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Association of polymorphisms in *HTR2A*, *HTR1A* and *TPH2* genes with suicide attempts in alcohol dependence: a preliminary report

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Abstract

We investigated a relationship between selected polymorphisms: rs6313 in *HTR2A*, rs6295 in *HTR1A* and rs1386494 in *TPH2*, and suicidal behavior in 150 alcohol-dependent patients. There was a significant association between more frequent C102C genotype in *HTR2A* and suicide attempts in alcoholic females. No differences in genotype distribution in *HTR1A* and *TPH2* SNPs were found between patients with and without suicide attempts.

Keywords

Alcoholism; Suicide; *HTR2A*

1. Introduction

Alcohol dependence is an individually and socially important public health problem, which is strongly associated with suicidal behavior. Suicide risk in alcohol-dependent subjects is 60–120 times greater than in the normal population (Sher, 2006). It has been proposed that the serotonergic system plays an important role in the mechanism of alcohol dependence, mainly due to its influence on the mesolimbic dopaminergic reward system (LeMarquand et al., 1994). Moreover, serotonin-related impulsivity and suicidal behavior have been linked to personality traits that increase susceptibility to alcohol dependence, especially to its particular subtypes (Lesch, 2005; Koller et al., 2006). On the other hand, alcohol use influences central serotonergic system leading to transient decreases in serotonin (5-HT) function during ethanol intoxication (Badawy, 1994), which could underlie and partly

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account for frequent suicide attempts and completed suicides during acute alcohol use (Cherpitel et al., 2004).

Although the studies in alcoholism and suicidality have shown a contribution of both genetic and environmental components to their development (Du et al., 2001; Dick and Foroud, 2003), the specific genes that modulate their risk as well as intermediate phenotypes have not been identified. Based on the published literature we have chosen candidate genes involved in 5-HT neurotransmission, whose function may be important for the development of alcoholism and suicidal behavior. These genes code for two serotonin receptors (*HTR2A* and *HTR1A*) and tryptophan hydroxylase 2 (*TPH2*), the key enzyme in the serotonin synthesis.

The 5-HT_{2A} receptor gene (*HTR2A*) is located on chromosome 13 (13q14–q21) and contains the T102C (rs6313) synonymous single nucleotide polymorphism, which has been associated with suicidal behavior (Du et al., 2000; Arias et al., 2001) and alcohol abuse (Hwu and Chen, 2000). The 5-HT_{1A} receptor gene (*HTR1A*) is located on chromosome 5 (5q11.2-13) and in recent studies it was shown that a common C(-1019)G polymorphism in the promoter region of this gene is likely associated with depression (Kishi et al., 2009), and suicide attempts (Lemondet et al., 2003; Wasserman et al., 2006; Serretti et al., 2009). Tryptophan hydroxylase-2 (*TPH2*) gene is located on chromosome 12 (12q21.1). A few genetic studies have already indicated associations between several *TPH2* gene polymorphisms and suicidal behavior (Zill et al., 2004; De Luca et al., 2005; Ke et al., 2006). The intronic polymorphism rs1386494 from the *TPH2* gene examined in our study was previously associated with the severity of treatment-resistant depression by Anttila et al. (2009), but there were also negative results (e.g., Illi et al., 2009).

Based on the review of relevant publications concerning the function of the described genes, it seemed justified to include these genes as potential candidates in our study on alcohol dependence and suicide attempts. Therefore, the aim of the present study was to investigate a possible role of variations in the *HTR2A* (T102C), *HTR1A* (C-1019G), and *TPH2* (rs1386494) genes in suicide attempts among patients with alcohol dependence. We have hypothesized that particular variants in each polymorphism (CC genotype in *HTR2A* rs6311; GG genotype in *HTR1A* rs6295 and AA genotype in *TPH2* rs1386494) are associated with suicide attempts in alcoholics. The paper is presenting preliminary results of this investigation.

2. Methods

2.1. Participants

The study included a sample of 150 alcohol-dependent (AD) patients (108 males and 42 females) diagnosed according to DSM-IV (APA, 2000) criteria consecutively admitted to residential addiction treatment programs at the Department of Psychiatry of the Medical University of Warsaw. All patients were unrelated Caucasians, of a Polish nationality and older than 18 years. Patients with significant psychiatric comorbidity were excluded from the study. The study population was later examined in two subgroups: 38 patients with and 110 patients without a history of at least one suicide attempt (SA; 2 patients did not respond to the questions about suicide). Patients were included in the study after written informed consent. The local Bioethics Committee of the Medical University of Warsaw and the Institutional Review Board at the University of Michigan approved the study protocol.

2.2. Measures

Patients were evaluated with the Mini International Neuropsychiatric Interview (M.I.N.I.) - a short, structured interview for both DSM-IV and ICD-10 psychiatric diagnoses (Sheehan et

al., 1998) and suicidal behavior. Participants were categorized as either having made a suicide attempt in their lifetime or not.

2.3. Genotyping

Peripheral blood samples were drawn from all subjects and the DNA of white blood cells was extracted using the standard procedure. The genotypes of the *HTR2A* T102C (rs6313), *HTR1A* C-1019G (rs6295) and *TPH2* (rs1386494) single nucleotide polymorphisms (SNPs) were analyzed in all patients using a Real-Time polymerase chain reaction method (RT-PCR). RT-PCR was performed by custom genetic analysis services, the LightCycler® 480 instrument available from Roche Applied Science, using Simple Probes, LightCycler® 480 Probes Master Mix and universal cycling conditions. LightSNiP (SimpleProbe) assays for SNPs were designed by TIB-MolBiol (Berlin, Germany).

2.4. Statistical analyses

All genotyping results were tested for Hardy–Weinberg equilibrium (HWE). The history of suicide attempts was the primary outcome phenotype. Genotype and allele frequencies were compared between groups by chi-square statistics and Fisher's exact test. Effect sizes for the chi-square tests were calculated using Phi estimates. Odds ratio (OR) values and 95% confidence intervals (CI) were calculated using SAS. No correction for multiple testing was performed. The significance level for all statistical tests was 0.05.

3. Results

All examined SNPs were in Hardy–Weinberg equilibrium. In the comparison of allele and genotype frequencies (Table 1), the *HTR2A* T102C polymorphism showed a trend of more frequent C102C genotype in AD individuals with vs. without suicide attempts ($\chi^2 = 3.61$; df = 1; $p = 0.057$). In the female AD subjects, the group with suicide attempts had a significantly higher frequency of the CC genotype (exact Fisher test, $p = 0.02$); there were no differences in males. The estimated odds ratio for completed suicides with the CC genotype in females was 6.72 (95% CI 1.50 – 30.10) with the effect size Phi = 0.41 for the chi-square test when subgroup analyses were done, and Phi = 0.16 for pooled group analysis. No significant differences in genotype or allele distributions for the *TPH2* rs1386494 and C(-1019)G *5HTR1A* polymorphisms between study groups were found.

4. Discussion

We performed an association study with the polymorphisms: rs6313 (T102C) in *HTR2A*, the rs6295 (C-1019G) in *HTR1A* and the rs1386494 (A/G) in *TPH2* in a sample of 150 alcohol-dependent inpatients. The genetic analyses revealed a tendency of more frequent CC genotype in the *HTR2A* T102C polymorphism in AD patients with vs. without suicide attempts, which was significant in female subjects ($p = 0.02$; no correction for multiple testing). The results are in line with our initial hypothesis and with previous research by Du et al. (2000) and Arias et al. (2001) who found a significant association between the 102C allele and suicidality in depressed patients. Also, 15 independent studies, which were included in a meta-analysis of Li et al. (2006), showed a lower T102 allele frequency in cases with suicidal behavior.

Our preliminary results suggest an association between the *HTR2A* T102C polymorphism and suicide attempts in alcohol dependence, especially in female subjects, which seems to be in contrast to results of Preuss and colleagues (2000). However, both studies had small sample sizes, and thus the divergent results might come from their limited power. Future studies of this polymorphism and the presence of the alcohol dependence remain an

interesting focus for investigations, especially in light of the recent findings that decreased 5-HT function mediated through 5-HT_{2A} receptors has been reported to elevate aldehyde dehydrogenase (ALDH) activity in the brain stem of alcohol treated rats and in vitro hepatocyte cultures (George et al., 2010).

In the present study the C(-1019)G *5HTR1A* polymorphism, which can have important influence on the function of the serotonin system, was not associated with suicide attempts in AD patients, which is consistent with the observation by Koller et al. (2006). Also, the analyses of allele frequency of the *TPH2* rs1386494 as well as the comparison genotypes in our study did not demonstrate a significant association with alcohol-related suicide behavior. These findings are parallel to the results of the study of Zill et al. (2007) who also did not find an association between alcohol dependence or alcoholism-related suicidal behavior and 20 SNPs in the *TPH2* gene.

Our results are interesting especially in light of a growing body of research that has provided evidence for important role of polymorphic variation in genes related to 5-HT receptors in mediating suicidal behavior in alcohol dependence. However, further investigation is warranted to allow for reliable conclusions considering the relatively small sample size, especially when subgroup analyses were done. Another limitation is that we did not assess nor exclude patients with personality disorders. Antisocial personality disorder and borderline personality disorder, for example, are associated with both alcohol dependence and suicide attempts. Our study cannot account for this possible confounder. Also, a limitation of the study is that the history of suicide attempts was obtained by self-report without corroboration from hospital records or third party interviews.

In conclusion, our results suggest that a natural variability in the genes involved in the action of 5-HT system might have an effect on development of suicidality in alcohol dependence. Particularly, a variant in 5-HT_{2A} receptor gene was associated with reported suicide attempts in female alcoholic patients. Therefore it seems important to continue examining the effects of genetic factors altering central 5-HT functioning on suicidal behavior of alcohol-dependent individuals in future studies on larger samples.

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Genotype and allele frequencies of the *HTR2A* T102C polymorphism (rs6313) in groups of alcohol-dependent patients with and without suicidal attempts (SA).

Table 1

<i>HTR2A</i> (rs6313)	Males		Females		Total	
	with SA	without SA	with SA	without SA	with SA	without SA
Genotypes						
CC	12 (46%)	31 (38%)	7 (58%) ¹	5 (17%) ¹	19 (50%) ²	36 (33%) ²
CT	11 (42%)	42 (52%)	3 (25%)	19 (66%)	14 (37%)	61 (55%)
TT	3 (12%)	8 (10%)	2 (17%)	5 (17%)	5 (13%)	13 (12%)
Total	26	81	12	29	38	110
Allele						
C	35 (0.67)	104 (0.64)	17 (0.71)	29 (0.5)	52 (0.68)	133 (0.60)
T	17 (0.33)	58 (0.36)	7 (0.29)	29 (0.5)	24 (0.32)	87 (0.40)

¹ genotype CC vs. (CT and TT) (Fisher's exact test; p = 0.02), OR = 6.72 (95% CI 1.50 – 30.10)

² genotype CC vs. (CT and TT) ($\chi^2 = 3.61$; df = 1; p = 0.057)