44)	1.43(1.10;1.88)	3.02(2.07;4.40)	$4.91x10^{-13}$
rs143207461	1734	549	205	70	314	134	21	23	C	5	1.12(0.83;1.50)	1.57(1.24;1.99)	4.00(2.16;7.43)	$2.16x10^{-15}$
Chr 10														
rs12244483	1708	540	230	79	297	120	38	37	T	6	1.05(0.8;1.40)	1.51(1.18;1.92)	3.27(2.02;5.29)	$1.20x10^{-13}$

rs12251673	1721	545	217	74	304	127	31	30	C	6	1.01(0.75;1.35)	1.54(1.21;1.95)	3.24(1.91;5.49)	$1.16x10^{-12}$
rs7068194	1722	545	217	74	304	127	31	30	T	6	1.01(0.75;1.35)	1.54(1.21;1.96)	3.25(1.92;5.50)	$1.17x10^{-12}$
rs7092757	1708	541	231	77	296	120	39	37	G	6	1.01(0.76;1.35)	1.51(1.18;1.92)	3.19(1.98;5.13)	$1.60x10^{-13}$
rs72826094	1847	589	91	28	321	145	12	12	A	20	1.02(0.65;1.59)	1.63(1.30;2.05)	4.55(1.92;10.81)	$4.27x10^{-14}$
Chr 11														
rs1002171	1716	535	223	84	306	128	29	29	G	6	1.10(0.84;1.46)	1.55(1.22;1.98)	3.46(2.02;5.94)	$1.17x10^{-12}$
rs2434468	1654	515	285	103	307	129	28	27	C	8	1.06(0.82;1.37)	1.55(1.22;1.97)	3.55(2.04;6.18)	$6.56x10^{-15}$
rs2511241	1658	526	281	93	301	124	34	33	C	8	1.06(0.82;1.38)	1.51(1.19;1.93)	3.69(2.21;6.16)	$1.53x10^{-18}$
rs3741392	1630	520	309	99	295	117	39	40	C	8	1.01(0.78;1.30)	1.49(1.17;1.91)	3.16(1.97;5.05)	$3.20x10^{-14}$
rs61899280	1726	546	213	73	310	134	25	23	C	6	1.07(0.80;1.43)	1.57(1.24;1.98)	3.96(2.18;7.19)	$3.63x10^{-17}$
Chr 12														
rs10506726	1733	537	206	81	317	135	17	22	T	6	1.22(0.92;1.62)	1.59(1.26;2.01)	5.04(2.57;9.86)	$1.95x10^{-21}$
rs11171745	1494	470	445	149	266	106	69	51	A	12	1.08(0.87;1.34)	1.45(1.12;1.88)	2.91(1.97;4.30)	$1.14x10^{-12}$
rs11171773	1691	536	248	83	302	128	33	29	A	7	1.06(0.81;1.40)	1.54(1.21;1.96)	3.33(1.98;5.61)	$9.35x10^{-13}$
rs116378618	1718	545	219	74	307	131	28	26	A	6	1.09(0.81;1.45)	1.56(1.23;1.98)	3.50(2.01;6.11)	$8.02x10^{-13}$
rs1689512	1494	470	445	149	266	106	69	51	G	12	1.08(0.87;1.34)	1.45(1.12;1.88)	2.91(1.97;4.30)	$1.14x10^{-12}$
rs17118317	1478	469	461	150	265	106	70	51	C	13	1.03(0.83;1.28)	1.44(1.11;1.86)	2.85(1.93;4.21)	$8.38x10^{-13}$
rs35436573	1604	507	335	112	288	119	47	38	A	9	1.03(0.81;1.32)	1.49(1.16;1.91)	3.13(1.99;4.92)	$3.83x10^{-14}$
rs4762693	1788	571	151	48	319	141	16	16	G	27	1.03(0.73;1.45)	1.61(1.27;2.02)	3.90(1.89;8.05)	$7.19x10^{-13}$
rs773643	1623	513	316	106	285	117	50	40	A	9	1.09(0.85;1.39)	1.49(1.16;1.91)	3.15(2.03;4.91)	$2.49x10^{-13}$
rs7956913	1488	473	448	146	266	106	69	51	D	12	1.03(0.83;1.29)	1.43(1.10;1.85)	2.91(1.97;4.30)	$8.69x10^{-14}$
Chr 13														
rs12872592	1367	430	572	189	261	99	74	58	G	15	1.04(0.85;1.28)	1.39(1.07;1.81)	3.00(2.05;4.38)	$5.58x10^{-16}$
Chr 14														
rs4981312	1410	452	526	167	254	99	80	58	G	15	1.01(0.82;1.24)	1.39(1.06;1.81)	2.80(1.94;4.04)	$1.15x10^{-13}$
rs7155978	1658	523	281	96	301	128	34	29	T	7	1.03(0.80;1.34)	1.54(1.21;1.96)	3.30(1.95;5.59)	$2.69x10^{-13}$
rs915064	1746	553	193	66	311	135	24	22	C	5	1.05(0.77;1.42)	1.58(1.25;2.00)	3.59(1.96;6.59)	$4.86x10^{-13}$
Chr 16														
rs1003341	1140	356	799	263	220	79	115	78	T	23	1.05(0.87;1.27)	1.29(0.96;1.73)	2.69(1.94;3.73)	$6.22x10^{-13}$
Chr 17														
rs3744761	1762	559	177	60	311	131	24	26	T	5	1.05(0.76;1.44)	1.57(1.24;1.98)	3.52(1.96;6.32)	$1.15x10^{-12}$
rs4362432	1660	516	279	103	300	121	35	36	A	8	1.17(0.90;1.50)	1.53(1.20;1.95)	3.52(2.15;5.76)	$4.68x10^{-14}$
Chr 20														
rs6032180	1607	508	332	111	285	117	50	40	T	9	1.06(0.83;1.35)	1.48(1.16;1.90)	3.23(2.06;5.07)	$4.64x10^{-15}$

MAF: minor allele frequency;

***** Synergy index results were considered significant at p-values <$2.4x10^{-7}$; Minimum number of subjects in each group:10

† Individuals who carry either one or two copies of the risk allele are considered to carry the risk variant

‡ Model adjusted for sex, age, education (categorical), physical activity (categorical), Mediterranean diet score and population structure (MDS1-3 continuous)

Table 3: Genes in proximity to the genetic variants included in the significant gene-smoking interaction results observed for C-IMT$_{max}$ with cut-off at the 75th percentile

	Position	Function	Gene in proximity to the genetic variant
Chr 1			
rs12134420	85272625	Intron variant	*BCL10*
rs2446622	161637183	Intergenic variant	*None*
rs72676073	67203930	Intron variant	*IL23R*
rs73009101	116825975	Intergenic variant	*None*
Chr 2			
rs6758414	120538909	Intergenic variant	
rs9789490	212992755	Upstream variant	*LOC102725082*
Chr 3			
rs9877192	188708468	Intron variant	*LPP*
Chr 4			
rs11736632	56306169	Intron variant	*CRACD; LOC105377664*
Chr 5			
rs13176964	175407619	Intergenic variant	*None*
rs2278392	148548662	Intron variant; upstream variant	*HTR4; LOC107986462; LOC105378221*
rs4867490	32919896	Intergenic variant	*None*
rs7722352	123450395	Intergenic variant	*None*
Chr 7			
rs28695838	52527273	Intergenic variant	*None*
Chr 8			
rs12545167	69595708	Intron variant	*SULF1*
rs752039	69601242	Intron variant	*SULF1*
rs4301463	130457363	Intergenic variant	*None*
rs6997802	130457843	Intergenic variant	*None*
Chr 9			
rs10810371	15290344	Intron variant	*TTC39B*
rs143207461	133514431	Upstream variant	*MYMK*
Chr 10			
rs12244483	30545968	Intergenic variant	*None*
rs12251673	6150108	Intron variant	*PFKFB3*
rs7068194	6149259	Intron variant	*PFKFB3*
rs7092757	30543292	Intergenic variant	*None*
rs72826094	113041729	Intron variant	*TCF7L2*
Chr 11			
rs1002171	71506525	Intergenic variant	*None*

rs2434468	43936390	Intergenic variant	*None*
rs2511241	73234296	Missense variant	*P2RY2*
rs3741392	64933558	Intron variant	*PPP2R5B*
rs61899280	46945082	Intron variant	*C11orf49*
Chr 12			
rs10506726	77073285	Intergenic variant	*None*
rs11171745	56118887	Intron variants	*ZC3H10*
rs11171773	56189702	Upstream variant	*SMARCC2; LOC107984468*
rs773643	56181404	Intron variant	*SMARCC2*
rs116378618	56166019	Intron variant	*SMARCC2*
rs1689512	56116853	Intron variant; upstream variant	*RPL41; ZC3H10*
rs17118317	56126591	Prime UTR* variant; upstream variant	*ZC3H10; ESYT1*
rs7956913	56129931	Intron variant	*ESYT1*
rs35436573	56159225	Intron variant	*MYL6*
rs4762693	21009309	Intron variant	*SLCO1B3-SLCO1B7*
Chr 13			
rs12872592	21154616	Prime UTR[a] variant	*SKA3*
Chr 14			
rs4981312	20675000	Intergenic variant	*None*
rs7155978	68785538	Intergenic variant	*None*
rs915064	62710859	Intron variant	*KCNH5*
Chr 16			
rs1003341	25537652	Intergenic variant	*None*
Chr 17			
rs3744761	45118646	Intron variant	*PLCD3*
rs4362432	45119179	Intron variant	*PLCD3*
Chr 20			
rs6032180	45428246	Intron variant	*LOC105372631*

* UTR: untranslated region

Figure 1: Visualization of 8 selected significant results from interaction analyzes. The bars show odds ratio point estimates for the risk of having C-IMT above the 75th percentile associated with a) the genetic risk variant without the presence of smoking, b) smoking without the presence of the genetic risk variant, and c) the genetic risk variant in combination with smoking. Reference category is non-smoking without the presence of the genetic risk variant. These 8 SNPs are located in coding genes previously linked to the development of atherosclerosis.