Alcohol Consumption in Healthy OPRM1 G Allele Carriers and Its Association with Impulsive Behavior

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Abstract

Aims: A link between alcohol use disorders (AUD) and impulsivity is well established. As there is evidence for the heritability of AUD, the investigation of the underlying genetic disposition for both conditions is an important issue. An association between AUD and a coding single nucleotide polymorphism (SNP) (rs1799971 encoding an Asn40Asp amino acid substitution, A118G) within the µ-opioid receptor 1 gene (OPRM1) has been reported. Therefore we tested the association between the OPRM1 A118G polymorphism and drinking as well as impulsive behavior in social drinkers.

Methods: A total of 214 healthy male social drinkers were recruited. Each participant was genotyped for the OPRM1 A118G variant. Alcohol use was assessed with items of the Alcohol Use Disorders Identification Test (AUDIT). Impulsivity was assessed using the UPPS impulsive behavior scale.

For statistical analyses, we considered correlations, t-tests and ordinal regression models using SPSS V21.

Results: In total, 49 out of 214 participants were carriers of the OPRM1 118G allele. On average the OPRM1 118G carriers showed a slightly higher propensity for alcohol drinking. Higher drinking frequency among the G allele carriers was linked with higher urgency and perseveration subscores of impulsivity.

Conclusion: Our results suggest a genetically influenced higher propensity for alcohol drinking among social drinkers carrying the 118G allele of the OPRM1 gene. The positive correlation between urgency and a higher drinking frequency among the OPRM1 118G hint towards a functional meaning of the opioid system in the regulation of impulsivity.
INTRODUCTION

Different aspects of impulsivity seem to play an important role in the development of alcohol dependence (Koob and Volkow, 2010). Therefore the influences of personality traits and impulsive behavior on the development of alcohol use disorders (AUD) have been investigated in several prospective studies (Dawes et al., 1997; Lejuez, 2010). As impulsivity is a heterogenic construct distinct impulsivity subtypes have been investigated in the context of alcohol dependence: Using the Whiteside four factor model for impulsivity (UPPS: Urgency, Lack of Premeditation, Lack of Perseverance, Sensation Seeking) an elevated level of impulsivity psychopathology in some alcohol abusers was found (Whiteside and Lynam, 2003). Further the results of a recently published meta-analysis confirmed that all four impulsivity traits were associated with a problematic alcohol consume (Coskunpinar et al., 2013; Stautz and Cooper, 2013). Therefore common neurobiological underpinnings for both AUD and impulsivity have been hypothesized, e.g. in the form of a dysfunctional dopamine system or variations in the endogenous opioid system (Brewer and Potenza, 2008). For the development of alcohol dependence there is evidence for a high heritability of 0.5–0.6 in twin studies and a gene-environment interaction from initiation to addiction (Ducci and Goldman, 2008). Several genes with a link to AUD have been characterized and some of their related disease modifying biological functions are well-known today (e.g. DRD2, ADH1B and ALDH2) (Koller et al., 2012). Otherwise for constructs like impulsivity or personality traits predisposing for a harmful substance use there is less genetic evidence. Although there are some well-established links between alcohol abuse and different facets of impulsivity in animals as well as in humans (Dick et al., 2010) the genetic underpinnings of impulsivity and related personality traits have only been established in some pilot studies. For example, Preuss et al. found that higher scores for impulsivity were associated with 5-HT2A 1438A alleles in alcohol-dependent subjects (Preuss et al., 2000). In another study, higher impulsivity scores among alcohol-dependent individuals with the A2/A2 and A1/A2 genotypes of the DRD2 dopamine D2 receptor gene were reported (Limosin et al., 2003). Recently Dick et al. found that the GABRA2 gene could mediate certain aspects of impulsivity in adolescent alcohol-dependent subjects (Dick et al., 2013).

Referring to these findings, efficient interventions for reducing both alcohol use and impulsive behavior are much needed in the treatment of alcohol-dependent patients. In a recent review Leeman et al. summarize that there is evidence for opioid antagonists to reduce alcohol use and enhance self-control (Leeman et al., 2014). Naltrexone is FDA-approved for treating alcohol dependence and in some clinical populations the subgroup of 118 G allele carriers of the µ-opioid receptor (OPRM1) gene polymorphism (the A118G SNP, rs1799971, encoding an Asn40Asp amino acid substitution) were more likely to respond to a treatment with Naltrexone (Chamorro et al., 2012). Referring to this, experimental findings have shown that 118 G allele carriers may experience a stronger attenuation of rewarding and stimulating alcohol effects of naltrexone (Ray and Hutchinson, 2007; Setiawan et al., 2011). With regard to impulsivity clinical evidence of the effect of opioid antagonists on impulse control comes from trials in disorders like kleptomania and pathological gambling (Grant et al., 2008, 2009).

Concerning the opioid system, there are experimental findings that the OPRM1 gene polymorphism contributes to impulsive behavior and reward learning in animals and humans: one study showed the relevance of the Opre1 gene for the control of behavioral impulsivity, as Oprm1 knockout mice displayed a remarkable decrease of premature responses in a response inhibition task (signal nosed poke task) compared to wild-type animals (Olmstead et al., 2009). In another study the µ-opioid receptor antagonist naltrexone improved amphetamine-induced inhibitory control deficits in rats and therefore indicated an important role in the regulation of impulsive choice (Wiskerke et al., 2011).

In humans the link between the µ-opioid system and its influence on impulsivity with respect to alcohol dependence has been investigated experimentally in an indirect way: in healthy subjects that performed a probabilistic reward task an aberrant reward responding of OPRM1 G allele carriers was found. Compared to AA homozygotes the latter showed a reduced response to rewarding stimuli (Lee et al., 2011). The authors conclude that this may correspond to a reduced phasic dopamine response during positive feedback modulated by the endogenous opioid system (Johnson and North, 1992; Lee et al., 2011). In another study the µ-opioid receptor antagonist naltrexone had a positive effect on impulsive choice in a subpopulation of abstinent alcohol-dependent patients. Therefore the authors inferred an influence of the opioid system in impulsive decision making (Mitchell et al., 2007). Recently Courtney et al. compared the influence of the OPRM1 A118G gene polymorphism on frontostriatal connectivity during a response inhibition task in both allele carrier groups (Courtney et al., 2013). Although they found no differences in inhibitory control between the groups their results conferred that the G-allele carriers show a more goal-directed alcohol use mediated through neural reward processes.

One difficulty for research related to substance abuse and impulsivity is that their relationship is reciprocal. Therefore it remains a problem to differ exactly whether impulsivity is a genetic determined personality trait or if impulsive behavior mainly is an outcome of long-term substance abuse. In other fields of addiction research, e.g. the abuse of stimulants, recently published data showed abnormalities in behavioral inhibition in stimulant-dependent individuals as well as in their biological siblings. These findings support the idea that substance use disorders may have underlying neurocognitive endophenotypes (Ersche et al., 2012). However, the transfer of a corresponding investigation into the field of alcohol research remains difficult, as individuals with a long and harmful alcohol consume often show severe cerebral pathologies that could lead to secondary alterations in response inhibition and impulsive behavior (Konrad et al., 2012).

Taken together there is evidence for common neurobiologic determinants of impulsivity and AUD. One genetic marker that might affect alcohol consumption as well as impulse control is the OPRM1 A118G polymorphism (Lee et al., 2011; Wiskerke et al., 2011). To our knowledge no studies have investigated the three way relationship between alcohol consumption, impulsivity and the OPRM1 A118G polymorphism among non-severely exposed individuals. For this task, we genotyped a cohort of healthy male social drinkers with respect to the OPRM1A 118G polymorphism and investigated the relationship between genotype, impulsivity and alcohol consumption.

MATERIALS AND METHODS

Study participants

The study participants were recruited in the context of the government funded OPIDOP study (EudraCT 2007-003806-88), a multicenter trial with the aim to investigate the effect of the central µ-opioid antagonist naltrexone on the dopaminergic system in OPRM1 118G-allele carriers. In a first step, we recruited subjects for genetic characterization of the OPRM1 allele and used the complete sample of participants for
the following investigation. Study participants were recruited by local advertising and announcement using a university Email server. Study subjects were paid for their participation. The whole investigation was conducted in the research center of the clinic for psychiatry and psychotherapy of the Mainz University Medical center after having been approved by the local ethics committee.

For inclusion, it was required that the participants had no current or previous psychiatric and substance abuse disorder. Before entering the study, subjects were characterized using a standardized psychiatric interview (‘Diagnostic System for Experts’ DIA-X) (Wittchen et al., 1998). Due to the OPIDOP study protocol all participants had to be male, non-smokers and between 21 and 45 years of age as experimental studies have shown that sex differences may play an important role in striatal dopamine release and its rewarding effects (Urban et al., 2010). After a short briefing about the study, written informed consent was obtained.

Genotyping
The OPRM1 genotypes (rs1799971) were determined by pyrosequencing. Patients DNA was isolated by a simple salting-out procedure and subsequently amplified by standard PCR amplification. PCR volume was 50 µl and contained 160 ng genomic DNA, 2 U recombinant Taq Polymerase (invitrogen, Darmstadt/DE), PCR buffer (10 mM Tris–HCl, 50 mM KCl, 2.5 mM MgCl2 pH 8.3), 200 mM dNTPs and 40 pmol of each primer (OPRM1_Py_F1bio CCCAGAAGCC CAGCAAT; OPRM1_Py_R1 CTGTCTCTCCGCCAGGTC). The forward primer was biotin-labeled. PCR conditions can be asked from the authors. Subsequent pyrosequencing using the Pyromark Pyrosequencer (Biotage-Qiagen) according to the manufacturer’s instructions. Data analysis was carried out with the PyroMark ID machine (Biotage-Qiagen) according to the manufacturer’s instructions. As intelligence may moderate impulsive behavior the intelligence quotient (IQ) was assessed with a multiple-choice verbal intelligence test (Mehrfachwahl-Wortschatz-Intelligenztest) (Lehrl, 2005; Buchmann et al., 2010). After a brief personally given oral explanation of the test, written informed consent was obtained.

Auditory
The Alcohol Use Identification Test is a self-rated inventory with 10 items to assess risky and harmful alcohol use as well as alcohol dependence. The Alcohol Use Disorders Identification Test (AUDIT) was developed and evaluated over a period of two decades and has been found to provide an accurate measure of alcohol abuse across gender, age and culture. For our analysis of a group of healthy social drinkers, we used the first three items of the AUDIT to assess hazardous drinking. Following Saunders et al., a score of 6 or more may indicate a risk of alcohol-related harm (Saunders et al., 1993). All questions have a set of responses to choose from and each response has a score ranging from 0 to 4: (a) How often do you have a drink containing alcohol? (b) How many drinks containing alcohol do you have on a typical day when you are drinking? (c) How often do you have six or more drinks on one occasion? The sum out of these three questions yields a score to represent hazardous drinking. We used a German version of the AUDIT as a written questionnaire after a brief personally given oral explanation of the test.

Statistical analyses
Statistical analyses were performed with the Statistical Package for the Social Sciences 21 (SPSS Inc., Chicago, IL, USA). To examine demographic differences between OPRM1A118G-allele carriers and the control subjects unpaired t-tests were conducted (age; IQ as value of the MWTB). Ordinal logistic regressions were performed to model the dependency of the odds ratio of drinking frequency on the two covariates: genotype and impulsivity variables: premeditation, urgency, sensation seeking and perseverance (leading to different regression models). All four subscales/dimensions of the UPPS were used separately. We chose a level of P < 0.05 to indicate noticeable results and refrained from significance-statements due to the high number of tests performed.

Results
We included 214 healthy male volunteers in our study who were genotyped for the OPRM1A118G polymorphism. In total, 49 out of the total of 214 participants were G-allele carriers. The demographic data are shown in Table 1. There were no differences in age and intelligence. The groups did not differ concerning a positive family history of AUD.

AUDIT and UPPS scores are shown in Table 2. OPRM1 118G-allele carriers had a nominally significant higher frequency of drinking (P = 0.039), but no higher alcohol intake per drinking day (P = 0.107). A trend towards a higher percentage in heavy drinking days was found among the 118G-allele carriers (P = 0.085). No

| Table 1. Demographics and distribution of our study group members |
|------------------|------------------|---------------|
|                  | A118G (n = 49)   | A118A (n = 165) | P      |
| Age (years)      | 25.26 (3.9)      | 25.89 (4.1)    | 0.337  |
| Verbal intelligence (MWTB) | 29.33 (3.6) | 29.73 (3.7) | 0.510  |
| Positive family history | 1.60 (0.5) | 1.51 (0.5) | 0.232  |

Numbers represent means and standard deviations in parenthesis. A118G = OPRM1 G allele carriers. A118A = OPRM1 allele homozygotes.
nominally significant difference for hazardous drinking was found between both groups.

For impulsive behavior the UPPS questionnaire showed nominally significant higher scores for lack of premeditation in G allele carriers ($P = 0.044$). No differences between the groups were found for urgency ($P = 0.815$), sensation seeking ($P = 0.584$) and (lack of) perseverance ($P = 0.210$). Because our study is explorative by nature, we did not perform a Bonferroni correction.

The results of the ordinal regression analyses are shown in Table 3. The four ordinal logistic regression models differed in the covariate representing the impulsivity that was included into the model besides genotype (accounting for the effect of different impulsivity manifestations on the genotype effect). There were only two participants with drinking frequency category four, so, we decided to merge the last two drinking frequency categories. Only the two regressions that included the covariate ‘urgency’ or the covariate ‘perseverance’ showed noticeable results (overall significance of the models were $P = 0.009$ and $P = 0.011$, respectively). In these regression models G allele carriers had a higher risk to drink more often than the homozygotes and higher impulsivity score increases these risk further. Concretely, a regression including ‘urgency’ leads to a 99% higher alcohol drinking risk of G allele carriers ($P = 0.034$) as compared to the homozygotes; the risk increases with 5.8% for every additional point in the ‘urgency’ score ($P = 0.034$). The regression with ‘perseverance’ slightly changed these estimates: ‘Perseverance’ leads to a 91% higher risk of frequently drinking alcohol in G allele carriers ($P = 0.046$) [7.0% increase for each point ($P = 0.045$)]. The regression confirms the result of the univariate tests that the G allele is also highly related to the frequency of drinking; in those cases within the cohort where the odds of drinking frequency are not explained by the genotype the considered impulsivity factor (‘urgency’ or ‘perseverance’) explains the differences.

Regarding the power of our study, we calculated the minimal detectable effect size regarding the difference in an impulsivity score between OPRM1 118G-allele carriers and homozygotes. Performing a two-sided independent $t$-test for an impulsivity score with power: 0.80, alpha level: 0.05, sample size of group 1: 49, sample size of group 2: 165, yields the effect size 0.53 (difference between the means divided by the pooled standard deviation). This means that we could detect a significant difference between the groups if one group had a mean score of at least 0.53 times the standard deviation above the other group.

**DISCUSSION**

The present study aims to assess the effect of the OPRM1A118G gene polymorphism on alcohol consumption and variations of trait impulsivity among male healthy social drinkers. There are two important findings in our study: first male social drinkers who are OPRM1 118G-allele carriers tend to drink more often alcohol and showed a higher lack of premeditation. Second, with regard to impulsive behavior, the G allele carrier group had a tendency to drink more frequently due to the impulsivity facets urgency and lack of perseverance.

Within our study population 49 out of 214 subjects were positive for the OPRM1 118G allele representing an allele frequency of 0.23. This is slightly higher than in the hapmap population of Caucasian individuals in North America and in Europe showing an allele frequency of 0.16 (hapmap.org). Genetic association studies that investigated the functional OPRM1 A118G gene polymorphism in AUD have yielded heterogeneous findings (Bergen et al., 1997; Kranzler et al., 1998; Franke et al., 2001; Chen et al., 2012; Koller et al., 2012). Some of them found an association between alcohol dependence and the OPRM1 A118G polymorphism with higher frequencies of the 118G allele among alcohol-dependent individuals (Kranzler et al., 1998; Koller et al., 2012). Other studies did not find a higher risk for alcohol dependence among the OPRM1 118G-allele carriers (Bergen et al., 1997; Franke et al., 2001). In native American tribes as well as in participants of Asian descend the OPRM1A118G polymorphism was associated with alcohol dependence (Chen et al., 2012; Ehlers and Gizer, 2013).

In a more indirect way the association between AUD and the OPRM1 A118G polymorphism has been studied by investigation of the relapse prevention effect of the µ-opioid antagonist naltrexone among OPRM1 118G-allele positive alcohol-dependent subjects. In this regard some but not all studies found a larger effect of naltrexone on several parameters of drinking in the AD subjects who were G allele carriers as compared to the homozygotes (Anton et al., 2008; Chamorro et al., 2012). From a pharmacological perspective there is evidence that individuals with the OPRM1 118G allele reported higher subjective feelings of intoxication, stimulation, sedation and euphoria in Caucasians and Asians (Ray and Hutchison, 2004; Mendez and Morales-Mulia, 2008; Ray et al., 2012) as well as enhanced dopamine rewarding effects in positron emission tomography (Ramchandani et al., 2011). Laboratory experiments have shown that the reinforcing effects of alcohol in healthy Caucasian males were
stronger in OPRM1 118G-allele carriers than in the AA homozygotes (Ray et al., 2013).

With regard to alcohol consumption we found a nominally significant higher drinking frequency among our subjects carrying an OPRM1 118G allele, assessed with the AUDIT questionnaire. There were no such differences for the categories total amount of alcohol consumed and the number of heavy drinking days. This drinking profile corresponds to experimental pharmacodynamic findings that in OPRM1 118G-allele carriers, alcohol had a higher potential to induce euphoria as well as intoxication (Ray and Hutchison, 2004). According to these results, individuals who are positive for the 118G allele tend to seek more often the reinforcing effects of alcohol but are not in need to consume higher doses to achieve a positive effect.

As mentioned above there is a strong empirical evidence for impulsivity as a risk marker for AUD and alcohol dependence (Ducci and Goldman, 2008). Among experts impulsivity is discussed as a heterogenic psychological construct. Therefore we used the German adaptation of the UPPS impulsive behavior scale as a standardized tool to examine the distinct facets of impulsive behavior (Whiteside and Lynam, 2001; Schmidt et al., 2008). However, some authors question the clinical relevance of all four facets of impulsivity for the understanding of impulsive behavior linked to substance use. Instead impulsivity related to substance use may rather be represented by two core processes of impulsivity: impulsive drug approach and a reduced capacity of inhibition of the latter (Gallo et al., 2014). In the UPPS impulsive behavior scale those two processes are represented by the facets of sensation seeking (impulsive drug approach) and lack of premeditation (capacity of inhibiting the approach). In this regard we found a nominally significant higher lack of premeditation among the OPRM1 118G-allele carriers compared to the AA homozygotes. Although our study design is explorative, our result of a higher lack of premeditation in G allele carriers may indicate a reduced capacity to inhibit the impulsive approach to substance use and therefore may represent a factor of vulnerability for developing alcohol dependence. In consequence patients who are OPRM1 118G-allele carriers may particularly benefit from opioid antagonists as the latter show a positive effect not only on drinking behavior but also on inhibitory control (Kieres et al., 2004; Mitchell et al., 2007; Olmstead et al., 2009; Wiskerke et al., 2011).

Surprisingly we found the two facets of impulsivity ‘urgency’ and ‘lack of perseverance’ to be crucial in explaining the higher drinking frequency in G allele carriers in our ordinal regression model. The latter do not represent one of the two core facets of impulsivity as proposed above in the model of impulsive behavior in substance abuse. However, two recently conducted meta-analyses found that all four UPPS facets of impulsivity were significantly correlated with alcohol use (Coskunpinar et al., 2013; Stautz and Cooper, 2013). It is remarkable that the strongest correlation were found for the facets of ‘urgency’ compared to ‘premeditation’ in G allele carriers linked with the impulsivity factor of urgency may indicate the use of alcohol as one strategy for the regulation of emotions in this group.

Taken together, our findings show a possible link between impulsivity and alcohol use in a genetically characterized group and therefore indirectly support former studies that found an elevated risk for AUD in subjects with an OPRM1 118G allele (Koller et al., 2012).

However, our study has some limitations. As the OPRM1 118G allele appears only in the minority of the population, we were not able to compare two groups that were equally in number. In addition due to the OPIODP study protocol only male participants were allowed to be included in the study limiting a generalization of the results. Although the number of participants was well above 200, the effect sizes and related P-values were moderate and did not remain significant after correction for multiple comparisons. Therefore our study does only permit limited generalization. An additional study with a high number of OPRM1 118G-allele carriers would be necessary for achieving more generalizable conclusions.

CONCLUSION

In summary we found a link between the impulsivity traits urgency and lack of perseverance and higher drinking frequency as a possible risk factor for problematic alcohol use in OPRM1 118G-allele carriers compared to AA homozygotes. Our findings may support former contributions in this field that individuals carrying the OPRM1 118G allele have an elevated risk for developing AUD due to a specific and genetically determined mechanism that moderates drinking habits and impulsive behavior.

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REFERENCES


