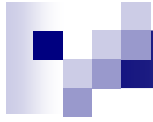


**A Hybrid Approach:  
Genome Wide Association  
and Candidate Gene Study  
for Nicotine Addiction**

**Laura Jean Bierut, MD  
and the NICSNP Consortium**



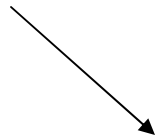
# Nicotine Dependence - A many step process

Initiation



# Nicotine Dependence - A many step process

Initiation

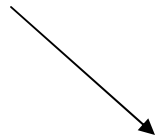


Smoking

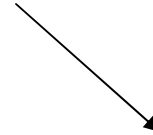


# Nicotine Dependence - A many step process

Initiation



Smoking



Nicotine Dependence



# NICSNP Project - A Hybrid Project

- Genome Wide Association Study
- Candidate Gene Study



# Nicotine Studies

- Collaborative Study of the Genetics of Nicotine Dependence  
Principal Investigator: Laura Jean Bierut (P01 CA 089392)
  - The Genetics of Vulnerability to Nicotine Addiction  
Principal Investigator: Pamela Madden (R01 DA 012854)
  - Genes for Smoking in Related and Unrelated Individuals  
Principal Investigator: Ovide Pomerleau (R01 DA 017640)
  - Pharmacokinetics of Nicotine in Twins  
Principal Investigator: Gary Swan (R01 DA 011170)
- NIDA Phenotypic Repository: John Rice



## Phenotype Definition

- Case: Nicotine dependent defined by a Fagerström Test for Nicotine Dependence (FTND)  $\geq 4$
- Control: Individual who has smoked 100 or more cigarettes and never had any symptoms of nicotine dependence (Lifetime FTND = 0).



# Subjects

- Sample: 1050 cases and 879 controls
- Case FTND: Mean 6.3 (range 4-10)
- Control FTND: 0





# Subjects

- Cases: 52% women and 48% men
- Controls: 66% women and 34% men
  
- Age: Mean 37 years
  
- All subjects are of European descent



# Overview -Genome Wide Association Study

- Genotyping of 2.4 million SNPs in pooled case (N=500) and control (N=500) samples.
- An evaluation of differences in allele frequencies between pools.
- Individual genotyping of the selected 40,000 SNPs in the case (N=1,000) and control (N=900) samples to further examine genetic association.



# Overview Candidate Gene Study

- Selection and ranking of candidate genes
- Examination of SNP coverage
- 4,000 SNPs allocated to candidate genes



# Candidate Genes

CHRNA3	Cholinergic Receptor, Neuronal Nicotinic, Alpha Polypeptide 3	15q24	CHRNA3 is an essential component of the nicotinic receptors mediating normal function of the autonomic nervous system.
CHRNA4	Cholinergic Receptor, Neuronal Nicotinic, Alpha Polypeptide 4	20q13.2-q13.3	Univariate family-based association tests demonstrated that variant alleles of the CHRNA4 gene were significantly associated with a protective effect against nicotine addiction.
CHRNA7	Cholinergic Receptor, Neuronal Nicotinic, Alpha Polypeptide 7	15q14	Defect at CHRNA7 associated with attentional disturbances in schizophrenia; heavy use of nicotine and nicotine dependency may represent self-treatment for the defect at CRHNA7.



## Candidate Genes

- Rank A: These genes will be followed with individual genotyping regardless of the pooled results.
  - Nicotinic Receptors
- Rank B: All the rest



## Data Analysis

- Pooled genotyping (N=500 cases and 500 controls) was used to identify SNPs most likely associated with nicotine dependence in the genome wide association study.
- Individual genotyping is then examined in the entire sample (N=1000 cases and 900 controls).



## Different Prior Probability

- The Genome Wide Association Study and the Candidate Gene Study have different prior probabilities.
- Correction for multiple testing varies between these two components of the study.



# Data Analysis

- There will be many significant differences between cases and controls.
- What findings will be “true” differences between cases and controls?
- More complicated analyses incorporating covariates.





# Analytic Model

- Logistic Regression Model
- Gender + Site + Genotype  
+Gender\*Genotype
- Gender + Site

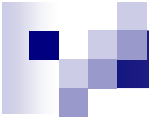
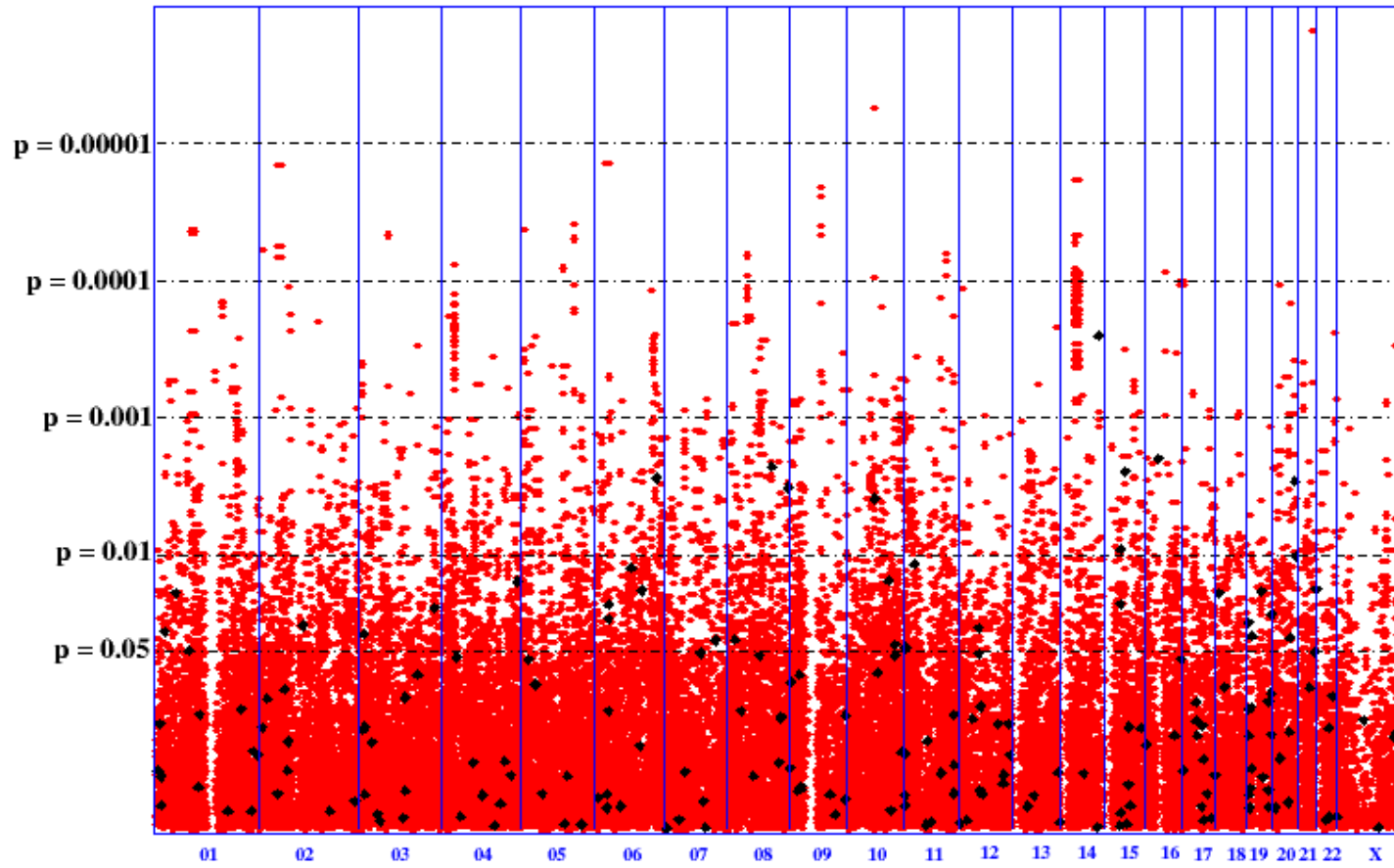
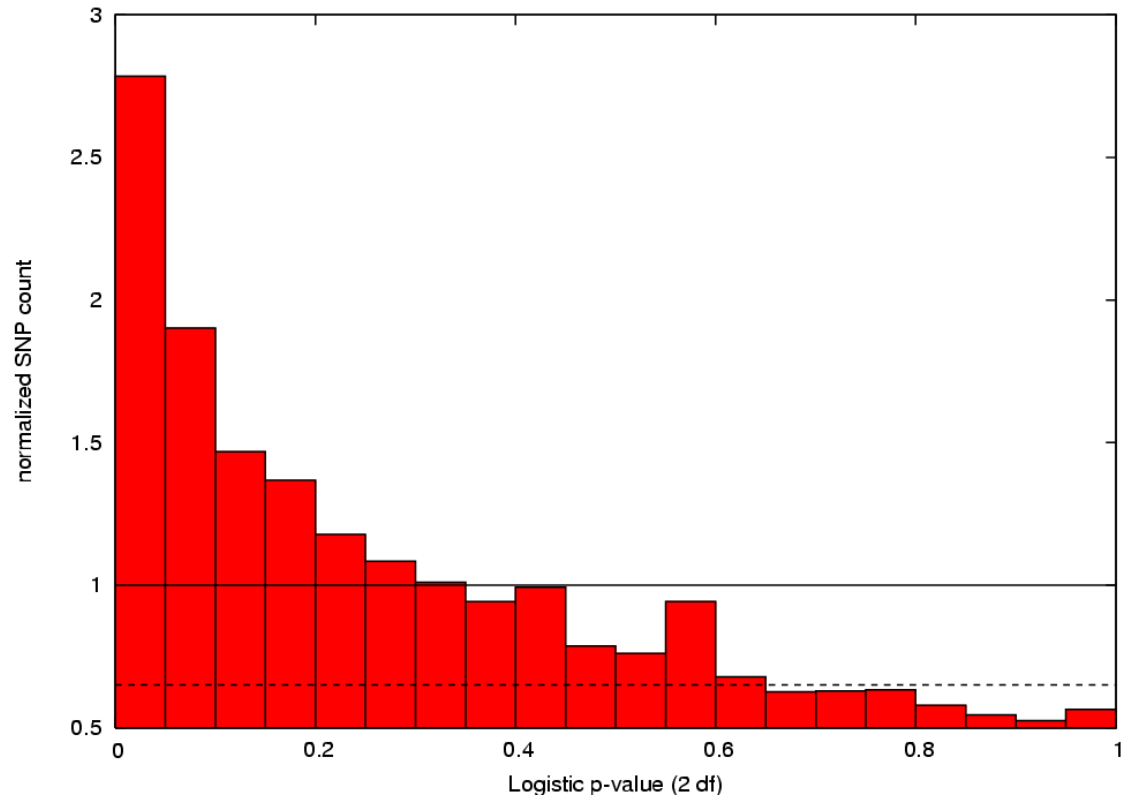
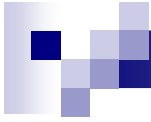


Figure 1







## Evidence of True Findings

- Enrichment of small p-values
- Sign agreement between pooled samples and additional samples (30 out of 35 SNPs)  
p-value  $< 10^{-4}$
- Convergence of results



# Summary

- Genome Wide Association Study -  
Nominates novel genes involved in nicotine dependence and a known candidate gene
- Candidate Gene Study - Numerous variants across several genes are associated with nicotine dependence



# Data Sharing

- Share with all in the NIDA Genetics Consortium.
- Website has been developed as a source of information.
- Share with the scientific community after 1 year.



## Future Directions

- Other replication samples will be key, including the study of parent offspring trios.
- Examine findings in different ethnic groups, such as the African American population.
- Move forward with laboratory studies.



# Thanks

- Dennis Ballinger, Andrew Bergen, Naomi Breslau, Gary Chase, Joseph Cubells, Danielle Dick, Dani Fallen, Mark Gold, Dorothy Hatsukami, Anthony Hinrichs, Eric Johnson, Ken Krauter, Mary Jeanne Kreek, Pamela Madden, Sharon Murphy, Cynthia Pomerleau, Ovide Pomerleau, John Rice, Huijun Ring, Nancy Saccone, Scott Saccone, Pak Sham, Gary Swan, Ming Tsuang, Michael Vanyukov, Kirk Wilhelmsen, Lei Yu, Hongyu Zhao and Joni Rutter