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Determination of shared genetic etiology and possible causal relations between tobacco smoking and depression

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Abstract

Backgrounds. Cigarette smoking is strongly associated with major depressive disorder (MDD). However, any genetic etiology of such comorbidity and causal relations is poorly understood, especially at the genome-wide level.

Methods. In the present *in silico* research, we analyzed summary data from the genome-wide association study of the Psychiatric Genetic Consortium for MDD ($n = 191\,005$) and UK Biobank for smoking ($n = 337\,030$) by using various biostatistical methods including Bayesian colocalization analysis, LD score regression, variant effect size correlation analysis, and Mendelian randomization (MR).

Results. By adopting a gene prioritization approach, we identified 43 genes shared by MDD and smoking, which were significantly enriched in membrane potential, gamma-aminobutyric acid receptor activity, and retrograde endocannabinoid signaling pathways, indicating that the comorbid mechanisms are involved in the neurotransmitter system. According to linkage disequilibrium score regression, we found a strong positive correlation between MDD and current smoking ($r_g = 0.365$; $p = 7.23 \times 10^{-25}$) and a negative correlation between MDD and former smoking ($r_g = -0.298$; $p = 1.59 \times 10^{-24}$). MR analysis suggested that genetic liability for depression increased smoking.

Conclusions. These findings inform the concomitant conditions of MDD and smoking and support the use of self-medication with smoking to counteract depression.

Introduction

The simultaneous occurrence of depression and smoking is a major public health concern. Substantial evidence supports a robust correlation between smoking and major depressive disorder (MDD), as well as the potential for bi-directional causal influences (Berlin & Covey, 2006; Hall & Prochaska, 2009; Liu et al., 2019; Payne, Ma, Crews, & Li, 2013; Zvolensky, Bakhshaie, Sheffer, Perez, & Goodwin, 2015). In addition to findings from epidemiological studies, genetic correlations between several smoking phenotypes and MDD have been observed in genetic studies (Liu et al., 2019; Wray et al., 2018). For example, Wray and colleagues reported the presence of significant positive genetic correlations between MDD and ever smoking (Wray et al., 2018). The most recent genome-wide association study (GWAS) on tobacco smoking and alcohol use revealed a negative genetic correlation for MDD with age at smoking initiation and positive genetic correlation between MDD and smoking initiation, cigarettes per day, and smoking cessation (Liu et al., 2019).

Genetic correlation can occur as a result of various processes. One common explanation is pleiotropy, which occurs when the same genes influence two or more traits or disorders. An alternative possibility is that two traits are causally related to each other. At least three hypotheses have been offered to explain the comorbidity between tobacco smoking and MDD. First, nicotine exposure and its metabolic process may influence neurobiological systems that have an etiologic role in MDD (Carmody, 1989). Second, MDD can lead to tobacco smoking via a self-medication process whereby tobacco smoking provides symptom relief for depression (Boden, Fergusson, & Horwood, 2010; Breslau, Peterson, Schultz, Chilcoat, & Andreski, 1998). Third, no causal relationship may exist, such that the observed correlation is attributable mainly to their shared genetic and environmental vulnerabilities (Breslau, Kilbey, & Andreski, 1993; Kendler et al., 1993). Given these options, it is important to elucidate the mechanisms of

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the comorbidity and determine its implications for the prevention and treatment of each disorder.

As is apparent from the literature, determination of the mechanisms underlying comorbidity between cigarette smoking and MDD has been inconsistent, in part influenced by differences in the intensity of smoking and the methods used in each research project (Johnson, Rhee, Chase, & Breslau, 2004; Payne et al., 2013). Evidence from longitudinal epidemiological studies suggests that both ever and current smoking contribute to depressed mood in adolescents (Goodman & Capitman, 2000; Wu & Anthony, 1999). On the other hand, there was no causal relation detected between MDD and daily smoking or nicotine dependence (Johnson et al., 2004; Windle & Windle, 2001). One approach to address such a confounding issue is to conduct an analysis with Mendelian randomization (MR), which enables the assessment of causal effects in observational datasets by employing genetic variants as instrument variables (Smith & Ebrahim, 2003). A recent report using this approach showed that lifetime smoking was a risk factor for MDD (Wootton et al., 2019); however, such findings have not yet been replicated.

In recent years, the availability of large-scale GWAS has yielded numerous new insights into the genetic architecture of both tobacco use and MDD (Li, 2018; Liu et al., 2019; Wray et al., 2018). These findings would help us to explore the shared genetic etiology and possible causal relations between tobacco smoking and MDD. To this end, in this study we first examined the pleiotropic effect for MDD and current/past tobacco smoking using publicly available GWAS summary statistics. Then we determined which biological processes or pathways were involved in such comorbidity. Finally, we used bidirectional MR methods to reveal the nature of the causal relationship between tobacco smoking and MDD.

Materials and methods

Data sources

MDD

We downloaded a combined meta-analysis from the Psychiatric Genetic Consortium (PGC) web site (https://www.med.unc.edu/pgc/results-and-downloads/mdd/) with a total of 130 664 major depression cases and 330 470 controls (online Supplementary Table S1) (Wray et al., 2018). The original report used 'major depression' to refer to cases that either meet the diagnostic criteria or alternative assessment methods. The 23andMe data (75 607 cases and 231 747 controls) were not available for downloading.

Tobacco smoking

The tobacco smoking data were obtained from UK Biobank, which displays self-reported smoking status. For the current study, we included current tobacco smoking (smoking on most or all days, smoking only occasionally, or not smoking, specified in field 1239) and past tobacco smoking (smoked on most or all days, smoked occasionally, tried just once or twice, or never smoked, specified in field 1249). We obtained the corresponding GWAS summary status from the LD hub (http://ldsc.broadinstitute.org/ldhub/), a centralized database of summary-level GWAS results gathered from publicly available sources (online Supplementary Table S1).

Assessment of shared association signals

To assess whether the MDD risk loci were significantly overrepresented in the genomic regions associated with smoking, we applied a custom variant set enrichment (VSE) analysis (Ahmed et al., 2017). By using genome-wide significant single nucleotide polymorphisms (SNPs) as tag variants for MDD, we obtained all associated variants within an LD block where each tag SNP is located. For each of these associated variant sets (AVSs), we computed its enrichment in genomic loci that show suggestive association signals ($p < 1 \times 10^{-5}$) for smoking phenotypes. We included two more datasets as negative controls: a GWAS of human height (Lango Allen et al., 2010) and a null GWAS dataset based on randomly distributed phenotypes. The magnitude of enrichment was estimated by random sampling of AVS 1000 times (denoted the matched random variant set; MRVS), and the overlap of MRVS with smoking-related loci was summarized. Finally, we calculated an exact p value for the significance of the enrichment by fitting a density function to the null distribution derived from the MRVS (Ahmed et al., 2017).

Identification of pleiotropic loci by Bayesian colocalization analysis

We aimed to identify genomic regions with a pleiotropic effect on both smoking and MDD. A Bayesian colocalization model was employed to estimate the posterior probability (PP) with the following hypotheses: (1) the region contains a genetic variant that influences traits A or B; (2) the region contains a genetic variant that influences both A and B; or (3) the region contains both a genetic variant that influences A and a separate genetic variant that influences B (Pickrell et al., 2016). Genomic regions were defined according to approximately independent LD blocks (Berisa & Pickrell, 2016). We inferred a genomic region where the presence of pleiotropism at the threshold of a PP was >0.9 for hypotheses 2 and 3.

Prioritization of common genes and functional enrichment analyses

The prioritization of common genes followed a three-step process. First, RefSeq-known genes within the GWAS overlap signals of MDD and colocalizing regions were annotated on a candidate genes list. Second, we used the GUILDify database (Guney, Garcia-Garcia, & Oliva, 2014) to retrieve phenotype-related genes (which served as a trained list) via keyword selection. Third, ToppGene software (Chen, Bardes, Aronow, & Jegga, 2009) was used to prioritize genes according to the candidate and trained lists simultaneously. The workflow of this part of the analysis is described in online Supplementary Fig. S1.

In the first step, we annotated 511 protein-coding genes. To obtain our trained list, we used the following keywords: neurotransmitters, serotonin, serotonergic system, serotonin-1A autoreceptors, 5-hydroxytryptamine (HT1A) receptor, serotonin transporter, glutamatergic transmission, glutamate reuptake, dopamine, noradrenergic and dopaminergic systems, hypothalamic–pituitary–adrenal axis, HPA axis, cortisol, corticosteroid receptor, glucocorticoid receptor, corticotropin-releasing hormone signaling system, neuroplasticity, brain-derived neurotrophic factor, nicotinic acetylcholine receptor (nAChR), serotonin receptor, gamma-aminobutyric acid (GABA) receptors, glutathione receptor signal, calcium signal, cAMP-mediated signal, mitogen-activated protein kinase (MAPK) signal, synaptic long-term potentiation, neurotrophin signal, tryptophan metabolism, interleukin (IL)-8 signal, cytochrome P450, CYP1A1, glutathione S-transferase Mu 1,

N-acetyltransferase, smoking, smoke, cigarette, tobacco use disorder, nicotine, nicotine dependence, addiction, long-term nicotine exposure, major depression, MDD, unipolar depression, and MDD. Only genes with a GUILDify score >0.1 remained on the final trained list. ToppGene was used to perform an annotation-based prioritization analysis by computing the semantic similarity between the candidate gene list and the trained list. The p value was estimated by random sampling of 5000 genes from the whole genome.

An overrepresentation enrichment of prioritized genes was implemented using the WebGestalt application (Zhang, Kirov, & Snoddy, 2005). For visualization of enrichment, we plotted $\log_2$ of the enrichment ratio and $-\log_{10}$ of the false discovery rate (FDR).

Genome-wide genetic correlation analysis by LD score regression

We used LD score regression (Bulik-Sullivan et al., 2015) to estimate genetic correlation (r_g) between MDD and smoking. The LD scores computed previously by the 1000 Genomes Projects reference panel of European ancestry were used to measure pair-wise LD r^2 among SNPs. Quality control steps were adopted from LD scores default procedures, including imputation quality >0.9 and minor allele frequency >0.1. Moreover, all SNPs retained for analysis were merged with SNPs in the HapMap 3 reference panel.

Inferring phenotype relations by variant effect size correlation

The rationale of this approach is as follows: if two traits are connected preliminarily, we would expect a correlated relation for the effect size of variants of the two phenotypes (Pickrell et al., 2016). Further, a simple determination of causal relations could be inferred according to the statement 'if a trait X causally influences trait Y, every risk variant for trait X should also influence trait Y.' However, the reverse is not true. Notably, when using observational data, strong statements about causality probably are impossible.

We first performed p value-informed LD pruning to obtain the independent GWAS SNPs ($p < 5 \times 10^{-8}$) for each trait. By scanning through all pairs of traits, we defined trait 1 as ascertainment, and extracted corresponding effect sizes [β or log(OR)] from trait 2. Then, the Spearman correlation was computed using the 'cor.test' R function.

Mendelian randomization

To identify potential causal effects for both smoking and depression, we performed bidirectional two-sample MR analysis using the TwoSampleMR (v. 0.4.22) R package (Hemani et al., 2018). The genetic instruments were identified using the following strategies. First, we filtered GWAS summary datasets to require genetic variants to be available in both smoking and depression. To obtain independent risk variants for each trait, we applied LD-based clumping for SNPs reaching genome-wide significance ($p < 5 \times 10^{-8}$) with an $r^2 < 0.05$ by PLINK (v. 1.07) (Purcell et al., 2007). The effect estimates and standard errors were extracted from relevant GWAS. Then, the common SNPs were harmonized using default parameters within the built-in 'harmonize data' function. After harmonization, the number of independent instrumental genetic variables was 360 for MDD, 24 for current smoking, and 85 for former smoking.

The inverse-variance weighted (IVW) method was proposed originally to average the causal estimates from each of the genetic variants (Burgess, Butterworth, & Thompson, 2013). Because of concern about the presence of pleiotropic elements for tobacco smoking and MDD, we employed a range of sensitivity analyses to assess the robustness of our findings when all the genetic variants are not valid instrumental variables (Bowden, Davey Smith, & Burgess, 2015; Burgess, Bowden, Fall, Ingelsson, & Thompson, 2017).

Results

Assessing known MDD risk loci in smoking behaviors

A previous large-scale meta-analysis of seven MDD cohorts identified 44 independent loci at a genome-wide significance level ($p < 5 \times 10^{-8}$) (Wray et al., 2018). We assessed the known MDD risk loci in two smoking-related traits available via the LD hub, which contained 832 summary-level GWAS results (Zheng et al., 2017). Of the 44 known MDD loci, 18 were nominally associated with at least one smoking phenotype (Fig. 1), including rs4869056 in *TENM2* at genome-wide significance for both current and former smoking (Table 1).

Further, we sought to determine whether the overlap of association signals was statistically significant. To this end, we implemented an enrichment test through the VSE R package (Ahmed et al., 2017). We found evidence of significant overrepresentation of MDD GWAS loci in the genomic regions that are suggestively linked to current and former smoking (Fig. 2). The significant overlap of association signals between MDD and smoking status suggests the presence of shared genetic etiologies in the two diseases.

Identification of pleiotropic loci that influence both tobacco smoking and MDD

Next, we aimed to identify genomic regions that contain shared causal variants for both tobacco smoking and MDD using a regional Bayesian colocalization test. For the analysis of MDD and current smoking, 12 regions were identified with a high PP, three of which were located in regions that are significantly or suggestively linked to smoking behaviors according to genome-wide linkage studies (online Supplementary Table S2) (Yang & Li, 2016). We identified lead SNPs in these regions that had genome-wide significance for current smoking (rs1549214, rs8033799, and rs7807019 $p < 5 \times 10^{-8}$) and suggestively significant associations with MDD ($p < 1 \times 10^{-5}$) (online Supplementary Table S2).

We also identified 17 genomic regions harboring causal variants common to both MDD and former smoking (online Supplementary Table S3). Among these, six regions were found in previously identified genome-wide linkage regions related to smoking (Yang & Li, 2016). A common signal was identified in *CTTNBP2*, which regulates postsynaptic excitatory synapse formation (Chen & Hsueh, 2012). The most strongly associated SNPs in this region showed suggestively significant ($p < 1 \times 10^{-5}$) associations with MDD (rs1548461; $p = 3.09 \times 10^{-6}$) and former smoking (rs10233018; $p = 1.28 \times 10^{-7}$), implying that it is unnecessary for SNPs in colocalizing regions to reach genome-wide significance for either trait.

Prioritization of common genes and functional enrichment analysis

We developed a functional annotation-based approach to prioritize common genes within colocalizing regions (online

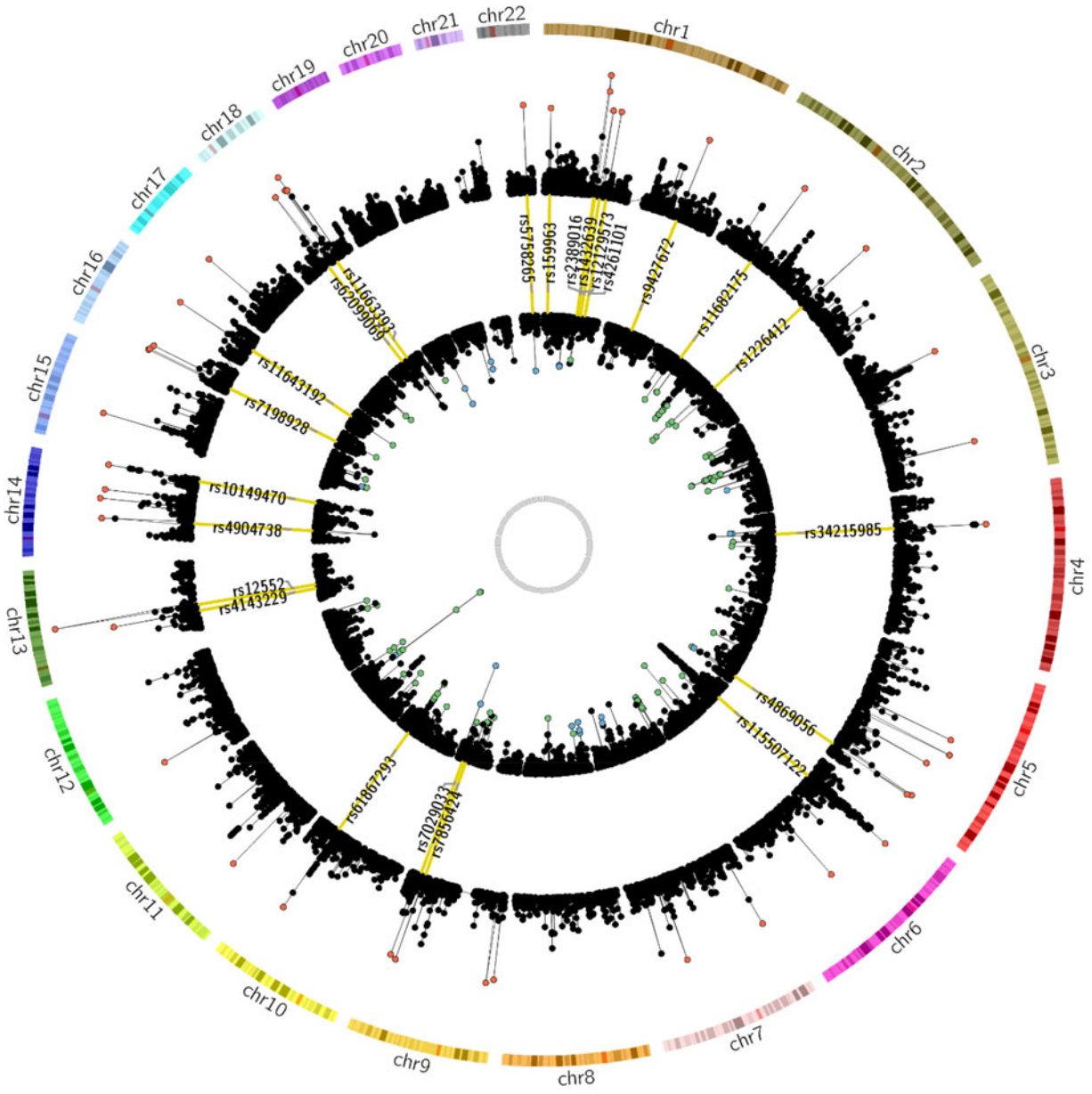

Fig. 1. Genetic susceptibility map for MDD and smoking behaviors. Outer ring defines the location of 22 human autosomes. Scatter plots in the second and fourth rings demonstrate analogy of the Manhattan plot for association results from MDD and smoking behaviors, respectively. The altitude of each dot represents statistical significance as $-\log_{10}(P)$. SNPs that reached genome-wide significance are colored red for MDD, blue for current tobacco smoking, and green for past tobacco smoking. Yellow bars in third ring mark 23 MDD risk loci at least nominally associated with smoking behaviors, and tag SNPs in these loci are labeled.

Supplementary Fig. S1). Briefly, a trained list containing 1162 genes associated significantly with pre-selected keywords was generated from the GUILDify database. We then performed prioritization analysis using ToppGene based on both the candidate genes list and the trained list. Finally, 43 functional genes were prioritized with FDR <0.05 (online Supplementary Table S4). The top ranked genes were: *AKT1*, *GABRB3*, *MAPK8*, *CXCL12*, *LDLRAP1*, *CD2AP*, *MAP3K7*, and *FGF8*.

Gene Ontology and KEGG pathway enrichment analyses were carried out for the prioritized common genes. We assessed the enrichment of functional terms using the Web-based tool WebGestalt, which revealed that regulation of membrane potential, GABA receptor activity, retrograde endocannabinoid signaling, GABA signaling pathway, positive regulation of ion transport, appendage development, nicotine addiction, threonine kinase signal, and ion channel binding were significantly overrepresented (Fig. 3). The number of enriched GO and KEGG terms associated with each common candidate gene was visualized by a chord plot (online Supplementary Fig. 2). It is worth noting that some prioritized genes were annotated into several enriched terms, such as *GABRB3*, *GABRA5*, *GABRG3*, *SOX11*, *SRI*, and *RIMS1*. These findings, taken together, show that the enrichments of common genes indicate the involvement of neural functions in the comorbidity of MDD and smoking.

Identification of phenotypic correlations using LDSC and correlated effect sizes

The overall patterns of genetic correlation (r_g) were estimated by two methods. At first, we used the LDSC method, which takes

Table 1. Significantly associated tag SNPs of major depression that reach nominal significances in smoking behaviors

SNP	MDD *p*	Past tob smk *p*	Current tob smk *p*	Gene context
rs10149470	***3.1 × 10^{-09}***	0.275	***0.012***	BAG5,4927; APOPT1, −11340
rs11643192	***3.4 × 10^{-08}***	***0.013***	***0.003***	PMFBP1, −7927; DHX3867465;
rs11663393	***1.6 × 10^{-08}***	0.132	***0.035***	(DCC); MIR4528, −148738
rs11682175	***4.7 × 10^{-09}***	***0.020838***	0.803	VRK2, −147192
rs12129573	***4 × 10^{-12}***	***4.5 × 10^{-05}***	***6.7 × 10^{-05}***	LINC01360, −3486
rs12552	***6.1 × 10^{-19}***	***1.4 × 10^{-04}***	0.532	(OLFM4); LINC0106580099
rs1432639	***4.6 × 10^{-15}***	***5.6 × 10^{-03}***	***0.002***	NEGR1, −64941
rs159963	***3.2 × 10^{-08}***	***9.4 × 10^{-03}***	***1.2 × 10^{-04}***	(RERE); SLC45A1100194
rs2389016	***1 × 10^{-08}***	***4.67 × 10^{-05}***	0.158	NULL
rs115507122	***3.3 × 10^{-11}***	***1.05 × 10^{-05}***	***0.003***	extended MHC
rs4143229	***2.5 × 10^{-08}***	0.810	***0.025***	(ENOX1); LACC1, −125620; CCDC12282689
rs4261101	***1 × 10^{-08}***	***0.002***	***0.003***	NULL
rs4869056	***6.8 × 10^{-09}***	***3.0 × 10^{-09}***	***1.0 × 10^{-09}***	(TENM2)
rs4904738	***2.6 × 10^{-09}***	***0.014***	0.094	(LRFN5)
rs5758265	***7.6 × 10^{-09}***	0.306	***1.3 × 10^{-06}***	NULL
rs61867293	***7 × 10^{-10}***	***0.048***	***0.001***	(SORCS3)
rs7198928	***1 × 10^{-08}***	0.097	***0.011***	(RBFOX1)
rs9427672	***3.1 × 10^{-08}***	***0.036***	***0.001***	DENND1B, −10 118

SNP, single nucleotide polymorphism; *p*, *p* value; MDD, major depression disorder; Current tob smk, Current tobacco smoking; Past tob smk, Past tobacco smoking.
p values less than 0.05 are given in Bold and italic. Gene context, distances between the Peak SNP and the closest genes are shown. Brackets indicate that the Peak SNP was within that gene. The closest genes upstream (taking strand into account, as a negative number indicating distance in bp between Peak SNP and the nearest gene boundary) and downstream (positive distance in bp) are also shown, if there is a flanking gene within 200 kb.

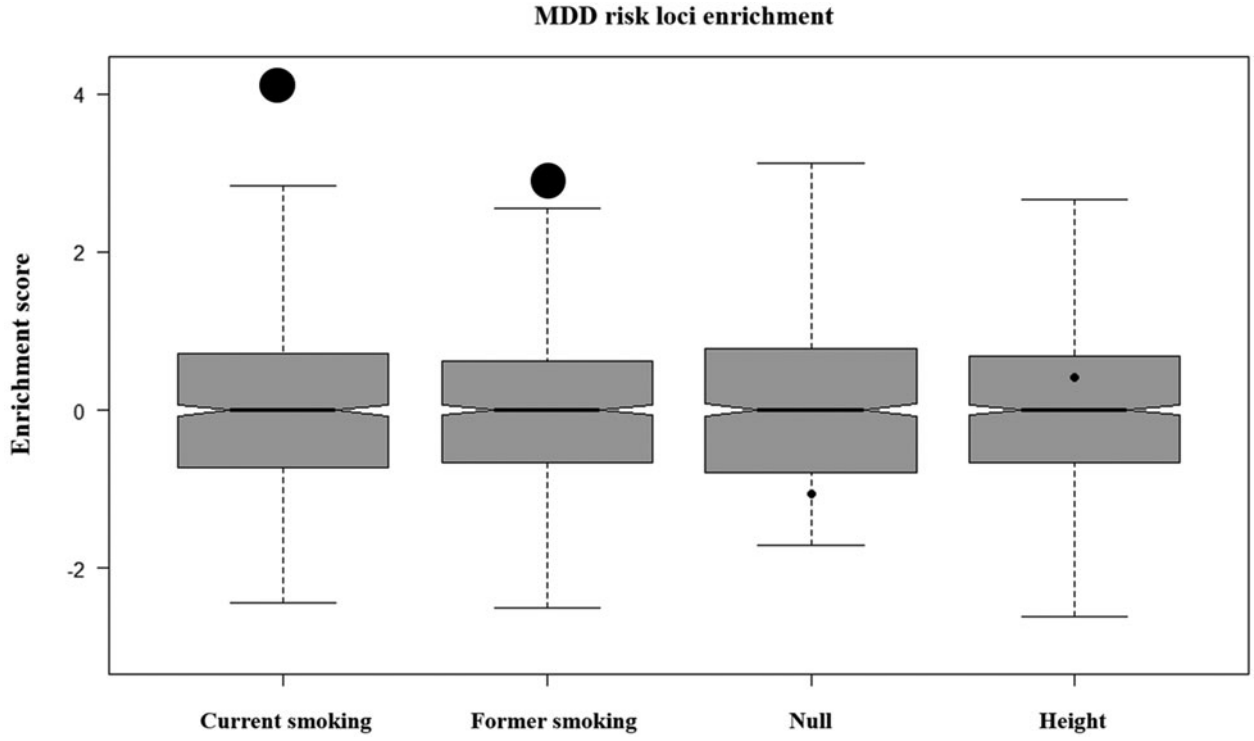

Fig. 2. Enrichment of MDD GWAS loci in smoking-related genomic regions ($p < 1 \times 10^{-5}$). Box and whisker plots show enrichment score distribution of the matched null set. Bar inside points to median score. Significant genome regions (Bonferroni-corrected $p < 0.01$) are in red.

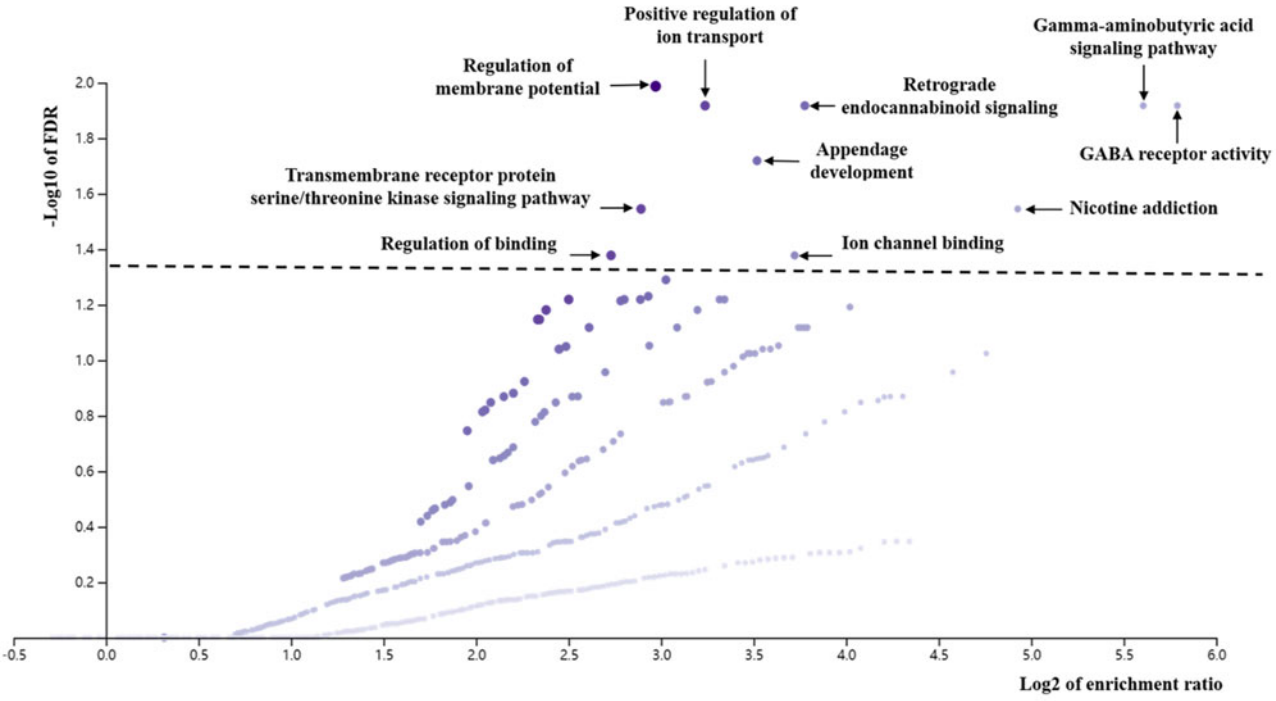

Fig. 3. Functional annotation of genes from common genomic regions. Each dot represents a GO term or a KEGG pathway. The red dashed line pinpoints the statistical threshold.

genome-wide SNPs into account. A strong positive correlation between MDD and current smoking ($r_g = 0.365$; $p = 7.23 \times 10^{-25}$) and a negative correlation between MDD and former smoking ($r_g = -0.2985$; $p = 1.59 \times 10^{-24}$) were observed (Fig. 4*a*). These significant genetic correlations again suggest a substantial overlap of genetic variants that predispose people/patients to both MDD and smoking.

If two traits share the same or similar molecular mechanisms, the effect size of a risk variant of the two phenotypes is expected to be correlated as well. As shown in Fig. 4*b*, genetic variants that increase the risk of MDD tended to increase the risk of current smoking ($\rho = 0.91$; $p = 6.76 \times 10^{-142}$). In contrast, a negative correlation between the variant effect size on MDD and former smoking was identified ($\rho = -0.093$; $p = 1.47 \times 10^{-164}$). We found that the directions of correlations for MDD with smoking status were consistent for genome-wide SNPs and GWAS-risk SNPs.

We observed asymmetry in the effect size correlations between MDD and smoking (Fig. 4*b*). Genetic variants ascertained as having effects on MDD had correlated effects on smoking phenotypes, whereas the reverse was not true. This suggests that a higher risk of MDD is a potential causal factor for smoking. However, the observation of asymmetry in the effect size could not account fully for causality given the lack of a randomized controlled trial (Pickrell et al., 2016).

Inferring casual relations by Mendelian randomization

Two-sample MR analysis using the IVW method yielded evidence that MDD increased the likelihood of current tobacco smoking (IVW: $\beta = 0.012$; $p < 0.0001$); however, the causal effect of MDD on past tobacco smoking was negative (IVW: $\beta = -0.054$; $p < 0.0001$) (Table 2). There was evidence of significant heterogeneity (online Supplementary Table S5), but MR Egger regression showed no evidence of directional pleiotropy (online Supplementary Table S6). Further, we performed several sensitivity analyses to assess the robustness of causal findings (Burgess et al., 2017). As shown in Table 2, the Egger regression method manifested a consistent effect direction from MDD to smoking, but the statistical significance became even weaker.

The causal effects of smoking status on the risk for MDD were null (Table 2). As expected, significant heterogeneities were observed, but the MR Egger intercepts indicated that the causal estimates were unbiased as a result of directional pleiotropy (online Supplementary Table S6).

Discussion

In this study, we first conducted a genome-wide overlap analysis for MDD and smoking using summary statistics from two large GWAS datasets. We revealed the genetic correlation and causal relationships between MDD and smoking, which present a comprehensive evaluation of shared genetic etiology. Very recently, Liu and colleagues published a large-scale GWAS study on several smoking phenotypes after we completed the analyses reported here (Liu et al., 2019). Interestingly, this newly published results were highly consistent with the findings reported here (online Supplementary Fig. S3).

This systematic approach helped identify genes and genetic mechanisms that confer comorbid effects. We identified tag SNPs significantly associated with MDD and smoking based on the overlap analysis and colocalization test; many of these reside in or near neurologically related genes. For example, variants in genes that encode the transmembrane protein and its receptor (*TENM2, SORCS3*) are significantly associated with MDD and smoking status. These genes are involved in neural development. *DCC* encodes the netrin 1 receptor, which directs axon growth and organizes neuronal connectivity by controlling target

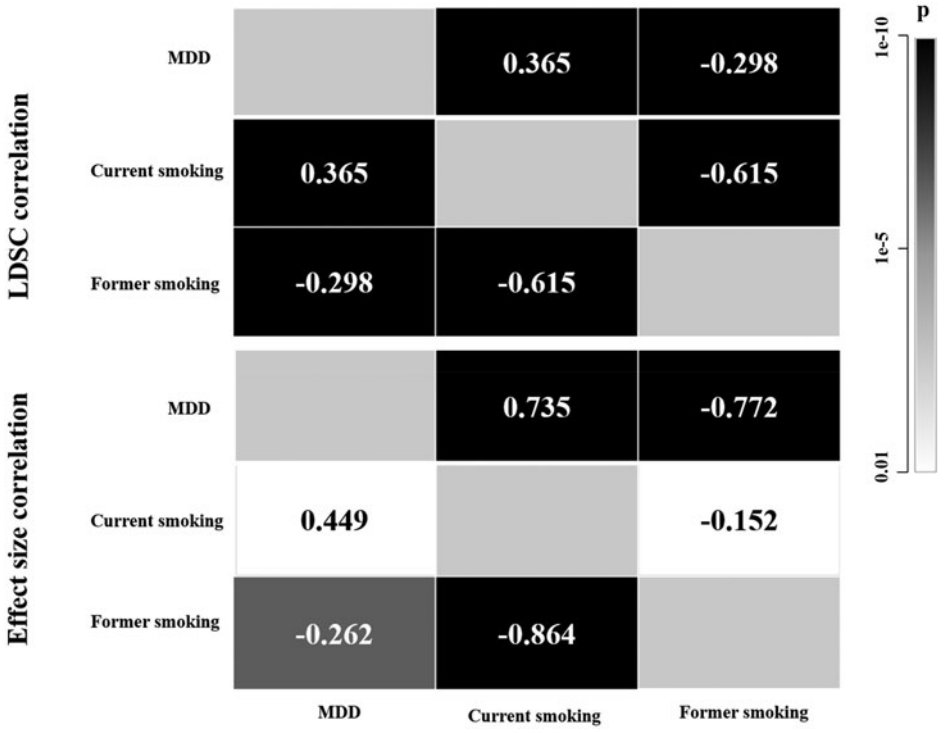

Fig. 4. Heatmap showing patterns of genetic correlations across MDD and smoking. Shown in color are all pairs in which this test had a *p* value <0.01. Darker colors indicate smaller *p* values, and color corresponds to the direction of correlation (red = positive; blue = negative).

Table 2. Two-sample MR analysis of the effect of major depression on smoking or vice versa

Effect	Exposure	Outcome	Method	No. SNPs	β	*p* value
MDD on smoking	MDD	Current tob smk	IVW	360	0.012	0.0001
			Weighted median	360	0.013	6.19×10^{-05}
			MR Egger	360	0.032	0.184
	MDD	Past tob smk	IVW	360	−0.054	0.0001
			Weighted median	360	−0.054	7.80×10^{-05}
			MR Egger	360	−0.208	0.045
Smoking on MDD	Current tob smk	MDD	IVW	24	−0.563	0.122
			Weighted median	24	−0.563	0.166
			MR Egger	24	0.899	0.575
	Past tob smk	MDD	IVW	85	0.097	0.147
			Weighted median	85	0.097	0.114
			MR Egger	85	−0.221	0.422

recognition, axon arborization, and synapse formation (Finci, Zhang, Meijers, & Wang, 2015). Neuronal growth regulator 1 (*NEGR1*) belongs to the immunoglobulin superfamily, suggesting a role in neuronal growth (Singh et al., 2018). Other examples are *NR4A2*, variations in which have been associated with disorders related to dopaminergic dysfunction; and *LRFN5*, which encodes a cell adhesion molecule that is highly expressed in the brain and promotes neurite outgrowth in hippocampal neurons.

Besides the significant findings in prioritizing common genes, we identified biological processes related to both depression and smoking. Regulation of both membrane potential and ion transport is involved in nerve impulse transmission and further controls the release and reception of neurotransmitter molecules. The increase or decrease of available neurotransmitters can change short-term synaptic status between enhancement and depression. Unequivocal studies suggest that dysfunction of synaptic plasticity with neuronal atrophy contributes to the pathophysiology of depression (Duman & Aghajanian, 2012; Duman, Aghajanian, Sanacora, & Krystal, 2016). The actions of nicotine in the brain are mediated by nAChRs, which regulate the release of a number of neurotransmitters, including glutamate and GABA (Wonnacott, 1997). A previous study found that repeated nicotine exposure decreases GABA release, which is mediated by $\alpha 6\beta 2$-containing nACh receptors located on presynaptic GABA neurons in the ventral tegmental area (Tang et al., 2011). The mechanism by which chronic nicotine exposure reduces GABAergic transmission could be alterations in $GABA_B$ receptor function or expression induced by nicotine (D'Souza & Markou,

2013). Further, it has been reported that N-methyl-D-aspartate receptors in GABAergic neurons could be blockaded by rapid-acting antidepressants (e.g. ketamine and scopolamine) (Chen, Ma, Fan, Yang, & Li, 2018; Gerhard, Wohleb, & Duman, 2016). These findings demonstrate the role of neural circuits and communications in the common molecular mechanisms of depression and smoking.

One of the significantly enriched KEGG pathways is retrograde endocannabinoid (eCB) signaling. Endocannabinoids also are key mediators of synaptic plasticity (Castillo, Younts, Chavez, & Hashimotodani, 2012). The molecular basis of eCBs regulating synaptic function is retrograde signaling in the central nervous system (Kano, Ohno-Shosaku, Hashimotodani, Uchigashima, & Watanabe, 2009). The activity of postsynaptic neurons produces eCB, which moves backward across the synapse, binds to presynaptic CB1Rs, and suppresses neurotransmitter release (Castillo et al., 2012). Multiple lines of evidence show dysregulation of the eCB system in neuropsychiatric conditions such as depression and addiction (Hillard, Weinlander, & Stuhr, 2012). Specifically, people with chronic stress or depression have lower eCB concentrations, implying impairment in endocannabinoid signaling in depressed individuals (Gorzalka & Hill, 2011). Further, animal studies have shown that central endocannabinoid signaling is reduced in several stress-based models of depression (Reich, Taylor, & McCarthy, 2009). Following the rationale above, several studies have revealed the antidepressant potential of the endocannabinoid system (Leite, Mocelin, Petersen, Leal, & Thiesen, 2009). According to these investigations, we hypothesized that the comorbidity observed between major depression and smoking is the treatment-related impact of nicotine, similar to the actions of eCBs.

The overall genetic correlations between depression and smoking have been observed previously (Bulik-Sullivan et al., 2015; Wray et al., 2018). Significant positive correlations were observed between MDD and ever/never smoking, which is consistent with epidemiological association studies. Further, the genetic architecture of major depression was negatively correlated with smoking cessation, indicating persons who were able to quit smoking have a lower risk of suffering from depression. The recently published and largest GWAS study of tobacco use, examining 1.2 million individuals, reported comprehensive genetic correlation profiles of depression and smoking (Liu et al., 2019), including significant positive genetic correlations of major depression with smoking initiation and smoking cessation.

For the current study, the genetic correlation between MDD and smoking is of interest. First, a positive correlation between major depression and current smoking indicates that genetic risk factors for current tobacco use and depression are positively correlated. These findings are in line with previous epidemiological observations that depressive symptoms and smoking have a high incidence of co-occurrence (Hu, Davies, & Kandel, 2006; Lasser et al., 2000). On the other hand, we found a significant negative correlation between MDD and former smoking, which enhanced findings by Wray et al. (2018). These findings suggest that smokers with a negative history for MDD are more likely to quit.

Wootton et al. (2019) demonstrated the causal relation between lifetime smoking and depression by using a comprehensive MR analysis. They found strong evidence that smoking is a risk factor for depression and also suggested that genetic liability for depression increases lifetime smoking likelihood. There are slight inconsistencies between the current study and Wootton findings because of the divergence in phenotype definition and genetic instruments employed. When tobacco smoking was considered as exposure, Wootton and colleagues combined various tobacco smoking measures into a lifetime smoking index, which represents a much broader definition of tobacco smoking than that used in this study. On the other hand, when depression was considered as exposure, both studies yielded the same conclusion, supporting the self-medication hypothesis.

The clinical implications that stem from these findings emphasize the importance of appropriate management of depression to facilitate successful tobacco dependence treatment. A substantial body of research points to the negative relation between depressive symptoms/disorders and successful quitting. Many factors likely play a role, including the direct effects of the genetic overlap examined in this study, as well of other important behavioral change mechanisms, such as the short-term reward/antidepressant effects of nicotine intake, value as a coping response, and degree of motivation to attempt quitting (Cohen & Lichtenstein, 1990; Hitsman, Borrelli, McChargue, Spring, & Niaura, 2003; Kopetz, MacPherson, Mitchell, Houston-Ludlam, & Wiers, 2017; Piper et al., 2017; Tulloch, Pipe, Clyde, Reid, & Els, 2016). The importance of pharmacologic choices in treating depressed smokers comes into play, supported by research indicating bupropion hydrochloride is of particular value in smokers who present with depressive symptoms, as well as recent provocative evidence that combining varenicline with antidepressants or nicotine replacement therapies may be useful (Ebbert, Burke, Hays, & Hurt, 2009; Ebbert et al., 2014; Issa, Abe, Moura, Santos, & Pereira, 2013; Koegelenberg et al., 2014). It has been suggested that when such individuals quit smoking, if the antidepressant regimen is not adequate, a likelihood of worsened depressive symptoms is observed. Finally, the fact that the metabolism of many psychiatric medications, including antidepressants (TCAs, SSRIs), is enhanced by smoking (via the ingestion of polycyclic aromatic hydrocarbons, which increase the expression of CYP P450 1A2), suggesting the importance of adjusting medication dosing. However, this explanation is not without problems, given evidence that sole use of antidepressants other than bupropion HCL have not been found to be effective in treating tobacco dependence (Shoaib & Buhidma, 2018). Further research is clearly needed to understand the complexity of this consideration.

There are a number of limitations to the current research that should be mentioned. First, because of the concerns on sample size, accuracy, and availability in the UK Biobank dataset, we only analyzed smoking status in this study and did not consider other smoking phenotypes such as the Fagerstrom test for nicotine dependence (FTND) and cigarettes per day (CPD). It is important to consider those quantitative measures on smoking such as FTND and CPD in future investigations as the relationship between smoking and depression depends on the severity of smoking (Payne et al., 2013). Second, there is a stark difference in the sample size among smoking phenotypes, which might contribute to discrepancies in statistical power. Thus, the main conclusions of this study were drawn on the basis of two relatively similar datasets: current smokers and former smokers. Third, the LD score regression analysis hinted at a minute genetic covariance intercept, indicating a small sample overlap between MDD and smoking. Although the genetic correlation estimations were not biased by sample overlap, the summary-based MR analysis would suffer from it. Fourth, information available on the summary-level GWAS data limited us to divide samples into

subgroups, which prevented us from studying the age-related heterogeneity.

In conclusion, this was a systematic analysis of the shared etiology and possible causal relations of smoking and depression employing large-scale GWASs. We found significant genetic overlap and correlations between MDD and smoking at both the SNP and gene level. Importantly, we identified potential comorbid molecular pathways enriched in the neurotransmitter system. The MR analysis suggests smoking behavior is a consequence of depression rather than a cause, supporting the role of self-medication for depressive symptoms. Overall, this study enhances the understanding of the genetic etiology of the relationship between depression and smoking.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S003329172000063X

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References

Ahmed, M., Sallari, R. C., Guo, H., Moore, J. H., He, H. H., & Lupien, M. (2017). Variant set enrichment: An R package to identify disease-associated functional genomic regions. *BioData Mining*, *10*, 9. doi: 10.1186/s13040-017-0129-5.

Berisa, T., & Pickrell, J. K. (2016). Approximately independent linkage disequilibrium blocks in human populations. *Bioinformatics (Oxford, England)*, *32*(2), 283–285. doi: 10.1093/bioinformatics/btv546.

Berlin, I., & Covey, L. S. (2006). Pre-cessation depressive mood predicts failure to quit smoking: The role of coping and personality traits. *Addiction*, *101*(12), 1814–1821. doi: 10.1111/j.1360-0443.2006.01616.x.

Boden, J. M., Fergusson, D. M., & Horwood, L. J. (2010). Cigarette smoking and depression: Tests of causal linkages using a longitudinal birth cohort. *British Journal of Psychiatry*, *196*(6), 440–446. doi: 10.1192/bjp.bp.109.065912.

Bowden, J., Davey Smith, G., & Burgess, S. (2015). Mendelian randomization with invalid instruments: Effect estimation and bias detection through Egger regression. *International Journal of Epidemiology*, *44*(2), 512–525. doi: 10.1093/ije/dyv080.

Breslau, N., Kilbey, M. M., & Andreski, P. (1993). Nicotine dependence and major depression. New evidence from a prospective investigation. *Archives of General Psychiatry*, *50*(1), 31–35. doi: 10.1001/archpsyc.1993.01820130033006.

Breslau, N., Peterson, E. L., Schultz, L. R., Chilcoat, H. D., & Andreski, P. (1998). Major depression and stages of smoking. A longitudinal investigation. *Archives of General Osychiatry*, *55*(2), 161–166. doi: 10.1001/archpsyc.55.2.161.

Bulik-Sullivan, B., Finucane, H. K., Anttila, V., Gusev, A., Day, F. R., Loh, P. R., … Neale, B. M. (2015). An atlas of genetic correlations across human diseases and traits. *Nature Genetics*, *47*(11), 1236–1241. doi: 10.1038/ng.3406.

Burgess, S., Bowden, J., Fall, T., Ingelsson, E., & Thompson, S. G. (2017). Sensitivity analyses for robust causal inference from mendelian randomization analyses with multiple genetic variants. *Epidemiology (Cambridge, Mass.)*, *28*(1), 30–42. doi: 10.1097/EDE.0000000000000559.

Burgess, S., Butterworth, A., & Thompson, S. G. (2013). Mendelian randomization analysis with multiple genetic variants using summarized data. *Genetic Epidemiology*, *37*(7), 658–665. doi: 10.1002/gepi.21758.

Carmody, T. P. (1989). Affect regulation, nicotine addiction, and smoking cessation. *Journal of Psychoactive Drugs*, *21*(3), 331–342. doi: 10.1080/02791072.1989.10472175.

Castillo, P. E., Younts, T. J., Chavez, A. E., & Hashimotodani, Y. (2012). Endocannabinoid signaling and synaptic function. *Neuron*, *76*(1), 70–81. doi: 10.1016/j.neuron.2012.09.020.

Chen, J., Bardes, E. E., Aronow, B. J., & Jegga, A. G. (2009). Toppgene Suite for gene list enrichment analysis and candidate gene prioritization. *Nucleic Acids Research*, *37*(Web Server issue), W305–W311. doi: 10.1093/nar/gkp427.

Chen, J., Ma, Y., Fan, R., Yang, Z., & Li, M. D. (2018). Implication of genes for the N-Methyl-D-Aspartate (NMDA) receptor in substance addictions. *Molecular Neurobiology*, *55*(9), 7567–7578. doi: 10.1007/s12035-018-0877-3.

Chen, Y. K., & Hsueh, Y. P. (2012). Cortactin-binding protein 2 modulates the mobility of cortactin and regulates dendritic spine formation and maintenance. *Journal of Neuroscience*, *32*(3), 1043–1055. doi: 10.1523/JNEUROSCI.4405-11.2012.

Cohen, S., & Lichtenstein, E. (1990). Perceived stress, quitting smoking, and smoking relapse. *Health Psychology*, *9*(4), 466–478. doi: 10.1037//0278-6133.9.4.466.

D'Souza, M. S., & Markou, A. (2013). The "stop" and "go" of nicotine dependence: Role of GABA and glutamate. *Cold Spring Harbor Perspectives in Medicine*, *3*(6), 1–18. doi: 10.1101/cshperspect.a012146.

Duman, R. S., & Aghajanian, G. K. (2012). Synaptic dysfunction in depression: Potential therapeutic targets. *Science (New York, N.Y.)*, *338*(6103), 68–72. doi: 10.1126/science.1222939.

Duman, R. S., Aghajanian, G. K., Sanacora, G., & Krystal, J. H. (2016). Synaptic plasticity and depression: New insights from stress and rapid-acting antidepressants. *Nature Medicine*, *22*(3), 238–249. doi: 10.1038/nm.4050.

Ebbert, J. O., Burke, M. V., Hays, J. T., & Hurt, R. D. (2009). Combination treatment with varenicline and nicotine replacement therapy. *Nicotine & Tobacco Research*, *11*(5), 572–576. doi: 10.1093/ntr/ntp042.

Ebbert, J. O., Hatsukami, D. K., Croghan, I. T., Schroeder, D. R., Allen, S. S., Hays, J. T., & Hurt, R. D. (2014). Combination varenicline and bupropion SR for tobacco-dependence treatment in cigarette smokers: A randomized trial. *JAMA*, *311*(2), 155–163. doi: 10.1001/jama.2013.283185.

Finci, L., Zhang, Y., Meijers, R., & Wang, J. H. (2015). Signaling mechanism of the netrin-1 receptor DCC in axon guidance. *Progress in Biophysics & Molecular Biology*, *118*(3), 153–160. doi: 10.1016/j.pbiomolbio.2015.04.001.

Gerhard, D. M., Wohleb, E. S., & Duman, R. S. (2016). Emerging treatment mechanisms for depression: Focus on glutamate and synaptic plasticity. *Drug Discovery Today*, *21*(3), 454–464. doi: 10.1016/j.drudis.2016.01.016.

Goodman, E., & Capitman, J. (2000). Depressive symptoms and cigarette smoking among teens. *Pediatrics*, *106*(4), 748–755. doi: 10.1542/peds.106.4.748.

Gorzalka, B. B., & Hill, M. N. (2011). Putative role of endocannabinoid signaling in the etiology of depression and actions of antidepressants. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *35*(7), 1575–1585. doi: 10.1016/j.pnpbp.2010.11.021.

Guney, E., Garcia-Garcia, J., & Oliva, B. (2014). GUILDify: A web server for phenotypic characterization of genes through biological data integration and network-based prioritization algorithms. *Bioinformatics (Oxford, England)*, *30*(12), 1789–1790. doi: 10.1093/bioinformatics/btu092.

Hall, S. M., & Prochaska, J. J. (2009). Treatment of smokers with co-occurring disorders: Emphasis on integration in mental health and addiction treatment settings. *Annual Review of Clinical Psychology*, *5*, 409–431. doi: 10.1146/annurev.clinpsy.032408.153614.

Hemani, G., Zheng, J., Elsworth, B., Wade, K. H., Haberland, V., Baird, D., … Haycock, P. C. (2018). The MR-Base platform supports systematic causal inference across the human phenome. *Elife*, *7*, e34408. doi: 10.7554/eLife.34408.

Hillard, C. J., Weinlander, K. M., & Stuhr, K. L. (2012). Contributions of endocannabinoid signaling to psychiatric disorders in humans: Genetic and biochemical evidence. *Neuroscience*, *204*, 207–229. doi: 10.1016/j.neuroscience.2011.11.020.

Hitsman, B., Borrelli, B., McChargue, D. E., Spring, B., & Niaura, R. (2003). History of depression and smoking cessation outcome: A meta-analysis. *Journal of Consulting and Clinical Psychology*, *71*(4), 657–663. doi: 10.1037/0022-006x.71.4.657.

Hu, M. C., Davies, M., & Kandel, D. B. (2006). Epidemiology and correlates of daily smoking and nicotine dependence among young adults in the United States. *American Journal of Public Health*, *96*(2), 299–308. doi: 10.2105/AJPH.2004.057232.

Issa, J. S., Abe, T. O., Moura, S., Santos, P. C., & Pereira, A. C. (2013). Effectiveness of coadministration of varenicline, bupropion, and serotonin reuptake inhibitors in a smoking cessation program in the real-life setting. *Nicotine & Tobacco Research*, *15*(6), 1146–1150. doi: 10.1093/ntr/nts230.

Johnson, E. O., Rhee, S. H., Chase, G. A., & Breslau, N. (2004). Comorbidity of depression with levels of smoking: An exploration of the shared familial risk hypothesis. *Nicotine & Tobacco Research*, *6*(6), 1029–1038. doi: 10.1080/14622200412331324901.

Kano, M., Ohno-Shosaku, T., Hashimotodani, Y., Uchigashima, M., & Watanabe, M. (2009). Endocannabinoid-mediated control of synaptic transmission. *Physiological Reviews*, *89*(1), 309–380. doi: 10.1152/physrev.00019.2008.

Kendler, K. S., Neale, M. C., MacLean, C. J., Heath, A. C., Eaves, L. J., & Kessler, R. C. (1993). Smoking and major depression. A causal analysis. *Archives of General Psychiatry*, *50*(1), 36–43. doi: 10.1001/archpsyc.1993.01820130038007.

Koegelenberg, C. F., Noor, F., Bateman, E. D., van Zyl-Smit, R. N., Bruning, A., O'Brien, J. A., … Irusen, E. M. (2014). Efficacy of varenicline combined with nicotine replacement therapy vs varenicline alone for smoking cessation: A randomized clinical trial. *JAMA*, *312*(2), 155–161. doi: 10.1001/jama.2014.7195.

Kopetz, C., MacPherson, L., Mitchell, A. D., Houston-Ludlam, A. N., & Wiers, R. W. (2017). A novel training approach to activate alternative behaviors for smoking in depressed smokers. *Experimental and Clinical Psychopharmacology*, *25*(1), 50–60. doi: 10.1037/pha0000108.

Lango Allen, H., Estrada, K., Lettre, G., Berndt, S. I., Weedon, M. N., Rivadeneira, F., … Hirschhorn, J. N. (2010). Hundreds of variants clustered in genomic loci and biological pathways affect human height. *Nature*, *467*(7317), 832–838. doi: 10.1038/nature09410.

Lasser, K., Boyd, J. W., Woolhandler, S., Himmelstein, D. U., McCormick, D., & Bor, D. H. (2000). Smoking and mental illness: A population-based prevalence study. *JAMA*, *284*(20), 2606–2610. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/11086367.

Leite, C. E., Mocelin, C. A., Petersen, G. O., Leal, M. B., & Thiesen, F. V. (2009). Rimonabant: An antagonist drug of the endocannabinoid system for the treatment of obesity. *Pharmacological Reports*, *61*(2), 217–224. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/19443932.

Li, M. D. (2018). *Tobacco smoking addiction: Epdemiology, genetics, mechanisms, and treatment*. Singapore: Springer.

Liu, M., Jiang, Y., Wedow, R., Li, Y., Brazel, D. M., Chen, F., … Vrieze, S. (2019). Association studies of up to 1.2 million individuals yield new insights into the genetic etiology of tobacco and alcohol use. *Nature Genetics*, *51*(2), 237–244. doi: 10.1038/s41588-018-0307-5.

Payne, T. J., Ma, J. Z., Crews, K. M., & Li, M. D. (2013). Depressive symptoms among heavy cigarette smokers: The influence of daily rate, gender, and race. *Nicotine & Tobacco Research*, *15*(10), 1714–1721. doi: 10.1093/ntr/ntt047.

Pickrell, J. K., Berisa, T., Liu, J. Z., Segurel, L., Tung, J. Y., & Hinds, D. A. (2016). Detection and interpretation of shared genetic influences on 42 human traits. *Nature Genetics*, *48*(7), 709–717. doi: 10.1038/ng.3570.

Piper, M. E., Schlam, T. R., Cook, J. W., Smith, S. S., Bolt, D. M., Loh, W. Y., … Baker, T. B. (2017). Toward precision smoking cessation treatment I: Moderator results from a factorial experiment. *Drug and Alcohol Dependence*, *171*, 59–65. doi: 10.1016/j.drugalcdep.2016.11.025.

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M. A., Bender, D., … Sham, P. C. (2007). PLINK: A tool set for whole-genome association and population-based linkage analyses. *American Journal of Human Genetics*, *81*(3), 559–575. doi: S0002-9297(07)61352-4 [pii] 10.1086/519795.

Reich, C. G., Taylor, M. E., & McCarthy, M. M. (2009). Differential effects of chronic unpredictable stress on hippocampal CB1 receptors in male and female rats. *Behavioural Brain Research*, *203*(2), 264–269. doi: 10.1016/j.bbr.2009.05.013.

Shoaib, M., & Buhidma, Y. (2018). Why are antidepressant drugs effective smoking cessation aids? *Current Neuropharmacology*, *16*(4), 426–437. doi: 10.2174/1570159X15666170915142122.

Singh, K., Loreth, D., Pottker, B., Hefti, K., Innos, J., Schwald, K., … Schafer, M. K. E. (2018). Neuronal growth and behavioral alterations in mice deficient for the psychiatric disease-associated Negr1 gene. *Frontiers in Molecular Neuroscience*, *11*, 30. doi: 10.3389/fnmol.2018.00030.

Smith, G. D., & Ebrahim, S. (2003). 'Mendelian randomization': Can genetic epidemiology contribute to understanding environmental determinants of disease? *International Journal of Epidemiology*, *32*(1), 1–22. doi: 10.1093/ije/dyg070.

Tang, A. H., Karson, M. A., Nagode, D. A., McIntosh, J. M., Uebele, V. N., Renger, J. J., … Alger, B. E. (2011). Nerve terminal nicotinic acetylcholine receptors initiate quantal GABA release from perisomatic interneurons by activating axonal T-type (Cav3) Ca(2)(+) channels and Ca(2)(+) release from stores. *Journal of Neuroscience*, *31*(38), 13546–13561. doi: 10.1523/JNEUROSCI.2781-11.2011.

Tulloch, H. E., Pipe, A. L., Clyde, M. J., Reid, R. D., & Els, C. (2016). The quit experience and concerns of smokers with psychiatric illness. *American Journal of Preventive Medicine*, *50*(6), 709–718. doi: 10.1016/j.amepre.2015.11.006.

Windle, M., & Windle, R. C. (2001). Depressive symptoms and cigarette smoking among middle adolescents: Prospective associations and intrapersonal and interpersonal influences. *Journal of Consulting and Clinical Psychology*, *69*(2), 215–226. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/11393599.

Wonnacott, S. (1997). Presynaptic nicotinic ACh receptors. *Trends in Neurosciences*, *20*(2), 92–98. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/9023878.

Wootton, R. E., Richmond, R. C., Stuijfzand, B. G., Lawn, R. B., Sallis, H. M., Taylor, G. M. J., … Munafo, M. R. (2019). Evidence for causal effects of lifetime smoking on risk for depression and schizophrenia: A Mendelian randomisation study. *Psychological Medicine*, 1–9. doi: 10.1017/S0033291719002678.

Wray, N. R., Ripke, S., Mattheisen, M., Trzaskowski, M., Byrne, E. M., Abdellaoui, A., … Major Depressive Disorder Working Group of the Psychiatric Genomics, C. (2018). Genome-wide association analyses identify 44 risk variants and refine the genetic architecture of major depression. *Nature Genetics*, *50*(5), 668–681. doi:10.1038/s41588-018-0090-3.

Wu, L. T., & Anthony, J. C. (1999). Tobacco smoking and depressed mood in late childhood and early adolescence. *American Journal of Public Health*, *89*(12), 1837–1840. doi: 10.2105/ajph.89.12.1837.

Yang, J., & Li, M. D. (2016). Converging findings from linkage and association analyses on susceptibility genes for smoking and other addictions. *Molecular Psychiatry*, *21*(8), 992–1008. doi: 10.1038/mp.2016.67.

Zhang, B., Kirov, S., & Snoddy, J. (2005). Webgestalt: An integrated system for exploring gene sets in various biological contexts. *Nucleic Acids Research*, *33*(Web Server issue), W741–W748. doi: 10.1093/nar/gki475.

Zheng, J., Erzurumluoglu, A. M., Elsworth, B. L., Kemp, J. P., Howe, L., Haycock, P. C., … Neale, B. M. (2017). LD Hub: A centralized database and web interface to perform LD score regression that maximizes the potential of summary level GWAS data for SNP heritability and genetic correlation analysis. *Bioinformatics (Oxford, England)*, *33*(2), 272–279. doi: 10.1093/bioinformatics/btw613.

Zvolensky, M. J., Bakhshaie, J., Sheffer, C., Perez, A., & Goodwin, R. D. (2015). Major depressive disorder and smoking relapse among adults in the United States: A 10-year, prospective investigation. *Psychiatry Research*, *226*(1), 73–77. doi: 10.1016/j.psychres.2014.11.064.