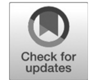
Smoking Modifies Pancreatic Cancer Risk Loci on 2q21.3



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ABSTRACT

Germline variation and smoking are independently associated with pancreatic ductal adenocarcinoma (PDAC). We conducted genome-wide smoking interaction analysis of PDAC using genotype data from four previous genome-wide association studies in individuals of European ancestry (7,937 cases and 11,774 controls). Examination of expression quantitative trait loci data from the Genotype-Tissue Expression Project followed by colocalization analysis was conducted to determine whether there was support for common SNP(s) underlying the observed associations. Statistical tests were two sided and $P < 5 \times 10^{-8}$ was considered statistically significant. Genome-wide significant evidence of qualitative interaction was identified on chr2q21.3 in intron 5 of the transmembrane protein 163 (TMEM163) and upstream of the cyclin T2 (CCNT2). The most significant SNP using the Empirical Bayes method, in this region that included 45 significantly associated SNPs, was rs1818613 [per allele OR in never smokers 0.87, 95% confidence interval (CI), 0.82–0.93; former smokers 1.00, 95% CI, 0.91–1.07; current smokers 1.25, 95% CI 1.12–1.40, $P_{\text{interaction}} = 3.08 \times 10^{-9}$). Examination of the Genotype-Tissue Expression Project data demonstrated an expression quantitative trait locus in this region for TMEM163 and CCNT2 in several tissue types. Colocalization analysis supported a shared SNP, rs842357, in high linkage disequilibrium with rs1818613 ($r^2 = 0.94$) driving both the observed interaction and the expression quantitative trait loci signals. Future studies are needed to confirm and understand the differential biologic mechanisms by smoking status that contribute to our PDAC findings.

Significance: This large genome-wide interaction study identifies a susceptibility locus on 2q21.3 that significantly modified PDAC risk by smoking status, providing insight into smoking-associated PDAC, with implications for prevention.

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Introduction

Pancreatic cancer is the seventh leading cause of cancer death worldwide (1). In 2018, 458,918 new cases of pancreatic cancer were diagnosed, and 432,242 individuals died from this disease in the world (1). The incidence of pancreatic cancer has significantly increased since the mid-1990s in the United States and worldwide (2, 3). The risk of pancreatic cancer increases with age, with the majority of cases diagnosed after age 55 years (1). Pancreatic ductal adenocarcinoma (PDAC) is the most common subtype and represents > 85% of all pancreatic cancers (1).

Inherited susceptibility plays an important role, as demonstrated by the high-risk of PDAC in individuals with a family history of pancreatic cancer, particularly those with multiple affected relatives (4). Pathogenic variants in *BRCA1, BRCA2, PALB2, ATM, CDKN2A, STK11*, and DNA mismatch repair genes are associated with increased PDAC (5), with recent studies demonstrating up to 10% of people with PDAC carry variants in these genes (6). Common variants also play a role in PDAC, with array-based heritability estimates of up to 21.2% (7). Our genome-wide association studies (GWAS) have identified 18 regions associated with PDAC. Associated gene regions include 1p36.33 (*NOC2L*), two independent loci at 1q32.1 (NR5A2), 2p13.3 (*ETAA1*), 3q29 (*TP63*), three loci at 5p15.33 (CLPTM1L-TERT), 7p12 (*TNS3*), 7p13 (*SUGCT*), 7q32.3 (*LINC-PINT*), 8q21.11 (*HNF4G*), 8q24.21(MYC), 9q34.2 (*ABO*), 13q12.2 (*PDX1*), 13q22.1 (*KLF5*), 16q23.1 (*BCAR1*), 17q12 (*HNF1B*), 17q25.1 (*LINC00673*), 18q21.32 (*GRP*), and 22q12.1 (*ZNRF3*; refs. 8–13).

Other risk factors for PDAC include cigarette smoking, diabetes, chronic pancreatitis, heavy alcohol use, and excess body weight (1). In particular, the association between smoking and PDAC is well established, with an estimated population attributable fraction in the United States of 12.1% (14). Both case–control and cohort studies have demonstrated an approximately 2-fold elevated risk for current smokers compared with never smokers (15, 16). A pooled analysis of data from 12 case–control studies within the Pancreatic Cancer Case-Control Consortium (PanC4) showed that compared with never smokers, the OR of PDAC was 1.17 [95% confidence interval (CI), 1.02–1.34] for former smokers and 2.20 (95% CI, 1.71–2.83) for current smokers (15, 16). A pooled nested case–control study within 12 cohorts in the Pancreatic Cancer Cohort Consortium (PanScan) showed an increased PDAC risk for current smokers compared with never smokers (OR = 1.77; 95% CI, 1.38–2.2; ref. 15). Although no overall association was observed for former smokers, former smokers who had quit less than 10 years had an elevated risk (OR = 2.19; 95% CI, 1.25–3.83) with the risk attenuating with time since cessation and approaching that of never smokers after 15 years (15).

Cigarette exposure has also been shown to cluster within families, and nicotine addiction has a strong heritable component (17, 18). Several genome-wide significant loci have been associated with distinct smoking-related traits (19–21). Established associations include a cluster of nicotinic acetylcholine receptor (nAChR) genes, *CHRNA5-CHRNA3-CHRNB4* located on chromosome 15q24 (20, 21). Despite the large number of associated loci, altogether the common genetic variants account only for 0.1% of the phenotypic variation in smoking cessation and 2.9% of the phenotypic variation in age at smoking initiation, indicating the highly polygenic nature of these traits (21).

Given the known the importance of genetic variation in modulating both pancreatic cancer and cigarette smoking, understanding whether the association of specific genetic variants differs by smoking status could increase our understanding of both of these traits. Candidate gene studies, mostly related to carcinogen metabolism, DNA repair, oxidative stress, and inflammation, have examined risk smoking interactions for PDAC with inconsistent results (22). A previous genome-wide gene-smoking interaction analysis for PDAC that included 2,028 cases and 2,109 controls from PanC4 did not find significant interactions (23); however, the small sample size did not have sufficient power to detect modest effect sizes. Therefore, in this study, we conducted genome-wide smoking interaction analysis of PDAC risk using genotype data from four previous GWAS conducted

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by the PanScan and PanC4 Consortia (8–12) and three alternative statistical methods that have robust power to detect gene-environment interactions (24). We also examined whether smoking modified the association of established PDAC susceptibility variants (8–13).

Materials and Methods

Study sample

Study participants were selected from four previously conducted GWAS from the PanScan and the PanC4. Details of these studies have been published previously (8–12). Each participating study obtained written informed consent from participants and approval from their local Institutional Review Board. The studies were conducted in accordance with recognized ethical guidelines (e.g., Declaration of Helsinki, CIOMS, Belmont Report, U.S. Common Rule). Both the Johns Hopkins School of Medicine (Baltimore, MD) Institutional Review Board and the NCI (Bethesda, MD) Special Studies Institutional Review Board approved the consortia study. Our study was based on 9,038 primary PDAC cases (ICD-O-3 code C250-C259) and 12,389 controls free of known PDAC. Participants with nonexocrine pancreatic tumors were excluded (histology types 8150, 8151, 8153, 8155, and 8240). We included only participants of European ancestry, to reduce confounding due to population stratification, and those with complete smoking data (Supplementary Fig. S1). Our final analytic dataset included 7,937 PDAC cases and 11,774 controls (Supplementary Table S1).

GWAS genotype data

Genotyping was conducted in four phases, PanScan I, PanScan II, PanScan III, and PanC4. The PanScan studies were genotyped at the NCI Cancer Genomics Research Laboratory at the NIH (Bethesda, MD) and genotyped on the Illumina HumanHap series arrays [Illumina HumanHap550 Infinium II (8), Human 610-Quad (11) for PanScan I and II, respectively, and the Illumina Omni series arrays (OmniExpress, Omni1M, Omni2.5, and Omni5M) for PanScan III (12)]. PanC4 was genotyped on the Illumina HumanOmniExpressExome-8v1 array at the Johns Hopkins Center for Inherited Disease Research (9). The data from the PanScan and PanC4 GWAS are available through dbGAP (accession numbers phs000206.v5.p3 and phs000648.v1.p1, respectively). Details on imputation and quality controls have been described previously (10). In brief, SNPs were excluded if they had call rates ≤ 98%, MAF ≤ 0.05, and Hardy–Weinberg equilibrium *P values*, $< 1.0 \times 10^{-6}$ in the controls. SNPs were prephased using SHAPEIT2 software (25). Genotype imputation was conducted using IMPUTE2 (26) with 1000 genomes phase 3 (27). Imputation was conducted separately for the PanScan I/II, PanScan III, and PanC4 GWAS. After imputation, we retained only SNPs with imputation quality scores >0.5 and MAFs >0.05. Our final analysis included 6,769,447 variants.

Smoking and demographic assessment

Smoking status was assessed through self- or proxy-report via self-administered questionnaires (15) or in-person interviews (15, 16). Baseline smoking status was used for the cohort studies. For the case–control studies, smoking status at diagnosis (for cases) or when the questionnaire was administered (for controls) was used (16). For these analyses, smoking was categorized as never, former, and current cigarette smoker. Never smokers were individuals who smoked fewer than 100 cigarettes in their lifetime or for less than 6 months. Former smokers were individuals who reported quitting cigarette smoking > 1 year prior to the administration of the questionnaire. Current smokers were individuals who reported current smoking at the time of the questionnaire or who reported quitting cigarette smoking within the past year. Data on age, sex, and other possible confounders were collected from questionnaires at baseline from each cohort study and when smoking was assessed from the case–control studies (15, 16).

Statistical analyses

We used the unconstrained maximum likelihood (UML), constrained maximum likelihood (CML), and Empirical Bayes (EB) methods to evaluate the interaction between smoking status and each individual genetic variant (24). The UML corresponds to standard logistic regression analysis of binary outcome data (case–control), which allows the joint distribution of underlying covariates of the model to remain completely unspecified. The CML exploits an assumption of independence between SNP and smoking status in the underlying population (28) The method, similar to the case-only method (29), can gain in inferential efficiency of the interaction parameter and yet it can be used to test or estimate all of the parameters of an underlying logistic model, including the main effect of a SNP and the exposure of interest. The EB is an intermediate between the two methods and allows for a data-adaptive relaxation of the gene-environment independence assumption. Because the EB provides a good compromise between bias and variance (30, 31), we used this as the primary method for evaluating interaction and the other two methods for sensitivity analysis.

The association analysis was conducted using CGEN software (Version 3.5.0; https://dceg.cancer.gov/tools/analysis/cgen), an R package for logistic regression analyses of SNP-environment interactions (32), using the “snp.score” option to incorporate the genotype probabilities from the imputed data in the analysis. This option calculates a score test (JScore), which tests for the joint effect of SNP and SNP-by-environment interaction under a logistic regression model.

The analyses were first conducted separately for the PanScan and PanC4 datasets and results were combined using meta-analysis. Smoking was included as categorical dummy variable with never smokers as reference. Association magnitude of each SNP was modeled according to additive SNP dosage. Imputed SNPs were incorporated through expected dosage using the snp.score function of the CGEN package (32). Interaction between SNP and smoking was modeled using two parameters (current, never) and (former, never). Each SNP genotype was coded using an underlying dosage model, coded in terms of observed/impute allele counts. The analysis was adjusted for age in decade, sex, and top eigenvectors (five for PanScan and nine for PanC4) from principal component analysis to control for ancestry (10). PanScan analyses were also adjusted for study and geographic region of the parent studies (12). For each SNP, we obtained the one-step maximum likelihood estimate of SNP and SNP-smoking interaction effects along with the associated variance-covariance matrix from the SNP-score function (24). We implemented a fixed-effects meta-analysis using these summary statistical analysis. Meta-analysis was performed for the joint association magnitudes of the SNP and the SNP by smoking, and interaction effect only. We then performed 2 degree-of-freedom tests for SNP by smoking interaction terms of the model as a way of identifying SNPs/regions, the effect of which may be modified by smoking. In addition, we performed 3 degree-of-freedom joint tests (33) that simultaneously test for both the main effect of a SNP and two SNP by smoking interaction terms. We also conducted linkage disequilibrium (LD) clumping at an r^2 threshold of 0.05 to identify independent regions of association (34). Statistical tests were two sided and $P \le 5 \times 10^{-8}$ statistically significant.

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Expression quantitative trait locus analysis

Using the NIH Genotype-Tissue Expression (GTEx) v8 (dbGaP Accession phs000424.v8.p2, https://gtexportal.org/home/; ref. 35), we examined expression quantitative trait locus (eQTL) to assess the *cis* effects of the SNPs in the 2q21.3 region on gene expression across multiple tissues. In addition, we created regional plots of the 2q21.3 region using the SNP2GENE function of FUMA (36).

The GTEx Project was supported by the Common Fund of the Office of the Director of the NIH, and by NCI, NHGRI, NHLBI, NIDA, NIMH, and NINDS. The data used for the analyses described in this article were obtained from: (https://gtexportal.org/home/) the GTEx Project Portal on June 30, 2019 and July 30, 2020 and/or dbGaP accession number phs000424.vN.pN on June 30, 2019.

Colocalization analysis

For each SNP, we first meta-analyzed estimate of interaction parameters across current and former smokers to obtain a single estimate of SNP by smoking interaction under a dose–response model for smoking with never former and current coded as 0, 1, and 2, respectively. We used single statistics to summarize the evidence of interaction of individual SNPs with respect to smoking status in the colocalization analysis for the ease of interpretation of final results. Estimates of interaction separately by smoking categories indicate the dose–response model is adequate. To perform colocalization analysis, we matched the reference and alternate allele across our genome-wide interaction study and the eQTL results using GTEx v7. We performed colocalization analysis using two methods, co-loc and eCAVIAR (37, 38). For eCAVIAR, we investigated the locus by considering 500 kb upstream and downstream of the most significant SNP, rs1818613, from the genome-wide interaction scan. In addition, we chose genes with at least one significant variant and set the maximum number causal variants to 3.

Results

We found genome-wide significance ($P < 5 \times 10^{-8}$) evidence for interaction between smoking and multiple SNPs located in a region on chromosome 2q21.2 on pancreatic cancer risk. **Figure 1** shows the Q-Q and Manhattan plot associated with genome-wide test for interaction using the EB method. Compared with the theoretical distributions, the lambda value of 0.93 showed reasonable control of type-1 errors.

Supplementary Fig. S2 shows the Q-Q and Manhattan plots for the CML and UML methods. The most significant SNP in this region was rs1818613 ($P = 3.08 \times 10^{-9}$). The SNP also achieved genome-wide significance ($P = 2.7 \times 10^{-9}$) for interaction in CML and was strong but not at the genome-wide significance threshold using UML (**Table 1**).

More than 45 additional SNPs within the approximately 100 kb region of high LD ($r^2 > 0.9$) also showed evidence of interaction (2 degree-of-freedom interaction EB, $P < 5 \times 10^{-8}$; **Fig. 2**, ref. 36). This region is located in intron 5 of the transmembrane protein 163 gene (*TMEM163*) and 100 kb upstream of transcription factor cyclin T2 (*CCNT2*). The pattern of the association was similar across the GWAS phases and in both cohorts and case–control study designs (Supplementary Tables S2 and S3).

Three genomic regions, 14q22.1 (lead SNP, rs8003600), 1q12 (rs3765814 *OPN3* intronic), and 16q12.1 (rs78013006 *LOC105371082* intronic) showed suggestive evidence for interactions using the EB approach (2 degree-of-freedom interaction EB $P \le 5 \times 10^{-6}$; Supplementary Table S4).

Established GWAS regions

Overall, no significant interactions by smoking status were observed for any of the 20 previously identified GWAS SNPs for PDAC in European populations (8–12). One SNP (rs16986825 *ZNRF3* on

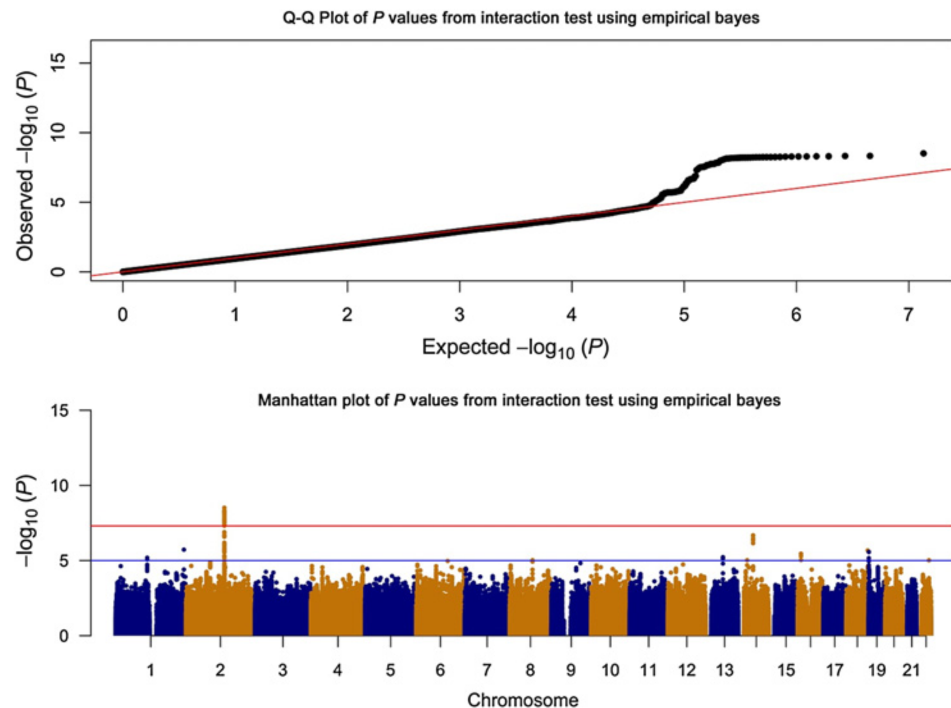

Figure 1.
Q-Q and Manhattan plots of interaction analysis using Empirical Bayes approach.

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Table 1. Region with genome-wide significant evidence for SNP by smoking interaction on risk of PDAC.

Chromosome Physical position SNP Ref/Effect alleles Ref Allele frequency Imputation quality Gene	Analytic Method	OR for rs1818613 (95% confidence interval) *P* Never smokers	 Former smokers	 Current smokers	$P_{Interaction}$[a]
2q21.3	CML	0.87	0.97	1.16	
135356285		(0.82–0.92)	(0.91–1.02)	(1.07–1.25)	2.7×10^{-9}
rs1818613		1.19×10^{-6}	0.24	3.3×10^{-4}	
G/T					
0.39	EB	0.87	1.00	1.25	
0.99		(0.82–0.93)	(0.93–1.07)	(1.12–1.40)	
TMEM163		0.001	0.94	1×10^{-4}	3.08×10^{-9}
(intronic)					
	UML	0.89	0.99	1.17	
		(0.84–0.96)	(0.92–1.06)	(1.08–1.28)	1.02×10^{-6}
		2.04×10^{-5}	0.74	2.6×10^{-4}	

Abbreviations: Physical position in Build 37: CML, constrained maximum likelihood; EB, Empirical Bayes; UML, unconstrained maximum likelihood.
[a]Based on two degrees of freedom χ^2 test. Analysis was adjusted for age, sex, ancestry (via principal components), and for PanScan study phase and site.

chr.22q12.1) had a $P = 0.005$ under the CML method (Supplementary Table S5).

eQTL and colocalization analysis

Examination of the GTEx8 data identified an eQLT (FDR < 0.05) in the same chromosome 2q21 region as our SNP-by-smoking interaction (**Fig. 2**; Supplementary Table S6). For rs1818613, under the additive model each copy of the T compared with the G allele was associated with increased expression of TMEM163 in heart atrial appendage ($\beta = 0.55$, $P = 1.2 \times 10^{-27}$), whole blood ($\beta = 0.40$, $P = 1.1 \times 10^{-21}$), esophagus muscularis ($\beta = 0.41$, $P = 7.0 \times 10^{-15}$) and pituitary ($\beta = 0.28$, $P = 1.8 \times 10^{-11}$) and decreased expression in testis ($\beta = -0.45$, $P = 3.1 \times 10^{-17}$) tissue. In addition, there was lower CCNT2 expression in tibial nerve tissue ($\beta = -0.13$, $P = 1.1 \times 10^{-9}$) and lung tissue ($\beta = -0.14$, $P = 1.5 \times 10^{-7}$) for the T allele compared with the G allele. At a lower significance, the T allele compared with the G allele of rs1818613 was also associated with expression in the brain, including higher expression of TMEM163 in the frontal cortex ($\beta = 0.21$, $P = 3.8 \times 10^{-6}$), CCNT2-AS1 in the cerebellar hemisphere ($\beta = 0.41$, $P = 2.3 \times 10^{-5}$) and cerebellum ($\beta = 0.35$, $P = 4.9 \times 10^{-5}$), and VDAC2P4 in the cerebellar hemisphere ($\beta = 0.38$ $P = 6.6 \times 10^{-5}$), and lower expression for *ZRANB3* in the hippocampus ($\beta = -0.30$, $P = 4.1 \times 10^{-5}$) and CCNT2 in the cortex ($\beta = -0.15$, $P = 6.1 \times 10^{-5}$).

Both co-loc and eCAVIAR colocalization analyses using GTEx 7 showed the posterior probability of a shared functional locus was extremely high. The most significant evidence was for rs842357, which is in strong LD with rs1818613 ($r^2 = 0.94$), and also had significant evidence of interaction with smoking (EB $P = 1.75 \times 10^{-08}$; **Fig. 3**).

The A compared with the G allele of rs842357 was associated with lower expression of TMEM163 in heart atrial appendage, tibial nerve, and stomach. The eCAVIAR posterior probability of a shared locus underlying both the SNP-by-smoking association and eQTL results for heart atrial appendage was 0.98. Additional evidence of colocalization (CLPP > 0.01) was observed for rs842357 and TMEM163 in brain anterior cingulate cortex BA24 and for CCNT2 in prostate, cells transformed fibroblasts, and small intestine terminal ileum (Supplementary Table S7). Interestingly, the A allele of rs843257 associated with decreased TMEM163 expression and increased CCNT2 expression compared with the T allele.

We used HaploReg (39) to inspect the region in LD ($r^2 > 0.8$) with rs842357 as the anchoring SNP, to identify variants altering transcription factor bindings (Supplementary Table S8). HaploReg includes a wide variety of functional annotations collected from Roadmap Epigenomics Project (40) as chromatin, DNase and promoter and enhancer histones marks. We observed H3K4me1 marks in this region, indicating this is an active enhancer region for tissues including pancreas as well as H3K4me3 marks in blood, heart, and thymus.

Discussion

We observed a qualitative interaction by cigarette smoking status for genetic variation and PDAC in a large LD block on chromosome 2 (2q21.3) in intron 5 of *TMEM163* and upstream of *CCNT2* such that alleles were associated with increased risk among current smokers and demonstrated a decreased risk among never smokers. The pattern of the interaction was consistent across three analytic methods and across study designs and individual GWAS. Our colocalization results support a single locus at the 2q21.3 region that underlies the interaction and an eQLT for TMEM163 and CCNT2 in this region. Given the qualitative nature of this interaction, it is not surprising we did not observe an association in this region when examining the main effect of SNPS in this region GWAS without modeling the interacting effect of smoking as the differing associations for smokers and nonsmokers would result in no overall association. To the best of our knowledge, this is the largest gene-by-smoking interaction study for PDAC conducted to date.

The *TMEM163* gene is conserved across many vertebrate species; it is highly expressed in specific brain regions and neuronal populations [glutamatergic and γ-aminobutyric acid (GABA)-ergic; ref. 41], and is modestly expressed in other tissues including the pancreas, pituitary, and testis (35). *TMEM*163 is a zinc binding and transporter protein involved in cellular zinc homeostasis and whose putative interaction with other zinc transporters and role in health and disease is not well understood (42). Zinc mediates a wide range of cellular processes and alternations in its homeostasis can disrupt cellular function (43).

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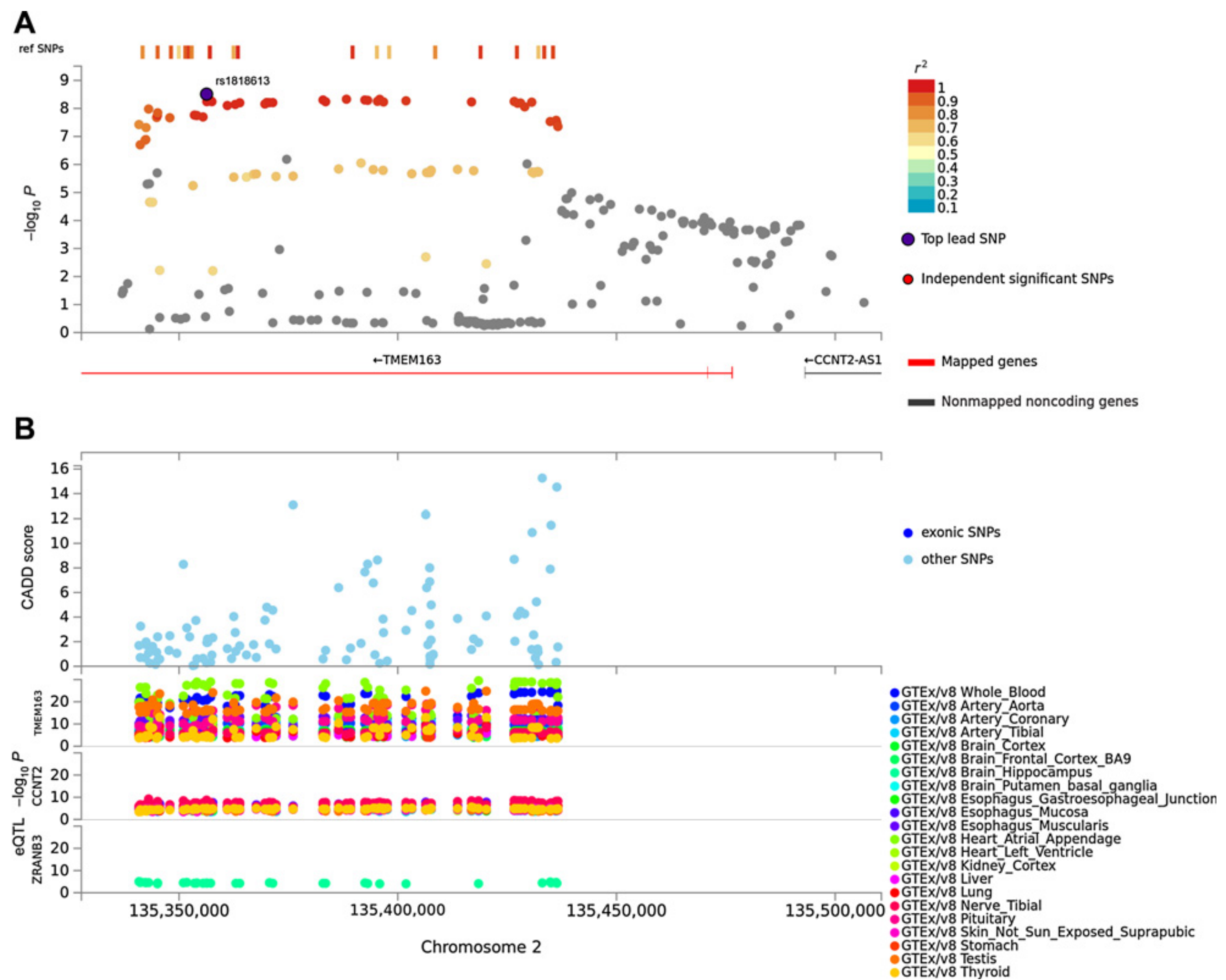

Figure 2.
Locus plot of 2q21.3 region for the interaction GWAS of pancreatic cancer by smoking using FUMA. **A,** Extended region of the *TMEM163* locus that prioritizes the *TMEM163* gene. **B,** Zoomed in regional plot of *TMEM163* locus with GWAS interaction *P* values (SNPs are colored on the basis of r^2), combined annotation dependent depletion (CADD score), and eQTL *P* value for the most significant associations. eQTLs are plotted per gene and colored on the basis of tissue types.

Dysregulation of other zinc transporters has been observed in PDAC such that zinc transporter upregulation has been associated with enhanced cancer cell migration and worse patient prognosis (43). Interestingly, a GWAS reported an association in the same 2q21.3 region in an intron of *TMEM163* gene for nicotine withdrawal in heavy smokers in a population-based Finnish Twin Cohort (19). Germline variation in 2q21 region has also been associated with Parkinson disease (44) and hematocrit concentrations (45) in populations of European ancestry and type 2 diabetes in Asian Indians (46) and a Mongolian population in China (47).

Any hypothesis regarding the underly mechanism of the observed interaction between genetic variation at the 2q21.3 region and cigarette smoking is speculative. Its qualitative nature suggests either a single mechanism that has protective effects in never smokers compared with adverse in current smokers or given the role of this region in regulating multiple proteins, the protective effect observed in never-smokers is overwhelmed by a second risk-increasing mechanism in the context of cigarette smoking. Colocalization of our association results with eQLT signals in TMEM163 and CCNT2 in several tissue types suggests gene regulation beyond the pancreatic gland may play a role. There was colocalization for the eQLT in TMEM163 in heart, nerve, and stomach. We observed high levels of H3K4me1 in this region for many tissues including pancreas, indicating this is an active enhancer region. Further work is needed to understand these findings. We also observed multiple eQTL signals in the brain and colocalization at a lower posterior probability for the brain anterior cingulate cortex, which functions in impulse control (48) and may play a role in nicotine dependence (49). This is in accord with the Finnish GWAS study, which linked variation in*TMEM163* to nicotine dependence (19) and might be contributing to our qualitative interaction and increased risk in smokers. Experimental studies have also shown CCNT2 to be differentially altered in the brain, especially the hypothalamus, in response to nicotine, nicotine withdrawal, and coinciding change in body weight (50). Future studies are needed to explore the 2q21.3 region in individuals with detailed smoking and other phenotype data as this may have utility for smoking cessation and cancer prevention. Metals found in cigarette smoke, such a cadmium, have been shown to compete with other zinc transporters (e.g., metallothionein and ZIP8)

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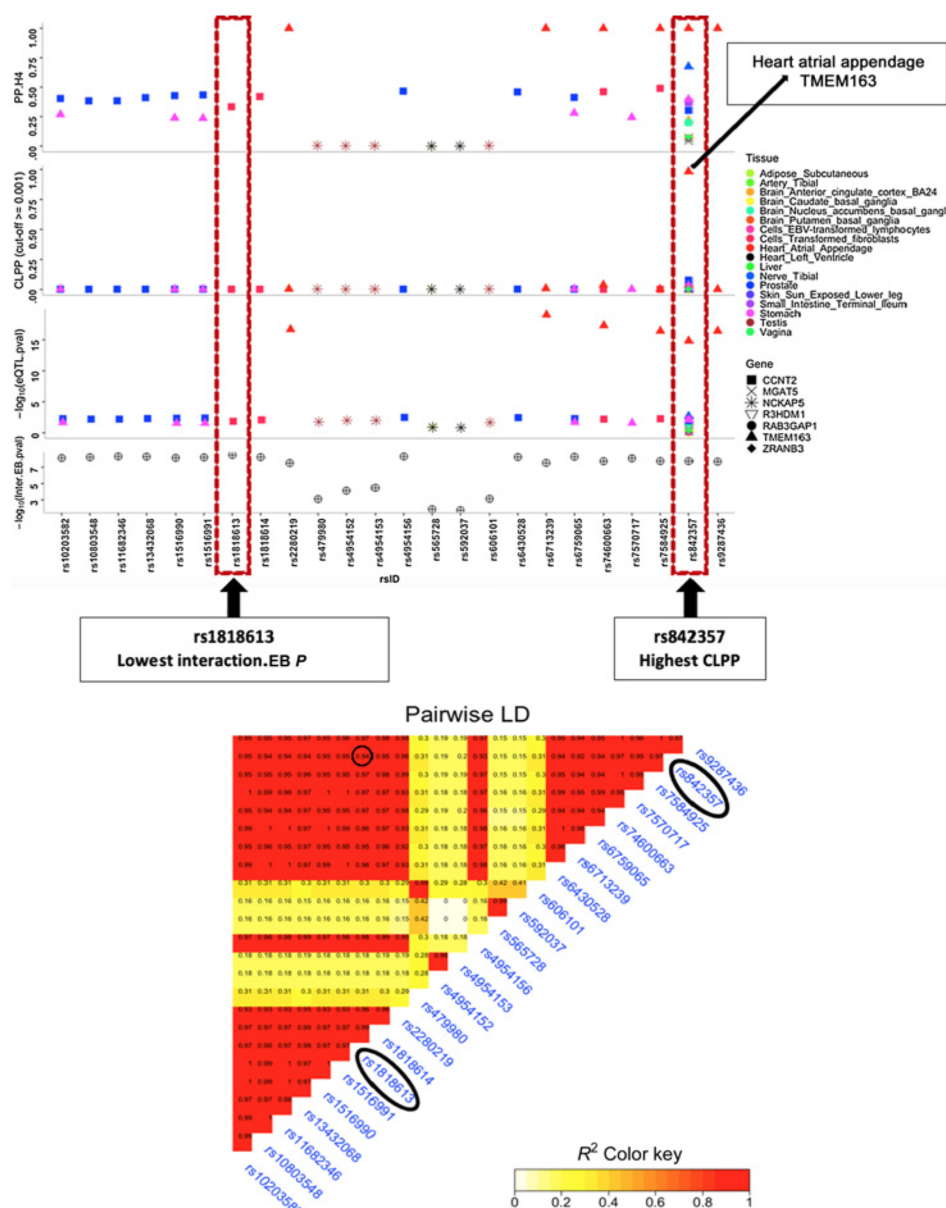

Figure 3.

Results of colocalization analysis using eCAVIAR and co-loc. SNPs with colocalization probability (CLPP) ≥ 0.001 are shown in this plot. PP.H4 denotes posterior probability of having a common causal SNP across eQTL and SNP X Smoking loci.

and increase chronic toxicity (51). It is possible a similar process could be contributing to the increased risk in smokers and qualitative interaction that we observe. Long-term cigarette smoke spreads smoke-related chemicals systemically in the bloodstream to target organs (52) and tobacco smoke inhalation causes pancreatic inflammation and damage to β-cells (53, 54). It is plausible that the interaction that we observe may be related to pancreatogenic disease processes in smokers (53, 55) that are not present in never smokers. Experimental studies are needed to explore these hypothesized mechanisms.

In conclusion, we identified a qualitative interaction for PDAC by cigarette smoking status at 2q21.3 in intron 5 of the *TMEM163* region. The colocalization results and eQTLs for *TMEM163* and *CCNT2* provides evidence of the importance of this gene region. Further studies are needed to replicate and understand the differential functional mechanisms by smoking status that contribute to our findings as these may have implications for cancer prevention.

Authors' Disclosures

No disclosures were reported.

Authors' Contributions

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