Pharmacogenetics of Tobacco Smoking and Lung Cancer

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GAME-ON Consortium
Smokers live 10 years less

Cigarette smokers

80% Persons who never smoked

57%

10 yr
Smoking: a leading cause of premature death

- **Currently:**
  >1 billion smokers globally
  6 million death each year

- **Projected:** 1 billion deaths during 21st century

<table>
<thead>
<tr>
<th>Disease</th>
<th>Current smoker</th>
<th>Never smoker</th>
<th>RR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic lung disease (J40–44)</td>
<td>1789</td>
<td>121</td>
<td>35.3 (29.2–42.5)</td>
</tr>
<tr>
<td>Cancer of lung (C34)</td>
<td>5633</td>
<td>698</td>
<td>21.4 (19.7–23.2)</td>
</tr>
<tr>
<td>Aortic aneurysm (I71)</td>
<td>330</td>
<td>164</td>
<td>6.32 (5.17–7.71)</td>
</tr>
<tr>
<td>Intestinal ischaemia (K55)</td>
<td>183</td>
<td>91</td>
<td>5.58 (4.27–7.29)</td>
</tr>
<tr>
<td>Cancer of mouth, pharynx, larynx, nasal cavity, or sinuses (C00–14,30–32)</td>
<td>204</td>
<td>91</td>
<td>4.83 (3.72–6.29)</td>
</tr>
<tr>
<td>Coronary heart disease (I21–25)</td>
<td>2726</td>
<td>1732</td>
<td>4.47 (4.19–4.77)</td>
</tr>
<tr>
<td>Cirrhosis or alcoholic liver (K70,74)</td>
<td>478</td>
<td>256</td>
<td>3.35 (2.84–3.94)</td>
</tr>
<tr>
<td>Cancer of bladder (C67)</td>
<td>178</td>
<td>156</td>
<td>3.29 (2.61–4.15)</td>
</tr>
<tr>
<td>Cancer of oesophagus (C15)</td>
<td>450</td>
<td>397</td>
<td>3.10 (2.68–3.58)</td>
</tr>
<tr>
<td>Pneumonia (J12–18)</td>
<td>494</td>
<td>408</td>
<td>3.09 (2.68–3.56)</td>
</tr>
<tr>
<td>Cerebrovascular disease (l60–69)</td>
<td>1528</td>
<td>1458</td>
<td>3.06 (2.83–3.31)</td>
</tr>
</tbody>
</table>

Pirie et al., 2010
I miss my lung, Bob.
Model of Nicotine Dependence - A many step process

Never Use

Initiation
First puff – First cigarette

Does everyone who regularly uses a nicotine become addicted?

Smoker
100 cigarettes lifetime

Non-dependent users

Nicotine Dependence
U.S. Population Screening and Nicotine Dependence

Screened
53,742

50.9%

Initiated Smoking
27,372

58.0%

Smoked 100+
Cigarettes
15,881

19.2%

35.2%

44.3%

No Symptoms
3,051

Some Symptoms
5,596

Nicotine Dependence
7,028

Collaborative Genetic Study of Nicotine Dependence, Bierut
Genetics of Smoking

Initiation
- Genetic: 44%
- Environmental: 56%

Dependence
- Genetic: 25%
- Environmental: 75%

Persistence
- Genetic: 70%
- Environmental: 30%

Quantity
- Genetic: 14%
- Environmental: 86%

Quitting
- Genetic: 40%
- Environmental: 60%

Twin, family and adoption studies in humans, together with animal studies, have provided the foundation for genetic effects on substance use, abuse and dependence.

Table 1 Heritability estimates for different drugs of abuse

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Heritability estimates</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Smoking</strong></td>
<td></td>
</tr>
<tr>
<td>Persistence</td>
<td>28–84%</td>
</tr>
<tr>
<td>Cigarette consumption</td>
<td>45–86%</td>
</tr>
<tr>
<td>Nicotine dependence</td>
<td>31–75%</td>
</tr>
<tr>
<td>Nicotine withdrawal symptoms</td>
<td>26–48%</td>
</tr>
<tr>
<td>Smoking cessation</td>
<td>50–58%</td>
</tr>
<tr>
<td><strong>Alcoholism</strong></td>
<td></td>
</tr>
<tr>
<td>Alcohol abuse/dependence</td>
<td>50–70%</td>
</tr>
<tr>
<td>Consumption levels</td>
<td>45–58%</td>
</tr>
<tr>
<td>Problem drinking</td>
<td>8–50%</td>
</tr>
<tr>
<td><strong>Opiates/heroin</strong></td>
<td></td>
</tr>
<tr>
<td>Abuse and/or dependence</td>
<td>43–60%</td>
</tr>
<tr>
<td><strong>Sedatives</strong></td>
<td></td>
</tr>
<tr>
<td>Abuse and/or dependence</td>
<td>29–58%</td>
</tr>
<tr>
<td><strong>Psychostimulants</strong></td>
<td></td>
</tr>
<tr>
<td>Abuse and/or dependence</td>
<td>42–74%</td>
</tr>
</tbody>
</table>

These studies have been reviewed in greater detail elsewhere. A list of the primary references can be found in Supplementary Table S2 online.

Chromosome 15q25 Is Important for Smoking
Nicotinic Receptors are Homo- or **Heteropentamers**

$\alpha_3$

$\beta_4$

$\alpha_5$

low level expression in brain
SNP rs16969968 affects maximal response to agonist

Chromosome 15q25.1 region contains specialized nicotinic receptor variants

**CHRNA3=27.87kb**
- 21 variants
  - Variant distribution: 5 @ 5’UTR; 8 @ exons and 8 @ flanking intron

**CHRNA5=28.53kb**
- 33 variants
  - Variant distribution: 13 @ UTR; 6 @ exons and 14 @ flanking intron

**LOC123688 =21.22KB**
- 15 variants
  - Variant distribution: 6 @ UTR; 6 @ exons and 3 @ flanking intron

**PSMA4=8.77 kb**
- 24 variants
  - Variant distribution: 7 @ UTR, 6 @ exons and 11 @ flanking intron

Wei et al. CEBP 2011 20:2603-9
Smoking Cessation

- 70% of smokers say they would like to quit
- 40% quit for at least 1 day each year, but 80% of them relapse within a month
- Only 3% of smokers quit successfully each year

Benowitz et al., 2010; Jha et al., 2013
Is *CHRNA5-A3-B4* involved in cessation?
Study Design

**U Wisconsin - TTURC**
- N=1073, European Ancestry
- Pharmacotherapy arms (Bupropion, NRT, combo) and one placebo arm
- Cessation

Abstinence at 60 days
Time to relapse over 60 days

**CHRNA5-A3-B4 Haplotypes**
- rs16969968
  Non-synonymous coding, Amino acid change in *CHRNA5*
- rs680244
  *CHRNA5* mRNA levels in brain and lung
- Combination of 2 variants
  - H1 (G_C, 20.8%)
  - H2 (G_T, 43.7%)
  - H3 (A_C, 35.5%)
Haplotypes predict cessation and response to medication

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>OR (Placebo)</th>
<th>OR (Treatment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1</td>
<td>0.98</td>
<td>1.11</td>
</tr>
<tr>
<td>H2</td>
<td>0.62</td>
<td>0.37</td>
</tr>
<tr>
<td>H3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Haplotype frequencies:
- H1 = G_C (20.8%)
- H2 = G_T (43.7%)
- H3 = A_C (35.5%)

Haplotypes predict abstinence in individuals receiving placebo medication

Haplotypes do not predict abstinence in individuals receiving active medication

The interaction of haplotypes and treatment is significant ($X^2=8.97$, df=2, $p=0.011$)
Response to Treatment Differs by Haplotype

a. Haplotype H1 (G_C)  
RH=0.83, p=0.36

b. Haplotype H2 (G_T)  
RH=0.48, p=2.7*10^{-8}

c. Haplotype H3 (A_C)  
RH=0.48, p=9.7*10^{-7}

Genetics can predict prognosis & inform treatment

- Smokers with the low risk haplotype (H1/G_C)
  - can quit more successfully without medication
  - do not benefit from medication

- Smokers with the high risk haplotype (H3/A_C)
  - have more difficulty quitting without medication
  - can benefit from medication with a 3-fold increase in cessation success
  - is associated with a 2-year delay in age of quitting
Chromosome 15q25 Is Important for Smoking
Manhattan plot for all CNPs and SNPs in the genome-wide analysis of Cigarettes per day

Kumasaka, Aoki, Okada, Takahashi, Ozaki, Mushiroda, Hirota, Tamari, Tanaka, Nakamura, Kamatani, Kubo, PLOS one 2012

Japanese population N=11696

Hits in green ($<10^{-5}$)

CYP2A6
Chromosome 22
$P<10^{-42}$

Nicotinic receptors
Chromosome 15
CYP2A6 Genotyping and Statistical Methods

CYP2A6 genotyping:

- Grouped by predicted metabolic activity

CYP2A6 normal (54%)

CYP2A6 reduced (46%)
a) Before conditioning

b) After conditioning on rs8102683 (CNV)

c) After conditioning on rs8102683 and rs11878604
Relative Nicotine (61 μM) metabolism by Expressed CYPs (Baculovirus): CYP2A6

Relative Velocity
(Cotinine formation)

Type of CYP
Genetically Reduced CYP2A6 increases nicotine plasma levels

Nicotine 4 mg base, oral  
Japanese subjects

Nicotine plasma (ng/ml) vs Time (hours)

- **Active Metabolizers**: *1/*1
  - Increase in nicotine levels over time
- **Normal Metabolizers**: *1/*4
  - Decrease in nicotine levels over time
- **Inactive Metabolizers**: *4/*4
  - Steady plasma levels

Ed Sellers
CYP2A6 Genotype: Fractional Clearance of Plasma NIC to COT
(Twin NIC infusion cohort)  Benowitz CPT 2006

Fractional Clearance of Plasma NIC to COT (Twin NIC infusion cohort)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Fractional Clearance (ml/min/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>*1/*1</td>
<td>15</td>
</tr>
<tr>
<td>*1/*12</td>
<td>12</td>
</tr>
<tr>
<td>*1/*9</td>
<td>13</td>
</tr>
<tr>
<td>*1/*2</td>
<td>8</td>
</tr>
<tr>
<td>*1/*4</td>
<td>6</td>
</tr>
<tr>
<td>*9/*9</td>
<td>4</td>
</tr>
<tr>
<td>*9/*12</td>
<td>4</td>
</tr>
<tr>
<td>*9/*4</td>
<td>4</td>
</tr>
</tbody>
</table>

Normal
Intermediate
Slow Metabolizers

P < 0.05
Does slow nicotine inactivation alter the amount smoked?

**Rationale:**
- Dependent smokers adjust their smoking behavior to maintain nicotine levels.
- Amount smoked $\approx 85\%$ genetic.

**Hypothesis:**
- Genetically slow nicotine metabolizers who are dependent smokers will smoke fewer cigarettes per day.
Slow metabolism decreases smoking
(# of cigarettes smoked and breath CO)

Rao et al., Mol Pharm 2002

Plasma NIC (ng/ml)  Cigarettes/ Day  Carbon Monoxide ppm

Not Significant  p < 0.001  p < 0.005

Normal Metabolizers
*1/*1 n=277

Slow Metabolizers
*1/*2 &*1/*4 n=14
Slow metabolizers smoke fewer cigarettes, Even at very early stages of smoking

Audrain-McGovern, Pediatrics 2007

![Graph showing smoking habits by metabolizer type and grade level.](chart.png)
Frequencies of CYP2A6 activity groups varies among ethnic groups (*2, *4-*10,*12, *17) N=2000

Malaiyandi et al, CPT 2005; Mwenifumbo et al., PG &G 2005, DAA 2007

Proportion of population %

- Poor metabolism
- Slow metabolism
- Intermediate metabolism

Japanese
- Korean
- Chinese
- African American
- Caucasian
Topography: CYP2A6 Slow metabolizers take smaller puffs

Andrew Strasser
NTR 2007

Mean Puff Volume (ml)

- Normal Metabolizers
  - P < 0.012
  - 54.3

- Slow Metabolizers
  - 44.9

Total Puff Volume (ml)

- Normal Metabolizers
  - P < 0.024
  - 676

- Slow Metabolizers
  - 512

P < 0.024
Pharmacogenetics of Treatment

Current treatment: low efficacy

- Genetic variation in response
- Tailor medication to genetic make-up
- Discover novel targets for drug development
Slow Metabolizers have better quit rates on placebo

Patterson et al 2008

% Quit

Placebo Arm

End of Treatment

Slow

32%

25%

20%

10%

Fast

1st Qrtl

2nd Qrtl

3rd Qrtl

4th Qrtl

3-HC: Cotinine Ratio in Quartiles

OR 0.27
Fast Metabolizers quit poorly on placebo, but respond well to Bupropion.

End of Treatment

3-HC: Cotinine Ratio in Quartiles

OR 4.6
Slow metabolizers respond well to Nicotine Patch

Lerman et al., Clin. Pharm. Ther. 2006, 2010

Replicated in multiple retrospective studies

Also looked at extended treatment
• even better for slow metabolizers
• no gain for fast metabolizers

Higher dose nicotine patch (pilot)
• may help faster metabolizers
Identification of a reliable marker for nicotine addiction treatment

Marker development
- Nicotine PK: heritability
- CYP2A6 variants
- CYP2A6 & metabolism
- Develop functional test-NMR

Proof of Association
- CYP2A6/NMR associations with response to Rx
- Replication in 4 independent trials

Proof of Efficacy and Utility
- Prospective stratified clinical trial
- Cost-effectiveness analysis
- Mechanistic studies

PNAT2: Clinical utility
2005-2010
2010-2015
Pharmacogenomic Trial Designs

**Restrospective**

ALL

- **DRUG A**
  - Responder
  - Nonresponder

- **DRUG B**
  - Responder
  - Nonresponder

**Prospective Stratified Test**

- Marker Positive
  - Drug A
  - Drug B

- Marker Negative
  - Drug A
  - Drug B

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**Best Uses:**
- Marker unknown at trial initiation
- Hypothesis generation
- Independent validation

**Limitations:**
- Unbalanced groups
- Reduced power
- Missing data

**Advantages:**
- Trial based on a priori hypothesis
- Allows for “enrichment”
- Balances Tx assignment
Pharmacogenomic Trial Designs

**Prospective Stratified**
- **Test**
  - Marker Positive
    - Drug A
    - Drug B
  - Marker Negative
    - Drug A
    - Drug B

**Prospective Screened**
- **ALL**
  - Genotype-Guided
    - Marker +
      - Drug A
    - Marker -
      - Drug B
  - Non-Guided
    - Drug A
    - Drug B

High ecological ('real world') validity

True Test of Whether Personalized Medicine is Effective?
Lost in Translation?

• Increase generalizability to clinical practice settings
• Demonstrate improvement of health outcomes and cost-effectiveness
• Establish evidence-based guidelines
• Enhance adoption in clinical practice

Khoury, Genomic Medicine, 2009
Prospective Randomized Trial

**Slow Metabolizers (oversampled)**

- **A** Placebo
- **B** Transdermal Nicotine
- **C** Varenicline
- **D** Placebo
- **E** Transdermal Nicotine
- **F** Varenicline

**Test**

- **N=675**

**Normal Metabolizers**

- **N=675**
Smokescreen® Genotyping Array
A platform for genetic research on smoking, addiction, and treatment approaches

Summary
- Genotyping array with 646,247 markers designed for studies of addiction, smoking, downstream consequences and treatment
- Developed as part of a SBIR contract with the National Institute on Drug Abuse (NIDA)
- High coverage in multiple populations (African, Asian, European)
- Available companion services by BioRealm
  - Unified quality control and analysis
  - Software interface to results
  - Genotyping at partner labs
GAME-ON OncoArray

**Common Content** – 40K
Fine-mapping of common cancer susceptibility loci (*TERT*, 8q24 (proximal and distal to *MYC*), *HNF1B, TET2, RAD51B, 11q13, MERIT40, MDM4)*
- Ancestry Informative Markers
- Cross-Site meta analysis
- Pharmacogenetic components
- eQTL (Height, Weight, BMI, WHR, Menarche, Menopause etc)
- Other cancers published GWAS variants
- Chromosome X and mitochondrial DNA variants

**GWAS Backbone**
- 260K
- Illumina Core

**OncoChip**
- 600K beadtypes

**Cancer Specific Variants**
- Lung
- Colon
- Breast
- Prostate
- Ovarian
  (proportional allocation)
Challenges in application of pharmacogenetics for smoking cessation

- Two different loci, each having multiple variants, influence smoking cessation. Genetic markers are effective for CHRNAs but serum markers may be better for characterizing CYP2A6 variation.
- Will smokers seeking to quit wait for test results before starting therapy?