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

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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.

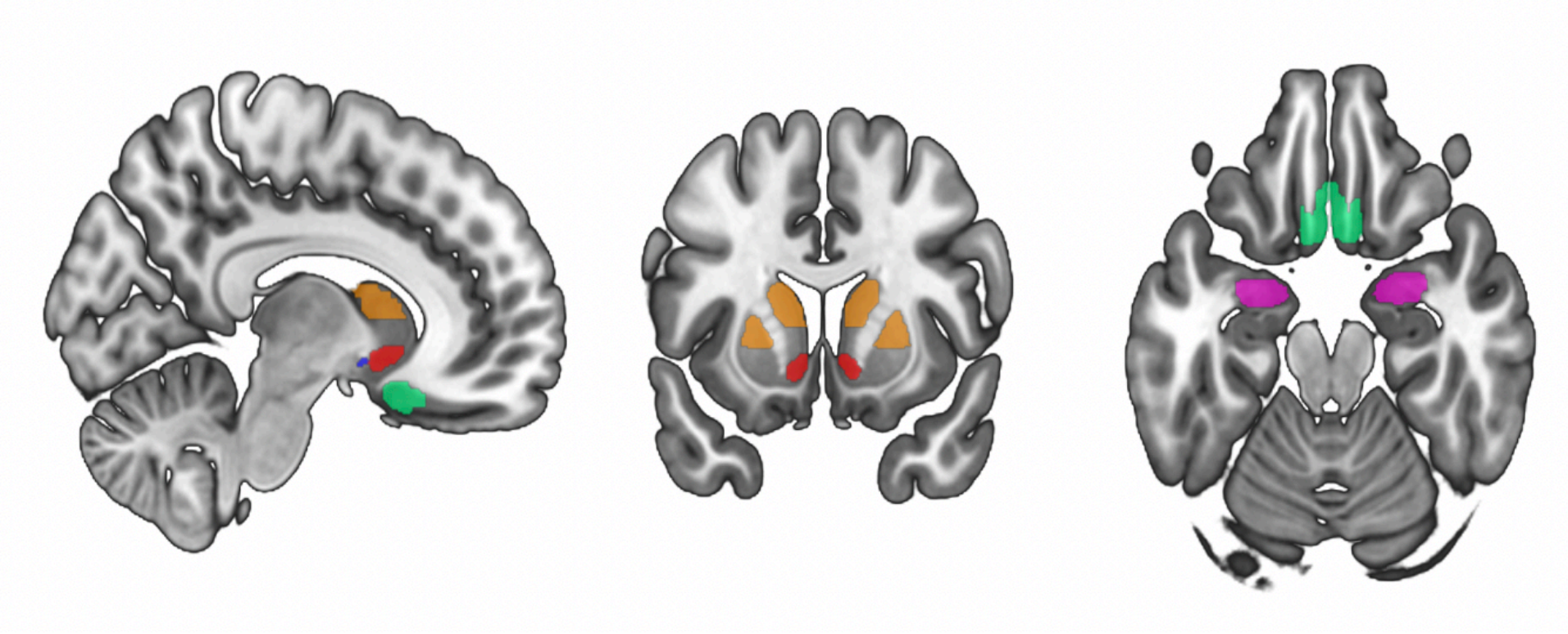
Genetic Analysis

- 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

Zhou H, Rentsch CT, Cheng Z, Kember RL, Nunez YZ, Sherva RM, Tate JP, Dao C, Xu K, Polimanti R, Farrer LA, Justice AC, Kranzler HR, Gelernter J; Veterans Affairs Million Veteran Program

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

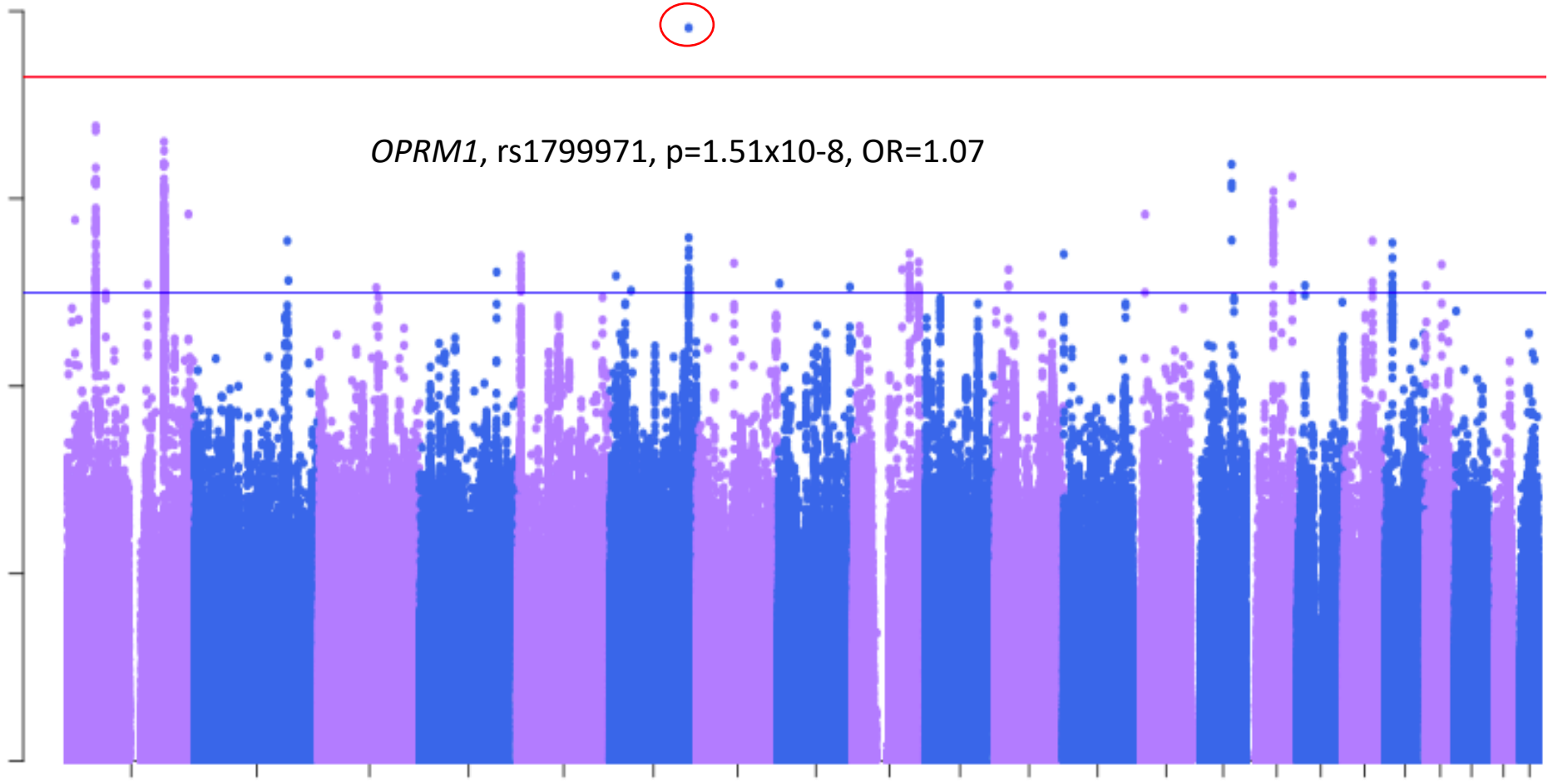
Results

- A functional coding variant (rs1799971, encoding Asn40Asp) in *OPRM1* (mu- opioid receptor gene, the main biological target for opioid drugs) was genome-wide significant (p=1.51x10-8) in the European-ancestry sample.
- Replicated in two independent samples
- Final meta-analysis p-value for this variant in all samples was 7.81×10^{-10}

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



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

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   1 cggatgagcc tctgtgaact actaaggtgg gaggggggcta tacgcagagg agaatgtcag
  61 atgctcagct cggtcccctc cgcctgacgc tcctctctgt ctcagccagg actggtttct
 121 gtaagaaaca gcaggagctg tggcagcggc gaaaggaagc ggctgaggcg cttggaaccc
 181 gaaaagtctc ggtgctcctg gctacctcgc acagcgtgcc cgcccggccg tcagtaccat
 241 ggacagcagc gctgccccca cgaacgccag caattgcact gatgccttgg cgtactcaag
 301 ttgctcccca gcacccagcc ccggttcctg ggtcaacttg tcccacttag atcgcaa
 361 gtccgaccca tgcggtccga accgcaccga cctgggcggg agagacagcc tgtg
 421 gaccggcagt ccctccatga tcacggccat cacgatcatg gccctctact ccatcgtgtg
 481 cgtggtgggg ctcttcggaa acttcctggt catgtatgtg attgtcagat acaccaagat
 541 gaagactgcc accaacatct acattttcaa ccttgctctg gcagatgcct tagccaccag
 601 taccctgccc ttccagagtg tgaattacct aatgggaaca tggccatttg gaaccatcct
 661 ttgcaagata gtgatctcca tagattacta taacatgttc accagcatat tcaccctctg
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1381 agtggataga actaatcatc agctagaaaa tctggaagca gaaactgctc cgttgcccta
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1501 aggttgcttc aagaatgtgt aggaggctct aattctctag gaaagtgcct gcttttaggt
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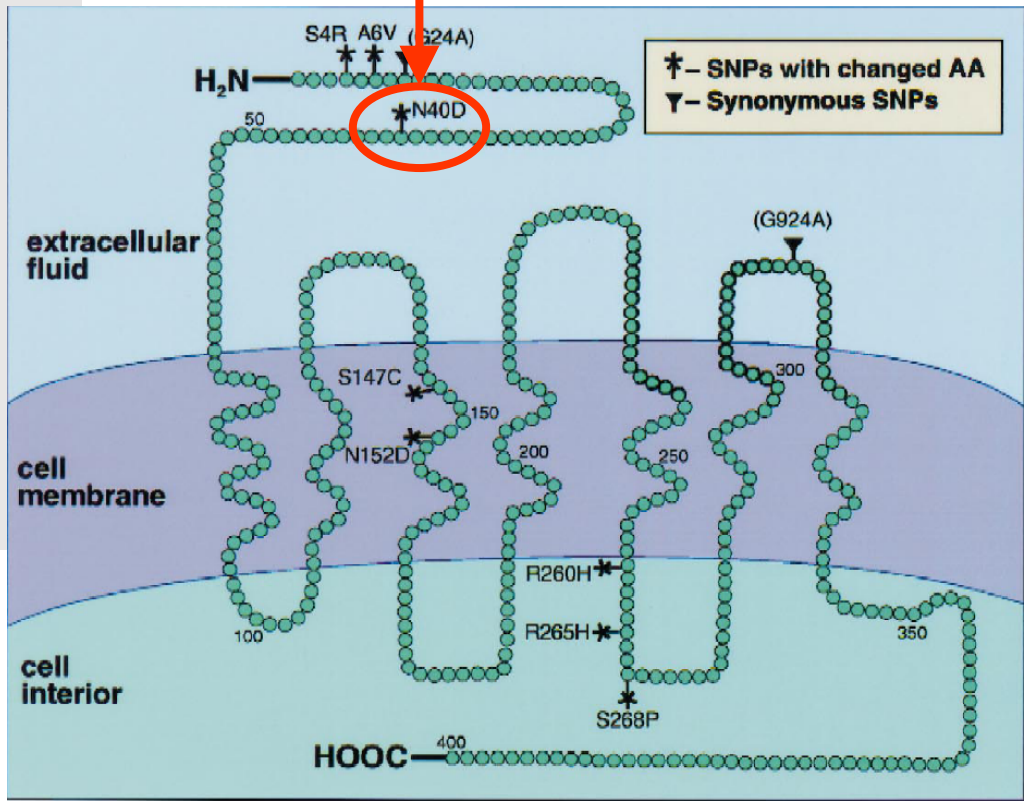


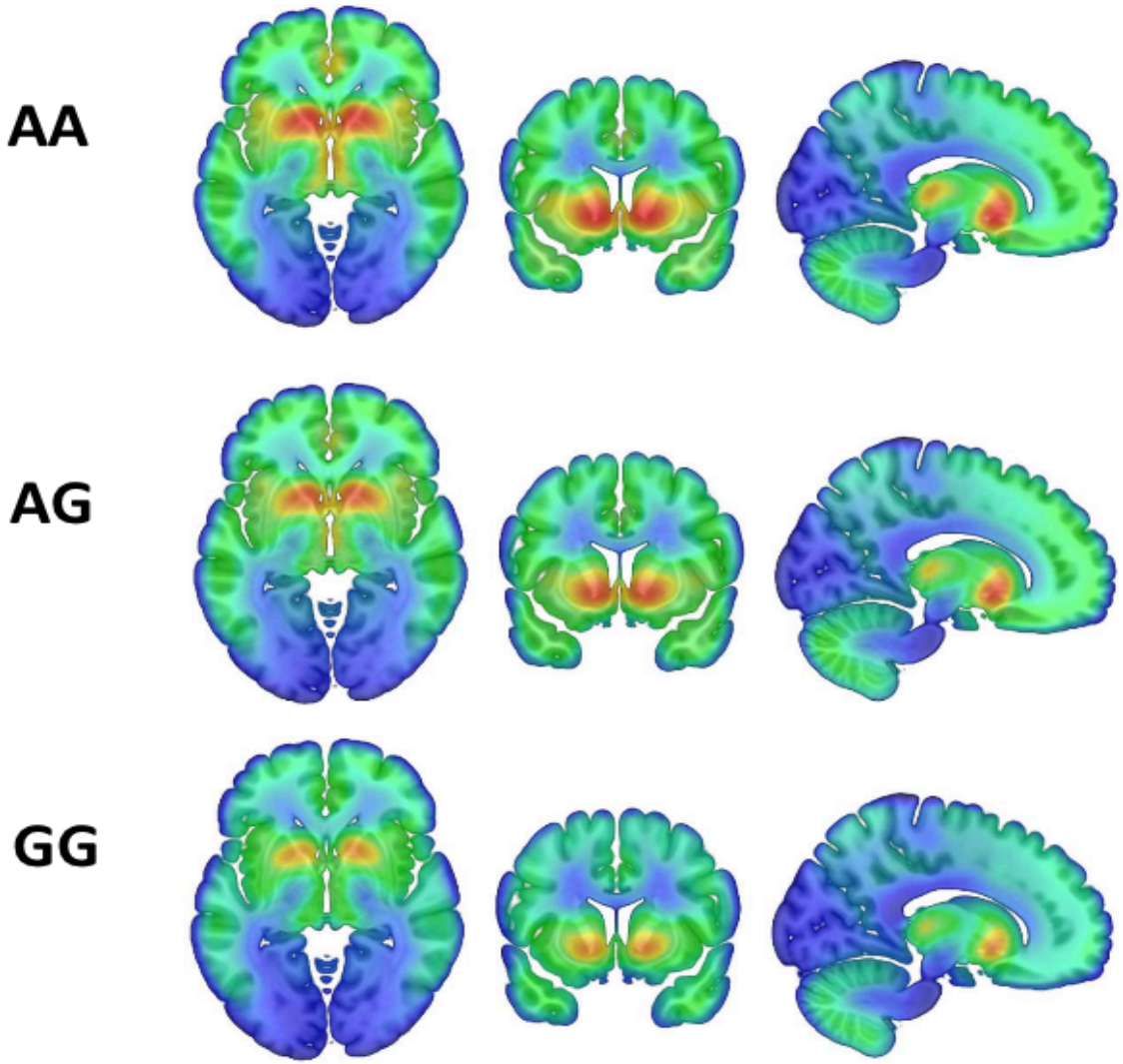
Diagnoses by A118G Genotype

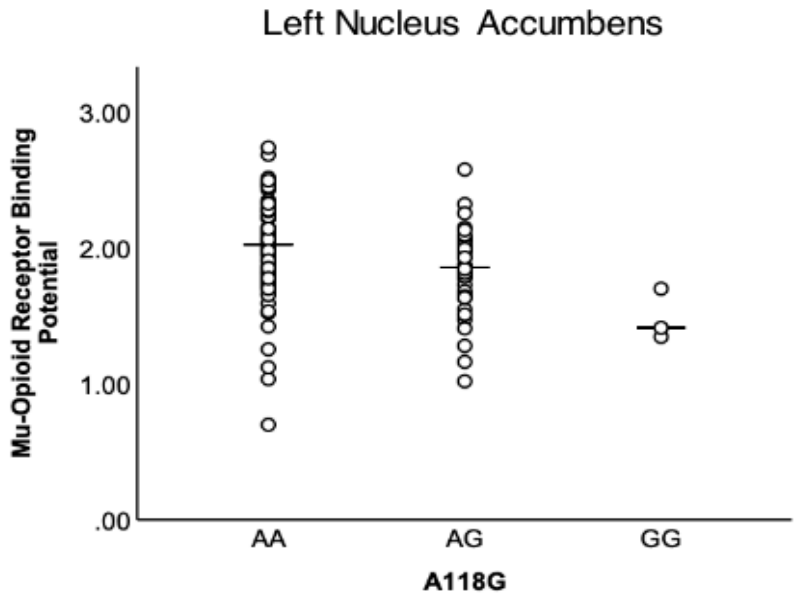
DIAGNOSIS	AA (n=100)	AG/GG (n=44)	TOTAL (n=144)
Controls (No Diagnosis)	50	24	74
Mood Disorder	20	8	28
Anxiety Disorder	13	2	15
Personality Disorder	8	0	8
Eating Disorder	0	1	1
Any Axis I or II Disorder	29	10	39
Chronic Non-Specific Back Pain	24	13	37
Any Chronic Pain, Axis I or II Disorder	50	20	70

Baseline Characteristics and psychophysiological responses during pain.

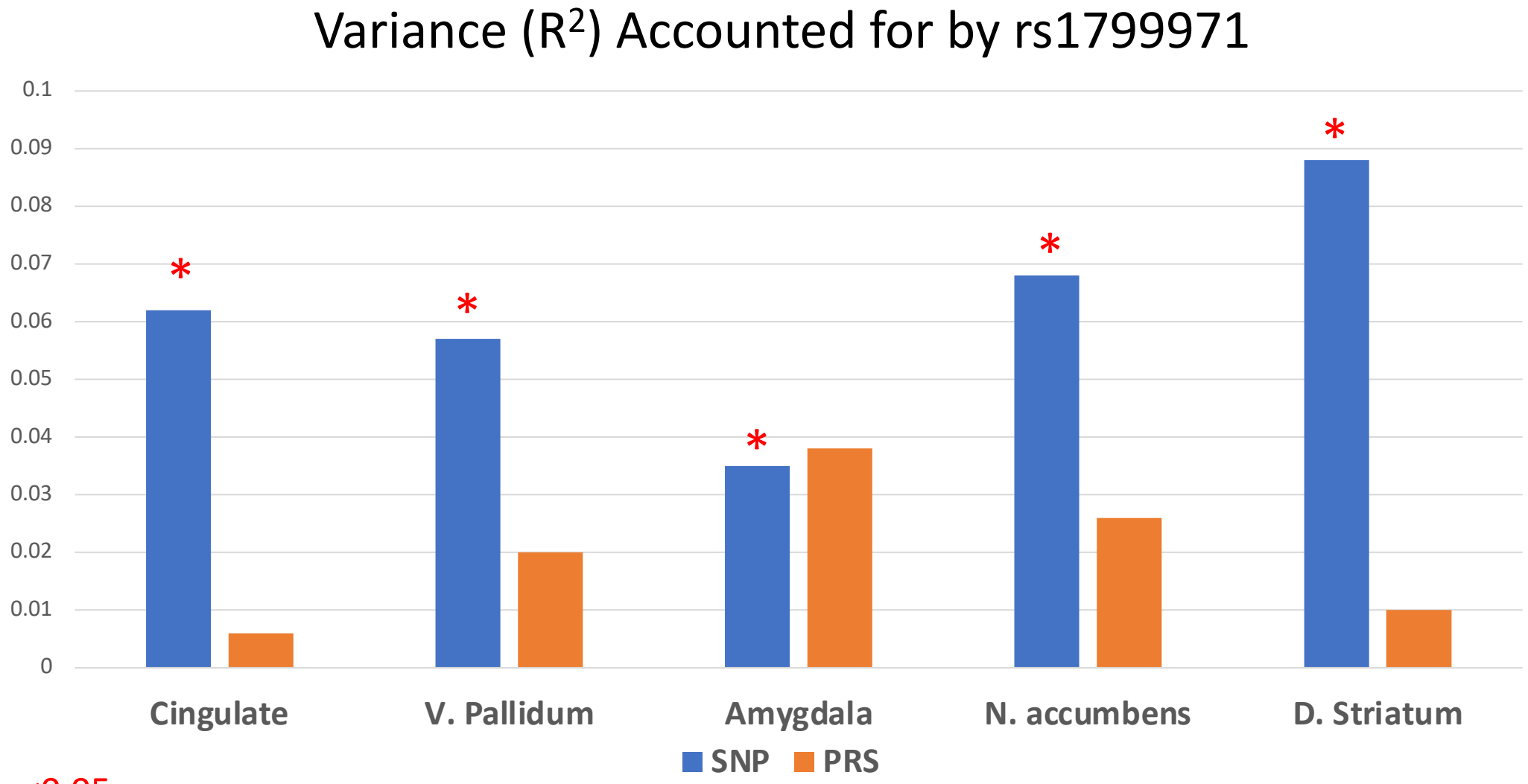
	AA			AG/GG				
	Males (n=30)	Females (n=43)	Total (n=73)	Males (n=15)	Females (n=21)	Total (n=36)	*F*	*p*
Baseline								
N	38	62	100	18	26	44	-	-
Age	32.9 ± 11.1	32.6 ± 11.1	32.7 ± 11.0	30.0 ± 10.1	34.4 ± 9.6	32.6 ± 9.9	0.59	0.62
Affective Ratings								
Positive Affect	21.6 ± 8.9	17.9 ± 9.7**	19.3 ± 9.5	23.4 ± 9.5	21.0 ± 9.6*	22.0 ± 9.5	2.21	0.09
Negative Affect	12.5 ± 10.1	8.7 ± 7.2**	10.2 ± 8.6	8.9 ± 5.9	7.9 ± 6.6*	8.3 ± 6.3	2.47	0.07
Experimental Pain								
Sensory Ratings								
Pain Intensity	37.9 ± 19.3*	39.9 ± 17.6*	39.1 ± 18.2	32.9 ± 13.5	39.0 ± 14.7**	35.3 ± 14.3	0.64	0.59
McGill Pain Sensory	15.7 ± 6.0*	16.0 ± 7.8*	15.9 ± 7.1	12.9 ± 5.5	16.4 ± 6.7**	14.9 ± 6.4	0.88	0.45
Average 15-sec VAS	30.2 ± 13.0*	32.4 ± 14.6*	31.5 ± 13.9	27.9 ± 11.6	32.5 ± 14.8	30.6 ± 13.6	0.51	0.68
Affective Ratings								
Pain Unpleasantness	36.0 ± 22.9*	46.1 ± 26.4*	42.0 ± 35.4	29.3 ± 15.5	42.9 ± 20.6**	36.9 ± 19.5	2.39	0.07
McGill Pain Affective	1.7 ± 2.4*	1.9 ± 2.3*	1.8 ± 2.3	0.7 ± 1.3	1.4 ± 2.0**	1.1 ± 1.7	1.29	0.28
ΔPANAS Positive	0.2 ± 3.9	0.3 ± 3.8*	0.3 ± 3.8	0.3 ± 3.5	-0.9 ± 5.8*	-0.3 ± 4.9	0.39	0.76
ΔPANAS Negative	0.2 ± 4.6	0.4 ± 3.1*	0.3 ± 3.8	0.4 ± 1.4	0.6 ± 7.0*	0.5 ± 5.3	0.03	0.99

Association of A118G with MOR BP_{ND}





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



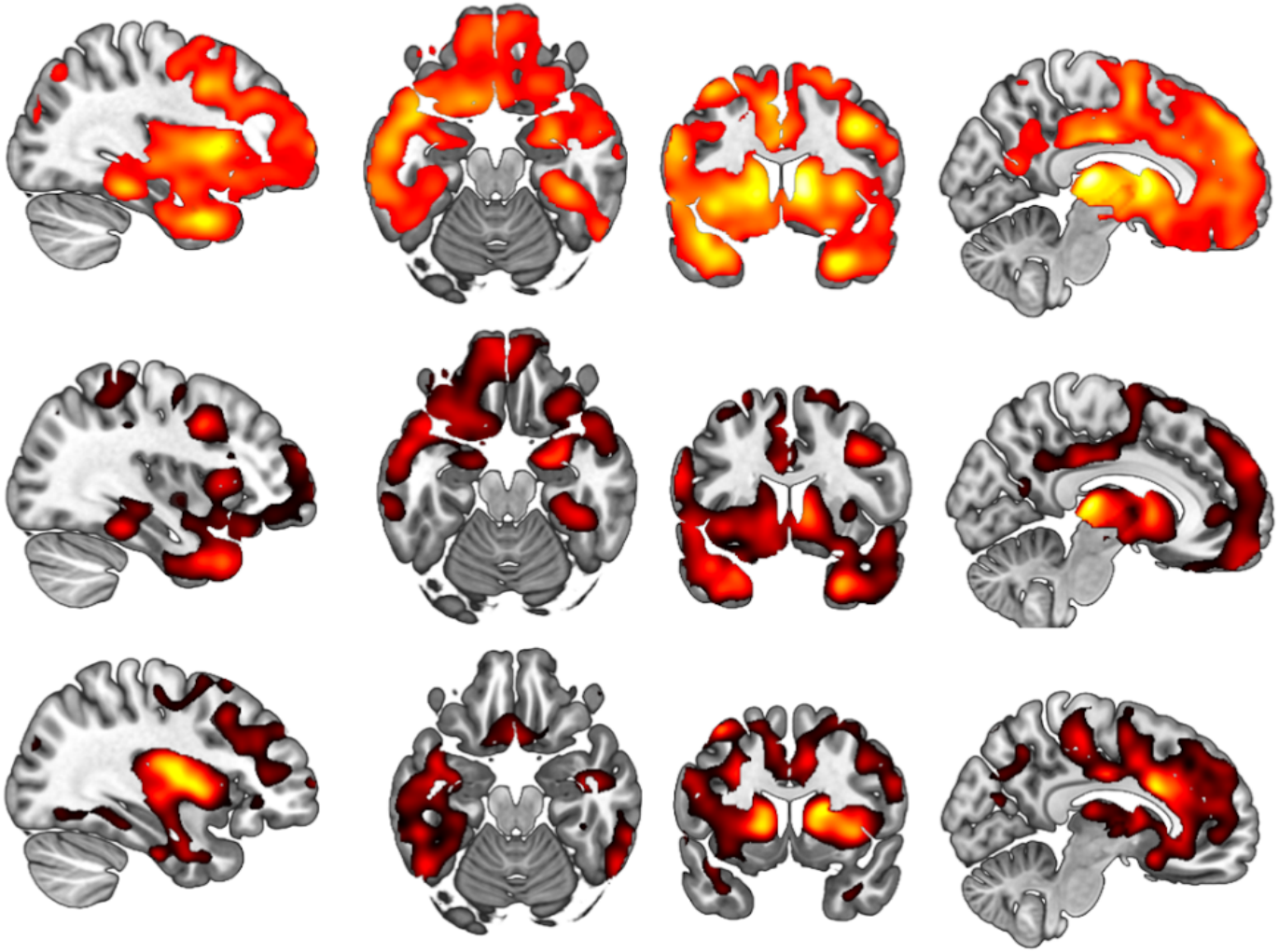
*q <0.05

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

All Subjects

Women

Men



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

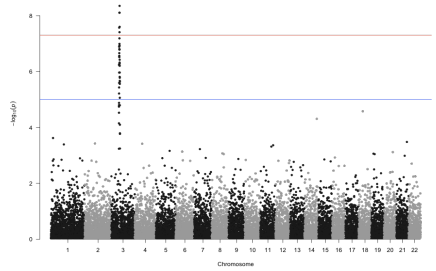
Polygenic Risk Scores

- 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

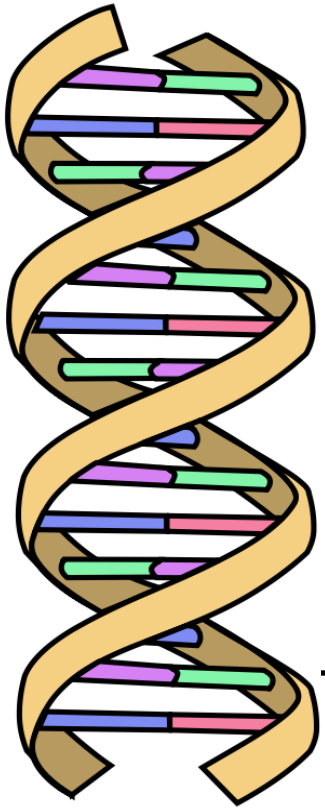


β_A=0.02 ⟵ ⟶ AA 2x.02 + AG 1x.02 +

β_G =-0.04 ⟵ ⟶ GT 1x-.04 + TT 0x-.04 +

β_C =-0.05 ⟵ ⟶ CG 1x-.05 + CC 2x-.05 +

β_T =0.09 ⟵ ⟶ AT 1x.09 + AA 1x.09



PRS = 0.04 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

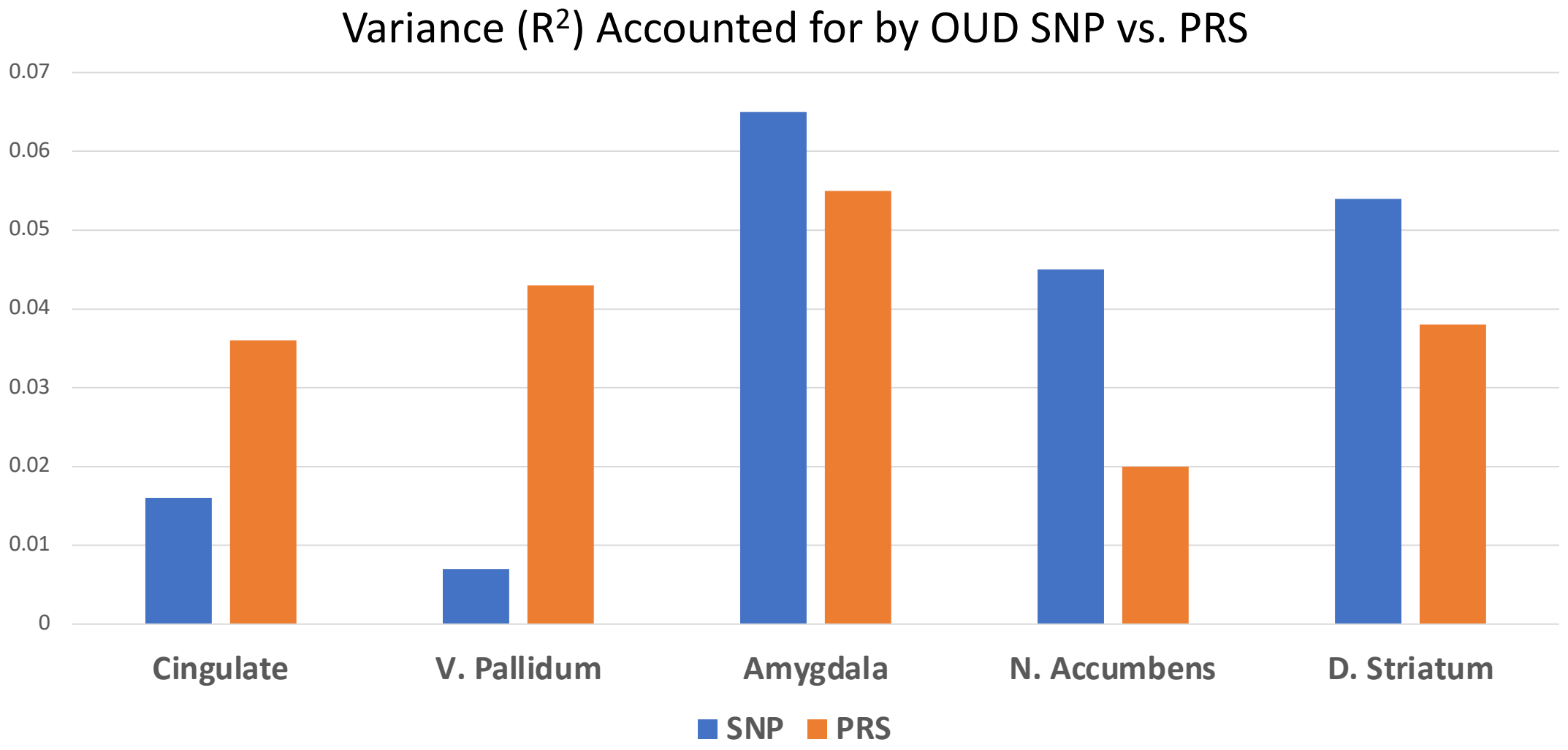
PRS method	Parameter	OR (95% CI)	P	AUC
PRS-CS	-	1.34 (1.08-1.67)	0.0083	0.7042
Clumping/thresholding	$p<1x10^{-6}$	0.84 (0.68-1.04)	0.1178	0.69
	$p<1x10^{-5}$	0.99 (0.80-1.23)	0.9212	0.687
	$p<1x10^{-4}$	1.20 (0.96-1.48)	0.1032	0.6911
	$p<1x10^{-3}$	1.19 (0.95-1.47)	0.1233	0.6903
	p<0.01	1.38 (1.11-1.72)	0.0032	0.708
	p<0.05	1.55 (1.25-1.92)	$7.49x10^{-5}$	0.7222
	p<0.1	1.52 (1.22-1.89)	0.0002	0.719
	p<0.5	1.51 (1.22-1.88)	0.0002	0.7149
	p<1	1.50 (1.20-1.86)	0.0003	0.7143

Case Prevalence Clumping/Thresholding PRS (p<0.05)

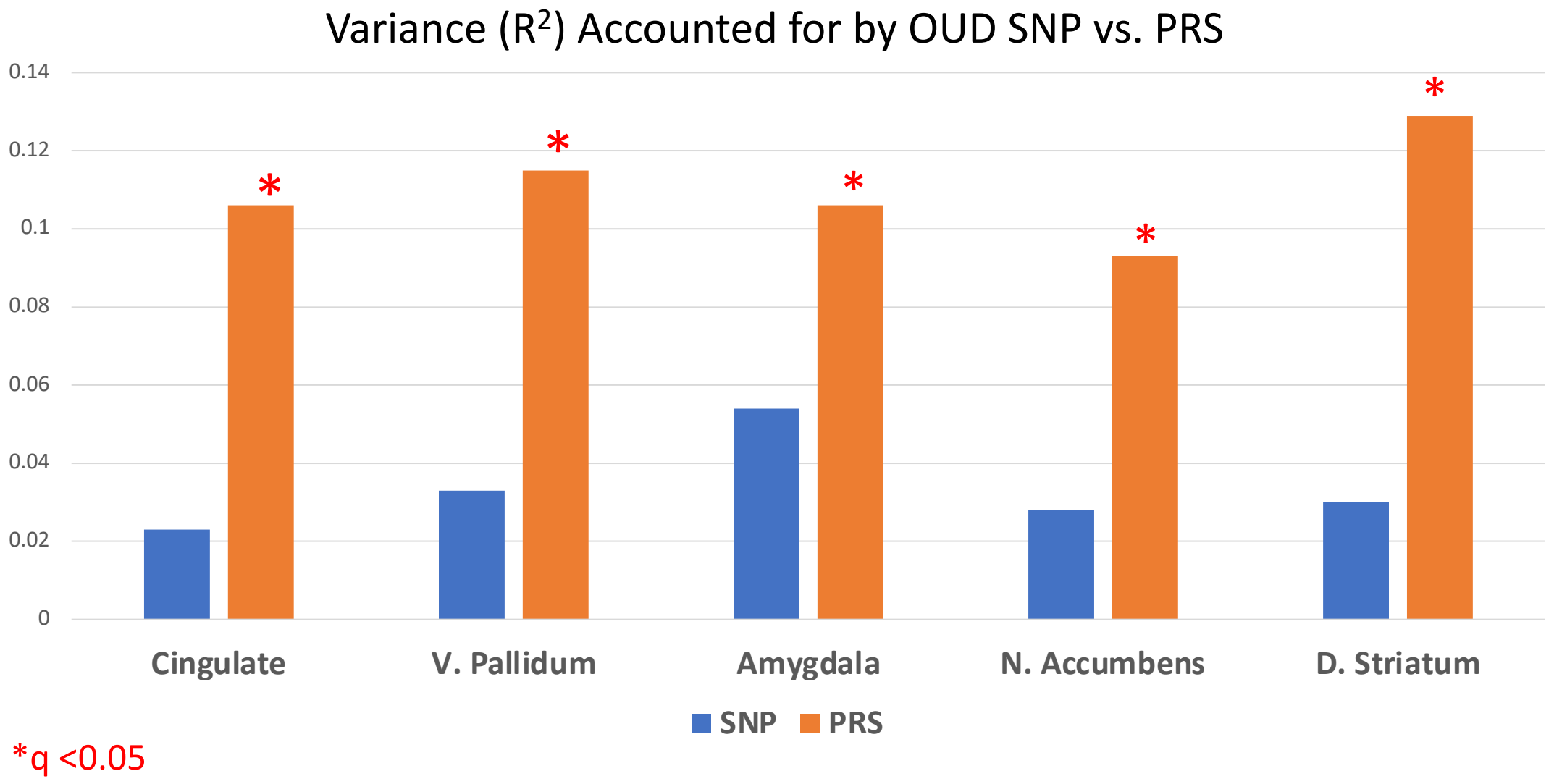
- Split PRS into deciles
- Calculated case prevalence per decile
- Compared top 10% of PRS to rest (90%): OR=2.05 (1.17-3.57), p=0.012

Decile	# cases	Percentage
1	5	0.49
2	5	0.49
3	4	0.39
4	8	0.79
5	2	0.20
6	10	0.98
7	7	0.69
8	19	1.87
9	9	0.88
10	16	1.57

Pain Challenge-Induced Changes in Receptor Availability: Total Sample (n=109)

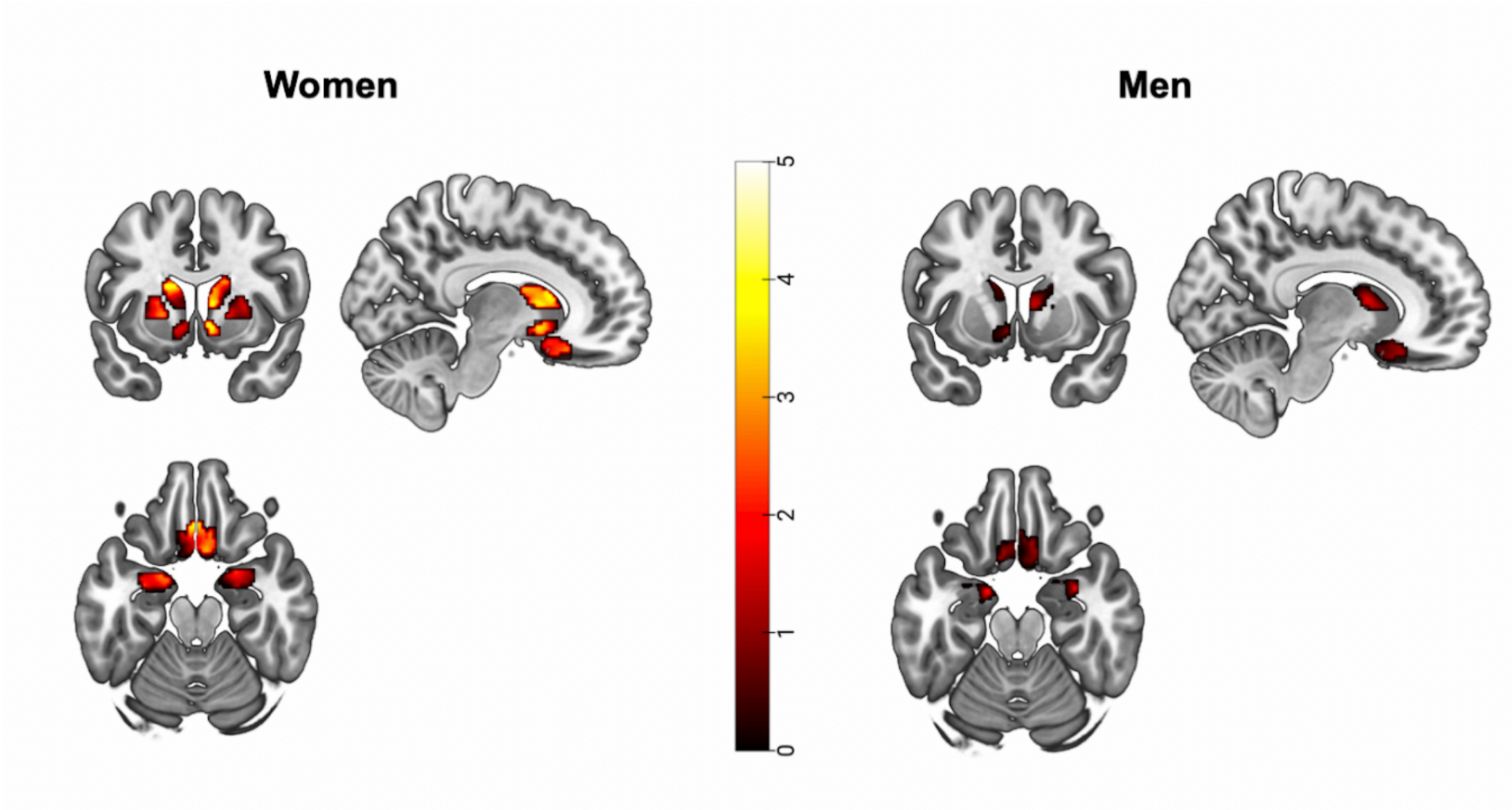


Pain Challenge-Induced Changes in Receptor Availability: Women (n=64)

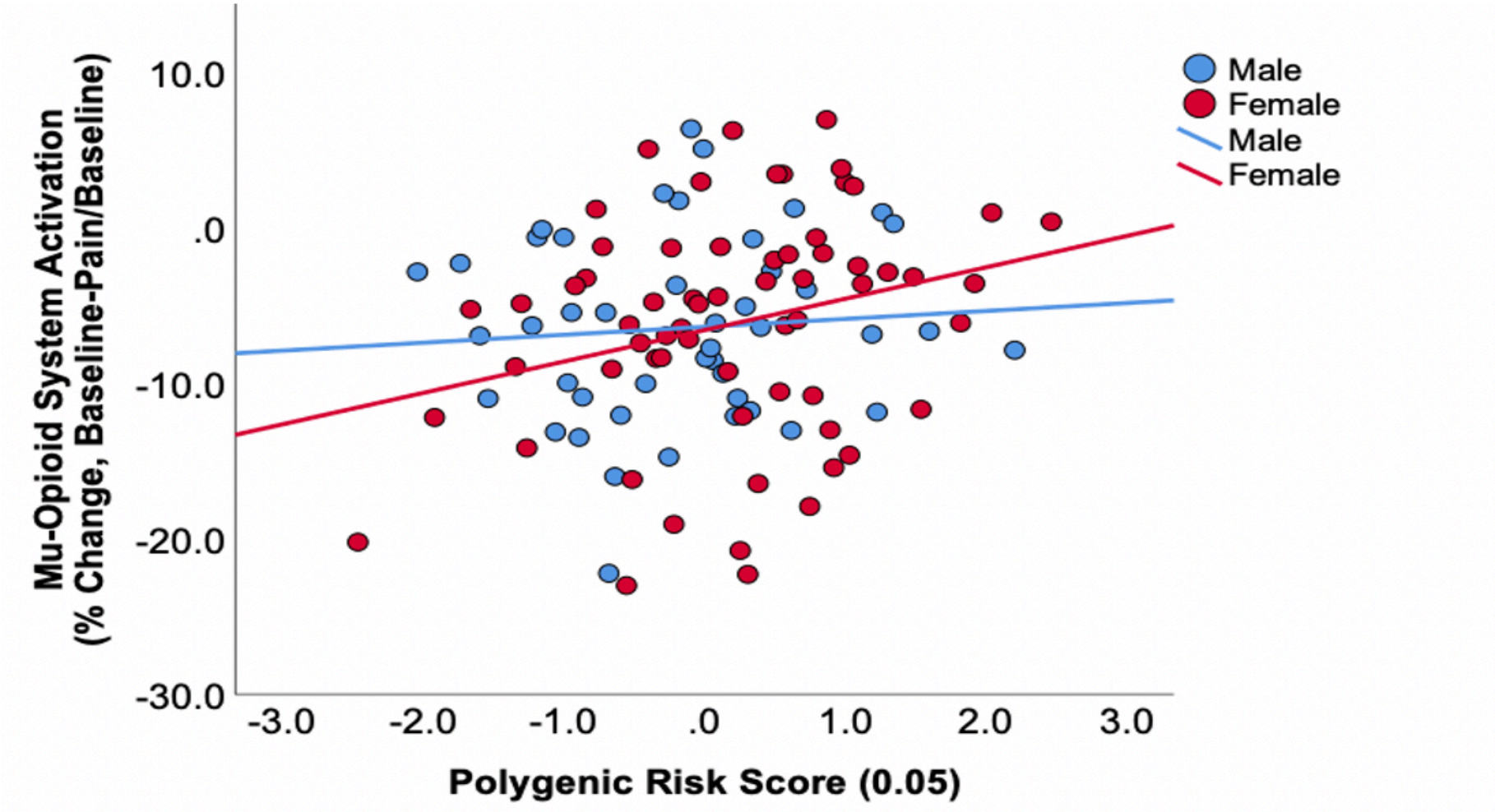


*q <0.05

BP_{ND} Reflecting Endogenous Opioid Release by Sex



Left Amygdala, Scatterplot, PRS 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