Gene-by-Environment Interactions in Chronic Obstructive Pulmonary Disease

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Outline
- COPD Background/Epidemiology.
- Gene x Environment Introduction.
- Alpha 1-antitrypsin Deficiency.
- Familial Aggregation of COPD.
- Linkage Analysis of COPD-related Phenotypes.
- Genetic Association Studies of COPD.

Chronic Obstructive Pulmonary Disease: Definition
- Chronic obstructive pulmonary disease (COPD) is a disease state characterized by airflow limitation that is not fully reversible. The airflow limitation is usually both progressive and associated with an abnormal inflammatory response of the lungs to noxious particles or gases (GOLD Project).
- COPD includes:
  - Emphysema
  - Chronic Bronchitis
  - Small Airways Disease
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Spirometry: Normal and COPD

Thanks to GOLD Project for Slide

* Thanks to ACCP/Chest Foundation Task Force on Women and Girls, Tobacco, and Lung Cancer for Slide

Trends in Cigarette Smoking Among Men and Women in the United States
Ages 18+ 1955-1997

Percentage smokers

Thanks to MMWR. 1999;48(43):988; National Center for Health Statistics 1988.
COPD Epidemiology/Significance

- 16 million Cases in U.S.
- Fourth Leading Cause of Death
- Substantial Morbidity

COPD: Evidence for Genetic Determinants

- A small percentage of COPD patients inherit severe alpha 1-antitrypsin deficiency.
- Studies of pulmonary function in the general population and in twins suggest a genetic contribution to variation in pulmonary function (Lewitter 1984, Redline 1987).
- Several studies have shown higher rates of airflow obstruction in relatives of COPD patients than in control subjects (Larson 1970, Kueppers 1977, Cohen 1980, McCloskey 2001).
Overview of Complex Trait Genetics

- Demonstrate Familial Aggregation of Complex Trait
- Positional Cloning
- Candidate Gene Approach
- Localize Susceptibility Gene(s)
- Identify Disease Susceptibility Gene and Functional Variants
- Diagnostics
- Determine Fraction of Explained Variation
- Treatment

Genotype-by-Environment Interaction

- General Definition: Environmental exposures have different phenotypic effects for different genotypes.
- Biological Definition: Nonadditive contribution of genetic and environmental factors to the phenotype.
- Statistical Definition: Lack of fit of a multiplicative model for the joint effect of genotype and environment on disease risk.

Example of Genotype-by-Environment Interaction: Phenylketonuria

<table>
<thead>
<tr>
<th>Environment</th>
<th>Genotype</th>
<th>Phenylalanine in Diet</th>
<th>Phenylalanine Removed from Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>PKU/+ or +/</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>PKU/PKU</td>
<td>Normal</td>
<td>Mental Retardation</td>
<td>Normal</td>
</tr>
</tbody>
</table>

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Do COPD and Smoking Fit the 2 x 2 Table?

<table>
<thead>
<tr>
<th>Environment</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Smoker</td>
<td>COPD Susceptibility Gene Present</td>
</tr>
<tr>
<td>Smoker</td>
<td>COPD/ ?Normal</td>
</tr>
</tbody>
</table>

- Major Problem: AAT is the only proven genetic risk factor

Application of the 2 x 4 Table in Case-Control COPD Genetic Association Studies

(Clayton and McKeeu, Lancet 2001; 358:1356)

<table>
<thead>
<tr>
<th>COPD CASES</th>
<th>CONTROLS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candidate Gene Present</td>
<td>Candidate Gene Absent</td>
</tr>
<tr>
<td>Non-Smoker</td>
<td>a</td>
</tr>
<tr>
<td>Smoker</td>
<td>c</td>
</tr>
</tbody>
</table>

Advantages:  
- a) Provides odds ratios for association between genotype and environment in cases only (ag/ce) and controls only (bh/df).
- b) Tests deviation from multiplicative or additive interaction model.

Problems:  
- a) Ignores smoking intensity.
- b) Ignores disease severity.
- c) Assumes a dominance model for susceptibility gene.

Types of Environmental Exposures

- Dichotomous Environmental Exposures
  - Smoking as Smoker/Non-smoker
    - G x E can be analyzed with contingency table.

- Continuous Environmental Exposures
  - Smoking as Pack-years
    - G x E expressed based on Norm of Reaction: The relationship of a phenotype to an environmental factor for a particular genotype.
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Genotype-by-Environment Interaction: Key Issues
- Environmental influence on a disease does not necessarily imply that G x E is present; G x E requires deviation from expected combined effects of genes and environment.
- Controversial as to whether deviation from additive or multiplicative model should be assessed.
- G x E depends on the scale of observation; some argue that finding any scale transformation that removes an interaction suggests that the interaction is not important.

Key Methods in Genetic Epidemiology
- Familial Aggregation Analysis:
  A group of methods that determine if a phenotype of interest clusters in families (e.g., increased risk to relatives, significant heritability).
- Linkage Analysis:
  A group of methods that analyze the distribution of DNA markers within families to determine if a particular region of the genome contains a gene related to the phenotype of interest.
- Association Studies:
  Methods that determine if a particular form of a DNA polymorphism occurs more frequently in subjects with a phenotype of interest; case-control or family data may be used.

Alpha 1-Antitrypsin: Background
- Alpha 1-antitrypsin, specified by the PI locus, is the major plasma protease inhibitor of leukocyte elastase.
- In Caucasian populations, the most common alleles are: M (0.95), S (0.02 to 0.03), and Z (0.01 to 0.02).
- Major PI alleles relate to SNPs.
- Isoelectric focusing of serum can accurately determine PI type.
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Alpha 1-Antitrypsin Deficiency and COPD
- Individuals with two Z alleles, or one Z and one null allele, are referred to as PI Z.
- PI Z individuals have approximately 15% of normal plasma alpha 1-antitrypsin levels.
- Z mutation is a functional variant causing protein polymerization in the endoplasmic reticulum of hepatocytes.
- PI Z is associated with increased risk for severe, early-onset COPD, but only 1-3% of COPD patients are PI Z.

Variable Expression of Lung Disease in PI Z Individuals
- Only 3 of 5 initial PI Z subjects described by Laurell and Eriksson (1963) had lung disease.
- Black and Kueppers (1978) demonstrated marked variability in pulmonary function and respiratory symptoms among PI Z nonsmokers.
- St. Louis AAT Study demonstrated marked variability in pulmonary function among PI Z subjects.

Pulmonary Function in Alpha 1-Antitrypsin Deficiency: Effects of Ascertainment

From Silverman EK et al., Ann Int Med 1989; 111:982-991

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Pulmonary Function in Alpha 1-Antitrypsin Deficiency: Effects of Cigarette Smoking

PI x Smoking Interaction in St. Louis AAT Study

FEV<sub>1</sub> in Swedish PI ZZ Nonsmokers

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**Alpha 1-Antitrypsin Deficiency: Augmentation Therapy**
- Biochemical efficacy has been shown.
- No supportive randomized clinical trial.
- Expensive!
- A pooled blood product (heat treatment kills hepatitis and HIV).
- Consider for Pi Z or Pi null-null patients with airflow obstruction.

**Who Should Be Tested for AAT Deficiency?**
  - Chronic bronchitis with airflow obstruction in a non-smoker.
  - Bronchiectasis without other risk factors.
  - Moderate/severe COPD by age 50.
  - Basilar predominance of emphysema.
  - Unremitting asthma, esp. under age 50.
  - Fam Hx of AAT def. or early COPD.
  - Cirrhosis without apparent risk factors.

**Is Current AAT Detection Working?**
- Estimated 80,000 Americans with Pi Z.
- Likely fewer than 5000 Pi Z subjects identified.
- Where are the other Pi Z subjects?
  - Severe, early-onset COPD but not tested for AAT deficiency.
  - COPD at later ages.
  - Healthy.
New Standards for the Diagnosis and Management of AAT Deficiency (2003)

- Task Force supported by ATS, ERS, ACCP, and Alpha 1 Foundation.
- Evidence-based presentation of broad range of AAT-related issues.
- Discussed recommendations for AAT Testing, recognizing that there are no studies of efficacy of genetic testing programs in AAT deficient subjects.
- Recommend AAT testing of all symptomatic adults with COPD, emphysema, and/or asthma with irreversible airflow obstruction.

Should All COPD Patients Be Tested for AAT Deficiency?

**Pro**
- High Prevalence of Undiagnosed AATD.
- Significant clinical burden.
- Non-randomized studies suggest benefits of Augmentation Therapy.
- Behavioral change opportunities.
- Economic benefits: Avoid unnecessary tests.

**Con**
- Augmentation Therapy has not been proven beneficial by RCT.
- Not known if smokers will quit if diagnosed with AATD.
- AAT Testing is an added expense.
- Adverse psychological consequences.
- Genetic discrimination.

Monogenic Syndromes with COPD

- Alpha 1-antitrypsin deficiency (Definite).
- Cutis laxa (Definite).
- Ehlers-Danlos (Possible: Pneumothorax, Bullae).
- Marfan Syndrome (Possible: Pneumothorax, Bullae).
- Birt-Hogg-Dube Syndrome (Possible: Pneumothorax, Bullae due to Folliculin mutations).
- Familial Spontaneous Pneumothorax (Possible: Due to Folliculin mutations).
- Down Syndrome (Possible: Cystic lung disease).
- Case Reports: Sialuria, Niemann-Pick Type C.
Familial Aggregation in COPD: Evidence for Gene-by-Smoking Interactions

Boston Early-Onset COPD Study Population

- **Proband Selection:** Subjects with severe COPD (FEV1 < 40%) at an early age (≤ 52 years), without alpha 1-antitrypsin deficiency.
- **Recruitment of Relatives:** All available first-degree relatives and spouses were invited to participate. All available aunts/uncles and grandparents were also included.
- **Linkage Data Set:** 72 early-onset COPD probands, 320 first-degree relatives (48 parents, 147 siblings, 125 children), 162 other relatives, and 31 spouses.

Spirometry in First-Degree Relatives of Early-Onset COPD Probands Compared to Control Subjects

Based on Silverman EK et al., Am J Respir Crit Care Med 1998; 157:1770-1778.
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Phenotypes with Smoking-Related Effects in First-Degree Relatives of Boston Early-Onset COPD Probands

- FEV₁
- FEV₁/FVC.
- Chronic Bronchitis.
- Bronchodilator Responsiveness.

Genetics of Pulmonary Function: COPD Patients
(McCluskey S, et al., AJRCCM 2001; 164: 1419-1424)

- Study assessing spirometry in 173 siblings of COPD patients.
- No airflow obstruction in nonsmoking siblings.
- In smokers, 31.5% of sibs had FEV₁ < 80% with FEV₁/FVC < 0.7, compared with 9.3% in matched controls.
- Odds ratio of airflow obstruction in smoking sibs compared to smoking controls was 4.7.

Linkage Analysis of COPD-related Phenotypes

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Genome Scan Linkage Analysis in Boston Early-Onset COPD Study Families

- Phenotypes:
  - Qualitative:
    - Mild-to-Severe Airflow Obstruction (FEV$_1$ < 80% and FEV$_1$/FVC < 90% pred).
    - Moderate-to-Severe Airflow Obstruction (FEV$_1$ < 60% and FEV$_1$/FVC < 90% pred).
  - Quantitative:
    - FEV$_1$, FEV$_1$/FVC (pre and post-BD).

- Statistical Methods:
  - Quantitative Phenotypes: Multipoint Variance Component Linkage Analysis (SOLAR).
  - Qualitative Phenotypes: Affected Relative Analysis (ALLEGRO).

Genome Scan in Early-Onset COPD Families: Genotyping and Data Cleaning

- DNA samples from 607 early-onset COPD pedigree members.
- 377 Short Tandem Repeat Markers genotyped by NHLBI Mammalian Genotyping Service (Mean spacing = 9.1 cM).
- Mendelian inconsistencies assessed with Relcheck/PedCheck.
  - Relcheck: Assesses Pedigree Inconsistencies (removed 22 subjects).
  - Pedcheck: Assesses Individual Marker Inconsistencies.

Original Genome Scan Linkage Results in Boston Early-Onset COPD Study

Note: Suggestive linkage corresponds to LOD score ≥ 1.9, and significant linkage corresponds to LOD score ≥ 3.3 in this extended pedigree study.

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Impact of Flanking STR Markers on Linkage Analysis of Quantitative Post-BD Spirometric Phenotypes in the Boston Early-Onset COPD Study

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Max LOD Score</th>
<th>FEV1 Post-BD</th>
<th>FEV1/FVC Post-BD</th>
</tr>
</thead>
<tbody>
<tr>
<td>2q (Genome Scan)</td>
<td>0.99</td>
<td>4.41</td>
<td></td>
</tr>
<tr>
<td>2q (+9 STRs)</td>
<td>1.31</td>
<td>4.30</td>
<td></td>
</tr>
<tr>
<td>8p (Genome Scan)</td>
<td>3.30</td>
<td>0.48</td>
<td></td>
</tr>
<tr>
<td>8p (+7 STRs)</td>
<td>1.95</td>
<td>0.30</td>
<td></td>
</tr>
<tr>
<td>12p (Genome Scan)</td>
<td>1.28</td>
<td>0.72</td>
<td></td>
</tr>
<tr>
<td>12p (+12 STRs)</td>
<td>2.06</td>
<td>2.51</td>
<td></td>
</tr>
<tr>
<td>19q (Genome Scan)</td>
<td>1.91</td>
<td>1.47</td>
<td></td>
</tr>
<tr>
<td>19q (+10 STRs)</td>
<td>2.14</td>
<td>1.87</td>
<td></td>
</tr>
</tbody>
</table>

Impact of Stratifying by Smoking on Linkage Analysis of Quantitative Post-BD Spirometric Phenotypes in the Boston Early-Onset COPD Study

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Max LOD Score (Flanking STR Markers Included)</th>
<th>FEV1 Post-BD</th>
<th>FEV1/FVC Post-BD</th>
</tr>
</thead>
<tbody>
<tr>
<td>2q (All Subjects)</td>
<td>1.31</td>
<td>4.30</td>
<td></td>
</tr>
<tr>
<td>2q (Smokers Only)</td>
<td>1.92</td>
<td>4.13</td>
<td></td>
</tr>
<tr>
<td>12p (All Subjects)</td>
<td>2.06</td>
<td>2.51</td>
<td></td>
</tr>
<tr>
<td>12p (Smokers Only)</td>
<td>3.26</td>
<td>3.04</td>
<td></td>
</tr>
<tr>
<td>19q (All Subjects)</td>
<td>2.14</td>
<td>1.87</td>
<td></td>
</tr>
<tr>
<td>19q (Smokers Only)</td>
<td>2.75</td>
<td>2.15</td>
<td></td>
</tr>
</tbody>
</table>

Chromosome 12 FEV1 Linkage with Flanking Markers Based on Smoking Adjustments

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Summary of Boston Early-Onset COPD Study Linkage Results
- Significant linkage of FEV₁/FVC to Chromosome 2q (LOD > 4); suggestive linkage to COPD-related phenotypes in several other genomic regions (Silverman, AJHG 2002; Palmer HMG 2003).
- Stratification by smoking status provided increased evidence for linkage on chromosomes 2q, 12p, and 19q, suggesting gene-by-smoking interactions (DeMeo AJRCCM 2004).
- On chromosome 12p, borderline significant linkage (LOD > 3) was seen for FEV₁ and FEV₁/FVC in smokers.
- On chromosome 19q, borderline significant linkage (LOD > 3) was seen for pre-bronchodilator FEV₁ in smokers (Celedon HMG 2004).

Linkage Analysis of Quantitative Spirometric Phenotypes in the General Population: Framingham Study Methods
- Sample: 1578 individuals from 330 pedigrees in the Framingham Study.
- Genotypes: 399 STR markers.
- Phenotypes:
  - Spirometric measurements of Original Cohort and Offspring Cohort of subjects in middle age.
  - Residual lung function measures for FEV₁, FVC, and FEV₁/FVC adjusted for age, height, weight, pack-years, and smoking status (not used as covariates in analysis).
- Linkage Analysis: Variance component method in SOLAR.

Linkage Analysis of Quantitative Spirometric Phenotypes in the General Population: Framingham Study Results
- Highest LOD Scores:
  - FEV₁: 2.4 on chromosome 6q.
  - FVC: 2.6 on chromosome 21p.
  - FEV₁/FVC: 1.4 on chromosome 6q.
- Modest Similarities to Boston Early-Onset COPD Study
  - FEV₁: chromosome 4 (1.6 in Fram, 1.2 in Bos).
  - FEV₁/FVC: chromosome 19 (1.3 in Fram, 1.5 in Bos).

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Linkage Analysis of Quantitative Spirometric Phenotypes in the General Population

<table>
<thead>
<tr>
<th>Author</th>
<th>Population (n)</th>
<th>FEV1 Region</th>
<th>Max LOD</th>
<th>FEV1/FVC Region</th>
<th>Max LOD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Joost (2002)</td>
<td>Framingham Study (n = 1578)</td>
<td>6q</td>
<td>2.4</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4p</td>
<td>1.6</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Wik (2003)</td>
<td>Family Heart Study (n = 2178)</td>
<td>--</td>
<td>--</td>
<td>4p</td>
<td>2.0**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>--</td>
<td>--</td>
<td>8p</td>
<td>1.6**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>--</td>
<td>--</td>
<td>11q</td>
<td>1.9**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>--</td>
<td>--</td>
<td>15q</td>
<td>1.6**</td>
</tr>
<tr>
<td>Malhotra (2003)</td>
<td>CEPH Pedigrees (n = 264)</td>
<td>--</td>
<td>--</td>
<td>2q</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>--</td>
<td>--</td>
<td>5q</td>
<td>2.2*</td>
</tr>
</tbody>
</table>

Notes: *Corresponds to a parametric heterogeneity LOD Score
**Based on Normalized Phenotypic Values

Potential Explanations for Differences in Linkage Analysis of Quantitative Spirometric Phenotypes in the General Population and in Early-Onset COPD Families

- Some regions of suggestive linkage will not contain real genetic determinants.
- Genetic determinants of normal spirometry may differ from disease.
- Multiple determinants may be present, but not adequate power to detect them all.
- Different determinants may be important in different populations.

Overview of COPD Fine Mapping

- Testing positional candidate genes from known pathophysiology.
- Testing positional candidate genes with differential expression.
- Systematic SNP screening of linked regions for association.

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Genetic Association Analysis of COPD

Case-Control Genetic Association Studies in COPD

<table>
<thead>
<tr>
<th>Category</th>
<th>Candidate Gene</th>
<th>Support Association</th>
<th>Do Not Support Association</th>
</tr>
</thead>
</table>

Note: Selected references which support or do not support an association to the specified locus are presented.

Case-Control Association Studies in COPD: Potential Causes of Inconsistent Results

- Genetic heterogeneity: Different genetic mechanisms in different populations.
- Phenotypic heterogeneity: Different definitions of cases/controls.
- Random error.
- Population stratification.
- Study design/analysis problems:
  - Failure to correct for multiple comparisons.
  - Poor control group selection.
  - Small sample sizes.

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Testing Candidate Genes in Boston Early-Onset COPD Families: Phenotypes

- Quantitative Phenotypes
  - FEV₁ (Pre and Post-Bronchodilator).
  - FEV₁/FVC (Pre and Post-Bronchodilator).

- Qualitative Phenotypes
  - Mild-to-severe airflow obstruction (FEV₁<80%, FEV₁/FVC<90% pred).
  - Moderate-to-severe airflow obstruction (FEV₁<60%, FEV₁/FVC<90% pred).

Testing Candidate Genes in Boston Early-Onset COPD Families: Statistics

- Family-based association analysis (FBAT) approach by Laird et al.

- New extensions in PBAT (Lange et al.)
  - Uses extended pedigree information.
  - Inclusion of G x E.

Testing Candidate Genes in COPD: Populations

- Boston Early-Onset COPD Study:
  - Family-based Association Analysis
    - Genome Scan Sample: 72 pedigrees with 585 subjects.
    - Total Sample: 127 pedigrees with 949 subjects.

- Case-Control Replication Sample
  - NETT Subjects: 304 severe emphysema patients.
  - Normative Aging Study Controls: 441 male smokers without airflow obstruction.
  - Note: No evidence for population stratification based on 44 unlinked SNPs (p > 0.05).
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Linkage of Chromosome 19 to Pre-BD FEV₁  

TGFβ1 and COPD

- Animal Models

- Prior Genetic Association Study in COPD (Wu et al., Thorax 2004)
  - Studied one non-synonymous SNP at codon 10 (Leu to Pro) in 165 COPD subjects, 140 blood donors, and 76 healthy smokers.
  - Lower Pro allele frequency in COPD subjects than blood donors or healthy smokers (p < 0.01).

PBAT Analysis of TGFB1 SNPs in the Boston Early-Onset COPD Study  
(Celedon 2004)

<table>
<thead>
<tr>
<th>TGFβ1 SNP</th>
<th>FEV1</th>
<th>FEV1/FVC</th>
<th>Mild-to-severe Obstruction</th>
<th>Mod-to-severe Obstruction</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2241712 (promoter)</td>
<td>0.03</td>
<td>N.S.</td>
<td>0.05</td>
<td>N.S.</td>
</tr>
<tr>
<td>rs1800469 (promoter)</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
<tr>
<td>rs192073 (Exon 1 Non-syn)</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
<tr>
<td>rs6957 (3’ genomic)</td>
<td>0.03</td>
<td>N.S.</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>rs2241718 (3’ genomic)</td>
<td>0.03</td>
<td>N.S.</td>
<td>0.04</td>
<td>0.04</td>
</tr>
</tbody>
</table>
Case-Control Association Analysis of TGFB1 SNPs in NETT and NAS

<table>
<thead>
<tr>
<th>TGFB1 SNP</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2241712 (promoter)</td>
<td>0.01</td>
</tr>
<tr>
<td>rs1800469 (promoter)</td>
<td>0.02</td>
</tr>
<tr>
<td>rs1982073 (Exon 1 Non-syn)</td>
<td>0.001</td>
</tr>
<tr>
<td>rs6957 (3' genomic)</td>
<td>N.S.</td>
</tr>
<tr>
<td>rs2241718 (3' genomic)</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

Is TGFB1 a COPD Susceptibility Gene?

- Positional candidate in a linkage region.
- Good pathophysiological rationale.
- One prior positive genetic association study.
- We found associations in both family-based and case-control studies, but only one SNP replicated.
- Conclusion: TGFB1 may be a COPD susceptibility gene, but additional study is required to find a functional SNP or SNPs.

Inclusion of Genotype-by-Environment Interactions in COPD

- Epidemiological Dogma:
  - Tests of Interactions are less powerful than tests of main effects.
  - Significant Interaction terms in models without significant main effects are not of great interest.
- Potential Reality for COPD: Testing for genotype-by-smoking interactions may be necessary to detect novel susceptibility genes.
- Challenge: G x E testing is not readily available in many linkage and association methods.
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G x E in COPD: Can We Use the Environment to Find Genes?

- Need to incorporate G x E in linkage analyses formally
- Will smoking-related linkage regions be replicated?
- Incorporation of G x E in association analyses:
  - Will power to find COPD genes be increased?
  - How will family-based and case-control results compare?
- Haplotype-based analyses of G x E in COPD (e.g. HaploGLM)
- Analysis of other environmental factors (e.g. second-hand smoke, occupational exposures)

Collaborators

- **Boston Early-onset COPD Study**: Frank Speizer, Jeffrey Drazen, Hal Chapman, Scott Weiss, John Reilly, Leo Ginns, Ed Campbell, Juan Celedon, Lyle Palmer, Vincent Carey, Jody Sylvia, David Kwiatkowski, Dawn DeMeo, Craig Hersh, Robert Mechem, Casey Keliefer, Tom Mariani, Steve Shapiro, Benjamin Raby, Irwin Buchwald

- **NETT Genetics Ancillary Study**: John Reilly, Deborah Russ, Scott Weiss, Steve Shapiro, David Kwiatkowski, Gus Lioijua, Craig Hersh, Dawn DeMeo, Joshua Benditt, Gerard Criner, Barry Male, Fernando Martinez, Paul Scanlon, Frank Sciurba, James Utz, Eric Hoffman, and the NETT Clinical Centers