Genetic Variability in CYP2E1 and Catalase Gene Among Currently and Formerly Alcohol-Dependent Male Subjects

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Abstract — Aims: The present study explored whether specific single-nucleotide polymorphisms in alcohol metabolic pathway are associated with alcohol dependence or alcohol-related psychopathological symptoms. Methods: Three groups of male unrelated subjects were included: 101 currently alcohol-dependent patients, 100 formerly alcohol-dependent subjects and 97 healthy controls. The following questionnaires were implemented: AUDIT, Zung Depression and Anxiety scale, Brief Social Phobia Scale, Yale-Brown Obsessive Compulsive Scale, Obsessive Compulsive Drinking Scale and Buss-Durkee Hostility Inventory. All the subjects were genotyped for CYP2E1 c.-1053C>T and CAT c.-262C>T. Results: Statistically significant differences in the distribution of genotypes and alleles for CAT c.-262C>T polymorphism were observed among the three investigated groups. We observed a higher frequency of CAT c.-262T allele in alcohol-dependent subjects (OR = 1.74, 95% CI = 1.164–2.610). Among currently dependent patients CAT c.-262T allele carriers had higher AUDIT scores (P = 0.023), while CYP2E1-1053T allele carriers had significantly higher YBOCS-obsession subscale scores (P = 0.005) and Zung Anxiety Scale scores (P = 0.011). Conclusions: Our findings suggest that the CAT c.-262C>T genetic polymorphism influences the susceptibility to alcohol dependence and severity of alcohol dependence, while CYP2E1 c.-1053C>T polymorphism influences the expression of obsessive-compulsive and anxiety symptoms.

INTRODUCTION

Alcohol dependence is a complex multifactorial disorder which affects millions of people worldwide and is both genetically and environmentally influenced (Dick and Bierut, 2006; Dick and Agrawal, 2008). Predisposition to alcohol dependence is influenced by personality characteristics such as sensation seeking, behavioral disinhibition and poor decision-making, but progression to dependence is based on the sensitivity to positive and negative effects of alcohol and the capacity to tolerate the aversive effects of alcohol (Licinio and Wong, 2009). Family, twin and adoption studies have convincingly demonstrated that genes play an important role in the development of alcohol dependence. Heritability estimates are in the range of 50–60% for both men and women (Mcgue, 1999; Ystrom et al., 2011). Several genes are known to contribute to this hereditability, including the genes involved in ethanol metabolism (Dick and Agrawal, 2008).

Three metabolic pathways participate in ethanol metabolism: alcohol dehydrogenase (ADH), microsomal ethanol oxidation system (MEOS) and catalase (CAT). ADH has a high affinity for ethanol; however, MEOS is induced by chronic ethanol consumption, while CAT has a prominent role in heavy ethanol consumers (Das and Vasudevan, 2007). Increased ethanol metabolism rates may contribute to the development of alcohol dependence: faster ethanol inactivation during long-term alcohol drinking may increase the motivation to consume more alcohol in order to maintain a desired level of ethanol at target sites (Gemma et al., 2006).

MEOS is involved in ethanol oxidation by the cytochrome P450 (CYP) enzymes, which include CYP2E1, CYP1A2 and CYP3A4 isoforms. The Rsal restriction fragment length polymorphism has been detected within the CYP2E1 gene (c.-1053C>T, rs2031920) (Gemma et al., 2006). Linkage and association analyses indicate that sequence changes within or near the CYP2E1 gene affect the level of response to alcohol, providing a predictor of alcoholism risk. Implicating CYP2E1 in the level of response to alcohol allows inferences to be made about how the brain perceives alcohol (Webb et al., 2011). There are some inconsistencies in the published literature: whilst the CYP2E1 -1053T allele was found to be a risk factor for alcohol dependence in some studies (Sun et al., 2001; Konishi et al., 2003; Kim et al., 2010; Webb et al., 2011), others did not detect this relationship (Pastorelli et al., 2001; Vidal et al., 2004; Cichoz-Lach et al., 2010; Celorio et al., 2012). Catanzano (Catanzano et al., 2012) found association between VNTR polymorphisms of the CYP2E1 gene and drinking habits in non-habitual drinkers.

CAT pathway plays a prominent role in the oxidation of ethanol in the brain (Zakhar, 2006). A common polymorphism in the promoter region of the catalase gene CAT c.-262C>T (rs1001179) (Forsberg et al., 2001) may influence alcohol metabolism and the level of response to alcohol (Hu et al., 2006). It was found that CAT levels were significantly higher in subjects carrying CAT c.-262T allele (Forsberg et al., 2001). CAT c.-262CC genotype is associated with a significant decrease of enzyme expression compared with TT and CT genotypes (Gavalas et al., 2004; Vidal et al., 2004). Although one study reported a relationship between CAT activity and alcohol intake (Koechling et al., 1995), the impact of this polymorphism in alcohol dependence has not yet been investigated well enough to draw definitive conclusions (Hu et al., 2006).

Ethanol metabolism has been associated with increased oxidative stress (Das and Vasudevan, 2007). During the catalytic cycle CYP2E1 generates reactive oxygen species (ROS), which can cause oxidative stress, triggering lipid peroxidation, protein inactivation, increased cytokine production, mitochondria and DNA damage leading to cell death (Gemma et al., 2006). Excessive oxidative stress results in damage to all major classes of macromolecules, and therefore affects several...
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fundamentally important cellular functions. Consequences that are especially detrimental to the proper functioning of the brain include mitochondrial dysfunction, altered neuronal signaling, and inhibition of neurogenesis. Each of these can further contribute to increased oxidative stress, leading to an additional burden on the brain (Hovatta et al., 2010). In addition to its role in ethanol metabolism, CAT represents an important enzymatic system able to inactivate ROS and their by-products (Gemma et al., 2006).

The present study explored whether specific single-nucleotide polymorphisms (SNPs) in the alcohol metabolic pathway, including CYP2E1 c.-1053C>T and CAT c.-262C>T, could be associated with alcohol dependence. We also examined whether some alcohol-related psychopathological symptoms are differently expressed according to genetic variability in alcohol-dependent subjects.

MATERIALS AND METHODS

Subjects

Three groups of subjects from Slovenian population were included in the study. All subjects were male, unrelated and aged from 18 to 65 years. The first group included currently alcohol-dependent patients, recruited from the Addiction Treatment Units at the University Psychiatric Clinic Ljubljana and Department of Psychiatry, University Clinical Center Maribor. The patients met the DSM IV criteria of alcohol dependence (American Psychiatric Association, 2000). The second group included formerly alcohol-dependent individuals, who maintained their full abstinence for more than 2 years and regularly attended the meetings of the support groups, relying on their reports and the reports of their group therapists. The third group included healthy, ethnically matched blood donor controls. A short structured clinical interview was administered by a psychiatrist to all blood donors in order to exclude possible Axis I disorders (including alcohol dependence and family history of alcohol dependence or other psychiatric disorders) according to DSM IV criteria.

Individuals with a present or past diagnosis of dependence or abuse of other substances (except nicotine), bipolar I disorder, major depression, schizophrenia, schizoaffective disorder, organic mental syndromes, head trauma, neurological disease or significant medical illness that could affect the central nervous system were excluded.

Informed consent was obtained from all participants after the nature of the study had been fully explained. The study was approved by the Slovenian National Medical Ethics Committee (approval no. 117/06/10 and 148/02/1011). The study was carried out in accordance with the latest version of the Declaration of Helsinki.

Clinical assessment

Clinical data were obtained from the medical records and from the medical interview. The following questionnaires were employed: Alcohol Use Disorders Identification Test (AUDIT) to define drinking habits and severity of alcohol problems and dependence (American Psychiatric Association and Rush, 2000), Zung Depression (Zung, 1965) and Anxiety (Zung, 1971) scale for mood traits, Brief Social Phobia Scale (BSPS) (Davidson et al., 1997) for social anxiety, Yale-Brown Obsessive Compulsive Scale (YBOCS) (Goodman and Price, 1992) and Obsessive Compulsive Drinking Scale (OCDS) (Anton, 2000) for obsessive–compulsive symptoms, and Buss-Durkee Hostility Inventory (BDHI) (Buss and Durkee, 1957) for aggressive and hostile traits. In the first group, the questionnaires were administered a minimum of 2 weeks following the admission to the Addiction Treatment Units, when the withdrawal symptoms had subsided. In the remaining two groups, the questionnaires were administered at the study entry. The rater was blinded to the genotyping results.

Laboratory assessment

In the group of currently dependent subjects and controls 5 ml of whole blood was collected for DNA isolation. In the group of formerly dependent subjects, a buccal swab was obtained for genomic DNA isolation. DNA extraction from blood samples was performed using the Qiagen DNA blood mini kit (Qiagen GmbH, Hilden, Germany) and from buccal swabs using the QIAamp DNA Mini Kit (Qiagen GmbH, Hilden, Germany). DNA concentration was determined by absorbance measurements.

Competitive allele-specific amplification (KASPar assay, KBioscience, Hoddesdon, Herts, UK) was used for genotyping CYP2E1 c.-1053C>T (rs2031920). The amplifications were performed in GeneAmp PCR System 9700 AB (Applied Biosystems, Foster City, California, USA) as recommended by the manufacturer (KBioscience). The level of fluorescence was measured on a 7500 Real Time PCR System AB and data were analyzed with 7500 System SDS Software (both Applied Biosystems, Foster City, CA, USA).

TaqMan® Pre-Designed SNP Genotyping Assay (C-11468118-10) was used for genotyping CAT c.-262C>T (rs1001179).

All genotyping reactions were repeated in 20% of the samples (selected randomly) to test for the typing accuracy and all the genotypes were concordant.

Statistical analysis

Statistical analyses were performed using Statistica package, version 7.0 (StatSoft Italia, Vignozza, Padua, Italy) for Windows®. Differences in the allele and genotype frequencies between currently dependent patients, formerly dependent subjects and healthy subjects as well as effects of such variants on categorical variables were calculated using the Pearson’s χ² statistics. The effects of the SNPs under investigation and continuous variables were investigated using ANOVA in the three groups separately. Factorial ANOVA was used to investigate the interaction between the two genetic variants of interest and continuous variables. All P-values were two-tailed and statistical significance was set at the 0.05 level. The study had sufficient power (0.80) to detect small-to-medium effect sizes (f² = 0.021). With these parameters we had a sufficient power (0.80) to detect a small-medium effect size (d = 0.355) that, as an illustration, would correspond to the probability of detecting a final difference of roughly two points on YBOCS.

RESULTS

In the present study we included 101 currently and 100 formerly alcohol-dependent individuals and also 97 healthy...
controls. Healthy controls were on average 10 years younger than currently or formerly dependent individuals ($P < 0.001$, $df = 2$, $F = 46.080$) and had a higher number of years of education ($P < 0.001$, $df = 2$, $F = 11.980$). A higher number of currently dependent patients were single compared with formerly dependent individuals or healthy controls and lower number of currently dependent patients lived in non-marital partnership ($P = 0.003$, $df = 2$, $F = 5.601$). The three groups of subjects did not differ significantly regarding their living environment. In currently dependent patients the average AUDIT score was $24.4 \pm 6.6$, while healthy controls had only slightly higher scores than formerly dependent individuals ($P < 0.001$, $df = 2$, $F = 792.1$).

The distributions of genotype and allele frequencies in our cohort are shown in Table 1. Whilst the distribution of genotypes for CAT c.-262C>T polymorphism was in Hardy–Weinberg equilibrium, the genotype distribution for CYP2E1 c.-1053C>T polymorphism deviated from it. This is likely due to the low frequency of CYP2E1 -1053T allele in our sample. CAT c.-262C>T polymorphism was relatively common, with minor allele frequency (MAF) from 21 to 38%, depending on the sample.

There was no difference in the distribution of genotypes or alleles for CYP2E1 c.-1053C>T polymorphism among the investigated groups. Statistically significant differences in the distribution of CAT genotypes ($P = 0.004$, Chi-square = 15.436, $df = 4$) and also of CAT alleles ($P = 0.001$, Chi-square = 15.261, $df = 2$) were observed among the three groups. To investigate the association with alcohol dependence, we merged the currently dependent and formerly dependent individuals, because these shared the diagnosis of alcohol dependence. This merged group differed significantly from the healthy controls regarding the frequency distribution genotypes and alleles of CAT c.-262C>T polymorphism ($P = 0.028$, Chi-square = 7.174, $df = 2$ and $P = 0.007$, Chi-square = 7.384, $df = 1$, respectively).

We observed a lower frequency of CAT -262T allele in the healthy control group, compared with the other two groups or the merged group of dependent subjects. Subjects with CAT -262TT genotype were at insignificantly higher risk to develop alcohol dependence (OR = 2.55, 95% CI = 0.852 to 7.636), while subjects with CAT -262T allele were at significantly higher risk to develop alcohol dependence (OR = 1.74, 95% CI = 1.164–2.610).

No significant associations were observed between the CYP2E1 genotypes and CYP2E1 alleles and the AUDIT score ($P = 0.088$, $df = 2$, $F = 2.493$ and $P = 0.051$, $df = 1$, $F = 3.848$, respectively) in currently dependent patients. In currently dependent patients a trend of association between the AUDIT score and CAT genotype was found in currently dependent patients ($P = 0.062$, $df = 2$, $F = 2.857$, $hp2 = 0.055$), while the association between CAT alleles and the AUDIT score was significant ($P = 0.023$, $df = 1$, $F = 5.250$, $hp2 = 0.026$). In currently dependent patients, carriers of CAT -262T allele had higher scores (mean score $25.7 \pm 5.9$ SD) than non-carriers (mean score $23.5 \pm 6.9$) on the AUDIT questionnaire which measures the severity of alcohol dependence.

The associations between the questionnaires and the distribution of genotypes and alleles for CYP2E1 c.-1053C>T polymorphism are presented in Table 2. In currently dependent patients, a trend of association was found between CYP2E1 genotype and the YBOCS-obsession subscale score and the $hp2$ ($P = 0.068$, $df = 2$, $F = 5.866$), but the most prominent association was observed between CYP2E1 alleles and the YBOCS-obsession subscale score ($P = 0.005$, $df = 1$, $F = 7.891$). In this group a trend of association was found also between the YBOCS-compulsion subscale score and the CYP2E1 genotype ($P = 0.055$, $df = 2$, $F = 2.996$), but not with CYP2E1 alleles.

A trend of association between CYP2E1 genotype and the Zung Anxiety Scale ($P = 0.062$, $df = 2$, $F = 2.853$) was found in currently dependent patients, while the association was significant for CYP2E1 alleles ($P = 0.011$, $df = 1$, $F = 6.532$).

No statistically significant association was found between CAT c.-262C>T polymorphism and psychopathological symptoms tested with the applied questionnaires in any of the groups.

**DISCUSSION**

The present study investigated the effects of the CYP2E1 c.-1053C>T and CAT c.-262C>T polymorphisms on alcohol dependence and psychopathological symptoms in homogenous
In the present study no association of the CYP2E1 c.-1053C>T polymorphism was found with the risk of alcohol dependence. The data published to date in this field are inconsistent, because the T allele was also associated with lower enzyme activity (Ahn et al., 2006; Bastaki et al., 2006). Our results are biologically plausible, presuming the association of the T-allele with higher levels of CAT transcription.

We observed that CAT -262T allele was associated with the severity of alcohol problems and dependence. One of the mechanisms in individuals with higher transcription of the CAT enzyme is increased ethanol metabolism, which may lead to increased motivation to consume more alcohol in order to maintain a desired level of ethanol at target sites (Gemma et al., 2006). CAT also plays an important role in motivation circuits in the brain. Rat model shows that catalase is responsible for up to 60% of the acetaldehyde formed in brain (Zimatkin et al., 1998), which has a key role in the motivational properties of ethanol and its activation of the mesolimbic dopaminergic system (Melis et al., 2007). This was confirmed in the latest studies in rat models where lower CAT activity blocked reward and alcohol self-administration (Quintanilla et al., 2006). In the present study no association of the CYP2E1 c.-1053C>T polymorphism was found with the severity of alcohol dependence.

Furthermore, in our currently alcohol-dependent patients the CYP2E1-1053C allele was associated with obsessive traits and to a lesser extent CYP2E1 -1053CC genotype was
associated with compulsive traits. Obsessive–compulsive disorder (OCD) has been linked to increased oxidative stress which may have a pathophysiological role in OCD (Ersan et al., 2006; Behl et al., 2010; Guldenpfennig et al., 2011; Orhan et al., 2012). During the catalytic cycle, CYP2E1 generates ROS such as H$_2$O$_2$, superoxide anion (O$_2^-$), hydroxyl (•OH) and substrate-derived radicals (1-hydroxyethyl radical) which can cause oxidative stress, triggering lipid peroxidation, protein inactivation, increased cytokine production, mitochondria and DNA damage leading to cell death (Gemma et al., 2006). The presence of CYP2E1 has been reported in neurons within the cerebral cortex, Purkinje and granule cell layers of the cerebellum, pyramidal neurons in hippocampal CA1, CA2 and CA3 regions in rat and human brains (Kumar et al., 2013).

Therefore, we could hypothesize that alcohol consumption may lead to an increase of ROS and may thus be connected with obsessive symptoms. The literature consistently suggests a high comorbidity of obsessive–compulsive traits and alcohol dependence (Regier et al., 1990; Echeburúa et al., 2007; Liang and Chikritzhs, 2011). On the other hand, OCD traits may be linked to an increased number of compulsive symptoms and behaviors (Regier et al., 1990) which can be connected to addictive behavior and lead to alcohol dependence. Therefore, it is not still clear whether the onset of obsessive–compulsive traits precedes or follows the onset of alcohol dependence (Suzuki et al., 2002). In our study the CYP2E1 -1053C allele was linked with higher level of anxiety in currently dependent subjects. Oxidative stress could be implicated in this state. Anxiety is a symptom of different psychiatric disorders such as generalized anxiety disorder, depression, panic attacks, phobias, OCD and posttraumatic stress disorders and could also be connected to oxidative stress. Rammal (Rammal et al., 2008) showed in a mouse model that high anxiety level significantly increased the generation of ROS in the peripheral blood. On the other hand, studies in animal models showed that ethanol exposure increased the intracellular redox state in the brain, resulting in the occurrence of depressive and anxiety-like behaviors (Brocardo et al., 2012). Nevertheless, the exact mechanisms that link oxidative stress to the pathogenesis of neuropsychiatric diseases remain largely unknown (Hovatta et al., 2010). Our results are biologically plausible, since the CYP2E1 -1053T allele is associated with higher enzyme transcription activity and therefore higher rates of ROS production (Gemna et al., 2006), which also supports our finding of more prominent obsessive–compulsive and anxiety symptoms in CYP2E1 -1053T-allele carriers. No human studies support a direct connection between CYP2E1 and obsessive–compulsive or anxiety symptoms; thus further investigation of the issue is needed.

Our study included a relatively homogeneous and small sample, it was limited to males, but all three groups of subjects came from similar environments. We have not included analyses for CYP2E1 VNTR polymorphism which appears to be important according to study by Naselli (Naselli et al., 2014). Although we did not test for genetic heterogeneity, such bias is unlikely, since we included only subjects from a small geographical area with ethnically homogeneous Caucasian population (Tiilikainen et al., 1997; Vidan-Jeras et al., 1998). The frequency distribution of CAT alleles in our cohort was similar to those observed in general Caucasian population (Bastaki et al., 2006; Suzen et al., 2010). CYP2E1 genotype frequencies did violate Hardy–Weinberg equilibrium, likely due to the low frequency of CYP2E1 -1053T alleles. The observed MAF for CYP2E1 -1053T allele was similar to the one documented in Caucasian population (http://snpedia.com/index.php/Rs2031920) and also in previous studies (Stephens et al., 1994; Pastorelli et al., 2001; Cichoń-Lach et al., 2010; Celorio et al., 2012). The main strength of our study is a thorough clinical assessment and inclusion of individuals with varying histories of alcoholism.

In conclusion, our findings suggest that the CAT c.-262C>T genetic polymorphism influences the susceptibility to alcohol dependence and severity of alcohol dependence, while the CYP2E1 c.-1053C>T polymorphism influences the expression of obsessive–compulsive and anxiety symptoms, possibly related to oxidative stress. However, further studies with larger cohorts are required to confirm these preliminary findings.

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