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ARTICLE

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Influence of *OPRM1* Asn^{40}Asp variant (A118G) on [^{11}C]carfentanil binding potential: preliminary findings in human subjects

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Abstract

The Asn40Asp variant (A118G) of the μ opioid receptor (*OPRM1*) gene is thought to contribute to the development and treatment of alcohol dependence. Employing positron emission tomography (PET), we first examined whether the single nucleotide polymorphism (SNP) modifies binding potential (BP_{ND}) of the μ-selective ligand [^{11}C]carfentanil in healthy control (Con) and 5-d abstinent alcohol-dependent (AD) subjects (unblocked basal scan). Second, we examined whether the allelic variants were associated with differences in *OPRM1* occupancy by naltrexone (50 mg) in AD subjects. Con and AD carriers of the G allele (AG) had lower global BP_{ND} at the basal scan than subjects homozygous for the A allele (AA). In AD subjects, naltrexone occupancy was slightly higher in AG subjects (98.9%) compared to AA subjects (93.1%), but this was not significant. We are the first to demonstrate using PET in healthy normal and AD subjects that the A118G SNP alters *OPRM1* availability.

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Introduction

Variations in the gene encoding the μ opioid receptor (*OPRM1*) may be relevant to the development and treatment of alcohol abuse and dependence. The most prevalent single nucleotide polymorphism (SNP) is a nucleotide exchange in exon 1 at the 118 position of the *N*-terminal domain of the *OPRM1* (rs1799971). *In vitro* studies have shown that the minor allele (G) of this SNP is associated with a lower level of receptor glycosylation and a reduced receptor half-life (Huang *et al.* 2012), increased binding affinity for β-endorphins (Bond *et al.* 1998) and reduced receptor expression (Zhang *et al.* 2005).

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The first demonstration of *in vivo* biological significance for the A118G SNP showed allele-specific cortisol responses to the opiate receptor antagonist naloxone. This result has been replicated in Caucasians (for review see Uhart & Wand, 2009). The minor allele (G) also has been associated with decreased pupil constriction to an opioid agonist, decreased analgesic response to electrical stimuli-induced pain and decreased opioid-induced respiratory depression in studies in healthy subjects as well as decreased clinical potency of opioid agonists (Lotsch & Geisslinger, 2011).

There is also evidence that the A118G SNP affects alcohol-related responses. In human laboratory studies in heavy drinkers who were administered alcohol, subjects who were carriers of the G allele report a heightened response to alcohol (Ray *et al.* 2011*a*) and greater urges to drink after exposure to alcohol-related cues (van den Wildenberg *et al.* 2007). The A118G SNP

may also influence alcohol treatment efficacy of the opioid antagonist naltrexone (Kranzler & Edenberg, 2010); alcohol-dependent (AD) subjects carrying the G allele were more likely to respond to naltrexone treatment than those homozygous for the A allele. Taken together, these data suggest that the A118 SNP influences responses to alcohol and naltrexone efficacy via functional differences in μ opioid receptor biology.

Based on the literature, it seemed likely that variations in the gene encoding the *OPRM1* may influence receptor availability in the human brain. If the A118G SNP genotype significantly contributes to individual differences in μ receptor availability, then such differences likely would not be limited to regional changes, but could be expected to occur across brain regions with high μ opioid receptor density (i.e. genotype differences in global *OPRM1* availability). Previously, we examined positron emission tomography (PET) imaging of regional differences in *OPRM1* binding potential (BP_{ND}) employing the radiotracer [^{11}C]carfentanil (CFN). We studied untreated, healthy control (Con) subjects as well as recently abstinent AD subjects before and during treatment with the FDA recommended therapeutic dose of naltrexone (50 mg/d; Weerts *et al.* 2008, 2011). We have genotyped most of these same AD and Con subjects for the A118G SNP and now present a secondary analysis to: (1) examine the effect of genotype on global *OPRM1* availability in recently abstinent AD subjects and Con subjects; (2) determine whether there were allele-specific effects (A *vs.* G) in AD subjects on the degree of *OPRM1* blockade by naltrexone.

Method

Subjects

Subjects in the current analyses included 25 AD and 28 Con subjects aged 21–60 yr. Subjects provided informed consent in the sober state, using an Institutional Review Board approved informed consent document. A detailed description of the assessment instruments used to determine study eligibility as well as demographics information on all subjects is provided in previous publications (Weerts *et al.* 2008, 2011). AD subjects were actively drinking prior to Clinical Research Unit (CRU) admission (see below) and met DSM-IV criteria for alcohol dependence based on an in-person interview using the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA-II). For all subjects, smoking status (smokers *vs.* non-smokers) and smoking intensity were also determined via the SSAGA-II and alcohol drinking intensity and patterns were characterized via the time line follow back (Sobell & Sobell, 1992). Smokers were defined as persons who reported smoking >100 cigarettes in their lifetime and currently smoked regularly (three or more cigarettes per day). Age-matched Con subjects were enrolled if they consumed less than eight drinks per week for women and <15 drinks per week for men and did not meet lifetime DSM-IV criteria for either alcohol abuse or dependence. Con and AD subjects were excluded if they met current or lifetime DSM-IV diagnostic criteria for another Axis I disorder, including other drug abuse/dependence (except nicotine), had a positive urine drug toxicology test at screening or hospital admission, had any ongoing health problems or reported maternal drinking during pregnancy. In addition, AD subjects were excluded if they reported alcohol-related seizures or the need for medication during previous detoxifications.

All subjects were admitted to the CRU after a negative breath alcohol test and completed scans under an in-patient protocol. During the in-patient stay, subjects could not smoke; nicotine-dependent subjects received a transdermal nicotine patch (21 mg) each day. Con subjects were admitted the night before the PET scan, were instructed not to drink for 48 h before admission and had to provide an alcohol-free breathalyser test at the time of admission. AD subjects completed detoxification and remained on the CRU for 19 d (Weerts *et al.* 2008). Pre-naltrexone PET imaging (basal scan) was conducted after alcohol withdrawal symptoms subsided (day 5) as determined by Clinical Institutes Withdrawal Assessment (CIWA-AR) scores. For AD subjects only, naltrexone (50 mg p.o.) was administered beginning on day 15 at 09:00 and 21:00 hours and then at 21:00 hours each day for the remainder of their CRU stay; nurses observed subjects swallowing the pill to ensure medication compliance. PET imaging during naltrexone maintenance (naltrexone scan) was conducted after subjects had received four doses of 50 mg (day 18). Previous studies have demonstrated that plasma levels of naltrexone and its active metabolite 6-β-naltrexol reach stability within 3 d of naltrexone dosing (Huang *et al.* 1997; McCaul *et al.* 2000).

DNA extraction and SNP analysis

Blood samples were genotyped for *OPRM1* polymorphism A118G SNP (rs1799971) as described previously (Chong *et al.* 2006). Subjects were categorized according to genotype (AA *vs.* AG).

Population stratification

To ensure adequate assessment and control of any relevant substructure that might be present in our population, DNA samples for each subject were genotyped using a panel of 23 microsatellite markers with high efficiency at clustering individuals into population subgroups (Smith *et al.* 2001; Yang *et al.* 2005). Short tandem repeat markers D1S252, D2S319, D12S352, D17S799, D8S272, D1S196, D7S640, D8S1827, D7S657, D22S274, D5S407, D2S162, D10S197, D11S935, D9S175 and D5S410 were selected from Applied Biosystems (USA) Linkage Mapping Set v. 2.5. Markers D7S2469, D16S3017, D10S1786, D15S1002, D6S1610 and D1S2628 were synthesized by Applied Biosystems with fluorescent dye PET® to allow genotyping in the same lane with the other markers.

Population stratification was determined using Structure software version 2.3.2 (http://pritch.bsd.uchicago.edu/structure.html) following the methods of Pritchard *et al.* (2000). The model included the possibility of admixture and correlated allele frequencies between populations; all other parameters were kept at default values.

PET imaging procedures

PET imaging procedures are described in detail previously (Weerts *et al.* 2011). Briefly, subjects underwent magnetic resonance imaging prior to PET imaging for anatomical identification of regions, atrophy correction and alignment of PET imaging planes within and across subjects. PET scans were acquired in 3D mode on a GE Advance PET scanner (GE Medical Systems, USA). A 10-min transmission scan was obtained using rotating germanium-68 rods before injection of [^{11}C]CFN. After i.v. bolus administration of the radiotracer [^{11}C]CFN, a set of 25 images with variable time periods (6×30, 5×60, 5×120, 9×480 s) was acquired over the 90-min scan. [^{11}C]CFN injected was 20.0 ± 0.6 mCi SA: $21{,}482.7\pm3310.1$ mCi/μmol for the Con group and 19.3 ± 0.5 mCi SA: $18{,}444.1\pm2849.6$ mCi/μmol for the AD group. The CFN dose did not exceed 0.04 μg/kg. Correcting for attenuation scatter and dead-time and physical decay (to the injection time), images were reconstructed in a $128\times128\times35$ matrix with a pixel size of $2\times2\times4.25$ mm with filtered back projection methods using a ramp filter (Kinahan & Rogers, 1989).

Derivation of PET outcome variables

BP_{ND} of [^{11}C]CFN was determined using reference tissue graphical analysis (RTGA) with occipital lobe as the reference region and setting the brain-to-blood clearance rate constant of the reference region (k_2^R) at 0.104/min (Endres *et al.* 2003). Estimates of BP_{ND} using RTGA have been shown to be highly correlated with those obtained from the arterial input-based kinetic model (Endres *et al.* 2003). We selected brain regions based on two criteria: regions with moderate to high BP_{ND} and/or regions thought to be involved in regulation of alcohol reward, learning and memory as well as dependence and withdrawal. The 14 volumes of interest (VOIs) were frontal lobe, temporal lobe, parietal lobe, fusiform gyrus, cingulate, hippocampus, amygdala, cerebellum, insula, ventral striatum, putamen, caudate nucleus, globus pallidus and thalamus. These VOIs encompassed a substantial portion of the brain containing μ opioid receptors (497 ± 59.7 ml).

For the current analysis, global BP_{ND} was calculated as a weighted average of all 14 VOIs. Naltrexone occupancy of *OPRM1* was calculated as [1 − (naltrexone scan global BP_{ND}/Basal scan global BP_{ND})].

Statistical analyses

Three main multi-linear models were constructed with basal global BP_{ND} as the dependent variable and genotype as the independent variable for all subjects (AD and Con, $n=53$), Con group only and AD group only. Alcohol dependence diagnosis (for the all-subject model only), gender and smoking status were added as covariates to the model based on significant effects for these variables on BP_{ND} in our previous study (Weerts *et al.* 2011). Naltrexone scan results (global BP_{ND} and naltrexone occupancy) were analysed using similar models with gender and smoking covariates.

Since the frequency of the 118G SNP varies across populations, we conducted a sensitivity analysis by adding ancestral population as a covariate to the above models. Also, since the G allele is very rare in African Americans, we completed a secondary analysis in the subset of European ancestry subjects in the study. We also completed a series of secondary analyses to examine other potential confounding factors that may have an effect on BP_{ND}. Because the number of drinking days per week was not balanced between the two genotype groups in AD subjects ($p=0.05$, see Results), we added it as covariate to the main models. Since smoking intensity may influence BP_{ND}, we also completed a secondary analysis where we replaced the smoking status (smoker *vs.* non-smoker) with cigarettes per day as a covariate in the main models. All statistical analyses were carried out using SAS version 9.2 (SAS Institute, USA).

Table 1. Age and distribution of gender, race and smoking status for AA and AG genotypes in alcohol dependent (AD) and healthy control (Con) subjects

	AA		AG	
	AD ($n=19$)	Con ($n=20$)	AD ($n=6$)	Con ($n=8$)
Mean age, yr (S.D.)[a]	43.4 (6.9)	44.1 (9.6)	45.3 (9.1)	44.9 (8.6)
Mean kg weight (S.D.)[a]	76.1 (14.3)	78.5 (14.7)	70.2 (9.8)	77.6 (11.6)
Gender (*n*)				
Male	14	12	4	6
Female	5	8	2	2
Self-reported race (*n*)				
Caucasian	10	9	5	6
Black	9	11	1	2
Ancestral markers (*n*)				
Group 2	9	10	5	6
Group 1	10	10	1	2
Smoking status (*n*)				
Non-smokers	4	16	1	4
Smokers	15	4	5	4
Mean cigarettes/d (S.D.)[b]	11.4 (10.3)	3.8 (7.0)	20.8 (13.6)	8.8 (8.8)
Mean drinks/d (S.D.)[a]	12.1 (7.5)	1.4 (1.7)	11.1 (4.6)	1.7 (1.7)
Mean drinking days/wk (S.D.)[c]	4.9 (2.1)	0.7 (1.5)	6.4 (0.6)	0.6 (0.9)

[a] No significant difference between groups.
[b] Smokers only included in means; no significant difference between groups.
[c] $p=0.052$ for AD AA compared to AD AG only.

Results

Two distinct genetic ancestral populations were identified and comparison of self-reported race and ancestral population groups showed comparable results: group 1 ($N=23$) was largely composed of individuals who self-reported their race as African–American (96%) and group 2 ($N=30$) was largely composed of individuals who self-reported their race as Caucasian (96.7%). There were 39 AA subjects, 14 AG subjects and no GG subjects. Mean ages, mean body weight, mean drinking days per week, mean drinks per drinking day and distribution of AA and AG subjects for gender, self-reported race, ancestral population and smoking status in AD and Con groups are shown in Table 1.

When genotype (AA *vs.* AG), gender (male *vs.* female), smoking status (smokers *vs.* non-smokers) and alcohol dependence diagnosis (AD *vs.* Con) were included in the model, there was a significant effect of genotype ($p=0.0011$) on the basal scan global BP_{ND} in all subjects. Specifically, global BP_{ND} was lower in carriers of the G allele (mean 0.479 ± 0.023 S.E.M.) of the A118G SNP compared to subjects who were homozygous for the A allele (mean 0.567 ± 0.016 S.E.M.). As shown in Supplementary Table S1 (available online), the genotype differences in BP_{ND} were observed across the 14 VOI used to determine global BPND in AD and Con subjects. The significant genotype difference in global BP_{ND} was preserved when we added ancestral population to the model ($p=0.0039$) and also when we included only Caucasian subjects of European ancestry in the overall model ($n=30$, $p=0.020$).

Adding smoking intensity (cigarettes per day) instead of smoking status in our overall model did not alter the genotype effect. Specifically, when genotype, gender, cigarettes per day and alcohol dependence diagnosis were included in the model, there was a significant effect of genotype ($p=0.0084$) on the basal scan global BP_{ND} in all subjects. Carriers of the G allele had lower global BP_{ND} (mean 0.483 ± 0.023 S.E.M.) than subjects who were homozygous for the A allele (mean 0.557 ± 0.015 S.E.M.). The genotype effect was also preserved when we included cigarettes per day and included only Caucasian subjects of European ancestry in the model ($p=0.044$).

Similar results were obtained when AD and Con subjects were analysed separately (Fig. 1*a*, *b*). After adjusting for gender and smoking status, global BP_{ND}

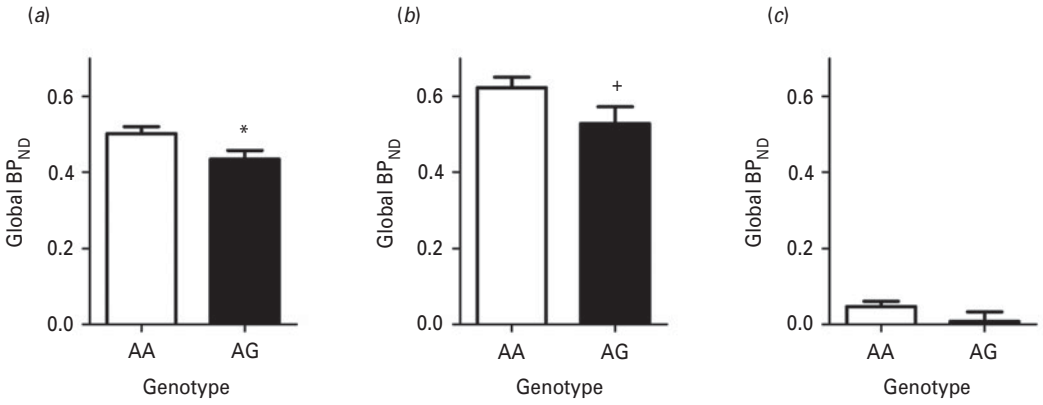

Fig. 1. Mean global binding potential (BP_{ND}) of [^{11}C]carfentanil, (*a*) for the basal scan in healthy controls, (*b*) for the basal scan in alcohol dependent (AD) subjects and (*c*) the naltrexone scan in AD subjects. Data shown are genotype group means ± S.E.M. adjusted for ancestral population, gender and smoking status. * $p = 0.027$, $^{+}$ $p = 0.062$.

was lower in Con subjects carrying the G allele ($p = 0.010$) and in AD subjects carrying the G allele ($p = 0.030$) compared to Con or AD subjects who were homozygous for the A allele. When ancestral population was added to the model, the global BP_{ND} in Con carriers of the G allele remained significantly lower than global BP_{ND} in Con subjects homozygous for the A allele ($p = 0.027$). However, there was now a trend for lower global BP_{ND} in AD carriers of the G allele compared to AD subjects homozygous for the A allele ($p = 0.062$). Likewise, when we limited our analysis to Con Caucasian subjects of European ancestry in the model, genotype differences were preserved in Con subjects ($n = 9$ AA, $n = 6$ AG, $p = 0.025$), with G carriers showing lower BP_{ND} than subjects homozygous for the A allele. Genotype differences were not maintained in AD Caucasian subjects of European ancestry ($n = 10$ AA, $n = 5$ AG, $p = 0.221$).

Naltrexone treatment resulted in a robust reduction in global BP_{ND} in all AD subjects regardless of genotype (Fig. 1*c*). Although not reaching statistical significance, global BP_{ND} was lower in G carriers compared to subjects homozygous for the A allele during naltrexone treatment. Examination of genotype differences in naltrexone occupancy yielded similar results. Naltrexone occupancy was higher in subjects who were carriers of the G allele (98.9% ± 4.0) than in subjects homozygous for the A allele (93.1% ± 2.2); this effect was not statistically significant ($p = 0.178$). Since our sample size was small, we completed a power calculation to determine the sample size necessary to observe a significant difference between allele groups in global BP_{ND} during naltrexone blockade, setting $p < 0.05$ and power > 0.8. Results showed that with a sample size of 88 subjects a significant difference in BP_{ND} would likely be detected if similar differences persisted.

In a secondary analysis, we also examined possible genotype differences in measures related to alcohol drinking, dependence and withdrawal. On the day of the scan, withdrawal symptoms were low, as indicated by pre-PET CIWA-AR mean scores (AA 0.7 ± 1.6 S.D. and AG 0.3 ± 0.8 S.D.) and did not differ significantly between genotype groups. Dependence severity as determined by the Alcohol Dependence Scale scores also did not differ between genotype groups (AD AG 20.5 + 7.1 SD *vs.* AD AA 16.8 + 4.9 S.D.). There was a trend ($p = 0.052$) for a genotype effect on mean number of drinking days per week in the 90 d that preceded hospital admission in AD subjects; AD carriers of the G allele reported drinking more days per week compared to AD subjects who were homozygous for the A allele (Table 1). When number of drinking days per week was included in the model with genotype, alcohol diagnosis, gender and smoking status, the significant genotype differences were preserved in all subjects ($p = 0.0018$) and also in AD ($p = 0.048$) and Con subjects ($p = 0.019$) when analysed separately.

Discussion

The current findings indicate that *OPRM1* A118G SNP influenced *OPRM1* availability in Con and AD subjects. Specifically, Con and AD subjects who were carriers of the G allele had lower global [^{11}C]CFN BP_{ND} at the basal scan than subjects who were homozygous for the A allele. It is notable that, despite the chronic history of heavy drinking and alcohol dependence in our AD subjects, the effects of the SNP

remained robust. Similar findings of lower BP_{ND} across multiple brain regions in G allele subjects were obtained in smokers under active and placebo nicotine administration procedures (Ray *et al.* 2011*b*).

There are at least two potential mechanisms for the observation of lower [^{11}C]CFN BP_{ND} in G carriers. First, G carriers may have fewer *OPRM1* receptors compared to carriers who are homozygous for the A allele. Using *in vitro* binding studies, Zhang *et al.* (2005) have shown a lower *OPRM1* Bmax in cell lines expressing the G allele compared to cell lines expressing the A allele. This may result from the alterations in glycosylation that reduce *OPRM1* half-life (Huang *et al.* 2012). Additionally, Bond *et al.* (1998) have shown that the G allele receptor has increased binding affinity for β-endorphins. This implies that the PET ligand [^{11}C]CFN will compete less efficiently with endogenous β-endorphins in G allele carriers.

Several recent alcohol treatment clinical trials have reported greater naltrexone efficacy in G allele carriers compared to patients who are homozygous for the A allele (Anton *et al.* 2008; Oslin *et al.* 2006). In laboratory studies, naltrexone decreased subjective responses to alcohol more in social drinkers who were carriers of the G allele (Ray *et al.* 2011*a*; Setiawan *et al.* 2011), although no genotype differences were found in treatment-seeking heavy drinkers (Tidey *et al.* 2008). It is plausible that these pharmacogenetic effects may result from allele specific differences in the degree of naltrexone blockade between allele groups. This scenario may be accounted for by the finding of decreased *OPRM1* Bmax in the G variant of the receptor compared to the receptor encoded by the A allele. That is, G-allele patients with lower *OPRM1* density will achieve more complete naltrexone blockade than patients who have higher *OPRM1* density at the recommended therapeutic dose (50 mg). Although allele group differences in naltrexone blockade did not achieve statistical significance in this study, we believe that it may be attributable to our relatively small sample of AD subjects. Our power estimate indicated that significant effects would be observed with the typical sample size enrolled in pharmacotherapy clinical trials. However, it is important to note that both allele groups achieved greater than 90% blockade of *OPRM1*. Therefore, even if effects were statistically significant, these small allele specific differences in naltrexone blockade seem unlikely to translate into a significant therapeutic advantage for G carriers. Other mechanisms must be at work.

This is the first demonstration in healthy social drinkers and AD subjects of the effects of the A118G SNP on *OPRM1* availability. Importantly, naltrexone blockade was >90% in both allele groups. Thus, previous observations of differences in therapeutic efficacy of naltrexone as a function of the A118G SNP cannot be readily explained by differences in the extent of naltrexone blockade at the *OPRM1*.

Supplementary material

For supplementary material accompanying this paper, visit http://dx.doi.org/10.1017/S146114571200017X.

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Dr Wand is the recipient of a gift fund from the Kenneth Lattman Foundation. He is an investigator in a post marketing study for Eli Lilly & Company, entitled The Global Hypopituitary Control and Complications Study (HypoCCS). He is an investigator in a post marketing study for Ipsen entitled Somatuline Depot (lanreotide) Injection for Acromegaly (SODA). Dr Wong is a consultant for Amgen. Between 2009 and the present, Dr Wong has received funding from the following companies: Acadia; Amgen; Avid; Biotie; Bristol Myers Squibb; GE; Intracellular; J&J; Lilly; Luhdeck; Merk; Orexigen; Otuska; Roche; Sanofi-Aventi; Sepracor. Dr McCaul was principal investigator on a contract (A Phase 2 Study of LY2196044 Compared with Naltrexone and Placebo in the Treatment of Alcohol Dependence) funded by Lilly Research Laboratories; Drs Weerts and Wand were co-investigators on this project.

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