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Association Between Smoking, Nicotine Dependence, and BDNF Val[66]Met Polymorphism with BDNF Concentrations in Serum

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Abstract

Introduction: Nicotine use is associated with the upregulation of brain-derived neurotrophic factor (BDNF) in serum. An association between smoking and the BDNF Val[66]Met polymorphism has also been found. The aim of this study is to examine the levels of serum BDNF in never-smokers, former smokers, and current smokers—with and without nicotine dependence—and to examine the interaction of the polymorphism and smoking status with serum BDNF.

Methods: We used baseline serum and gene data of BDNF on 2,088 participants from the Netherlands Study of Depression and Anxiety (NESDA) to investigate smoking-BDNF association while controlling for potential confounding variables. Nicotine dependence was assessed with the Fagerstrom Test for Nicotine Dependence (FTND).

Results: Smokers with and without nicotine dependence had higher levels of serum BDNF than former and never-smokers. Nicotine dependence and number of cigarettes smoked per day did not add to the prediction of serum BDNF; however, total number of smoking years was a significant predictor of serum BDNF. There was no association of BDNF Val[66]Met, nor an interaction of this polymorphism and smoking status, with serum BDNF.

Conclusions: Current smoking and higher number of smoking years are associated with higher levels of serum BDNF, and this is independent of the BDNF genotype. Nicotine dependence itself is not associated with a further increase or decrease of serum BDNF. Longitudinal investigations that address changes in serum BDNF in incident smokers and/or in quitters may be useful to understand the association of smoking with BDNF.

Introduction

Brain-derived neurotrophic factor (BDNF), a small dimeric protein, is a member of the neurotrophin family of growth factors.[1] It is densely expressed in the central and the peripheral nervous system, and is the most abundant of the neurotrophins in the brain with high concentrations in the hippocampus and cerebral cortex.[2,3] It is involved in the growth, development, regeneration, survival, maintenance, and function of neurons.[4,5] It is also involved in the modulation of neurotransmitter release across several neurotransmitter systems with

key effects on serotonergic[6], dopaminergic[7], and glutamatergic neurotransmitter systems[8–10], and in the plasticity mechanisms such as long-term potentiation[11], a cellular mechanism underlying learning and memory.

Peripheral BDNF is highly concentrated in platelets[12–14], with approximately 50–200-fold higher circulation in serum than in plasma.[15,16] The difference between the levels of serum and plasma BDNF could reflect the release of BDNF from platelets during blood clotting.[12] In animals, the brain and peripheral BDNF levels undergo similar changes during growth and developmental process, and BDNF levels in serum correlate positively to cortical BDNF.[17] This may indicate that peripheral BDNF levels are reflective of BDNF levels in the brain.

The BDNF protein is encoded by the *BDNF gene* which, in humans, is located on chromosome 11.[18] The single nucleotide polymorphism (SNP) rs6265 in BDNF gene results in an amino acid Valine-to-Methionine substitution at codon 66 (Val^{66}Met).[19]

As already mentioned, BDNF expression in the brain is regulated by the serotonergic[6] and the dopaminergic[20] neurotransmitter systems which are known to be involved in nicotine use and addictive behaviors.[21–25] For instance, studies have indicated that nicotine exposure increases brain serotonin secreation[26], that the serotonin transporter gene is associated with smoking behavior[27–30], and that nicotine withdrawal results in a decrease of dopamine in the nucleus accumbens.[25]

Evidence from animal studies indicates that high levels of brain BDNF may be associated with drug addiction. Nicotine infusion in neonatal piglets significantly increases the expression of BDNF mRNA and protein in the hippocampus[31], and hippocampal BDNF mRNA expression is enhanced or reduced, after chronic or acute administration of nicotine, respectively.[32]

Given the difficulty of the direct examination of brain BDNF in humans, the levels of BDNF have been primarily studied in the periphery, mainly in the blood serum. In a Chinese sample of chronic schizophrenic inpatients (N = 139; 102 smokers) with no drug or alcohol dependence, smokers had higher levels of serum BDNF than nonsmokers. The number of cigarettes smoked per day was positively correlated with serum BDNF levels.[33] In a subsample of this study that has investigated the determinants of serum BDNF in individuals with no current diagnoses of major depression or anxiety disorder, a positive association of serum BDNF and smoking was found, suggesting that smoking is associated with increasing serum BDNF levels.[34] In summary, these findings suggest that the effect of nicotine use on central and peripheral BDNF expression depends on the amount of smoking. Higher number of cigarettes smoked per day and chronic nicotine exposure might be associated with upregulation of serum BDNF levels.

There is also some evidence of an association of BDNF Val^{66}Met polymorphism with smoking[35,36], with the frequency of the *Met* allele of the polymorphism being higher in current and former smokers than in never-smokers.[35] However, another study failed to replicate these findings.[37]

The aim of this study was to examine serum BDNF levels in never-smokers, former smokers, and current smokers with and without nicotine dependence, and to investigate the association of smoking severity and chronicity with serum BDNF levels. Further, the effect of BDNF Val^{66}Met polymorphism in this association will also be examined. As has been pointed out earlier, brain serotonin and dopamine, which are involved in addictive behaviors, regulate BDNF expression in the brain. However, the role of BDNF in relation to smoking behavior has not been well-explored. The rationale for investigating these associations is to robustly replicate previous findings of higher serum BDNF in smokers and to explore whether the association is particularly evident in *nicotine-dependent* smokers. To our knowledge, no previous study has investigated the association of serum BDNF levels with smoking, taking into account smokers who quit, and nicotine-dependent smokers. Moreover, there is no study investigating the association of chronic cigarette use with serum BDNF levels, and whether the BDNF Val^{66}Met polymorphism may moderate the association between smoking and BDNF serum levels.

We hypothesize that (a) both groups of current smokers, that is, nondependent and nicotine-dependent smokers, have higher levels of serum BDNF than the nonsmoking groups of former- and never-smokers; (b) nicotine-dependent smokers have higher serum BDNF than nondependent smokers; (c) former and never-smokers will be comparable in serum BDNF levels; (d) number of cigarettes smoked per day, total smoking years, and nicotine dependence will be positively correlated with and will predict serum BDNF. We will adjust the analyses for several potential confounding variables, including the presence of depressive and anxiety disorders, which have been shown to be associated with BNDF[38–41] as well as with smoking behavior.[42–45]

Methods

Participants and Data

Participants were selected from the Netherlands Study of Depression and Anxiety (NESDA), an on-going prospective cohort study which started in September 2004. Recruitment took place in mental health care organizations, primary care, and in the general population. The baseline NESDA sample consists of 2,981 participants (66.4% females) between 18 and 65 years of age, with a current diagnosis of anxiety and/or depression (57%), with a history of these disorders (21%) and with no lifetime history of these disorders (22%). Exclusion criteria were primary diagnosis of a psychotic disorder, addiction disorder, obsessive–compulsive disorder, or bipolar disorder. Approval of the NESDA protocol was obtained from the Ethical Review Board of the VU University Medical Center and from the local review boards of participating centers. All participants signed informed consent for the study after full information about the study was provided to them. Further details on the rationale, objectives, design and sample of NESDA were published elsewhere.[46]

In this study, we selected participants for whom data on serum BDNF and BDNF gene Val^{66}Met polymorphism were available (N = 2,088). The sample was stratified into never-smokers, former smokers, and current smokers without and with nicotine dependence.

Measures

Smoking and Nicotine Dependence

Smoking behavior was assessed by a questionnaire. The Fagerstrom test for nicotine dependence (FTND) was used to assess nicotine dependence.[47] The reliability and internal consistency of FTND have been shown in previous research.[48] The FTND assesses daily smoking rate, the interval between waking up and the first cigarette, frequency of smoking after waking up, difficulty refraining from smoking in places where it is forbidden, and despite medical illness, and also difficulty delaying the first cigarette in the morning. The sum score of the FTND ranges from 0 to 10. We grouped the participants into four smoking groups of never-smokers (those who had no lifetime history of smoking), former smokers (those who had

stopped smoking definitively), nondependent smokers (those current smokers who had scored less than four on FTND), and nicotine-dependent smokers (those current smokers who had scored four or higher on FTND).[49,50]

Potential Confounding Variables

The current (6-month recency) diagnoses of major depression and anxiety disorders were ascertained using the Composite International Diagnostic Interview (CIDI version 2.1). The CIDI is a structured interview designed to assess diagnoses of psychiatric disorders according to DSM-IV criteria. The CIDI has high inter-rater reliability, high test–retest reliability, and high validity for depressive and anxiety disorders.[51] The Alcohol Use Disorder Identification Test (AUDIT) was used to assess alcohol intake.[52] The International Physical Activity Questionnaire (IPAQ) was used to measure self-reported physical activity. IPAQ estimates weekly energy expenditure based on daily physical activities.[53] Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared (weight/height^2). Number of past-year negative life events were assessed with the Brugha questionnaire.[54] Data on the use of antidepressants were acquired through drug container observation and self-report. Use of an antidepressant was defined as intake of minimally the daily dose as recommended by the World Health Organization during the last month on at least 50% of the days. The duration of use was expressed in months.[55] All methods were standardized through periodical external quality assessments by the Dutch Foundation for Quality Assessment in Clinical Laboratories. Other covariates under study were age, sex, and education. These covariates were chosen due to their theoretical relevance to smoking and BDNF.[56–58]

Serum BDNF

Blood (50 ml) was drawn (between 07:30 and 09:30 hr) after an overnight fast, and serum was separated and stored at −85°C until it was assayed. EmaxImmuno Assay system from Promega was used to measure BDNF protein levels according to the manufacturer's protocol (Madison, WI, USA). In order to increase the detectable BDNF in a dilution-dependent way, the undiluted serum was treated with acid. Grenier Bio-One high affinity 96-well plates were used. Serum samples were diluted 100 times, and the absorbency was read in duplicate using a Bio-Rad Benchmark microplate reader at 450 nm. Serum BDNF protein levels were expressed in nanograms per milliliter (ng/ml). The intra- and inter-assay coefficients of variation were found to be within 3 and 9%, respectively. Prior to analyses, BDNF values that were three standard deviations (*SD*) above the mean (n = 5, 0.35%) were trimmed to a value of the mean plus three *SD*s. One BDNF value (0.07%) was below the reliable detection limit of the ELISA kit of 1.56 ng/ml and was set at the lower detection limit of 1.56 ng/ml. Persons with missing and nonmissing BDNF were not significantly different from each other in age, sex, and diagnoses (ps > .05).

Genotyping

Venous blood samples were collected at baseline (between 08:30 and 09:30 hr) 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.[59] To extract the Val^{66}Met polymorphism from the whole genome data, PLINK software (http://pngu.mgh.harvard.edu/~purcell/plink/) was used. The imputation accuracy of rs6265 (Val^{66}Met polymorphism) is 99.9 % (r2hat = 0.999).

The current sample consists of 64.8% Val^{66}Val and 3.4% Met^{66}Met homozygotes, whereas 31.8% were Val^{66}Met heterozygotes. We combined the low-frequency homozygous Met^{66}Met carriers with the heterozygous Val^{66}Met carriers, as done before.[60]

Statistical Analyses

Data were checked for outliers and coding errors. Preliminary analyses indicated no serious violation of the assumptions of univariate and regression analyses. Between-group differences on demographic, health, and clinical characteristics were determined using one-way ANOVAs (with post hoc tests for significant F-statistic) and chi-square test for independence. The Hardy–Weinberg equilibrium for the BDNF polymorphism was tested using a chi-square test for goodness-of-fit. Estimates of the main and interaction effects of smoking status and BDNF Val^{66}Met polymorphism on serum BDNF levels were determined using univariate ANCOVA. The model was adjusted for the potential confounding effects of the variables on which the smoking groups differed. These covariates were age, education, alcohol use, BMI, number of negative life events in the past year, and antidepressants use. Significant effects were further followed by similar ANCOVA, while adjusting for the above-mentioned covariates. Correlation coefficients of serum BDNF with number of cigarettes smoked per day, total smoking years, and nicotine dependence were calculated. Finally, multiple linear regression was run to see how much of the variance in serum BDNF is explained by smoking severity, which was assessed by number of cigarettes smoked per day and nicotine dependence and chronicity, as assessed by total years of smoking. The independent variables/covariates were entered by fitting three models. In the first model, we entered age, sex, education, and number of past-year negative life events. The second model added alcohol use, BMI, antidepressant use, and the diagnosis of an affective disorder. In the third model, we added number of cigarettes smoked per day, total smoking years, and nicotine dependence. Thus, the estimates provided from the final model included all variables. Analyses were run in SPSS (v. 19.0) for Windows. Statistical significance was set at $p < .05$. Eta squared, partial eta squared, and Cramer's V were used as estimates of effect size.

Results

Participants' Demographic and Clinical Characteristics

Of the 2,088 participants, 27.0 % were never-smokers, 33.0 % were former smokers, and 40.0 % were current smokers. Of the current smokers, 36.7 % were nicotine dependent. The genotype distributions in the four smoking groups did not deviate significantly from the Hardy–Weinberg Equilibrium (never-smokers: p = .7; former smokers: p = .4; nondependent smokers: p = .3; nicotine-dependent smokers: p = .7). Table 1 presents the demographic, health, and clinical characteristics of the participants stratified according to their smoking status. ANOVA revealed significant group differences in age (F (3, 2084) =35.0, η^2 = .05), years of education (F (3,2084) = 19.3, η^2 = .03), alcohol use (F (3, 2064) = 49.8, η^2 = .07), BMI (F (3,2082) = 6.4, η^2 = .01), and number of past-year negative life events (F (3,2084) = 9.3, η^2 = .01), while a nonsignificant

Table 1. Baseline Demographic and Health Behavior Characteristics of the Participants Stratified According to Their Smoking Status

Demographic, health, and clinical characteristics	Smoking status				
			Current smokers		
	Never-smokers (n = 564)	Former smokers (n = 690)	Nondependent (n = 528)	Nicotine-dependent (n = 306)	p
Age (mean, *SD*)	39.5 (13.5)	45.9 (12.0)	39.8 (12.8)	42.4 (11.4)	< .001
Sex, female (n, %)	402 (71.3)	458 (66.4)	339 (64.2)	191 (62.4)	.03
Education in years (mean, *SD*)	12.6 (3.2)	12.5 (3.3)	11.8 (3.2)	11.1 (3.2)	< .001
Alcohol (mean, *SD*)	3.3 (3.4)	4.7 (4.1)	6.5 (5.5)	6.1 (5.8)	< .001
Physical activity (mean, *SD*)[a]	3.7 (3.0)	3.7 (3.0)	4.0 (3.5)	3.6 (3.5)	.256
BMI (mean, *SD*)	25.3 (4.9)	26.2 (4.9)	25.0 (5.1)	25.8 (5.1)	< .001
Number of past-year negative life events (mean, *SD*)	0.8 (1.0)	0.8 (1.0)	1.0 (1.2)	1.1 (1.2)	< .001
Use of antidepressants (n, %)	147 (24.1)	186 (30.4)	163 (26.7)	115 (18.8)	.001
Current diagnosis of an affective disorder (n, %)	306 (54.3)	386 (55.9)	341 (64.6)	208 (68.0)	< .001

[a]Mean met-minutes (ratio of energy expenditure during activity to energy expenditure at rest) divided by 1,000.

Table 2. Mean (SE) Serum BDNF Levels in the Study Sample Stratified on the Basis of Their Smoking Behavior

Smoking status	Mean	*SE*	95% CI
Never-smokers	8.9	.15	8.6, 9.2
Former smokers	8.7	.13	8.4, 8.9
Nondependent smokers	9.5	.15	9.2, 9.8
Nicotine-dependent smokers	9.5	.20	9.1, 9.9

CI = confidence interval.

group difference in physical activity (p > .05). Post hoc comparisons indicated that former smokers were significantly older than the other three groups, and nicotine-dependent smokers were older than nondependent and never-smokers. The latter two groups were not different significantly in age. Never-smokers and former smokers had significantly more years of education, drank less alcohol, and they experienced less number of stressful life events in the past-year than the two current-smoking groups. The BMI of nondependent and never-smokers was lower than former smokers. Chi-square test indicated that groups differed significantly in sex distribution (χ^2 (3, 2088) = 9.3, Cramer's V = .07), use of antidepressants (χ^2 (3, 2088) = 15.5, Cramer's V = .09) and current diagnosis of an affective disorder (χ^2 (3, 2088) = 24.3, Cramer's V = .11).

Association Between Smoking Status and BDNF Genotype with Serum BDNF

Univariate ANCOVA revealed that, after adjusting for covariates, that is, age of the participant at baseline, education, alcohol, BMI, number of past-year negative life events, and antidepressant use, the main effect of smoking status on serum BDNF was significant (F (3, 2052) = 7.5; p < .001; partial η^2 = 0.01) suggesting that the four smoking groups had significantly different serum BDNF levels. The main effect of BDNF genotype and its interaction effect with smoking status on BDNF levels were nonsignificant (ps > .05). Follow-up analyses, adjusted for the above-mentioned covariates, revealed that serum BDNF of the two nonsmoking groups, that is, never-smokers (mean = 8.8, *SD* = 3.1) and former smokers (mean = 8.9, *SD* = 3.3) were significantly lower than the two current smoking groups: nondependent smokers (mean = 9.4, *SD* = 3.6) and nicotine-dependent smokers (mean = 9.5, *SD* = 3.6). Never-smokers were not significantly different from former smokers in serum BDNF levels (p > .05). Similarly, both the current smoking groups were comparable in serum BDNF (p > .05). Table 2 presents the estimates of the mean values (and their associated *SE* and 95% confidence intervals) of serum BDNF in the four smoking groups.

Pearson product-moment correlation showed a significant positive correlation of serum BDNF with total years of smoking (r = 0.14, N = 2088, p < .001), while a nonsignificant correlation with number of cigarettes smoked per day and nicotine dependence (ps > .05).

Regression analysis indicated that the first model with age, sex, education, and number of past-year negative life events explained 2.7 % of the variance in serum BDNF (p < .001). The second model that added alcohol use, BMI, antidepressant use, and the presence of an affective disorder, to the previous model, and the final model that added total years of smoking, cigarettes smoked per day, and nicotine dependence to the previous models, did not explain additional significant variance in serum BDNF (ps > .05). Age and total smoking years were significant predictors of serum BDNF, however, cigarettes smoked per day and nicotine dependence did not further predict serum BDNF (Table 3).

Discussion

We examined the levels of serum BDNF in never-smokers, former smokers, and current smokers with and without nicotine dependence, while controlling for potential confounding variables. As we expected, nondependent and nicotine-dependent current smokers had higher levels of serum BDNF than the two nonsmoking groups of former and never-smokers who were comparable with regard to serum BDNF levels. Inconsistent with our hypothesis, the two current smoking groups with and without nicotine dependence did not differ in serum BDNF. Moreover, we did not find nicotine dependence and number of cigarettes smoked per day to be significant predictors of serum BDNF. Thus, smoking severity was not associated with serum BDNF levels. However, total smoking years were a significant predictor of serum BDNF, indicating an

Table 3. Regression of Smoking Status on Serum BDNF[a]

Predictors	*B*	*SE*	β	*p*
Age	0.03	0.01	.11	.01**
Sex	0.09	0.27	.01	.74
Education	0.01	0.04	.01	.85
Past-year negative life events	−0.13	0.11	−.04	.25
Alcohol use	0.004	0.03	.01	.88
BMI	0.01	0.03	.02	.59
Antidepressant use	0.06	0.13	.02	.62
Diagnostic status of an affective disorder	0.04	0.26	.01	.87
Number of cigarettes smoked per day	−0.01	0.02	−.02	.68
Total smoking years	0.02	0.01	.10	.05*
Nicotine dependence	−0.13	0.12	−.05	.26

[a]Data have been shown only for the final model including all variables.
**$p \leq .01$; *$p \leq .05$.

influence of smoking chronicity on serum BDNF. Further, we did not find an interaction of BDNF genotype and smoking status on serum BDNF, which suggests that BDNF Val[66]Met polymorphism did not moderate the association between smoking and serum BDNF.

Animal research has shown that BDNF mRNA and protein expression in the hippocampus is enhanced after nicotine infusion[31], and that chronic nicotine administration in the hippocampus enhances BDNF mRNA expression, while acute nicotine administration reduces it.[32] This suggests that the association between upregulation of BDNF and nicotine use might be related to the amount and duration of smoking. It has been suggested that acute nicotine might increase 5-HT release in the hippocampus[61] and 5-HT_{2A} receptors regulate BDNF expression negatively, thus acute nicotine could decrease hippocampal BDNF gene expression by indirectly activating 5-HT_{2A} receptors. Alternatively, acute nicotine has inhibitory effects on BDNF mRNA. However, after chronic administration, tolerance may develop to the inhibitory effect of nicotine on BDNF mRNA expression[32], which may lead to gradual increase of BDNF levels in chronic smokers.

In humans, research on the link/associations between smoking and BDNF is sparse. There is some evidence that smokers have higher levels of serum BDNF than nonsmokers[33]. These preclinical and clinical studies are consistent with our findings of increased levels of serum BDNF in smokers. However, a causal association between smoking and BDNF cannot be established from our findings because of the cross-sectional design of the current study. Longitudinal investigations that examine changes over time in serum BDNF levels after smoking initiation or quitting are warranted in shedding more light on direction of the smoking–BDNF link.

Our findings are inconsistent with one study showing that nicotine-dependent smokers, with no history of psychiatric or substance-related disorder, had lower levels of serum BDNF as compared to nonsmokers.[62] However, one reason of this discrepency in findings might be an unreliable estimate because of the low sample size (16 nicotine-dependent smokers, and 13 nonsmokers) of this study.

An important limitation of the present study is that it is cross-sectional, so a causal association between BDNF and smoking cannot be established. Secondly, serum BDNF levels may not accurately reflect central BDNF levels, although previous animal research has shown a strong correlation between serum BDNF levels and cortical BDNF.[17] Thirdly, results of the present study on serum BDNF cannot be generalized to the studies conducted on BDNF stored in plasma or platelets because plasma BDNF is circulated in platelets with 200 fold less concentration than serum BDNF. Finally, the effect of other hormones, receptors or neurotransmitters and their interaction with serum BDNF were not taken into account which might have influenced our results.[63] Despite these limitations, the present study, with a fairly large sample size, highlights the need of investigating longitudinally the link between smoking and BDNF in humans, taking into account nicotine dependence. We were also able to control our analyses for the diagnosis of an affective disorder (depression or anxiety). This is important as stress, depression and anxiety have often been associated with central and peripheral reductions of BDNF levels in animals and humans.[38,40,41,64–66]

This study has important implications for future research on the neurobiology of addictive behaviors. Animal studies on whether a change in BDNF in the brain and the periphery is associated with addiction are needed, as well as human studies that longitudinally investigate whether quitting smoking and/or smoking initiation has an effect on serum BDNF levels. Finally, the tendency to smoke may result from a complex interaction of many genes and biological markers, in addition to environmental factors, which should be investigated in future research.

To conclude, current smokers have higher levels of BDNF as compared to the nonsmoking individuals and this is not due to the effect of nicotine dependence and/or BDNF Val[66]Met polymorphism. Moreover, higher levels of serum BDNF were positively associated with chronic cigarette smoking. Future studies that longitudinally address changes in BDNF in incident smokers and persons who quit smoking are needed to better understand the nature of the relationship between smoking and serum BDNF concentrations.

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Declaration of Interests

None declared.

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