A genetic determinant of the striatal dopamine response to alcohol in men

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Excessive alcohol use, a major cause of morbidity and mortality, is less well understood than other addictive disorders. Dopamine release in ventral striatum is a common element of drug reward, but alcohol has an unusually complex pharmacology, and humans vary greatly in their alcohol responses. This variation is related to genetic susceptibility for alcoholism, which contributes more than half of alcoholism risk. Here, we report that a functional OPRM1 A118G polymorphism is a major determinant of striatal dopamine responses to alcohol. Social drinkers recruited based on OPRM1 genotype were challenged in separate sessions with alcohol and placebo under pharmacokinetically controlled conditions, and examined for striatal dopamine release using positron emission tomography and [11C]-raclopride displacement. A striatal dopamine response to alcohol was restricted to carriers of the minor 118G allele. To directly establish the causal role of OPRM1 A118G variation, we generated two humanized mouse lines, carrying the respective human sequence variant. Brain microdialysis showed a fourfold greater peak dopamine response to an alcohol challenge in h/mOPRM1-118GG than in h/mOPRM1-118AA mice. OPRM1 A118G variation is a genetic determinant of dopamine responses to alcohol, a mechanism by which it likely modulates alcohol reward. Molecular Psychiatry (2011) 16, 809–817; doi:10.1038/mp.2010.56; published online 18 May 2010

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Introduction

Alcohol use is a major cause of disability worldwide,¹ but available treatments have limited efficacy.² Development of novel, mechanism-based pharmacotherapies will require an improved understanding of the neurobiology that underlies addictive properties of alcohol.³ Compared to other addictive drugs, alcohol has a complex pharmacology. Sedative, ataxic and anxiolytic alcohol effects are primarily mediated through GABA and glutamate signaling. In contrast, rewarding properties of alcohol such as euphoria and psychomotor stimulation are thought to involve endogenous opioids and mesolimbic dopamine (DA). In response to alcohol, μ-opioid receptor (OPRM1) activation in the ventral tegmental area suppresses the activity of inhibitory GABAergic interneurons, resulting in disinhibition of DA neurons and DA release from their terminals in the ventral striatum.⁴ Accordingly, OPRM1 blockade is a treatment for alcohol dependence.³

Humans vary substantially in their alcohol responses, and this variability is related to genetic susceptibility for alcohol use disorders, which accounts for more than half the disease risk in this condition.⁵,⁶ Striatal DA release is a common element of drug reward,⁷ and alcohol-induced DA release has been shown both in rodents⁸,⁹ and in humans.¹⁰ There is, however, marked individual variation in alcohol-induced behavioral responses thought to be related to DA activation, such as psychomotor stimulation. Functional variation in opioid genes may contribute to this variation by modulating alcohol-induced DA release. A functional OPRM1 118G variant¹¹ confers enhanced subjective alcohol responses,¹² and a functionally equivalent rhesus macaque 77G variant¹³ confers enhanced alcohol-induced psychomotor stimulation.¹⁴ Despite this, it remains controversial whether the OPRM1 A118G polymorphism is a risk factor for alcohol use disorders.¹⁵

Here, we asked whether OPRM1 A118G modulates striatal DA release in response to alcohol. We first
carried out a human positron emission tomography (PET) study using displacement of the DA-D2 ligand \(^{11}C\text{-}\text{raclopride}\), an indirect measure of endogenous DA release, and found that 118G carriers had a markedly more vigorous striatal DA response to alcohol compared to subjects homozygous for the major 118A allele. In parallel, we isolated the influence of \textit{OPRM1} A118G variation by generating humanized mice, carrying the human exon 1 of the \textit{OPRM1} gene, either as the major 118A allele or with the 118G SNP introduced through site-directed mutagenesis. Direct microdialysis measures of the response to a rewarding dose of alcohol showed a fourfold higher peak DA response to the alcohol challenge.

Materials and methods

Additional details for all methods are provided in Supplementary Information.

**PET study**

Subjects were healthy male social drinkers, consuming less than 20 standard drinks per week. They were nonsmokers who had never smoked or had quit at least a year before enrolling in the study. Because the ventral striatum shrinks an average of 3.6–3.7% per decade, the age range was restricted to 21–45 years to reduce age-related variability. Furthermore, because rhesus macaque \textit{OPRM1} genotype predominately modulates psychomotor responses to alcohol in males, and because recent human data indicate only borderline detectable striatal DA release in response to alcohol in females, the study population was restricted to males. Subject demographics are provided in Supplementary Table S1. Allele frequencies of \textit{OPRM1} 118G in Caucasian populations are around 15\% and in 118G homozygotes are rare. Subjects were recruited into two approximately equally sized groups: (1) individuals homozygous for the major 118A allele (118AA genotype; \(n=16\)) of the \textit{OPRM1} polymorphism; (2) individuals carrying one or two copies of the variant 118G allele (118AG or 118GG genotype; because no subject with the rare 118GG genotype was in fact found, this group is hereafter called 118AG; \(n=12\)) of the \textit{OPRM1} polymorphism. Genotyping was performed on DNA from whole blood. SNP rs1799971 (A/G) was genotyped using the assay-on-demand (assay ID: C_8950074_1) from Applied Biosystems (Foster City, CA, USA). The alleles were discriminated by post-PCR plate read on ABI Prism 7900HT Sequence Detector System (Applied Biosystems).

Because blood alcohol concentrations (BACs) after oral alcohol administration are highly variable, we used a physiologically based pharmacokinetic model, and intravenous alcohol infusion to achieve highly standardized brain alcohol exposure successfully used in prior studies. Subjects underwent three infusion sessions, on separate days, separated by approximately 1 week.

A baseline session was carried out outside the PET scanner to ensure that subjects tolerated the alcohol infusion without experiencing nausea or marked sedation, and allow them to habituate to the procedure. All subjects received intravenous alcohol that raised BACs from 0 to 80 mg per 100 ml within 15 min. This is a peak BAC commonly achieved by social drinkers, although the infusion procedure produced this level at a faster rate than is typically achieved with oral alcohol use. The exposure profile was chosen based on prior findings showing that it robustly activates the ventral striatum in a manner that can be detected by functional brain imaging. Subjective response to alcohol was measured using a modified Drug Effects Questionnaire obtained before the beginning of the infusion and every 7.5 min during the infusion. Breath alcohol concentrations were monitored every 7.5 min during the infusion, and every 30 min thereafter. Blood samples for BACs were collected before starting the infusion, and then at 15, 30 and 45 min.

In the second and third sessions, subjects received infusions of alcohol or placebo in counterbalanced order while undergoing a PET scan with \(^{11}C\text{-}\text{raclopride}\) (GE Advance Tomograph, Waukesha, WI, USA). An 8-min transmission scan was first acquired with two rotating rod sources for the purpose of attenuation correction. Next, a 10 mCi dose of \(^{15}O\text{-}\text{water}\) was administered over 1 min through an IV line to assess regional perfusion and coregister the PET image with a brain MRI obtained from the subject during a separate visit. Following this, the infusion (6\% v/v ethanol or normal saline) was started. At 15 min after the start of the infusion, a 12 mCi dose of \(^{11}C\text{-}\text{raclopride}\) was administered over 1 min. Dynamic PET scans began with the \(^{11}C\text{-}\text{raclopride}\) injection and continued for 90 min. A schematic of the PET sessions is shown in Figure 1d.

PET analysis was based on a region of interest methodology. A structural MR scan was coregistered with the subject’s PET scans. Regions of interest were drawn in the primary areas of postsynaptic DA release, namely the anterior ventral striatum, posterior ventral striatum, caudate and putamen (Supplementary Figure S1). The anterior ventral striatum was defined as striatum anterior and inferior to the anterior commissure. The posterior ventral striatum was defined as striatum posterior and inferior to the anterior commissure. The PET data from the placebo session were used as a measure of baseline raclopride binding potential (BP), using the simplified reference tissue model. Percent change in binding potential (\(\%\text{BP}\)) following alcohol was calculated as \((\text{BP}_{\text{placebo}} - \text{BP}_{\text{alcohol}})/\text{BP}_{\text{placebo}}\). Reduction in raclopride binding is attributed to competition with DA endogenously released by the alcohol challenge, and the \(\%\text{BP}\) has been shown to be proportional to the magnitude of DA release.

PET data were examined for homogeneity of variance and analyzed using general linear models (Statistica 6.0; StatSoft, Tulsa, OK, USA). For the
D. BP, we performed a repeated measures analysis of covariance, with genotype and testing sequence as between subjects variables, region of interest as the within subjects variable and drinking frequency (measured by the Lifetime Drinking History questionnaire30) as the covariate. Subjective responses to alcohol were analyzed using repeated measures analysis of variance with genotype and testing sequence as between subjects variables and time point as the within subjects variable. Post hoc comparisons were conducted using Newman–Kuel tests. Effect sizes were calculated using $q^{2}$ from Statistica, and Cohen’s f calculated using GPower 3.1.0 (Franz Faul, Universität Kiel, Germany).

Humanized mouse lines

At the OPRM1 locus, linkage disequilibrium exists between OPRM1 A118G and other markers.31 We therefore generated two humanized mouse lines, where the OPRM1 exon 1 was replaced by the corresponding human sequence. One of the lines (h/mOPRM-118AA) is homozygous for the major human 118A allele. For the other (h/mOPRM1-118GG), we used site-directed mutagenesis to introduce a G in position 118. Thus, the lines are genetically identical, with the exception of the A→G substitution (Figures 2a–c). The lines are on a C57BL/6 background, where robust voluntary alcohol consumption is observed.32 Generation of the h/mOPRM1-118AA and h/mOPRM1-118GG lines is detailed in Supplementary Information. Insertion of sequence corresponding to human OPRM1 exon 1 is shown in Figure 2c. Determination of the respective h/mOPRM1-118 genotype was carried out using the PCR method described for the human PET study. First-generation h/mOPRM1-118AA and h/mOPRM1-118GG mice obtained from a cross of the founder lines were assessed with regard to overall health and behavior using an established testing battery.33 Supplementary Table S2 shows that there were no genotype differences, with the exception of some excessive running in GG subjects on introduction into the novel environment, and a modest but statistically significant increase in locomotion in h/m118AA males compared to h/m118GG males. Male mice aged 13–16 weeks were used for the binding and signaling experiments, and male mice aged 24–32 weeks were used for the microdialysis experiments.

Figure 1 Human PET study. Axial view of group maps showing change of [11C]-raclopride binding potential (ΔBP; nCi ml$^{-1}$) between placebo and alcohol sessions in (a) AA individuals and (b) AG individuals. Color bars indicate corresponding ΔBP values. Reduction in raclopride binding is attributed to competition with dopamine released by the alcohol challenge; thus, a negative ΔBP indicates an increase in endogenous dopamine release. (c) Relative change in binding potential (%ΔBP) for [11C]-raclopride between alcohol and placebo sessions in four striatal regions of interest. Data are least square means (± s.e.m.). Main genotype effect: $P=0.006$; $^*P<0.05$ on post hoc tests within individual regions. AVS, anterior ventral striatum; PVS, posterior ventral striatum. (d) Schematic of PET sessions, and blood alcohol concentration profiles over time during the alcohol session (mean ± s.e.m.). There was no significant difference between genotypes ($F[1,24]=0.51$, $P=0.48$).
Receptor binding studies
Membrane binding was assessed in preparations from CHO-K1 cells transfected with OPRM1 receptor cDNA and incubated with 2 nM \[^{3}H\]-d-Ala\(^2\), N-MePhe\(^4\), Gly-ol-]enkephalin (DAMGO) (37 Ci mmol\(^{-1}\); NEN, Boston, MA, USA) and nonradioactive naloxone, morphine and \(\beta\)-endorphin (\(\beta\)-End) ranging from 0.01 to 1000 nM. The mixture was incubated at
room temperature, filtered through Whatman GF/B filters (FP-100; Brandel, Gaithersburg, MD, USA), washed with ice-cold Tris-HCl and assayed for radioactivity by liquid scintillation. Data were analyzed by nonlinear analysis using the Prism computer program (GraphPad Software, La Jolla, CA, USA).

Autoradiographic binding was studied on 20 μm coronal cryosections as described,44 using 5 nM [3H]DAMGO (48.9 Ci mmol⁻¹; NEN). Nonspecific binding was determined from adjacent sections in the presence of the radioligand plus 10 μM naloxone. Radiolabeled, dried tissue sections and [3H]microscales (Amersham, Uppsala, Sweden) were apposed to tritium-sensitive screens (Fujifilm imaging plates, Valhalla, NY, USA), and images were obtained using a Fujifilm BAS 5000 PhosphorImager.

Agonist-induced GTP[γ-35S] activity was evaluated as described39 on 20 μm coronal sections, by incubating GTP[γ-35S] (0.04 nM) with 3 μM DAMGO in assay buffer at 25 °C for 2 h. Agonist studies were also performed in the presence of the antagonist naloxone to verify the receptor specificity of G-protein activation. Basal activity was assessed in each experiment with GDP in the absence of agonist, and nonspecific binding was evaluated in the presence of 10 μM unlabeled GTP[γ-35S] without GDP. Slides were rinsed, dried and exposed to tritium-sensitive screens (Fujifilm imaging plates).

Images were analyzed using MCID Image Analysis Software (Imaging Research, Linton, UK). Regions of interest were defined by anatomical landmarks.36 Agonist-stimulated activity in brain sections was calculated by subtracting the optical density in basal sections (incubated with GDP alone) from that of agonist-stimulated sections; results were expressed as percentage of basal activity.

Electrophysiology
Enzymatically dissociated trigeminal ganglion neurons were used. Ca²⁺ currents were recorded using whole-cell patch clamping as described.27 Currents were acquired with the Axopatch 200B amplifier (Molecular Devices, Sunnyvale, CA, USA), filtered and cell membrane capacitance and pipette series resistance were electronically compensated. The concentration–response relationships were determined by sequential application of the respective agonist in increasing concentrations. Two to three different concentrations were used with each cell to minimize desensitization. The results were pooled and the concentration–response curves were fit to the Hill equation. Data and statistical analyses were performed with IGOR Pro (Lake Oswego, OR, USA) and Prism 4.0 (GraphPad Software) software packages, respectively.

Microdialysis
Under isofluorane anesthesia, mice were implanted with microdialysis probes as previously described,38 aimed at the nucleus accumbens and secured to the skull using a skull screw and cement. Histologically verified placement within the nucleus accumbens for each subject is shown in Figure 3a. During a 12 h postsurgical recovery period, probes were slowly perfused with artificial cerebrospinal fluid. The perfusion flow rate was then increased to 0.6 μl min⁻¹ for 2 h to reach an interstitial equilibrium before sample collection. Dialysate fractions were subsequently collected, during the dark cycle, at 10 min intervals during a 60 min baseline period and, after an injection of EtOH (2 g kg⁻¹, i.p.), during a 120 min postinjection period. Samples were analyzed for DA and 5-HT using high-performance liquid chromatography coupled with electrochemical detection.

After confirmation by analysis of variance of no group differences in baseline DA or 5-HT concentrations, we converted data to percent change from the average baseline concentration obtained before pretreatment. Within- and between-group analyses were performed by two-way analysis of variance with repeated measures over time, with separate analyses performed for the DA and 5-HT data.

Results

Human PET study
BACs at the end of the alcohol infusion during the PET session were, on average, within 1 mg per 100 ml
of the target level, confirming the high level of accuracy and precision of the method. There were no differences between BACs achieved in the AA and AG groups (analysis of variance: \(F[1, 24] = 0.287, P = 0.597\)). Baseline BP for \(^{11}\text{C}\)-raclopride, as measured on the saline infusion session, also did not differ between genotypes (AA: 3535.8 ± 107.8; AG: 3722.7 ± 124.5 nCi ml\(^{-1}\), mean ± s.e.m.; \(F[1,26] = 1.3, P = 0.27\)), making preexisting differences in D2 receptor densities unlikely.

Across the four striatal subregions of interest (anterior ventral striatum, posterior ventral striatum, caudate and putamen; Supplementary Figure S1), the change in BP (\(\Delta\text{BP}\)) attributable to the alcohol challenge ((\(\text{BP}_{\text{placebo}} - \text{BP}_{\text{alcohol}}\))/\(\text{BP}_{\text{placebo}}\)) differed markedly as a function of genotype, with AG subjects consistently showing signs of greater DA release than AA individuals (main genotype effect: \(F[1,22] = 9.3, P = 0.006\)). Genotype accounted for 29.7% of the variance in response, measured as \(\eta^2\) translating into an effect size of Cohen’s \(f = 0.65\), that is between ‘moderate’ and ‘large’. There was also a significant genotype × region interaction (\(F[3,66] = 3.8, P = 0.01\)); accordingly, post hoc tests showed that the main genotype effect was predominantly driven by significant differences in the anterior and posterior ventral striatum, regions most closely associated with drug reward (Figures 1a–c). The highest response to alcohol was observed in the anterior ventral striatum of the AG group, where \(\Delta\text{BP}\) was approximately 9%.

This is more than half of the response seen with amphetamine,\(^{16}\) a drug that potently enhances synaptic DA by acting on the DA transporter. This relative potency is in agreement with direct measures obtained by brain microdialysis in experimental animals.\(^{9,16}\) In contrast, AA subjects did not show an alcohol-induced displacement of the radioligand in any region. Inclusion of the BAC at 45 min as a covariate in the statistical analysis of the %\(\Delta\text{BP}\) data did not influence the main effect of genotype (\(F[1,19] = 9.7, P = 0.0057\)). The BAC at 45 min per se showed a trend for a significant effect on the %\(\Delta\text{BP}\) (\(F[1,19] = 3.86, P = 0.064\)).

Similar to other imaging genetic studies,\(^{39,40}\) subjective ratings showed higher variability than the biological measure. The only effect detected was a genotype × time interaction, with AG subjects reporting lower levels of intoxication than AA subjects at the later time points, suggestive of faster acute tolerance development (see Supplementary Materials and Supplementary Figure S2E).

### Characterization of humanized receptors and mouse lines

When expressed in CHO cells, both humanized receptors showed binding of the classical OPRM1 selective ligand [\(^{1}\text{H}\)]-DAMGO, displaced by the endogenous OPRM1 agonist \(\beta\)-End with expected low nanomolar IC\(_{50}\), and with no difference between genotypes (\(F[4,46] = 1.3, P = 0.3\); Figure 2d). Signaling,
assessed as modulation of Ca\(^{2+}\) currents in acutely isolated trigeminal ganglion neurons, was also normal in response to both DAMGO and β-End, once again without any genotype differences (DAMGO: F[4,12] = 1.8, P = 0.2; β-End: F[4,12] = 1.2, P = 0.4; Figures 2e–f). Finally, \(^{[3]H}\)-DAMGO binding on mouse brain sections did not show genotype differences in receptor densities across a number of brain regions examined, including ventral and dorsal striatum and the ventral tegmental area (Supplementary Figure S5).

**DA responses to alcohol in humanized mouse lines**

Microdialysis probes placement in ventral striatum/nucleus accumbens is shown in Figure 3a. Baseline DA levels did not differ between genotypes (F[1,22] = 6.9 x 10^-6, NS) and were 2.42 ± 0.4 nM for h/mOPRM1-118AA and 2.43 ± 0.6 nM for h/mOPRM1-118GG mice, respectively (n = 12 per group). Alcohol injection (2 g kg\(^{-1}\), a dose that robustly induces conditioned place preference in C57BL/6 mice) increased dialysate DA in both lines (main effect: F[6,132] = 12.4; P < 0.0001) however, response differed markedly between genotypes (main genotype effect: F[1,22] = 7.2; P < 0.05; genotype x time interaction: F[6,132] = 12.4; P < 0.0005; Figure 3b). Peak DA increase was nearly fourfold greater in GG mice than in AA animals. The genotype effect was specific for DA release induced by alcohol; although alcohol also significantly increased 5-HT (main effect: F[6,132] = 15.6; P < 0.0001), this was not affected by genotype (main genotype effect: F[1,22] = 3.6; NS; genotype x time interaction: F[6,132] = 0.92; NS; Figure 3c).

**Discussion**

Taken together, our data strongly support a causal role of the OPRM1 118G allele to confer a more vigorous DA response to alcohol in the ventral striatum. These findings may be related to observations of differential subjective alcohol effects as a function of OPRM1 A118G genotype in humans,\(^{12}\) and of marked increased psychomotor responses to alcohol in rhesus males carrying the functionally equivalent 77G variant.\(^{14}\) Interestingly, we also found that subjective feelings of intoxication over time differed as a function of genotype. Carriers of the 118G allele showed signs of rapid acute tolerance, similar to that previously described in men at genetic risk for alcoholism.\(^{31}\) The use of a reverse-translational approach allowed us to effectively isolate the influence of the human 118G variant from that of other polymorphisms with which it might be in linkage disequilibrium.

Excessive activation of brain reward systems by alcohol in OPRM1 118G carriers might be expected to confer susceptibility for alcohol use disorders. Although we in fact found evidence in support of this notion in an ethnically homogenous Caucasian sample,\(^{42}\) others have not. One study found that other polymorphisms within the same haplotype block, but not A118G, were associated with diagnoses of substance dependence,\(^{31}\) whereas a meta-analysis of all available data for A118G was negative.\(^{43}\) This apparent discrepancy closely parallels recent findings with the 5-HT transporter gene promoter length polymorphism (5-HTTLPR). A robust role of 5-HTTLPR variation has been found using an imaging based endophenotype that is closely related to negative affect\(^{39,44}\) and these findings are paralleled by experimental data in a nonhuman primate model.\(^{45,46}\) Nevertheless, findings from 5-HTTLPR association studies in depression have not held up on meta-analysis.\(^{47,48}\) The reasons for these discrepancies are presently unknown, much debated and critical to resolve. One important possibility is that current diagnostic categories pool genetically heterogeneous phenocopies, as has specifically been proposed in depression.\(^{49}\) If so, the use of biological endophenotypes, such as in this study, can potentially aid the dissection of these populations into genetically and pathophysiologically more homogeneous categories.

Irrespective of its possible role as a susceptibility factor, excessive reward-related DA response to alcohol in carriers of OPRM1 118G might be of pharmacogenetic importance in treatment of excessive alcohol use. Indeed, two studies have found that the presence of the 118G allele was associated with a therapeutic response to the opioid antagonist naltrexone.\(^{50,51}\) Although one study failed to find such an effect,\(^{52}\) recent nonhuman primate data provide strong evidence for this notion under closely controlled experimental conditions.\(^{53}\) Our data are consistent with a selective role of endogenous opioids for alcohol reward in OPRM1 118G carriers, and provide a possible biological mechanism for the preferential naltrexone response in this population. However, the dissociation between objective measures of alcohol-induced DA release and subjective reports of alcohol effects may argue against a simplistic view of alcohol-induced DA release as an immediate mediator of drug reward. Instead, these data may be more compatible with a view of ventral striatal DA activation as a signal predictive of alcohol reward, and perhaps involved in learning processes important for addiction.\(^{54}\) Of note, the level of alcohol-induced DA activation in our study was overall lower than previously reported.\(^{10}\) A difference between the two studies is that alcohol administration was oral in the prior report, while we used intravenous administration. This may indicate that striatal DA response to alcohol is only in part driven by direct pharmacological actions of alcohol, while an additional component of the activation is evoked by smell or taste cues.

Our data show that the 118G allele, which encodes an amino-acid substitution in the N-terminal extracellular loop of the receptor, confers a higher striatal DA-activation in response to alcohol regardless of whether it occurs in the context of other markers within its haplotype block or not. However, the molecular mechanism that mediates functional consequences of the 118G variant remains controversial. Initially described as a gain-of-function mutation...
due to increased affinity for β-End.11 **OPRM1** 118G has since been proposed to instead confer decreased expression in several systems, including human postmortem brain tissue,55 and a mouse model where expression in several systems, including human has since been proposed to instead confer decreased
introduced the N→D substitution in the context of a murine N-terminal receptor protein sequence. In contrast, we generated mice expressing humanized receptors throughout this region by replacing all of exon 1, the main region of divergence between the two species (Figure 2a). Using this model, our present findings show that a key functional phenotype associated with the 118G variant, alcohol-induced striatal DA release, is replicated across species in the absence of effects on receptor affinity, binding density or signaling. A remaining possibility is altered oligomerization, a mechanism that is critical for opioid receptor desensitization, endocytosis and re-insertion.57 **OPRM1** A118G modifies a glycosylation site in the N-terminal extracellular loop of the receptor, and could thereby influence receptor dimerization and trafficking.

Functionally equivalent **OPRM1** variants have evolved independently in two primate lineages, the rhesus macaque and man.11 In both species, minor allele carriers are more sensitive to disruption of social attachment bonds.58,59 In rhesus, this is accompanied by bold/exploratory traits, whereas lower conscientiousness has been observed in human 118G carriers.13,58,60 These traits may have been advantageous in the evolutionary history of the respective species, and recent studies suggest that the 118G allele has been under positive selection.61 In contrast, the ability of these variants to modulate DA responses to alcohol is likely a coincidental evolutionary by-product.

Identifying genetic sources of individual variation in complex behavioral phenotypes has proven challenging. Our findings support the notion that use of biological intermediate phenotypes and translational approaches can contribute important data to facilitate this difficult endeavor.

**Conflict of interest**

The authors declare no conflict of interest.

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