Smoking and the course of anxiety and depression

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CHAPTER 4

Effect of Variation in BDNF Val^{66}Met Polymorphism, Smoking, and Nicotine Dependence on Symptom Severity of Depressive and Anxiety Disorders

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Abstract

Background. *Smoking, especially nicotine dependence is associated with more severe symptoms of depression and anxiety disorders. However, the mechanisms underlying this association are unclear. We investigated the effect of BDNF Val^{66}Met polymorphism on the severity of depressive and anxiety symptoms in never-smokers, former smokers, and current smokers with and without nicotine dependence.*

Methods. *Participants from the Netherlands Study of Depression and Anxiety (NESDA) with a current diagnosis of depression and/or an anxiety disorder and with available BDNF Val^{66}Met polymorphism data were included (N=1,271). Dependent variables were severity of symptoms and independent variables were smoking status and BDNF genotype. Age, sex, education, recent negative life events, alcohol use, body mass index, and physical activity were treated as covariates.*

Results. *In Val^{66}Val carriers, nicotine-dependent smokers had more severe symptoms of depression and anxiety than non-dependent smokers, former smokers and never-smokers, whereas the latter three groups did not differ on severity of symptoms. In Met^{66} carriers, there were no differences among the four smoking groups on severity of depression and anxiety. Nicotine dependence was the strongest predictor of severity of symptoms only in Val^{66}Val carriers.*

Conclusions. *In patients with an affective disorder, the relationship between nicotine dependence and symptom severity may be moderated by the BDNF Val^{66}Met polymorphism. These results may suggest that inherent genetic differences may be crucial for the worse behavioral outcome of nicotine, and that Val^{66}Val carriers may benefit most in mental health from smoking cessation.*

Introduction

Epidemiological and clinical research has provided substantial evidence of an association of smoking with depression and anxiety disorders[1-5]. Nicotine-dependent smokers have relatively high rates and more severe symptoms of depression and/ or anxiety as compared to non-dependent smokers, former- and never-smokers[6-13]. Twin studies have indicated that the association between smoking / nicotine dependence and depression/ anxiety can be explained by genetic[14-18], and / or environmental factors[14, 18-20]. However, the biological mechanisms underlying this association are not yet clear.

Brain-derived neurotrophic factor, (BDNF), an important regulatory protein and densely-expressed neurotrophin in the central and the peripheral nervous system, is a member of the nerve growth factor family[21, 22]. It supports the survival, differentiation, and maintenance of neurons in the nervous system[23]. It also regulates neurotransmitter systems dopamine[24] and serotonin[25] and is involved in neuroplasticity mechanisms such as long-term potentiation that underlies learning and memory[26-28]. The BDNF protein is encoded by the *BDNF gene* which, in humans, is located on chromosome 11[29]. The single nucleotide polymorphism (SNP) rs6265 in BDNF gene results in an amino acid Valine-to-Methionine substitution at codon 66 (Val[66]Met)[30].

The Val[66]Met polymorphism in the brain-derived neurotrophic factor (BDNF) gene may be a plausible candidate gene polymorphism underlying smoking-depression/ anxiety association. An association of this polymorphism has been found with affective disorders, and to some extent, also with smoking and other addictive behaviors. For example, previous research points to the involvement of variation in BDNF Val[66]Met polymorphism in the pathophysiology of depression and anxiety disorders. Studies using magnetic resonance imaging found that Met[66] allele carriers with depression had smaller hippocampal volumes than Val[66]Val carriers[31]. Smaller hippocampal volume is also a general characteristic of major depression[32]. A meta-analysis showed that the polymorphism was associated with major depressive disorder (MDD) only in

men, and that the MDD cases more often carried the Met[66] allele and were often in the homozygous Met[66]Met genotype group[33]. However, findings from two cohorts and a meta-analysis indicated that Val[66]Met polymorphism is unlikely to play a role in the genetic susceptibility to depression in a large sample[34]. One possible reason of this lack of association might be the non-clinical nature of the sample and self-report assessment of depression[34]. Few studies investigated the relationship of combination of gene markers including BDNF Val[66]Met polymorphism with depression. Though single-loci analyses did not show evidence of a significant positive association of the polymorphism with major depression, haplotype analysis of the combination of markers including BDNF rs6265[35-37] produced significant associations with major depression. A recent mega-analysis of genome-wide association studies, however, failed to reveal an important role of this and other polymorphisms in major depression[38].

No association of BDNF Val[66]Met polymorphism has been found with anxiety disorders including generalized anxiety disorder[39], panic disorder[40-42] and post-traumatic stress disorder[43] except in one community sample of children and adolescents[44].

Few studies examined the relevance of this polymorphism with addictive behaviors. For example, heroin-dependent Met[66] carriers were more often involved in drug-seeking behaviors and more cigarette use than Val[66]Val homozygotes[45]. In another study it was found that Met[66] carriers drank more alcohol per week, were more anxious to tolerate pressure and stress, and showed higher anticipatory cortisol response to stress than Val[66]Val carriers[46]. A recent study found that variation in BDNF Val[66]Met polymorphism might be involved in smoking in schizophrenic patients[47]. In a healthy adult German sample (N = 320), the frequency of the Met[66] allele of BDNF gene was higher in current and former smokers than in never-smokers[48]. However, another study failed to replicate these findings[49]. In a recent study with healthy Chinese male population (322 smokers, 306 non-smokers), age at the onset of smoking was associated with BDNF Val[66]Met polymorphism such that smokers with the Met[66] allele initiated smoking significantly earlier than the Val[66]Val

homozygous group[50]. Thus, Met[66] variant of the BDNF gene seems to be involved in addictive behaviors.

In summary, although several studies supported the association of BDNF Val[66]Met polymorphism with clinical major depressive disorder, a large mega-analysis failed to identify a role of this polymorphism in depression. There is also some evidence of an association of the Met[66] allele with smoking, although the number of studies and the sample sizes are limited. Smoking does appear to affect symptom severity in depression and anxiety disorders. It has been argued that psychiatric disorders may only be understood if both genetic and environmental factors are taken into account in the statistical models[38], and that, in order to elucidate the underlying molecular mechanisms that may explain the association between phenotypes, gene-by-environment interaction studies may be more useful[51].

In the present study, we investigated the interaction between the BDNF gene Val[66]Met polymorphism and smoking status on depressive and anxiety symptoms in a clinical sample. In a previous report, we found that nicotine-dependent smokers had more severe symptoms of depression and anxiety disorders than non-dependent smokers, former smokers, and never-smokers[52]. The current study investigated a possible involvement of the BDNF gene Val[66]Met polymorphism in this association.

Methods

Participants and Data

Participants were selected from the Netherlands Study of Depression and Anxiety (NESDA), an on-going naturalistic prospective cohort study. NESDA started in September 2004 and investigates the long-term course and consequences of depression and anxiety disorders by examining clinical, psychosocial, biological and genetic determinants. At baseline, NESDA consisted of 2,981 participants (66.4 % females) between 18 to 65 years of age. Participants were recruited from mental health care, primary care, and the general population in order to represent the entire range of psychopathology. The sample consisted of persons with a current diagnosis of anxiety and/or depression (57 %), persons with a remitted history of the disorders (21 %) and healthy controls (22 %). The two exclusion criteria were (i) a primary diagnosis of a psychotic disorder, addiction disorder, obsessive-compulsive disorder, or bipolar disorder and (ii) not being fluent in Dutch. Data were collected on demographic, clinical, psychosocial, psychological, and physiological variables, and health behaviors including alcohol intake, smoking, drug use, and physical activity. For data collection, self-report questionnaires, interviews, a medical examination, a cognitive computer task, and blood and saliva samples were gathered. NESDA protocol was approved by the Ethical Review Board of the VU University Medical Center and the local review boards of participating centers. All participants provided written informed consent. Further details on the rationale, objectives, design and sample of NESDA were published elsewhere[53].

In the present study, we selected participants who had a current (past 6 months) diagnosis of depression and/or an anxiety disorder at the baseline assessment and for whom information on BDNF genotype was available (N= 1,271).

Measures

Smoking

Smoking behavior was measured by a questionnaire that covered past and current smoking behavior. Participants were classified into four groups based on their smoking status: never-smokers, former smokers, non-dependent smokers and nicotine-dependent smokers. Former smokers were those who stopped smoking definitively, and never-smokers were those who had no lifetime history of smoking. Nicotine dependence was assessed with the Fagerstrom Test for Nicotine Dependence (FTND)[54]. The reliability and internal consistency of FTND have been shown in previous research[55]. The FTND (score range: 0-10) assesses daily smoking rate, the interval between waking up and smoking the first cigarette, frequency of smoking after waking up, difficulty refraining from smoking in places where it is forbidden, and despite medical illness, and difficulty giving up the first cigarette in the morning. Current smokers with a score of 4 or higher on the FTND in the present study were defined as nicotine-dependent smokers[12, 56].

Psychopathology

The Composite International Diagnostic Interview (CIDI version 2.1)[57] was used to assess the DSM-IV criteria for anxiety and depressive disorders. The CIDI has high inter-rater reliability, high test-retest reliability and high validity for depressive and anxiety disorders[57].

Severity of depressive symptoms was assessed by the Inventory of Depressive Symptomatology (IDS). The IDS (score range: 0–84) is a 30-item self-report inventory which has shown high correlations with observer rated scales[58]. The 21-item Beck Anxiety Inventory (BAI; score range: 0-62) was used to assess severity of anxiety symptoms[59]. The symptoms of fear were measured with the 15-item Fear Questionnaire[60]. We used two sub-scales of the Fear Questionnaire: (i) FQ items for social fear symptoms, and (ii) FQ items for agoraphobia symptoms[60]. The sum score of both sub-scales ranges from 0 to 40. BAI and both subscales of FQ have sufficient internal consistency[59, 61].

Covariates

The Alcohol Use Disorder Identification Test (AUDIT) was used to assess alcohol intake[62]. The International Physical Activity Questionnaire (IPAQ) was used to measure physical activity. IPAQ estimates weekly energy expenditure based on daily physical activities[63]. Negative life events in the past year were assessed with the List of Threatening Events Questionnaire (LTE-Q)[64]. These events reflect the occurrence of stressful events such as serious personal illness or injury, death or loss of a loved one, and financial problems in the past year. Body mass index (BMI) was calculated (kg/m^2). Other covariates under study were age, sex, and education. These covariates were chosen because of their association with severity of affective symptoms[65-70].

Genotyping

Venous blood samples were collected at baseline (between 0830 and 0930 hours) after overnight fasting and DNA was isolated using the FlexiGene DNA AGF3000 kit (Qiagen, Valencia, CA, USA) on an AutoGenFlex 3000 workstation (Autogen, Holliston, MA, USA). DNA concentrations were determined using the PicoGreens dsDNA Quantitation kit from Molecular Probes. Genotyping of the participants was conducted by Perlegen Sciences (Mountain View, CA, USA) using four proprietary, high-density oligonucleotide arrays. Detailed description of how genotyping was performed has been published elsewhere[71]. To extract the Val^{66}Met polymorphism from the whole genome data, PLINK software version 1.07 (http://pngu.mgh.harvard.edu/~purcell/plink/) was used. The imputation accuracy of rs6265 (Val^{66}Met polymorphism) was 99.9 % (r2hat = 0.999).

The current sample consists of 65.8 % Val^{66}Val and 3.5 % Met^{66}Met homozygotes, whereas 30.7 % were Val^{66}Met heterozygotes. We combined the low-frequency Met^{66}Met with Val^{66}Met and referred to the group as Met^{66} carriers [46].

Statistical Analyses

Preliminary analyses were conducted to ensure no violation of the assumptions of univariate and multivariate analysis. Normality of distributions, linearity, and multicollinearity, heterogeneity of variance, outlying scores, and coding errors were checked. The Hardy-Weinberg equilibrium for the BDNF polymorphism was tested using a chi-square test for goodness of fit. Differences on demographic and clinical characteristics between the groups stratified according to their smoking status were determined with one-way between-groups ANOVA and chi-square test for independence. The association of BDNF Val^{66}Met polymorphism was examined, separately, with smoking status, nicotine dependence, and with the symptoms of depression and anxiety disorders, using chi-square test for independence, independent-samples t-test, and multivariate ANCOVA, respectively. Next, the estimates of the interaction effects of genotype and smoking status on the symptoms of depression and anxiety disorders were computed with multivariate ANCOVA while controlling for the covariates on which the groups differed significantly. In order to control for the potentially confounding influences of the covariates on the main and the interaction effects of the BDNF genotype and smoking status, we entered all the covariate x smoking status and the covariate x BDNF gene interaction terms in the same model that tests the BDNF gene x smoking status interaction term, along with the simple effects of the covariates[72, 73]. Significant interaction effects were followed up by univariate ANCOVAs, run separately for Val^{66}Val and Met^{66} carriers. Eta squared and partial eta squared were used as the measures of effect size, and alpha level of 0.05 was used. Finally, the predictors of symptom severity were examined separately for Val^{66}Val and Met^{66} carriers using multiple linear regression analyses. Two models were fitted in each regression analysis. In the first model, we entered age, sex, education, past year negative life events, alcohol intake and body mass index, and in the second model we added nicotine dependence. Thus the estimates provided from the final model included all variables. Analyses were run in PASW (V. 19.0) for windows.

Results

Preliminary analyses did not indicate any serious violation of the assumptions of univariate and multivariate tests. The genotype distributions in the four smoking groups did not deviate significantly from the Hardy-Weinberg Equilibrium (never-smokers: $p = 0.5$; former smokers: $p = 0.6$; non-dependent smokers: $p = 0.3$; nicotine-dependent smokers: $p = 0.7$).

Sample Characteristics

Of the 1,271 participants, 24.6 % were never-smokers, 31.0 % were former smokers, and 44.4 % were current smokers. Of the current smokers, 46.5 % were nicotine-dependent. The smoking groups differed significantly with respect to age ($F_{(3, 1267)} = 26.1$; $p < 0.001$), education ($F_{(3, 1267)} = 12.1$; $p < 0.001$), past year negative life events ($F_{(3, 1267)} = 6.6$; $p < 0.001$), alcohol intake ($F_{(3, 1252)} = 33.8$; $p < 0.001$), and body mass index ($F_{(3, 1266)} = 5.0$; $p < 0.01$). No group differences were found in physical activity, sex distribution, and BDNF genotype (ps > 0.05). Post-hoc comparisons between the smoking groups for significant association of continuous variables are presented in table 1.

Association of BDNF Genotype with Smoking, and with Symptom Severity

No association of smoking status with BDNF genotype was found. The four smoking groups did not differ significantly in allele distribution ($p > 0.05$). We also examined allele distribution by collapsing the four smoking groups into two groups of current smokers (non-dependent and dependent) and non-smokers (former and never-smokers), however, the difference did not reach significance ($p > 0.05$).

Multivariate ANCOVA revealed that the main effect of BDNF $Val^{66}Met$ polymorphism on the severity of symptoms of depression, general anxiety, social anxiety and agoraphobia was non-significant (ps > 0.05).

Table 1. Participants' characteristics stratified according to their smoking status

Sociodemographic variables and health behaviors	Never-smokers N = 313		Former smokers N = 394		Current smokers				Effect size[2]	Tukey[3]
					Non-dependent N = 302		Nicotine-dependent N = 262			
Age (Mean, SD)	38.7	12.8	45.3	11.8	38.4	11.9	41.7	10.7	0.06***	FS>D>FS,NS
Sex, F (N, %)	220	70.3	262	66.5	208	68.9	164	62.6	ns	
Education, in years (Mean, SD)	12.2	3.2	12.1	3.3	11.8	3.1	10.7	3.1	0.03***	D<nD,FS,NS
Past year negative life events (Mean, SD)	0.9	1.1	0.9	1.0	1.0	1.2	1.3	1.4	0.02***	D>nD,FS,NS
Alcohol use (Mean, SD)	2.9	3.4	4.8	4.6	6.5	5.5	6.5	6.7	0.07***	nD,D>FS>NS
Physical activity (Mean, SD)[1]	3.4	2.9	3.6	3.0	3.9	3.5	3.6	3.6	ns	
Body mass index (Mean, SD)	25.6	5.2	26.4	5.1	24.8	4.8	25.5	5.6	0.01**	nD<FS
BDNF genotype (N, %)									ns	
Val[66]Val carriers	211	67.4	268	68.0	183	60.6	174	66.4		
Met[66] carriers	102	32.6	126	32.0	119	39.4	88	33.6		

***$p < 0.001$, **$p < 0.01$, *$p < 0.05$

[1]Mean met-minutes (ratio of energy expenditure during activity to energy expenditure at rest) divided by 1000

[2]ns: non-significant

[3]Only significant results are shown; NS: never-smokers, FS: former smokers, nD: non-dependent, D: nicotine-dependent

Interaction Effect of BDNF Genotype and Smoking Status on Symptom Severity

A multivariate ANCOVA showed that the BDNF genotype and smoking status interaction effect was significant for symptoms of depression ($F_{(3, 1230)} = 3.1$; $p < 0.05$; partial $\eta 2 = 0.01$) and anxiety ($F_{(3, 1230)} = 2.8$; $p < 0.05$; partial $\eta 2 = 0.01$), while non-significant for symptoms of social anxiety and agoraphobia ($ps > 0.05$).

The subsequent univariate ANCOVAs revealed that in $Val^{66}Val$ carriers, the main effect of smoking status on symptoms of depression ($F_{(3, 812)} = 8.7$; $p < 0.001$; partial $\eta 2 = 0.03$) and general anxiety ($F_{(3, 813)} = 5.6$; $p = 0.001$; partial $\eta 2 = 0.02$) was significant. Pairwise comparisons showed that nicotine-dependent smokers had significantly more severe symptoms of depression and general anxiety than non-dependent smokers, former smokers, and never-smokers ($ps < 0.05$). Never-smokers, former smokers, and non-dependent current smokers did not differ significantly from each other ($ps > 0.05$) on severity of depressive and anxiety symptoms (table 2).

In Met^{66} carriers**,** the main effect of smoking status on severity of symptoms of depression and general anxiety was non-significant in the univariate ANCOVA ($ps > 0.1$), suggesting that smoking groups carrying Met^{66} allele did not differ significantly from each other on severity of symptoms of depression and general anxiety (table 2).

Table 2. Estimates of the severity of symptoms of depression and anxiety disorder in different smoking groups stratified according to their BDNF genotype[a]

Severity of symptoms	Never-smokers			Former smokers			Current smokers (nD)[b]			Current smokers (D)[b]			Partial η^2
	Mean	SE	95% CI	Mean	SE	95% CI	Mean	SE	95% CI	Mean	SE	95% CI	
	BDNF Val[66]Val carriers												
Symptoms of depression	23.1	4.2	14.9, 31.3	26.0	1.3	23.4, 28.7	33.1	2.1	29.0, 37.2	43.8	4.9	34.2, 53.4	.03***
Symptoms of anxiety	11.3	3.6	4.2, 18.3	14.2	1.1	11.9, 16.4	18.9	1.8	15.4, 22.4	27.1	4.2	18.8, 35.3	.02***
	BDNF Met[66] carriers												
Symptoms of depression	25.7	5.4	15.0, 36.3	28.1	2.0	24.1, 32.0	30.2	2.4	25.5, 34.9	32.9	6.1	21.0, 44.9	ns
Symptoms of anxiety	13.3	4.6	4.3, 22.4	15.4	1.7	12.0, 18.7	17.5	2.0	13.6, 21.5	18.1	5.1	8.0, 28.3	ns

***$p \leq 0.001$
[a]Adjusted for covariates and their interactions with smoking and genotype.
[b]nD: non-dependent; D: nicotine-dependent.
ns: non-significant.

Predictors of Symptom Severity

Regression analyses indicated that in the $Val^{66}Val$ group, the first model with age, sex, education, negative life events, alcohol intake, and body mass index explained 7.6 % of the variance in severity of depression ($p < 0.001$). The second model that added nicotine dependence accounted for an additional 5 % of the significant variance ($R^2 = 0.125$; $p < 0.001$). In Met^{66} carriers, only the first model with age, sex, education, negative life events, alcohol intake, and body mass index explained significant variance ($R^2 = 0.07$, $p < 0.05$).

The same pattern emerged with anxiety severity as the dependent measure. In the $Val^{66}Val$ group, the first model explained 6.4 % variance in severity of anxiety ($p = 0.001$), and the second model with nicotine dependence accounted for an additional 5 % of the significant variance ($R^2 = 0.12$; $p < 0.001$). In Met^{66} carriers, only the first model explained significant variance ($R^2 = 0.10$; $p < 0.01$).

Nicotine dependence was the strongest significant predictor of the symptoms of depression and anxiety only in Val66Val carriers (table 3).

Table 3. Regression of nicotine dependence on the severity of symptoms stratified according to the BDNF genotype

	BDNF Val[66]Val carriers				BDNF Met[66] carriers*			
Predictors	**B**	**SE**	**B**	***p***	**B**	**SE**	**β**	***p***
				Severity of depression				
Age	-0.07	0.06	-.07	ns	0.01	0.07	.01	ns
Sex	0.78	1.39	.03	ns	-0.03	1.82	.001	ns
Education	-0.49	0.21	-.13	*	-0.67	0.27	-.18	*
Negative life events	0.55	0.51	.06	ns	0.06	0.77	.01	ns
Alcohol intake	0.01	0.12	.004	ns	-0.04	0.17	-.02	ns
BMI	0.38	0.13	.16	**	0.31	0.17	0.13	ns
Nicotine dependence	1.11	0.26	.24	***	0.58	0.34	.12	ns
				Severity of general anxiety				
Age	-0.05	0.05	-.05	ns	-0.01	0.06	-.01	ns
Sex	-0.10	1.18	-.004	ns	-1.40	1.56	-.06	ns
Education	-0.45	0.17	-.14	*	-0.90	0.23	-.27	***
Negative life events	0.53	0.44	.06	ns	-0.26	0.67	-.03	ns
Alcohol intake	0.07	0.10	.04	ns	0.05	0.14	.02	ns
BMI	0.20	0.11	.10	ns	0.21	0.14	.11	ns
Nicotine dependence	0.96	0.22	.24	***	0.41	0.29	.10	ns

***$p < 0.001$; **$p < 0.01$; *$p < 0.05$
ns: non-significant

Discussion

We previously reported that nicotine-dependent smokers had more severe symptoms of depression and anxiety disorders than non-dependent smokers, former smokers, and never-smokers[52]. In the present study, we examined the role of the BDNF Val^{66}Met polymorphism in this association.

The BDNF polymorphism had no direct effect on smoking status and on the severity of affective symptoms in the present sample. However, we did observe an interaction of smoking status and BDNF polymorphism with the symptoms of depression and anxiety. In Val^{66}Val carriers, nicotine-dependent smokers had more severe symptoms of depression and anxiety disorder than non-dependent smokers, former smokers and never-smokers, whereas the latter three groups were comparable in symptom severity. In Met^{66} carriers no differences among the four smoking groups were found in symptoms of depression and anxiety. We also found that after controlling for the potential confounding variables, nicotine dependence was the strongest predictor of the symptoms of depression and anxiety only in Val^{66}Val homozygotes. These findings suggest that genetic predisposition and nicotine dependence may act interdependently in the severity of symptoms of affective disorders.

These findings need to be replicated as there is no previous report on the interacting effects of BDNF gene and smoking status on depressive and anxiety symptoms. Most of the previous literature is based on association studies that look for a direct relationship of BDNF Val^{66}Met polymorphism with depression, or with smoking, and most of the studies examined genotype or allele frequencies. In order to elucidate the underlying molecular mechanisms through which variation in the BDNF gene may lead to severity of symptoms, gene-by-environment interaction studies may be more useful[38, 51].

The observed association might be interpreted in the context of previous MRI research which shows that Val[66]Val genotype is associated with reduced hippocampal gray matter volumes in healthy Caucasian participants[74]. Further, reduced hippocampal gray matter volume has also been associated with major depression[32]. Thus, nicotine-dependent smokers who carry Val[66]Val variant of the BDNF gene may have reduced hippocampal gray matter volume and thus may experience more severe symptoms than Met[66] carriers whose brain morphology may not be depression-vulnerable. This speculation has been supported by animal research. In a recent animal model of depression, nicotine administration exacerbated depressive-like behavior in inbred Wistar-Kyoto (WKY) rats, which had reduced hippocampal volume but not in the control Wistar rats with comparatively large hippocampal volume[75]. Research has also shown greater genotypic variability among WKY rats compared to other inbred strains[76, 77]. It has been proposed that the differential responses of WKY and Wistar rats to nicotine may reflect genetic differences[75]. Thus, the current findings suggest that genetic differences are important determinants to explain worse behavioral outcome of nicotine in some individuals but not in others. However, this theory needs to be confirmed in future MRI and genetic investigations.

This study has some limitations which should be taken into account. Firstly, we investigated only one polymorphism, therefore it is likely that other polymorphisms in the BDNF gene or other genes are involved. Secondly, the study is cross-sectional and causality cannot be inferred. Thirdly, our findings may not be generalizable to other ethnic groups or homogenous age groups.

A strength of our study is the relatively large sample size compared to prior research in this area. We investigated patients with a diagnosed psychiatric disorder, unlike most previous studies that used samples from general population. The depression and anxiety disorder diagnoses were made according to DSM-IV criteria, whereas most previous studies assessed symptoms using self-report measures. The study sample is ethnically homogenous and we were able to control for a large number of covariates.

Despite a well-established association between smoking and depression and anxiety disorders, the mechanisms underlying this association have rarely been investigated from a genetic perspective. Only few studies have investigated the interacting effects of genetic predisposition and depressive symptoms on smoking. The two short alleles of DRD4 gene are associated with self-medicating smoking practices in depressed individuals[78]. Similarly, smokers with severe depressive symptoms were progressing to a higher level of smoking only if they had DRD2A1 allele[79]. However, these studies support the notion of smoking as a self-medicating agent to alleviate depressive symptoms. The theory, that smoking is a vulnerability factor in depression and anxiety has recently been investigated using the rs1051730 SNP variant located in the nicotine acetylcholine receptor gene cluster on chromosome 15. Self-reported smoking was positively associated with the prevalence of both anxiety and depression, and the measured polymorphism was positively associated with smoking. However, no association of the polymorphism with either anxiety or depression was found among smokers suggesting that smoking is not a causal factor of anxiety and depression[80]. Our results, however, are supportive of the 'smoking as a vulnerability factor in depression/ anxiety' theory. We found a genotype-dependent dose-response relationship between nicotine dependence and symptom severity. Though, this theory could not be elucidated in the current study because of its cross-sectional nature, it may provide a starting point for understanding the neurobiological links between smoking or nicotine dependence and affective disorders.

This study has clinical implications, for example, Val^{66}Val carriers may benefit most from smoking cessation or lowering amount of smoking. It could

still be that smoking in this group is a failed attempt at self-medication. Thus, future study could focus on immediate versus longer-term subjective effects of lighting a cigarette in this group. If it turns out that smoking reduces anxiety in the short-term, this group may particularly benefit from other interventions that reduce anxiety while they attempt to quit.

This study, also, has important implications for future molecular research on smoking-psychopathology association. Given the high prevalence of depression and anxiety in smokers, it is important to focus on investigating the genetic and biological influences to elucidate the mechanisms underlying the association between smoking and affective disorders. Similarly, ethnicity should be given consideration because of the ethnic differences in genotype and allele frequencies[42]. Moreover, DSM assessment for nicotine dependence may be more useful for identifying smokers vulnerable to depression and anxiety[81]. Understanding of genetic influences on smoking-psychopathology association may be significant for guiding smoking prevention and intervention programs in identifying smokers, particularly those with nicotine dependence who are vulnerable to adverse outcomes

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