Genetic Approaches to Alcoholism, Alcohol Abuse Susceptibility, and Therapeutic Response

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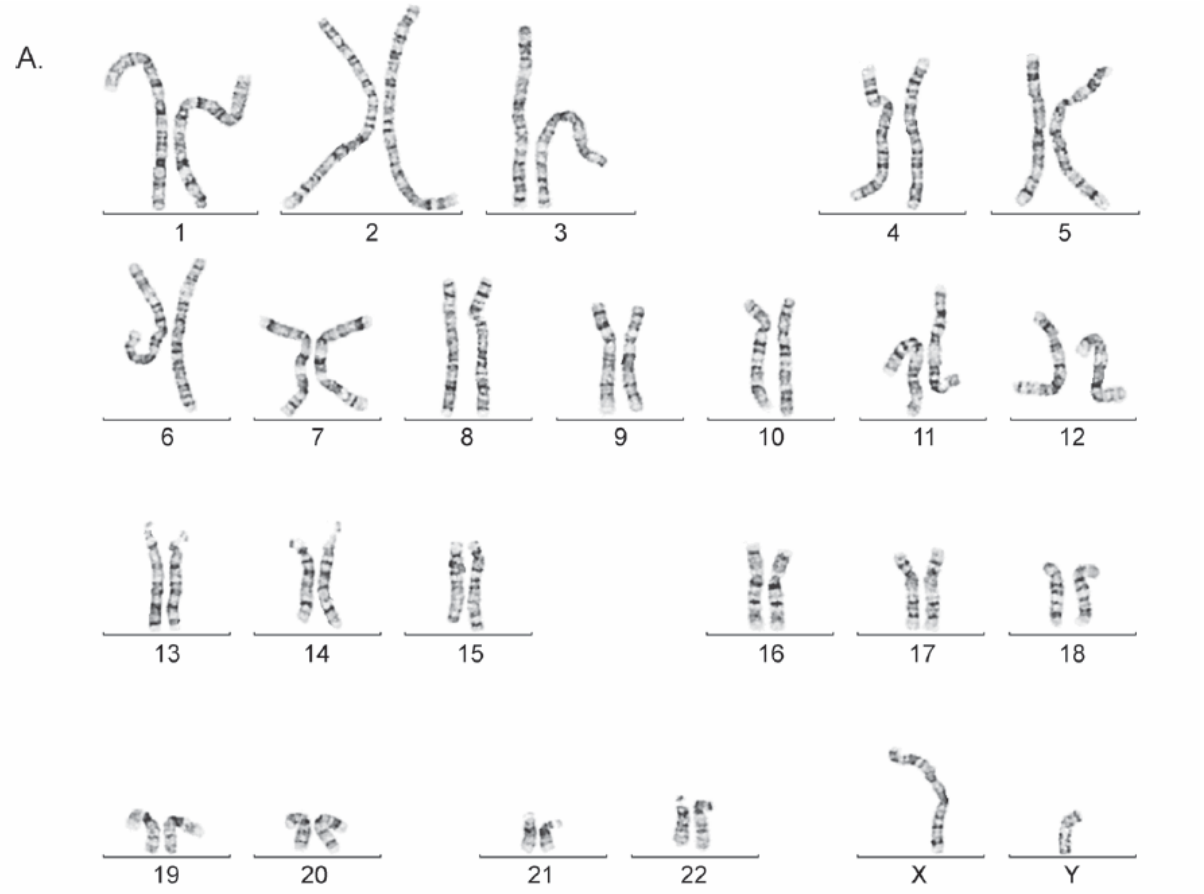
Craig Venter Genome

2,810,000,000 bases, 7.5-fold coverage

- 3,213,401 SNPs
  - 85% in dbSNP
- 58,823 block substitutions
- 292,102 heterozygous indels
- 559,473 homozygous indels
- numerous Copy Number Variants



Levy S, Sutton G, Ng PC, Feuk L, Halpern AL, et al. (2007) PLoS Biol 5(10): e254 doi:10.1371/journal.pbio.0050254

Approach to Variant Discovery

- Intermediate clinical phenotypes: levels of response to alcohol challenge
  - San Diego cohorts with Marc Schuckit
  - Clinically characterized for acute response to alcohol challenge
    - Level of “high”
    - Body Sway
    - Questionnare-based history of acute responses
- Two cohorts
  - Original prospective study
    - 453 probands (plus spouses, children)
    - 1/2 with family history, 1/2 without
    - Followed for >25 years
  - Sib pairs
    - 511 siblings (all family history+)
    - 323 parents
    - 143 extended family members

Approach to Variant Discovery

- Initial genetic markers come from *candidate genes*
  - Animal models: Worms, flies, and mice
  - Map location
  - Biochemistry of cellular responses to alcohol
- 150 genes selected for initial exploration
- Exons sequenced in sib pairs to identify both common and rare variants
  - Common variants to show association with modest increase in relative risk of disease (1.15 - 1.4 fold)
  - Rare variants expected to show higher levels of relative risk (2 - 5 fold)

Sequencing in Clinically Characterized Cohorts

- 145 genes resequenced in half or all San Diego Sibs
  - Coding region + flanking sequence
  - If no signal from first half, second half not sequenced
- Approximately 1 million bases assayed
  - Good heterozygote detection (PHRED 40 - 50)
- 90% of coding sequence examined
- 2440 SNPs identified

Alleles per Individual

- Average subject carries 157 alleles (variants) of the *candidate genes*
- 91% of these alleles are **common** SNPs, 9% are **rare** SNPs
- Average subject carries 14 rare SNPS among *candidate genes*
- Average of 143 common SNPs among *candidate genes*
- Average subject has at least one **non-synonymous** SNP in 13% of *candidate genes*
- Thus, total **non-synonymous** SNPs per subject = ~2600

Overall Association Statistics in Discovery Set

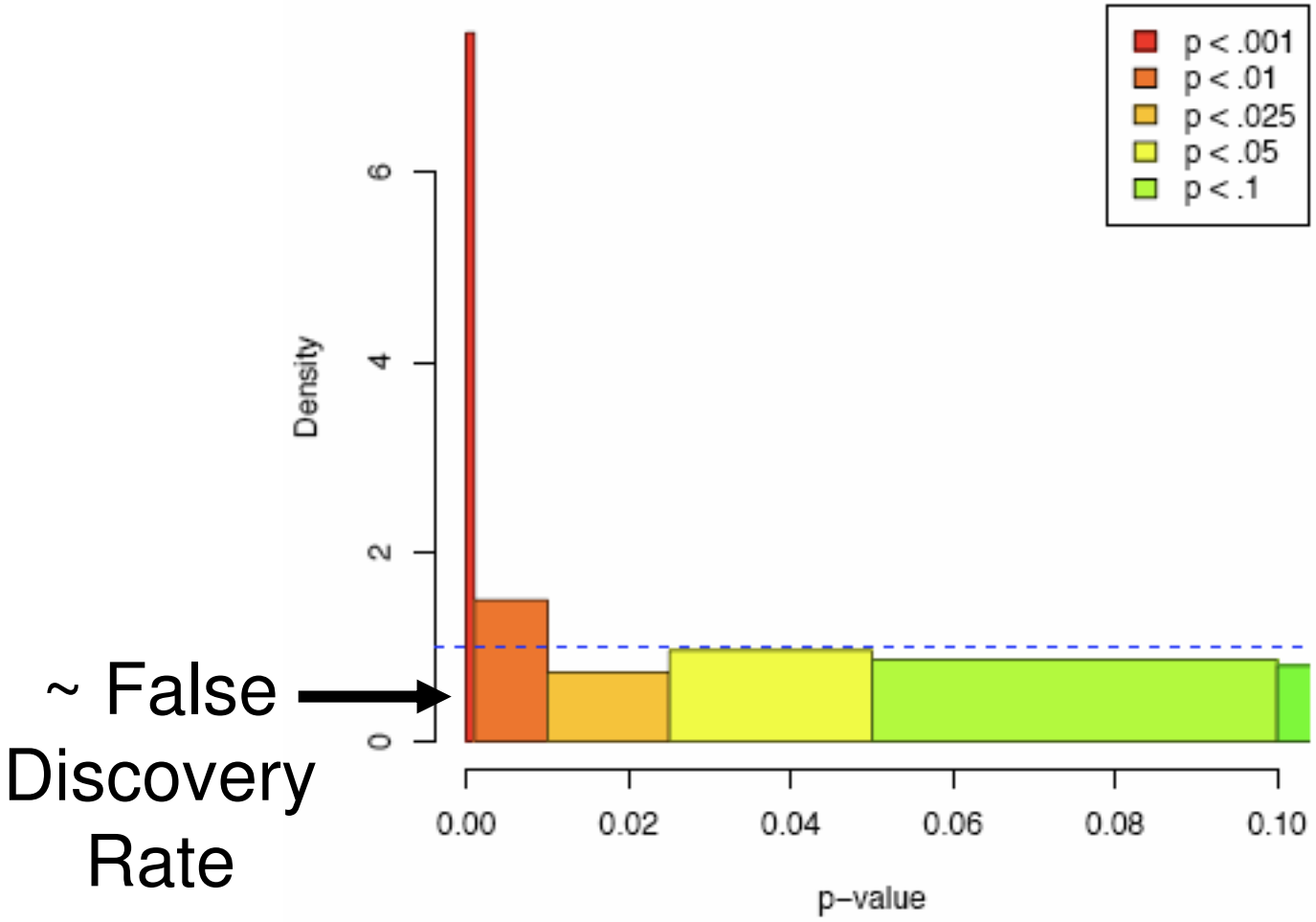
- Full set of siblings from San Diego Sib-Pair cohort
- No specific genetic model in analysis
- Statistical tests include hom-hom genotype comparisons, het-hom comparison, and marker effect
- Tibshirani Q-values used to evaluate proportion of False discoveries
  - Controls for multiple sampling

Tibshirani Q-Values

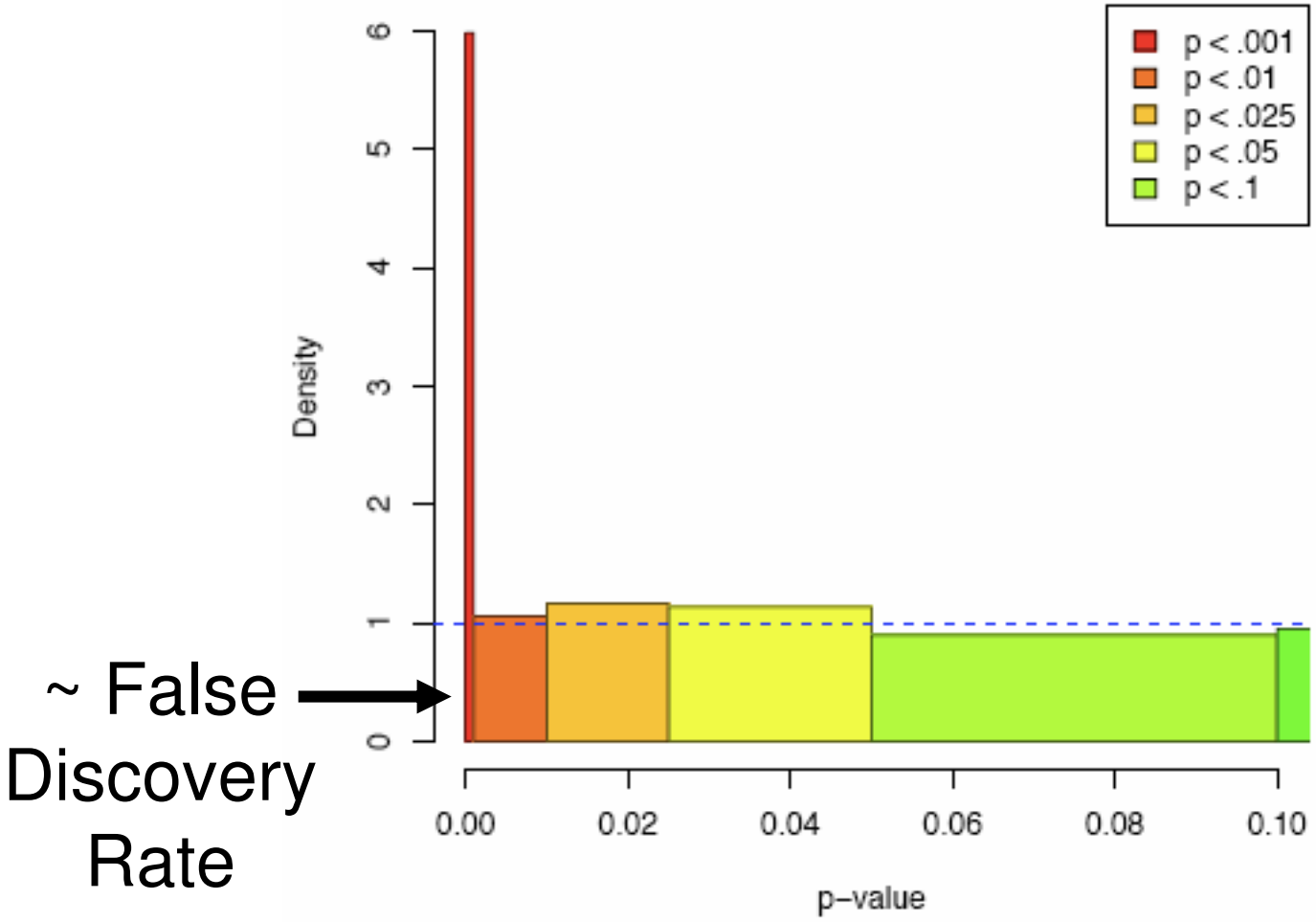
- The q value is a measure in terms of the False Discovery Rate (FDR).
- For example, a false positive rate of 5% means that on average 5% of the truly null features in the study will be called significant.
- A FDR of 5%means that among all features called significant, 5%of these are truly null on average.
- (*Storey and Tibshirani, PNAS 2003 100, 9443)*

- *ALLOWS US TO CALCULATE PROPORTION OF FALSE DISCOVERIES FOR EACH P-VALUE*

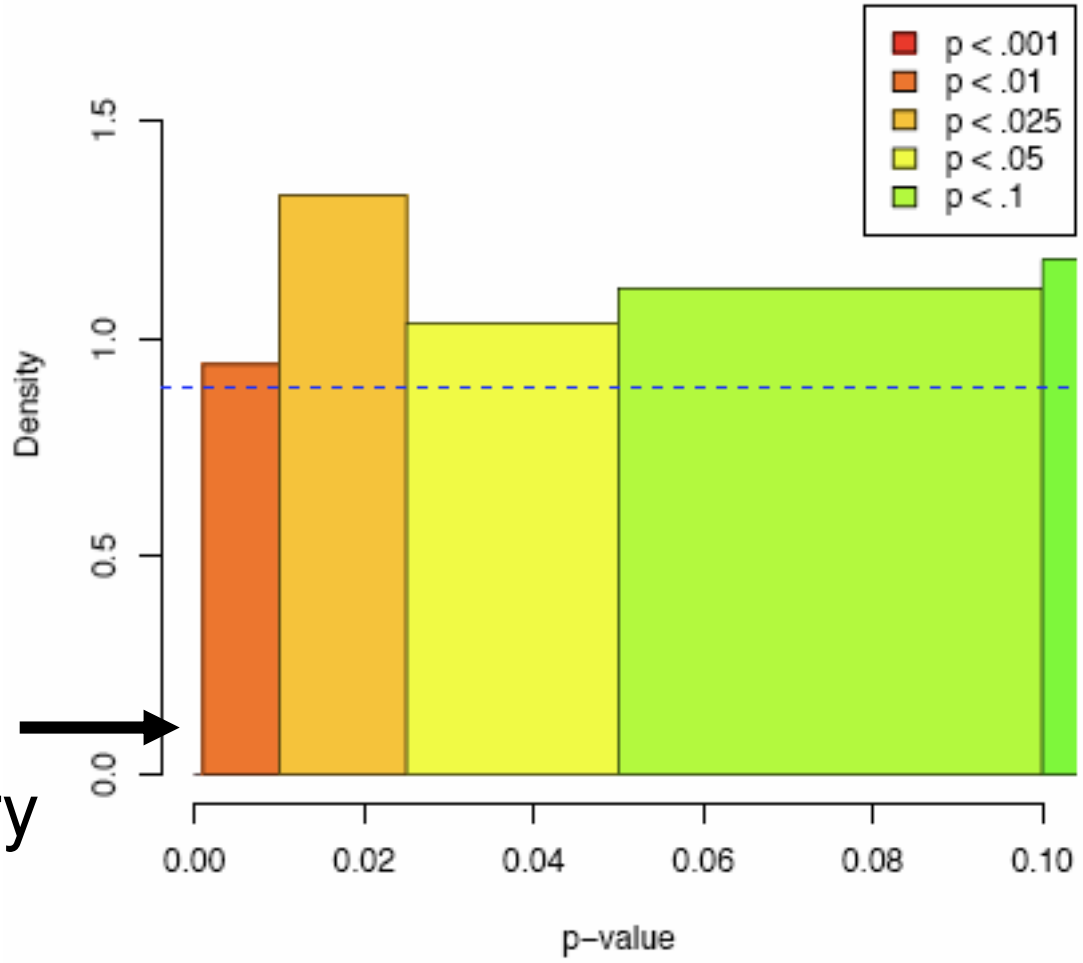
False Discovery Estimates
Body Sway A/P



False Discovery Estimates
Body Sway Lateral

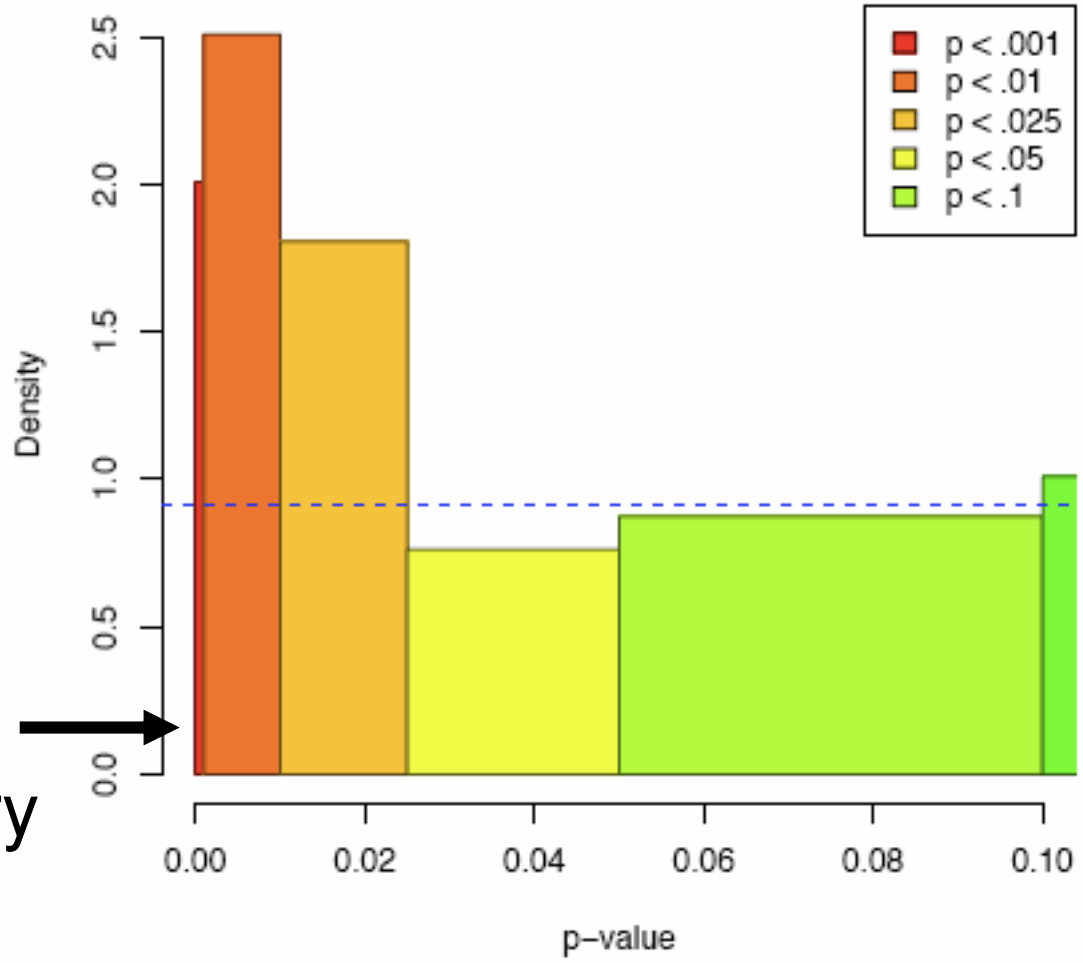


False Discovery Estimates
SHAS



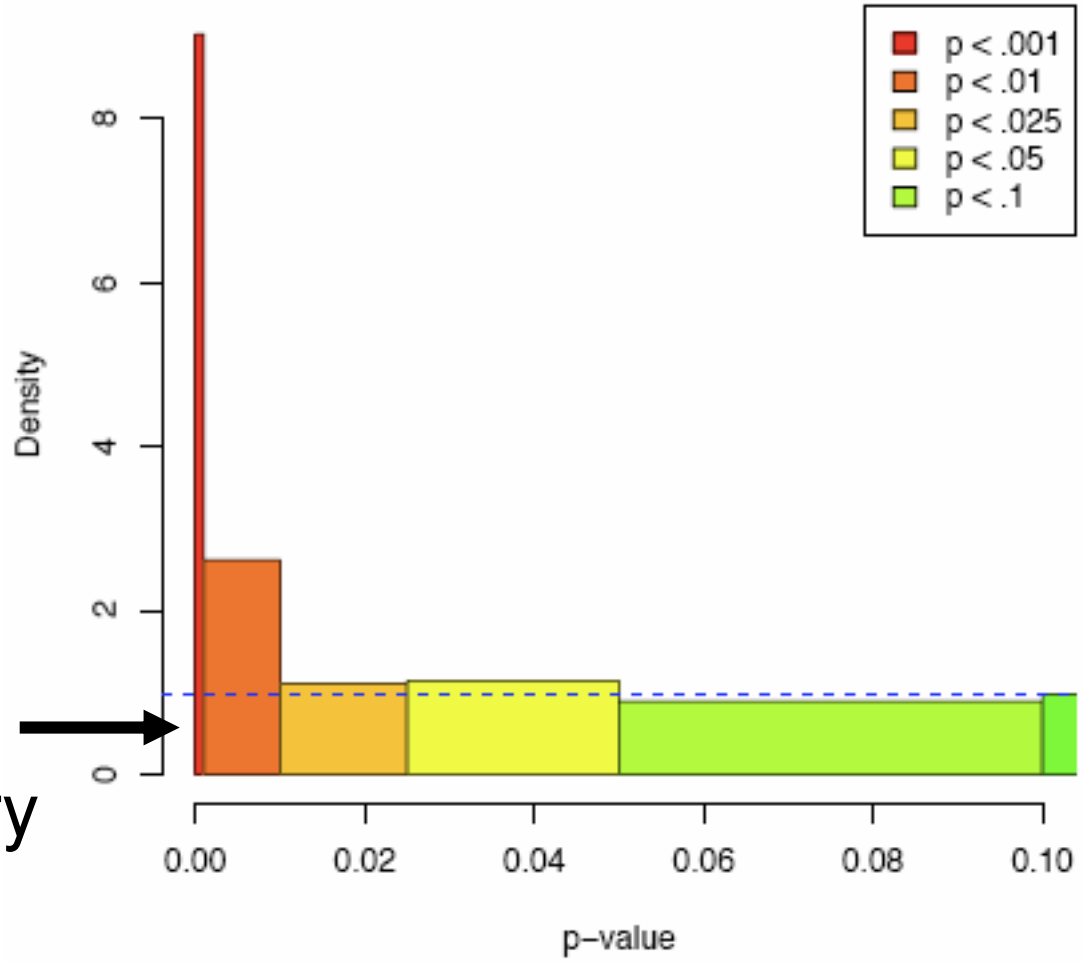
~ False Discovery Rate

False Discovery Estimates
SRE First



~ False Discovery Rate

False Discovery Estimates
SRE Total



~ False Discovery Rate

Statistical Summary
Rare vs Common Variants

- Lateral and anterior-posterior Body Sway show significant associations only with rare variants
- Self reported experience and self reported “high” by acute challenge both have a mix of rare and common variants
- Is Body Sway a less complex phenotype, closer to primary biology?

Whole Genome Scans

- Whole genome scans can discover genes with common alleles of low relative risk
- We have, therefore, looked at the San Diego Sibs and Prospective probands (370,000 SNP Illumina) as well as a Costa Rican population (50,000 SNP Affymetrix) are each showing multiple SNPs with strong associations

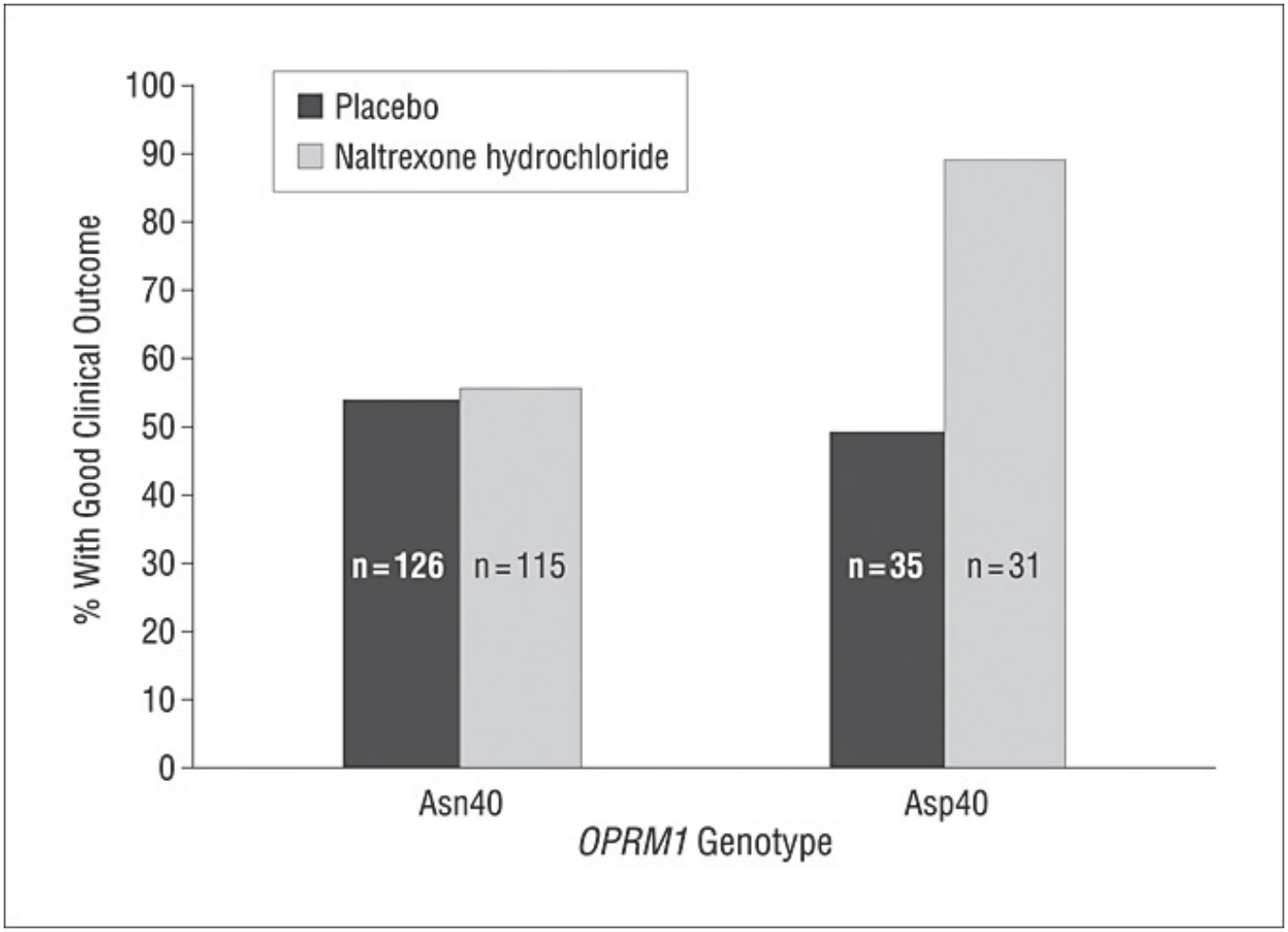
Whole Genome Scans

- As with Genes and Environment, multiple Rare SNPs versus Common SNPs **not *either/or*** - but **rather *both/and***
- In summary, it appears that multiple, low frequency SNPs will be important in primary biology
- Furthermore, it also appears that the SNPs are distributed over many genes
- Because of relatively high concordance (25%) of fraternal (as opposed to identical at 50%) twins, likely that only a few (1-3) of the rare variants may be necessary for individual susceptibility

Therapeutic Response

- Identification of genetic components of therapeutic responses
- For example with Naltrexone, which targets morphine receptor, and is approved for treatment of alcohol abuse
- Arg40asp variant of morphine receptor gene suggests clinical difference in response to naltrexone therapy

Good clinical outcome based on OPRM1 and medication group in those receiving medical management alone (no combined behavioral intervention) (test of genotype x medication interaction, P = .005)



Anton, R. F. et al. Arch Gen Psychiatry 2008;65:135-144.

ARCHIVES OF
GENERAL PSYCHIATRY

Fig 1

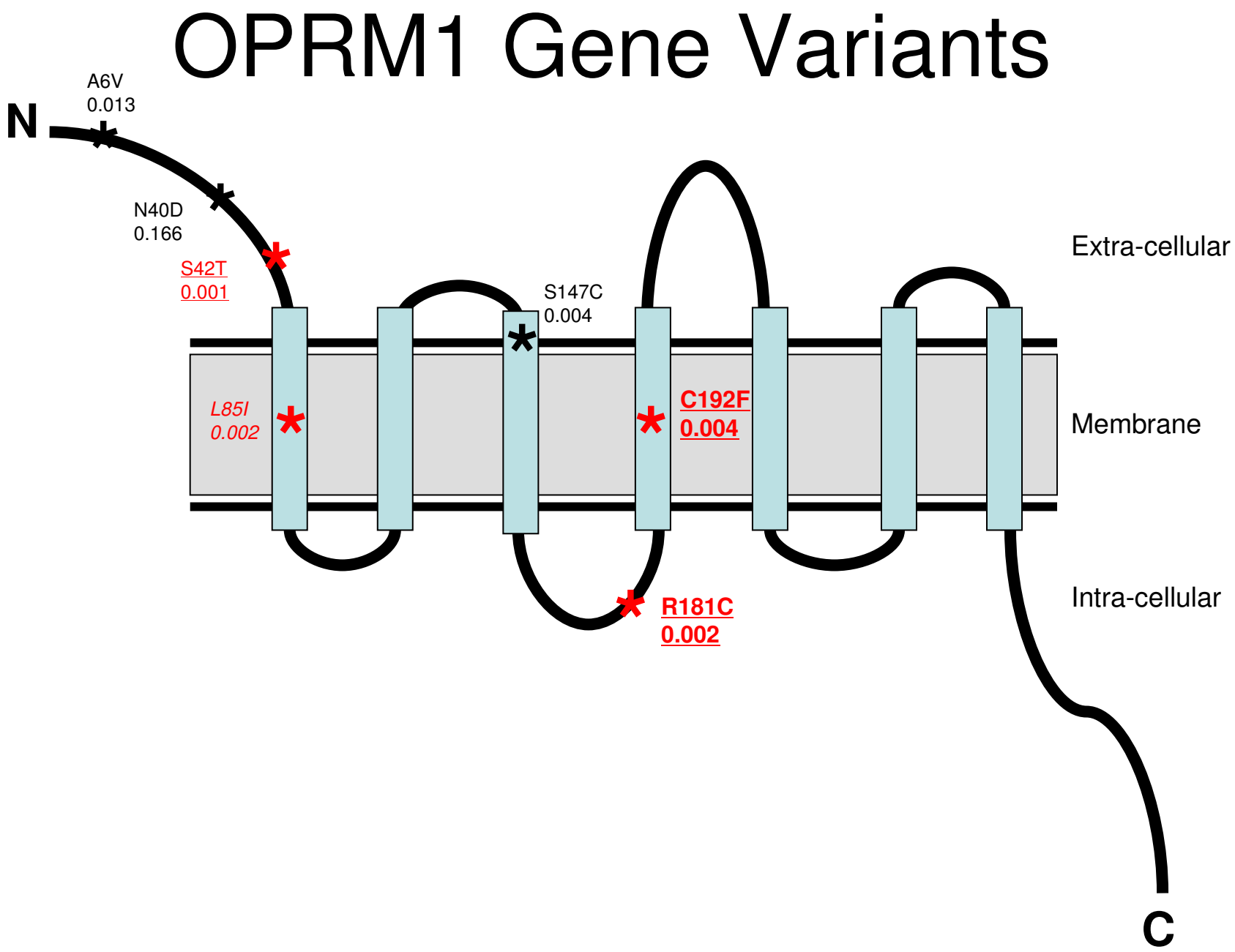
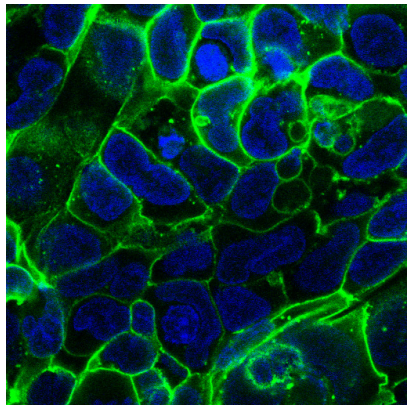

OPRM1 Gene Variants
N
A6V
0.013
N40D
0.166
S42T
0.001
S147C
0.004
L85I
0.002
C192F
0.004
R181C
0.002
Extra-cellular
Membrane
Intra-cellular
C

Fig 2

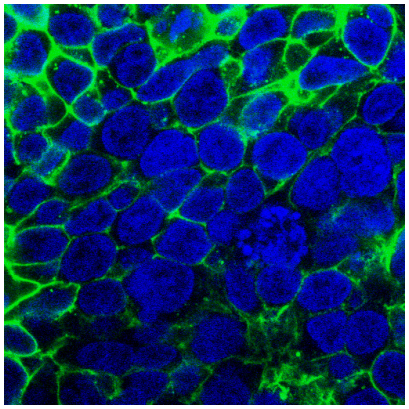
Trafficking of OPRM1

Response to Ligands

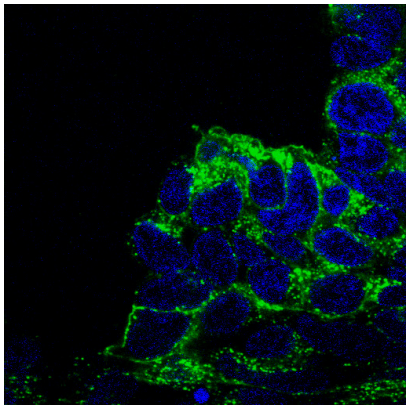
Wild type MOR1A



No treatment

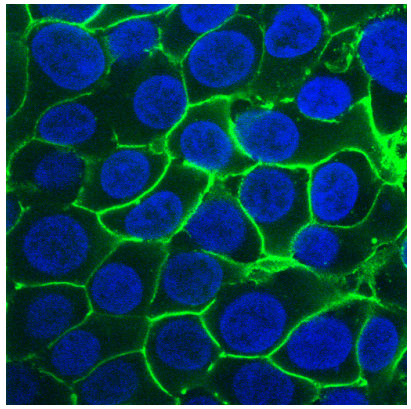


10uM Morphine

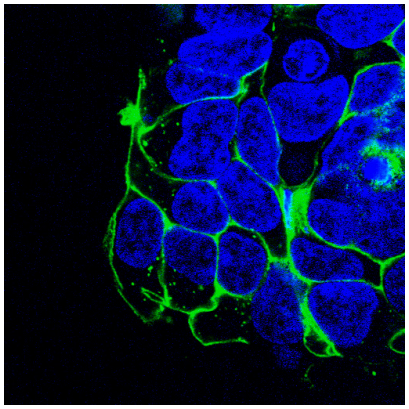


10uM DAMGO

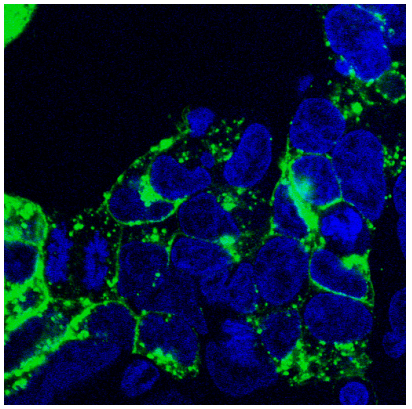
Wild type MOR1



No treatment



10uM Morphine



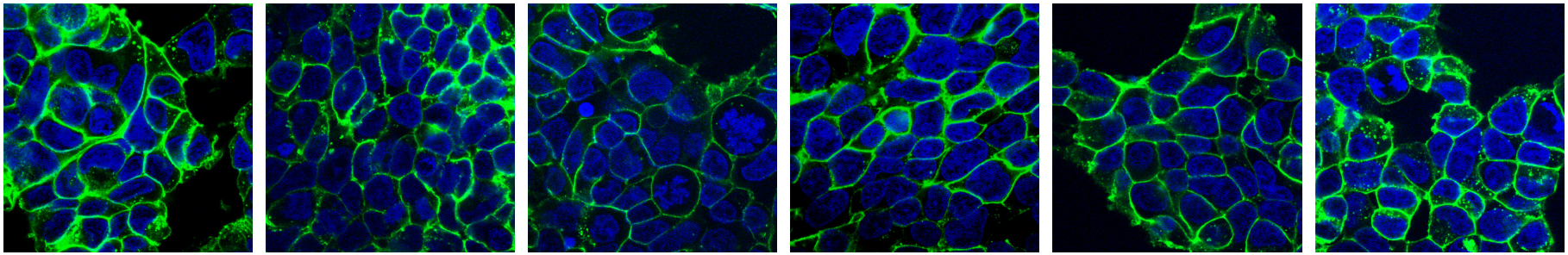
10uM DAMGO

Fig 3

OPRM1 Variants

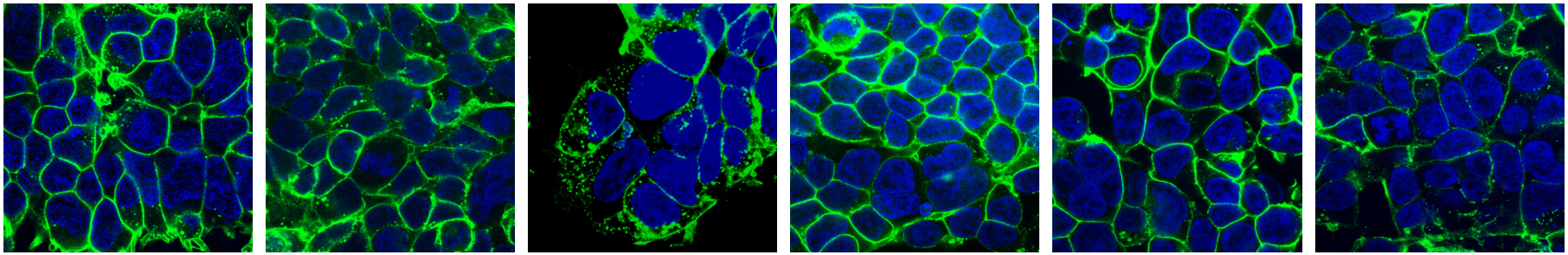
Response to Ligands

A: No treatment



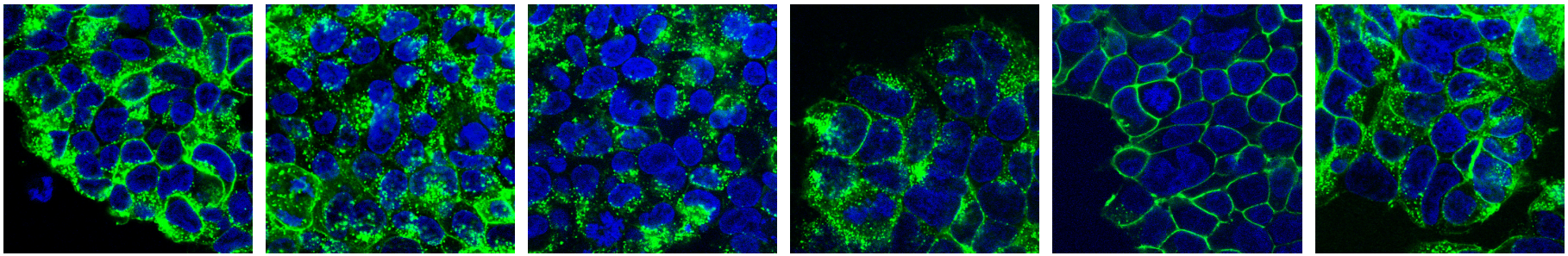
A6V **S42T** **L85I** **S147C** **R181C** **C192F**

B: 10uM Morphine, 30 min



A6V **S42T** **L85I** **S147C** **R181C** **C192F**

C: 10uM DAMGO, 30 min



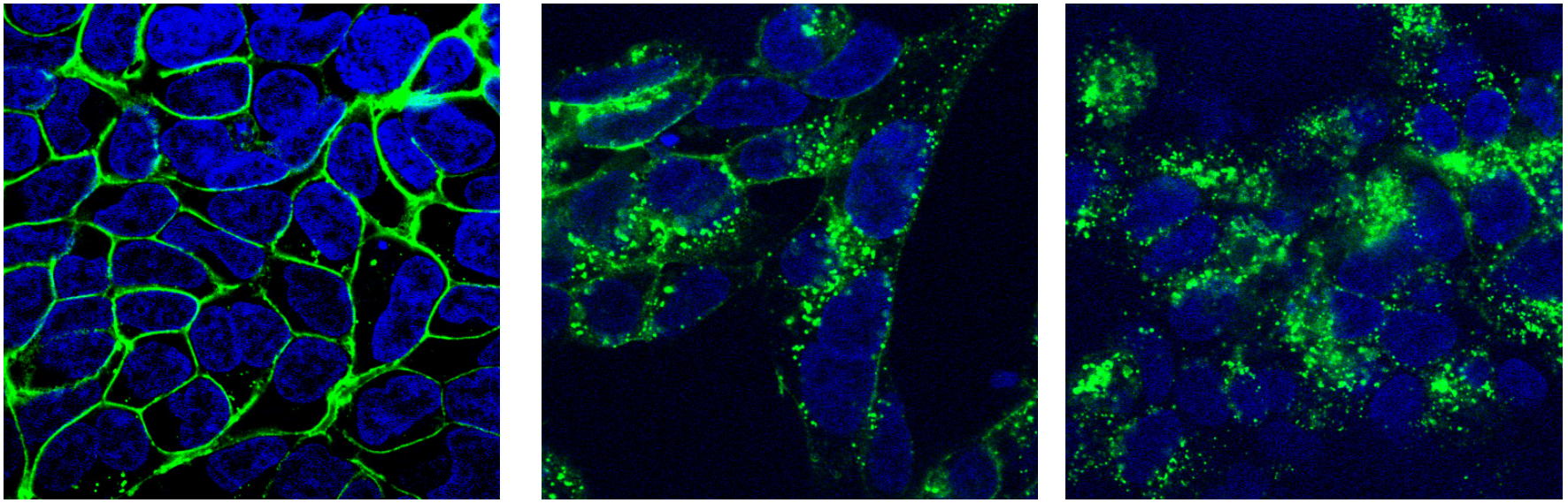
A6V **S42T** **L85I** **S147C** **R181C** **C192F**

Fig 4

OPRM1 L85I

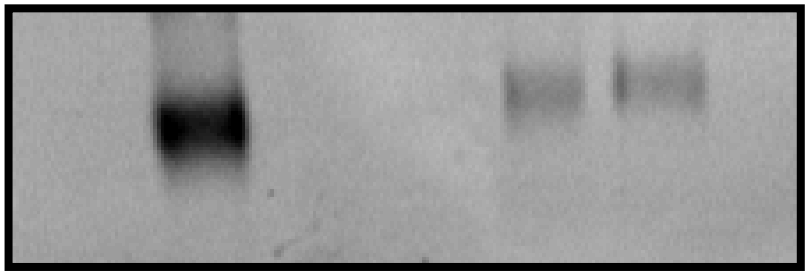
Response to Ligands

A



No treatment | 10μM Morphine (MS) | 10μM DAMGO (DG)

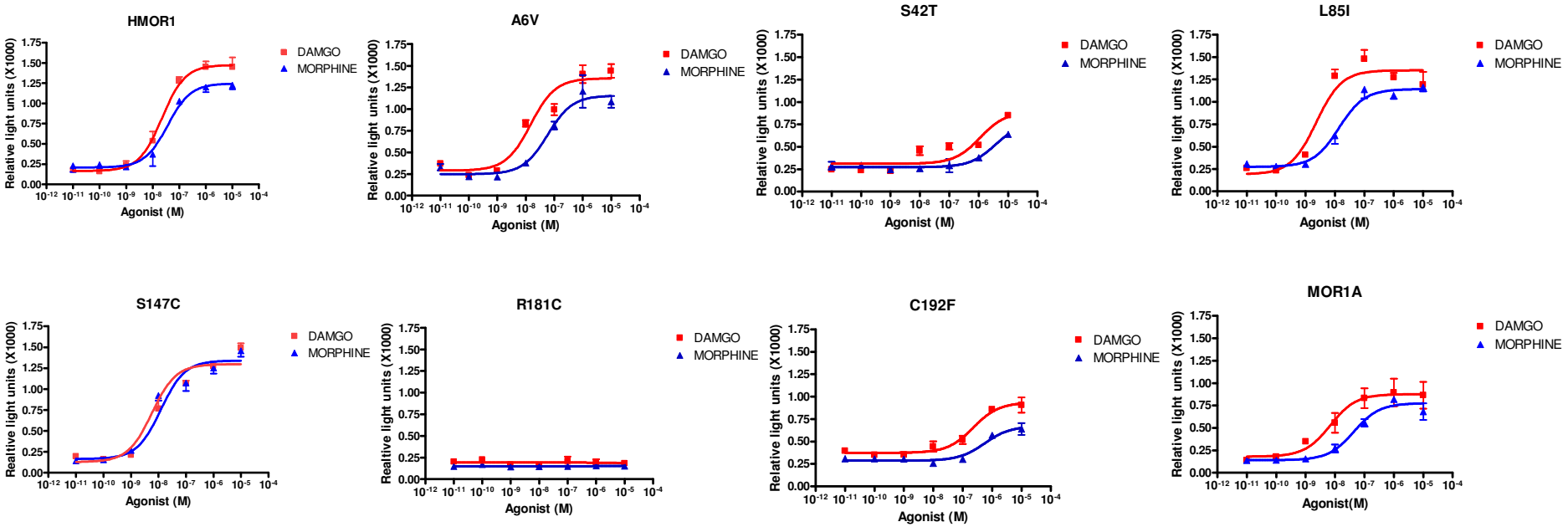
B



30 min agonist exposure, biotin label

Fig 5

A



B

EC50 values (nM)

	DAMGO	Morphine
HMOR1	40.67 + 12.658	48.0 + 5.099
A6V	13.28 + 0.792	51.62 + 9.02
S42T	>150	>150
L85I	15.61 + 2.134	80.97 + 10.55
S147C	13.133 + 6.46	4.833 + 0.09
R181C	0	0
C192F	>150	>150
HMOR1A	35.403 + 4.107	39.433 + 9.077

OPRM1 Variants

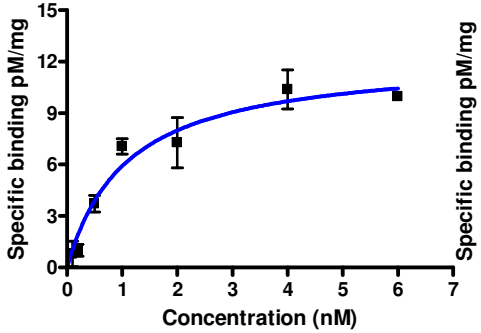
Receptor Activity

Fig 6

OPRM1 Variants

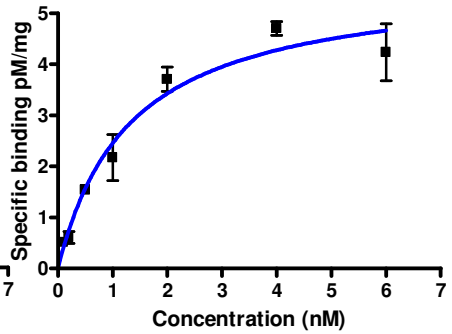
Ligand Binding

HMOR1



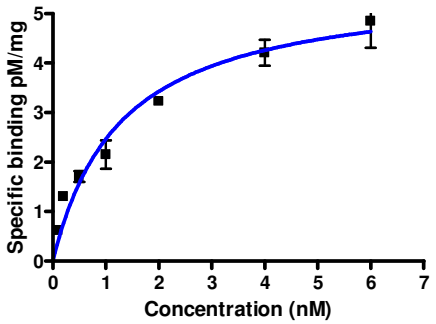
Kd: 1.107nM + 0.195
Bmax: 12.34pM/mg + 1.298

HMOR1A



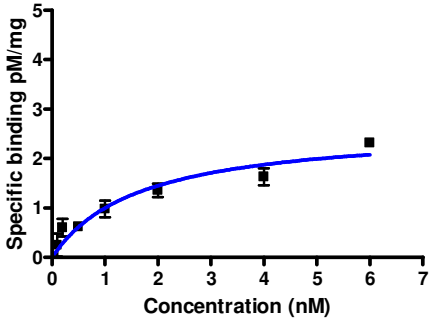
Kd: 1.79nM + 0.725
Bmax: 4.9pM/mg + 0.89

A6V



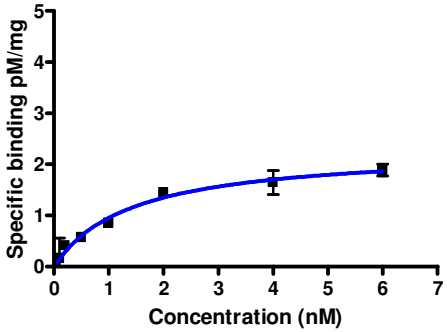
Kd: 1.347nM + 0.366
Bmax: 5.808pM/mg + 0.766

S42T



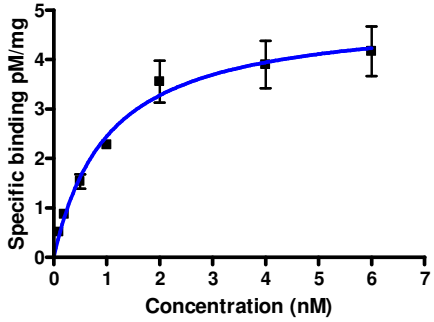
Kd: 1.730nM + 0.279
Bmax: 2.651pM/mg + 0.046

L85I



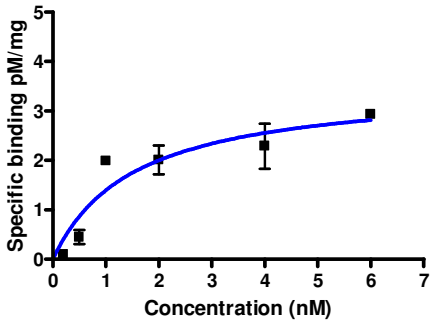
Kd: 1.423 + 0.276
Bmax: 2.304pM/mg + 0.604

S147C



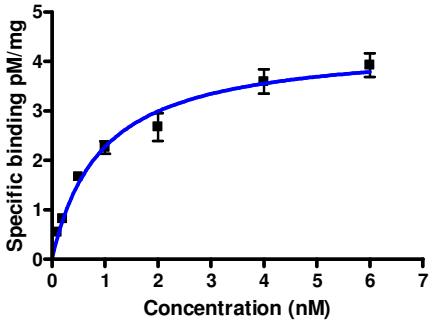
Kd: 1.023nM + 0.213
Bmax: 4.97 pM/mg+ 0.95

R181CMOR1



Kd: 1.664 nM + 0.369
Bmax: 5.777 pM/mg + 0.459

C192F



Kd: .0.9428 nM + 0.154
Bmax: 4.396 pM/mg + 0.48

The $1,000 Genome

- The ability to obtain entire genome sequence of I individuals seems “almost so close you can reach out and touch it)
- A conundrum - most variants are rare, thus hard to get association signals
  - However, each gene may show several or many rare variants
  - If shown to be functional, can be grouped for analysis
- **Families** will regain importance for analysis of **rare variants**, the likely basis for most familial susceptibility to alcohol abuse and alcoholism

Ernest Gallo Clinic and Research Center
Human Genetics Group

- Wet Lab
  - **Ajay Ravindranathan**
  - Amy Choi
  - **Anastasia Constantinescu**
  - Andrew Lee
  - Chris Walker
  - **Geoff Joslyn**
  - **Margaret Robertson**
  - Ray Chui
  - Ray White
- Silicon Lab
  - **Gerry Brush**
  - Kaleas Johnson
  - Larry Kline