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BDNF *Val*^{66}*Met* genotype is associated with drug-seeking phenotypes in heroin-dependent individuals: a pilot study

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ABSTRACT

Brain-derived neurotrophic factor (BDNF) *Val*^{66}*Met* genotype has been associated with neurobehavioral deficits. To examine its relevance for addiction, we examined BDNF genotype differences in drug-seeking behavior. Heroin-dependent volunteers ($n = 128$) completed an interview that assessed past-month naturalistic drug-seeking/use behaviors. In African Americans ($n = 74$), the *Met* allele was uncommon (carrier frequency 6.8%); thus, analyses focused on European Americans ($n = 54$), in whom the *Met* allele was common (carrier frequency 37.0%). In their natural setting, *Met* carriers ($n = 20$) reported more time- and cost-intensive heroin-seeking and more cigarette use than *Val* homozygotes ($n = 34$). BDNF *Val*^{66}*Met* genotype predicted 18.4% of variance in 'weekly heroin investment' (purchasing time × amount × frequency). These data suggest that the BDNF *Met* allele may confer a 'preferred drug-invested' phenotype, resistant to moderating effects of higher drug prices and non-drug reinforcement. These preliminary hypothesis-generating findings require replication, but are consistent with pre-clinical data that demonstrate neurotrophic influence in drug reinforcement. Whether this genotype is relevant to other abused substances besides opioids or nicotine, or treatment response, remains to be determined.

Keywords BDNF, genotype, cigarette, drug-seeking, heroin, opioid.

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Genetic association studies of addictive disorders typically attempt to identify polymorphisms underlying initial vulnerability (e.g. Enoch *et al.* 2009; Saccone *et al.* 2009; Yuferov *et al.* 2010). Yet, genetic influences may operate at various stages of the addiction cycle (Li & Burmeister 2009; Khokhar *et al.* 2010). Little is known about which biologically plausible functional genotypes alter *persistence* of addictive behaviors. Improved knowledge could help predict resistance to, or benefit from, treatments. Unlike studies of vulnerability, genetic studies of addictive persistence target individuals who are already drug-dependent because the goal is to understand genotypic and phenotypic heterogeneity within the clinical population.

Substance use disorders are complex syndromes that are not ideally suited for genetic studies (Wong & Schumann 2008). Phenotype selection requires a targeted approach (Gottesman & Gould 2003; Ducci & Goldman 2008; Lerman, Perkins & Gould 2009). Assessing an intermediate phenotype (versus a broader phenotype such as a nosological condition) is preferable because a circumscribed measure will tend to be more reliable (which improves power to find associations with the genotype) and perhaps more closely related to genetic underpinnings than a multifactor syndrome. In the present research, we selected *drug-seeking behavior* (intermediate phenotype) by heroin-dependent, out-of-treatment volunteers because (1) individual drug-seeking patterns are periodic (within days) and stable (between days) due to physical dependence and motivation to avoid opioid withdrawal signs/symptoms (Koob and Le Moal, 2001); and (2) this characteristic pattern

enables investigation to focus on predictive validity, i.e. whether genetic heterogeneity within this group explains phenotypic variance. Our approach emphasizes the drug user's habitual purchasing *costs* (time and money), which capture behavioral *investment* in this drug-seeking repertoire. We control for enabling environmental factors (e.g. income, drug cost and supply), which are presumably orthogonal to genetic influence. Finally, because cigarette smoking is highly prevalent among heroin-dependent individuals, we examined whether genotypic effect is limited to opioid-seeking behavior or may also apply to nicotine-reinforced behavior (as cigarette smoking also follows a highly period and stable pattern), i.e. behavioral specificity.

Brain-derived neurotrophic factor (BDNF) is the most widely distributed neurotrophin and is involved in neurogenesis, differentiation, survival and synaptic plasticity (Lu 2003; Lipsky & Marini 2007; Russo *et al.* 2009). BDNF secretion is activity-dependent—e.g. increased by cognition and exercise, and decreased by stressors—and modulates neurotransmission in dopamine, glutamate, gamma-aminobutyric acid and serotonin systems (e.g. Goggi *et al.* 2002; Carvalho *et al.* 2008). The human BDNF gene encodes a 247 amino acid pre-protein (pro-BDNF) that is cleaved to form an evolutionarily conserved 120 amino acid mature protein (Maisonpierre *et al.* 1991). A single nucleotide polymorphism (SNP; *rs*6265) results in methionine substitution for valine at codon 66 (*Val*^{66}*Met*). The evolutionarily recent, less-frequent *Met* allele alters the pro-BDNF protein sequence, which disrupts trafficking and results in less activity-dependent BDNF secretion without affecting the mature BDNF sequence (Egan *et al.* 2003; Lu 2003; Chen *et al.* 2004).

As might be expected from its neurotrophic physiological influence, the BDNF *Val*^{66}*Met* genotype has unsurprisingly been associated with pleiotropic effects. *Met* allele carriers exhibit several reliable intermediate phenotypes: reduced gray matter volume in the hippocampus (Pezawas *et al.* 2004; Szeszko *et al.* 2005; Bueller *et al.* 2006; Frodl *et al.* 2007 [European/Caucasian]) and dorsolateral prefrontal cortex (e.g. Hariri *et al.* 2003), and impaired hippocampal-dependent memory function (Egan *et al.* 2003; Hariri *et al.* 2003 [Caucasian]). In the realm of substance use, the BDNF ^{66}*Met* allele has been associated with headache-related overuse of non-opioid analgesics (Di Lorenzo *et al.* 2008), increased risk of nicotine dependence (Lang *et al.* 2007 [German]) and earlier onset of alcohol dependence (Matsushita *et al.* 2004 [Japanese]), but decreased risk of dependence on heroin (Cheng *et al.* 2005 [Han Chinese]) and protection against post-treatment alcohol relapse (Wojnar *et al.* 2009 [Polish]). *Met* allele frequency and informativeness varies significantly by ancestry, with highest prevalence in Asians, moderate prevalence for Europeans and lowest among American Indians and individuals of African descent (http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs=rs6265; Petryshen *et al.* 2010). Accordingly, association studies should test phenotypic relationships with the BDNF *Val*^{66}*Met* genotype within clearly defined ancestral groups.

In addition to the above evidence that the *Val*^{66}*Met* genotype has neurobiological and clinical relevance, findings from animal studies further suggest that BDNF physiology influences opioid-dependence behaviors. Chronic opioid exposure alters BDNF/TrkB receptor-mediated dopamine function in the ventral tegmental area (VTA; Bolanos & Nestler 2004; Russo *et al.* 2009) and its projection to the nucleus accumbens, the key neural circuitry underlying opioid reinforcement. Experimental infusions of BDNF directly into the VTA produce drug-seeking (Lu *et al.* 2004) and biochemical changes (Berhow *et al.* 1995; Sklair-Tavron *et al.* 1996; Vargas-Perez *et al.* 2009). Discontinuation of chronic opioid exposure leads to increased BDNF mRNA expression in brain regions underlying physical dependence and drug-seeking (Numan *et al.* 1998; Hatami *et al.* 2007). BDNF mRNA expression in prefrontal cortex is upregulated following exposure to psychostimulants and morphine, but to a lesser extent with nicotine (Le Foll, Diaz & Sokoloff 2005).

Given that the BDNF ^{66}*Met* allele has been linked to impaired hippocampal and frontal-cortical morphology and learning/memory problems, and thus *impaired behavioral flexibility*, we theorized that the *Met* allele might confer *resistance to environmentally or pharmacologically induced changes in drug-seeking/use*. If true, then the *Met* allele could have opposite effects at different stages of addiction, i.e. protecting against initial vulnerability (thus explaining counterintuitive results by Cheng *et al.* 2005) while making it more difficult to modify chronic drug-seeking/use (i.e. harder to *un*learn). Thus, we asked: Do heroin-dependent *Met* allele carriers exhibit greater drug-seeking behavior in the context of non-drug environmental alternatives? Our aim was to determine the extent of BDNF *Val*^{66}*Met* associations with behavioral investment in opioid-seeking.

MATERIALS AND METHODS

Participants

This investigation encompasses three source studies approved by Investigational Review Boards at Wayne State University and the University of Michigan (for methodological details, see Greenwald & Hursh 2006; Greenwald & Steinmiller 2009; Greenwald 2010), and conducted in accordance with the Declaration of Helsinki. Certificates of confidentiality were obtained from the National Institute on Drug Abuse. Male and female volunteers, 18–55

years old, were recruited from the Detroit area using newspaper ads and word-of-mouth. Ethnicity and race were not exclusion factors, but, due to low frequency of other racial/ethnic groups, only African American (AA) and European American (EA) subjects were included in the present data analyses. Those identifying themselves as heroin-dependent and not seeking treatment were instructed to call for a telephone interview.

The screening process included informed consent, providing demographic data, comprehensive substance use and medical histories, and an interview lasting 20–30 minutes that was used to obtain specific data about past-month income, drug purchasing and use factors (see below). All participants reported current daily heroin use, provided a urine sample that was positive for opioids (> 300 ng/ml), and were diagnosed with current opioid dependence based on clinician interview using Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria. Urine samples were also tested for methadone, cocaine, benzodiazepines, cannabinoids and barbiturates. Volunteers had to provide an alcohol-free breath sample (< 0.002%). Participants were paid $30 for completing the first screening visit, and those who continued in screening toward qualifying for laboratory-based studies could earn $25 more over two subsequent screening visits.

Genotyping

The Golden Gate drug addiction Illumina panel (Hodgkinson *et al*. 2008) was used to genotype blood samples provided by each participant. Whole blood (6 ml/subject) was collected into ethylenediaminetetraacetic acid tubes and DNA was extracted using Oragene DNA self collection kit (Qiagen, Valencia, CA, USA) (formerly Gentra Puregene kit).

Due to the relatively rare BDNF *rs6265 Met/Met* genotype (see Table 1), all analyses contrasted *Met* carriers (*Met/Met* + *Met/Val*) with *Val* homozygotes. To examine the specificity of the BDNF *rs6265* polymorphism, four BDNF variants with relatively high minor allele frequencies (> 0.25) located in the 3′ untranslated (UTR) region (*rs1519480*, *rs7124442*, *rs7934165* and *rs11030121*) were also included in the analyses. All of these 3′ UTR SNPs were in linkage disequilibrium (LD) with the functional SNP (*rs6265*), and all were in LD with each other. Furthermore, none of the 3′ UTR SNPs was significantly related to the phenotypes tested here. For this reason, these 3′ UTR SNPs are not mentioned further.

Phenotyping measures

Phenotypes were derived from a semi-structured interview that was previously validated with heroin abusers (Roddy & Greenwald 2009; Roddy, Steinmiller & Greenwald 2011). Participants were asked a series of interrelated questions to ascertain past-month sources and amounts of income (legal and illegal), heroin price, estimated purity, all drug and non-drug expenditures, drug-acquisitive behaviors (e.g. purchase time, amount spent and purchasing frequency) and heroin consumption (e.g. bags per day, including the distribution of use throughout the day). To examine the behavioral specificity of BDNF genotype on drug-seeking, we also ascertained daily cigarette use, alcohol use and illegal drug use as part of a comprehensive substance use history questionnaire.

Data analyses

BDNF *Val*^{66}*Met* genotype and allelic distributions were computed for each ancestral group (Table 1). Genotype frequencies were tested for Hardy–Weinberg equilibrium using a web-based calculator (http://scienceforall.org/2010/06/20/hardy-weinberg-equilibrium-calculator/). Analyses were conducted using SPSS v.19 (SPSS Inc., Chicago, IL, USA). BDNF genotype comparisons on categorical variables [other BDNF genotypes, race, gender, route of heroin use (injection versus non-injection), ever overdosed on heroin, and lifetime psychiatric diagnoses] were performed using χ^2 tests. BDNF genotype comparisons for continuous variables were conducted using one-way analysis of variance (ANOVA; Table 2). Descriptive statistics are presented as means ± 1 standard deviation (SD). For all analyses, the criterion for null hypothesis rejection was set at nominal $P < 0.05$.

Variables that were not normally distributed (see below) were $\log_{10}$-transformed for correlation and regression analyses. See Figure 1 for distributions of heroin-purchasing measures. We conducted tests of BDNF genotype effect for key measures of drug-seeking/use behavior, and for control variables that—while not hypothesized as related to the genotype—might need to

Table 1 Brain-derived neurotrophic factor *Val/Met* genotype distributions and allele frequencies [% within group (row)].

Genotype (n) *rs6265*	*Met/Met* *A/A*	*Met/Val* *A/G*	*Val/Val* *G/G*	*Minor allele* *A (Met)*	*Major allele* *G (Val)*
Black (74)	0 (0.0)	5 (6.8)	69 (93.2)	5 (3.4)	143 (96.6)
White (54)	3 (5.6)	17 (31.5)	34 (63.0)	23 (21.3)	85 (78.7)
Overall (128)	3 (2.3)	22 (17.2)	103 (80.5)	28 (10.9)	228 (89.1)

Table 2 Characteristics of European American participants ($n = 54$), by brain-derived neurotrophic factor *Val*^{66}*Met* genotype.

Measure	*Met carrier* $n = 20$	*Val/Val* $n = 34$	*Effect size partial* η^2 *(power)*	χ^2 *[1,54] or* $F[1,53]$ *(P =)*
Demographics				
Gender (% male)	70	71		0.01 (0.964)
Education (years)	12.2 (0.8)	12.5 (0.5)	0.018 (0.16)	0.94 (0.336)
Estimated IQ	107.1 (7.6)	109.0 (10.4)	0.010 (0.11)	0.50 (0.481)
History of heroin use				
Duration of regular use (years)	19.8 (10.9)	15.6 (9.5)	0.040 (0.30)	2.17 (0.147)
# times tried to quit	19.8 (29.5)	14.4 (24.5)	0.010 (0.11)	0.54 (0.468)
Ever overdosed (%)	45	44		0.01 (0.950)
Current heroin use				
Injection use (%)	100	88		2.54 (0.111)
# suppliers	3.2 (2.0)	3.1 (1.4)	0.000 (0.05)	0.01 (0.945)
Heroin unit price ($)	10.00 (2.58)	9.74 (3.81)	0.001 (0.06)	0.08 (0.784)
Purchase time (minutes)	81.5 (66.7)	52.1 (60.8)	0.111 (0.71)[a]	6.52 (0.014)
Unit purchase amount ($)	46.50 (20.01)	31.77 (21.21)	0.135 (0.80)[a]	8.12 (0.006)
# weekly purchases	11.9 (6.3)	14.2 (9.8)	0.010 (0.11)[a]	0.51 (0.478)
Daily use (# bags)	6.3 (3.4)	5.1 (3.2)	0.047 (0.35)[a]	2.57 (0.115)
Other recent drug use				
Cigarette use (# per day)	17.9 (7.2)	13.2 (8.9)	0.073 (0.50)	4.03 (0.050)
Alcohol use (# past 30 days)	1.8 (3.6)	2.1 (3.8)	0.001 (0.06)	0.06 (0.805)
Cocaine use # past 30 days	3.0 (6.8)	5.7 (7.9)	0.031 (0.24)	1.64 (0.206)
Positive urinalysis (%)	28	68	7.53 (0.006)	
Marijuana use # past 30 days	0.2 (0.5)	1.5 (4.4)	0.032 (0.25)	1.71 (0.197)
Positive urinalysis (%)	11	32	2.83 (0.092)	
Past-month income/expenses				
Total income ($)	2368 (1204)	1829 (1287)	0.064 (0.46)[a]	3.57 (0.064)
Proportion income spent on:				
Heroin	76.1 (14.6)	76.5 (19.5)	0.000 (0.05)	0.01 (0.942)
Cigarettes	4.8 (3.2)	2.7 (2.9)	0.109 (0.70)	6.36 (0.015)
Food	5.3 (4.8)	5.7 (6.3)	0.001 (0.06)	0.05 (0.820)
Shelter/utilities	4.2 (6.8)	5.1 (8.2)	0.003 (0.07)	0.16 (0.693)
Lifetime DSM-IV diagnoses (%)				
Antisocial personality disorder	50 (16)	20 (25)		
Anxiety disorder (any type)	13 (16)	12 (25)		
Major depressive disorder	31 (16)	12 (25)		
Alcohol use disorder	69 (16)	64 (25)		
Cocaine use disorder	63 (16)	68 (25)		
Cannabis use disorder	64 (14)	36 (24)		

[a]Effect sizes and power are for $\log_{10}$-transformed variables. See text. Psychiatric diagnostic data (based on SCID) were available for fewer participants than in the overall sample. Sample sizes are shown in parentheses adjacent to each percentage for each diagnosis. Substance use disorders refer to meeting criteria for lifetime abuse or dependence. Antisocial personality disorder refers to cases that also met criteria for childhood conduct disorder. Due to the smaller group sizes for DSM-IV diagnoses, statistical differences were not evaluated. DSM-IV = Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition; IQ = intelligence quotient

be included as covariates in regression analyses; Table 2 lists these variables. Pearson's correlations were computed among behavioral economic measures (Fig. 2), including drug supply factors ($\log_{10}$ past-month income, number of heroin suppliers and unit cost), heroin-purchasing pattern ($\log_{10}$ time, $\log_{10}$ amount and $\log_{10}$ frequency; percent of income spent on heroin), heroin consumption ($\log_{10}$ total daily bags used) and non-heroin expenses (percent of income spent on food, shelter/utilities, and cigarettes). This was also done to identify control variables for regression analyses in which we predicted different heroin-seeking phenotypes (Table 3). We derived a novel heroin-purchasing summary score, 'weekly heroin investment' [$\log_{10}$ purchase time × purchase amount × number of weekly purchases], expressed in dollar-minutes weekly.

RESULTS

Participant characteristics

Table 1 presents BDNF genotype and allelic frequencies for *rs*6265 (*Met* carrier versus *Val/Val*) for AAs ($n = 74$),

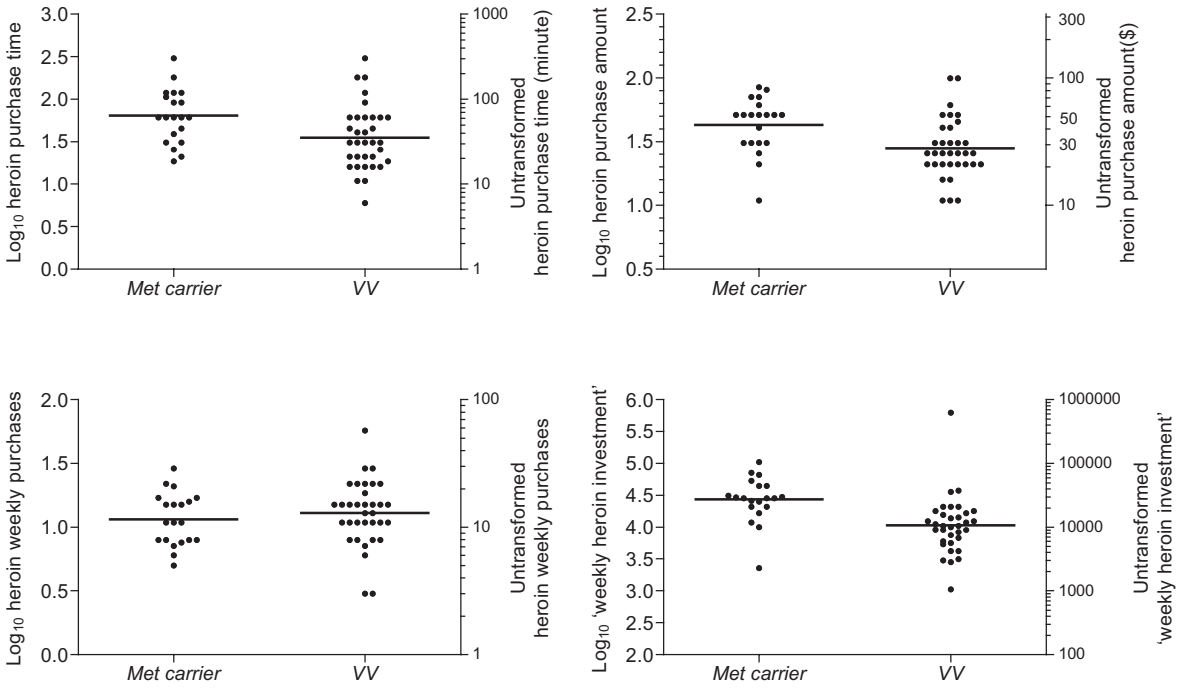

Figure 1 Response distributions for European American participants ($n = 54$) with means (horizontal bars) by brain-derived neurotrophic factor *rs*6265 genotype [*Met* carriers ($n = 20$) versus *Val* homozygotes ($n = 34$)] for heroin-seeking phenotypes: purchase time (*upper left panel*), purchase amount (*upper right panel*), weekly purchases (*lower left panel*) and the empirically derived index 'Weekly Heroin Investment' (*lower right panel*), which is the product score of purchase time × purchase amount × weekly purchases (measured in dollar-minutes weekly). For each measure, the $\log_{10}$-transformed scores are shown on the left ordinate, and the corresponding untransformed scores are illustrated on the right ordinate. For all measures except weekly heroin purchases, *Met* carriers significantly differed from *Val* homozygotes

EAs ($n = 54$) and overall sample ($n = 128$). Genotype frequencies for *rs*6265 did not deviate significantly from Hardy–Weinberg equilibrium in the European- or African-descent groups or the overall sample. Allele frequencies significantly differed between races, with the *Met* allele extremely rare in AA. Separate analyses were performed for EAs and AAs. For the present purposes, we primarily report results for EAs and include data for AAs in the supplementary materials.

BDNF *Val*^{66}*Met* effect on drug-seeking/use

Univariate analyses

Some continuous measures of heroin-seeking and income were not normally distributed. Figure 1 shows the distributions of responding by BDNF *rs*6265 genotype (*Met* carriers versus *Val* homozygotes) for three primary heroin-seeking phenotypes: typical purchase time (minute), average purchase amount (dollars), number of weekly purchases and an empirically derived index referred to as 'weekly heroin investment' that is the product of these three measures (purchase time × purchase amount × weekly purchases). Figure S1 compares the distributions of these four phenotypes in EAs and AAs. Figure 2 illustrates the covariance among these phenotypes in EAs, while demonstrating BDNF *Val*^{66}*Met* genotype differences in these response distributions.

One-way ANOVAs found significant BDNF *rs*6265 genotype differences such that *Met* carriers had longer purchase times and higher unit purchase amounts than *Val* homozygotes (see Table 2). Similar non-significant tendencies (P's < .15) were observed for *Met* carriers to report longer duration of heroin use, more likely to inject heroin, and more daily bags consumed. *Met* carriers also reported marginally higher total past-month income. Table S1 provides comparable data for AA subjects.

Multivariate analyses

Multivariate ANOVA (MANOVA) was used to ascertain whether results remained significant for the four primary outcomes (purchase time, unit purchase amount, weekly purchases and daily bags consumed) when adjusting for multi-collinearity among these measures (Fig. 2); the 'heroin investment' measure was excluded because it was derived directly from the first three measures. The MANOVA confirmed that the BDNF genotype effect remained significant, Hotelling $F(4,49) = 3.17$, $P = .022$.

Stepwise multiple regression analyses were used to determine whether *Val*^{66}*Met* genotype and control variables predicted measures of heroin-seeking in the natural

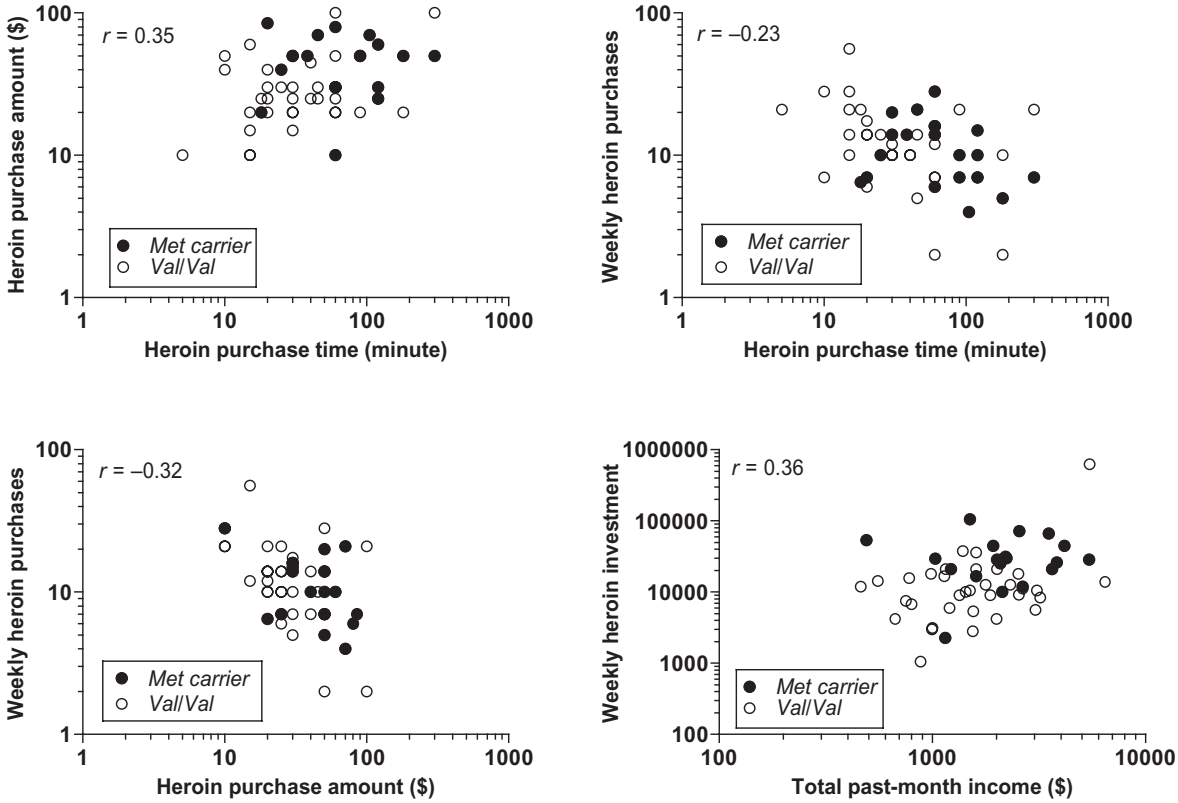

Figure 2 Relationships between drug-seeking phenotypes in European American participants (total $n = 54$). Each panel illustrates a significant ($P < 0.05$) overall correlation between two heroin-seeking phenotypes (*upper left*: purchase time × purchase amount; *upper right*: purchase time × weekly purchases; *lower left*: purchase amount × weekly purchases), as well as differences in the response distributions between brain-derived neurotrophic factor (BDNF) *Met* allele carriers ($n = 20$; closed circles) and *Val/Val* genotype ($n = 34$; open circles). *Lower right panel*: combined prediction of 'weekly heroin investment' by BDNF genotype and past-month income (see Table 3, regression analysis)

Table 3 Summary of stepwise multiple regressions, European Americans ($n = 54$).

Predictors	ΔR^2	*Cum. Adjust.* R^2	β	*T value*	*P value*
Log_{10} heroin purchase time					
BDNF *rs*6265 Met carrier	0.111	0.094	0.334	2.554	0.014
Log_{10} heroin unit purchase amount					
Total past-month income	0.158	0.142	0.325	2.569	0.013
BDNF *rs*6265 Met carrier	0.076	0.204	0.285	2.249	0.029
Log_{10} number of weekly heroin purchases					
Number of suppliers	0.108	0.090	0.355	2.784	0.008
Age	0.071	0.146	–0.267	–2.092	0.041
Log_{10} 'Weekly Heroin Investment' (purchase time × amount × frequency)					
BDNF *rs*6265 Met carrier	0.184	0.169	0.361	2.882	0.006
Total past-month income	0.068	0.223	0.270	2.159	0.036
Log_{10} daily bags of heroin consumed					
Total past-month income	0.483	0.473	0.655	6.637	0.0001
Age	0.040	0.503	–0.203	–2.058	0.045

ΔR^2 refers to unadjusted change in variance accounted for by the individual predictor variable. Cum. Adjust. R^2 refers to the cumulative (stepwise) adjusted variance accounted for. β refers to the standardized beta coefficient. *t*-Test residual degrees of freedom in steps 1 and 2 of all models above are 52 and 51, respectively. BDNF = brain-derived neurotrophic factor.

environment. Initial analyses were stratified by ancestral race (EA versus AA), after we determined this factor explained significant variance on some measures. In AAs, the infrequent *Met* allele was not related to any heroin-seeking measure but due to its rarity, there was little power in the AA sample to detect such an effect. Thus, final regression analyses focused on EAs. Covariates in all these analyses were age, number of heroin suppliers, current injection heroin use and total past-month income.

Table 3 shows that, in EAs ($n = 54$), BDNF *Met* allele carriers ($n = 20$) had significantly longer heroin-purchasing times and higher purchase amounts than *Val* homozygotes ($n = 34$), which accounted for 11.1% and 7.6% of variance in these outcomes, respectively. BDNF *Met* carriers had significantly higher 'weekly heroin investment' scores than *Val* homozygotes (see Fig. 1), which accounted for 18.4% of variance in this measure. Higher total income was the primary significant predictor of greater heroin unit purchase amounts and daily bags consumed (explaining 15.8% and 48.3% of variance in these two measures, respectively), whereas income was a secondary predictor of the 'weekly heroin investment' score (6.8%, in contrast to 18.4% explained by BDNF genotype; see lower right panel of Fig. 2 and Table 3).

In parallel regression analyses, BDNF genotype was not significantly related to number of weekly heroin purchases or number of daily bags consumed. Rather, the number of weekly purchases was higher for subjects with more heroin suppliers and those who were younger (explaining 10.8% and 7.1% of the incremental variance, respectively).

BDNF Val^{66}Met genotype and cigarette/nicotine use

Prevalence of smoking is very high among heroin-dependent individuals, including this sample, providing the opportunity to examine whether BDNF genotype impacts this other stable form of (legal) drug use. The influence of BDNF genotype on cigarette purchasing and use was examined in EAs ($n = 54$). Eighty-seven percent reported daily cigarette use. Two stepwise multiple regressions (which included non-smoking participants) were used to predict the proportion of past-month income spent on cigarettes and the number of cigarettes smoked daily (BDNF genotype groups significantly differed in univariate analyses; see Table 2), controlling for age, route of heroin use, total past-month income and daily bags of heroin consumed. Relative to *Val* homozygotes, *Met* carriers reported spending a significantly higher proportion of income on cigarettes (standardized $\beta = 0.33$, $t = 2.52$, adjusted $r^2 = 0.092$) and smoking significantly more cigarettes daily (standardized $\beta = 0.27$, $t = 2.01$, adjusted $r^2 = 0.073$). No other predictors were significant.

DISCUSSION

The BDNF ^{66}*Met* allele has been repeatedly associated with neurobehavioral deficits, including hippocampal and frontal-cortical volume loss, and impaired learning/memory. The *Met* allele leads to less BDNF secretion and reduced neurotrophic influence, which may decrease organismic behavioral flexibility or adaptive fitness. In this study, we theorized that the ^{66}*Met* allele may confer *resistance to environmentally or pharmacologically induced changes in drug-seeking, or reduced behavioral flexibility*, once addictive behavior has progressed to a chronic stage. To test this hypothesis, we assessed several related phenotypes using a validated semi-structured interview method to assess past-month drug-seeking/use.

Consistent with previous large population-based data, *Met* allele frequency was much greater among our European-ancestral than African-ancestral subjects (21% versus 3%). Important to recognize is that prior associations between BDNF *Val^{66}Met* genotype and neurobehavioral deficits were observed exclusively among European/Caucasian samples, where the *Met* allele is more informative.

Among EAs in this study, the ^{66}*Met* allele was significantly associated with increases in several drug-seeking behaviors. Bivariate relationships between *Val^{66}Met* genotype and drug-seeking/use were initially observed for heroin purchase time, purchase amount and an empirically derived 'weekly heroin investment' score (purchase time × amount × weekly frequency); effect sizes were moderate. In stepwise multiple regression analyses that controlled for other factors that showed zero-order correlations with the BDNF genotype (younger age, higher income, more heroin suppliers and injection route of heroin use), these bivariate relationships remained significant. A significant genotype effect was not observed for number of weekly purchases or daily bags of heroin consumed. *Val^{66}Met* genotype accounted for unique variance (change in r^2 value) of 7.6%, 11.1% and 18.4% in heroin unit purchase amount, purchase time and weekly heroin investment, respectively. Thus, BDNF *Val^{66}Met* genotype was more closely related to measures of *drug-seeking* than *consumption*. This is critical for selecting an appropriate phenotype: heroin and other illegal drug users (including many in our sample) often obtain some drug free (e.g. shared by others at no cost), or through bartering (e.g. providing sex or transportation for drugs). These behavior patterns were assessed in our interview because we noted in our validation studies the potential for dissociation between drug-seeking and drug consumption.

Chen *et al.* (2006) generated a transgenic mouse model of the BDNF *Val^{66}Met* polymorphism. *Met*-homozygous animals—who exhibit 50% lower BDNF levels and ≈30% less activity-dependent $BDNF_{Met}$ release

from neurons—demonstrate greater anxiety- and depression-like behaviors (without alterations in locomotion), and loss of hippocampal volume and less dendritic complexity in dentate gyrus neurons. Thus, it may be useful to test associations with addictive behavior in this model. We predict that in mice trained to self-administer heroin-like opioids, *Met*/*Met* (versus *Val*/*Val*) mice would exhibit higher breakpoints, be less responsive to medication and environmental-incentive induced disruptions of this behavior, and reinstate (following extinction) opioid self-administration more readily.

A limitation of this study is that the sample size was rather small, so hypotheses related to epistatic effects could not be tested with adequate statistical power. The preliminary results of this study are thus hypothesis generating, given the large number of tests performed on key phenotypes (which exhibited multi-collinearity with one another) and control variables, and—despite surviving multivariate adjustment—need to be confirmed. Nevertheless, we believe that several of these results are likely correct due to BDNF's biological plausibility as a potential mediator or moderator of addictive behavioral processes. Specifically, there is growing evidence that BDNF is involved in behavioral sensitization following repeated exposure to abused drugs including opioids. For instance, BDNF interacts closely with the dopamine D3 autoreceptor (DRD3; Le Foll *et al*. 2005), which controls phasic dopamine activity (Sokoloff *et al*. 2006) and is implicated in conditioned drug-seeking behavior (Everitt & Robbins 2000). Striatal BDNF/DRD3 interactions could be one candidate mechanism by which drug-seeking is sensitized and instantiated as habitual behavior (Vanderschuren & Kalivas 2000; Gerdeman *et al*. 2003). This suggests important avenues for research, including whether dopamine polymorphisms could act in epistasis with BDNF to modulate drug-seeking.

Given the potentially widespread neurotrophic influence of BDNF, an important question concerns the generality of its effects (behavioral specificity). In this study, we observed in EAs that BDNF *Val*^{66}*Met* genotype also accounted for 9.2% of variance in purchasing (percent of income spent) and 7.3% of variance in use (daily number) of cigarettes. These findings in our predominantly male subject sample are consistent with a study of nicotine-dependent smokers (Beuten *et al*. 2005) and a recent large-scale genome-wide association study (The Tobacco and Genetics Consortium 2010). Although an association of *Val*^{66}*Met* with smoking severity was not found in the study of Beuten *et al*., a haplotype showed a significant relationship in male EA smokers, but not female EAs or AA smokers. Other evidence points to a role of BDNF, D3 and D1 receptor polymorphisms in nicotine addiction, specifically with quantity of tobacco smoked (Novak *et al*. 2010). The BDNF *Val*^{66}*Met* genotype thus seems to produce an effect on drug-seeking/use in EA populations that is broader than for one particular class of abused substances and may therefore be of general importance to addiction.

On the other hand, urinalysis and self-report data were collected at screening to ascertain recent use of drugs besides opioids. Results indicated that relative to BDNF *Val* homozygotes, significantly fewer *Met* allele carriers tested positive for cocaine. A similar non-significant trend was observed for cannabis use. This finding does not support the idea that the BDNF *Met* allele is related to generally greater substance use. Rather, these data are consistent with the hypothesis that the *Met* allele is a risk factor for promoting the *engrained use of preferred drugs*—in this case, heroin and cigarettes.

Given the present preliminary findings and published data, our working hypothesis is that the ^{66}*Met* allele, which leads to decreased BDNF secretion and less neuroadaptation due to lower rates of cell proliferation, impairs behavioral flexibility once drug self-administration becomes habitual. This neurotrophic environment may promote a range of neurobehavioral deficits. With specific regard to addiction, reduced BDNF function and its sequelae may strengthen reinforcing efficacy of preferred drugs relative to non-drug alternatives or despite pharmacotherapy. In short, selected forms of drug-seeking behavior may become entrenched and more resistant to change in BDNF *Met* carriers. We found reliable ^{66}*Met* genotype differences across opioid-seeking phenotypes (i.e. increased purchase time and increased purchase amount) and across drug classes (greater opioid and nicotine use). Taken together with prior findings, the robust effects of this genotype may implicate its broader importance for understanding and treating addictive behavior and underlying processes such as impairments in learning/memory. If results of future work confirm the influence of this neurotrophic genotype, ^{66}*Met* allele carriers might require higher levels of intervention (e.g. cognitive behavior therapy or pharmacotherapy) to overcome their chronic drug use pattern. Although we observed behavioral effects of the *Val*^{66}*Met* genotype in a restricted sample (small in size and primarily for EAs), this converging pattern of association increases confidence that the results are meaningful. Further research could be theoretically and clinically useful by determining whether this hypothesis applies to the habitual use of other substances.

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Conflict of Interest

The authors declare no conflict of interest with respect to the conduct or content of this work.

Authors Contribution

MKG was responsible for the study concept, design, analysis and drafting the manuscript. CLS contributed to study implementation, interviewing, data coordination and management. ES conducted the genotyping analyses. LL contributed to psychiatric screening and edited the manuscript. MB was responsible for the genotyping approach, overseeing analysis and editing the manuscript. All authors have critically reviewed content and approved final version submitted for publication.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1 Response distributions with means by BDNF rs6265 genotype and race for heroin-seeking phenotypes.
Table S1 Characteristics of African American participants ($n = 74$), by BDNF Val66Met genotype