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Examining the association of *NRXN3* SNPs with borderline personality disorder phenotypes in heroin dependent cases and socio-economically disadvantaged controls☆

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ABSTRACT

Background: Borderline personality disorder (BPD) and substance use disorders frequently co-occur; their dual presence predicts poor prognosis. The genetic underpinnings of BPD have not been well-characterized and could offer insight into comorbidity. The current report focuses on the association of neurexin 3 (*NRXN3*) single nucleotide polymorphisms (SNPs) with BPD symptoms in heroin dependent cases and controls.

Methods: The sample of the Comorbidity and Trauma Study, a genetic association study of heroin dependence, consists of Australian heroin dependent cases ascertained from opioid replacement therapy clinics and controls ascertained in nearby economically disadvantaged neighborhoods. The assessment included a screening instrument for BPD, used previously in Australian population surveys. Genotypic and BPD phenotypic data were available for 1439 cases and 507 controls. We examined the association of 1430 candidate gene SNPs with BPD phenotypes.

Results: One or more *NRXN3* SNPs were nominally associated with all BPD phenotypes; however, none met the conservative significance threshold we employed to correct for multiple testing. The most strongly associated SNPs included rs10144398 with identity disturbance ($p = 4.9 \times 10^{-5}$) and rs10151731 with affective instability ($p = 8.8 \times 10^{-5}$). The strongest association with screening positive for BPD was found for the *NRXN3* SNP, rs10083466 ($p = .0013$). Neither the correlation of BPD phenotypes nor the linkage disequilibrium relationships of the SNPs account for the number of observed associations involving *NRXN3* SNPs.

Conclusions: Our findings provide intriguing preliminary evidence for the association of *NRXN3* with BPD phenotypes. The strongest associations were found for traits (i.e., affective instability; identity disturbance) also observed with other disorders.

1. Introduction

Borderline personality disorder (BPD) is a public health issue of great concern. Although BPD is a relatively uncommon disorder with a 1-2% general population prevalence (Jackson and Burgess, 2000; Torgersen, 2005), those afflicted have markedly increased morbidity and mortality that includes risk for suicide (Duberstein and Conwell, 1997; Skodol et al., 2002b; Black et al., 2004). The long term outcome of BPD is characterized by severe and persistent

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impairment in social functioning (Skodol et al., 2002a; Gunderson et al., 2011) and utilization of substantial mental health resources (Skodol et al., 2002b). Despite its vast impact on the lives of patients, their families and on the health system, our understanding of factors contributing to BPD liability and the treatment options available to patients remain fairly limited (Feurino and Silk, 2011).

Additionally, epidemiological research supports that comorbid substance use disorders, including opioid dependence, are very prevalent in BPD with more than 40% of patients with BPD also having a diagnosis of a drug use disorder (Trull et al., 2000; Zanarini et al., 2011; Trull et al., in press). Borderline traits, and the disorder itself, are also overrepresented in patients with opioid dependence with rates of 44.1% for BPD in patients seeking buprenorphine treatment (Trull et al., 2000; Sansone et al., 2008) and this comorbidity is a predictor of poor outcome (Moos et al., 2001; Darke et al., 2005, 2007). The endogenous opioid system dysfunction hypothesis has been proposed as a potential explanation of the pathophysiology of BPD and the high rates of comorbidity with opioid dependence (Bandelow et al., 2010; New and Stanley, 2010). In support of this hypothesis, differences in μ-opioid receptors and in the function of the endogenous opioid system between patients with BPD and healthy controls have been reported (Prossin et al., 2010). Hence, characterizing the genetic underpinnings of BPD in a sample of cases with heroin dependence may prove to be of particular clinical importance.

Genetic and environmental contributions to the liability to BPD have been estimated (Leichsenring et al., 2011). In twin and family studies, the heritability of the disorder ranged from 35% to 69% (Torgersen et al., 2000; Distel et al., 2008; Kendler et al., 2008; Torgersen et al., 2008). Several candidate gene association studies have been conducted for BPD or for correlated personality traits; however, findings thus far remain unreplicated (Siever et al., 2002; Joyce et al., 2006; Ni et al., 2006; McCloskey et al., 2009; Wilson et al., 2009; Maurex et al., 2010; Nemoda et al., 2010; Perez-Rodriguez et al., 2010; Stoltenberg et al., 2011). The recent revision of the diagnostic classification of psychiatric illness to produce DSM-V included active discussion of a transition from a strictly categorical approach according to which a psychiatric illness is either present or not in any given individual to a more continuous, dimensional approach (Kupfer et al., 2002). This transition has been considered particularly pertinent for personality disorders, including BPD (Regier, 2007), and has been reinforced by a methodological shift in the study of the genetics of BPD where more emphasis has been applied on the identification of endophenotypes (Siever et al., 2002). In this context, most examinations of BPD's genetic underpinnings have traditionally utilized broad, categorical approaches. More recently, studies have examined the heritability of individual borderline personality traits and not the diagnosis per se, based on the rationale that this approach may improve understanding of component phenotypes (Siever et al., 2002; McCloskey et al., 2009).

The current investigation examines the association of single nucleotide polymorphisms (SNPs) with individual borderline personality traits in the Comorbidity and Trauma Study (CATS), a genetic association study of opioid dependence. This report focuses on neurexin 3 (*NRXN3*) SNPs for which evidence of nominal association was observed for all examined BPD phenotypes. *NRXN3* polymorphisms have been previously reported to be associated with substance dependence (Liu et al., 2005; Hishimoto et al., 2007; Lachman et al., 2007; Docampo et al., 2012). A recent study observed an association of *NRXN3* polymorphisms with attentional impulsivity and alcohol problems in men (Stoltenberg et al., 2011).

2. Methods

The methods for CATS have been described in detail elsewhere (Maloney et al., 2009; Shand et al., 2011). A brief summary is provided below.

Table 1
Borderline personality items (true–false) and prevalence of endorsement (%).

I can't decide what kind of person I want to be. (undecided)	44.9
I go to extremes to try and keep people from leaving me. (extreme)	28.5
I get into very intense relationships that don't last. (intense)	34.7
I argue or fight when people try to stop me from doing what I want. (argue)	56.0
I've never threatened suicide or injured myself on purpose. (threat)	38.9
I don't stick with a plan if I don't get results right away. (stick)	45.3
Sometimes I get so angry I break or smash things. (angry)	38.9
I often feel "empty" inside. (empty)	62.4
I'm very moody. (moody)	50.7
I take chances and do reckless things. (reckless)	57.6
Total symptoms endorsed (sxtotal)	mean 4.6 (SD 2.8)
Screened positive (screen)[a]	52.3

[a] Endorsed ≥3 symptoms, their persistence for most of adult life, and interference from them.

2.1. Sample

Cases were recruited from opioid replacement therapy (ORT) clinics in the greater Sydney region and were required to be age 18 or older, understand English, and have participated in ORT for opioid dependence. Participants reporting recent suicidal intent or current psychosis were excluded. Individuals recruited from geographic areas in proximity to ORT clinics, termed "neighborhood controls," were excluded for recreational opioid use more than five times lifetime (data were included from 23 controls who denied opioid use more than 5 times at screening, but reported greater use with no dependence symptoms at interview). All other inclusion and exclusion criteria were identical to cases. Data are reported here from heroin dependent cases ($N = 1439$; 39.1% female) and neighborhood controls ($N = 507$; 55.4% female) for whom both genotypic and BPD data were available. The mean age of cases [36.4 years (SD 8.6)] was significantly greater ($p = .0006$) than that of controls [34.6 years (SD 10.5)]. A significantly greater percentage of controls than cases reported have completed high school and received a diploma (54.4% versus 17.5%; $p < .0001$). Although both populations are primarily of European ancestry, both groups also contained individuals of Asian ancestry. Institutional review board (IRB) approval was obtained from University of New South Wales, Washington University School of Medicine, Queensland Institute of Medical Research, and all New South Wales area health service ethics committees governing participating clinics. Data were collected between 2004 and 2008. Participants provided written informed consent and were reimbursed AU$50.00.

2.2. Assessment

All interviews were conducted in person by experienced interviewers. Diagnostic sections on illicit drug and alcohol dependence were modified from the Semi-Structured Assessment for the Genetics of Alcoholism – Australia (SSAGA-OZ; Bucholz et al., 1994; Hesselbrock et al., 1999); the nicotine dependence section was modified from the Nicotine Addiction Genetics Study assessment (Saccone et al., 2007; Agrawal et al., 2008). Additionally, a screener for BPD, adopted from the International Personality Examination (IPDE; Loranger et al., 1994), was also administered. It utilizes the ICD-10 criteria to determine whether 10 borderline traits are characteristic of the participant's usual personality (World Health Organization, 1992). The individual BPD items and the two summary phenotypes derived from them are displayed in Table 1. This has also been used in the Australian National Survey of Mental Health and Well-Being and is considered to provide an adequate screening assessment for BPD (Lewin et al., 2005). Participants who endorsed having at least 3 out of the 10 assessed BPD symptoms, and reported that these symptoms persisted for most of their adult life and caused significant interference with their lives and activities, were classified as screening positive for BPD. The threshold of 3 symptoms is based on Loranger and colleagues' work (1994) and has been adapted elsewhere (Lewin et al., 2005; Maloney et al., 2009). An additional measure of self-reported impulsivity, the 30-item Barratt Impulsiveness Scale (BIS; Patton et al., 1995; Maloney et al., 2009; Stanford et al., 2009), was added to the study's assessment protocol after data collection had commenced; it is available on 1521 participants.

2.3. Marker selection

The pair-wise option of Tagger (de Bakker et al., 2005), implemented in Haploview (Barrett et al., 2005) with a threshold of $r^2 \geq 0.8$ for most genes was used to select a custom set of 1536 SNPs. Since *NRXN3* is a large gene, we primarily selected SNPs for genotyping that are in relatively low linkage disequilibrium (LD) to provide coverage in an efficient manner with some redundancy allowed for areas

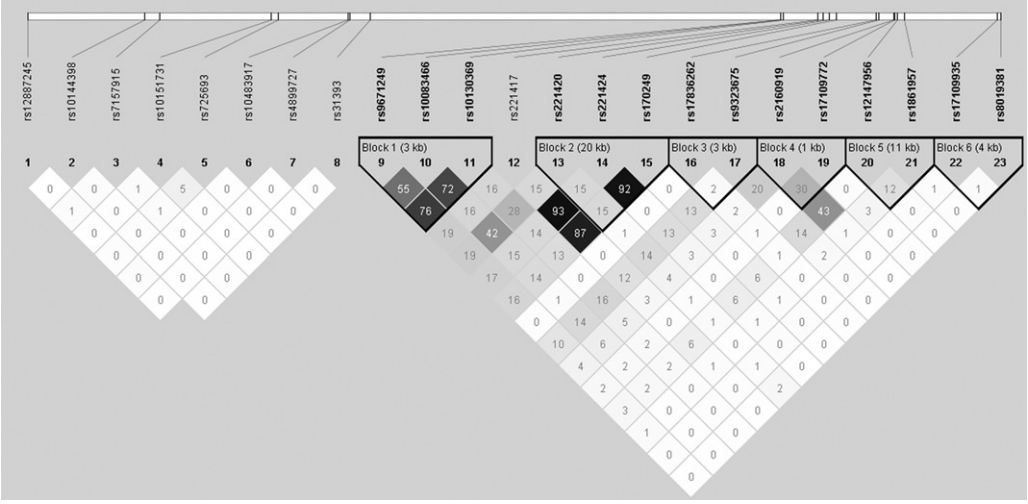

Fig. 1. Linkage disequilibrium analysis of select *NRXN3 SNPs* (r^2 values are shown).

where prior reports had observed associations (see Fig. 1). The set provided coverage of 72 candidate genes selected on the basis of relevance for heroin dependence, 47 additional SNPs previously reported to be associated with heroin dependence, and 30 ancestry-informative markers (AIMs).

2.4. Genotyping

Genotyping was performed on an Illumina BeadStation using GoldenGate technology (Peters et al., 2008). DNA samples from CEPH trio 1334 obtained from the Coriell Cell Repository served as internal quality controls for clustering and reproducibility. Primary genotypic data analyses with Illumina BeadStudio software were followed by visual inspection and assessment of data quality and clustering.

2.5. Statistical analyses

2.5.1. Data cleaning. Details of data cleaning have been reported previously (Nelson et al., 2012). The mean call rate for SNPs remaining after data cleaning exceeded 99.9%. The 1430 SNPs that remained after data cleaning included 23 *NRXN3* polymorphisms. All 1430 SNPs were examined for association with BPD and related phenotypes (Table 2).

2.5.2. Admixture. Principal components analysis (PCA) was conducted using the smartpca program in the Eigensoft 3.0 package (Patterson et al., 2006) to determine whether correction for admixture was necessary. The kill r^2 setting of 0.8 was used to remove SNPs in high LD with others in the panel. Data from 1123 of the 1430 SNPs were retained. PCA found that comparisons of cases to neighborhood controls did not require inclusion of principal components as covariates.

Table 2
NRXN3 SNPs genotyped.

SNP	Location	Classification	Minor Allele
rs12887245	77,901,980	Intronic	A
rs10144398	78,081,097	Intronic	A
rs7157915	78,103,930	Intronic	C
rs10151731	78,275,372	Intronic	C
rs725693	78,285,563	Intronic	C
rs10483917	78,392,399	Intronic	A
rs4899727	78,394,606	Intronic	G
rs31393	78,426,368	Intronic	G
rs9671249	79,053,658	Intronic	G
rs10083466	79,054,471	Intronic	A
rs10130369	79,057,542	Intronic	A
rs221417	79,111,379	Intronic	A
rs221420	79,119,291	Intronic	G
rs221424	79,128,774	Intronic	G
rs170249	79,139,680	Intronic	A
rs17836262	79,200,816	Intronic	C
rs9323675	79,204,384	Coding	A
rs2160919	79,227,549	Intronic	A
rs17109772	79,229,104	Intronic	G
rs12147956	79,232,192	Intronic	G
rs1861957	79,243,988	Intronic	A
rs17109935	79,386,064	Intronic	G
rs8019381	79,390,336	Intronic	A

2.5.3. Association. Logistic regression analyses were performed in PLINK (Purcell et al., 2007) to examine the association between the log-additive effects of minor allele dosage and binary outcome measures (i.e., individual BPD items, screening positive for BPD). Linear regression was used to examine association with BPD symptom count. Heroin dependence status was included as a covariate in all analyses, and sex served as an additional covariate for analyses involving X chromosome SNPs. After applying the most conservative correction for multiple testing, a strict Bonferroni correction that assumes all SNPs and BPD variables are independent measures, the significance threshold was revised to 2.91×10^{-6} (i.e., .05/1430 SNPs/12 phenotypic comparisons).

3. Results

The prevalence of BPD symptoms and of screening positive for BPD is shown in Table 1. All symptoms were endorsed at rates far exceeding those of general population samples, as expected. Overall, 58.6% of opioid dependent cases and 34.3% of neighborhood controls screened positively for BPD. The increased prevalence of BPD in these samples has been discussed in more detail previously (Maloney et al., 2009). All symptoms other than "intense relationships" were also more commonly reported by opioid dependent cases than controls. The strongest associations with opioid dependence were found for "emptiness" (OR 2.84; 95%CI 2.31-3.50) and screening positively for BPD (OR 2.71; 95%CI 2.19-3.36). Opioid dependent individuals also endorsed a significantly higher number of BPD symptoms than controls (4.98 versus 3.41; $t = 11.39$; $p < 0.001$). All genetic association analyses thus include a control for opioid dependence status. Compared with anticipated rates in the general population, the neighborhood controls, thus not surprisingly showed an overrepresentation of individuals screening for BPD, as they were drawn from neighborhoods with higher exposure opportunity to and rates of substance use (excluding opioid dependence, but including subjects with dependence on licit and other illicit drugs).

The genetic association analyses found that two *NRXN3* SNPs were the strongest observed associations: rs10144398 with "undecided" (i.e., identity disturbance; 4.9×10^{-5}) and rs10151731 with "moody" (i.e., affective instability; 8.8×10^{-5}). Interestingly, one or more *NRXN3* SNPs were found to be nominally associated with each examined BPD phenotype (Table 3). The LD relationships (Fig. 1) explained some (e.g., involving rs10083466, rs10130369, and rs9671249), but not all (e.g., not rs10144398 and rs17109935) instances in which we found multiple *NRXN3* SNPs associated with

Table 3
Association of *NRXN3* SNPs with individual and total borderline personality disorder symptoms (*p* values are shown).

SNP	Angry	Argue	Empty	Extreme	Intense	Moody	Reckless	Stick	Threat	Undecided	Sxtotal	Screen
rs12887245	.79	.13	.36	.83	.07	.70	.23	.005	.92	.07	.06	.16
rs10144398	.79	.99	.83	.16	.15	.76	.32	.016	.61	.000049	.047	.68
rs7157915	.74	.62	.46	.88	.56	.96	.66	.72	.95	.31	.97	.36
rs10151731	.70	.44	.09	.48	.21	.000088	.37	.85	.59	.15	.029	.22
rs725693	.95	.49	.11	.10	.78	.07	.037	.14	.40	.78	.43	.59
rs10483917	.17	.78	.012	.95	.26	.16	.15	.09	.003	.82	.023	.96
rs4899727	.007	.58	.45	.74	.50	.18	.08	.23	.65	.27	.09	.39
rs31393	.41	.26	.53	.15	.13	.89	.58	.70	.34	.51	.15	.35
rs9671249	.57	.51	.35	.51	.51	.65	.83	.21	.93	.85	.74	.03
rs10083466	.52	.42	.09	.27	.35	.09	.12	.25	.35	.59	.046	.0013
rs10130369	.86	.27	.49	.59	.92	.96	.93	.46	.60	.80	.83	.04
rs221417	.52	.13	.56	.82	.99	.77	.86	.76	.76	.97	.81	.86
rs221420	.98	.06	.82	.40	.49	.52	.65	.86	.29	.83	.28	.24
rs221424	.34	.07	.77	.82	.91	.64	.76	.92	.82	.86	.88	.62
rs170249	.32	.09	.87	.99	.69	.62	.96	.82	.73	.60	.87	.57
rs17836262	.04	.48	.06	.39	.12	.025	.045	.004	.97	.23	.006	.12
rs9323675	.88	.03	.40	.16	.26	.32	.93	.07	.78	.78	.61	.40
rs2160919	.67	.67	.88	.36	.62	.48	.052	.35	.72	.36	.68	.39
rs17109772	.84	.38	.92	.87	.08	.73	.07	.71	.028	.37	.23	.43
rs12147956	.58	.11	.89	.34	.46	.35	.21	.50	.45	.83	.55	.08
rs1861957	.24	.19	.50	.34	.67	.96	.96	.09	.27	.96	.99	.99
rs17109935	.74	.53	.018	.036	.08	.48	.09	.008	.55	.027	.035	.15
rs8019381	.55	.82	.14	.17	.04	.044	.045	.44	.89	.88	.08	.12

Table 4
Odds ratios for nominally associated *NRXN3* SNPs (beta values provided for sxtotal).

Phenotype	SNP	OR	SNP	OR	SNP	OR	SNP	OR
Angry	rs4899727	0.64	rs17836262	0.81				
Argue	rs9323675	0.81						
Empty	rs10483917	0.55	rs17109935	1.25				
Extreme	rs17109935	1.22						
Intense	rs8019381	1.26						
Moody	rs10151731	1.42	rs17836262	0.80	rs8019381	1.26		
Reckless	rs725693	1.15	rs17836262	0.82	rs8019381	1.27		
Stick	rs17836262	0.75	rs12887245	1.43	rs17109935	1.26	rs10144398	0.84
Threat	rs10483917	0.43	rs17109772	1.32				
Undecided	rs10144398	0.75	rs17109935	1.21				
Screen	rs10083466	0.78	rs10130369	0.85				
Sxtotal	rs17836262 −0.36; rs10483917 −0.71; rs10151731 0.25; rs17109935 0.24; rs10083466 −0.20; rs10144398 −0.19							

Table 5
Pearson correlations of individual borderline personality disorder items, screen, and total symptoms.[a]

	Angry	Argue	Empty	Extreme	Intense	Moody	Reckless	Stick	Threat	Undecided	Screen	Sxtotal	BIS total
Angry	–	0.28	0.24	0.22	0.18	0.35	0.28	0.20	0.19	0.21	0.31	0.56	0.28
Argue		–	0.24	0.25	0.25	0.30	0.31	0.23	0.15	0.19	0.35	0.57	0.28
Empty			–	0.29	0.28	0.41	0.27	0.29	0.28	0.33	0.53	0.64	0.33
Extreme				–	0.35	0.26	0.21	0.22	0.20	0.28	0.35	0.57	0.26
Intense					–	0.25	0.25	0.21	0.17	0.24	0.35	0.56	0.27
Moody						–	0.33	0.21	0.22	0.27	0.45	0.64	0.32
Reckless							–	0.22	0.16	0.19	0.39	0.57	0.36
Stick								–	0.13	0.29	0.39	0.53	0.34
Threat									–	0.15	0.31	0.47	0.21
Undecided										–	0.39	0.56	0.31
Screen											–	0.67	0.40
Sxtotal												–	0.52
BIS total													–

[a] All values are significant at $p < 0.0001$.

the same BPD phenotype. Supplementary Tables 1–5[1] display the 10 SNPs most significantly associated with each dependent variable. Interestingly, *NRXN3* SNPs were included among the top hits for 7 of the dependent variables ("screen", "sxtotal", "undecided", "empty", "moody", "threat", "stick"). Several *NRXN3* SNPs were associated with multiple BPD phenotypes. For each such SNP, the direction of risk remained the same across phenotypes (Table 4). Although all individual BPD items were significantly correlated (Table 5), the correlations are modest (ranging from 0.15 to 0.41) and thus did not definitively account for the multiple associations involving individual SNPs. Higher correlations were observed between the individual and summary items ("sxtotal" and "screen") as well as between the summary items.

Other genes with SNPs associated with multiple items include *GRIN2A*, which was represented among the top 10 SNPs for 6 BPD phenotypes. The strongest associations observed for *GRIN2A* SNPs

[1] Supplementary material can be found by accessing the online version of this paper. Please see Appendix A for more information.

included rs4782080 with "intense" ($p = 1.4 \times 10^{-4}$) and rs7191784 with "angry" ($p = 8.1 \times 10^{-4}$). Although only a single strong association involving an *NPY* SNP was noted, rs3025120 was strongly associated with "threat" ($p = 1.2 \times 10^{-4}$; OR 1.91).

4. Discussion

We report evidence of association involving multiple SNPs within the *NRXN3* gene and BPD phenotypes. The strongest associations we observed, while not meeting the stringent Bonferroni correction for multiple testing that we conservatively employed, have p values that, in a candidate gene association study, are worthy of further investigation.

Neurexins are presynaptic, transmembrane, cell adhesion proteins that are believed to play an important role in functional synapse formation and maturation (Dean and Dresbach, 2006; Südhof, 2008; Knight et al., 2011). The pre-synaptic neurexins interact widely with post-synaptic neuroligins to stabilize transient points of contact between axons and dendrites (Krueger et al., 2012). Numerous isoforms and splice variants that exist for each neurexin and neuroligan are believed to play important roles in determining key elements of synaptic differentiation, a process that is now recognized to be much more complex than previously appreciated and also involve several other families of proteins (Krueger et al., 2012).

A prior report (Stoltenberg et al., 2011) had observed gender-specific associations of *NRXN3* polymorphisms with impulsivity, a cardinal feature of BPD (American Psychiatric Association, 2000) and a well-known risk factor for addiction (Verdejo-García et al., 2008). We performed several post hoc analyses to determine whether our findings could be explained by the hypothesis that *NRXN3* polymorphisms serve as vulnerability factors for impulsivity and thus ultimately for a variety of externalizing disorders, including BPD and substance use disorders. We first determined that BIS total score is significantly correlated with all BPD phenotypes observing the strongest correlations with the two composite measures, sxtotal and screening positively (see Table 5). We next conducted an examination of the association of BIS total score with *NRXN3* polymorphisms, and found that two SNPs were nominally associated: rs17836262 ($p = 0.021$) and rs10083466 ($p = 0.030$). Although these associations are modest in magnitude, it is noteworthy that these same SNPs were the most highly associated with the two variables representing broader BPD constructs (the former with sxtotal and the latter with screen). A recent report (Docampo et al., 2012) also observed an association of rs1424850, a *NRXN3* SNP in high LD with rs10083466, with smoking behavior. Consistent with our results, the effects of the minor allele were also protective in their report. Since Stoltenberg et al. (2011) had observed an association involving a *NRXN3* SNP, rs11624704, and attentional impulsivity in men, we examined whether any *NRXN3* SNPs was associated with BIS attentional impulsivity in our sample. We observed modest nominal association involving rs17109772 ($p = 0.04$) in men and rs10083466 in women ($p = 0.03$). Because rs11624704 is not in strong LD with any SNPs we genotyped, our data cannot address replication of the prior report (Stoltenberg et al., 2011). Three SNPs that we genotyped (rs10151731, rs10144398, and rs725693) are in low-level LD with rs11624704. The strongest associations that we observed involved the former two SNPs; in addition, the latter SNP has the strongest (nominal) association with recklessness in our sample. Overall, the results of these post hoc analyses suggest that impulsivity and the composite BPD phenotypes are modestly associated with a similar set of *NRXN3* SNPs. The strongest associations that we observed involve other single-item phenotypes, moody and undecided, for which impulsivity does not appear to explain our findings. Thus, additional research will be necessary to characterize these phenotypes more clearly.

Prior reports have found evidence of association of *NRXN3* SNPs with alcohol, nicotine, and illicit drug use phenotypes (Liu et al., 2005; Hishimoto et al., 2007; Stoltenberg et al., 2011; Docampo et al., 2012). BPD has been observed in epidemiologic and clinical samples to be associated with significant risk for licit and illicit drug dependence. Investigators have posited a role for dysfunction in opioid receptor neurotransmission in BPD (Stanley and Siever, 2010). Despite extensive coverage of opioid receptor and related genes in our study, our findings of scattered nominal associations offer at best minimal support for this hypothesis.

4.1. Limitations

A number of methodological limitations should be considered when interpreting our findings. Foremost, we acknowledge that this investigation was not designed to comprehensively examine BPD liability. The assessment was not meant to diagnose BPD but rather as a screening instrument for the disorder. However, it has been deemed to be an adequate assessment for BPD despite its omission of assessment for DSM-IV criterion 9, which refers to transient, stress-related paranoid ideation or severe dissociative symptoms, and has been used in other studies (Lewin et al., 2005). Additionally, the threshold used for a positive screen (i.e., 3 endorsed symptoms out of a total of 10) is low in comparison to the 5 out of 9 symptoms required by DSM-IV. This low threshold may also be contributing to the somewhat higher than expected prevalence of positive screening for BPD of our samples in comparison to related samples. We conducted a post hoc factor analysis of BPD symptoms to extract an estimate of variance common to these items and found that a one factor solution fit the data well. When we performed association analyses for the BPD symptom factor score, the results were nearly identical to those for our sxtotal variable (e.g., lowest p value = 0.0058 for rs17836262) suggesting that the latter variable is a reasonable composite measure of BPD symptoms. The psychometric properties of the individual true-false BPD items that we used as binary dependent variables have not been carefully examined. Because of these limitations, we adopted what many might consider as overly conservative threshold for statistical significance, reporting even our strongest findings as suggestive of nominal association. We acknowledge that a less conservative approach (e.g., false discovery rate) would have provided a less stringent threshold for statistical significance.

Another important potential limitation is the ability to generalize from our sample of opioid dependent cases and neighborhood controls to clinical or general population samples ascertained explicitly for BPD. Although we acknowledge that our results will need to be replicated in other samples, we also believe that the associations with BPD symptom constructs in a sample not ascertained for BPD may actually be more broadly useful in improving understanding of risk across a spectrum of externalizing disorders with overlapping symptom profiles. It is possible that we may be underestimating the association with correlated phenotypes by including heroin dependence as a covariate in all analyses. Finally, *NRXN3* is a large gene for which we genotyped tagged SNPs that were selected to provide reasonable coverage. The relative lack of LD observed across the vast majority of these SNPs precludes performing haplotype-based analyses that could help pinpoint causal variants. It is very likely that additional variants (i.e., not genotyped in the current report) that are more strongly associated with BPD phenotypes exist; however, further investigation will be necessary to identify them.

4.2. Conclusion

In conclusion, our findings provide intriguing preliminary evidence for association of *NRXN3* SNPs with multiple BPD phenotypes. Further research and replication of these findings are warranted to determine their true clinical significance.

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Contributors

ECN, MTL, ACH, LD, NGM, and GWM were responsible for the study concept and design. ECN performed the data analyses. AA assisted with data analysis and interpretation of findings. VNP and ECN drafted the manuscript. TJT, ALG, MTL, ACH, AA, AKH, LW, AAT, PAFM, EM, LD, NGM, and GWM provided critical revision of the manuscript and contributed to its intellectual content. All authors critically reviewed content and approved the final version of the manuscript.

Conflict of interest

No conflict declared.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.drugalcdep.2012.11.011.

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