

Interactions between DAT1 and OPRM1, subcortical volume and later alcohol use in alcohol naïve youth.

Emma Jane Rose¹, Valerie Darcey², John VanMeter², and Diana Fishbein¹

1: Bennett Pierce Prevention Research Center, The Pennsylvania State University, University Park, PA

2: Center for Functional and Molecular Imaging, Georgetown University, Washington, DC.

Introduction:

The risk for adolescent alcohol use arises from a developmental trajectory characterized by impulsivity, risky decision-making, reward sensitivity and executive dysfunction¹, and is likely related to genetic risk for alcohol abuse and its impact on neurodevelopment. However, the relationship between genetic risk for alcohol abuse and brain structure preceding use is not well understood. Here we considered the impact of 2 'risk' variants - a dopamine transporter gene (DAT1) variable number tandem repeat (VNTR; rs28363170) and an opioid receptor mu 1 (OPRM1) single nucleotide polymorphism (SNP; rs1799971) - on brain structure pre-alcohol and its relationship to subsequent alcohol use. DAT1 A9 carriers have lower dopamine levels and are more reward sensitive than A10 homozygotes and have an increased risk of alcoholism², while the OPRM1 G allele has been associated with the reinforcing effects of alcohol³. These variants also have an interactive effect on the subjective response to alcohol⁴.

Methods:

Drug and alcohol naïve pre-adolescents were recruited to a prospective, longitudinal study of neurodevelopmental trajectories mediating risk for alcohol use. At baseline, high-resolution T1 MPRAGE images were collected on a 3T scanner. Alcohol use was determined at 18-month follow-up using the revised Drug Use Screening Inventory and a Tobacco, Alcohol and Drug use screener and was defined as one or more instances of drinking. The sample (N=57) included 11 (~19%) who reported alcohol use and 46 who remained alcohol naïve. For genotyping, DNA was extracted from saliva using a modification of a previously described method⁵. Taqman SNP genotyping assay was performed using an allelic discrimination assay protocol. The VNTRs were amplified using fluorescent primers and VNTR fluorescent-labeled products were analyzed using the 3730XL DNA Analyzer. Genotypes were identified using Genotyper v5.0. Imaging data were preprocessed in Freesurfer using standard processing pipeline parameters for subcortical volumes (SV). Whole-brain GLM analysis considered associations between DAT1 and OPRM1 genotypes, SV at baseline and alcohol use at follow-up. These analyses included standard covariates (e.g. gender, intracranial volume) and those related to alcohol use and/or structural variability in the sample (e.g. IQ, age, pubertal stage, family history of drug abuse).

Results:

Although non-significant, the alcohol use group had a greater percentage of G allele carriers for OPRM1 and A9 carriers for DAT1 (Fig.1). GLM models revealed significant interactions ($p < 0.05$) between DAT1 and use on SV in the right (r.) thalamus, left (l.) caudate, r.caudate, r.putamen, r.pallidum and l.amygdala (Fig.2). In all, greater volumes in users vs. non-users in A9 carriers and the opposite pattern in A10 carriers drove this interaction. There were also significant interactions between OPRM1, DAT1 and alcohol use in SV in r.pallidum, l.hippocampus, l. & r.

amygdala and r. nucleus accumbens (NAcc; Fig. 2). This 3-way interaction was due to a similar pattern of SV for G allele carriers as seen for the DAT1 x use interaction, while A allele carriers failed to show a differential effect of DAT1 (Fig.3).

Conclusions:

These data suggest a differential impact of DAT1 and OPRM1 on SV in regions that support reward processing (e.g. caudate, putamen & NAcc.) and which show structural deficits post-alcohol use (e.g. hippocampus)⁶. In an extension of prior work our data indicate that the impact of DAT1 and OPRM1 on subjective response to alcohol may be related to structural variability in reward pathway regions that precedes alcohol use. Despite small samples and related power issues, there was a striking consistency in these effects across regions, and so these effects warrant exploration in larger samples. Moreover, the relationship between DAT1 and OPRM1 mediated structural variability pre-use and the escalation from use, to abuse and/or dependence should be explored.

1. Sullivan EV, Pfefferbaum A. Neurocircuitry in alcoholism: a substrate of disruption and repair. *Psychopharmacology (Berl)*. 2005;180(4):583-594.
2. Dreher JC, Kohn P, Kolachana B, Weinberger DR, Berman KF. Variation in dopamine genes influences responsivity of the human reward system. *Proc Natl Acad Sci U S A*. 2009;106(2):617-622.
3. Ray LA, Hutchison KE. A polymorphism of the mu-opioid receptor gene (OPRM1) and sensitivity to the effects of alcohol in humans. *Alcohol Clin Exp Res*. 2004;28(12):1789-1795.
4. Ray LA, Bujarski S, Squeglia LM, Ashenhurst JR, Anton RF. Interactive effects of OPRM1 and DAT1 genetic variation on subjective responses to alcohol. *Alcohol Alcohol*. 2014;49(3):261-270.
5. Freeman B, Smith N, Curtis C, Hockett L, Mill J, Craig IW. DNA from buccal swabs recruited by mail: evaluation of storage effects on long-term stability and suitability for multiplex polymerase chain reaction genotyping. *Behav Genet*. 2003;33(1):67-72.
6. Welch KA, Carson A, Lawrie SM. Brain structure in adolescents and young adults with alcohol problems: systematic review of imaging studies. *Alcohol Alcohol*. 2013;48(4):433-444.

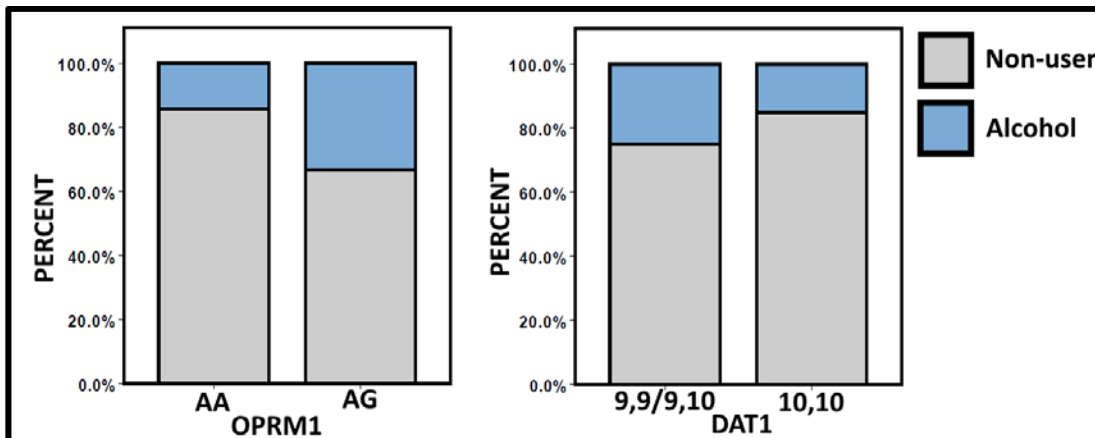


Figure 1: OPRM1 and DAT1 genotype distributions (percent) by alcohol use status. Note: although non-significant ($p > 0.05$) there was a greater percentage of G vs. A allele carriers for OPRM1 and A9 carriers for DAT1 in the alcohol use group.

	DAT1 x Alcohol Use Status	OPRM1 x DAT1 x Alcohol Use Status
RIGHT THALAMUS	$F_{(1,45)} = 4.41, p=0.041$	ns
LEFT CAUDATE	$F_{(1,45)} = 7.72, p=0.010$	ns
RIGHT CAUDATE	$F_{(1,45)} = 8.37, p=0.006$	ns
RIGHT PUTAMEN	$F_{(1,45)} = 6.35, p=0.015$	ns
RIGHT PALLIDUM	$F_{(1,45)} = 8.24, p=0.006$	$F_{(1,45)} = 4.49, p=0.039$
LEFT HIPPOCAMPUS	ns	$F_{(1,45)} = 4.38, p=0.042$
LEFT AMYGDALA	$F_{(1,45)} = 4.28, p=0.044$	$F_{(1,45)} = 4.71, p=0.035$
RIGHT AMYGDALA	ns	$F_{(1,45)} = 5.57, p=0.022$
RIGHT NUCLEUS ACCUMBENS	ns	$F_{(1,45)} = 9.73, p=0.003$

Figure 2: Summary of significant interactions between genotype (DAT1 (N: 9,9/9,10=24, 10,10=33) and/or OPRM1 (N: AA=42, AG=15)) and alcohol use on subcortical volumes ($p < 0.05$).

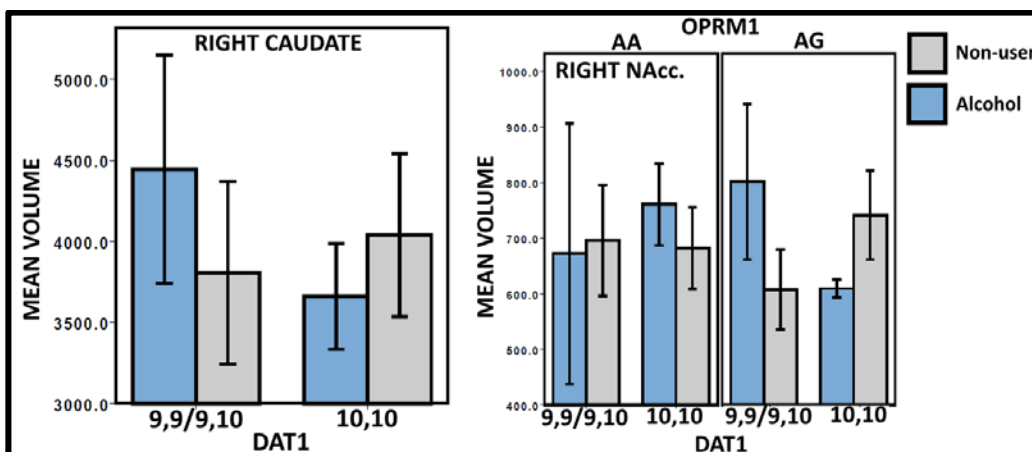


Figure 3: Examples of the patterns of subcortical volumes showing interactions between DAT1 and alcohol use (i.e., right caudate) or for DAT1 x OPRM1 x alcohol use (i.e., right nucleus accumbens). These patterns were the same for all regions showing an effect (although not shown here for space considerations)