DISC1 as a Possible Genetic Contribution to Opioid Dependence in a Polish Sample

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ABSTRACT. Objective: Disrupted-in-schizophrenia 1 (*DISC1*) has been linked to vulnerability to a variety of psychiatric disorders and neuropsychiatric phenotypes. However, *DISC1* has not been frequently examined as a potential risk factor for substance dependence. An association between opioid dependence and *DISC1* rs2738888 polymorphism has been recently reported. In addition, opioid dependence was associated with rs6419156 located close to the protein phosphatase 3 catalytic subunit alpha isoform (*PPP3CA*) gene. The aim of the present study was to examine the associations between opioid dependence with rs2738888 and rs6419156 in an independent sample. **Method:** The selected polymorphisms were genotyped in a sample of 392 individuals (69.9% male) diagnosed as alcohol- and/or opioid-dependent. A control

group ($n = 257$; 67.7% male) was derived from the Polish National Health Survey ($N = 14,350$). **Results:** The frequency of rs2738888 C allele was higher in controls than in opioid-dependent cases ($OR = 0.65$, $p = .045$). Phenotypic-oriented analyses performed within opioid-dependent individuals revealed the association between lifetime suicide attempt and rs2738888. The C allele of rs2738888 had a protective effect on lifetime suicide attempt in opioid-dependent patients ($OR = 0.25$, $p = .003$). Rs6419156 was not associated with substance dependence in the examined sample. **Conclusions:** The *DISC1* may play an important role in vulnerability to opioid dependence. In addition, *DISC1* may also be a genetic risk factor for suicide attempt in opioid-dependent individuals. (*J. Stud. Alcohol Drugs*, 77, 220–226, 2016)

SUBSTANCE DEPENDENCE is a complex condition with a well-established genetic contribution. Twin and adoption studies revealed that the heritability of substance-specific addictions reached 40%–80% (Agrawal et al., 2012). Although substance dependence and substance-related disorders are global public health problems, we are still far from understanding the specific genetic background of vulnerability to dependence.

Genome-wide association studies (GWASs), which are able to analyze hundreds of thousands of single-nucleotide polymorphisms (SNPs) across the whole genome in thousands of participants, are considered the best way to study complex psychiatric conditions, including substance dependence (Hindorff et al., 2009). Recently, several GWASs have

been published for alcohol dependence (Gelernter et al., 2014a; Kapoor et al., 2014; Treutlein et al., 2009; Wetherill et al., 2015), nicotine dependence (Gelernter et al., 2015; Loukola et al., 2014), cannabis dependence (Agrawal et al., 2011), and opioid dependence (Nielsen et al., 2010). However, the GWAS sometimes did not provide conclusive evidence for genetic factors previously reported as associated with dependence in single-candidate gene studies. Therefore, missing heritability needs more research and different genetic approaches.

Recently, a new GWAS of substance dependence was reported (Gelernter et al., 2014b). The discovery sample consisted of European Americans (EA) and African Americans (AA) recruited for studies of drug (opioid or cocaine) or alcohol dependence. The most notable results were found in the AA group and described the association between opioid dependence and gene variants involved in potassium signaling pathways. Interestingly, an additional association between opioid dependence and disrupted-in-schizophrenia 1 (*DISC1*) gene polymorphism rs2738888 was detected in both the AA and EA subgroups. This association was identified in the first of three phases of the study. In the final

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meta-analyses, the authors focused on associations mapped to calcium and potassium pathways and did not provide detailed information about results related to *DISC1* derived from replication samples. *DISC1* encodes a protein that is known to be involved in a wide range of functional roles in the central nervous system. Neural proliferation and migration, neurite outgrowth, neuronal signaling, and synaptic plasticity are among the most frequently cited targets of the *DISC1* protein (Lipina & Roder, 2014). *DISC1* has been broadly reported as a genetic risk factor across a spectrum of psychiatric disorders, including schizophrenia and bipolar disorder (Soares et al., 2011).

Gelernter et al. (2014b) also identified, in the case-control model, an association between opioid dependence and SNP rs6419156 located approximately 196 kb upstream of the protein phosphatase 3 catalytic subunit alpha isoform (*PPP3CA*) gene. *PPP3CA* encodes the alpha catalytic subunit of a Ca^{2+} /calmodulin-dependent serine/threonine phosphatase, which is also called calcineurin. Genetic alterations in *PPP3CA* have been previously linked with vulnerability to misuse of multiple substances (Chiocco et al., 2010). It was reported that calcineurin may influence reward- and memory-related phenotypes in addiction thought to link dopaminergic and glutamatergic signals (Chiocco et al., 2010; Gerdjikov & Beninger, 2005).

Replication has become a gold standard in genetic association studies and also is extremely important to further investigate emerging results from GWASs. A successful replication provides an independent verification of the discovered associations (Greene et al., 2009). The purpose of the present study was to examine the previously reported association between opioid dependence and the polymorphisms described above, rs2738888 and rs6419156, in the independent data set.

Method

The study included a sample comprised of 392 substance-dependent individuals diagnosed as alcohol and/or opioid dependent according to the *International Classification of Diseases, 10th Edition* (ICD-10), criteria (World Health Organization, 2013). Only patients over age 18 were included. The exclusion criteria were severe cognitive impairment and current psychosis precluding ability to provide valid consent. Cognitive impairment was confirmed with the Wechsler Adult Intelligence Scale (Wechsler, 2008). Eligibility for the study was assessed during psychiatric examination at the entry to treatment program. The detailed protocol for this study was approved by the Research Ethics Committee at the Medical University of Warsaw.

Cohort 1 consisted of 246 alcohol-dependent patients entering abstinence-based, drug-free treatment programs in Warsaw, Poland. Cohort 2 was recruited in a methadone maintenance treatment clinic in Warsaw and consisted of

146 opioid-dependent individuals. Patients were diagnosed as alcohol or drug dependent by experienced psychiatrists working at the substance use disorder clinics. The MINI International Neuropsychiatric Interview (Sheehan et al., 1998) was used to diagnose alcohol or drug dependence and to assess co-occurring psychopathology. The severity of alcohol and drug dependence was evaluated further using the Severity of Dependence Scale (Gossop et al., 1995) and the Michigan Alcohol Screening Test (Selzer, 1971). In addition, the presence of depressive symptoms was assessed in Cohort 1 with the Beck Depression Inventory (BDI; Beck et al., 1961) and in Cohort 2 with the Patient Health Questionnaire (PHQ-9; Wittkamp et al., 2007). The standard cutoff scores were used for border severity of depression: 10 points or more on the BDI and 5 points or more on the PHQ-9, whereas, for severe depression, the cutoffs on each measure were 29 and 20 points, respectively. Lifetime history of suicide attempt was assessed with the Suicidality Module of the MINI.

Participants were asked to complete a questionnaire that included items regarding demographics, education, economic status, history of psychiatric and somatic treatment, psychiatric comorbidities, suicidal behavior, sexual and physical abuse, and family history of substance use disorders, psychiatric disorders, or suicidal behavior. Questions were administered using a modified version of the University of Arkansas Substance Abuse Outcomes Module, a self-administered scale (Rost et al., 1996), which was translated into Polish.

The control sample came from a previously described National Health Survey WOBASZ study (Broda & Rywik, 2005; Rywik et al., 2005). This Polish multi-center population health survey was designed to assess the cardiovascular risk in the community. The WOBASZ study included a representative random sample of the Polish population. Participants were randomly chosen from the permanent residents, ages 20–74 years. All individuals were asked to complete a questionnaire that included information about alcohol consumption. Of 14,350 WOBASZ participants, those who drank alcohol not more than once a month and no more than one standard drink (10 g of alcohol) were classified as nondrinkers and selected as a control group for the present study ($n = 257$). The control group was assessed for depressive symptoms using the BDI. No further information about psychiatric comorbidity or lifetime suicide attempts was collected. The WOBASZ study was approved by The Medical Ethics Committee of the National Institute of Cardiology in Warsaw.

When comparing the combined substance-dependent sample with the control group, we did not find any significant differences with regard to sex ($p = .342$) or age ($p = .275$). However, Cohort 1 was older than controls, whereas Cohort 2 was younger than controls. The characteristics of substance-dependent participants and controls are shown in Table 1.

TABLE 1. Characteristics of substance-dependent participants and controls

Characteristics	Substance-dependent individuals (Cohort 1 and Cohort 2 samples)	Cohort 1	Cohort 2	Controls
Gender: male/female (% male), p^a	274/118 (69.9), $p^a = .342$	173/73 (70.3), $p^a = .226$	101/45 (69.2), $p^a = .760$	174/83 (67.7)
Age, M (SD), p^a	40.2 (9.8), $p^a = .275$	43.7 (10.1), $p^a < .001$	34.4 (5.9), $p^a < .001$	40.0 (8.7)
Severity of alcohol dependence (MAST); median (IQR), p^b	29 (17–39)	34 (27–41), $p^b < .001$	13 (10–22)	
No. of alcohol-dependent individuals, n (%)	357 (91)	246 (100)	111 (76)	
No. of opioid-dependent individuals, n (%)	146 (37)	0 (0)	146 (100)	
Depressive symptoms (BDI); median (IQR)		18 (10–26)		5 (2–11)
Depressive symptoms (PHQ-9); median (IQR)			12 (7–18)	
Border of depression (BDI of ≥ 10 or PHQ-9 of ≥ 5), n (%), p^a , p^b	294 (75), $p^a < .001$	178 (72), $p^a < .001$, $p^b = .302$	116 (80), $p^a < .001$	66 (26)
Severe depression (BDI of ≥ 29 or PHQ-9 of ≥ 20), n (%), p^a	74 (19), $p^a < .001$	50 (20), $p^a < .001$, $p^b = .267$	24 (16), $p^a < .001$	4 (2)
Lifetime history of suicide Attempt, n (%), p^b	121 (30.9)	68 (27.6), $p^b = .113$	53 (36.3)	

Notes: **Bold** indicates $p < .05$. MAST = Michigan Alcohol Screening Test; IQR = interquartile range; no. = number; BDI = Beck Depression Inventory; PHQ-9 = Patient Health Questionnaire. a Comparison with controls; b comparison between Cohort 1 and Cohort 2.

All cases and controls were White with Polish nationality. Participation was voluntary. All individuals gave written, informed consent for participation and for collecting blood or saliva samples for DNA isolation and genetic testing.

Genotyping

The genomic DNA from substance-dependent individuals and controls was extracted using standard salting procedures. DNA samples were anonymized immediately after collection. The selected polymorphisms, rs2738888 and rs6419156, were genotyped using Real-time TaqMan Allelic Discrimination Assay using pre-designed primers obtained from Applied Biosystems (Pre-designed TaqMan SNP Genotyping Assays, 7500 Real-time PCR System, Applied Biosystems, Assay ID C__15928618_10 and C__29234191_10, respectively) on an ABI PRISM 9700 platform (Applied Biosystems). The results were analyzed using 7500 System SDS Software (Applied Biosystems).

Among the cases, the genotyping success cell rate was 98% (384 samples + 8 undetermined samples) for rs2738888 and 99.5% (390 samples + 2 undetermined samples) for rs6419156. Among the controls, the genotyping success cell rate was 99.2% (255 samples + 2 undetermined samples) for rs2738888 and 98.1% (252 samples + 5 undetermined samples) for rs6419156. Our study could detect with power

of .8 ($\alpha = .05$) an allelic association conferring an odds ratio (OR) of 0.65.

Statistical analysis

All genotype distributions were tested for Hardy–Weinberg equilibrium using the Web-Assotest program (<http://www.ekstroem.com/assotest/assotest.html>). IBM SPSS Statistics for Windows, Version 20 (IBM Corp., Armonk, NY) was used to compare the distribution of alleles and genotypes among substance-dependent individuals and controls and to perform genotype–phenotype oriented analysis. When analyzing possible differences in the distribution of alleles and genotypes between substance-dependent participants and controls, three comparisons were made: Cohort 1 versus controls, Cohort 2 versus controls, and the combined substance-dependent group versus controls. The chi-square and Fisher's exact test were applied for categorical variables. The Mann–Whitney U test or Kruskal–Wallis test was used for continuous variables with nonparametric distribution after checking for normality by the Kolmogorov–Smirnov test. In addition, linear regression was used for multivariate analyses. Because the association between opioid dependence and rs2738888 or rs6419156 was previously reported (Gelernter et al., 2014b) and hypothesized, the correction for two tests was not applied. However, in genotype–phenotype oriented

TABLE 2. Distribution of genotypes and analysis of the association between rs2738888 and rs6419156 and substance use disorders

SNP	Group	Genotypes			Allele		HWE <i>p</i>	Allelic comparison ^a OR [CI], <i>p</i>
		TT (%)	CT (%)	CC (%)	T (%)	C (%)		
rs2738888	Substance-dependent individuals, <i>n</i> = 384	283 (73.7)	88 (22.9)	13 (3.4)	654 (85.2)	114 (14.8)	.067	0.80 [0.59, 1.09], .154
	Cohort 1, <i>n</i> = 243	174 (71.6)	59 (24.3)	10 (4.1)	407 (83.7)	79 (16.3)	.092	0.89 [0.64, 1.24], .506
	Cohort 2, <i>n</i> = 141	109 (77.3)	29 (20.6)	3 (2.1)	247 (87.6)	35 (12.4)	.521	0.65 [0.43, 0.99], .045
	Controls, <i>n</i> = 255	171 (67.1)	77 (30.2)	7 (2.7)	419 (82.2)	91 (17.8)	.633	
		GG (%)	AG (%)	AA (%)	G (%)	A (%)		
rs6419156	Substance-dependent individuals, <i>n</i> = 390	243 (53.4)	125 (38.5)	22 (8.1)	611 (78.3)	169 (21.7)	.271	0.98 [0.75, 1.28], .880
	Cohort 1, <i>n</i> = 244	154 (63.1)	77 (31.6)	13 (5.3)	385 (78.9)	103 (21.1)	.413	0.95 [0.70, 1.28], .725
	Cohort 2, <i>n</i> = 146	89 (61.0)	48 (32.9)	9 (6.1)	226 (77.4)	66 (22.6)	.466	1.03 [0.73, 1.46], .850
	Controls, <i>n</i> = 252	151 (61.4)	91 (33.4)	10 (5.2)	393 (78.0)	111 (22.0)	.415	

Notes: **Bold** indicates $p < .05$. SNP = Single-nucleotide polymorphism; HWE = Hardy–Weinberg equilibrium; OR = odds ratio; CI = confidence interval.
^aComparison with controls.

analyses, a Bonferroni correction was used with α -corrected = .0125 (for four tests: two SNPs plus two cohorts).

Results

The genotype distribution in cases and controls was in Hardy–Weinberg equilibrium (Table 2). When comparing the substance-dependent group, Cohort 1, and Cohort 2 with controls, no significant differences in either genotype or allele distributions for rs6419156 were observed. In addition, no significant differences in either genotype or allele distributions for rs2738888 were found between substance-dependent group or Cohort 1 and controls. However, the frequency of rs2738888 C allele among opioid-dependent cases in Cohort 2 (12.4%) was lower than among controls (17.8%, OR = 0.65, 95% CI [0.43, 0.99] $p = .045$). This allelic association between rs2738888 and opioid dependence remained significant in the logistic regression model after adjusting for age and gender (OR = 0.59, 95% CI [0.38, 0.92], $p = .021$).

Given the previously reported association between *DISC1* polymorphisms and schizophrenia or depression, phenotype-oriented analyses were performed within the opioid-dependent group (Cohort 2) to explore this subject further (Table 3). The *DISC1* polymorphism was associated with severe depression (OR = 0.13, 95% CI [0.02, 0.98], $p = .015$; comparing the allelic distribution in opioid-dependent individuals with severe depression to opioid-dependent individuals without severe depression) and with lifetime history of suicide attempt (OR = 0.25, 95% CI [0.09, 0.66], $p = .003$; comparing the allelic distribution in opioid-dependent individuals with lifetime suicide attempt to opioid-dependent

individuals without lifetime suicide attempt). The association between rs2738888 and lifetime suicide attempt in the opioid-dependent sample remained significant after Bonferroni correction. It is interesting to note that all opioid-dependent patients with severe depression and lifetime suicide attempt ($n = 14$) had TT genotype (100%).

No association between suicide attempts or depression and the *DISC1* polymorphism was detected in the alcohol-dependent group (Table 3).

Discussion

The present analyses found an association between opioid dependence and *DISC1* polymorphism. The rs2738888 C allele showed a protective effect against opioid dependence. These results, in an independent sample of Polish adults with alcohol and/or opioid dependence, are consistent with the results of the GWAS reported recently by Gelernter et al. (2014b), in which the “C” allele was protective (personal communication from J. Gelernter). An additional contribution of the present study is the phenotype-oriented analysis, which indicates a possible association between rs2738888 and lifetime suicide attempt in opioid-dependent patients.

DISC1 was initially identified in a large Scottish family, in which balanced (1;11)(q42.1; q14.3) translocation segregated with schizophrenia, bipolar disorder, and recurrent major depression (Millar et al., 2000). Since then, *DISC1* has been linked in genetic association studies to vulnerability to a wide range of psychiatric disorders including schizophrenia, bipolar disorder, major depression, schizoaffective disorders, autism, Asperger syndrome, Huntington’s disease, and Alzheimer’s disease (Boxall et al., 2011; Brandon & Sawa,

TABLE 3. Phenotypic-oriented analyses for rs2738888

Group	Phenotype	Genotype			Allele		OR [CI], <i>p</i> C vs. T
		TT (%)	CT (%)	CC (%)	T (%)	C (%)	
Cohort 1	Severe depression (BDI $\geq$ 29), <i>n</i> = 50	33 (66.0)	17 (34.0)	0 (0.0)	83 (83.0)	17 (17.0)	0.96 [0.53, 1.73], .885 ^a
	No severe depression (BDI < 29), <i>n</i> = 183	133 (72.7)	40 (21.9)	10 (5.50)	306 (83.6)	60 (16.4)	
	Lifetime history of suicide attempt, <i>n</i> = 68	48 (70.6)	19 (27.9)	1 (1.5)	115 (84.6)	21 (15.4)	1.10 [0.64, 1.91], .725 ^b
	No history of suicide attempt, <i>n</i> = 167	120 (78.9)	38 (22.8)	9 (5.4)	278 (83.2)	56 (16.8)	
Cohort 2	Severe depression (PHQ-9 $\geq$ 20), <i>n</i> = 24	23 (95.8)	1 (4.2)	0 (0)	45 (97.3)	1 (2.1)	0.13 [0.02, 0.98], .015 ^a
	No severe depression (PHQ < 20), <i>n</i> = 116	85 (73.3)	28 (24.1)	3 (2.6)	200 (85.3)	34 (14.7)	
	Lifetime history of suicide attempt, <i>n</i> = 50	45 (90%)	5 (10%)	0 (0%)	95 (95%)	5 (5%)	0.25 [0.09, 0.66], .003 ^b
	No history of suicide attempt, <i>n</i> = 91	63 (69.2)	24 (26.4)	4 (4.4)	150 (82.4)	32 (17.6)	

Notes: **Bold** indicates $p < .05$. OR = odds ratio; CI = confidence interval; BDI = Beck Depression Inventory; PHQ-9 = Patient Health Questionnaire. ^aComparison of substance-dependent individuals with severe depression (Cohort 1 or 2, respectively) and substance-dependent individuals without severe depression (Cohort 1 or 2, respectively); ^bcomparison of substance-dependent individuals with lifetime suicide attempt (Cohort 1 or 2, respectively) and substance-dependent individuals without lifetime suicide attempt (Cohort 1 or 2, respectively).

2011; Porteous et al., 2011; Thomson et al., 2013; Young-Pearse et al., 2010). In addition, *DISC1* has been reported as a risk factor for some neuropsychiatric phenotypes, such as anxiety, neuroticism, emotional stability, chronic fatigue syndrome, and social anhedonia (Chubb et al., 2008; Thomson et al., 2013). It is important to note that a genome scan meta-analysis, which included 32 independent genome-wide linkage scan analyses, did not provide evidence for linkage between *DISC1* and schizophrenia (Ng et al., 2009). Also, a meta-analysis carried out by Mathieson et al. (2012) using 10 candidate gene studies and three GWASs did not confirm an association between *DISC1* and schizophrenia. Moreover, *DISC1* has not reached significance in a GWAS of bipolar disorder (Sklar et al., 2011) or major depression (Ripke et al., 2013). Those negative results could be attributable to phenotypic heterogeneity of psychiatric disorders.

To the best of our knowledge, *DISC1* has not been frequently examined as a possible genetic risk factor for dependence. However, Sawamura et al. (2005) reported that a *DISC1* isoform subcellular distribution in the orbitofrontal cortex in autopsied brains from patients with drug or alcohol dependence was changed in comparison with normal control brains. Similar increases in *DISC1* isoform nuclear pool were observed in brain samples from individuals diagnosed with schizophrenia or major depression (Sawamura et al., 2005). Recently published results of deep resequencing of *DISC1* (Xie et al., 2014) indicated that rare variants in *DISC1* are associated with opioid dependence. Interestingly, a novel GWAS association between alcohol dependence and *CCDC88A* (coiled-coil domain containing 88A) on chromosome 2, which interacts with *DISC1*, was also recently found (Gelernter et al., 2014a).

Two decades of intensive research on *DISC1* function in the central nervous system indicates that this protein seems to have numerous effects in a variety of psychiatric diagnoses and neuropsychiatric phenotypes. *DISC1* is a scaffold multifunctional protein that plays an important role in neurogenesis (neuronal proliferation, differentiation, maturation, integration, migration, growth, and synaptogenesis), neuronal signaling, and neuroplasticity (Wu et al., 2013). The biological functions of *DISC1* are enriched by extensive interactions with multiple proteins (Thomson et al., 2013). We hypothesized that *DISC1* may influence vulnerability to substance use disorders via regulation of glutamatergic and dopaminergic neurotransmission (Lipina & Roder, 2014; Niwa et al., 2010; Wei et al., 2014).

The results of the present study suggest a possible association between a *DISC1* polymorphism and lifetime suicide attempt in patients with opioid dependence. There is growing evidence that vulnerability to suicidal behavior is inherited independently from psychiatric disorders (Mann, 2003). We hypothesized that *DISC1* may be associated with lifetime history of suicide attempts in opioid-dependent individuals via interactions with N-methyl-D-aspartate receptors (NMDA receptors). Previous research identified a reduction in the potency of NMDA receptors in the hippocampus of suicide victims when compared with sudden-death controls (Sowa-Kućma et al., 2013). In addition, genetic alternation of glutamatergic receptors has been linked with suicide attempts (Sokolowski et al., 2013).

Although *PPP3CA* seems to be another possible risk factor for opioid dependence, the results of the present study did not replicate the association between opioid dependence and SNP rs6419156 that was reported by Gelernter et al.

(2014b). This lack of association may be due to a relatively small sample size. In addition, because the risk allele was different in the two racial groups (EA and AA) in Gelernter's study (2014b), it might be difficult to detect an association in a different population.

Several limitations of the present study are important to consider. First, the study sample was small; however, the study could detect an allelic association conferring $OR = 0.65$ with power of 0.8. The present research focused only on two selected SNPs that were previously reported to be associated with opioid dependence, and correcting for two tests was not necessary. However, for genotype–phenotype analyses a Bonferroni correction was used. Psychiatric diagnoses, including opioid dependence, were not available for the control group; we were able to include only those who were classified as non-alcohol drinkers. This limitation could lead to underdiagnosis of opioid dependence.

In conclusion, the results of the present study suggest that *DISC1* rs2738888 polymorphism is associated with opioid dependence. This genetic marker may also be important for understanding the risk for lifetime suicide attempt in opioid-dependent individuals. More research is needed to understand the possible contribution of *DISC1* to opioid dependence. This widely studied gene, with multiple functions and interactions across the central nervous system, should be examined in future work as a possible genetic determinant of opioid dependence.

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