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Loss of Cardio-Protective Effects at the *ADAMTS7* Locus Due to Gene-Smoking Interactions

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Abstract

***Background*—**Common diseases such as coronary heart disease (CHD) are complex in etiology. The interaction of genetic susceptibility with lifestyle factors may play a prominent role. However, gene-environment interactions for CHD have been difficult to identify. Here, we investigate interaction of smoking behavior, a potent lifestyle factor, with genotypes that have been shown to associate with CHD risk.

***Methods*—**We analyzed data on 60,919 CHD cases and 80,243 controls from 29 studies for gene-smoking interactions for genetic variants at 45 loci previously reported to associate with CHD risk. We also studied 5 loci associated with smoking behavior. Study specific gene-smoking interaction effects were calculated and pooled using fixed-effects meta-analyses. Interaction analyses were declared to be significant at a *P-value* < 1.0×10^{-3} (Bonferroni correction for 50 tests).

***Results*—**We identified novel gene-smoking interaction for a variant upstream of the *ADAMTS7* gene. Every T allele of rs7178051 was associated with lower CHD risk by 12% in never-smokers (P-value: 1.3×10^{-16}) compared to 5% in ever-smokers (P-value: 2.5×10^{-4}) translating to a 60% loss of CHD protection conferred by this allelic variation in people who smoked tobacco (*Interaction P-value*: 8.7×10^{-5}). The protective T allele at rs7178051 was also associated with reduced *ADAMTS7* expression in human aortic endothelial cells and lymphoblastoid cell lines. Exposure of human coronary artery smooth muscle cells to cigarette smoke extract led to induction of *ADAMTS7*.

***Conclusions*—**Allelic variation at rs7178051 that associates with reduced *ADAMTS7* expression confers stronger CHD protection in "never-smokers" compared to "ever-smokers". Increased vascular *ADAMTS7* expression may contribute to the loss of CHD protection in smokers.

Key Words: coronary artery disease; Genome Wide Association Study; smoking; ADAMTS7, Gene-environment interaction

Clinical Perspective

What is new?

- Using data on 60,919 CHD cases and 80,243 controls, this study conducted gene-environment interaction analyses to investigate effect modification by smoking behavior at established CHD and smoking related loci.
- Cardio-protective effects associated with allelic variation at the *ADAMTS7* locus were attenuated by 60% in people who smoked tobacco compared to those who did not smoke.
- Allelic variation at *ADAMTS7* associated with reduced CHD risk was associated with reduced *ADAMTS7* expression in human aortic endothelial cells and lymphoblastoid cell lines.
- Exposure of human coronary artery smooth muscle cells to cigarette smoke extract led to induction of *ADAMTS7*.

What are the clinical implications?

- These human genomic data provide new insights into potential mechanisms of CHD in cigarette smokers.
- Findings from this study also point towards the directional impact of the *ADAMTS7* locus on CHD.
- ADAMTS7 and its substrates have a specific role in cigarette smoking related CHD.
- Inhibition of ADAMTS7 is a novel potential therapeutic strategy for CHD that may have particular benefits in individuals who smoke cigarettes.

Coronary heart disease (CHD) is a complex disorder resulting from the interplay of lifestyle and genetic factors.[1, 2] Yet, gene-environment interactions for CHD have been difficult to identify. Cigarette smoking is one of the strongest lifestyle risk factors for CHD but the underlying molecular mechanisms of CHD in humans who smoke remain uncertain.[3-5] Cigarette smoking accounts for one-fifth of all CHD events globally and is responsible for ~1.6 million deaths attributable to CHD annually.[6] Genome-wide association studies (GWAS) have improved our understanding on the genetic predisposition to both CHD and smoking behavior.[7-10] Joint or interactive effects of genetic variation and smoking behavior in the etiology of CHD, however, remain poorly understood. GWAS can provide new opportunities to investigate gene-smoking interactions.

We hypothesized that genetic predisposition to CHD is modified by cigarette smoking at loci discovered by GWAS to be associated with either CHD or smoking behavior. We conducted a focused experiment at 50 main-effect loci (45 for CHD and 5 for smoking behavior) using genetic data and information on smoking behavior in 60,919 CHD cases and 80,243 controls from 29 different studies. We report novel findings on gene-smoking interactions in CHD.

Methods

Summary of study Design

All studies participating in the CARDIoGRAMplusC4D consortium[7-9] that had information available on smoking status, CHD risk and genotypes at the 50 CHD and smoking behavior-associated loci were invited to participate. The current study had five inter-related components (**Supplementary Figure-1)**. First, as part of the quality control, we investigated the association of smoking status with CHD risk within each study. Second, we performed an updated analysis of all

the SNPs (± 50 KB) at the 45 established CHD loci to identify the variant with the strongest CHD association in our study population at each established CHD locus. Effect estimates from each study in association with CHD risk were obtained and pooled to identify the strongest CHD associated variant ("lead variant"). Third, we investigated gene-smoking interactions at these 45 CHD loci and at 5 loci related to smoking behavior. Fourth, for loci demonstrating differential CHD associations by smoking status, we mapped the interaction region, examined linkage disequilibrium (LD) across the region and performed conditional analyses to identify independent genetic signals. Finally, for loci exhibiting gene-smoking interaction in CHD, we assessed eQTL data for association of variants with expression of local genes in available datasets and examined expression of these genes in multiple cell types that play prominent roles in smoking-CHD pathobiology.

Harmonization of phenotypes and genotypes

Summary level estimates for each study were shared via a secure FTP site. We used "ever-smoking" as a primary exposure and data were harmonized by uniformly characterizing participants in each study into two categories, "ever-smokers" and "never-smokers". "Ever-smokers" were defined as those who had smoked more than 100 cigarettes in a lifetime. For case-control studies, information on "ever smoking" status collected at the time of enrollment was used for the current analyses; whereas for prospective cohort studies, information on smoking status obtained at the baseline visit was used for the current investigation. CHD was defined based on evidence from angiography or history of verified myocardial infarction (MI), percutaneous coronary interventions (PCI) or coronary artery bypass grafting (CABG) as published in CARDIoGRAMplusC4D projects.[7-9] Genotype data generated through GWAS (directly genotyped or imputed) or cardio-metabochip (directly genotyped only) arrays were obtained from each study and all genetic data were aligned using the build-37 reference panel. Imputed SNPs were removed if they had any of the

following: (i) a minor allele frequency of <1%; (ii) info score of <0.90; or (iii) confidence score <0.90. For each study, GWAS data were imputed using the Phase II CEU HapMap reference population.[11] Standard quality control criteria were applied by each participating study, as described previously.[7] All participating studies in the CARDIoGRAMplusC4D consortium were approved by their locally relevant institutional review boards and all participants gave written informed consent before their enrollment in each study. [7-9]

Statistical Analysis

Gene-smoking interaction analyses

Initial quality control and association of established CHD loci with CHD risk

As part of an initial quality control, effect estimates from each study were obtained for "ever-smoking" status and CHD risk using a case-control logistic regression model adjusted for age and sex. Each participating study also assessed and, if needed, controlled for population stratification by including principal components as covariates in the regression model as described earlier.[7-9] To identify variant(s) with the most significant association with CHD risk at established CHD loci in our study population, logistic regression analyses were conducted by each participating study for all the SNPs flanking (±50 kb) the lead variant previously reported at each CHD locus. Effect estimates and standard errors were obtained and meta-analyzed using a fixed-effects inverse variance approach. All lead variants identified through these analyses were further investigated for gene-smoking interactions in CHD. One lead variant per locus was selected for primary gene-smoking interaction analyses.

Investigation of the APOE locus

Although *APOE* has been recently established as a GWAS locus,[7] previous studies prior to GWAS have suggested that CHD risk is higher among carriers of the ε4 allele at the *APOE* locus in

smokers than in non-smokers.[12-14] Because the ε2, ε3 and ε4 alleles at the *APOE* locus are not captured by the GWAS platform, we specifically conducted genotyping for rs429358 and rs7412 variants to capture the three epsilon (ε) alleles in 13,822 participants (including 7,286 first-onset myocardial infarction cases) in the PROMIS study.[15]

Gene-smoking interaction analyses at CHD and smoking loci

To assess gene-smoking interactions, analyses were conducted within each study, adjusted for age, sex and other study specific covariates (e.g., principal components), and variants were analyzed in association with CHD separately in "ever-smokers" and "never-smokers". Results from the two groups were then used to test for interaction within each study. For the 50 variants, an interaction test statistic was calculated within each study using the following equation as adapted from *Teslovich TM* et.al.[16]

$$\frac{(\beta n - \beta e)}{\sqrt{SEn^2 + SEe^2}}$$

where β_n and β_e are the beta coefficients for the SNP in never-smokers and ever-smokers respectively, SEn and SEe are the standard errors for the log-ORs estimated for never-smokers and ever-smokers, respectively. Study specific interaction beta(s) and se(s) were calculated within each study and were pooled across studies using a fixed-effects meta-analysis. Interaction analyses were declared to be significant at a P-value of $<1.0\text{x}10^{-3}$ (Bonferroni correction for 50 tests).

Conditional analyses on chr.15q25.1

At chr.15q25.1, we observed two variants exhibiting gene-smoking interactions for CHD. The proximity of these two signals raised the possibility that the observed interactions may represent a single interaction locus across the entire region. To investigate this possibility we undertook conditional analyses using an approximate conditional and joint analyses approach, also known as GCTA (Genome-wide Complex Trait Analysis), as described previously.[17-22] Briefly, this method

leverages summary-level statistics from a meta-analysis and uses LD corrections between SNPs estimated from a reference sample. Such an approach has been shown to yield similar results to that obtained from conditional analyses conducted on individual participant data and has been successfully implemented in several other studies that have fine-mapped loci for other complex traits.[17-22] Using this approach, we first conducted separate conditional analyses at the chr.15q25.1 locus to identify main-effect variant(s) independently associated with CHD and smoking behavior, respectively. We used the meta-analyzed data for CHD main effects in the CARDIoGRAMplus4D consortium to identify SNPs independently associated with CHD risk and we used the genetic meta-analysis data from the Tobacco and Genetics Consortium (TGC) in 140,000 participants to identify variants independently associated with smoking behavior. We then estimated the effects of these independent variants on CHD risk stratified by smoking status and mutually adjusted the effects of these variants for each other.

Analysis of eQTLs and regulatory features at the chr15q25.1 gene-smoking interaction locus

eQTL analyses

We mined publicly available databases to identify genotype-related expression differences (eQTLs) in *ADAMTS7* and the *CHRNB4-A3-A5* gene cluster in order to understand the directionality of the association of expression of these genes with CHD and smoking behavior. Specifically, we investigated data available from the GTEx consortium,[23] the HapMap consortium (restricted to European populations),` and the Multiple Tissue Human Expression Resource (MuTHER).[24] We also analyzed expression data in 147 donor HAoEC lines.[25] We used a nominal P-value of 0.002 to account for multiple testing involved in the eQTL analyses.

Regulatory features of the chr. 15q25.1 region

Data from ENCODE[26] were explored as described in eMethods. ChIP-seq experiments were

performed on confluent HCASMC (Cell Applications 350-05a & Lonza CC-2583; cultured in SmGM-2 BulletKit media; Lonza) as described.[27] TCF21 (Abcam ab49475), Jun (Santa Cruz Biotechnology sc-1694), JunD (Santa Cruz Biotechnology sc-74), and CEBP (Santa Cruz Biotechnology sc-150) transcription factor binding was interrogated and H3K27ac data were acquired using the same ChIP protocol with an anti-H3K27ac antibody (Abcam; ab4729). Reads were aligned to the human genome (GRCh37p13) using STAR.[28]

Analyses of *ADAMTS7* and *CHRNB4-A3-A5* gene expression in vascular cells and tissues

ADAMTS7 and CHRNB4-A3-A5 gene expression in vascular cells

ADAMTS7 and *CHRNB4-A3-A5* mRNA levels were measured in cultured human coronary artery smooth muscle cells (HCASMC; Lonza CC-2583, Lonza Walkersville, MD), human coronary artery endothelial cells (HCAEC, Lonza CC-2585), human aortic smooth muscle cells (HAoSMC, Lonza CC-2571), human aortic endothelial cells (HAoEC, Lonza CC-2535), human aortic adventitial fibroblasts (HAoAF, Lonza CC-7014), and human acute monocytic leukemia cell line (THP-1, ATCC TIB-202). Further details are provided in eMethods.

ADAMTS7 and CHRNB4-A3-A5 gene expression in response to cigarette smoke extract

HCASMC were grown to confluence and cigarette smoke extract experiments performed at passage-7. Cigarette smoke extract was custom-prepared by Arista Laboratories (Richmond, VA). Briefly, the condensate was generated by smoking Marlboro Red King Size Hard Pack cigarettes on an analytical smoke machine under International Organization for Standardization smoking conditions. The smoke condensate was collected on 92 mm filter pads and extracted from each pad in DMSO by shaking to obtain a solution of ~20 mg/mL final concentration of the total particulate matter. Serum starved (24 hrs) HCASMC were treated with 0.5% or 1.0% cigarette smoke extract

(v/v) for 4, 12, and 24 hrs in serum reduced conditions (0.5% FBS in DMEM). Details on RNA preparation and q-PCR are provided in **Supplementary Methods**.

Results

Description of the participating studies

Of the 37 studies participating in the CARDIoGRAMplusC4D consortium, information on "ever-smoking" was available in 30 studies, yielding a total sample size of 60,919 CHD cases and 80,243 controls. All studies recruited participants of European ancestry, except PROMIS (South Asian),[15] LOLIPOP (South Asian)[29] and FGENTCARD (Lebanese).[30] Number of CHD cases and controls and percentages that were "ever-smokers" are provided in **Supplementary Table 1**. As expected, in all the participating studies, association of "ever-smoking" status with CHD risk was directionally consistent with an increased risk of CHD **(Supplementary Figure 2)**.

New variants associated with CHD at established loci

Supplementary Figure 3 and **Supplementary Table 2** present effect estimates for the association with CHD for (i) the most significant variant that we identified at known CHD loci in the current CARDIoGRAMplusC4D consortium analysis as well as for (ii) the top SNP previously reported at each of these established CHD loci. Of the 45 established CHD loci, we identified 32 for which we discovered a more statistically significant SNP in association with CHD risk in our dataset than the prior reported top variant. All of these 32 SNPs were in moderate to high LD (r^2 >0.6) with the previously published variants.[7-9] In our primary gene-smoking interaction analyses, at each of the CHD loci, we, therefore, used the SNP with the most significant CHD association **(Supplementary Figure 3** and **Supplementary Table 2)**. Because the smoking behavior phenotype (captured as

cigarettes per day [CPD]) was not available in all CARDIoGRAMplusC4D studies, we used the top variant previously reported for CPD[10] at each locus (**Supplementary Figure 4**).

Analyses of the APOE locus.

The effect of rs6857, the lead CHD variant at the *APOE* locus, was similar in "ever-smokers" compared to "never-smokers" (**Supplementary Table 3)**. Specifically, the CHD OR for the T allele at rs6857 was found to be 1.10 (P-value 7.93×10^{-4}) in "never-smokers" (12,159 CHD cases and 22,932 controls) which was quantitatively similar to the CHD OR of 1.09 (P-value: 8.68×10^{-5}) observed in "ever-smokers" (23,753 CHD cases and 24,019 controls) (interaction P-value: 0.76) (**Supplementary Figure 5a**). Investigation in the PROMIS study of the *APOE* epsilon genotypes yielded consistent findings; the OR for CHD among ε4 carriers in "never-smokers" was 1.13 compared to the CHD OR of 1.07 observed in "ever-smokers" (interaction P-value: 0.82) (**Supplementary Figure 5a**).

Novel gene-smoking interaction effects on CHD at chromosome 15q25.1

Of the 50 loci, we identified effect-modification by "ever-smoking" status on CHD risk for the lead variants at two distinct loci, rs7178051, in proximity of *ADAMTS7* (an established CHD locus), and rs1051730, in proximity of *CHRNB4-A3-A5* (an established smoking behavior locus) (**Supplementary Table 3**). Although associated with different traits and located in distinct LD blocks, these two variants reside ~224 KBs apart on chr.15q25.1 and are in weak linkage disequilibrium (LD) ($r^2 = 0.22$), raising the question of whether these two chr.15q25.1 gene-smoking interactions on CHD are independent of each other.

At the *ADAMTS7* CHD locus, the T allele at the rs7178051 variant was found to be more strongly and inversely associated with CHD risk in never-smokers (OR: 0.88; P-value: 7.02×10^{-16}) compared to a much weaker effect in ever-smokers (OR: 0.95; P-value: 8.64×10^{-4}) (P-value of

interaction: $8.57x10^{-5}$) (**Table 1**). Thus, the protective impact of the rs7178051 T allele observed in never-smokers was halved in people who smoked (**Figure-1**). This difference is not related to power differences within strata because for this variant, there were less data available in the never-smoking group (21,232 CHD cases and 38,713 controls) compared to the ever-smoking group (39,585 CHD cases and 40,749 controls). There was no substantial evidence of heterogeneity for the interaction beta across the participating studies (Heterogeneity chi-squared = 36.23 (d.f. = 25); P-value for the χ^2 test of heterogeneity = 0.06; I^2 = 31.0%; tau-squared (τ^2 = 0). We further conducted sensitivity analyses using a random effect model; the results remained unchanged and the interaction beta remained significant (**Supplementary Figure 5b**). Although the frequency of rs7178051 was 39% in Europeans compared to 28% in South Asians, further analyses stratified by ancestry (i.e., European versus non-Europeans) showed similar results (**Supplementary Figure 5c**). Other variants discovered through prior CHD GWAS at the *ADAMTS7* locus (e.g., rs7173743, rs4380028, rs3825807) were in moderate to high LD (r^2 >0.50) with rs7178051 and were also found to display a similar pattern of gene-smoking interaction effects (**Table 1**).

At the *CHRNB4-A3-A5* smoking locus, the A allele at the rs1051730 variant had an inverse trend (not significant after adjustment) of association with CHD in never-smokers (OR: 0.96; P-value: $1.56x10^{-2}$) and a positive trend (not significant after adjustment) in ever-smokers (OR: 1.03; P-value: $1.53x10^{-2}$) (P-value of interaction: $2.37x10^{-4}$) (**Table 1 and Supplementary Table 3**). For this variant, data on 20,559 CHD cases and 38,198 controls were available in the never-smoking group whereas 38,923 CHD cases and 40,371 controls were available in the ever-smoking group. Similar gene-smoking interaction patterns were observed for other variants (e.g., rs2036527, rs8034191) that have been previously reported for CPD behavior at the *CHRNB4-A3-A5* gene cluster (**Table 1**).

Further interrogation of the chr15q21.1 region encompassing rs7178051 and rs1051730 across three distinct LD blocks **(Figure 1)** revealed multiple additional variants for which we observed gene-smoking interactions in CHD (**Table 1** and **Figure 1**). Indeed, several SNPs (e.g., rs7178051, rs10083696, rs7176187, rs6495335, rs4887077) had genome-wide significant associations with CHD in "never-smokers" but had weaker and less significant associations with CHD in "ever-smokers" (**Figure 1**). Alleles clustered specifically around *ADAMTS7* rather than at the *CHRNB4-A3-A5* genes appear to be protective of CHD in "never-smokers" but have attenuated protective effects in "ever-smokers" (**Figure 2**).

Conditional analyses

To investigate the possibility that the two chr.15q25.1 gene-smoking interactions might represent a single interaction locus across the entire region we undertook an approximate conditional and joint analyses[17-22] using summary data derived from CARDIoGRAMplus4D for CHD and from the TGC for smoking behavior. In-addition to rs7178051, we identified one other variant, rs11072794 in low LD with rs7178051 (r^2=0.20) that was associated independently with CHD **(Figure 3a;** red triangles**)** (**Figure 3b & Supplementary Figure 6b;** red triangles). We also confirmed two variants (rs1051730 and rs684513) located in two different LD blocks that were independently associated with smoking behavior in the TGC data[10] **(Figure 3d & Supplementary Figure 6b;** grey circles**)**.

In analyses of the CHD variants, both rs7178051 and rs11072794 remained strongly associated with CHD after adjusting for the top CPD variants (rs1051730 and rs684513) (**Figure 3d**, red triangles) whereas their weak association with CPD was lost after adjusting for the top CPD variants (**Figure 3d;** grey circles); e.g., the P-value for rs7178051 association with CPD was $1x10^{-5}$ in unadjusted analyses but attenuated to 0.55 after adjusting for rs1051730 and rs684513. In analyses of the CPD variants, both rs1051730 and rs684513 remained strongly associated with CPD

after adjusting for the top CHD variants (rs7178051 and rs11072794) (**Figure 3b,** grey circles) whereas their weak association with CHD was lost after adjusting for the top CHD variants **(Figure 3b,** red triangles**)**. As expected, conditional analyses that included all four of these variants resulted in a null association of the region with both CHD and CPD **(Supplementary Figure 6b)**. To underscore the validity of the conditional approach using summary data, we used individual participant data from an expanded PROMIS sample involving 9,025 MI cases and 8,506 controls. We found that the OR conferred by allelic variation at rs7178051 remained associated with MI risk independent of the two CPD variants (rs1051730 and rs684513) and rs11072794 (the second CHD SNP) (**Supplementary Figure 6c**). Conversely, the apparent effect of allelic variation at rs1051730 (the top CPD variant) on CHD risk was lost when we adjusted for the other three variants, rs7178051, rs11072794 and rs684513 (**Supplementary Figure 6c**).

Next, using summary level data we examined the association of each of these four variants with CHD risk separately in "ever-smokers" and "never-smokers" while mutually adjusting for the other three variants (**Figure 4 & Supplementary Figure 7**). In these analyses, only allelic variation at rs7178051 was found to have independent genome-wide significant effects on CHD in never-smokers. rs7178051 was also the only one of these four variants with significant differences in the effect estimate for gene-CHD associations between the two smoking groups (P-value for the χ^2 test of heterogeneity: 5.4×10^{-5}) after adjusting for the effects of other variants (rs11072794, rs1051730 and rs684513). These conditional analyses suggest that (a) variants located near the *ADAMTS7* gene but not *CHRNB4-A3-A5* genes have independent effects on CHD, (b) a single independent gene-smoking interaction signal for CHD exists on chr.15q.25.1 which is localized at the *ADAMTS7* CHD locus (marked by rs7178051) and (c) an apparent interaction signal observed at the

nearby *CHRNB4-A3-A5* CPD locus (marked by rs1051730) is not independent of the *ADAMTS7* (rs7178051) interaction signal.

To assess the robustness of conditional analyses methodology that uses summary level data (i.e., GCTA)[17-22], we conducted sensitivity analyses in the PROMIS dataset (9,025 MI cases and 8,506 controls). We assessed the association of rs7178051 (top CHD SNP) and rs1051730 (top CPD SNP) after mutually adjusting for each other by conducting (i) standard logistic regression using individual participant data and (ii) summary level data in PROMIS using the GCTA method (**Supplementary Table 4**). The top CHD SNP was found associated with CHD risk in PROMIS independent of the top CPD variant using both the methods, in-contrast the effect on CHD of the top CPD SNP attenuated sharply when adjusted for the top CHD SNP – the effect estimates obtained using the two methods were very similar (**Supplementary Table 4**).

Finally, to further demonstrate that the gene-smoking interaction effect in CHD at rs7178051 is independent of the *CHRNB4-A3-A5* CPD locus, we conducted sensitivity analyses in the PROMIS study by restricting our gene-environment interaction analysis to subjects who do not carry the minor alleles of rs1051730 and rs684513 (the two SNPs associated with CPD) at the *CHRNB4-A3-A5* locus. The T allele at the rs7178051 variant was associated with CHD only in never-smokers (OR: 0.88; P-value: 0.01) compared to a weaker and non-significant association in ever-smokers (OR: 0.94; P-value: 0.21) (**Supplementary Table 5**). The effect estimates obtained in each of the categories defined by smoking status in PROMIS were similar to the effect estimates obtained in our overall meta-analyses that utilized data in all participants (**Supplementary Table 5**).

Analysis of eQTLs and regulatory features at the chr15q25.1 gene-smoking interaction locus. We mined publicly available eQTL data from the HapMap consortium,[11] GTEx consortium[23] and the MuTHER consortium[24] as well as data from 147 HAoEC lines[25] to examine the association between mRNA expression of *ADAMTS7* and *CHRN* genes with CHD, CPD and gene-smoking interaction SNPs at the chr15q25.1 locus. SNP-mRNA associations with p-values <0.002 (correction for 20 tests) are presented **(Figure 5)**. The top two CHD variants (rs7178051, rs11072794) are associated with reduced *ADAMTS7* expression (e.g., rs11072794 p=6.01x10^{-21} in MuTHER LCL, n=850; and rs7178051 p=0.0029 in HAoEC, n=147) but have no association with expression of *CHRN* genes in any cell or tissue examined. In contrast, the top two CPD variants (rs1051730 and rs684513) were associated with *CHRN* gene expression (e.g., rs1051730 p=6.9x10^{-7} for CHRNA5 in GTEx skeletal muscle and nerve tissue) but have no association with *ADAMTS7* in these cells or tissues. These findings complement conditional analyses suggesting that gene-CHD and gene-smoking interaction effects on CHD are likely mediated by *ADAMTS7* whereas the smoking behavior effect appears to be mediated through the *CHRNA3-5* gene cluster.

In analysis of data from the ENCODE project[26] and for human aortic tissue in NIH Roadmap Epigenomics project, *ADAMTS7* was associated with RNAseq reads and an active transcription mark, H3K36me3, whereas *CHRN* genes had low/absent RNAseq reads and were positive for repressive marks, H3K27me3 and H3K9me3 **(Supplementary Figure 8)**. In HCASMC ChIPseq data, rs7178051 the top CHD and gene-smoking CHD interacting SNP, is located in a region with active regulatory marks H3K4me1 and H3K4me3 as well as transcription factor binding site for TCF21, an important HCASMC transcription factor also associated with CAD. This ChIPseq pattern was observed also in human aortic tissue (**Figure 6**). These regulatory data suggest active transcription of *ADAMTS7*, but not *CHRN* genes, in vascular cells and aortic tissue

and reveal that rs7178051, the lead gene-smoking CHD interaction SNP, overlaps active transcription marks and transcription factor binding regions in HCASMC.

***ADAMTS7* and *CHRNB4-A3-A5* expression in vascular cells and their response to cigarette smoke extract**

In order to explore which genes at the chr15q25.1 locus are expressed in CHD-relevant vascular cells, we performed q-PCR of *ADAMTS7* and the *CHRNB4-A3-A5* genes in primary human vascular cells and in the THP1 human monocyte cell line **(Supplementary Figure 9 & Figure 5)**. Whilst *ADAMTS7* mRNA was expressed abundantly in all vascular cell types, mRNA was below detection or expressed at a very low level for each of the genes in the *CHRNB4-A3-A5* cluster in any of these cell types **(Supplementary Figure 9)**. Next, we explored the effect of cigarette smoke extract on gene expression in HCASMC, a cell type of particular relevance to vascular responses to cigarette smoke products[31, 32] as well as to *ADAMTS7* vascular functions in atherosclerosis and CHD.[33] In primary HCASMC, cigarette smoke extract exposure increased *ADAMTS7* mRNA levels by over 2-fold **(Figure 5)** but did not affect expression of the *CHRN* genes (not shown). Thus, in contrast to *CHRN* genes, *ADAMTS7* is both expressed and modulated by cigarette smoke extract in CHD-relevant vascular cells providing biological support for *ADAMTS7,* but not CHRN genes, in the gene-smoking interaction at chr15q25.1.

Discussion

We conducted a gene-environment interaction study at fifty loci associated with either CHD or smoking behavior and found evidence of effect-modification of genotype-related CHD risk by smoking-behavior at the chr.15q21.1 CHD locus. Specifically, we observed highly significant attenuation of the cardio-protective effects associated with alleles at this locus in people who

smoked cigarettes. Conditional analyses identified an LD block located at the *ADAMTS7* gene that accounted for both the main effect on CHD as well as the gene-smoking interactions in CHD. Data from expression and cell studies support our genetic analysis, suggesting that the underlying mechanism relates to genotype differences in the effect of smoking on expression of *ADAMTS7* in vascular tissue.

Our findings have novel mechanistic and clinical implications. These human genomic data provide new insights into the mechanism of CHD in cigarette smokers. Identification of gene-smoking interaction at the chr15q21.1 locus suggests a specific role in smoking-related CHD for ADAMTS7 and its substrates, vascular matrix and vascular smooth muscle cell biology more broadly. Such insights can help to prioritize translational strategies for smoking-related CHD and present opportunities to study lifestyle interventions and pharmacological strategies to lower CHD in individuals who smoke cigarettes. Thus, inhibition of ADAMTS7 represents a novel potential therapeutic strategy for CHD that may have particular benefits in individuals who smoke cigarettes. All smokers should receive counseling for smoking cessation yet such broad public health strategies have failed to reach or impact smoking behavior in a large portion of nicotine-addicted individuals. Our data provides a human genomic context for consideration of targeting specific genetically at-risk individuals via intensified preventive strategies and development of novel pharmacological treatments.

Our study also represents a realistic strategy for performing gene-environment interaction studies using contemporary genetic data. We illustrate that identifying joint effects of genetic and lifestyle factors in CHD requires very large sample sizes, yet such analyses are biologically informative when studies are adequately powered. In this context, an important observation in our large sample is the lack of effect modification by smoking behavior on CHD at the *APOE* locus, a

previously reported smoking interaction locus.[12-14] This finding is consistent with a recent meta-analysis that found no evidence of effect modification by smoking for *APOE* genotypes and CHD risk.[34] These studies raise concerns that most prior gene-environment interactions studies in CHD have been prone to the same biases (i.e., limited statistical power and false positive associations) as candidate gene studies investigating main effects in the pre-GWAS era. The present study differs from previous studies by being much larger and, importantly, it includes genomic and functional follow-up data supporting the plausibility of the observed gene-environment interaction.

ADAMTS7 (or the A disintegrin and metalloproteinase with thrombospondin motifs-7) is a member of the ADAMTS family of secreted zinc metalloproteases.[35, 36] We previously discovered and replicated genetic variation at the *ADAMTS7* locus in association with coronary atherosclerosis and MI.[7-9] Both *in vivo* and *in vitro* studies suggest that ADAMTS7 modulates VSMC phenotype switching and migration and that this may be mediated via cartilage oligomeric matrix protein (COMP) or thrombospondin-1 (TSP-1),[32,33] i.e. putative ADAMTS7 substrates expressed in vascular tissue. Genetic variation at *ADAMTS7,* however, has no relationship with traditional risk factors or mechanistic biomarkers; hence the directional impact of *ADAMTS7* expression on CHD risk and the underlying biological mechanisms have been unclear.[32]

Our gene-smoking interaction analyses provide novel insights into the directional impact of the *ADAMTS7* locus on CHD, the underlying mechanisms of CHD in smokers, and how such findings ultimately might translate towards achieving health benefits in society. Our human eQTL interrogations reveal that common alleles that relate to lower CHD risk at the *ADAMTS7* locus are also associated with reduced *ADAMTS7* expression, implying an atherogenic role of the gene. This is supported by our recent *in vivo* experimental studies; *Adamts7* deficiency protected against diet-induced atherosclerosis in both the *Ldlr*$^{-/-}$ and *ApoE*$^{-/-}$ mouse models, reduced neointima formation

following arterial injury, and decreased VSMC migration *in vitro*.[33] In our smoking-stratified analyses, we observed CHD protective effect which was attenuated in smokers. Thus, smoking exposure may overcome the genetic effect of protective alleles that act by reducing *ADAMTS7* expression. Such a possibility is supported by our HCASMC data that reveals increased *ADAMTS7* expression in HCASMC exposed to cigarette smoke extract. These human genome-smoking studies are the first to implicate a specific locus as causal in mediating increased risk of CHD in smokers. Additional translational studies are needed to establish the precise mechanisms of atheroprotection for alleles at the *ADAMTS7* locus, how cigarette smoking impacts these genetic effects, and whether deletion or inhibition of ADAMTS7 *in vivo* attenuates the specific acceleration of atherosclerosis conferred by cigarette smoking.

Strengths and limitations of our study merit consideration. This is a large study that conducted gene-smoking interaction analyses in CHD by using GWAS data. We observed substantial heterogeneity across study samples in our initial quality control analyses of "ever-smoking" status with CHD risk. This is similar, however, to the heterogeneity reported in a recent meta-analysis that pooled risk ratios from all the past prospective studies with information on association of "ever-smoking" with incident CHD events.[5] We recognize that other smoking related phenotypes are important e.g., "current smoking" may have a more pronounced role than "ever-smoking" in plaque rupture and thrombosis in patients with MI. We were however unable to distinguish between "former" versus "current" smokers within "Ever Smokers" in our current analyses; furthermore we were not able to analyze graded exposure to cigarette smoking such as "pack-years". Given the use of multiple studies and meta-analyses of data, we used only one analytical approach to investigate gene-smoking interactions. This approach, however, was feasible and powerful in this large-scale consortium setting. While we used a fixed effects approach in our

meta-analyses, a random effects meta-analysis yielded qualitatively similar results (data not shown). The lack or replication is partially offset by a large sample size, consistency across study cohorts and racial groups and supplemental genomic and experimental evidence supporting biological plausibility. This approach is also consistent with recent recommendations[37] which favor use of a powerful discovery experiment using all data rather than reducing power by splitting available dataset for discovery and validation. While our *in vitro* studies support a role for ADAMTS7 in the gene-smoking interaction, it will be important to confirm that *Adamts7* deficiency protect against cigarette-smoke acceleration of atherosclerosis in rodent models.

Our interaction analyses, conditional analyses, eQTL interrogations and cell studies suggest that *ADAMTS7*, but not the *CHRNB4-A3-A5* gene cluster, is likely causal at 15q21.1 for gene-smoking interaction effects in CHD. Yet, analyses are not definitive. Although top interacting SNPs and CHD SNPs (e.g., rs7178051) were associated with *ADAMTS7,* but not *CHRNB4-A3-A5,* expression in LCLs, large-scale eQTL data and allele specific expression data (e.g., via RNA sequencing) are not available for vascular tissues limiting causal inference. In our small HCAEC datasets, we did however find that alleles at rs7178051 associate with *ADAMTS7* expression but not with any *CHRNB4-A3-A5* genes suggesting, at least in one vascular cell type, that the gene-smoking interaction is mediated via *ADAMTS7.*

Conclusions

We provide novel evidence for allelic variation exhibiting gene-smoking interaction in CHD at the chr.15q21.1 locus. The protective effect conferred by variation at this locus in never-smokers is markedly attenuated in people who are ever-smokers. Stepwise conditional analyses, gene expression data in vascular cells, eQTL interrogation, and cigarette smoke extract exposure in HCASMC suggest that *ADAMTS7* accounts for both the gene-smoking interaction in CHD and the

CHD main effect on chr.15q21.1. Our findings reveal interactions of genetic variants and key lifestyle determinants in the etiology of CHD, provide new insights into the potential mechanisms of CHD in cigarette smokers, and facilitate precision medicine advances in cigarette-smoking related CHD. Our work motivates future large-scale studies investigating joint effects of genes and environment in CHD using existing complex-disease consortia datasets and genome-wide discovery approaches. This will provide opportunities to detect additional and novel loci displaying gene-environment interactions revealing genetic contexts for targeting intensive lifestyle interventions and novel therapeutics.

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Table 1. Novel genotype-smoking interaction findings in coronary heart disease at the chromosome 15q25.1 locus

					Never Smokers					Ever Smokers					
Variant	**Association**	**allele**	**LD with rs7178051***	**LD with rs1051730^**	**N cases**	**N controls**	**N Total**	**Beta (SE)**	**P-value**	**N cases**	**N controls**	**N Total**	**Beta (SE)**	**P-value**	**P-value interaction**
*rs7178051[4]	CHD (NPR)	T/C	-	0.22	21232	38713	59945	-0.13 (0.01)	1.30E-16	39585	40749	80334	-0.05 (.01)	2.49E-04	8.57E-05
†rs1051730[16]	SB (known)	A/G	0.22	-	20559	38198	58757	-0.04 (0.02)	0.02	38923	40371	79294	0.03 (0.01)	0.02	2.37E-04
rs7173743[1]	CHD (Known)	C/T	0.61	0.18	21050	37955	59005	-0.11 (0.01)	2.73E-13	39044	39559	78603	-0.04 (0.01)	8.60E-04	9.29E-05
rs10083696[2]	CHD (Novel)	A/G	1.0	0.22	19721	36206	55927	-0.11 (0.02)	1.60E-12	38807	40018	78825	-0.05 (0.01)	2.72E-04	5.15E-05
rs7176187[3]	CHD (Novel)	T/C	1.0	0.24	21232	38713	59945	-0.12 (0.01)	7.02E-16	39585	40749	80334	-0.04 (0.01)	8.64E-04	6.93E-05
rs6495335[5]	CHD (Novel)	G/T	1.0	0.22	20144	37217	57361	-0.13 (0.02)	2.39E-15	36448	38203	74651	-0.04 (0.01)	1.69E-03	9.51E-04
rs4380028[6]	CHD (Known)	T/C	1	0.22	21232	38713	59945	-0.12 (0.01)	2.20E-15	39585	40749	80334	-0.04 (.01)	1.03E-03	5.44E-04
rs3825807[7]	CHD (Known)	G/A	0.52	0.43	17137	28633	45771	-0.09 (0.02)	2.82E-08	30071	29014	59086	-0.03 (0.01)	0.04	2.6E-03
rs3813565[8]	CHD (NPR)	T/G	0.43	0.56	19466	35830	55296	-0.08 (0.02)	5.08E-07	36642	37759	74401	-0.01 (0.01)	0.42	3.05E-04
rs11638490[9]	CHD (NPR)	T/C	0.44	0.51	20465	37897	58362	-0.08 (0.01)	6.90E-08	38533	39690	78223	-0.01 (0.01)	0.28	2.25E-04
rs11072791[11]	CHD (NPR)	A/C	0.44	0.51	19289	35944	55233	-0.08 (0.02)	2.83E-07	35245	36635	71880	-.005 (0.01)	0.68	1.06E-04
rs922692[12]	CHD (NPR)	A/C	0.44	0.50	20559	38198	58757	-0.08 (0.01)	2.81E-07	38923	40371	79294	-0.01 (0.01)	0.29	2.75E-04
rs11638372[13]	CHD (NPR)	T/C	0.44	0.50	21232	38713	59945	-0.08 (0.01)	6.92E-08	39585	40749	80334	-0.01 (0.01)	0.23	3.16E-04
rs4887077[14]	CHD (NPR)	T/C	0.44	0.50	21232	38713	59945	-0.08 (0.01)	4.71E-08	39585	40749	80334	-0.02 (0.01)	0.20	3.92E-05
rs12899135[15]	CHD (NPR)	G/A	0.39	0.56	20377	37440	57817	-0.07 (0.02)	3.97E-06	38382	39181	77563	-0.01 (0.01)	0.58	4.54E-04
rs684513[18]	SB (Known)	C/G	0.01	0.10	12517	21054	33572	-0.01 (0.02)	0.67	24641	24487	49129	0.03 (0.02)	0.18	0.08
rs2036527[19]	SB (Known)	A/G	0.17	0.90	20559	38198	58757	-0.04 (0.02)	0.02	38923	40371	79294	0.03 (0.01)	0.02	2.14E-04
rs10519203[20]	CHD (NPR)	G/A	0.19	0.93	21232	38713	59945	-0.04 (0.01)	5.93E-03	39585	40749	80334	0.03 (0.01)	0.03	1.27E-04
rs8034191[21]	SB (Known)	C/T	0.19	1.0	19251	32131	51382	-0.05 (0.02)	2.62E-03	34925	34047	68972	0.02 (0.01)	0.06	3.91E-05

CHD = coronary heart disease; SB = smoking behavior; NPR: Not a previously reported variant with disease risk

*lead variant in association with CHD in our dataset; † lead variant in association with SB

[1-21]each number refers to the physical location of the variant in figure

Figure Legends

Figure 1. (a) Regional association analyses at the chromosome 15q25.1 locus in association with CHD risk stratified by smoking status. Association P-values for genetic variants with CHD risk in "never-smokers" (green squares) and "ever-smokers" (red triangles). (b) Longitudinal bars represent gene-smoking CHD interaction P-values at the chromosome 15q25.1 locus; bars in blue are P-values for variants listed in Table-1 and each variant has been assigned a unique identification number based on its physical location; (c) LD-blocks at the 15q25.1 locus visualized through HAPLOVIEW using LD estimates in the HapMAP-2 CEU reference population.

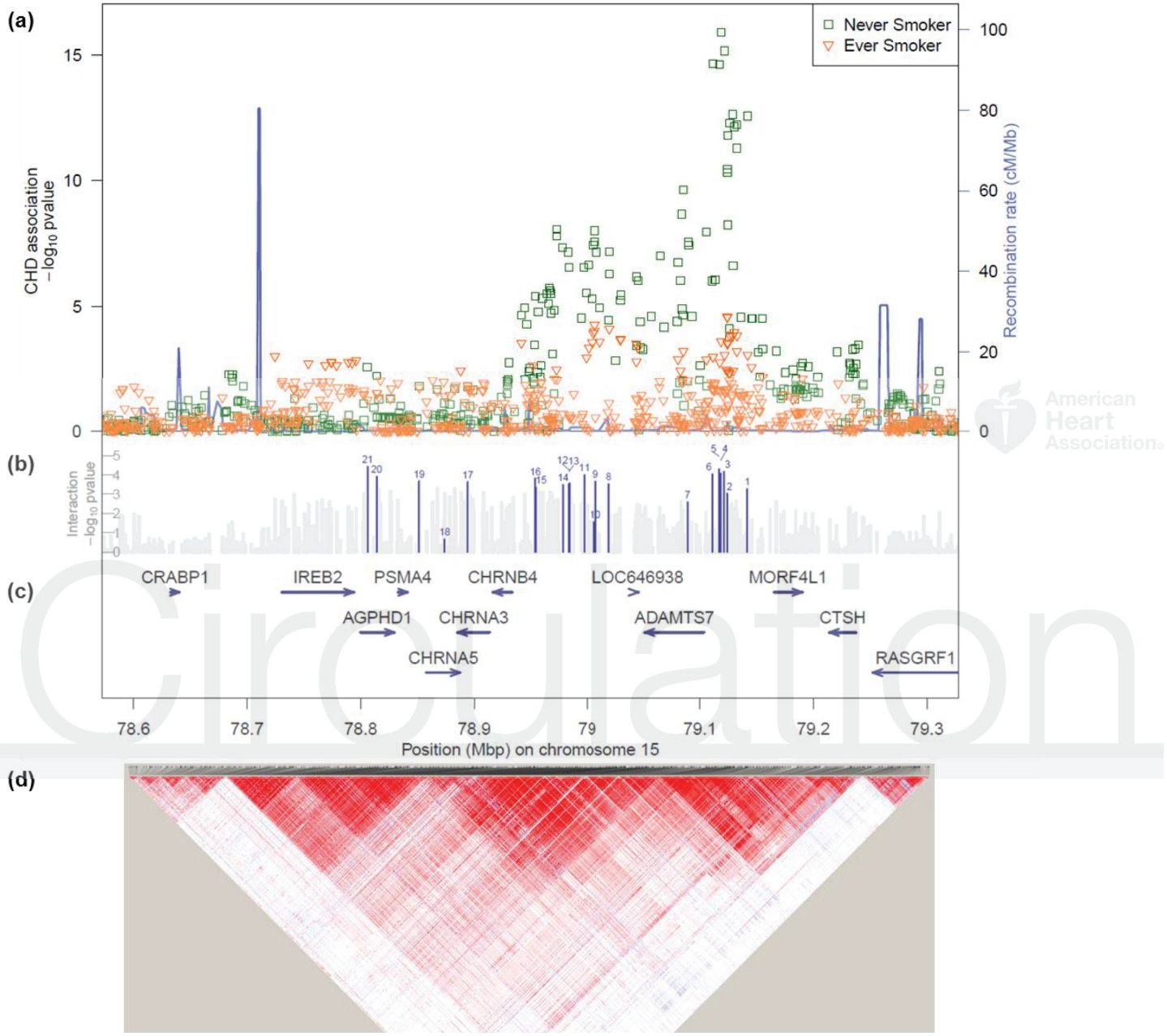
Figure 2. Several variants at chromosome 15q21.1 have stronger effects on CHD risk in "never-smokers" compared to "ever-smokers". Variants with the strongest interaction P-value are displayed.

Figure 3. Step-wise conditional analysis of genetic variation at the chromosome 15q21.1 locus with CHD (red triangles) and smoking behavior (cigarettes per day, CPD; grey circles). At the chromosome 15q21.1 locus, analyses adjusted for rs7178051 and rs11072794 completely attenuated the gene-CHD associations whereas gene-smoking remained unchanged. Analyses adjusted for rs1051730 and rs684513 completely attenuated the gene-smoking associations whereas gene-CHD effect remained unchanged.

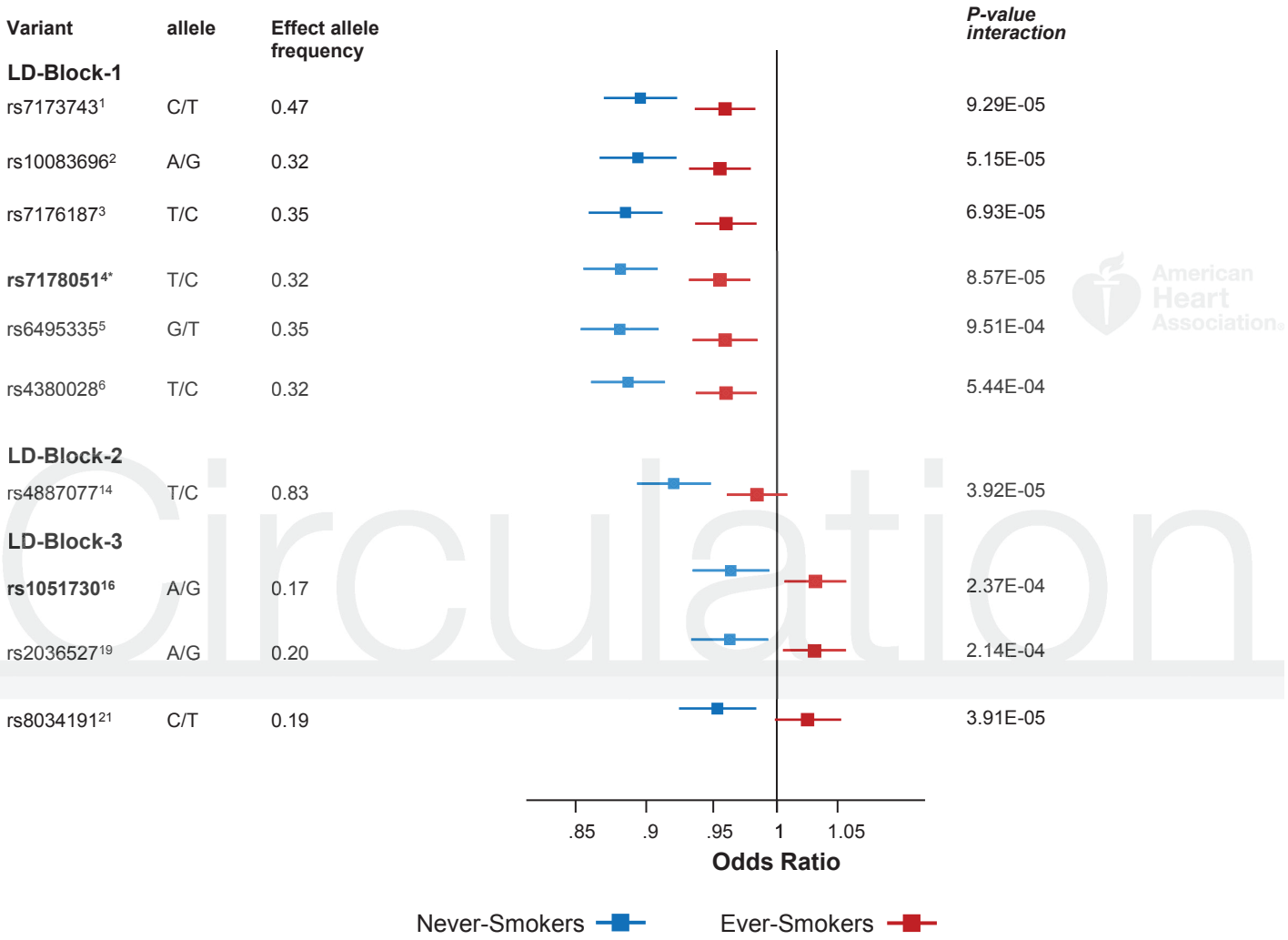
Figure 4. Analyses mutually adjusted for rs7178051, rs11072794, rs1051730 and rs684513 at 15q21.1 on CHD and smoking behavior; gene-CHD interaction analyses were only found significant for rs7178051. Analyses on the left panel show associations of rs7178051, rs11072794, rs1051730 and rs684513 with CHD risk mutually adjusted for each other. Analyses on the right panel show associations of rs7178051, rs11072794, rs1051730 and rs684513 with smoking behavior mutually adjusted for each other.

Figure 5. (a) *ADAMTS7* and *CHRNB4-A3-A5* mRNA levels were measured in HCASMC. Cells were cultured to confluence, total RNA was extracted and cDNA generated. q-PCR was performed for *ACTB, GAPDH, TBP, ADAMTS7, CHRNB4, CHRNA3, CHRNA5* (95°C 15s, 60°C 1min). Delta Cts were calculated as follows: $(Ct_{ACTB} + Ct_{GAPDH} + Ct_{TBP})/3 - Ct_{TARGET\ GENE}$). Fold changes are derived from delta delta Cts based on formula $FC = 2^{-dCt}$. (b) Confluent HCASMC were exposed to cigarette smoke extract. Serum starved (x24 hrs.) confluent HCASMC were treated with 0.5% or 1.0% cigarette smoke extract (v/v) for 4, 12, and 24 hrs. in serum reduced conditions (0.5% FBS in DMEM). Total RNA was extracted, cDNA generated preparation and q-PCR performed for *ADAMTS7* by Taqman and normalized to *GAPDH*. The Average Ct for ADAMTS7 at baseline was 28.25. Results were presented as means ± SEM, and data were analyzed using Student's t-Test. (c) expression and eQTL Data from the GTEx consortium, the HapMap consortium (restricted to European populations), the Multiple Tissue Human Expression Resource (MuTHER) and in 147 donor HAoEC lines. Association of the independent lead variants identified in our conditional analyses with expression of *ADAMTS7* and genes in the *CHRNB4-A3-A5* cluster. A P-value threshold of 0.002 was set to account for multiple testing involved in the eQTL analyses.

Figure 6. Genome browser view of regulatory features at rs7178051 on Chr15q21.1. ChIP-seq experiments were performed on confluent HCASMC for TCF21, Jun, JunD, CEBP and H3K4me1, H3K27me3, H3K27ac. DNAaseI hypersensitivity data for human AoSMC were acquired from the ENCODE project. Human aortic tissue H3K4me1, H3K9me3, H3K27me3, and H3K36me3 ChIP-seq data were acquired from the NIH Roadmap Epigenomics Project. HCASMC = human coronary artery smooth muscle cells; AoSMC = human aortic smooth muscle cells.



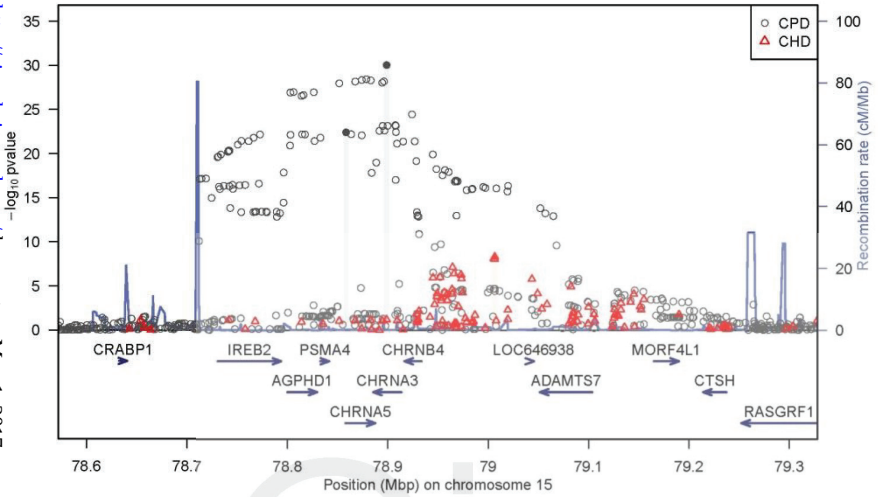
1-rs7173743; 2-rs10083696; 3-rs7176187; 4-rs7178051; 5-rs6495335; 6-rs4380028; 7-rs3825807; 8-rs3813565; 9-rs11638490; 10-rs11072794; 11-rs11072791; 12-rs922692; 13-rs11638372; 14-rs4887077; 15-rs12899135; 16-rs17487514; 17-rs1051730; 18-rs637137; 19-rs2036527; 20-rs10519203; 21-rs8034191. LD 1-3 indicate three separate linkage disequilibrium blocks in European ancestry at the chromosome 15q25.1 locus.



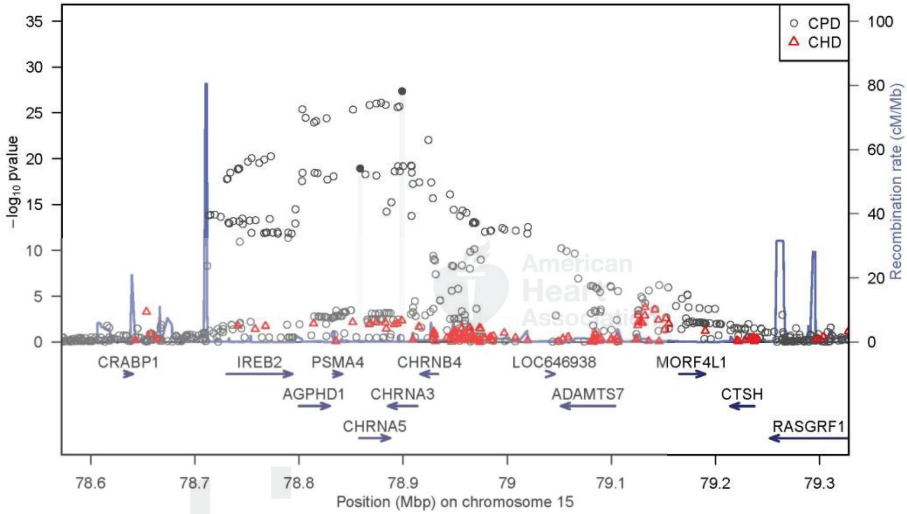
rs7178051 is the lead variant identified in association with CHD in our study population; whereas rs1051730 is the lead variant previously identified in association with smoking behavior .
Variants are ordered based on their base pair position in **Figure-1**.

Stepwise conditional analyses for CHD risk and CPD behavior adjusting for top CHD variants at chromosome 15q21.1

(a) analyses conditioned on rs7178051

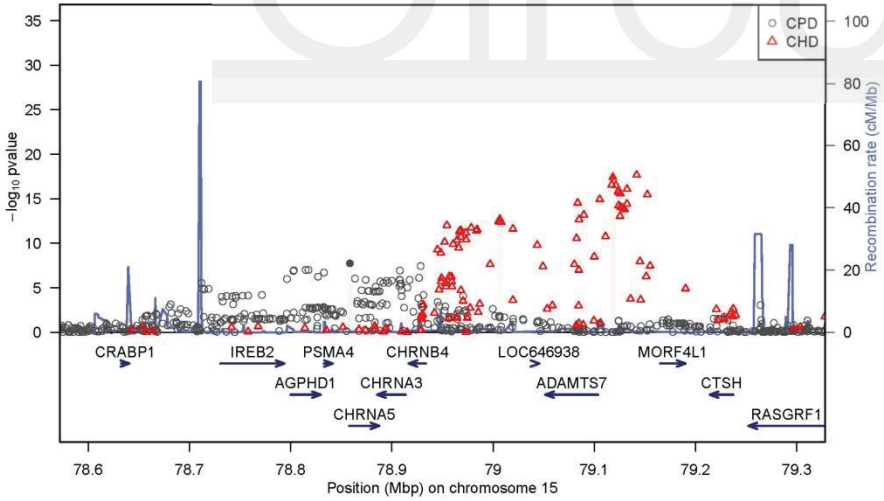


(b) analyses conditioned on rs7178051 and rs11072794

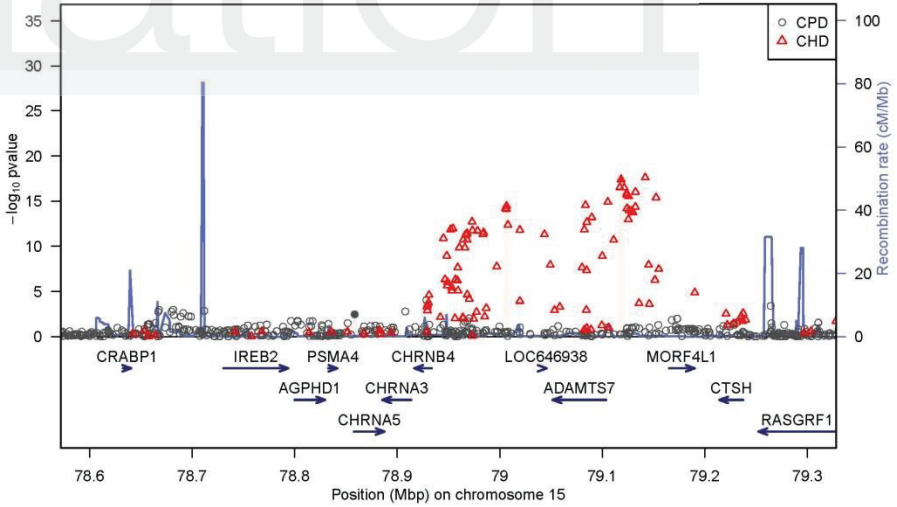


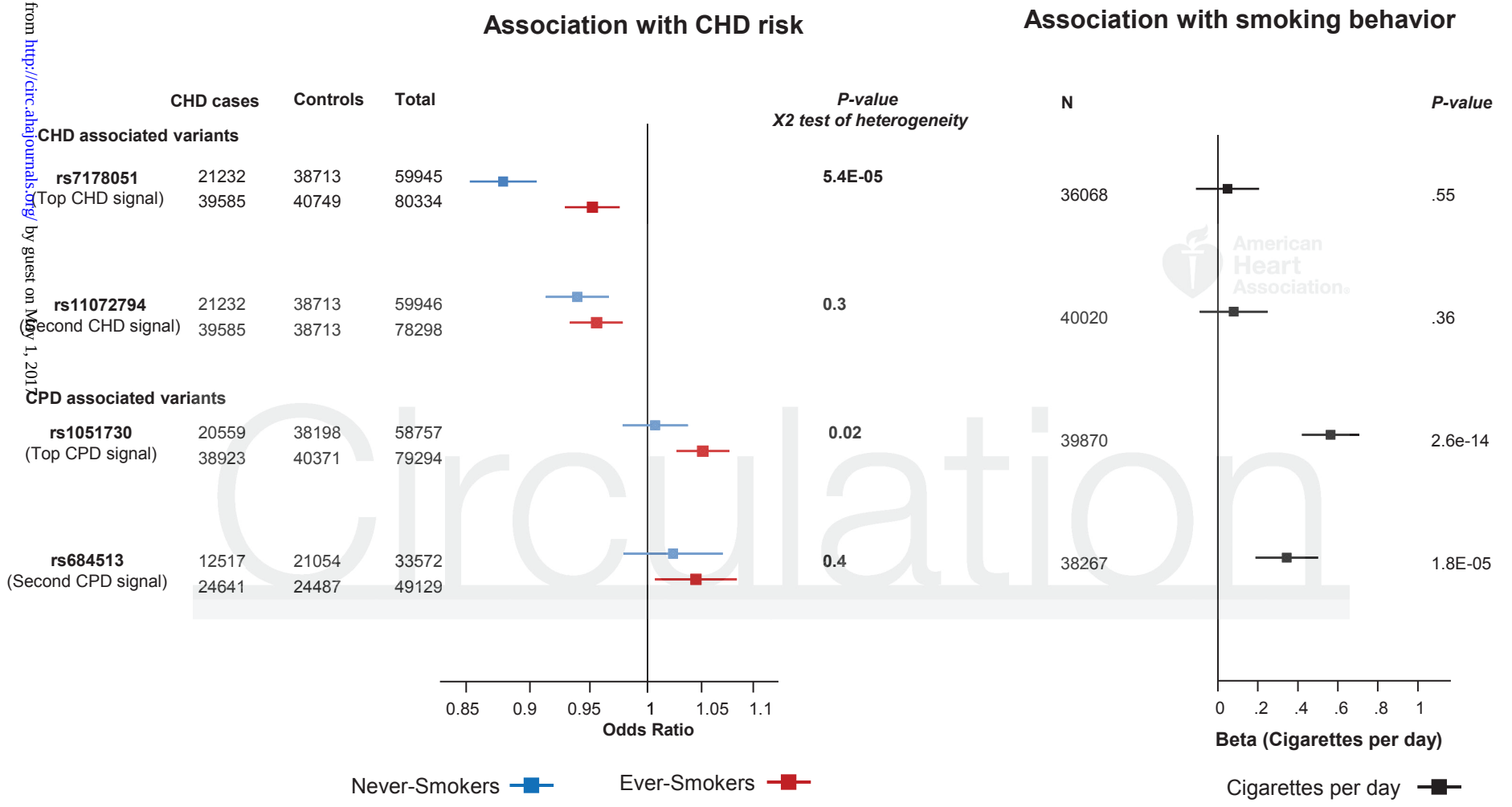
Stepwise conditional analyses for CHD risk and CPD behavior adjusting for top CPD variants at chromosome 15q21.1

(c) analyses conditioned on rs1051730

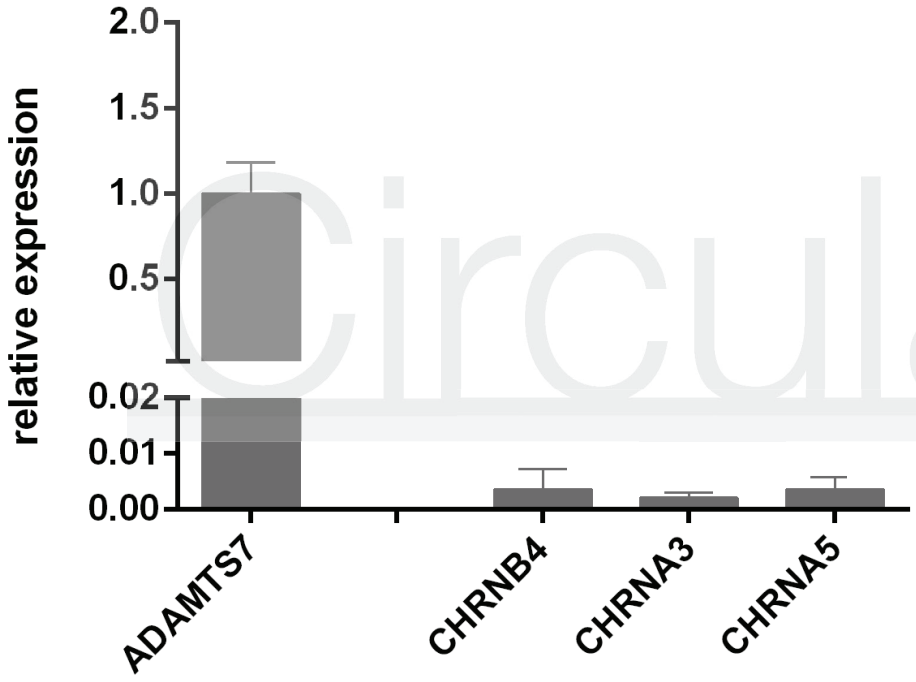


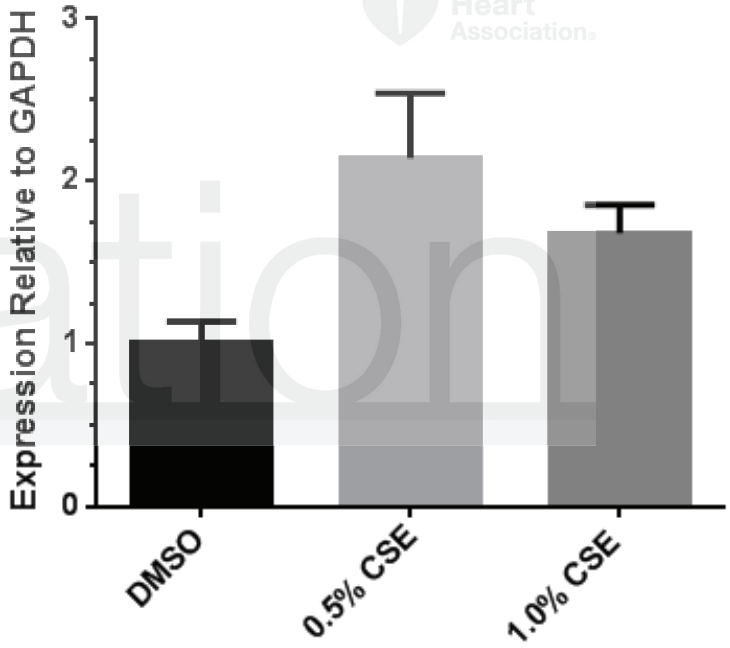
(d) Analyses conditioned on rs1051730 and rs684513

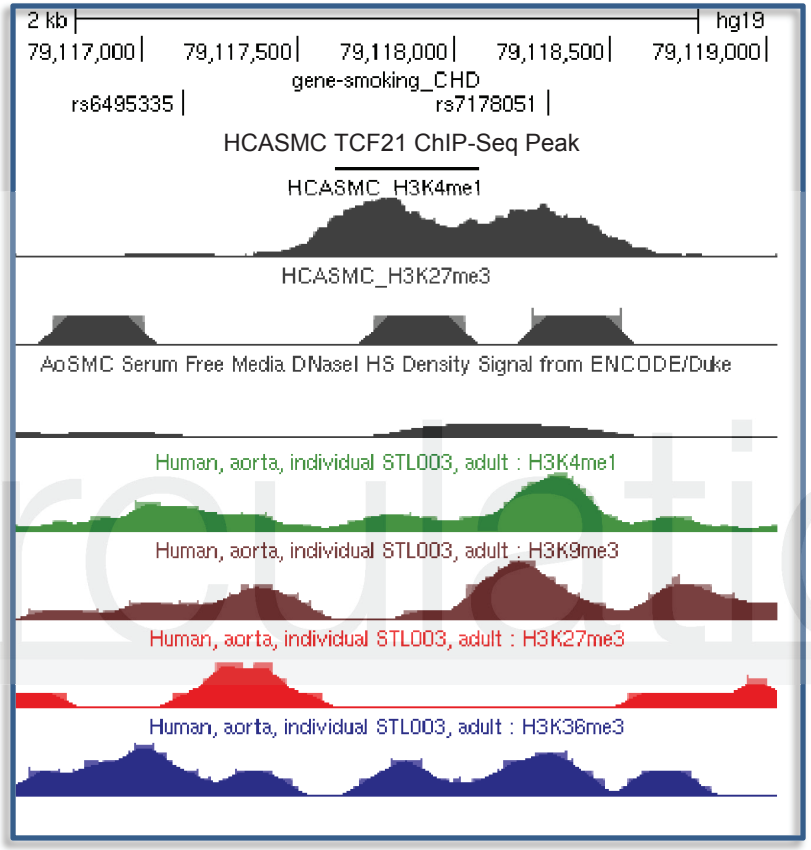




Gene-CHD and gene-smoking analyses for rs7178051 were adjusted for rs11072794, rs1051730 and rs684513;
Gene-CHD and gene-smoking analyses for rs11072794 were adjusted for rs7178051, rs1051730 and rs684513;
Gene-CHD and gene-smoking analyses for rs1051730 were adjusted for rs7178051, rs11072794 and rs684513;
Gene-CHD and gene-smoking analyses for rs684513 were adjusted for rs7178051, rs11072794 and rs1051730.







Loss of Cardio-Protective Effects at the *ADAMTS7* Locus Due to Gene-Smoking Interactions

Danish Saleheen, Wei Zhao, Robin Young, Christopher P. Nelson, Weang Kee Ho, Jane F. Ferguson, Asif Rasheed, Kristy Ou, Sylvia T. Nurnberg, Robert C. Bauer, Anuj Goel, Ron Do, Alexandre F. R. Stewart, Jaana Hartiala, Weihua Zhang, Gudmar Thorleifsson, Rona J. Strawbridge, Juha Sinisalo, Stavroula Kanoni, Sanaz Sedaghat, Eirini Marouli, Kati Kristiansson, Jing Hua Zhao, Robert Scott, Dominique Gauguier, Svati H. Shah, Albert Vernon Smith, Natalie Van Zuydam, Amanda J. Cox, Christina Willenborg, Thorsten Kessler, Lingyao Zeng, Michael A. Province, Andrea Ganna, Lars Lind, Nancy L. Pedersen, Charles C. White, Anni Joensuu, Marcus Edi Kleber, Alistair S. Hall, Winfried März, Veikko Salomaa, Christopher O'Donnell, Erik Ingelsson, Mary F. Feitosa, Jeanette Erdmann, Donald W. Bowden, Colin N. A. Palmer, Vilmundur Gudnason, Ulf de Faire, Pierre Zalloua, Nicholas Wareham, John R. Thompson, Kari Kuulasmaa, George Dedoussis, Markus Perola, Abbas Dehghan, John C. Chambers, Jaspal Kooner, Hooman Allayee, Panos Deloukas, Ruth McPherson, Kari Stefansson, Heribert Schunkert, Sekar Kathiresan, Martin Farrall, Philippe M. Frossard, Daniel J. Rader, Nilesh Samani, Muredach P. Reilly, EPIC-CVD, PROMIS and CARDIoGRAMplusC4D
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SUPPLEMENTAL MATERIAL

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Supplementary Data

ADAMTS7 and *CHRNB4-A3-A5* gene expression in vascular cells:

Cells were cultured to confluence in media under conditions recommended by the suppliers (Lonza and ATCC). Total RNA from cultured cells was extracted using Trizol (Invitrogen P/N 15596-018). DNAse digestion was performed with the Turbo DNAfree kit from Ambion (P/N AM1907). cDNA was generated according to the manufacturer's protocol with the SuperScript® III First-Strand Synthesis System (Invitrogen 18080-051). Real-time quantitative PCR (q-PCR) measurements were performed on an Applied Biosystems 7900HT Fast Real-Time PCR System using the TaqMan® Gene Expression Master Mix (P/N 4369016) and the following TaqMan probes: ACTB (Hs01060665_g1), GAPDH (Hs02758991_g1), TBP (Hs00427620_m1), ADAMTS7 (Hs00276223_m1), CHRNB4 (Hs00609520_m1), CHRNA3 (Hs01088199_m1), CHRNA5 (Hs00181248_m1). The standard cycling protocol was 95°C 10min, 40x (95°C 15s, 60°C 1min). Delta Cts were calculated as follows: $(Ct_{ACTB} + Ct_{GAPDH} + Ct_{TBP})/3 - Ct_{TARGET\ GENE})$. Fold changes are derived from delta delta Cts based on formula $FC = 2^{-dCt}$. Graphs were generated using GraphPad Prism 6.04.

ADAMTS7 and *CHRNB4-A3-A5 gene* expression in response to cigarette smoke extract (CSE):

RNA preparation and q-PCR were conducted as described above except RNA was extracted using RNeasy Mini Kit from Qiagen (Valencia, CA), reverse transcription was done using High-Capacity cDNA Reverse Transcription Kit from Life Technologies (Grand Island, NY), and cDNA samples were quantified for expression of *ADAMTS7* and *CHRNB4-A3-A5* genes by Taqman and normalized to *GAPDH*. Graphs were generated using GraphPad Prism 6.04. Results were presented as means ± SEM, and data were analyzed using Student's t-Test.

Regulatory features of the chr. 15q25.1 region: UCSC browser images were integrated using data from the ENCODE project (http://genome.ucsc.edu/cgibin/hgTracks?db=hg19&hubUrl=http://ftp.ebi.ac.uk/pub/databases/ensembl/encode/integration_data_jan2011/hub.txt, PMID 22955616) and the NIH Roadmap Epigenomics Project (http://genome.ucsc.edu/cgibin/hgTracks?db=hg19&hubUrl=http://vizhub.wustl.edu/VizHub/RoadmapRelease4.txt, PMID 25693563).

Supplementary Figure 1. Flow chart of study strategy. The current study had five inter-related components. First, as part of the quality control, we investigated the association of smoking status with CHD risk within each study. Second, for all the SNPs (± 50 KB) at the 45 established CHD loci, effect estimates from each study in association with CHD risk were obtained and pooled to identify the strongest variant ("lead variant") at all the established CHD loci. Third, we conducted gene-smoking interaction analyses for 45 CHD variants with the most significant association with the CHD risk in our study population as well as for 5 variants previously reported in association with smoking behavior. Fourth, for loci demonstrating differential CHD associations by smoking status, we mapped the interaction region, examined linkage disequilibrium (LD) across the region and performed conditional analyses to identify independent genetic signals. Finally, for loci exhibiting interaction, we assessed their eQTL patterns of local genes in available datasets and examined expression of these genes in in multiple cell types that play prominent roles in smoking-CHD pathobiology.

Supplementary Table 1. Description of the participating studies with information available on "ever-smoking" status, CHD risk and genotypes at the 50 candidate loci. Information on "ever-smoking" was available in 29 studies, yielding a total sample size of 60,919 CHD cases and 80,243 controls. All studies recruited participants of European ancestry, except in PROMIS (South Asian), LOLIPOP (South Asian) and FGENTCARD (Lebanese).

Supplementary Figure 2. Association of "ever-smoking" status with CHD in participating studies. As expected, in all the participating studies, association of "ever-smoking" status with CHD risk was directionally consistent with an increased risk of CHD.

Supplementary Figure 3. Comparison of the lead variants with the top previously reported CHD variants at the candidate loci. Effect estimates for SNP association with CHD for (i) the most significant SNP that we identified at established CHD loci in the current study population (larger than any previously published) as well as for (ii) SNPs previously reported at these established CHD loci in prior GWA studies. Of the 45 established CHD loci, we identified 32 for which we found a more significant SNP in association with CHD risk in our dataset than the previously reported variant.

Supplementary Figure 4. Association of reported variants with smoking behavior in the Tobacco Genetics Consortium (n=140,000). Data on rs302543 was not available in sufficient studies; hence was not analyzed in the current gene-CHD smoking interaction analyses.

Supplementary Table 2. Association of top variants at established CHD loci in our study population. Effect estimates for SNP association with CHD for the most significant SNP that we identified at established CHD loci in the current study population (larger than any previously published) as well as for SNPs previously reported at these established CHD loci in prior GWA studies. Of the 45 established CHD loci, we identified 32 for which we found a more significant SNP in association with CHD risk in our dataset than the previously reported variant.

Supplementary Table 3. Stratified (Never-smokers" and "Ever-Smokers") and Gene-smoking interaction analyses in CHD for the CHD and smoking behavior loci. Of the 50 candidate variants, we identified effect-modification by "ever-smoking" status on CHD for the lead variants at two distinct loci, rs7178051, at the *ADAMTS7* CHD locus, and rs1051730, at the *CHRNB4-A3-A5* genes smoking behavior locus). Although associated with different traits and located in distinct LD blocks, these two variants reside only ~224 KBs apart on chr.15q25.1 and indeed are in weak linkage disequilibrium (LD) (r2 = 0.22).

Supplementary Figure 5a. Association by smoking status of the *APOE* εpsiolon genotypes with CHD in PROMIS. The OR for CHD among ε4 carriers in "never-smokers" was 1.10 which was similar to the CHD OR of 1.11 observed in "ever-smokers".

Supplementary Figure 5b. Forest plot displaying interaction beta across the participating studies.

Supplementary Figure 5c. Forest plot displaying interaction beta across the participating studies by ethnicity

Supplementary Figure 6. (a) Unadjusted associations of chromosome 15q21.1 variants with CHD (red triangles) and smoking behavior (cigarettes per day, CPD; grey circles); (b) analyses adjusted for rs7178051, rs11638490, rs1051730 and rs684513 in association with CHD and CPD; (c) analyses of rs7178051 and rs1051730 with MI risk in PROMIS (9,025 MI cases and 8,506 controls)

Supplementary Figure 7. Unadjusted effects of 15q21.1 lead variants on CHD stratified by smoking status in the CARDIoGRAMplusC4D consortium and analyses of variants with smoking behavior in the Tobacco and Genetics Consortium (TGC) in 140,000 participants.

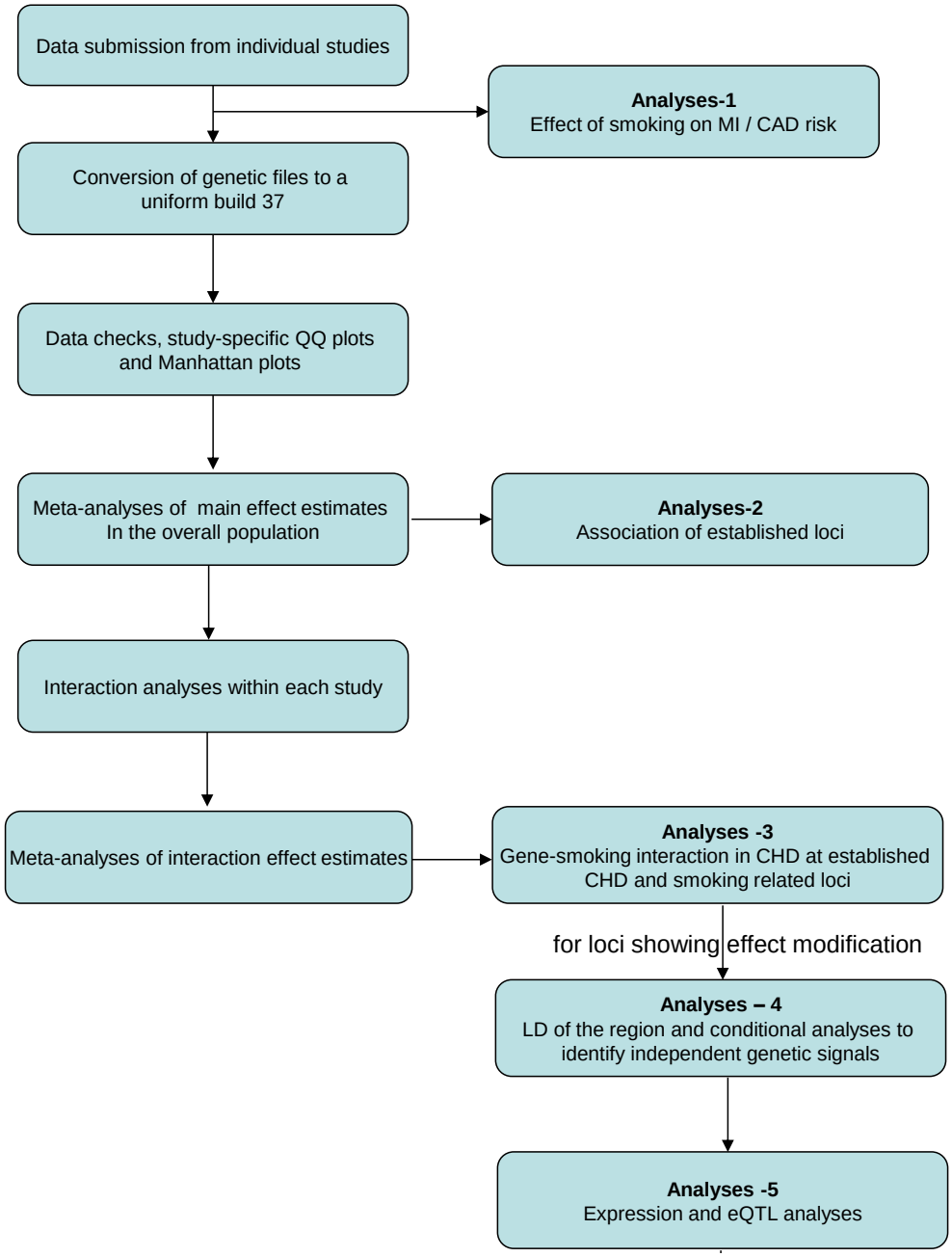
Supplementary Table 4. Association of rs7178051 with MI risk in PROMIS in participants by smoking status who do not carry the minor allele for rs1051730 and rs684513 variants

Supplementary Table 5. Association of rs7178051 (top CHD SNP) and rs1051730 (top CPD SNP) mutually adjusted for each other in 9,025 MI cases and 8,506 controls in PROMIS

Supplementary Figure 8. Genome browser view of regulatory features at the CHD and smoking behavior loci on Chr15q21.1. ChIP-seq experiments were performed on confluent HCASMC for TCF21, Jun, JunD, CEBP and H3K4me1, H3K27me3, H3K27ac. DNAaseI hypersensitivity data for human AoSMC were acquired from the ENCODE project. Human aortic tissue H3K4me1, H3K9me3, H3K27me3, and H3K36me3 ChIP-seq data were acquired from the NIH Roadmap Epigenomics Project. *ADAMTS7* was associated with RNAseq reads and an active transcription mark, H3K36me3, whereas the *CHRNB4-A3-A5* genes had low/absent RNAseq reads and were positive for repressive marks H3K27me3 and H3K9me3 HCASMC = human coronary artery smooth muscle cells; AoSMC = human aortic smooth muscle cells. TF = transcription factor.

Supplementary Figure 9. *ADAMTS7* and *CHRNB4-A3-A5* mRNA levels were measured in HCASMC, HCAEC, HAoSMC, HAoEC, HAoAF, and the THP-1 cell line. Cells were cultured to confluence, total RNA was extracted and cDNA generated. q-PCR was performed for *ACTB, GAPDH, TBP, ADAMTS7, CHRNB4, CHRNA3, CHRNA5*. Delta Cts were calculated as follows: $(Ct_{ACTB} + Ct_{GAPDH} + Ct_{TBP})/3 - Ct_{TARGET\ GENE})$. Fold changes are derived from delta delta Cts based on formula $FC = 2^{-dCt}$. Graphs were generated using GraphPad Prism 6.04.

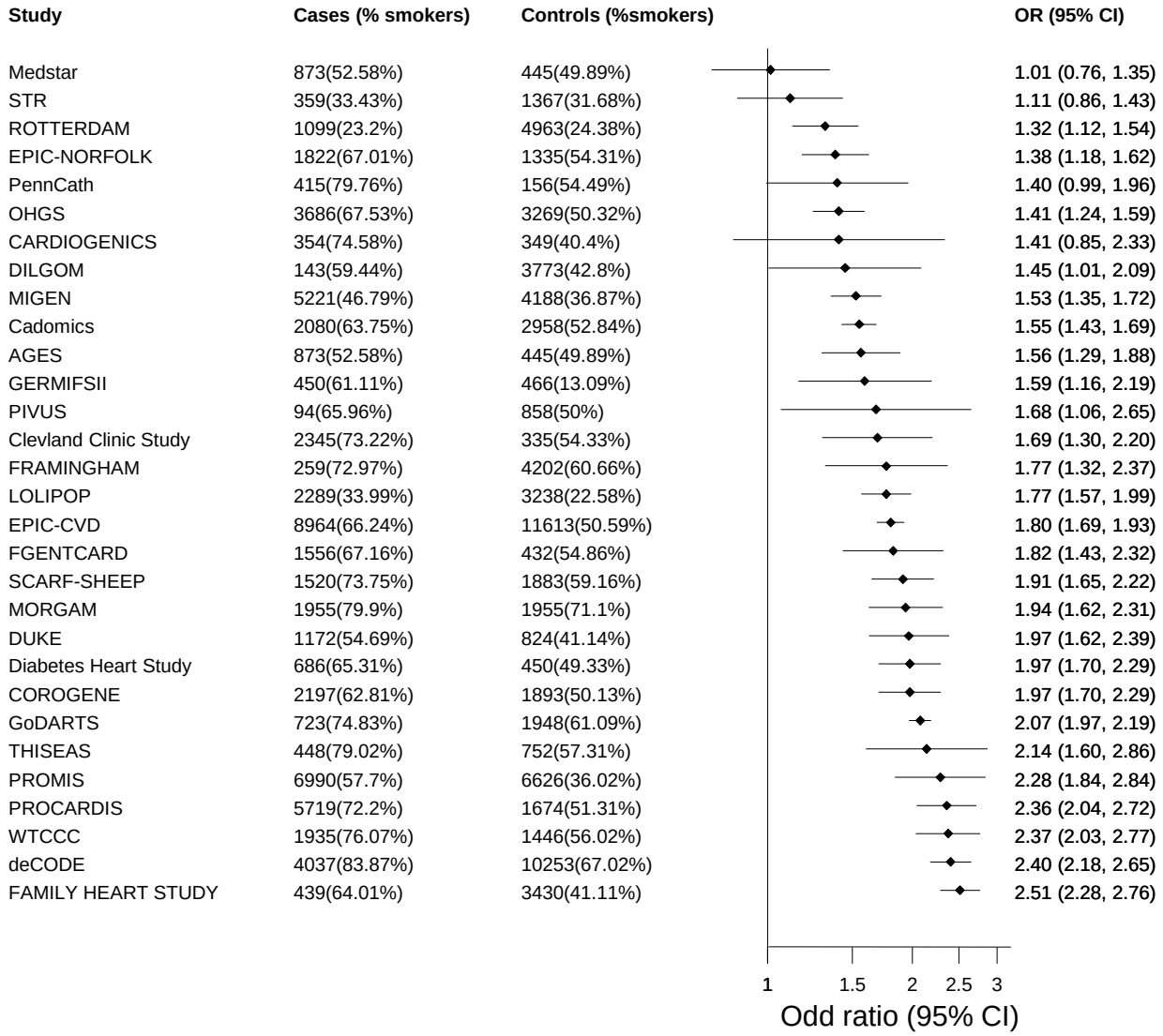
Supplementary Figure 1. Flow chart of study strategy



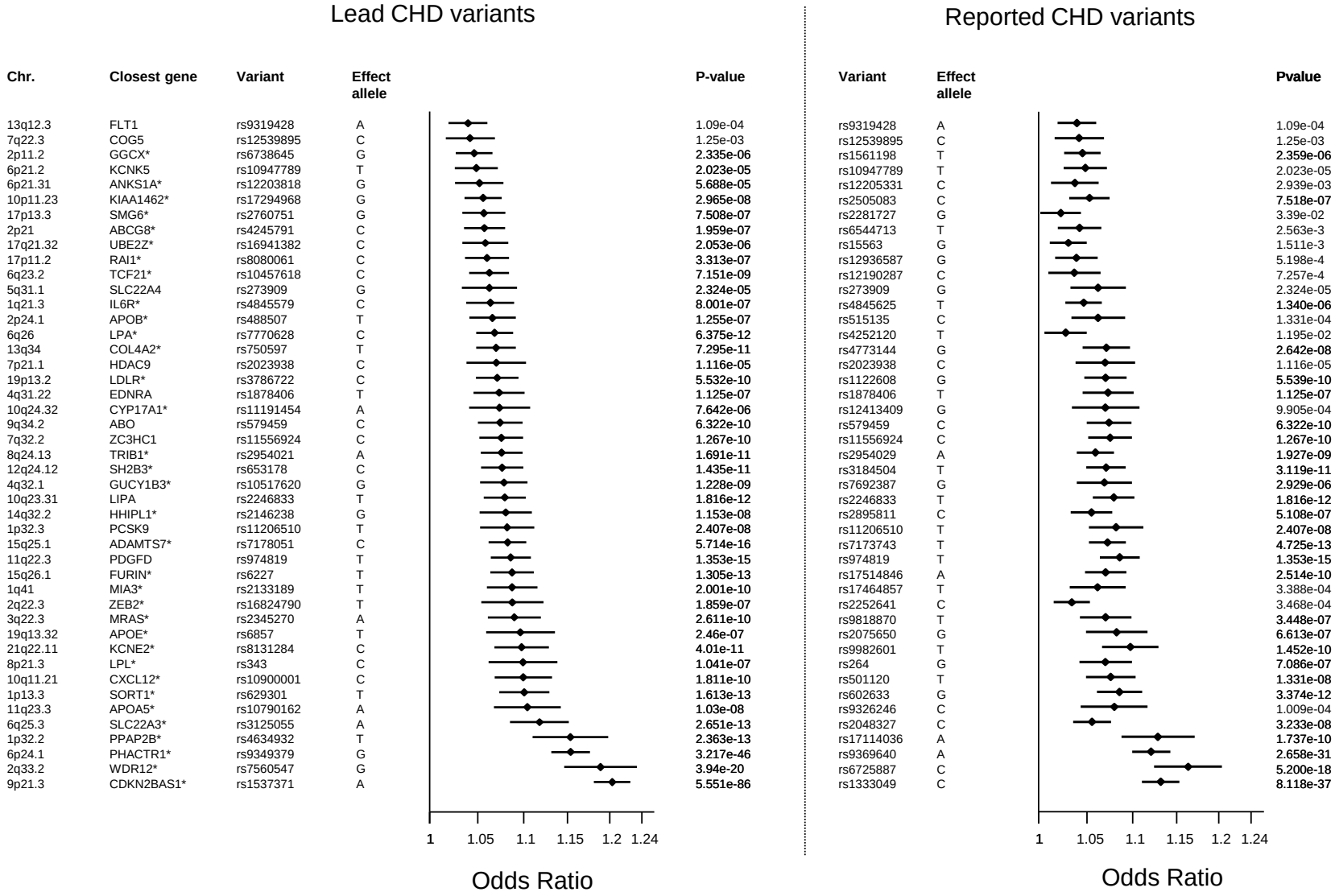
Supplementary Table 1. Description of the participating studies with information available on "ever-smoking" status, coronary heart disease risk and genotypes at the 50 candidate loci

Study Name	Location	Ethnicity	Age (years) Mean (SD)	Platform	Number of CHD cases	Number of controls	Ever smokers in CHD cases (%)	Ever smokers in controls (%)
Medstar	USA	European	48.9(6.4)/59.7(8.9)	GWAS	873	445	52.5	49.8
STR	USA	European	78.9(9.7)/73.1(11.0)	Metabochip	359	1367	33.4	31.6
ROTTERDAM	Netherlands	European	41.6(6.0)/51.0(11.8)	GWAS	1099	4963	23.2	24.4
EPIC-Norfolk	UK	European	71.8(8.18)/60.3(9.3)	Metabochip	1822	1335	67.0	54.3
PennCath	USA	European	52.7 (7.6)/61.7(9.6)	GWAS	415	156	79.8	54.5
OHGS	Canada	European	49.0(7.3)/74.5(5.5)	GWAS	3686	3269	67.5	50.3
CARDIOGENICS	Europe	European	57.0(8.8)/53.5(7.0)	GWAS	354	349	74.6	40.4
DILGOM	FINLAND	European	56.6(9.5)/51.7(13.6)	Metabochip	143	3773	59.4	42.8
MIGEN	USA & Europe	European	42.4 (6.6)/43.0(7.8)	GWAS	5221	4188	46.79	36.87
CADOMICS	Germany	European	59.3(10.8)/59.3(10.8)	GWAS	2080	2958	63.75	52.84
AGES	ICELAND	European	76.4 (5.4)/79.1(5.5)	GWAS	873	445	52.58	49.89
GERMIFSII	Germany	European	55.0(6.8)/51.1(12.9)	GWAS	450	466	61.1	13.09
PIVUS	Sweden	European	65.0(7.2)/70.2(0.2)	Metabochip	94	858	65.96	50
Cleveland Clinic	USA	European	61.7(11.1)/73.0(5.7)	GWAS	2345	335	73.22	54.33
FRAMINGHAM	USA	European	64.5(12.8)/75.2(12.2)	GWAS	259	4202	72.9	60.6
LOLIPOP	UK	South Asian	59.3(9.7)/52.4(10.2)	GWAS	2289	3238	33.9	22.5
EPIC-CVD	Europe	European	71.8(8.18)/60.3(9.3)	Metabochip	8964	11613	66.24	50.59
FGENTCARD	Lebanon	Middle-eastern	61.0(11.1)/55.6(11.6)	GWAS	1556	432	67.1	54.8
SCARF-SHEEP	Sweden	European	57.6(7.3)/50.5(7.0)	Metabochip	1520	1883	73.7	59.2
MORGAM	Europe	European	64.5(7.3)/60.9(7.8)	Metabochip	1955	1955	79.9	71.1
DUKE	USA	European	57(9.7)/63(8.7)	GWAS	1172	824	54.69	41.1
Diabetes Heart Study	USA	European	59.3(10)/61.5 (9.35)	Metabochip	686	450	65.3	49.3
COROGENE	FINLAND	European	66.0(11.8)/56.7(11.3)	GWAS	2197	1893	62.8	50.1
GoDARTS	Scotland	European	61.5(10.5)/61.8(9.5)	Metabochip	723	1948	74.8	61.1
THISEAS	GREECE	European	57.6(7.3)/50.5(7.0)	Metabochip	448	752	79.02	57.3
PROMIS	Pakistan	South Asian	54.2(10.6)/53.5(10.0)	GWAS	6990	6626	57.7	36.02
PROCARDIS	Europe	European	53.6(8.1)/60.9(13.1)	GWAS	5719	1674	72.2	51.3
WTCCC	United Kingdom	European	53.5(9.6)/44(0)	GWAS	1935	1446	76.07	56.02
deCODE	Iceland	European	74.8(11.8)/53.7(21.5)	GWAS	4037	10253	83.87	67.02
Family Heart Study	USA	European	64.5+12.8/75.2(12.2)	GWAS	439	3430	64.01	41.11

Supplementary Figure 2. Association of “ever-smoking” status with coronary heart disease in participating studies



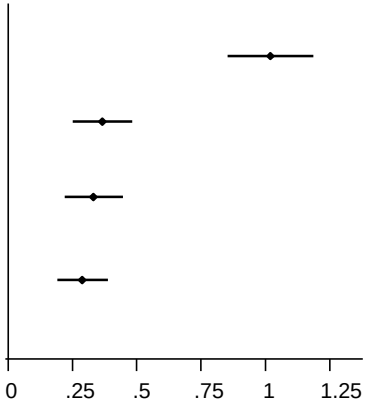
Supplementary Figure 3. Comparison of the lead variants with the top previously reported coronary heart disease variants at the candidate loci



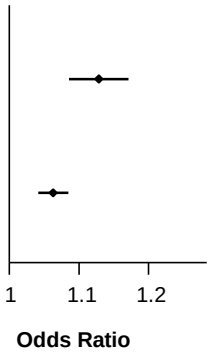
*lead variant observed in our study population differed with the reported variant

Supplementary Figure 4. Association of reported variants with smoking behavior in the Tobacco Genetics Consortium

CHR	SNP	Gene	EA	N	Beta (95% CI)	*P-value*
15q25.1	rs1051730	CHRNA3	A	73853	1.02 (0.85, 1.19)	2.75x10-73
10q23.32	rs1329650	LOC100188947	G	73853	0.37 (0.25, 0.48)	5.67x10-10
19q13.2	rs3733829	CYP2A6	G	73853	0.33 (0.22, 0.45)	1.04x10-8
8p11.21	rs6474412	CHRNB3	T	84956	0.29 (0.19, 0.39)	1.04x10-8



CHR	SNP	Gene	EA	N	OR (95% CI)	*P-value*
Ever-versus never smokers						
9q34.2	rs3025343	DBH	A	143023	1.13 (1.09, 1.17)	3.56x10-8
Former never current smokers						
11p14.1	rs6265	BDNF	C	64924	1.06 (1.04, 1.08)	1.84x10-8



Information on rs3025343 was not available in all participants in the CARDIoGRAMplusC4D consortium; hence excluded from analyses. EA. Effect allele

Supplementary Table 2. Association of top variants at established coronary heart disease loci in our study population

		Lead variant observed in our study population at established CHD Loci						Association of reported variant at established CHD loci in our study population					
Chr	**Locus**	**SNP**	**Position (Mb)**	**Effect allele**	**Beta**	**SE**	**P-value**	**SNP**	**Position (Mb)**	**Effect allele**	**Beta**	**SE**	**P-value**
13q12.3	FLT1	rs9319428	28973621	A	0.04	0.01	1.09E-04	rs9319428	28973621	A	0.04	0.01	1.09E-04
7q22.3	COG5	rs12539895	107091849	C	0.04	0.01	1.25E-03	rs12539895	107091849	C	0.04	0.01	1.25E-03
2p11.2	GGCX*	rs6738645	85783128	G	0.05	0.01	2.34E-06	rs1561198	85809989	T	0.04	0.01	2.36E-06
6p21.2	KCNK5	rs10947789	39174922	T	0.05	0.01	2.02E-05	rs10947789	39174922	T	0.05	0.01	2.02E-05
6p21.31	ANKS1A*	rs12203818	35251317	G	0.05	0.01	5.69E-05	rs12205331	34898455	C	0.04	0.01	2.94E-03
10p11.23	KIAA1462*	rs17294968	30300420	G	0.05	0.01	2.97E-08	rs2505083	30335122	C	0.05	0.01	7.52E-07
17p13.3	SMG6*	rs2760751	2028106	G	0.05	0.01	7.51E-07	rs2281727	2117945	G	0.02	0.01	3.39E-02
2p21	ABCG8*	rs4245791	44074431	C	0.06	0.01	1.96E-07	rs6544713	44073881	T	0.04	0.01	2.56E-04
17q21.32	UBE2Z*	rs16941382	45043508	C	0.06	0.01	2.05E-06	rs15563	47005193	G	0.03	0.01	1.51E-03
17p11.2	RAI1*	rs8080061	17776389	C	0.06	0.01	3.31E-07	rs12936587	17543722	G	0.04	0.01	5.20E-04
6q23.2	TCF21*	rs10457618	134188626	C	0.06	0.01	7.15E-09	rs12190287	134214525	C	0.04	0.01	7.26E-03
5q31.1	SLC22A4	rs273909	131667353	G	0.06	0.01	2.32E-05	rs273909	131667353	G	0.06	0.01	2.32E-05
1q21.3	IL6R*	rs4845579	151770138	C	0.06	0.01	8.00E-07	rs4845625	154422067	T	0.05	0.01	1.34E-06
2p24.1	APOB*	rs488507	21393689	T	0.06	0.01	1.26E-07	rs515135	21286057	C	0.06	0.01	1.33E-05
6q26	LPA*	rs7770628	161018174	C	0.07	0.01	6.38E-12	rs4252120	161143608	T	0.03	0.01	1.20E-02
13q34	COL4A2*	rs750597	111029256	T	0.07	0.01	7.30E-11	rs4773144	110960712	G	0.07	0.01	2.64E-08
7p21.1	HDAC9	rs2023938	19036775	C	0.07	0.02	1.12E-05	rs2023938	19036775	C	0.07	0.02	1.12E-05
19p13.2	LDLR*	rs3786722	11161537	C	0.07	0.01	5.53E-10	rs1122608	11163601	G	0.07	0.01	5.54E-10
4q31.22	EDNRA	rs1878406	148393664	T	0.07	0.01	1.13E-07	rs1878406	148393664	T	0.07	0.01	1.13E-07
10q24.32	CYP17A1*	rs11191454	104660004	A	0.07	0.02	7.64E-06	rs12413409	104719096	G	0.07	0.02	9.91E-05
9q34.2	ABO	rs579459	136154168	C	0.07	0.01	6.32E-10	rs579459	136154168	C	0.07	0.01	6.32E-10
7q32.2	ZC3HC1	rs11556924	129663496	C	0.07	0.01	1.27E-10	rs11556924	129663496	C	0.07	0.01	1.27E-10
8q24.13	TRIB1*	rs2954021	126482077	A	0.07	0.01	1.69E-11	rs2954029	126490972	A	0.06	0.01	1.93E-09
12q24.12	SH2B3*	rs653178	112007756	C	0.07	0.01	1.44E-11	rs3184504	111884608	T	0.07	0.01	3.12E-11
4q32.1	GUCY1B3*	rs10517620	156676558	G	0.08	0.01	1.23E-09	rs7692387	156635309	G	0.07	0.01	2.93E-06
10q23.31	LIPA	rs2246833	91005854	T	0.08	0.01	1.82E-12	rs2246833	91005854	T	0.08	0.01	1.82E-12
14q32.2	HHIPL1	rs2895811	100133942	C	0.05	0.01	5.11E-07	rs2895811	100133942	C	0.05	0.01	5.11E-07
1p32.3	PCSK9	rs11206510	55496039	T	0.08	0.01	2.41E-08	rs11206510	55496039	T	0.08	0.01	2.41E-08
15q25.1	ADAMTS7*	rs7178051	79118296	C	0.08	0.01	5.71E-16	rs7173743	79141784	T	0.07	0.01	4.73E-13
11q22.3	PDGFD	rs974819	103660567	T	0.08	0.01	1.35E-15	rs974819	103660567	T	0.08	0.01	1.35E-15
15q26.1	FURIN*	rs6227	91425232	T	0.08	0.01	1.31E-13	rs17514846	91416550	A	0.07	0.01	2.51E-10
1q41	MIA3*	rs2133189	222814442	T	0.08	0.01	2.00E-10	rs17464857	222762709	T	0.06	0.01	3.39E-05
2q22.3	ZEB2*	rs16824790	146106518	T	0.08	0.02	1.86E-07	rs2252641	145801461	C	0.03	0.01	3.47E-04
3q22.3	MRAS*	rs2345270	137325390	A	0.09	0.01	2.61E-10	rs9818870	138122122	T	0.07	0.01	3.45E-07
19q13.32	APOE*	rs6857	45392254	T	0.09	0.02	2.46E-07	rs2075650	45395619	G	0.08	0.02	6.61E-07
21q22.11	KCNE2*	rs8131284	35607496	C	0.09	0.01	4.01E-11	rs9982601	35599128	T	0.09	0.01	1.45E-10
8p21.3	LPL*	rs343	19810787	C	0.09	0.02	1.04E-07	rs264	19813180	G	0.07	0.01	7.09E-07
10q11.21	CXCL12*	rs10900001	44695585	C	0.09	0.01	1.81E-10	rs501120	44753867	T	0.07	0.01	1.33E-08
1p13.3	SORT1*	rs629301	109818306	T	0.10	0.01	1.61E-13	rs602633	109821511	G	0.08	0.01	3.37E-12
11q23.3	APOA5*	rs10790162	116639104	A	0.10	0.02	1.03E-08	rs9326246	116611733	C	0.08	0.02	1.01E-05
6q25.3	SLC22A3*	rs3125055	160736787	A	0.11	0.02	2.65E-13	rs2048327	160863532	C	0.05	0.01	3.23E-08
1p32.2	PPAP2B*	rs4634932	56996191	T	0.14	0.02	2.36E-13	rs17114036	56962821	A	0.12	0.02	1.74E-10
6p24.1	PHACTR1*	rs9349379	12903957	G	0.14	0.01	3.22E-46	rs9369640	12901441	A	0.11	0.01	2.66E-31
2q33.2	WDR12*	rs7560547	203757916	G	0.17	0.02	3.94E-20	rs6725887	203745885	C	0.15	0.02	5.20E-18
9p21.3	CDKN2BAS1*	rs1537371	22099568	A	0.18	0.01	5.55E-86	rs1333049	22125503	C	0.12	0.01	8.12E-37

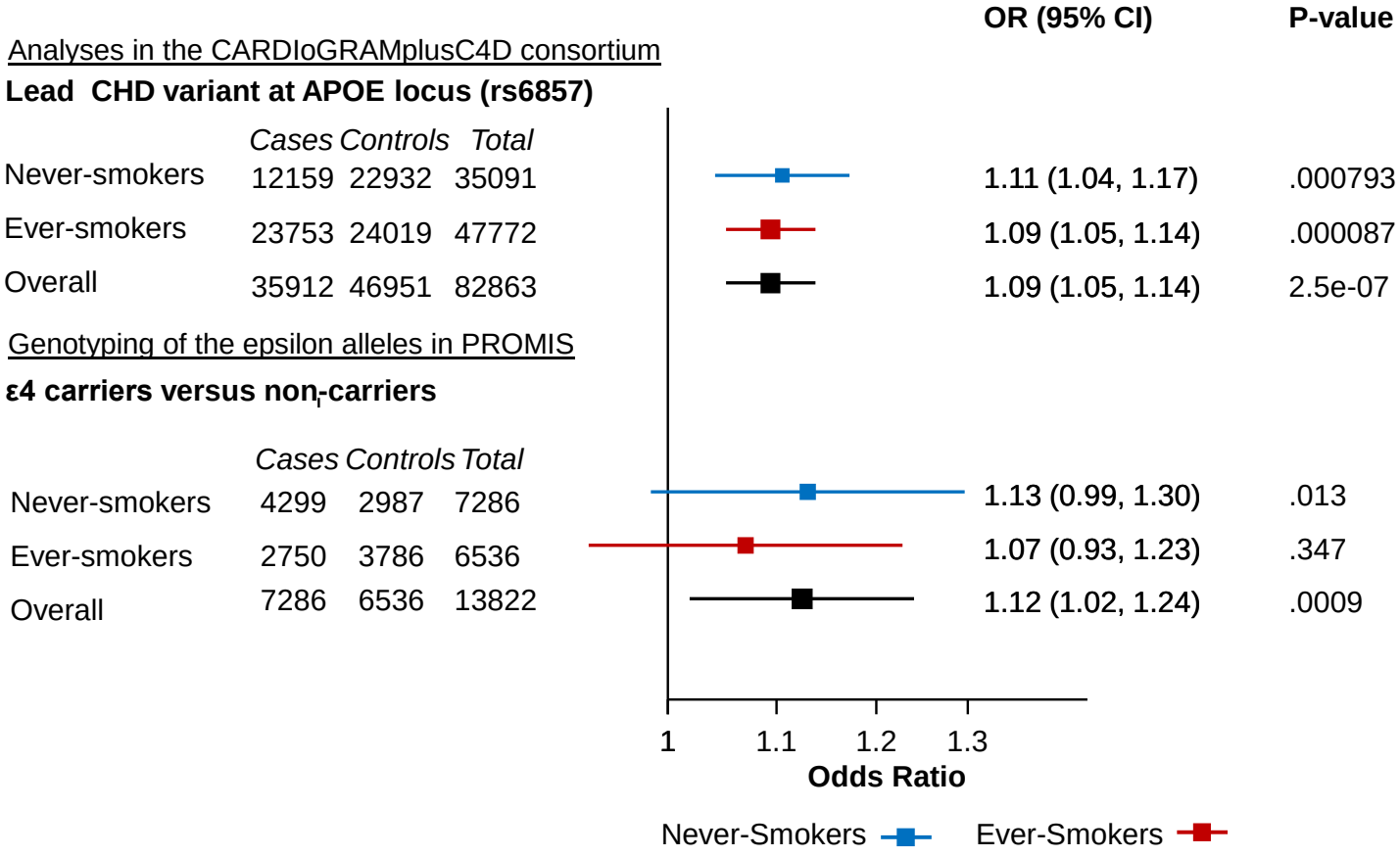
***lead variant observed in our study population differed with the reported variant**

Supplementary Table 3. Stratified (Never-smokers" and "Ever-Smokers") and Gene-smoking interaction analyses in coronary heart disease for the coronary heart disease and smoking behavior loci

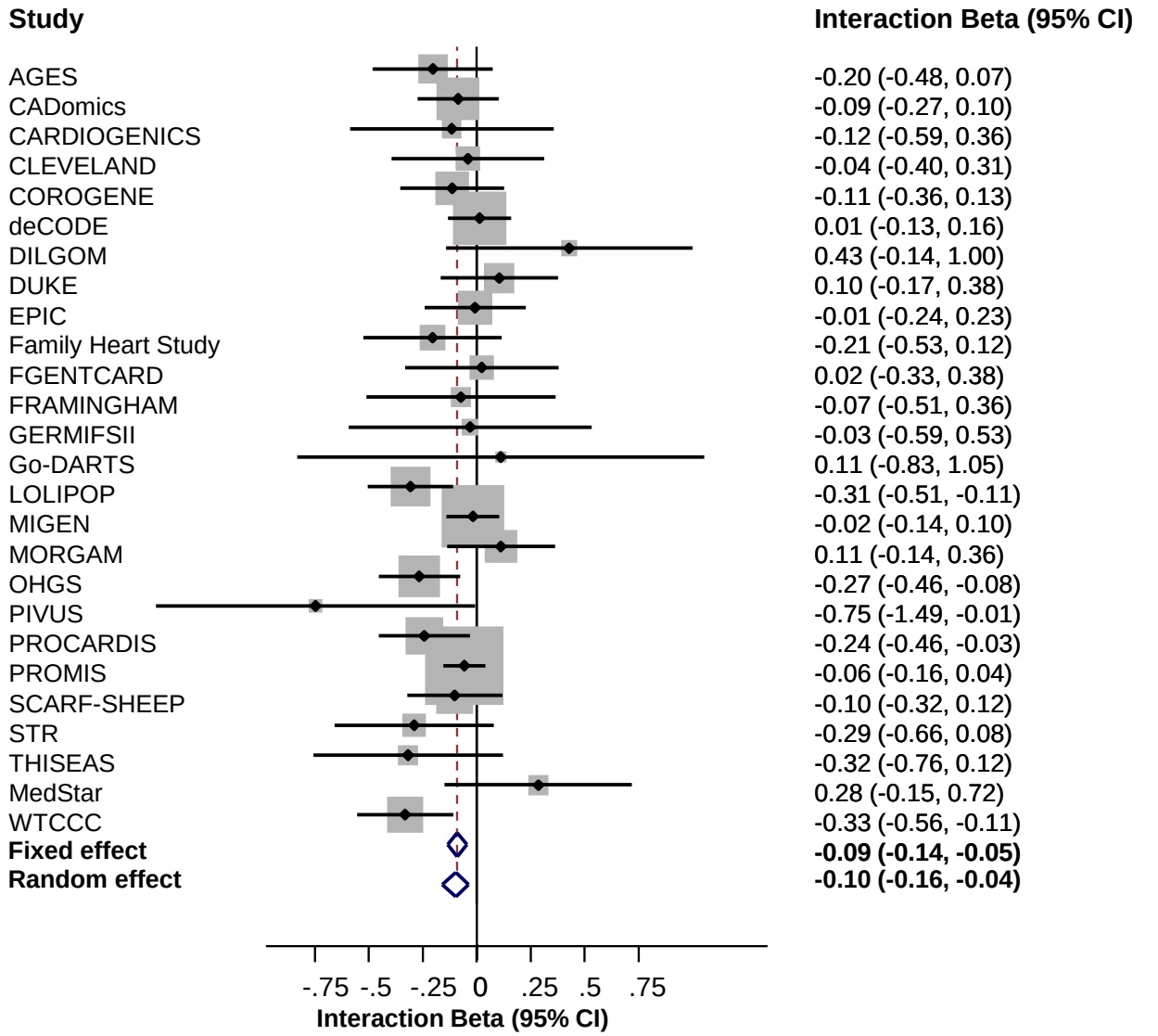
				Never-Smokers						Ever-Smokers						
Chr	Locus	variant	Allele (E/R)*	Cases	Controls	Total	Beta	SE	P-value	Cases	Controls	Total	Beta	Se	P-value	INTERACTION P-value
CHD RELATED LOCI																
15q25.1	**ADAMTS7**	**rs7178051**	**T/C**	**21232**	**38713**	**59945**	**-0.13**	**0.02**	**1.30E-16**	**39585**	**40749**	**80334**	**-0.05**	**0.01**	**2.49E-04**	**8.57E-05**
14q32.2	HHIPL1	rs2895811	T/C	16542	29114	45656	-0.08	0.02	2.68E-06	31524	30816	62340	-0.04	0.01	8.16E-03	9.45E-03
1q41	MIA3	rs2133189	T/C	14475	23848	38323	0.12	0.02	6.31E-09	24428	22522	46950	0.06	0.02	7.19E-04	0.05057
6p21.2	KCNK5	rs10947789	T/C	21232	38713	59945	0.02	0.02	1.56E-01	39585	40749	80334	0.06	0.01	1.37E-05	0.0949
10q24.32	CYP17A1	rs11191454	A/G	18470	29062	47532	0.10	0.02	7.84E-05	38155	35437	73592	0.05	0.02	1.12E-02	0.1088
12q24.12	SH2B3	rs653178	T/C	18206	32975	51181	-0.10	0.02	3.44E-09	33647	34874	68521	-0.05	0.01	8.95E-05	0.1131
5q31.1	SLC22A4	rs273909	A/G	19247	33076	52323	-0.09	0.02	5.05E-05	38049	36478	74527	-0.04	0.02	3.18E-02	0.1144
11q22.3	PDGFD	rs974819	T/C	20559	38198	58757	0.11	0.02	5.73E-11	38923	40371	79294	0.07	0.01	7.70E-07	0.1426
9q34.2	ABO	rs579459	T/C	21232	38713	59945	-0.06	0.02	1.37E-03	39585	40749	80334	-0.08	0.02	7.52E-08	0.1598
13q12.3	FLT1	rs9319428	A/G	21232	38713	59945	0.06	0.02	3.13E-04	39585	40749	80334	0.03	0.01	4.07E-02	0.1619
2p11.2	GGCX	rs6738645	T/G	20377	37440	57817	-0.02	0.02	1.35E-01	38382	39181	77563	-0.06	0.01	1.07E-06	0.1687
2p21	ABCG8	rs4245791	T/C	18906	34620	53526	-0.07	0.02	1.35E-05	37169	38266	75435	-0.04	0.01	1.52E-03	0.2016
17p13.3	SMG6	rs2760751	A/G	18484	31315	49799	-0.07	0.02	8.19E-05	33722	32479	66201	-0.05	0.01	1.76E-03	0.2017
21q22.11	KCNE2	rs8131284	T/C	20827	32578	53405	-0.12	0.02	1.25E-07	39353	37234	76587	-0.08	0.02	2.36E-05	0.2254
15q26.1	FURIN	rs6227	T/C	17094	31503	48597	0.11	0.02	1.08E-09	30978	32908	63886	0.07	0.01	4.87E-06	0.2261
1p32.3	PCSK9	rs11206510	T/C	19080	30813	49893	0.07	0.02	1.91E-03	33026	28327	61353	0.09	0.02	2.76E-06	0.2742
6q23.2	TCF21	rs10457618	T/C	20553	37945	58498	-0.05	0.02	4.98E-03	38909	40166	79075	-0.07	0.01	2.07E-07	0.315
6q25.3	SLC22A3	rs3125055	A/T	19496	31336	50832	0.13	0.02	3.32E-08	37193	35724	72917	0.10	0.02	8.24E-07	0.3191
2q33.2	WDR12	rs7560547	A/G	11452	16774	28226	-0.15	0.03	5.62E-07	23827	22810	46637	-0.19	0.02	8.83E-15	0.3584
6q26	LPA	rs7770628	T/C	21050	37955	59005	-0.06	0.01	9.27E-05	39044	39559	78603	-0.07	0.01	1.30E-08	0.3634
13q34	COL4A2	rs750597	A/T	21232	38713	59945	-0.07	0.02	4.00E-06	39585	40749	80334	-0.06	0.01	3.46E-06	0.3881
7p21.1	HDAC9	rs2023938	T/C	20178	31202	51380	-0.05	0.02	6.02E-02	39137	36961	76098	-0.08	0.02	3.44E-05	0.3987
10q11.21	CXCL12	rs10900001	C/G	20029	30278	50307	0.09	0.02	1.04E-04	38286	35107	73393	0.10	0.02	3.91E-07	0.4158
1p13.3	SORT1	rs629301	T/G	17401	28346	45747	0.11	0.02	9.86E-08	32124	30718	62842	0.09	0.02	2.42E-07	0.419
4q31.22	EDNRA	rs1878406	T/C	20568	31970	52538	0.08	0.02	8.06E-05	39177	37144	76321	0.06	0.02	2.96E-04	0.4329
17p11.2	RAI1	rs8080061	T/C	16313	26850	43163	-0.04	0.02	1.63E-02	28987	28172	57159	-0.07	0.02	3.06E-06	0.4536
1p32.2	PPAP2B	rs4634932	T/C	14428	21600	36028	0.18	0.03	3.47E-09	29796	27171	56967	0.12	0.03	4.61E-06	0.4825
3q22.3	SOX14	rs2345270	A/G	13811	22661	36472	0.07	0.02	4.94E-04	24704	21225	45929	0.10	0.02	8.78E-08	0.4994
10q23.31	LIPA	rs2246833	T/C	17742	32340	50082	0.09	0.02	1.29E-07	32175	34064	66239	0.07	0.01	1.85E-06	0.5051
1q21.3	IL6R	rs4845579	T/C	20933	37873	58806	-0.06	0.02	9.71E-04	39323	39994	79317	-0.06	0.02	2.39E-04	0.5327
4q32.1	GUCY1B3	rs10517620	A/G	19324	32464	51788	-0.08	0.02	7.83E-06	34925	34047	68972	-0.07	0.02	3.04E-05	0.6146
6p21.31	ANKS1A	rs12203818	A/G	18318	34529	52847	-0.05	0.02	1.61E-02	36527	37975	74502	-0.05	0.02	1.25E-03	0.6371
7q32.2	ZC3HC1	rs11556924	T/C	15973	28497	44470	-0.08	0.02	3.03E-06	30026	30932	60958	-0.06	0.01	6.85E-06	0.6466
17q21.32	UBE2Z	rs16941382	T/C	21013	37746	58759	-0.06	0.02	8.19E-04	39402	40002	79404	-0.05	0.02	6.75E-04	0.7015
8p21.3	LPL	rs343	A/C	16096	25458	41554	-0.10	0.03	3.00E-04	35930	33164	69094	-0.09	0.02	8.97E-05	0.7019
11q23.3	APOA5	rs10790162	A/G	18776	28882	47658	0.11	0.03	2.31E-05	38184	34895	73079	0.09	0.02	9.36E-05	0.7106
8q24.13	TRIB1	rs2954021	A/G	16449	27132	43581	0.08	0.02	3.67E-06	29253	28441	57694	0.07	0.01	9.83E-07	0.7373
19p13.2	LDLR	rs3786722	A/C	21232	38713	59945	-0.07	0.02	7.01E-05	39585	40749	80334	-0.07	0.01	1.92E-06	0.7522
2p24.1	APOB	rs488507	T/G	20559	37688	58247	0.06	0.02	1.06E-03	38310	39748	78058	0.07	0.02	3.32E-05	0.7576
19q13.32	APOE	rs6857	T/C	12159	22932	35091	0.10	0.03	7.93E-04	23753	24019	47772	0.09	0.02	8.68E-05	0.7612
10p11.23	KIAA1462	rs17294968	A/G	21232	38713	59945	-0.05	0.02	4.26E-04	39585	40749	80334	-0.05	0.01	1.87E-05	0.8262
7q22.3	COG5	rs12539895	A/C	18206	32975	51181	-0.05	0.02	2.02E-02	33647	34874	68521	-0.04	0.02	2.34E-02	0.8558
6p24.1	PHACTR1	rs9349379	A/G	19819	33838	53657	-0.15	0.02	9.37E-21	37887	37825	75712	-0.14	0.01	3.62E-27	0.9004
9p21.3	CDKN2BAS1	rs1537371	A/C	21232	38713	59945	0.19	0.01	2.45E-37	39585	40749	80334	0.18	0.01	1.73E-50	0.9201
2q22.3	ZEB2	rs16824790	T/C	15330	27420	42750	0.09	0.03	1.13E-03	30004	33753	63757	0.08	0.02	4.67E-05	0.9841
SMOKING RELATED LOCI																
15q25.1	**CHRNA3**	**rs1051730**	**A/G**	**20559**	**38198**	**58758**	**-0.04**	**0.02**	**1.65E-02**	**38923**	**40371**	**79294**	**0.03**	**0.01**	**1.53E-02**	**2.37E-04**
10q23.32	LOC100188947	rs1329650	T/G	19339	32588	68973	-0.01	0.02	0.49	34925	34047	68973	-0.022	0.0141	0.1032	**0.5291**
19q13.2	CYP2A6	rs3733829	A/G	18875	31953	50829	0.01	0.02	0.465	33453	33237	66691	-0.005	0.01	0.7142	0.4924
11p14.1	BDNF-AS	rs6265	T/C	21232	38713	59946	-0.06	0.02	1.72E-03	39585	40749	80334	-0.03	0.02	3.79E-02	0.4163
8p11.21	CHRNB3	rs6474412	T/C	19269	30935	50205	-0.01	0.02	6.15E-01	34736	31498	66234	-0.01	0.02	6.61E-01	0.7113

*(E/R) – (effect allele / reference allele)

Supplementary Figure 5a. Association by smoking status of the *APOE* locus with coronary heart disease in the CARDIoGRAMplusC4D consortium and PROMIS

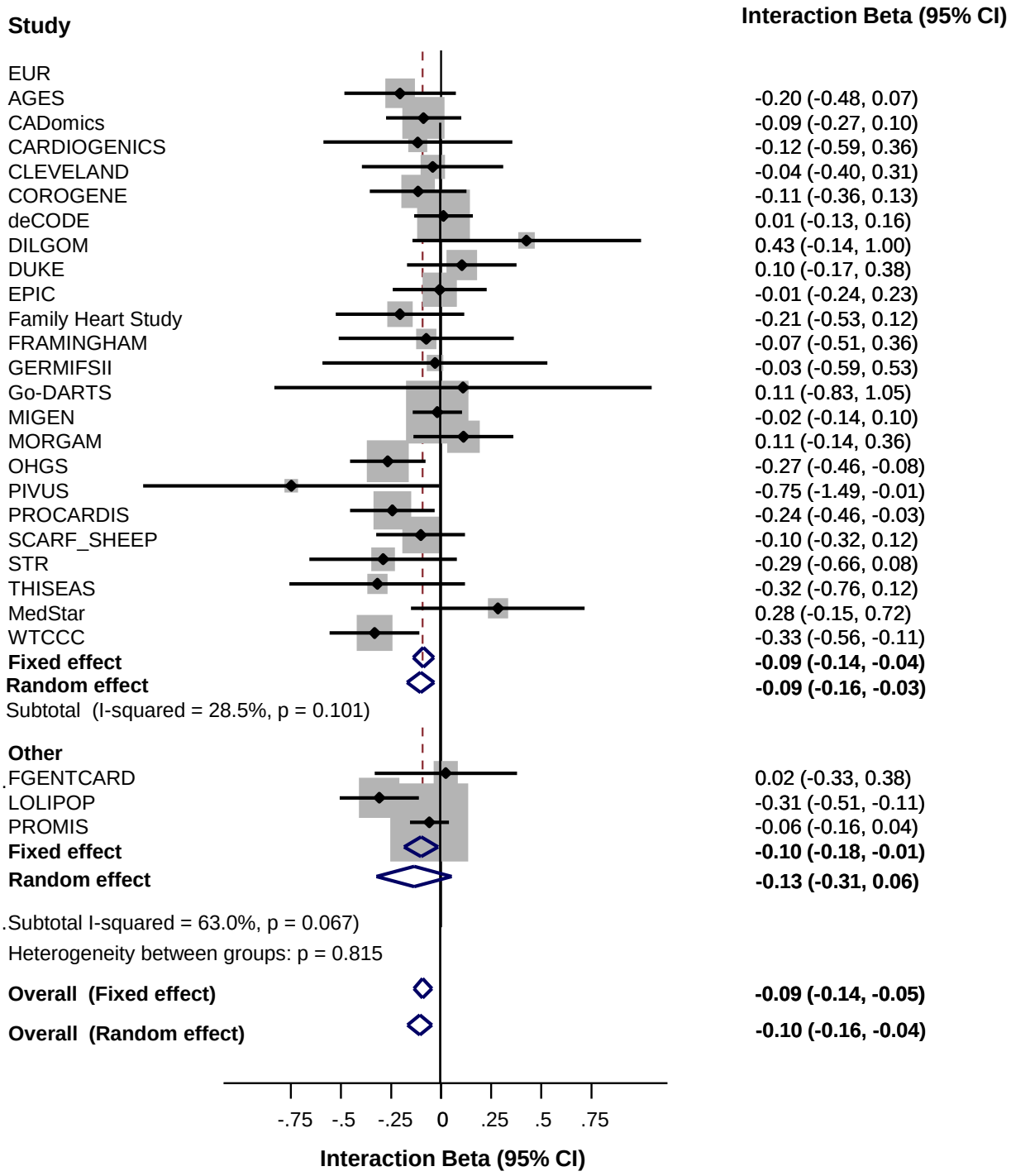


Supplementary Figure 5b. Forest plot displaying interaction beta across the participating studies



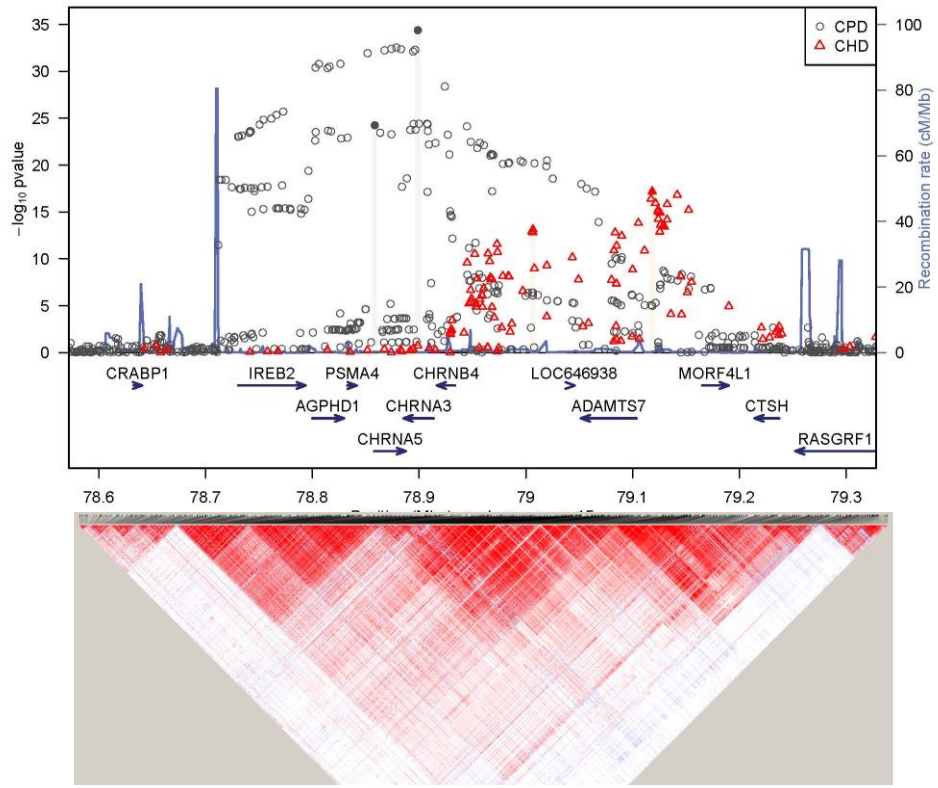
Overall (I-squared = 31.0%, p = 0.068)

Supplementary Figure 5c. Forest plot displaying interaction beta across the participating studies by ethnicity

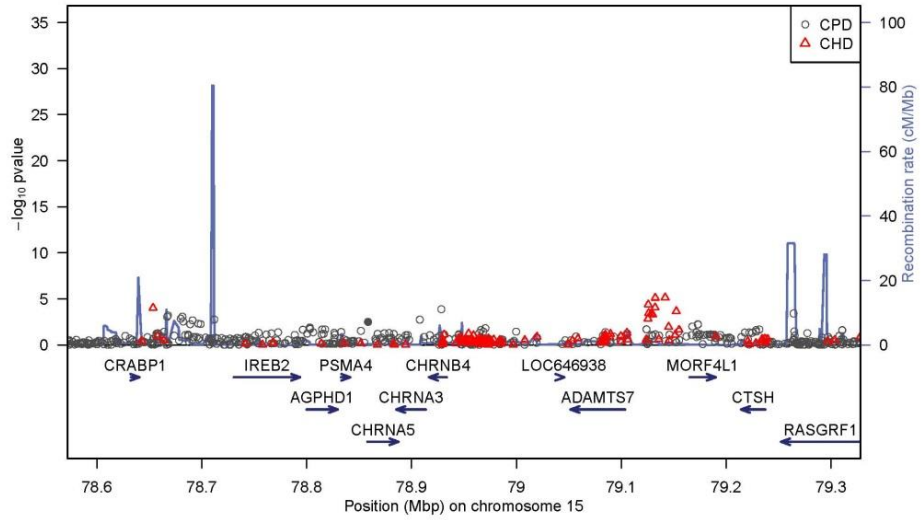


Supplementary Figure 6. (a) Unadjusted and (b) adjusted associations of chromosome 15q21.1 variants with coronary heart disease (CHD, red triangles) and smoking behavior (cigarettes per day, CPD; grey circles)

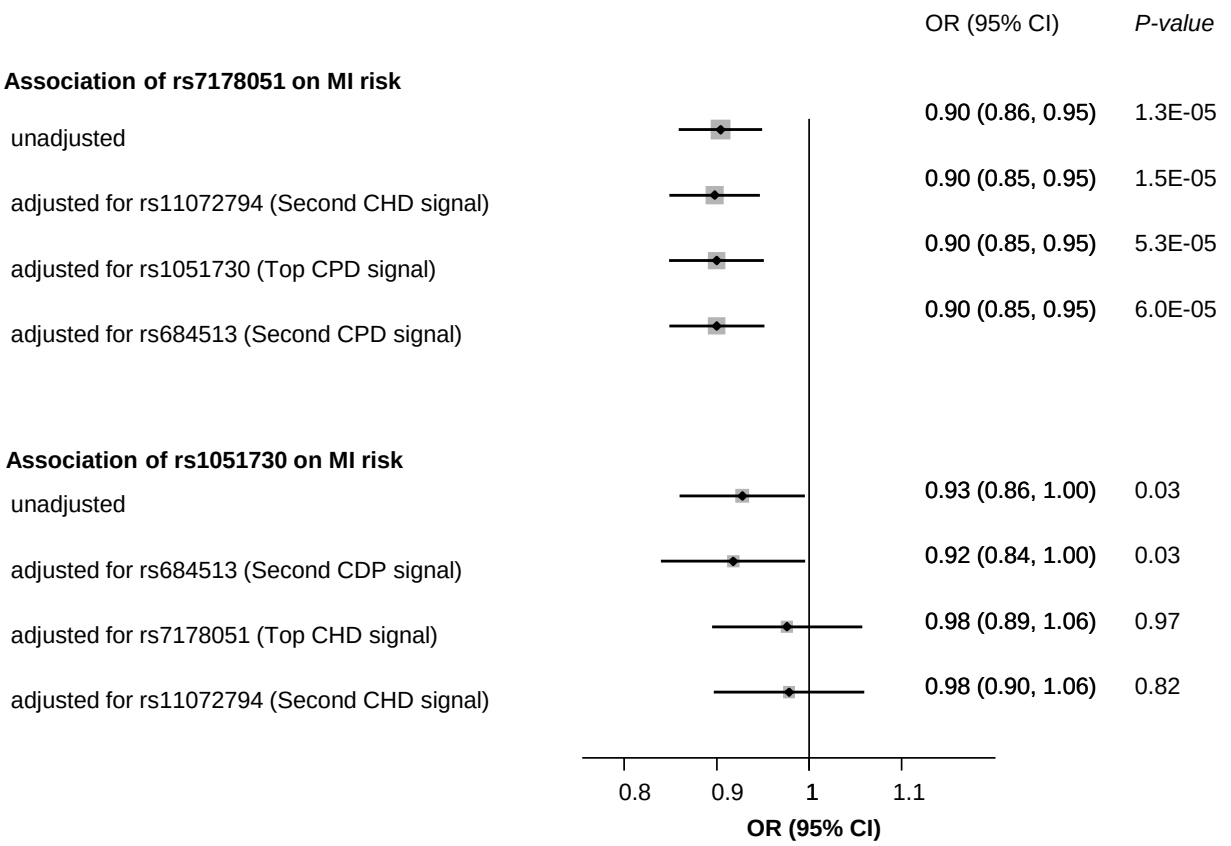
(a) Main effects on CHD risk and CPD behavior (unconditional)



(b) analyses conditioned on rs717805, rs11072794, rs1051730 and rs684513

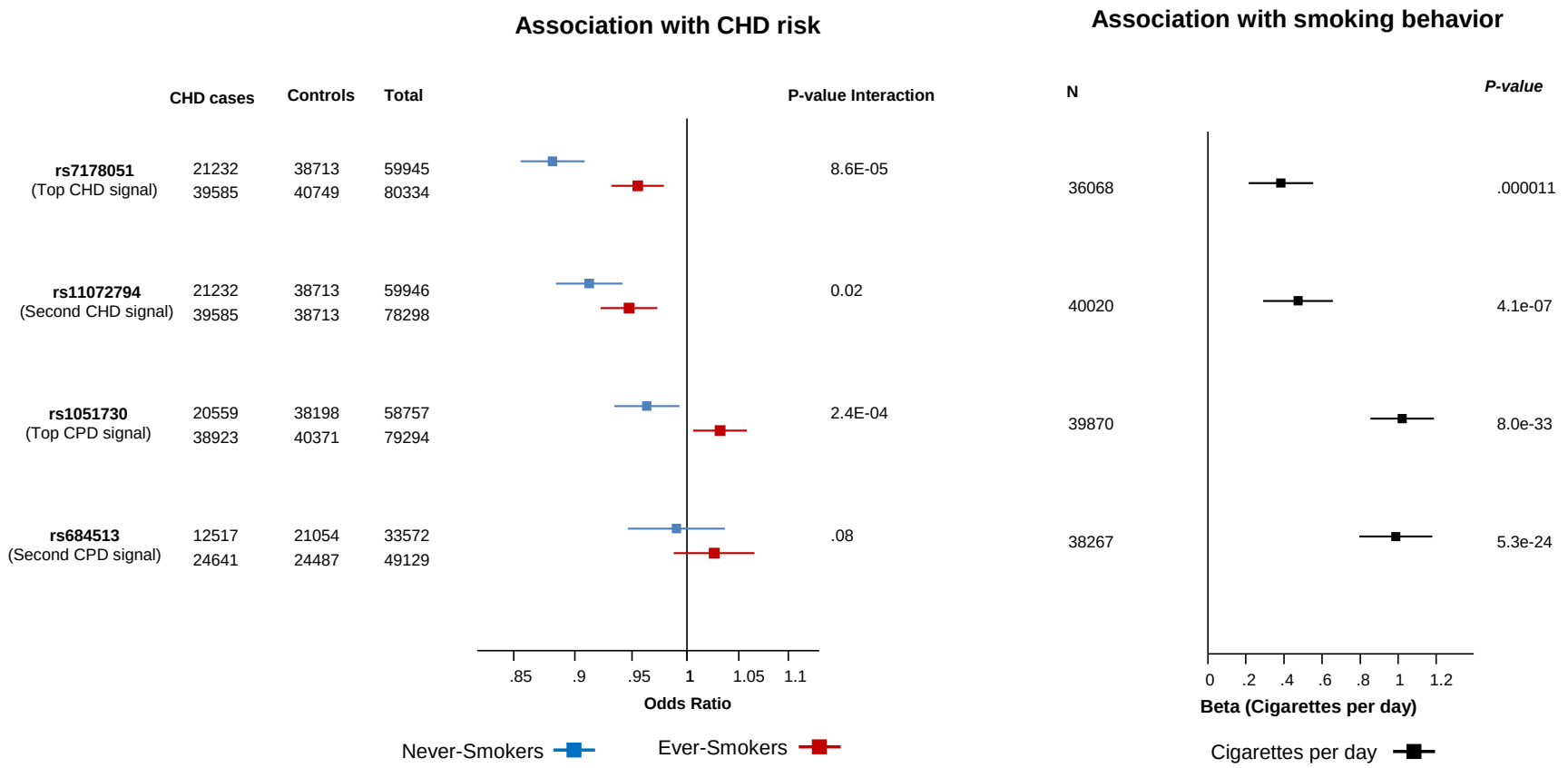


Supplementary Figure 6c – Analyses of rs7178051 and rs1051730 with MI risk in PROMIS (9,025 MI cases and 8,506 controls)



The current analyses used data from a customized cardiometabochip that was genotyped in 9,025 MI cases and 8,506 controls from the PROMIS study

Supplementary Figure 7. Unadjusted effects of 15q21.1 lead variants on coronary heart disease and smoking behavior



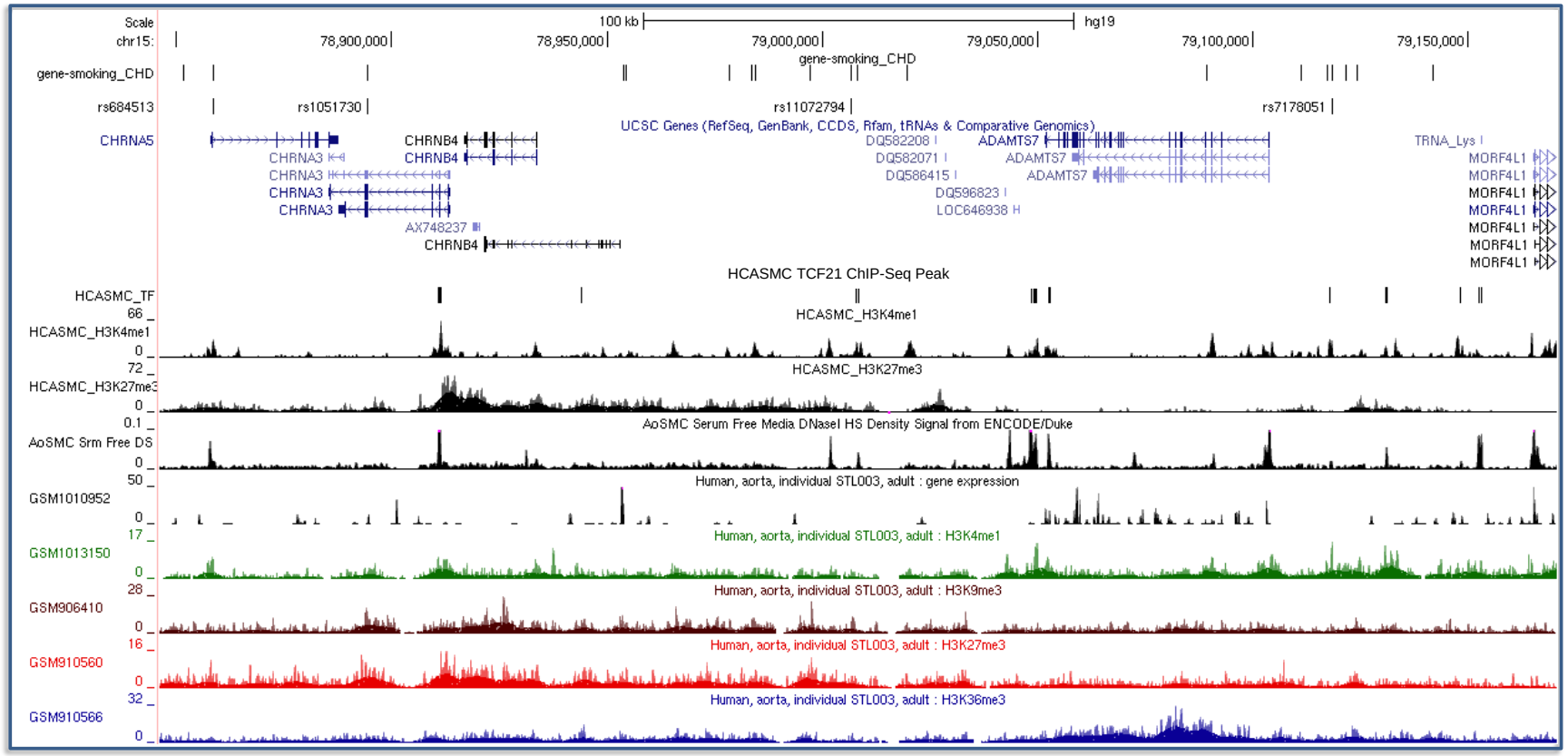
Supplementary Table 4. Association of rs7178051 (top CHD SNP) and rs1051730 (top CPD SNP) mutually adjusted for each other in 9,025 MI cases and 8,506 controls in PROMIS

PROMIS (9,025 MI cases and 8,506 controls)			
	OR	Se	P-value
rs7178051 (unadjusted)	0.90	0.02	1.31E-05
adjusted for rs1051730 using logistic regression	0.91	0.02	6.78E-05
adjusted for rs1051730 using GCTA	0.91	0.02	2.60E-05
rs1051730 (unadjusted)	0.96	0.02	0.076
adjusted for rs7178051 using logistic regression	1.00	0.03	0.875
adjusted for rs7178051 using GCTA method	1.00	0.02	0.80

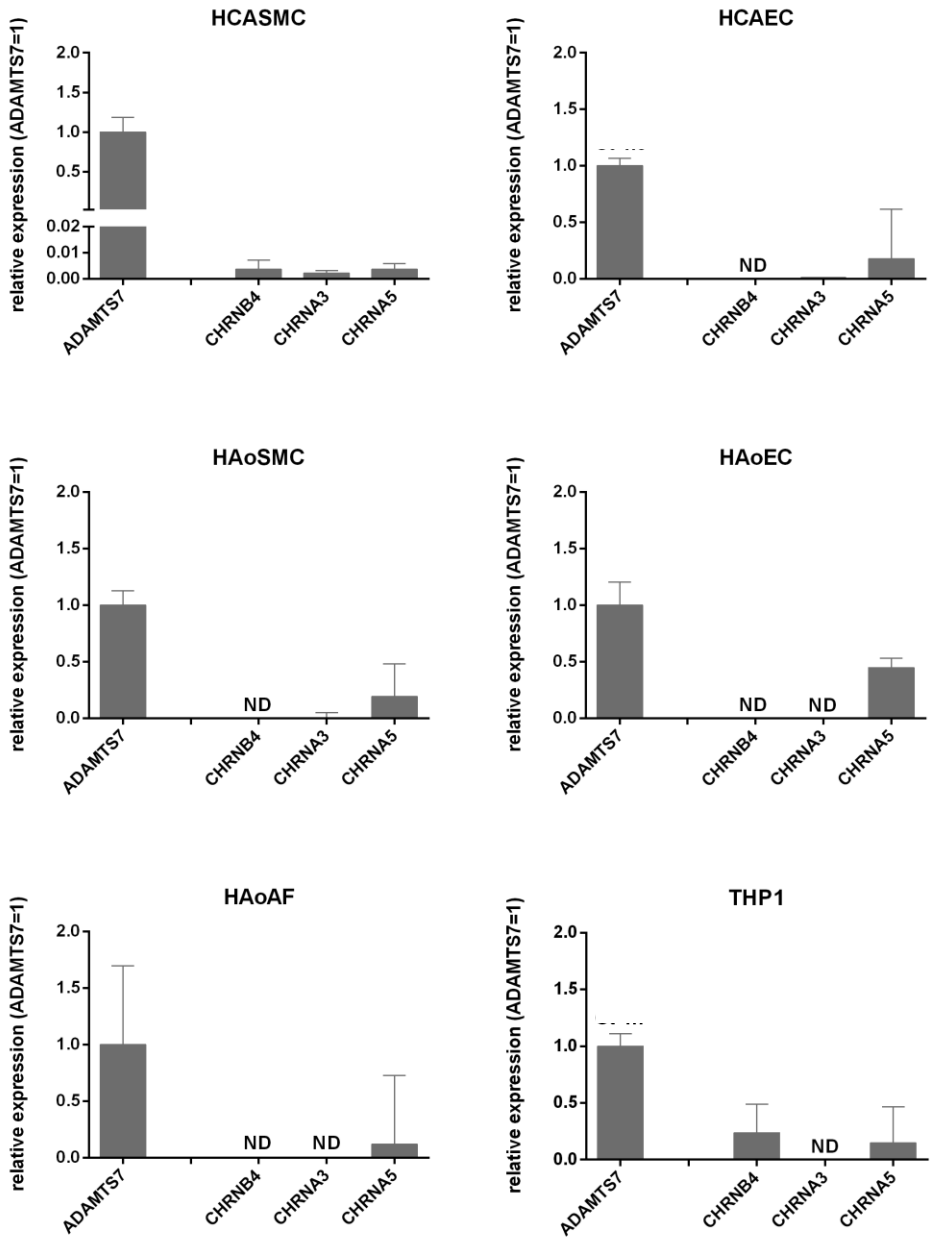
Supplementary Table 5. Association of rs7178051 with MI risk in PROMIS in participants by smoking status who do not carry the minor allele for rs1051730 and rs684513 variants

	PROMIS			
	Cases	**Controls**	**OR**	**P-value**
Never-smokers	2110	2787	0.88	0.01
Ever-smokers	2982	2026	0.94	0.21
Current meta-analyses utilizing data from all participants				
Never-smokers	21232	38713	0.88	1.30×10^{-16}
Ever-smokers	39585	40749	0.95	2.49×10^{-04}

Supplementary Figure 8. Genome browser view of regulatory features at Chr15q21.



Supplementary Figure 9. Expression of *ADAMTS7* and *CHRNB4-A3-A5* mRNAs in HCASMC, HCAEC, HAoSMC, HAoEC, HAoAF and THP-1 cells.



HCASMC = human coronary artery smooth muscle cells; HCAEC = human coronary artery endothelial cells; HAoSMC = human aortic smooth muscle cells; HAoEC = human aortic endothelial cells; HAoAF = human aortic adventitial fibroblasts; THP-1 = human acute monocytic leukemia cell line; ND = not detected.