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Research article

Association study of *BDNF* and *DRD3* genes with alcohol use disorder in Schizophrenia

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ABSTRACT

Alcohol use disorder (AUD) is a leading risk factor of disease burden in the world. It is also commonly comorbid with over 20% of schizophrenia patients. The brain-derived neurotrophic factor (BDNF) and dopamine D3 receptor (DRD3) have been implicated in alcohol drinking behaviour. Previous genetic studies of the *BDNF* and *DRD3* genes produced mixed findings; however, only one study investigated two *BDNF* genetic markers with alcohol dependence in schizophrenia patients. We investigated 15 single-nucleotide polymorphisms (SNPs) in *DRD3* and four SNPs in *BDNF* for possible association with alcohol abuse or dependence in schizophrenia patients of European ancestry (N = 195). The patients were assessed for the occurrence of alcohol abuse or alcohol dependence using the Structured Clinical Interview for DSM-IV Axis I Disorders, Patient Edition (SCID-I/P). We found the *BDNF* Val66Met to be associated with alcohol dependence (p = 0.004). We also found haplotypes across *BDNF* to be nominally associated with alcohol dependence. Analyses of *DRD3* markers and haplotypes yielded mostly negative findings. Our findings support a role of the *BDNF* gene in alcohol dependence in schizophrenia patients. Larger samples are required to confirm our findings, particularly those of *BDNF* haplotypes.

1. Introduction

Alcohol use disorder (AUD) is a prevalent and debilitating condition affecting approximately 6.2% of adults and 2.5% of youth in the U.S. (Substance Abuse and Mental Health Services Administration (SAMHSA), 2015). Approximately 3.3 million deaths worldwide have been attributed to harmful alcohol use [1], making it one of the leading risk factors for disease burden globally [2]. AUD, in turn, leads to significant medical complications including liver diseases, gastritis, pancreatitis, cardiomegaly, hypoglycemia, Wernicke-Korsakoff syndrome (thiamine deficiency), and cerebellar ataxia. In fact, about 3.8% of global deaths and 4.6% of global disability-adjusted life years are attributable to AUD [3]. Excessive alcohol consumption is estimated to have cost the U.S. $223.5 billion in 2006, 72.2% of which was from lost productivity, 11.0% from healthcare costs, and 9.4% from costs to the criminal justice system [4].

In addition to its medical complications, AUD can also lead to cognitive, behavioural, and psychological impairments. In fact, there is a significant comorbidity rate between AUD and many psychiatric conditions. One study found that among individuals with alcohol abuse, the one-year prevalence of schizophrenia, and mood and anxiety disorders ranged between 24 and 37%, with odds ratios between 2.6–3.8 [5]. It is speculated that AUD and other psychiatric disorders have a bidirectional relationship, wherein patients attempt to cope with their disorder through alcohol use, which in turn leads to further neurological damage and more symptoms of mental illness [6]. Another possibility is that these disorders share a common underlying etiology, with the same set of neurobiological and cognitive impairments manifesting themselves through low mood, anxiety, psychosis, or substance abuse [5].

One psychiatric condition that has particularly significant overlap with AUD is schizophrenia. AUD is the most common comorbidity found in patients with schizophrenia [7], with a concurrent comorbidity rate of 9.4% and median lifetime prevalence of 20.6% [8].

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AUD is known to complicate treatment of schizophrenia as it often leads to more social, legal, and medical problems [9]. In fact, having comorbid AUD is associated with higher risk of homelessness, suicidality, impulsivity, hostility, and committing violent crimes; greater severity of negative and positive symptoms; higher rates of relapse following remission; medication non-adherence; more frequent hospitalizations; poor psychosocial outcomes; and higher prevalence of chronic health conditions such as coronary artery disease and hypertension [10]. Patients with comorbid AUD and schizophrenia may also be a potential subgroup requiring specific treatment targets.

In terms of etiology, AUD is an extremely complex and poorly understood disorder with likely contributions from genetic, cognitive, behavioural, psychological, and environmental factors [11]. Overall, according to a meta-analysis of twin and adoption studies AUD is approximately 50% heritable, with modest shared environmental effects (10%) [12]. Based on such findings, genome-wide association studies have identified potential genetic loci associated with AUD, with the most robust ones being in the GABAergic system *GABRA2* and alcohol-metabolizing enzymes *ALDH2*, *ADH1B*, and *ADH1C* [13,14]. Stress-related genes such as *CRHR1* have also been found to be associated with AUD in the context of early life trauma [13].

More recently, two genes involved in neural plasticity and transmission have been studied as potential candidate genes for the risk of AUD: brain-derived neurotrophic factor (*BDNF*) and dopamine D3 receptor (*DRD3*). BDNF is involved in regulation of nerve cell proliferation and survival, in addition to supporting proper dopaminergic function [15–17]. DRD3 is found particularly in the nucleus accumbens of the limbic system that plays a major role in reward and reinforcement [18,19]. Ethanol and cocaine has been shown to increase BDNF [20,21], and BDNF signaling through the BDNF receptor TrkB leads to increased dorsal striatal D3 receptor levels [20,22]. D3 receptor in turn may regulate ethanol consumption in a homeostatic manner [20,22]. While several polymorphisms of both genes have been identified, two functional single-nucleotide polymorphisms (SNPs) in particular have been extensively explored: *BDNF* Val66Met (rs6265) and *DRD3* Ser9Gly (rs6280).

The rs6265 SNP resides in exon 2 of the *BDNF* gene, and leads to a valine-to-methionine conversion in residue 66 of precursor BDNF peptide (pro-BDNF) [23]. While this conversion is not included in the mature form of *BDNF*, the Met variant is associated with impaired dendritic trafficking of *BDNF* mRNA [24,25] as well as decreased activity-dependent secretion of pro-BDNF [26,27]. On the other hand, the rs6280 SNP is in exon 1 of the *DRD3* gene, and results in a serine-to-glycine substitution at the extracellular N-terminal domain of the D3 receptor [28]. This gain-of-function variation has been known to increase dopamine binding affinity of *DRD3* [29], thus causing greater dopamine-mediated cAMP response and prolonged mitogen-associated protein kinase signaling [30].

The rs6265 and rs6280 SNPs have both been implicated in various forms of substance dependence including methamphetamine, heroin, cocaine, and nicotine [31–34]. However, studies on the role of *BDNF* and *DRD3* in AUD have led to more mixed results [35].

Most studies only examined the well-known SNPs rs6265 and rs6280. In addition, few studies explored the role of *BDNF* and *DRD3* in the context of comorbid AUD and other psychiatric disorders such as schizophrenia. This is particularly relevant given that *BDNF* and *DRD3* may be implicated in schizophrenia risk and prognosis [36–43].

Despite the potential role of *BDNF* and *DRD3* in substance abuse as well as schizophrenia, few studies have examined these genes in the context of comorbid AUD and schizophrenia. Krebs et al. [44] found that while there was no significant difference in the frequency of rs6280 polymorphism of *DRD3* between schizophrenia patients and controls, the homozygosity was significantly more prevalent in schizophrenia patients with lifetime substance use comorbidity compared to those without history of substance use or controls. The authors concluded that the *DRD3* rs6280 polymorphism may be associated with predisposition to substance abuse in the context of schizophrenia, but not schizophrenia itself [44]. The substances of abuse or dependence included alcohol, but the authors did not investigate the possible association between rs6280 and alcohol use disorder. The only study that specifically investigated *DRD3* or *BDNF* genes in alcohol use disorder was Cheah et al. [45] where the authors genotyped two *BDNF* SNPs (rs6265 and rs7103411) in a sample consisting of three groups: schizophrenia, alcohol-dependent without schizophrenia, and healthy controls. Overall, the A allele of rs6265 and C allele of rs7103411 were associated with comorbid alcohol dependence as well as risk-taking behaviour after drinking in the schizophrenia subgroup. In addition, the rs6265-rs7103411 A-C haplotype was associated with comorbid alcohol dependence and schizophrenia. Interestingly, no association was found when these SNPs were tested in the non-schizophrenia alcohol-dependent group [45]. This finding raises the possibility that while these polymorphisms may not play an important role in AUD itself, they could be involved in the development of comorbid AUD in the context of schizophrenia.

Such findings show that *BDNF* and *DRD3* may be implicated in the complex comorbidity between AUD and schizophrenia, by modulating susceptibility to substance abuse and dependence among patients with schizophrenia. This is of particular importance considering that comorbid diagnosis of AUD is associated with poorer prognosis and treatment outcomes among schizophrenia patients. Therefore, in this study, we aimed to better elucidate the genetic etiology of concurrent AUD and schizophrenia by determining whether single-nucleotide polymorphisms (SNPs) in *BDNF* and *DRD3* are associated with AUD among patients with schizophrenia. Nineteen tag polymorphisms across the *BDNF* and *DRD3* genes were examined, using a homogeneous sample of schizophrenia patients of European ancestry.

2. Methods

2.1. Subjects

We included in our sample 195 unrelated schizophrenia (SCZ) or schizoaffective disorder patients of European ancestry (66% males, age (average +/− standard deviation): 37.93 +/− 10.69 years) were recruited from the Centre for Addiction and Mental Health in Toronto, Canada as previously described [41,46,47]. The Structured Clinical Interview for DSM-IV (SCID-IV; First et al., 1996) was used as the primary diagnostic tool by clinical research staff. Patients who satisfied the DSM-IV diagnostic criteria for SCZ or schizoaffective disorder were included (APA, 1994), while patients with history of major substance abuse (excluding alcohol), major neurological disorders, and head injury with significant loss of consciousness were not included in the study. History of alcohol abuse and alcohol dependence was assessed based on the SCID-IV interview and the information was available for 155 patients. Alcohol abuse was defined as: having failed to complete duties; alcohol use in physically dangerous situations; social, interpersonal, or legal problems resulting from alcohol use. Alcohol dependence was defined as: exhibiting tolerance, withdrawal, alcohol use despite problems, reduced activities, an increase in time spent on alcohol use, wanting or desiring to quit, or drinking more than planned. Within our schizophrenia sample, 38 patients had a lifetime history of alcohol abuse, 26 had a lifetime history of alcohol dependence, and 21 had a history of both at some point in their lives. Our sample of has over 80% power to detect an odds ratio of 2.40 (two-sided alpha 0.05, additive model, minor allele frequency 0.2) (QUANTO). This study has been approved by CAMH Research Ethics Board, and the participants have provided informed consent to the work.

2.2. DNA isolation and polymorphism genotyping

Blood specimens were acquired by venipuncture into two 10-mL EDTA tubes. We purified genomic DNA from blood lymphocytes using