Comparing an Opioid Use Disorder-Associated SNP with a Polygenic Risk Score as Predictors of Mu-Opioid Receptor Binding Potential

Background

• Opioid use disorder (OUD) is a common, often fatal disorder that is polygenic and moderately heritable.
• Substantial sex differences exist in OUD prevalence and risk factors.
• Only one replicable OUD-related variant has been identified through genome-wide association studies (GWAS): rs1799971(A118G) in OPRM1, which encodes the μ-opioid receptor (MOR).
• Polygenic risk scores (PRS) could account for OUD risk beyond that accounted for by rs1799971.
Aims

• Elucidate the mechanism(s) by which genetic factors contribute to OUD risk

• Evaluate sex differences both during a control (pre-challenge) condition and an acute stress paradigm
PET Study Sample

- **144 individuals of European ancestry** (88 females, 56 males; age 18-55) who underwent $^{11}$C carfentanil PET brain imaging in one of 5 studies conducted at the University of Michigan

- **Inclusion Criteria**: Right-handed, non-smokers who drank <10 standard drinks of alcohol per week, performed physical exercise no more than 1 h/d, with no history of recreational drug use

- **Exclusion Criteria**: Reported use of any centrally acting medications, including nicotine, during the past 2 months
Scan Sessions

• Participants underwent one (n=69) or two (n=75) 90-min PET scans to measure pre-challenge receptor availability and changes in receptor availability during moderate levels of sustained pain.

• The pain condition consisted of the introduction of noxious hypertonic (5%) saline into the relaxed masseter muscle at low volume to maintain a standardized target pain level of 40 on a 100-mm VAS over 20 min.
Genetic Analysis

• Genotyping used the Infinium PsychArray
• PCA for ancestral matching and population stratification adjustment
• PRS for OUD at an *a priori* p-value threshold (p<0.05) calculated using summary statistics from Zhou et al. (2020) and PRSice 2.0
• Follow-up tests examined PRS for height, major depression, and chronic pain in similar models.
Analysis of Scan Data

• MOR non-displaceable binding potential ($BP_{ND}$) measured in five addiction-related regions of interest (ROIs) using the positron emission tomography radioligand [11C]carfentanil

  • Nucleus accumbens
  • Ventral pallidum
  • Amygdala
  • Subgenual anterior cingulate
  • Dorsal striatum
Regions of Interest in Sagittal, Frontal, and Transverse Planes

Red=Nucleus Accumbens, Blue=Ventral Pallidum, Purple=Amygdala, Green=Subgenual Cingulate Cortex, Orange=Dorsal Striatum
Analysis of Scan Data

• Linear mixed model association testing of $BP_{ND}$ with rs1799971 and PRS as independent variables and age and the first 10 ancestry PCs as covariates

• Analyses conducted on the entire sample and separately by sex

• Benjamini-Hochberg false discovery rate correction ($q<0.05$) for multiple testing
Association of *OPRM1* functional coding variant with opioid use disorder. A genome-wide association study


*JAMA Psychiatry*

Jun 3:e201206, 2020
Samples

• Meta-GWAS of OUD in MVP, Yale-Penn, and SAGE samples
  • European ancestry: 8,529 affected individuals and 71,200 opioid-exposed controls
  • African ancestry: 4,032 affected individuals and 26,029 opioid-exposed controls

Zhou et al. 2020
Results

• A functional coding variant (rs1799971, encoding Asn40Asp) in \textit{OPRM1} (mu- opioid receptor gene, the main biological target for opioid drugs) was genome-wide significant ($p=1.51 \times 10^{-8}$) in the European-ancestry sample.

• Replicated in two independent samples

• Final meta-analysis p-value for this variant in all samples was $7.81 \times 10^{-10}$

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Zhou et al. 2020
GWAS of OUD (MVP, Yale-Penn, and SAGE Samples)
N=10,544 European-ancestry cases and 72,163 opioid-exposed controls

OPRM1, rs1799971, p=1.51x10^-8, OR=1.07

Zhou et al., JAMA Psychiatry, 2020
Functional Variation at *OPRM1*

- *OPRM1* (6q24-25) encodes the μ-opioid receptor, a 7-transmembrane, G-protein-coupled receptor.
- Rs1799971 is an A118G single nucleotide polymorphism in *OPRM1* that encodes an amino acid substitution in the 40th residue of the receptor protein: Asn40Asp
- The SNP has functional effects in model systems, the most consistent finding being a loss of function.
Variation at the OPRM1 Locus

```
1  cggatgagcc  tctgtgaact  actaaggttg  gagggggcta  tacgcagagg  agaatgtcag
61  atgctcagct  cgctcccttc  cgctcaacgc  tctctctgt  ctcagccagg  actgtggtct
121  gtaagaaaca  gacgagagct  tggcagcggc  gaaagggagg  ggtgggctgc  cagctcaggt
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241  ggacagcgcg  gctgccccca  gaaacgggct  caatggctct  gatgcctcag  gcgtcagcag
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1561  ctctccactt  ctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctc
```
## Diagnoses by A118G Genotype

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<tr>
<th>DIAGNOSIS</th>
<th>AA (n=100)</th>
<th>AG/GG (n=44)</th>
<th>TOTAL (n=144)</th>
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<tr>
<td>Controls (No Diagnosis)</td>
<td>50</td>
<td>24</td>
<td>74</td>
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<tr>
<td>Mood Disorder</td>
<td>20</td>
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<td>Anxiety Disorder</td>
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<td>Personality Disorder</td>
<td>8</td>
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<td>8</td>
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<tr>
<td>Eating Disorder</td>
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<td>1</td>
<td>1</td>
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<tr>
<td>Any Axis I or II Disorder</td>
<td>29</td>
<td>10</td>
<td>39</td>
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<tr>
<td>Chronic Non-Specific Back Pain</td>
<td>24</td>
<td>13</td>
<td>37</td>
</tr>
<tr>
<td>Any Chronic Pain, Axis I or II Disorder</td>
<td>50</td>
<td>20</td>
<td>70</td>
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Baseline Characteristics and psychophysiological responses during pain.

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<tr>
<th></th>
<th>AA</th>
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<th>AG/GG</th>
<th></th>
<th>F</th>
<th>p</th>
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<tr>
<td></td>
<td>Males (n=30)</td>
<td>Females (n=43)</td>
<td>Total (n=73)</td>
<td>Males (n=15)</td>
<td>Females (n=21)</td>
<td>Total (n=36)</td>
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<td>Baseline</td>
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<tr>
<td>N</td>
<td>38</td>
<td>62</td>
<td>100</td>
<td>18</td>
<td>26</td>
<td>44</td>
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<tr>
<td>Age</td>
<td>32.9 ± 11.1</td>
<td>32.6 ± 11.1</td>
<td>32.7 ± 11.0</td>
<td>30.0 ± 10.1</td>
<td>34.4 ± 9.6</td>
<td>32.6 ± 9.9</td>
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<td>Affective Ratings</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Positive Affect</td>
<td>21.6 ± 8.9</td>
<td>17.9 ± 9.7**</td>
<td>19.3 ± 9.5</td>
<td>23.4 ± 9.5</td>
<td>21.6 ± 9.6*</td>
<td>22.0 ± 9.5</td>
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<tr>
<td>Negative Affect</td>
<td>12.5 ± 10.1</td>
<td>8.7 ± 7.2**</td>
<td>10.2 ± 8.6</td>
<td>8.9 ± 5.9</td>
<td>7.9 ± 6.6*</td>
<td>8.3 ± 6.3</td>
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<td>Experimental Pain</td>
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<td>Sensory Ratings</td>
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<tr>
<td>Pain Intensity</td>
<td>37.9 ± 19.3*</td>
<td>39.9 ± 17.6*</td>
<td>39.1 ± 18.2</td>
<td>32.9 ± 13.5</td>
<td>39.0 ± 14.7**</td>
<td>35.3 ± 14.3</td>
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<tr>
<td>McGill Pain Sensory</td>
<td>15.7 ± 6.0*</td>
<td>16.0 ± 7.8*</td>
<td>15.9 ± 7.1</td>
<td>12.9 ± 5.5</td>
<td>16.4 ± 6.7**</td>
<td>14.9 ± 6.4</td>
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<td>Average 15-sec VAS</td>
<td>30.2 ± 13.0*</td>
<td>32.4 ± 14.6*</td>
<td>31.5 ± 13.9</td>
<td>27.9 ± 11.6</td>
<td>32.5 ± 14.8</td>
<td>30.6 ± 13.6</td>
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<tr>
<td>Affective Ratings</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pain Unpleasantness</td>
<td>36.0 ± 22.9*</td>
<td>46.1 ± 26.4*</td>
<td>42.0 ± 35.4</td>
<td>29.3 ± 15.5</td>
<td>42.9 ± 20.6**</td>
<td>36.9 ± 19.5</td>
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<tr>
<td>McGill Pain Affective</td>
<td>1.7 ± 2.4*</td>
<td>1.9 ± 2.3*</td>
<td>1.8 ± 2.3</td>
<td>0.7 ± 1.3</td>
<td>1.4 ± 2.0**</td>
<td>1.1 ± 1.7</td>
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<tr>
<td>ΔPANAS Positive</td>
<td>0.2 ± 3.9</td>
<td>0.3 ± 3.8*</td>
<td>0.3 ± 3.8</td>
<td>0.3 ± 3.5</td>
<td>-0.9 ± 5.8*</td>
<td>-0.3 ± 4.9</td>
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<tr>
<td>ΔPANAS Negative</td>
<td>0.2 ± 4.6</td>
<td>0.4 ± 3.1*</td>
<td>0.3 ± 3.8</td>
<td>0.4 ± 1.4</td>
<td>0.6 ± 7.0*</td>
<td>0.5 ± 5.3</td>
</tr>
</tbody>
</table>
Association of A118G with MOR BP_{ND}

AA

AG

GG

Left Nucleus Accumbens

Mu-Opioid Receptor Binding Potential

A118G
Pre-Challenge Scan (Receptor Availability): n=144

Variance ($R^2$) Accounted for by rs1799971

*q <0.05
A118G Results: Two group comparison, AA > AG + GG (p=0.05, uncorrected)

V. pallidum, amygdala, and N. accumbens significant only in women and striatum only in men
Polygenic Risk Scores

- Very little of the heritability is explained by the significant GWAS SNP
- SNPs that are non-significant contain real signal
  - Why are they not significant?
    - Very small effect sizes, stringent multiple-testing correction
- What if we want to predict the phenotype in a different sample?
  - Calculate polygenic risk scores!
PRS methods

• Used summary statistics provided by Hang Zhou from OUD meta-analysis

• Used two methods to develop PRS: PRS-CS (1 score) and clumping/thresholding with a number of p-value cut offs (9 scores)
Polygenic Risk Scores

\[ \beta_A = 0.02 \]
\[ \beta_G = -0.04 \]
\[ \beta_C = -0.05 \]
\[ \beta_T = 0.09 \]

\[ \text{PRS} = 0.04 \]
\[ \text{PRS} = 0.01 \]
Penn Medicine BioBank (PMBB)

- Provides researchers with centralized access to a large number of blood and tissue samples with attached health information.
- Facility banks blood specimens (i.e., whole blood, plasma, serum, buffy coat, and DNA isolated from leukocytes) and tissues (i.e., formalin-fixed paraffin embedded, fresh and flash frozen).
- ~60,000 individuals
- Multiple ancestries
Determining the Best PRS

- To determine best PRS, tested for association of PRS with OUD phenotype
- OUD phenotype determined by ICD-9 and -10 codes (summary table from Zhou et al.)
- ICD-9 and -10 codes restricted to subset of encounters that represent encounters with a physician
- In 52,354 PMBB individuals, 566 have at least 1 code for OUD
- In 10,182 EUR individuals with genetic data, 85 have at least 1 code for OUD
- Logistic regression model to test for association between PRS and OUD phenotype, with age, sex and PCs 1-10 as covariates
Determining the best PRS

<table>
<thead>
<tr>
<th>PRS method</th>
<th>Parameter</th>
<th>OR (95% CI)</th>
<th>P</th>
<th>AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRS-CS</td>
<td>-</td>
<td>1.34 (1.08-1.67)</td>
<td>0.0083</td>
<td>0.7042</td>
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<tr>
<td>Clumping/thresholding</td>
<td>p&lt;1x10⁻⁶</td>
<td>0.84 (0.68-1.04)</td>
<td>0.1178</td>
<td>0.69</td>
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<tr>
<td></td>
<td>p&lt;1x10⁻⁵</td>
<td>0.99 (0.80-1.23)</td>
<td>0.9212</td>
<td>0.687</td>
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<tr>
<td></td>
<td>p&lt;1x10⁻⁴</td>
<td>1.20 (0.96-1.48)</td>
<td>0.1032</td>
<td>0.6911</td>
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<tr>
<td></td>
<td>p&lt;1x10⁻³</td>
<td>1.19 (0.95-1.47)</td>
<td>0.1233</td>
<td>0.6903</td>
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<tr>
<td></td>
<td>p&lt;0.01</td>
<td>1.38 (1.11-1.72)</td>
<td>0.0032</td>
<td>0.708</td>
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<td></td>
<td>p&lt;0.05</td>
<td>1.55 (1.25-1.92)</td>
<td>(7.49\times10^{-5})</td>
<td>0.7222</td>
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<td></td>
<td>p&lt;0.1</td>
<td>1.52 (1.22-1.89)</td>
<td>0.0002</td>
<td>0.719</td>
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<tr>
<td></td>
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<td>1.51 (1.22-1.88)</td>
<td>0.0002</td>
<td>0.7149</td>
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<td>p&lt;1</td>
<td>1.50 (1.20-1.86)</td>
<td>0.0003</td>
<td>0.7143</td>
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Case Prevalence Clumping/Thresholding PRS (p<0.05)

• Split PRS into deciles
• Calculated case prevalence per decile
• Compared top 10% of PRS to rest (90%): OR=2.05 (1.17-3.57), p=0.012

<table>
<thead>
<tr>
<th>Decile</th>
<th># cases</th>
<th>Percentage</th>
</tr>
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<tr>
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<td>5</td>
<td>0.49</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>0.49</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>0.39</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>0.79</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>0.20</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>0.98</td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td>0.69</td>
</tr>
<tr>
<td>8</td>
<td>19</td>
<td>1.87</td>
</tr>
<tr>
<td>9</td>
<td>9</td>
<td>0.88</td>
</tr>
<tr>
<td>10</td>
<td>16</td>
<td>1.57</td>
</tr>
</tbody>
</table>
Pain Challenge-Induced Changes in Receptor Availability: Total Sample (n=109)

Variance ($R^2$) Accounted for by OUD SNP vs. PRS

- Cingulate
- V. Pallidum
- Amygdala
- N. Accumbens
- D. Striatum

SNP vs. PRS comparison chart showing the variance accounted for in different brain regions.
Pain Challenge-Induced Changes in Receptor Availability: Women (n=64)

Variance (R²) Accounted for by OUD SNP vs. PRS

*q <0.05
$B_{PD}$ Reflecting Endogenous Opioid Release by Sex
Left Amygdala, Scatterplot, PRS Mu-Opioid System Activation

The scatterplot illustrates the relationship between Polygenic Risk Score (PRS) and Mu-Opioid System Activation. The x-axis represents the Polygenic Risk Score (0.05), while the y-axis shows the % Change, Baseline-Pain/Baseline. The data points are color-coded by gender: blue for males and red for females. The graph shows a positive correlation between PRS and Mu-Opioid System Activation.
Conclusions

• We replicated the association of the G allele with lower MOR receptor availability during the pre-challenge scan.

• There were no significant associations of the PRS with pre-challenge receptor availability.

• In women only, during a pain stimulus (which releases endogenous opioids), the OUD PRS was significantly associated with changes in opioid system activation.

• Parallel analyses of PRS for height, chronic pain, and MDD showed no effects on receptor availability at either timepoint.

• Both the effects of the SNP and of the PRS were most evident in women, who comprised 60% of the sample.
Possible Future Directions

• Prospective replication study in patients from the PMBB who are at the extremes of OUD PRS
  • Use either the acute pain paradigm or a pharmacological challenge such as amphetamine to activate the opioid system

• Evaluate effects in detoxified, opioid-free OUD patients