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Brain-Derived Neurotrophic Factor rs6265 (Val66Met) Polymorphism Is Associated with Disease Severity and Incidence of Cardiovascular Events in a Patient Cohort

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Abstract

Background—The rs6265 (Val66Met) single-nucleotide polymorphism in the *BDNF* gene has been related to a number of endophenotypes that have in turn been shown to confer risk for atherosclerotic cardiovascular disease (CVD). To date, however, very few studies have examined the association of the Val66Met SNP with CVD clinical outcomes.

Methods—In a cohort of 5510 Caucasian patients enrolled in the CATHeterization GENetics (CATHGEN) study at Duke University Hospital between 2001 and 2011, we determined the

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severity of coronary artery disease (CAD) and CVD event incidence through up to 11.8 years of follow-up. We examined the association of Val66Met genotype with time-to-death or MI, adjusting for age, sex, CAD risk variables, and CAD severity measures.

Results—The Val/Val genotype was associated with a higher risk than Met carriers for clinical CVD events ($P = 0.034$, $HR = 1.12$, 95% $CI = [1.01, 1.24]$). In addition, compared to Met carriers, individuals with the Val/Val genotype had a greater odds of having more diseased vessels (NDV) ($OR = 1.17$, 95% $CI [1.06, 1.30]$, $P = 0.002$), and lower left ventricular ejection fraction (LVEF) ($\beta = -0.72$, 95% $CI [-1.42, -0.02]$, $P = 0.044$).

Conclusions—The Val/Val genotype was associated with greater severity of CAD and incidence of CVD-related clinical events in a patient sample. If these findings are confirmed in further research, intervention studies in clinical groups with the Val/Val genotype could be undertaken to prevent disease and improve prognosis.

Keywords

BDNF Val66Met; rs6265; CVD event; death; myocardial infarction

INTRODUCTION

It has been proposed that genetic variants can affect the development and clinical course of cardiovascular disease (CVD) by a number of behavioral and biological pathways that are ultimately involved in CVD pathogenesis and precipitation of clinical CVD events (1). These pathways may include an inclination toward increased HPA-axis activation in response to psychosocial stress, obesity, inflammation, and more severe addiction to tobacco. The gene that encodes brain-derived neurotrophic factor (BDNF) is a promising candidate to be associated with the development and course of CVD. BDNF protein is an important neurotrophin, regulating survival and growth of neurons and synapses (2), and protecting against stress-induced neuronal damage (3). The BDNF rs6265 G/A variant has been shown to be functional, changing a valine at position 66 of the BDNF protein to a methionine (Val66Met, (4,5)) and has, therefore, received considerable attention with respect to the above risk factors.

A number of epidemiological studies, for example, have found that Val carriers have higher body mass index (BMI) (6–9) than Met carriers. In a study of the association between the Val66Met polymorphism and metabolic risk variables among patients with fibromyalgia syndrome (10), the Val/Val genotype was associated with higher mean BMI and high-sensitivity C-reactive protein (hsCRP) levels than did those carrying the Met genotype. Jiang et al. (11) also reported that among patients with unstable angina pectoris (UAP), Val/Val genotype was associated with higher plasma hsCRP levels than Met carriers. In our previous work (12), we found a consistent pattern in two independent samples that the depressive symptom levels as a function of chronic stress increased significantly more among persons with the Val/Val genotype than Met carriers. We have also found that blood cortisol levels are higher in Val/Val females during a laboratory stress protocol of anger and sad recall than in Met females in a sample of experimental study (13). The latter finding may be relevant to CVD in that chronically increased HPA axis function, indicated by elevated hair cortisol

levels, also has been associated with increased risk of acute myocardial infarction (MI) in men (14). Moreover, larger salivary cortisol responses to mental stress in men and women have been associated with increased levels of coronary artery calcification (15). Studies also have shown a larger HPA axis and cardiovascular responses to acute psychosocial stress in Val/Val males (16,17). The higher plasma hsCRP level is an independent predictor of cardiovascular events (18,19). Given the above, we hypothesized that the Val/Val genotype would be associated with increased severity of coronary artery disease (CAD) and incidence of CVD mortality and morbidity in a large sample of Caucasian patients, who have been evaluated in the cardiac catheterization laboratory at Duke University Hospital (Durham, NC).

METHODS

Participants

The collection of data on CATHeterization GENetics (CATHGEN) subjects has been described in detail elsewhere (20–22). Briefly, CATHGEN study participants were recruited through the cardiac catheterization laboratories at Duke University Hospital (Durham, NC, USA), with approval from the Duke Institutional Review Board, and all participants signed informed consent. Although the CATHGEN study is comprised a variety of ethnic groups, the majority of subjects are Caucasian (75.0%). To avoid the confounding effects of population stratification while maximizing our power to detect genetic associations, we chose to analyze the Caucasian participants only. In the present study we included 5510 Caucasian participants who had data on the variables interest and covariates.

Genotyping

Genomic DNA was extracted from frozen whole blood samples collected from the participants during cardiac catheterization. The *BDNF* Val66Met (rs6265) SNP was genotyped using the Taqman genotyping assay (Life Technologies) and the Type-It Fast Probe PCR kit (Qiagen). For each 5 µl reaction a solution containing 2µl Type-It Fast Probe PCR Master Mix (Qiagen), 0.05 µl 80x Taqman assay (Life Technologies), and 0.5 µl Q-solution (Qiagen) was added to 3 ng of dried DNA template. The temperature program was 95 °C for 5 minutes, then 50 cycles of 95 °C for 15 seconds and 60°C for 30 seconds, run on the GeneAmp PCR system 9700 (Life Technologies). Fluorescence was read and analyzed on the ViiA 7 instrument (Life Technologies). PCR reactions were performed in 384 well format with 4 negative controls. The SNP genotype call rate was above 95%, and the genotype frequencies were in Hardy-Weinberg equilibrium ($p = 0.866$).

Disease Severity: Number of diseased vessels and left ventricular ejection fraction

Severity of CAD was determined as an ordinal variable defined as the number of diseased vessels (NDV) with significant (≥ 75%) stenosis as determined by the interventional cardiologist performing the procedure. The greater the value of NDV (0–3), the more severe the CAD. Left ventricular ejection fraction (LVEF) is expressed as the percentage of blood pumped out of the left ventricle on systole. LVEF was measured either during cardiac catheterization, or chart review for an echocardiogram, nuclear study or cardiac MRI done within 1 year on either side of the catheterization.

Events

As part of the ongoing follow-up of the Duke Databank for Cardiovascular Diseases (DDCD), CATHGEN participants are surveyed annually for CVD events and the participant list is compared to the National Death Index. The events were defined as death from any cause or myocardial infarction (MI) (number of days from cardiac catheterization to the first subsequent MI had to be ≥ 7 to avoid any effects from cardiac catheterization), and they were followed for up to 11.8 years with a median follow-up time of 5.9 years.

Risk Factors

Body mass index (BMI), and binary indicators of history of cigarette smoking, dyslipidemia, hypertension, and history of diabetes were all assessed at the time cardiac catheterization, *i.e.*, at the beginning of the study. No data were available to provide a measure of life stress or depression.

Statistical Analyses

All analyses were performed using SAS version 9.4 (SAS Inc., Cary, NC). Patients who were homozygous for the Met allele were combined with the heterozygous individuals as the Met group to be compared with homozygous Val/Val patients. The statistics for demographic, risk factor, and disease severity variables were analyzed between Val66Met genotypes (Val/Val vs. Met) using linear, binary logistic or ordinal logistic regression adjusting for age and sex, depending on the measurement characteristics of the outcome variable. Cox regression models were estimated using defined events as the response variable. Participants who were lost to follow-up or who had not experienced an event by the last date of follow-up were coded as censored. Our primary analysis was to test the Val66Met genotype association with death or MI using proportional hazards models, carried out in three incremental stages: Model 1: adjusting for age and sex; Model 2: adding adjustment for the risk factors of BMI, history of smoking, history of diabetes, history of dyslipidemia, history of hypertension; Model 3: adding adjustment for the potential mediating mechanisms of LVEF, and NDV. Proportional hazards assumption was met for all models. We also evaluated the association of Val66Met with NDV and LVEF using ordinal logistic regression model and linear regression, respectively. Using the SPPOWER simulation algorithm available in the rms package in R, we estimated that we had 80% power at $\alpha = 0.05$ to detect a hazard ratio of at least 1.30 favoring the Val/Val group compared to Met carriers.

RESULTS

The clinical characteristics of the study sample are presented in Table 1 stratified by Val/Val and Met carriers (Met/Met + Val/Met). None of the demographic or CVD risk factors were statistically different between Val66Met genotypes. However, CAD severity was significantly higher in Val/Val carriers than Met group, as measured by higher NDV (OR = 1.17, 95% CI [1.06, 1.30], $P = 0.002$), and lower LVEF ($\beta = -0.72$, 95% CI [-1.42, -0.02], $P = 0.044$). We observed 1,622 clinical events over a median follow-up time of 5.9 years (interquartile range = 3.9–8.6). The unadjusted proportion of individuals experiencing

clinical events was slightly higher in the Val/Val group (N=1093, 30.1%) compared to the Met group (N=529, 28.1%), but this difference was not statistically significant ($P = 0.104$).

The fitted survival curves for both genotypes are presented in Figure 1, quantified in the Cox regression model. Parallel to the raw event frequency counts, Val/Val carriers had the more rapid time-to-event rate. Table 2 displays the results of hazard ratios (HR) in the age/sex only adjusted (Model 1) and additional covariate adjusted Cox regression models (Model 2 and Model 3). In the age/sex only adjusted Cox model (see Table 2, Model 1), Val/Val carriers were at higher risk than Met carriers for the clinical CVD events of all-cause death or MI (HR = 1.12, 95% CI = [1.01, 1.24], $P = 0.034$). After adjusting for additional risk factors (Model 2) including BMI, history of diabetes, history hypertension, history of dyslipidemia, and smoking history (see Table 2, Model 2), the increased risk for Val/Val still remained (HR=1.11, 95% CI = [1.00, 1.24], $P = 0.04$). However, after further adjusting for additional CAD severity indicated by NDV and/or LVEF, the effect of Val66Met on the clinical events was no longer significant (HR=1.08, 95% CI = [0.97, 1.20], $P = 0.148$). Subsidiary analyses including one disease severity measure at a time both resulted in the effect of Val66Met being reduced and no longer statistically significant (HR for Val66Met with NDV only HR = 1.09, 95% CI = [0.98, 1.21], $P = 0.096$; HR for LVEF only, HR = 1.10, 95% CI = [0.99, 1.22], $P = 0.072$). In terms of the effects of the covariates in the models, with the exception of history of hypertension and sex, all of the adjustment covariates were associated with CVD events in the fully adjusted (Model 3, adjusted for risk factors, NDV, and LVEF) Cox regression model (see Table 2, Model 3). Older age, history of smoking, history of diabetes, and a higher number of coronary arteries were associated with increased rates of events, while higher BMI, higher LVEF, and history of dyslipidemia were associated with a lower risk of events.

The ordinal logistic regression model using Val66Met to predict NDV, adjusted only for age and sex, showed that the Val/Val subjects had a greater risk for higher CAD severity (higher number of diseased vessels) than Met subjects (OR=1.17, 95% CI = [1.06, 1.30], $P = 0.002$). After adjusting for additional risk factors, the point estimate for Val66Met was slightly reduced, but remained significantly related to NDV (OR=1.15, 95% CI = [1.04, 1.28], $P = 0.008$). The linear regression model of LVEF adjusted for age and sex also indicated that Val/Val carriers had a higher risk of higher CAD severity (lower LVEF) than Met carriers ($\beta = -0.72$, 95% CI = [-1.42, -0.02], $P = 0.044$). After adjusting for additional risk factors, the higher risk of LVEF for Val/Val remained statistically significant ($\beta = -0.70$, 95% CI = [-1.40, -0.001], $P = 0.0497$). However, adjusting for both risk factors and NDV, the effect of Val66Met on LVEF became nonsignificant ($\beta = -0.52$, 95% CI = [-1.21, -0.17], $P = 0.142$).

DISCUSSION

The *BDNF* Val66Met polymorphism was associated with CAD severity and clinical CVD events over a median 5.9-year follow-up period in our clinical sample. Specifically, Val/Val homozygotes had more diseased vessels, lower LVEF, and a 12% higher hazard of clinical CVD events compared to Met carriers. Adjustment for demographics and conventional risk factors did not explain the association between the genetic variant and clinical events, ruling

out confounding with these factors. However, adjustment for the two disease severity markers did reduce the gene-event association, while the variant was associated with both of the disease markers. Taken together, these observations suggest that one way the variant may be influencing event risk is by way of its impact on atherogenesis. As we have noted, our hypothesis that Val66Met would be related to cardiac clinical events was based on prior literature that showed associations between the variant and risk factors for CAD, including depressive symptoms (12), and larger HPA axis reactivity (13,16,17). The present finding lends further support to this proposition. These findings also are consistent with at least one study of Val66Met with respect CAD, which found that the Val/Val genotype, compared to Met carriers, was associated with a higher occurrence of unstable angina pectoris in the population of Han Chinese (11).

The current findings may have important implications for the development of targeted preventive interventions and treatments. It has been shown, for example, that cognitive behavioral stress management (CBSM) training produces reduced anxiety and cortisol levels that are maintained over a 12-month follow-up period in women undergoing treatment for breast cancer (23) as well as reduced depression and blood pressure and heart rate both at rest and during acute mental stress in men recovering from coronary artery bypass surgery (24). Two randomized controlled trials of CBSM training delivered in small groups of CHD patients found that those receiving CBMS training had reduced mortality and recurrent clinical events (25,26). A clinical trial of such cognitive behavioral stress management training in CHD patients homozygous for the *BDNF* Val allele could, for example, result in improved prognosis.

There are several limitations to the present study that should be noted. Indices of HPA axis function were not available in the sample, making it impossible to directly test the role of increased HPA axis function as a mechanism mediating adverse outcomes among those with the Val/Val genotype. The absence of adequate measures of depressive symptom levels in the patients makes it impossible to test the association of depression with HPA axis function and/or clinical events. Other factors such as physical exercise, life styles and gene-gene interaction could also impact the outcomes. Future studies in African Americans need to be done to see if Val/Val associations with disease severity and prognosis are similar to those found in Caucasian CHD patients. Although compelling evidence indicates that this functional *BDNF* Val66Met coding variant should be considered a prime candidate influencing the development and the course of CHD, further investigation of the genetic sequence in the remainder of the gene and in the nearby regions should be performed to determine whether it is the causal variant creating that CHD risk and whether better genetic markers of that risk exist. Despite these limitations, the consistency of the association of the Val66Met Val/Val genotype with more severe documented coronary atherosclerosis, reduced LVEF and increased CHD event rates makes a strong case that the Val66Met polymorphism deserves continuing attention in the search for genetic variants that potentiate effects of environmental stress on the development and course of CHD. Further replication and studies on the underlying mechanisms should be done -- for instance, the effects of genetic variants might be via methylation profiling and/or gene expression on the endophenotypes in the development of CVD.

In conclusion, we found that a functional *BDNF* SNP, Val66Met, that has been previously associated with increased cortisol response to stress, depression, and metabolic traits linked to CVD, was associated with increased CAD severity and incidence of death or MI, independent of traditional CHD risk factors in a large clinical sample of Caucasian patients. If these findings are replicated in further research, behavioral and pharmacologic interventions to reduce HPA axis reactivity and depression in Val/Val carriers could be potentially used as a means of improving CVD-related prognosis in patients, and ultimately in primary prevention.

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Figure 1. Fitted survival curves illustrating the risk of death or myocardial infarction for *BDNF* Val66Met genotype
The difference between Val/Val and Met carriers based on the Cox regression model of CVD events, adjusted for age and sex only ($P = 0.034$) was statistically significant, but became non-significant adjusted for risk factors and CAD severity ($P = 0.148$).

Table 1

Characteristics stratified by Val66Met genotype.

	Val/Val N=3627 (65.8%)	Met N=1883 (34.2%)	P-value
Female, N (%)	1247(34.4)	636 (33.8)	0.664 ^a
Age in years, mean(SD)	62.6 (11.4)	62.5 (11.7)	0.854 ^b
Body mass index (kg/m ²), mean (SD)	29.7 (6.8)	29.5 (6.7)	0.269 ^c
Left ventricular ejection fraction, mean (SD)	56.6 (13.0)	57.3 (12.5)	0.044 ^c
History of hypertension, N (%)	2440 (67.3)	1226 (65.1)	0.111 ^d
History of hyperlipidemia, N (%)	2302 (63.5)	1195 (63.5)	0.978 ^d
History of diabetes, N (%)	925 (25.5)	478 (25.4)	0.923 ^d
History of smoking, N (%)	1880 (51.8)	933 (50.0)	0.085 ^d
Number of events, N (%)	1093 (30.1)	529 (28.1)	0.104 ^d
Number of diseased vessel, N (%)	0		
	1	833 (23.0)	459 (24.4)
	2	662 (18.3)	310 (16.5)
	3	890 (24.5)	415 (22.0)

^aDerived from binary logistic regression model adjusted for age only^bDerived from linear model adjusted for sex only^cDerived from linear model adjusted for age and sex^dDerived from binary logistic regression model adjusted for age and sex^eDerived from ordinal logistic regression model adjusted for age and sex

Table 2

Cox regression models predicting death or myocardial infarction (N=5,510, N events =1,622)

	Model 1			Model 2			Model 3		
	HR	95% CI	P	HR	95% CI	P	HR	95% CI	P
Val66Met (Val/Val vs. Met)	1.12	1.01, 1.24	0.034	1.11	1.00, 1.24	0.04	1.08	0.97, 1.20	0.148
Age (years)	1.05	1.04, 1.05	<0.0001	1.05	1.04, 1.05	<0.0001	1.04	1.04, 1.05	<0.0001
Sex (male vs. female)	1.27	1.14, 1.41	<0.0001	1.20	1.08, 1.33	0.0009	0.98	0.87, 1.09	0.685
BMI (kg/m ²)				0.99	0.98, 1.00	0.005	0.99	0.98, 1.00	0.015
History of smoking				1.47	1.33, 1.63	<0.0001	1.41	1.27, 1.56	<0.0001
History of dyslipidemia				0.77	0.70, 0.86	<0.0001	0.75	0.67, 0.83	<0.0001
History of hypertension				1.06	0.95, 1.19	0.308	1.05	0.94, 1.17	0.414
History of diabetes				1.64	1.47, 1.82	<0.0001	1.53	1.37, 1.71	<0.0001
NDV							1.15	1.10, 1.20	<0.0001
LVEF							0.98	0.98, 0.98	<0.0001

HR = Hazard ratio; 95% CI = 95% Confidence Interval; BMI = Body mass index; NDV = number of diseased vessels; LVEF = Left ventricular ejection fraction.

Model 1: adjusted for age and sex only;

Model 2: adjusted for age, sex, and risk factors (history of smoking, dyslipidemia, hypertension, diabetes, body mass index);

Model 3: adjusted for age, sex, risk factors (history of smoking, dyslipidemia, hypertension, diabetes, body mass index), and CAD severity (NDV and LVEF).