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

<table>
<thead>
<tr>
<th>Gene</th>
<th>Description</th>
<th>Chromosome</th>
</tr>
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<tbody>
<tr>
<td>CHRNA3</td>
<td>Cholinergic Receptor, Neuronal Nicotinic, Alpha Polypeptide 3</td>
<td>15q24</td>
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<tr>
<td></td>
<td>CHRNA3 is an essential component of the nicotinic receptors mediating normal function of the autonomic nervous system.</td>
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<td></td>
<td>Univariate family-based association tests demonstrated that variant alleles of the CHRNA4 gene were significantly associated with a protective effect against nicotine addiction.</td>
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<tr>
<td>CHRNA4</td>
<td>Cholinergic Receptor, Neuronal Nicotinic, Alpha Polypeptide 4</td>
<td>20q13.2-q13.3</td>
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<tr>
<td></td>
<td>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.</td>
<td></td>
</tr>
<tr>
<td>CHRNA7</td>
<td>Cholinergic Receptor, Neuronal Nicotinic, Alpha Polypeptide 7</td>
<td>15q14</td>
</tr>
</tbody>
</table>
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).

Skol et al, 2006
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
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

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