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SMOKING AND OBESITY ASSOCIATED BDNF GENE VARIANCE PREDICTS TOTAL AND CARDIOVASCULAR MORTALITY IN SMOKERS

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Abstract

Objective

The brain-derived neurotrophic factor (BDNF) locus has been implicated in psychiatric and substance related disorders. Recent genome-wide association studies (GWAS) have shown strong associations between single nucleotide polymorphisms in BDNF, smoking behavior and high BMI. Our aim was to test if genetic BDNF variation alters the risk of smoking related morbidity and mortality.

Design

Cox proportional hazards models were used to relate the BDNF rs4923461(A/G) polymorphisms to all-cause, cancer and cardiovascular mortality and CVD incidence adjusted for age, sex, BMI and smoking quantity.

Setting

The Malmö Diet and Cancer Study (MDCS), a population based prospective cohort study (n=30 447).

Patients

We obtained complete data of 25 071 subjects of whom 6507 were current smokers and 18 564 non smokers who underwent a baseline examination 1991-1996.

Main Outcome Measures

During a mean follow-up time of 12 years, 1049 deaths (346 cardiovascular deaths and 492 cancer deaths) and 802 incident CVD events occurred among current smokers.

Results

The major allele (A) of rs4923461was significantly associated with ever having smoked (*P*=0.03) and high BMI (P=0.001). The A-allele was associated with risk of all-cause (HR=1.12, 95% CI 1.00-1.25, *P*<0.05) and CVD (HR=1.23, 95% CI 1.01-1.49, *P*=0.04) mortality. There was no significant association between the rs4923461 and cancer mortality or CVD incidence.

Conclusions

Our data suggests that smoking- and obesity-associated variation of the BDNF gene affects the risk of death, especially due to cardiovascular causes, in smokers. Determination of BDNF genotype in smokers may guide the intensity of smoke cessation interventions needed.

INTRODUCTION

Cigarette smoking accounts for several adverse health effects. Despite increasing awareness, more than 1 billion people worldwide smoke tobacco daily. Recent reports estimate that smoking accounts for nearly one of every five deaths each year in the United States. In addition to a distinct correlation with a number of cancer diagnoses, smoking is also estimated to augment the risk of coronary heart disease and stroke by 2 to 4 times, when compared to nonsmokers.(1)

Variance in smoking behavior is influenced by both psychosocial factors and genetic disposition, however tools for assessing future smoking related complications are lacking.(2, 3) Previous genome-wide association studies (GWAS) have identified multiple loci related to different smoking phenotypes. These include the brain-derived neurotrophic factor (BDNF) locus on chromosome 11 which showed a strong association with smoking initiation.(4) Furthermore, prior studies have showed a strong connection between the BDNF locus and body mass index BMI.(5, 6)

The protein BDNF belongs to a neurotrophin family and plays a critical role in regulating neuron protective mechanisms such as survival, function, development and plasticity of the cell.(7) The distribution in key regions of the central nervous system regulating mood and behavior has resulted in extensive studies related to several psychiatric disorders. Associations to substance related disorders; eating disorders and schizophrenia have been confirmed in a meta-analysis of case-control studies (8) and animal studies (9) and have suggested an important role in drug addiction by acting upon the reward system of the brain. Hypothesis that BDNF might be associated with nicotine addiction has further been suggested and differences in plasma levels of

BDNF have been seen in smokers compared to nonsmokers, indicating that chronic smoking leads to a down-regulation of the protein.(10)

Given the association with smoking behavior and substance related disorders, the purpose of this study was to test the hypothesis that genetic variations in the BDNF locus alter the risk of smoking related complications among smokers in the Malmö Diet and Cancer Study (MDCS); a population based prospective cohort study.

METHODS

Study Population

The population-based Malmö Diet and Cancer study (MDCS) included 12121 men born 1923-1945 and 18326 women born 1923-1950 from Malmö, Sweden. Participants attended baseline examinations between 1991 to1996.

All participants provided written informed consent and study protocols were approved by the ethical committee at Lund University, Lund, Sweden.

We selected the BDNF rs4923461, the lead SNP in previous GWAS for BMI (6), as it is in almost complete linkage disequilibrium (www.HapMap.org) with the SNPs shown to be smoking associated in recent GWAS (4). Genotyping of rs4923461 was successfully performed in 27 508 subjects out of 28 564, success rate 96.3%. Complete data for age, sex, BMI and smoking status, was available in 25 789 individuals. This cohort was used in analysis of genotype and ever smoker status. (Table 1)

Complete data for age, sex, BMI, smoking status as well as quantity (cigarettes per day, CPD) (11) was available for 25 071 individuals. This cohort was used in analysis of genotype and prediction of smoking related complications. (Table 1)

Socioeconomic status (SES) was defined by the highest level of education and the participants were divided into three groups. The low SES group had not completed elementary school, corresponding to a maximum of 6 to 8 years of education; the middle SES group had 9 to 12 years of education and the high SES group reported a university degree or studies at least one year after GCE (General Certificate of Education).(12)

Smoking status

Study participants (n=25 789) were classified as current smokers (n=7225) if they reported smoking regularly (n=6057) or sometimes within the past year (n=1168) and as non smokers (n=18 564) if they reported never having smoked (n=9773) or having quit smoking at least one year before interview (n=8791). Further on, a combined group of ever smokers was formed, consisting of the current and previous smokers (n=16 016).

After further adjustment for smoking quantity, complete data in current smokers was registered for 6507 current smokers of which 5647 subjects reported to smoke regularly and 860 to smoke occasionally. We have no data on smoking status after the baseline exam.

DNA extraction and genotyping

Genotyping of BDNF rs4923461 was performed using TaqMan (Applied Biosystems) with primers and conditions according to the manufacturer´s recommendation.

Clinical Endpoints

Four endpoints were examined; total mortality, cardiovascular mortality, cancer mortality and first incidence of cardiovascular disease (CVD).

Information on total mortality, cardiovascular mortality and cancer mortality during follow-up was retrieved by linking the 10-digit civil registration number with the Swedish National Cause of Death Register (SNCDR). Mortality was classified as attributable to cardiovascular causes when the main International Classification of Diseases (ICD) code was 390–459 (ICD 9) or I00-I99 (ICD 10) and was attributable to cancer when the ICD code was 140–239 (ICD 9) or C00-C99 (ICD 10) on the cause of death certificate.

CVD was defined as fatal-or non-fatal myocardial infarction (MI) or stroke or death due to ischemic heart disease from the Swedish Hospital Discharge Register or SNCDR. MI was defined as codes 410 (ICD9) or I21 (ICD10), death due to ischemic heart disease using codes 412 and 414 (ICD9) or I22-I23 and I25 (ICD10) and stroke using codes 430,431,434 and 436 (ICD9) or I60-I61, I63 and I64 according to ICD10.

Follow-up extended until January 1st 2007. Mean (SD) follow-up in analysis of mortality and analysis of first incident CVD was 12 (3) years.

Statistical Analysis

SPSS version 19.0 (IBM Corporation, Route 100 Somers, NY 10589) was used for all calculations.

Continuous variables are reported as means (SD) and dichotomous variables as numbers (%).

Cross-sectional relationships between genotype and smoking status were evaluated with crude and multivariate adjusted logistic regression. Relationships between genotype and BMI were tested with crude and multivariate adjusted linear regression models.

We calculated crude and multivariate adjusted hazard ratios (HR) and 95% confidence intervals (95% CI) for genotype in relation to incidence of the four different endpoints during follow-up using Cox Proportional Hazards Model.

A two-sided p-value of < 0.05 was considered as statistically significant.

A post hoc power analysis showed that the power among current smokers to detect the observed BDNF genetic effect on total mortality was 82%.

RESULTS

Baseline characteristics

Approximately 60% of the MDCS participants were women. The mean (SD) age was 57 **(**8) years, mean BMI 26 (4) kg/ m² and the mean value of number of cigarettes per day in current smokers were 13(7). The participating men demonstrated a mean age of 59 (7) years, a mean BMI of 26 (4) kg/ m² and a mean intake of 16 (9) cigarettes per day. The genotype distribution in the population did not deviate from Hardy Weinberg Equilibrium (P=0.09).

BDNF rs 4923461 Polymorphism Related to Ever Having Smoked and BMI

In an additive model, the major allele (A), which previously has been associated with smoking and with high BMI, was significantly associated with increased odds of ever having smoked (OR=1.050, 95% CI=1.004-1.098, *P*=0.032). After adjustment for age and sex, the association

was still significant (OR=1.050, 95% CI=1.003-1.099, *P*=0.035) as well as after additional adjustment for BMI (OR= 1.052; 95% CI= 1.006-1.101; *P*=0.028). Also, the major allele was significantly associated with BMI in crude analysis (β=0.145±0.044, *P*=0.001) as well as after age and sex adjustment (β =0.155, ±0.044, *P*<0.001).

BDNF rs 4923461 Polymorphism and Smoking Related Complications in Current Smokers

Total mortality

During follow-up 1049 (16.1%) deaths occurred among smokers.

Additive models, with the major allele (A) coded and adjustment for age and sex (Model 1), showed that each copy of the smoking associated allele was associated with significantly increased risk of death (Hazard ratio; 95% confidence interval) (1.131; 1.013-1.263). After further adjustment including age, sex and BMI (Model 2) the increased risk of death remained significant (1.131; 1.013-1.263) as well as after additional adjustment for smoking quantity (Model 3) (1.118; 1.002-1.249). Multivariable-adjusted hazard ratios per genotype with the GG genotype as the reference and *P*-values for trend are shown in Table 2.

Death from Cardiovascular Disease

Among current smokers 346 (5.3%) cardiovascular deaths occurred. In all three additive models (Models 1-3), higher hazard ratios were significantly related to presence of the A-allele (1.247; 1.024-1.518), (1.235; 1.014-1.503) and (1.225; 1.006-1.492), respectively. Multivariable-adjusted hazard ratios per genotype and *P*-values for trend are shown in Table 2.

Death from Cancer

A number of 492 (7.6%) deaths from cancer were reported during follow-up. No significant association between the A-allele and risk of cancer mortality was observed in additive models (Models 1-3) (1.142; 0.971-1.343), (1.145; 0.973-1.346) and (1.127; 0.959-1.326) respectively.

A significant association indicating an increased risk of cancer mortality among the major homozygotes (A/A) compared to the minor homozygotes (G/G) was seen in Models 1-3. Multivariable-adjusted hazard ratios per genotype and *P*-values for trend are shown in Table 2.

First Incidence in Cardiovascular Disease

When analyzing the BDNF polymorphism in relation to first incident CVD event, a total of 6321 cases with complete data were registered and included 802 (12.7%) first CVD events. No significant association of BDNF rs4923461 was present in the additive models (Models 1-3) (1.092; 0.963-1.238), (1.079; 0.952-1.224) and (1.070; 0.944-1.213), respectively. Multivariable-adjusted hazard ratios per genotype and *P*-values for trend are shown in Table 2.

Additional adjustments

The exposure for cigarette smoke was in addition to CPD calculated as "pack years". The results were unchanged, here demonstrated with Model 3 for the endpoints total mortality and CVD mortality: (HR: 1.117; 95% CI: 1.000-1.248; *P*: 0.049) (1.229; 1.009-1.498; 0.040). The variable socioeconomic status had little impact on the results, why it is not included in the tables.

BDNF rs 4923461 Polymorphism and Smoking Related Complications in Non Smokers

We reproduced Cox regression analyses for subjects reporting to be never or previous smokers, excluding the variable CPD. In this group, 1871 (10.1%) deaths occurred, 603 (3.2%) cardiovascular deaths and 877 (4.7%) cancer deaths. A total of 1606 (8.9%) first CVD events were reported.

There was no association between rs4923461 and any of the four endpoints in non-smokers. A borderline significant inverse association between the A-allele and cancer mortality (0.898; 0.802-1.006) was observed (Table 3).

To distinguish the group of previous smokers (n=8791) from never smokers, this group was analyzed separately, but no associations were seen in this subgroup either (data not shown).

DISCUSSION

We show here that a genetic variation of the previously smoking and high BMI associated BDNF locus predicts an increased risk of dying among smokers, especially the risk of death from cardiovascular diseases. Our research also confirms the recent associations to regular smoking (4) and increasing BMI.(6) Thus, genetic variation of the BDNF locus not only increases the likelihood of being a smoker, but also confers an increased risk of death among smokers. Assuming that the genetic BDNF association with mortality is attributable to a lesser likelihood of smoking cessation during long term follow-up, our results may have clinical implications warranting more intense smoking cessation interventions in subjects at such increased genetic risk.

The associations between the genetic BDNF variation and the outcomes total and cardiovascular mortality are independent of traditional risk factors such as age, sex, BMI and smoking quantity. This indicates that, despite the association between the BDNF locus and BMI, the association with mortality in smokers is not mediated by obesity and its complications, such as diabetes mellitus but rather with prolonged exposure to smoking due to lesser likelihood of smoking cessation during follow-up. This interpretation is further supported by total lack of association between the BDNF locus and mortality among never or previous smokers. However, we cannot exclude other causes of the increased mortality rates among smokers carrying the risk allele. For example, genetic BDNF variation has also been linked to other substance abuse disorders and

psychiatric diseases (8) with well lower life expectancies. Unfortunately we do not have records of substance abuse or psychiatric disorders and therefore these subjects could not be excluded from analyses.

We could not predict new events of CVD in our cohort. One theory being, that smokers have had their first event before baseline exams and therefore were excluded in our analysis of incident CVD. Another explanation, as discussed above, is that the first event more often had a severe outcome in patients who use tobacco.

While the ample size of our cohort provides us with adequate statistical power to detect associations, the wide confidence intervals demand a need for caution when drawing conclusions. In order to improve precision in our results, it is possible that a genetic smoking propensity score based on GWAS identified SNPs may have greater predictive accuracy. (13) Moreover, chronic obstructive pulmonary disease (COPD) is a frequent consequence of both smoking and CVD which we did not take into account.

Tobacco consumption conveys negative consequences both for the health of the individuals as well as increasing national health care costs. Although there has been substantial progress in preventing the spread of tobacco use worldwide, it is still the single most preventable cause of death in the United States (14). Nicotine replacement therapies (NRT) have shown low quitting rates pointing out the need for new methods of intervention (15). The progress of applicable techniques for genetic decoding, continuously introduce new possibilities for identifying individuals with a higher risk of being smokers. In conclusion genetic BDNF variation predicts risk of death in smokers. Our results suggest that future treatment may involve the molecular

consequences of this genetic variation or guide the intensity of smoke cessation interventions needed by determination of BDNF genotype in smokers.

CONTRIBUTORSHIP

Conception and design, or analysis and interpretation of data: Halldén, Sjögren, Hedblad, Engström, Melander.

Drafting the article or revising it critically for important intellectual content: Halldén, Sjögren, Hedblad, Engström, Narkiewicz, Hoffmann, Wahlstrand, Hedner, Melander.

Final approval of the version to be published: Halldén, Melander.

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COMPETING INTERESTS

None declared.

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TABLE 1

Distribution of genotypes in the study population

	A/A	A/G	G/G
All*	16 414	8375	1000
(n=25 789)	(63.5%)	(32.5%)	(4.0%)
Ever smokers	10 256	5171	589
(n=16 016)	(64.0%)	(32.5%)	(3.5%)
Non smokers	6158	3204	411
(n=9773)	(63.0%)	(33.0%)	(4.0%)
All†	15 959	8146	966
(n=25 071)	(63.5%)	(32.5%)	(4.0%)
Current smokers	4192	2074	241
(n=6507)	(64.5%)	(32.0%)	(3.5%)
Non smokers	11 767	6072	725
(n=18 564)	(63.5%)	(32.5%)	(4.0%)

* In cohort with complete data for sex, age, rs4923461, BMI and smoking status

† In cohort with complete data for sex, age, rs4923461, BMI, smoking status and smoking quantity

TABLE 2

Multivariable-adjusted Hazard Ratios per Genotype in Current Smokers

Genotype	**G/G**	**A/G**	**A/A**	
		HRs 95% CI		*P*trend
Total mortality				
Model 1	1.0 (ref)	1.091 (0.776-1.534)	1.224(0.894-1.732)	**0.029**
Model 2	1.0(ref)	1.091 (0.776-1.534)	1.224 (0.893-1.732)	**0.029**
Model 3	1.0 (ref)	1.101 (0.783-1.548)	1.236 (0.887-1.720)	**0.047**
CVD Mortality				
Model 1	1.0 (ref)	1.061 (0.582-1.934)	1.372 (0.768-2.451)	**0.028**
Model 2	1.0 (ref)	1.057 (0.580-1.928)	1.352 (0.756-2.416)	**0.036**
Model 3	1.0 (ref)	1.062 (0.582-1.936)	1.344(0.752-2.402)	**0.047**
Cancer Mortality				
Model 1	1.0 (ref)	1.788 (0.970-3.295)	1.880(1.030-3.249)*	0.108
Model 2	1.0 (ref)	1.791 (0.972-3.302)	1.888(1.035-3.444)*	0.103
Model 3	1.0 (ref)	1.815 (0.984-3.345)	1.872(1.026-3.415)*	0.147
First Incident CVD				

Model 1	1.0 (ref)	0.940 (0.647-1.366)	1.063 (0.740-1.527)	0.171
Model 2	1.0 (ref)	0.939 (0.644-1.360)	1.045 (0.727-1.501)	0.233
Model 3	1.0 (ref)	0.936 (0.644-1.361)	1.034 (0.720-1.486)	0.291

***P*<0.050**

Adjustments:

Model 1: Age and sex.

Model 2: Age, sex and BMI.

Model 3: Age, sex, BMI and cigarettes per day (CPD)

TABLE 3

Multivariable-adjusted Hazard Ratios per Genotype in Non Smokers

Genotype	**G/G**	**A/G**	**A/A**	
		HRs 95% CI		*P*trend
Total mortality				
Model 1	1.0 (ref)	1.090(0.853-1.393)	1.024 (0.806-1.302)	0.414
Model 2	1.0 (ref)	1.080 (0.845-1.381)	1.015 (0.799-1.290)	0.386
CVD Mortality				
Model 1	1.0 (ref)	0.861 (0.571-1.299)	0.933 (0.627-1.388)	0.646
Model 2	1.0 (ref)	0.851 (0.564-1.283)	0.923 (0.621-1.373)	0.655
Cancer Mortality				
Model 1	1.0 (ref)	1.238 (0.860-1.780)	1.028 (0.719-1.469)	0.064
Model 2	1.0 (ref)	1.228 (0.854-1.767)	1.019 (0.713-1.457)	0.059
First Incident CVD				
Model 1	1.0 (ref)	0.883 (0.688-1.134)	0.902 (0.708-1.149)	0.850
Model 2	1.0 (ref)	0.872 (0.679-1.120)	0.890 (0.698-1.133)	0.782

Adjustments:

Model 1: Age and sex.

Model 2: Age, sex and BMI.