Published in final edited form as:

J Psychiatr Res. 2013 April ; 47(4): 527–533. doi:10.1016/j.jpsychires.2012.12.004.

The CC genotype in the T102C *HTR2A* polymorphism predicts relapse in individuals after alcohol treatment

Andrzej Jakubczyk¹, Anna Klimkiewicz¹, Maciej Kopera¹, Aleksandra Krasowska¹, Małgorzata Wrzosek², Halina Matsumoto¹, Margit Burmeister³, Kirk J. Brower³, and Marcin Wojnar^{1,3}

¹Department of Psychiatry, Medical University of Warsaw, Warsaw, Poland ²Department of Pharmacogenomics, Medical University of Warsaw, Warsaw, Poland ³Department of Psychiatry, University of Michigan, Ann Arbor, MI, USA

Abstract

The serotonin system is hypothesized to contribute to predisposition and course of alcohol dependence. However, the potential association between the T102C polymorphism (rs6313) in the type 2A serotonin receptor (*HTR2A*) gene and treatment outcomes in alcohol dependence has not been investigated. The aim of the study was to assess the contribution of this genetic polymorphism as a predictor of relapse in relation to other previously identified predictors. A sample of 254 alcohol dependent subjects, were recruited in alcohol treatment centers in Warsaw, Poland and prospectively assessed at baseline and follow-up after 12 months. At baseline, information about demographics, psychopathological symptoms and alcohol problems was obtained. The stop-signal task was performed and blood samples for genetic analysis of *HTR2A* T102C (rs6313) were collected. Relapse was defined as any drinking during the follow-up period. The statistical analysis showed that the CC genotype was significantly associated with increased relapse. Other significant factors were baseline depressive symptoms, number of drinking days during the 3 months prior to the baseline assessment, severity of alcohol-related problems, and a lifetime history of impulsive suicide attempts. Logistic regression analysis with and without the genetic factor revealed that adding the genetic factor increased the R square value by about 4%, with the CC genotype in the T102C polymorphism being the strongest predictor of relapse (OR=2.32). The significant influence on relapse of the CC genotype, which is associated with fewer 5-HT_{2A} receptors in the central nervous system, suggests the possibility that this genetic polymorphism could influence response to serotonergic medications.

© 2012 Elsevier Ltd. All rights reserved.

Corresponding author: Marcin Wojnar, M.D., Ph.D., Department of Psychiatry, Medical University of Warsaw, Nowowiejska 27, 00-665 Warsaw, Poland. Tel.: +48600822669 Fax: +48228251315; marcin.wojnar@wum.edu.pl.

Contributors

Marcin W, KB, and MB designed the study and wrote the protocol. AJ, Marcin W, MK, KB and Aleksandra K managed the literature search. Małgorzata W, HM, and MB designed and directly supervised genotyping. AJ, KB and Marcin W performed all statistical analyses and summarized the results. Marcin W, AJ, Anna K, KB and MB analyzed and interpreted clinical and genotyping results. AJ, Aleksandra K, Anna K, MK and Marcin W conducted the stop-signal task and data collection. AJ, MW and KB wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Keywords

Alcohol dependence; Serotonin; *HTR2A*; Genetic polymorphism; Relapse

1. Introduction

Alcohol use is a major factor contributing to global health burden as well as social and economic harm. About 3 to 8% of all deaths worldwide are directly or indirectly associated with drinking alcohol (Rehm et al. 2009). From a global perspective, health harms of alcohol use are more severe than those associated with smoking (Rehm et al. 2011). These statistics support growing interest in developing successful treatment programs for alcohol dependence but, despite consistent effort, the disorder is still characterized by persistent vulnerability to relapse even after specialized treatment (MATCH 1997).

The serotonin system may contribute to both predisposition and course of addictions, including alcohol dependence (Kirby et al. 2011). One of the major putative links between low serotonin activity and alcohol drinking is impulsivity (Kirby et al. 2011; Stoltenberg et al. 2012). Both behavioral impulsivity (impulsive action, inability to stop an action that has already been started) and cognitive impulsivity (inability to predict or disregard of the consequences of one's behavior) have been shown to be important predictors of severe course and relapse in alcohol-dependent patients (Rubio et al. 2008; Kirby et al. 2011; Evren et al. 2012). Consequently, impulsivity is a reasonable and potential target in alcohol treatment (Lejuez et al. 2010; Conrod et al. 2006; Conrod et al. 2011).

One important behavioral manifestation of impulsivity is suicidal behavior. Lifetime risk of making a suicide attempt in alcohol-dependent patients is high and estimated to be 7 – 15%; about 60% of these attempts are impulsive (Wojnar et al. 2008). In a study by Spokas et al. (Spokas et al. 2012), participants who made an impulsive attempt were more likely to be diagnosed with an alcohol use disorder than those who made a premeditated attempt. In addition, we previously reported that post-treatment relapse rates to drinking were higher in alcohol-dependent individuals with a history of impulsive suicide attempts (Wojnar et al. 2008).

Genetic associations with risk of relapse in alcohol dependence have been reported for *BDNF* (Wojnar et al. 2009), *GABRA2* (Bauer et al. 2007; Bauer et al. 2012) and *5-HTTLPR* (Pinto et al. 2008) polymorphisms. In addition, early pharmacogenetic studies suggest a moderating effect of polymorphisms in the serotonin transporter gene on the efficacy of both ondansetron (Johnson et al. 2011) and sertraline (Kranzler et al. 2011) in reducing the severity of alcohol drinking during treatment.

Our recent study of alcohol-dependent individuals revealed a significant association between the CC polymorphism in the type 2A serotonin receptor (*HTR2A*) gene and behavioral impulsivity (Jakubczyk et al. 2012) as measured by the stop-signal task, an objective measure of motor impulsivity. In other studies, the “high-risk” CC genotype, associated with decreased 5-HT_{2A} receptors in the central nervous system, was also associated with indirect behavioral manifestations of impulsivity, such as aggressive behavior in male alcoholics (Hwu and Chen 2000), more frequent nicotine dependence (do Prado-Lima et al. 2004), and suicidal ideation in individuals with a major depressive episode (Du et al. 2000).

However, to our best knowledge, the potential association between *HTR2A* polymorphisms and treatment outcomes in alcohol dependence has not been investigated. Taking into consideration our previous results (Jakubczyk et al. 2012) suggesting a significant association between *HTR2A* polymorphism and behavioral impulsivity in alcohol-

dependent patients, as well as results of other studies suggesting an important role of impulsivity in predicting relapse (Evren et al. 2012), we decided to investigate a direct association between the rs6313 polymorphism in *HTR2A* and relapse. We hypothesized that the CC genotype would predict relapse in alcohol-dependent patients. We also assessed other well-recognized predictors of relapse (depressive symptoms, suicide attempts, sleep problems, severity of alcohol dependence) (Brower 2003; Bottlender and Soyka 2005; Boschloo et al. 2012) in order to compare their relative contribution to relapse in relation to this genetic polymorphism.

2. Materials and Methods

2.1 Subjects

Alcohol-dependent patients were recruited from residential alcohol treatment centers and outpatient clinics in Warsaw, Poland. All treatment programs were abstinence-based and drug-free. Of 389 subjects who consented to be in the study, 386 (99.2%) completed the baseline questionnaire, of which 357 (92.5%) patients had valid genetic data for the *HTR2A* T102C polymorphism. Follow-up data were available for 274 (71.0%) of the original 386 patients and for 254 (71.1%) of the 357 patients with genetic data, which constitutes the sample for this study. There were no significant differences in terms of demographic characteristics, behavioral impulsivity (stop-signal task), depressive symptoms, lifetime history of suicide attempts, and severity of consequences of drinking between patients who were (n=254) and were not (n=103) followed. However, patients not followed drank significantly more alcohol during the past 3 months ($p=0.0002$), and were less likely than the followed group to have the CC genotype (24.3% vs. 38.6%, chi square = 7.73, df=2, $p=.026$).

The study was carried out in accordance with the ethical principles described in 1964 Declaration of Helsinki and was approved by the Medical School Institutional Review Board at the University of Michigan and the Bioethics Committee at the Medical University of Warsaw. All subjects were informed about the objective and course of the study and gave written informed consent for participation, which was confidential and voluntary. The study group included only subjects with a current DSM-IV diagnosis of alcohol dependence, which was assessed clinically by a multidisciplinary team of a psychiatrist and an addiction therapist. Agitated patients, individuals under 18 years of age, and those with active withdrawal or psychotic symptoms were excluded. All subjects scored 25 or higher on the Mini-Mental State Exam (Folstein et al. 1975).

2.2 Assessment procedures

The study employed a prospective design and the protocol was divided into two parts: baseline assessment and follow-up after a median of 12 months (interquartile range: 9–14 months). At baseline, all participants were asked to complete a questionnaire that included information about demographics, psychopathological symptoms and alcohol problems. The stop-signal task was performed in the presence of a research assistant who provided instructions and any necessary help during the test. Blood samples for genetic analysis were collected by a trained nurse. After finishing the alcohol treatment program the patients were followed for approximately 12 months and alcohol drinking was evaluated. Where possible, information about drinking was obtained from family members; if contact with the patient was impossible, family remained the only source of data. A complete follow-up assessment and questionnaire was obtained directly from about 50% of patients. The rest of the data concerning drinking was obtained either from family members or by an abbreviated phone call to patient. To utilize drinking data for as many patients as possible, relapse was defined as any drinking during the period between the baseline and follow-up assessments.

Regardless of agreement, if either the patient or the family reported drinking, then the patient was considered to have relapsed. If neither the patient nor family could be contacted, then the patient was not included in the analyses.

2.3 Measures

- I. The level of behavioral impulsivity was measured using the stop-signal task (Logan et al. 1984). An objective measure of impulsivity, administered via a computer program, in which (based on the concept of behavioral impulsivity) the ability of stopping a response that has already been started was evaluated. The result of the test is presented as a *stop reaction time* (StopRT) (Band et al. 2003). This value (in milliseconds) is a useful indicator of executive functions: the longer the stop reaction time the higher the level of behavioral impulsivity. The stop-signal task is an independent, objective method of assessing the level of impulsivity that reflects real-time brain activity; therefore, it is arguably closer to a genetic effect and a better candidate for an endophenotype than a self-administered questionnaire.
- II. The history and severity of alcohol problems was evaluated using the Short Inventory of Problems (SIP) (Alterman et al. 2009) and a single question from the modified version of the Substance Abuse Outcomes Module (Smith et al. 1996) asking about the duration of drinking problems.
- III. The severity of alcohol dependence was assessed using the Michigan Alcoholism Screening Test (MAST) (Selzer 1971). The Alcohol Timeline Follow-Back interview (Sobell et al. 1988) was used to quantify how many days the patient drank alcohol during the 3 months prior to the baseline assessment.
- IV. Depressive symptoms were evaluated with the Beck Depression Inventory (BDI) (Beck et al. 1996) and severity of sleep problems by the Athens Insomnia Scale (Fornal-Pawlowska et al. 2011).
- V. History of suicide attempts was evaluated with questions from the Mini International Neuropsychiatric Interview (Sheehan et al. 1998). In individuals with a past suicide attempt, trained interviewers asked patients if the attempt was made after alcohol use and how long they had spent planning their most recent suicide attempt. Those attempts with less than 30 min of planning prior to the suicide attempt were categorized as “impulsive suicide attempts”.
- VI. Relapse was assessed by a single question asking if the patient had drunk any alcohol during the follow-up period. Patients who drank any alcohol during the period between baseline and follow-up assessment were considered to have relapsed.

2.4 Genotyping

DNA was extracted using the Gentra PureGene blood kit using manufacturer's instructions (Qiagen). The single nucleotide polymorphism, rs6313 (T102C), in the *HTR2A* gene (13q14-q21) (Erdmann et al. 1996) was analyzed in all patients using the LightSNiP typing assay (TIB-MolBiol, Berlin, Germany) by analyzing the melting curves with the LightCycler 480 instrument/system (Roche Diagnostics). A subset of 200 subjects were also genotyped at the University of Michigan using inventoried Taqman assays according to the manufacturer's conditions and solutions (Applied Biosystems ABI, Foster City, CA), and run on the University of Michigan DNA Core facility's ABI PRISM 7900HT sequence analyzer. Genotype concordance was 100%.

2.5 Statistical analysis

The statistical analysis was performed using Statistica Software, version 10.0 as well as SPSS version 18.0 for logistic regression analysis. The analyses focused on examining associations between the genetic polymorphism of the *HTR2A* gene and relapse following alcohol treatment program. Based on theoretical background and our previous results with regard to impulsivity (Jakubczyk et al. 2012), we analyzed two groups, individuals with the CC genotype and T-allele carriers (CT and TT). We also assessed associations between relapse and other known factors, including impulsivity, history of suicide attempts, severity of alcohol dependence, sleep problems, severity of depressive symptoms, and severity of consequences of alcohol drinking.

All continuous data were tested for normal distribution using the Kolmogorov-Smirnov test. For normally distributed variables, data are presented as arithmetic means and standard deviations (mean $\pm$ SD); and differences by relapse status were tested by analysis of variance. For variables not normally distributed, data are presented as medians and quartiles (25; 75); and differences by relapse status were tested by the Mann-Whitney U test. To test associations between genotypes and relapse as well as history of suicide attempts, chi square tests were performed. A value of $p < 0.05$ was considered statistically significant. All variables that were significant in the primary analyses were entered into a logistic regression analysis in order to ascertain the independent significance of predictors of relapse in alcohol-dependent patients. In order to evaluate the influence of the genetic factor, the analysis was performed in two blocks. In the first block all independent variables were entered without the genotype. In the second block, the binary genetic factor was entered while keeping all the previous factors in the multivariate model. The Nagelkerke R square values of both models were compared and subtracted to estimate the additional variance explained by the genetic factor as a predictor of relapse.

3. Results

3.1 Demographic and clinical characteristics

The mean age of participants (N=254) was 44.2 ± 10.2 years; 74.4% were men and 25.6% were women. All patients were Caucasian. The median age of onset of drinking problems was 24.5 (18, 29) years with mean duration of alcohol dependence in the study group of 19.6 ± 10.6 years. About 49% of the subjects participated previously in at least one alcohol treatment program during their lifetime, and 60.2% reported alcohol problems in at least one parent. One-third of the study group (33.8%) declared a history of at least one suicide attempt during the lifetime; 63.9% of these attempts were impulsive and 66.2% of all attempts were made after alcohol drinking.

The genotype percentages in the *HTR2A* T102C polymorphism rs6313 were: CC – 38.6%, CT – 47.6%, TT – 13.8%. The genotypes were in Hardy-Weinberg equilibrium ($p=0.81$). There were no differences in the distribution of the genotypes ($\chi^2 = 2.92$; $p = 0.23$) between men and women in *HTR2A*. The allele frequencies were: C – 0.624, T – 0.376.

3.2 Relapse

A total of 145 (57.1%) patients relapsed. The statistical analysis revealed a significant association between the *HTR2A* T102C polymorphism and relapse (chi square =7.07, df=1, $p=0.008$). The CC genotype was associated with more frequent relapse (see Table 1). Other factors that were significantly associated with relapse included a history of impulsive suicide attempts (chi square = 4.5, df=1, $p=0.03$), although a history of suicide attempts in general was not (chi square= 2.19, df=1, $p=0.14$), baseline depressive symptoms (BDI score; $F=11.253$, df=1, $p=0.0009$; see Table 2), baseline severity of alcohol-related problems (SIP

score; $F=7.482$, $df=1$, $p=0.007$; see Table 2), and number of drinking days during the 3 months prior to the baseline assessment ($F=7.608$, $df=1$, $p=0.006$; see Table 2).

We found no association between relapse and behavioral impulsivity (StopRT; $F=1.626$, $df=1$, $p=0.2$), severity of alcohol dependence (MAST score; $F=0.53$, $df=1$, $p=0.47$), duration of alcohol dependence ($F=0.217$, $df=1$, $p=0.64$), severity of sleep problems (AIS score; Mann-Whitney U test, $p=0.17$; see Table 2), age ($F=2.519$, $df=1$, $p=0.11$) or gender (chi square = 2.385, $df=1$, $p=0.12$). There was no significant association between the follow-up interval period and relapse in analyzed group (Mann-Whitney U Test, $p=0.10$). Comparison of patients who relapsed and remained abstinent after alcohol treatment is presented in Table 2.

When entered into logistic regression analysis in the first multivariate model (without the genetic factor), only severity of depressive symptoms at baseline assessment (OR=1.04; 95% CI: 1.01 – 1.06; $p=0.013$) and number of drinking days prior to the baseline assessment remained significant predictors of relapse (OR=1.01; 95% CI: 1.00 – 1.02; $p=0.012$). The model was significant (chi square = 23.14, $df=5$, $p<0.0005$) with more than 12% of the variance explained (Nagelkerke R square = 0.125). The first model is presented in Table 3.

In the second block (comprising all factors from the first block with the genetic factor), the *HTR2A* polymorphism was a significant predictor of relapse (OR=2.264; 95% CI: 1.3–4.1) and increased the adjusted variance by about 4 percentage points. The final model was significant ($p<0.0005$; chi square = 30.92; $df=6$) with about 16% of the variance explained (Nagelkerke R square = 0.164). The sensitivity and specificity of the final model for predicting relapse were 67.5% and 63.6%, respectively. Overall, the model correctly classified 65.7% of the sample. The second multivariate model is presented in Table 3.

4. Discussion

Our results indicate a significant association between the *HTR2A* T102C (rs6313) polymorphism and risk of relapse after treatment in alcohol-dependent patients. As hypothesized, the high-risk CC genotype turned out to be significantly associated with relapse. Patients with the CC genotype were 2.3 times more likely to relapse than those with a T allele. To our best knowledge, this is the first report showing a direct association between the rs6313 polymorphism and return to drinking after treatment for alcohol dependence.

The T102C *HTR2A* polymorphism we analyzed is synonymous, i.e., it does not change the amino acid sequence (Polesskaya and Sokolov 2002). However, it is associated with the quantity of the mRNA and protein made, with the T allele associated with an increase in the number of 5-HT_{2A} receptors in the central nervous system (Polesskaya and Sokolov 2002). Mechanisms underlying these differences in expression remain unclear, although it has been speculated that the T102C polymorphism may affect the stability of its mRNA, (Polesskaya and Sokolov 2002), or methylation in the promoter region of the *HTR2A* gene (Polesskaya et al. 2006). Regardless of mechanism, subjects with the CC genotype in the *HTR2A* T102C polymorphism, who were more prone to relapse in our study, likely had lower expression of the gene, fewer 5-HT_{2A} receptors, and lower serotonin activity. Notably, major allele homozygotes turned out to be the high-risk group, which is consistent with the fact that more than half of the patients (57.1%) relapsed. In comparison to other European groups, in which C allele frequencies range from 0.535 to 0.562 and T allele frequencies range from 0.438 to 0.465, our sample had a higher frequency of C allele (0.624) and lower frequency of the T allele (0.376) (http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=6313). Likewise, our sample had a higher frequency of the CC genotype (0.386) than other European groups (0.274 to 0.333). The higher frequencies of the C allele and CC genotype

in this study likely reflect alcohol dependence. In a study by Wrzosek et al. (2012) on a group of 150 alcohol-dependent patients (which is a subsample of our study group), the frequencies of the C allele and CC genotype in the alcohol-dependent group were significantly higher than in controls. Therefore, these studies suggest that the CC genotype is both a marker for alcohol dependence and a risk factor for worse treatment outcome.

In addition to the CC genotype, other significant correlates of relapse in the final model were baseline drinking frequency and severity of depressive symptoms, and their effect did not change much with the addition of the genotype, indicating they are independent predictors. For each additional drinking day in the 3 months prior to baseline, patients were 1.017 times more likely to relapse. On average, those who relapsed drank 8.7 more days during the 3 months prior to baseline than those who did not relapse. Therefore, on average they were 1.148 times more likely to relapse because of their more frequent drinking prior to baseline. Similarly, those who relapsed scored 4.6 points higher on the BDI than those who did not. Therefore, on average they were 1.175 times more likely to relapse due to their higher levels of depression at baseline. By comparison, genotype was the strongest predictor of relapse in this study (OR=2.264).

The latter results confirm a commonly discussed finding in the literature that depressive symptoms are an important risk factor of relapse in alcohol dependence (Bottlender and Soyka 2005; Boschloo et al. 2012). However, when the regression analysis was run separately by gender (data not shown), it appeared that depressive symptoms remained a significant predictor of relapse only in the group of male patients. These results are consistent with other studies reporting that depressive symptoms (Palfai et al. 2007) and a diagnosis of depression (Rounsaville et al. 1987) were predictors of poor treatment outcomes only in men, whereas in women baseline depression may have even had a positive influence on treatment effectiveness for alcohol dependence (Rounsaville et al. 1987). However, the association between the *5HT2A* polymorphism and relapse as a function of gender needs to be studied with a larger group of female alcohol-dependent patients. The relatively small number of women may be considered a limitation of the current study.

Regarding drinking frequency in the 3 months prior to the baseline assessment, the means of 31.9 and 23.2 days for the groups that relapsed and abstained, respectively, may be considered low for individuals admitted to treatment for alcohol dependence. These low numbers may be associated with conditions for alcohol treatment in Poland. Specifically, many patients were required to attend Alcohol Anonymous meetings and ambulatory therapy prior to qualifying for admission to inpatient treatment programs.

We previously observed a significant association between impulsive suicide attempts and relapse (Wojnar et al. 2008), which is replicated here with a twice larger sample size, but only in the bivariate analysis and not in the final model. On the other hand, in our preliminary study of 154 alcohol-dependent patients, of whom 113 had genetic data for rs6313, we did not find an association between the *5HT2A* T102C polymorphism and relapse (Wojnar et al. 2009). The difference in sample size is a possible explanation for this discrepancy. We also previously reported in a subsample of these subjects a significant association between the CC genotype and behavioral impulsivity (Jakubczyk et al. 2012), but this objective measure of impulsivity (StopRT) did not predict relapse in this study. Finally, in contrast to other studies, we did not find a relationship between insomnia and relapse (Foster and Peters 1999; Brower 2003).

Our results confirm in a larger group of patients our previous results that alcohol-dependent patients are likely to make at least one suicide attempt during their lifetime (Wojnar et al. 2008). In our study almost one-third of all patients had a history of at least one suicide

attempt and more than two-thirds of these attempts were impulsive. Moreover, almost 70% of all suicide attempts were made under the influence of alcohol.

While it is not clear how the T102C variant affects relapse risk, it is worth pointing out that the CC genotype has previously been associated with impulsive behavior (Jakubczyk et al. 2012), and early onset alcoholism (compared to controls) (Wrzosek et al. 2011), while the C allele has been associated with suicide attempts (Arias et al. 2001). Although the exact mechanism of action is unclear, this variant is associated in several publications with impulsivity, suicidality, and alcoholism severity, which are all in turn predictors of relapse, and hence our finding of a direct association with relapse is consistent with the previous literature.

The results of our study have to be interpreted in the context of its limitations. The follow-up rate was 71.1%, considered satisfactory. Notably, the relapse assessment with a single item may be considered a limitation of the study. However, this was the single outcome variable for which we had the most confidence; especially when drinking information from only the family was available. In addition, this single measure of drinking outcome is consistent with other studies in the literature (Bauer 2001; Bowden-Jones et al. 2005; Cardenas et al. 2011). The followed and not followed subjects did not differ in terms of demographic characteristics, depressive symptoms, history of lifetime or impulsive suicide attempts, or severity of consequences of drinking. However, subjects who were lost to follow-up drank significantly more frequently during the past 3 months, and, curiously, had a lower frequency of the CC genotype, such that the genotype frequencies were not in Hardy-Weinberg equilibrium in the not followed group ($p=0.009$), whereas the frequency of genotypes in the followed group were in Hardy-Weinberg equilibrium ($p=0.808$). As genotyping error is unlikely to differentially affect one sample group, this may indicate some kind of heterogeneity within the not followed group. However, in the absence of data, it is difficult to draw any conclusions about the drop out sample.

Finally, impulsivity is considered to be a multidimensional construct (Moeller et al. 2001) with behavioral, cognitive, attentional and emotional domains. In our study we analyzed only the behavioral dimension. Other dimensions of impulsivity should also be analyzed in studies of alcohol-dependent individuals.

In conclusion, our results point to the significance of the T102C *HTR2A* polymorphism in relation to relapse among alcohol-dependent patients. The novel and important finding is the direct association between the CC genotype and higher risk of relapse after alcohol treatment while controlling for other known risk factors. Although the CC genotype is related to behavioral impulsivity, it did not appear to exert its effect on relapse via this mechanism given the lack of a relationship between behavioral impulsivity and relapse in this study. Nevertheless, future research should look for a potential mediating effect of other impulsivity constructs and measures. This finding has potential clinical implications. It highlights the importance of the serotonin system with a special interest in optimizing 5-HT_{2A} receptor transmission for relapse prevention. For example, alcohol-dependent patients with the CC genotype may have fewer 5-HT_{2A} receptors and some antidepressants, such as citalopram, can down-regulate 5-HT_{2A} receptors. Thus, patients with the CC genotype may potentially do worse than T allele carriers with this class of antidepressant, analogous to other serotonin system genetic variants (McMahon et al. 2006; Kranzler et al. 2011), although this is unlikely to be the explanation here, as the patients were not treated with antidepressants. In an experimental study on rodents, 5-HT_{2A} antagonists appeared to reduce the level of behavioral impulsivity (Fletcher et al. 2007). The question whether it is in up-regulation mechanism or through activating the dopamine system remains open.

Nevertheless, this type of selective intervention based on *HTR2A* pharmacogenetics remains a potentially fruitful area of investigation in alcohol-dependent patients.

Acknowledgments

We would like to thank Mrs. El bieta Sliwerska for technical assistance as well as all members of the research team in Poland (especially, Izabela Nowosad, MD; Katarzyna Kositorna, MS; Anna Wnorowska, MD; Aleksandra Konopa, MD) as well as the medical staff and patients at “Kolska”, “Pruszkow”, “Petra” and “Solec” Addiction Treatment Centers in Warsaw for their support of this research.

Role of Funding Source

This study was supported by the Polish Ministry of Science and Higher Education grant 2P05D 004 29, the Fogarty International Center/NIDA International Substance Abuse Research Program grant D43-TW05818, the Fogarty International Center/NIAAA International Collaborative Alcohol & Injury Research Training Program grant D43-TW007569 and NIAAA grant R21 AA016104.

Authors have not signed an agreement with any sponsor of the study reported in this paper that has a clause which prevents them from publishing both positive and negative results, from collaborating with other investigators to pool data across sites, or that forbids them from publishing without the approval of the sponsor. The views expressed in this report are those of the authors and do not necessarily represent those of the supporting agencies. The supporting agencies had no further role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

References

- Alterman AI, Cacciola JS, Ivey MA, Habing B, Lynch KG. Reliability and validity of the alcohol short index of problems and a newly constructed drug short index of problems. *Journal of Studies on Alcohol and Drugs*. 2009; 70:304–307. [PubMed: 19261243]
- Arias B, Gasto C, Catalan R, Gutierrez B, Pintor L, Fananas L. The 5-HT(2A) receptor gene 102T/C polymorphism is associated with suicidal behavior in depressed patients. *American Journal of Medical Genetics*. 2001; 105:801–804. [PubMed: 11803534]
- Band GP, van der Molen MW, Logan GD. Horse-race model simulations of the stop-signal procedure. *Acta Psychologica (Amst)*. 2003; 112:105–142.
- Bauer LO. Predicting relapse to alcohol and drug abuse via quantitative electroencephalography. *Neuropsychopharmacology*. 2001; 25:332–340. [PubMed: 11522462]
- Bauer LO, Covault J, Gelernter J. GABRA2 and KIBRA genotypes predict early relapse to substance use. *Drug and Alcohol Dependence*. 2012; 123:154–159. [PubMed: 22129841]
- Bauer LO, Covault J, Harel O, Das S, Gelernter J, Anton R, Kranzler HR. Variation in GABRA2 predicts drinking behavior in project MATCH subjects. *Alcoholism: Clinical and Experimental Research*. 2007; 31:1780–1787.
- Beck, AT.; Steer, RA.; Brown, G. Brown manual for the beck depression inventory II. San Antonio, TX: Psychological Corporation; 1996.
- Boschloo L, Vogelzangs N, van den Brink W, Smit JH, Beekman AT, Penninx BW. Predictors of the 2-year recurrence and persistence of alcohol dependence. *Addiction*. 2012
- Bottlender M, Soyka M. Efficacy of an intensive outpatient rehabilitation program in alcoholism: predictors of outcome 6 months after treatment. *European Addiction Research*. 2005; 11:132–137. [PubMed: 15990430]
- Bottlender M, Soyka M. Outpatient alcoholism treatment: predictors of outcome after 3 years. *Drug and Alcohol Dependence*. 2005; 80:83–89. [PubMed: 15878808]
- Bowden-Jones H, McPhillips M, Rogers R, Hutton S, Joyce E. Risk-taking on tests sensitive to ventromedial prefrontal cortex dysfunction predicts early relapse in alcohol dependency: a pilot study. *Journal of Neuropsychiatry and Clinical Neuroscience*. 2005; 17:417–420.
- Brower KJ. Insomnia, alcoholism and relapse. *Sleep Medicine Reviews*. 2003; 7:523–539. [PubMed: 15018094]

- Cardenas VA, Durazzo TC, Gazdzinski S, Mon A, Studholme C, Meyerhoff DJ. Brain morphology at entry into treatment for alcohol dependence is related to relapse propensity. *Biological Psychiatry*. 2011; 70:561–567. [PubMed: 21601177]
- Conrod PJ, Castellanos-Ryan N, Mackie C. Long-term effects of a personality-targeted intervention to reduce alcohol use in adolescents. *Journal of Consulting and Clinical Psychology*. 2011; 79:296–306. [PubMed: 21500886]
- Conrod PJ, Stewart SH, Comeau N, Maclean AM. Efficacy of cognitive-behavioral interventions targeting personality risk factors for youth alcohol misuse. *Journal of Clinical Child and Adolescent Psychology*. 2006; 35:550–563. [PubMed: 17007600]
- do Prado-Lima PA, Chatkin JM, Taufer M, Oliveira G, Silveira E, Neto CA, Haggstram F, Bodanese LC, da Cruz IB. Polymorphism of 5HT2A serotonin receptor gene is implicated in smoking addiction. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*. 2004; 128B:90–93.
- Du L, Bakish D, Lapierre YD, Ravindran AV, Hrdina PD. Association of polymorphism of serotonin 2A receptor gene with suicidal ideation in major depressive disorder. *American Journal of Medical Genetics*. 2000; 96:56–60. [PubMed: 10686553]
- Erdmann J, Shimron-Abarbanell D, Rietschel M, Albus M, Maier W, Korner J, Bondy B, Chen K, Shih JC, Knapp M, Propping P, Nothen MM. Systematic screening for mutations in the human serotonin-2A (5-HT2A) receptor gene: identification of two naturally occurring receptor variants and association analysis in schizophrenia. *Human Genetics*. 1996; 97:614–619. [PubMed: 8655141]
- Evren C, Durkaya M, Evren B, Dalbudak E, Cetin R. Relationship of relapse with impulsivity, novelty seeking and craving in male alcohol-dependent inpatients. *Drug and Alcohol Review*. 2012; 31:81–90. [PubMed: 21450046]
- Fletcher PJ, Tampakeras M, Sinyard J, Higgins GA. Opposing effects of 5-HT(2A) and 5-HT(2C) receptor antagonists in the rat and mouse on premature responding in the five-choice serial reaction time test. *Psychopharmacology (Berl)*. 2007; 195:223–234. [PubMed: 17673981]
- Folstein MF, Folstein SE, McHugh PR. “Mini-mental state” A practical method for grading the cognitive state of patients for the clinician. *Journal of Psychiatric Research*. 1975; 12:189–198. [PubMed: 1202204]
- Fornal-Pawlowska M, Wolynczyk-Gmaj D, Szelenberger W. Validation of the Polish version of the Athens Insomnia Scale. *Psychiatria Polska*. 2011; 45:211–221. [PubMed: 21714210]
- Foster JH, Peters TJ. Impaired sleep in alcohol misusers and dependent alcoholics and the impact upon outcome. *Alcoholism: Clinical and Experimental Research*. 1999; 23:1044–1051.
- Hwu HG, Chen CH. Association of 5HT2A receptor gene polymorphism and alcohol abuse with behavior problems. *American Journal of Medical Genetics*. 2000; 96:797–800. [PubMed: 11121184]
- Jakubczyk A, Wrzosek M, Lukaszewicz J, Sadowska-Mazuryk J, Matsumoto H, Sliwerska E, Glass J, Burmeister M, Brower KJ, Wojnar M. The CC genotype in HTR2A T102C polymorphism is associated with behavioral impulsivity in alcohol-dependent patients. *Journal of Psychiatric Research*. 2012; 46:44–49. [PubMed: 21930285]
- Johnson BA, Ait-Daoud N, Seneviratne C, Roache JD, Javors MA, Wang XQ, Liu L, Penberthy JK, DiClemente CC, Li MD. Pharmacogenetic approach at the serotonin transporter gene as a method of reducing the severity of alcohol drinking. *American Journal of Psychiatry*. 2011; 168:265–275. [PubMed: 21247998]
- Kirby LG, Zeeb FD, Winstanley CA. Contributions of serotonin in addiction vulnerability. *Neuropharmacology*. 2011; 61:421–432. [PubMed: 21466815]
- Kranzler HR, Armeli S, Tennen H, Covault J, Feinn R, Arias AJ, Pettinati H, Oncken C. A double-blind, randomized trial of sertraline for alcohol dependence: moderation by age of onset [corrected] and 5-hydroxytryptamine transporter-linked promoter region genotype. *Journal of Clinical Psychopharmacology*. 2011; 31:22–30. [PubMed: 21192139]
- Lejuez CW, Magidson JF, Mitchell SH, Sinha R, Stevens MC, de Wit H. Behavioral and biological indicators of impulsivity in the development of alcohol use, problems, and disorders. *Alcoholism: Clinical and Experimental Research*. 2010; 34:1334–1345.

- Logan GD, Cowan WB, Davis KA. On the ability to inhibit simple and choice reaction time responses: a model and a method. *Journal of Experimental Psychology and Human Perception and Performance*. 1984; 10:276–291.
- MATCH P. Matching Alcoholism Treatments to Client Heterogeneity: Project MATCH posttreatment drinking outcomes. *Journal of Studies on Alcohol*. 1997; 58:7–29. [PubMed: 8979210]
- McMahon FJ, Buervenich S, Charney D, Lipsky R, Rush AJ, Wilson AF, Sorant AJ, Papanicolaou GJ, Laje G, Fava M, Trivedi MH, Wisniewski SR, Manji H. Variation in the gene encoding the serotonin 2A receptor is associated with outcome of antidepressant treatment. *American Journal of Human Genetics*. 2006; 78:804–814. [PubMed: 16642436]
- Moeller FG, Barratt ES, Dougherty DM, Schmitz JM, Swann AC. Psychiatric aspects of impulsivity. *American Journal of Psychiatry*. 2001; 158:1783–1793. [PubMed: 11691682]
- Palfai TP, Cheng DM, Samet JH, Kraemer KL, Roberts MS, Saitz R. Depressive symptoms and subsequent alcohol use and problems: a prospective study of medical inpatients with unhealthy alcohol use. *Journal of Studies on Alcohol and Drugs*. 2007; 68:673–680. [PubMed: 17690800]
- Pinto E, Reggers J, Gorwood P, Boni C, Scantamburlo G, Pitchot W, Ansseau M. The short allele of the serotonin transporter promoter polymorphism influences relapse in alcohol dependence. *Alcohol and Alcoholism*. 2008; 43:398–400. [PubMed: 18364363]
- Polesskaya OO, Aston C, Sokolov BP. Allele C-specific methylation of the 5-HT2A receptor gene: evidence for correlation with its expression and expression of DNA methylase DNMT1. *Journal of Neuroscience Research*. 2006; 83:362–373. [PubMed: 16358338]
- Polesskaya OO, Sokolov BP. Differential expression of the “C” and “T” alleles of the 5-HT2A receptor gene in the temporal cortex of normal individuals and schizophrenics. *Journal of Neuroscience Research*. 2002; 67:812–822. [PubMed: 11891796]
- Rehm J, Mathers C, Popova S, Thavorncharoensap M, Teerawattananon Y, Patra J. Global burden of disease and injury and economic cost attributable to alcohol use and alcohol-use disorders. *Lancet*. 2009; 373:2223–2233. [PubMed: 19560604]
- Rehm J, Zatonski W, Taylor B, Anderson P. Epidemiology and alcohol policy in Europe. *Addiction*. 2011; 106 (Suppl 1):11–19. [PubMed: 21324017]
- Rounsaville BJ, Dolinsky ZS, Babor TF, Meyer RE. Psychopathology as a predictor of treatment outcome in alcoholics. *Archives of General Psychiatry*. 1987; 44:505–513.
- Rubio G, Jimenez M, Rodriguez-Jimenez R, Martinez I, Avila C, Ferre F, Jimenez-Arriero MA, Ponce G, Palomo T. The role of behavioral impulsivity in the development of alcohol dependence: a 4-year follow-up study. *Alcoholism: Clinical and Experimental Research*. 2008; 32:1681–1687.
- Selzer ML. The Michigan alcoholism screening test: the quest for a new diagnostic instrument. *American Journal of Psychiatry*. 1971; 127:1653–1658. [PubMed: 5565851]
- Sheehan DV, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E, Hergueta T, Baker R, Dunbar GC. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *Journal of Clinical Psychiatry*. 1998; 59(Suppl 20):22–33. quiz 34–57. [PubMed: 9881538]
- Smith, GE.; Ross, R.; Rost, KM. Psychiatric outcomes module: substance abuse outcomes module (SAOM). In: Sederer, LI.; Dickey, B., editors. *Outcome assessment in clinical practice*. Baltimore: Williams and Wilkins; 1996. p. 85–88.
- Sobell LC, Sobell MB, Leo GI, Cancilla A. Reliability of a timeline method: assessing normal drinkers' reports of recent drinking and a comparative evaluation across several populations. *British Journal of Addiction*. 1988; 83:393–402. [PubMed: 3395719]
- Spokas M, Wenzel A, Brown GK, Beck AT. Characteristics of individuals who make impulsive suicide attempts. *Journal of Affective Disorders*. 2012; 136:1121–1125. [PubMed: 22119087]
- Stoltenberg SF, Christ CC, Highland KB. Serotonin system gene polymorphisms are associated with impulsivity in a context dependent manner. *Progress in Neuropsychopharmacology and Biological Psychiatry*. 2012; 39:182–191.
- Wojnar M, Brower KJ, Strobbe S, Ilgen M, Matsumoto H, Nowosad I, Sliwerska E, Burmeister M. Association between Val66Met brain-derived neurotrophic factor (BDNF) gene polymorphism and post-treatment relapse in alcohol dependence. *Alcoholism: Clinical and Experimental Research*. 2009; 33:693–702.

- Wojnar M, Ilgen M, Jakubczyk A, Wnorowska A, Klimkiewicz A, Brower KJ. Impulsive suicide attempts predict post-treatment relapse in alcohol-dependent patients. *Drug and Alcohol Dependence*. 2008; 97:268–275. [PubMed: 18556146]
- Wojnar M, Ilgen M, Czy E, Strobbe S, Klimkiewicz A, Jakubczyk A, Glass J, Brower KJ. Impulsive and non-impulsive suicide attempts in patients treated for alcohol dependence. *Journal of Affective Disorders*. 2008; 115:131–139. [PubMed: 18835498]
- Wrzosek M, Jakubczyk A, Matsumoto H, Lukaszewicz J, Brower KJ, Wojnar M. Serotonin 2A receptor gene (HTR2A) polymorphism in alcohol-dependent patients. *Pharmacological Reports*. 2012; 64:449–453. [PubMed: 22661198]
- Wrzosek M, Lukaszewicz J, Serafin P, Jakubczyk A, Klimkiewicz A, Matsumoto H, Brower KJ, Wojnar M. Association of polymorphisms in HTR2A, HTR1A and TPH2 genes with suicide attempts in alcohol dependence: a preliminary report. *Psychiatry Research*. 2011; 190:149–151. [PubMed: 21621273]

Table 1Relationship between rs6313 polymorphism in the *HTR2A* gene and relapse

	Relapse N (%)	No relapse N (%)
CC	62 (24.4)	36 (14.2)
CT or TT	72 (28.3)	84 (33.1)

HTR2A – serotonin type 2A receptor gene.

Chi square=7.07; df=1; p=0.008

Table 2

Comparison of patients who relapsed or not after alcohol treatment

	Relapse	No relapse	P value
Number of drinking days in 3 months prior to baseline assessment (TLFB)	31.9 ($\pm$ 24.1)	23.2 ($\pm$ 23.9)	0.006
Behavioral impulsivity (StopRT in msec)	217.0 ($\pm$ 59.4)	207.3 ($\pm$ 59.4)	0.20
Depressive symptoms (BDI)	20.9 ($\pm$ 11.9)	16.3 ($\pm$ 10.3)	0.0009
Sleep problems (AIS)	8.0 (4;11.5)	7.0 (4;10)	0.12
Consequences of drinking (SIP)	25.1 ($\pm$ 10.9)	21.4 ($\pm$ 11.2)	0.007
Severity of alcohol dependence (MAST)	35.1 ($\pm$ 10.0)	34.3 ($\pm$ 8.8)	0.47
Duration of alcohol dependence (years)	19.7 ($\pm$ 10.7)	19.1 ($\pm$ 10.3)	0.64
Age	43.2 ($\pm$ 10.4)	45.1 ($\pm$ 9.9)	0.11
Follow-up period (months)	12 (9.5; 14)	11 (8.;13.8)	0.10
Gender (men/women)	111/34	78/31	0.12

Normally distributed values are presented as means and standard deviations (mean $\pm$ SD) and analyzed by analyses of variance.

Non-normal variables are presented as median and quartiles (25;75) and analyzed by independent samples Mann-Whitney U tests.

P values < 0.05 are bolded.

AIS – Athens Insomnia Scale, BDI – Beck Depression Inventory, SIP – Short Inventory of Problems, TLFB – Alcohol Timeline Follow-Back interview

Table 3

Multifactorial models of logistic regression analysis for the prediction of relapse after alcohol treatment: model I – without genetic marker, model – II with genetic marker.

	Model I			Model II		
	OR	95% CI	p	OR	95% CI	p
Drinking days, past 3 months (TLFB)	1.014	1.00–1.03	0.012	1.017	1.01–1.03	0.005
Impulsive suicide attempts	1.63	0.8–3.3	0.175	1.494	0.7–3.08	0.28
Severity of depressive symptoms (BDI)	1.036	1.01–1.06	0.013	1.038	1.01–1.07	0.01
Consequences of drinking (SIP)	1.003	0.97–1.03	0.81	1.001	0.97–1.03	0.952
Gender	0.666	0.36–1.23	0.196	0.72	0.38–1.34	0.3
HTR2A T102C polymorphism (CC vs. T allele)	-	-	-	2.264	1.3–4.1	0.006
Model I: R square Nagelkerke = 0.125 chi square = 23.140; df=5; p<0.0005						
Model II: R square Nagelkerke = 0.164 chi square = 30.918; df=6; p<0.0005						

TLFB – Alcohol Timeline Follow-Back interview, BDI – Beck Depression Inventory, SIP – Short Inventory of Problems, 95% CI – 95% confidence interval

p values under 0.05 are bolded