Smoking: Biochemical Dependence and Genetics of Lung Cancer

Letty F. Schuman, MD
November 5, 2012
Objectives

- Discuss a brief history of tobacco.
- Discuss the biochemical dependence of nicotine.
- Discuss genetic polymorphisms of CYP2A6 and nicotine dependence.
- Understand the molecular abnormalities of KRAS and p53 in lung cancer.
- Discuss genetic polymorphisms (CYP1A1 and CYP2E1) and lung cancer susceptibility.
2.5 million year old fossilized tobacco leaves discovered in northeastern Peru in 2010

6000 BC: Tobacco plant first cultivated in the Americas

Circa 1 BC: Indigenous Americans begin smoking tobacco and using for medical use (tobacco enemas)

1492: Columbus discovers tobacco in Cuba and takes to Europe
- 1600: Tobacco introduced in India by Portuguese where it quickly becomes valuable in barter trade
- 1604: King James I writes *A Counterblaste to Tobacco* (1st anti-smoking literature)
- 1612: Tobacco first grown commercially in Americas
- 1761: First study of effects of tobacco by John Hill in England → snuff users at risk for nasal cancers
- 1826: Nicotine 1st isolated from tobacco plant

![Nicotine chemical structure](image)
1913: Birth of the “modern” cigarette (pre-blended and packaged) by RJ Reynolds, introduces Camel brand

1938: “Smoking Causes Cancer” by Drs. Alton Ochsner and Michael DeBakey - 1st link between smoking and lung cancer

1964: Surgeons General’s Reports determines that smoking causes lung cancer in men

1966: Health warnings on cigarette packs

1971: Television ads for cigarettes taken off air in U.S.
1982: Surgeon General reports that second hand smoke may cause lung cancer

1985: Lung cancer becomes the #1 killer of women (previously breast cancer)

1994: CEOs of cigarette companies testify before Congress that it is their opinion that nicotine is not addictive

2012: Per WHO, tobacco kills nearly 6 million people/year; 100 million deaths in 20th century
Nicotine

- Alkaloid found in leaves of tobacco plant, *Nicotiana tabacum*
- Approximately 0.6-3% of the dry weight of tobacco
- Average cigarette contains 10-15 mg of nicotine with smokers inhaling 1-3 mg (=10 puffs of cigarette)
  - 60 mg can be fatal
- Major addictive component of tobacco
Nicotine Dependence

- After inhalation, nicotine
  - Travels to the lungs → alveoli
  - Absorbed into the bloodstream via capillary air exchange
  - Crosses brain-barrier within 10-20 seconds
• Neurons communicate via synapses
• Electrical signal from neuron #1 triggers release of neurotransmitters into the synaptic space
• Neurotransmitter attaches to specific receptor on dendrite of neuron #2 causing a cellular response
Acetylcholine Receptors

- Acetylcholine is a major natural neurotransmitter in the brain
  - Binds to acetylcholine receptors
  - Produce feelings of pleasure, reward, alertness, concentration, reduced anxiety
- Causes conformation change in receptor and opens channel pore
- Allows positively charged ions to flow across membrane (Na+ inward, K+ outward) for positive net flow inward
- Causes depolarization → electrical impulse carried down the nerve
- After a few milliseconds, the channel closes and the receptor is temporarily unresponsive to any neurotransmitters
Nicotinic Acetylcholine Receptors

- Nicotine mimics the actions of acetylcholine
  - Binds directly to certain acetylcholine receptors (nicotinic acetylcholine receptors, or nAChRs)
    - Pentameric ion channel (3α, 2β) with 12 different possible subunit combinations, α2-α10, β2-β4
  - Nicotine activates more nAChRs than acetylcholine due to higher nicotine concentrations causing heightened responses
• Nicotine also triggers release of another neurotransmitter, Dopamine, the “pleasure neurotransmitter”

• Contributes to development and maintenance of rewarding behaviors within the nucleus accumbens and frontal cortex
Nicotine Dependence

- Effects are short lived
  - Distribution $\frac{1}{2}$ life of 15-20 minutes
  - Elimination $\frac{1}{2}$ life of 1-2 hours

- When there is no nicotine, the cell does not get as excited when it binds with normal neurotransmitter (ACh)
  - This makes the smoker want to have another cigarette to achieve the same effect.

- Withdrawal symptoms (headaches, tremors, shakiness, irritability, etc.) when nicotine absent
Nicotine Dependence

- Chronic nicotine exposure causes desensitization of nAChRs
  - Decrease or loss of biological response following prolonged or repetitive stimulation

- Chronic nicotine exposure increases (upregulates) the number of nAChRs with high-affinity binding to nicotine (~90% α4β2 receptors)

- More nicotine to overcome desensitization and more nicotinic receptor binding to experience same effects (tolerance)
Figure 1.12 PET images of nicotinic receptors in the human brain. The increased number of nicotinic receptors (red and yellow) in the smoker’s brain is dramatic. Top view (left), side view (middle), front view (right).
Genetics of Nicotine Dependence

- In vivo, ~80% of nicotine is oxidized to cotinine (inactive metabolite)
- ~90% of oxidation is mediated by CYP2A6 enzyme in the liver
  - CYP2A6 part of the superfamily of cytochrome P450 enzymes that metabolize ~75% of drugs
- Negative correlation between nicotine plasma levels and cigarette cravings
- Positive correlation between nicotine elimination rate and cigarette cravings
CYP2A6 Polymorphisms

- At least 26 known polymorphisms in the CYP2A6 gene (differences in coding regions, e.g. single base pair exchange)
  - Some with known affects on enzyme activity → nicotine metabolism → smoking behaviors/dependence
- For example, CYP2A6*2, *4, *9, *12 associated with decreased or absent nicotine metabolism ("poor metabolizers")
CYP2A6 Polymorphisms

- Poor metabolizers are shown to have:
  - Decreased risk for smoking
  - Lower cigarette consumption
  - Shorter duration of smoking
  - Increased ability to quit
  - More prevalence in nonsmoking populations

- With prolonged or elevated nicotine levels
  - Less craving for cigarettes
  - May experience adverse side effects (nausea and dizziness)
CYP2A6 Polymorphisms

- Frequency of CYP2A6 polymorphisms vary in different ethnic groups
  - Asian population has very low prevalence of certain CYP2A6 polymorphisms (CYP2A6*2, *12) and may contribute to high number of smokers/smoking related lung cancers
  - Hispanic population very high prevalence of CYP2A6*4 polymorphism and smoking is lower than in the general population
Is Nicotine a Carcinogen?

- Short answer: No
- Not given a cancer rating by the International Agency for Research on Cancer (IARC)
  - Not analyzed as a carcinogen in pure form
- IARC Classification System
  - Group I – Carcinogenic to humans (108 agents)
  - Group 2A – Probably carcinogenic (64 agents)
  - Group 2B – Possibly carcinogenic (272 agents)
  - Group 3 – Not classifiable as to its carcinogenicity (508) agents
  - Group 4 – Probably not carcinogenic (1 agent)
Tobacco Smoke Carcinogens

- Polycyclic aromatic hydrocarbons (PAHs)
  - Benzo(a)pyrene (pro-carcinogen) → Benzo(a)pyrene diol-epoxide (carcinogen)
    - IARC Group I Carcinogen
    - Intercalates in DNA by covalent binding (DNA adduct)
    - Preferentially forming DNA adducts at guanine nucleotides
      - Guanine (G) DNA adduct distorts double-helical DNA structure, and is misread most often as a thymine (T)
      - Original G-C base pair ultimately replaced by a T-A base pair
Lung Cancer

- “Cancer is the result of molecular changes that occur in the cell, resulting in the deregulation of pathways that control normal cellular growth, differentiation, and apoptosis.”

- Pathways contain proteins commonly altered or lost in cancer

- Proto-oncogenes and tumor suppressor genes
  - KRAS and p53
Proto-oncogenes, K-ras

Mediates cell entrance into G1 phase of cell cycle
KRAS Proto-oncogene

- Proto-oncogene is a native growth promoting gene
- Oncogene is the altered form of proto-oncogene (mutation or increased expression) which can cause cancer
- KRAS gene is a proto-oncogene whose protein, K-Ras, functions as a GTPase that converts GTP → GDP
K-ras Protein

- K-ras acts as a molecular on/off switch
  - When turned on, recruits and activates proteins for growth factor production
- K-ras binds to GTP and upon conversion to GDP, K-ras turned off
  - Conversion usually slow process, regulated by GTPase-activating proteins (GAPs)
  - Guanine nucleotide exchange factors (GEFs) activate GTPases by stimulating release of GDP from K-ras to allow binding of GTP
KRAS Oncogene

- KRAS oncogene produces K-ras protein that binds to GTP and still transmits signal but no longer functions as GTPase
  - Permanent activation of K-ras proteins causes cell growth and division

- KRAS gene mutations are exclusively missense mutations (single nucleotide is changed) at codons 12, 13, or 61
  - Most commonly substitution of guanine to thymine (G to T)
  - Benzo(a)pyrene diol epoxide guanine DNA adduct → thymine replacement
  - K-ras mutations in >50% of non-small cell lung carcinomas
Cell Cycle

Proto-oncogenes, K-ras
Mediates cell entrance into G1 phase of cell cycle

Tumor suppressor gene, p53
Prevents progression through G1 phase
Initiation of G1 Phase

- Initiated by extracellular signals inducing transcription of cyclinD
- Part of family of proteins, cyclins, that control the progression of cell through the cell cycle
- Forms a complex with cdk4 or 6 (cyclin-dependent kinase) = cdk-cyclin complex
- Enables kinase activity (phosphorylation) of Retinoblastoma protein (pRB) within the Rb-E2F complex
  - Rb – tumor suppressor protein that prevents E2F transcription factor from interacting with cell and cause proliferation
Progression of G1 Phase

- Regulated by proteins called ‘inhibitors of cdk-cyclin complexes,’ in 2 families
  - Inhibitor of cdk4 (INK4) family
  - Kinase inhibitory protein (KIP) family

- INK4 family of proteins (p15, p16, p18, and p19) binds to and inhibits cdk4 or 6
  - Prevents formation of cdk-cyclin complex, phosphorylation of pRB, and activation of E2F
p53 Tumor Suppressor Gene

- KIP family of proteins (p21, p27, and p57) inhibits the formation of cdk-cyclin complexes
  - Prevents phosphorylation of pRB, and activation of E2F
- KIP family of proteins is regulated by the p53 tumor suppressor gene
  - “Guardian of the genome” - initiates growth arrest for DNA repair in the presence of damaged DNA before passage to S phase
    - Once cell passes through to S phase, it is committed to mitoses, therefore activation of p53 results in apoptosis
**p53 Tumor Suppressor Gene**

- p53 is located on the short arm of chromosome 17 (17p13.1)
- p53 gene mutations are most commonly missense mutations along “hotspots” of highly conserved regions (exons 5-8)
  - Most commonly substitution of guanine to thymine (G to T)
    - Benzo(a)pyrene diol epoxide guanine DNA adduct → thymine replacement
    - Mutation inactivates p53 tumor suppressor protein
- p53 mutations in >50% of non-small cell lung carcinomas and ~70% in small cell lung carcinomas
Exogenous growth factor binding to growth factor receptor

Ras → G0

(Myc) → (Myc) → Max

Cdk4,6
Cyclin D
G1

Cdk2
Cyclin A, B
G2

S

E2F
P
RB

E2F
P
RB

Cdk2
Cyclin E

p16/INK4

p21/KIP

P53
Lung Cancer Susceptibility

- Lung cancer is most lethal cancer worldwide despite improvements in diagnostic and therapeutic techniques
- Smoking is major cause of lung cancer
  - Not all smokers develop cancer
  - Genetic susceptibility to lung cancer likely exists
    - Polymorphisms in genes controlling carcinogen metabolism
- Encourage lifestyle changes/avoidance of tobacco
- Appropriate/increased screening
- Earlier detection/treatment
Cytochrome P450

- Tobacco procarcinogens are metabolized by superfamily of cytochrome P450 (CYP) enzymes by oxidation
  - P450s account for ~75% of drug metabolism
- P450s show extensive structural polymorphisms (differences in coding regions)
- Polymorphisms in CYP1A1 and CYP2E1 and genetic susceptibility to lung cancer studied with conflicting results
  - Meta-analyses to help define association
Meta-analysis

- “A quantitative statistical analysis of several separate but similar experiments or studies in order to test the pooled data for statistical significance.”
  - Merriam-Webster dictionary

- Systematic review – “Comprehensive review of all relevant studies on a particular topic/question ..and summarizing the findings.”
  - George Washington University (http://www.gwumc.edu/library/tutorials/studydesign101/systematicreviews.html)
CYP1A1 MspI polymorphisms and lung cancer risk: An updated meta-analysis involving 20,209 subjects

Ya-nan Ji, Qin Wang, Xin-qing Lin, Li-jun Suo

A B S T R A C T

Published data describing the association between CYP1A1 MspI gene polymorphism and lung cancer risk are inconclusive. To determine a more conclusive relationship, we performed an updated meta-analysis of all eligible studies and conducted the subgroup analysis by stratification according to the ethnicity source, histological types of lung cancer, gender and smoking status of case and control populations. A total of 51 studies comprising 20,209 subjects were included in the analysis. A significantly elevated lung cancer risk was associated with two variant genotypes (for TT vs CC: OR = 1.24, 95% CI = 1.11–1.40; for CT and TT combined vs CC: OR = 1.19, 95% CI = 1.12–1.27) in the overall population. In the stratified analysis, significantly higher risks associated with lung cancer were found in Asians, Caucasians, lung SCC, lung AC and the male population. In contrast, negligible risks were found in the mixed population, lung SCLC and the female population. Additionally, a significant association was found in the smoker population, whereas no association was found in non-smoker populations. This meta-analysis suggests that the MspI polymorphisms of CYP1A1 correlate with increased lung cancer susceptibility, and that there is an interaction between the CYP1A1 polymorphism and smoking. However, the associations vary in different ethnic populations, histological types of lung cancer and the gender of case and control populations.

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Cytochrome P450 1A1 (CYP1A1)

- CYP1A1 metabolizes several procarcinogens, particularly PAHs (benzo(a)pyrene) whose products form DNA adducts → lung cancer
- Single nucleotide polymorphism (SNP) has been identified and studied in CYP1A1 gene
  - T → C exchange in MspI restriction site in the 3’ non-coding region
  - MspI restriction site polymorphism results in 3 genotypes
    - Predominant homozygous m1 allele (wild)
    - Heterozygous m1/m2
    - Rare homozygous m2 allele
- Past published data conflicting in describing association between CYP1A1 MspI gene polymorphism and lung cancer risk
- 51 studies examined with 7,993 lung cancer cases and 12,216 controls
Summary of Odds Ratios (OR) and P values

Table 2
Summary of the ORs for various contrasts of CYP1A1 Mspl gene polymorphisms in the meta-analysis.

<table>
<thead>
<tr>
<th>Subgroup analysis</th>
<th>Studies</th>
<th>T allele vs C allele</th>
<th></th>
<th>TT vs CC</th>
<th></th>
<th>(CT + TT) vs CC</th>
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<td></td>
<td></td>
<td>OR (95%) $P_h$</td>
<td></td>
<td>OR (95%) $P_h$</td>
<td></td>
<td>OR (95%) $P_h$</td>
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<tr>
<td>Total</td>
<td>51</td>
<td>1.16 (1.10–1.29) 0.002</td>
<td></td>
<td>1.24 (1.11–1.40) 0.003</td>
<td></td>
<td>1.19 (1.12–1.27) 0.000</td>
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<td>Ethnicity</td>
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<td>Asian</td>
<td>27</td>
<td>1.26 (1.14–1.42) 0.002</td>
<td></td>
<td>1.22 (1.10–1.40) 0.004</td>
<td></td>
<td>1.30 (1.18–1.44) 0.002</td>
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<td>Caucasian</td>
<td>12</td>
<td>1.24 (1.10–1.36) 0.036</td>
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<td>1.23 (1.08–1.34) 0.053</td>
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<td>1.29 (1.13–1.46) 0.046</td>
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<td>Mixed population</td>
<td>12</td>
<td>1.08 (0.90–1.18) 0.330</td>
<td></td>
<td>1.05 (0.89–1.28) 0.140</td>
<td></td>
<td>1.02 (0.92–1.14) 0.330</td>
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<td>Histological type</td>
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<td>SCC</td>
<td>14</td>
<td>1.84 (1.56–2.24) 0.000</td>
<td></td>
<td>1.77 (1.48–2.01) 0.005</td>
<td></td>
<td>1.81 (1.52–2.14) 0.000</td>
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<tr>
<td>AC</td>
<td>13</td>
<td>1.28 (1.05–1.44) 0.002</td>
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<td>1.34 (1.11–1.52) 0.014</td>
<td></td>
<td>1.19 (1.01–1.41) 0.000</td>
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<td>SCLC</td>
<td>9</td>
<td>0.96 (0.71–1.30) 0.456</td>
<td></td>
<td>0.88 (0.68–1.06) 0.864</td>
<td></td>
<td>0.98 (0.73–1.32) 0.976</td>
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<td>Gender</td>
<td></td>
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<td>Male</td>
<td>3</td>
<td>1.42 (1.04–1.78) 0.210</td>
<td></td>
<td>1.39 (1.23–1.79) 0.210</td>
<td></td>
<td>1.46 (1.07–1.98) 0.380</td>
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<td>Female</td>
<td>8</td>
<td>0.94 (0.79–1.16) 0.000</td>
<td></td>
<td>0.98 (0.88–1.26) 0.003</td>
<td></td>
<td>0.91 (0.77–1.08) 0.000</td>
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<td>Smoking status</td>
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<td>Smokers</td>
<td>9</td>
<td>1.78 (1.46–2.18) 0.003</td>
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<td>1.62 (1.33–1.96) 0.000</td>
<td></td>
<td>1.75 (1.44–2.13) 0.003</td>
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<td>Non-smokers</td>
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<td>1.16 (0.98–1.48) 0.168</td>
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<td>1.18 (0.95–1.58) 0.086</td>
<td></td>
<td>1.14 (0.95–1.36) 0.114</td>
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</table>

- **CC** = Predominant homozygous m1 allele (wild)
- **CT** = Heterozygous m1/m2
- **TT** = Rare homozygous m2 allele
Association between CYP2E1 genetic polymorphisms and lung cancer risk: A meta-analysis

Yadong Wang a,*, Haiyan Yang b, Li Li a, Haiyu Wang a, Congke Zhang a, Gongju Yin a, Baoyu Zhu a

a Henan Center for Disease Control and Prevention, Zhengzhou 450016, PR China
b Department of Epidemiology and Health Statistics, School of Public Health, Zhengzhou University, Zhengzhou 450001, PR China

ABSTRACT

Genetic variations in metabolic genes are thought to modify the metabolic process of carcinogens and are suggested to be related to cancer risk. However, epidemiological results are not always consistent. In this meta-analysis, we assessed reported studies of associations between polymorphisms of CYP2E1 Rsal/PstI and Dral, and the risk of lung cancer. We found decreased lung cancer risk among subjects carrying CYP2E1 Rsal/PstI c1/c2 and c1/c2 + c2/c2 genotype [odds ratio (OR) = 0.80, 95% confidence interval (CI): 0.72–0.89 and OR = 0.82, 95% CI: 0.72–0.93, respectively], using 4436 cases and 6385 controls from 26 studies. We also observed a decreased lung cancer risk among subjects carrying c1/c2 and c1/c2 + c2/c2 genotypes in the Asian population and on the basis of population control in stratified analysis. We found a protective effect of the CYP2E1 Dral CC and CD + CC polymorphisms for lung cancer (OR = 0.58, 95% CI: 0.41–0.81 and OR = 0.84, 95% CI: 0.73–0.96, respectively). The meta-analysis suggests that CYP2E1 Rsal/PstI and Dral polymorphisms may affect the susceptibility of lung cancer, and a study with a larger sample size is needed to further evaluate gene–environment interaction on CYP2E1 polymorphisms and lung cancer risk.

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Cytochrome P4502E1 (CYP2E1)

- CYP2E1 is an enzyme involved in oxidation (metabolic inactivation) of low molecular weight carcinogens, such as nitrosamines in cigarette smoke.
- Located on chromosome 10q24.3-qter
  - 18,754 bp long
  - 9 exons
  - 8 introns
- Contains 6 restriction fragment length polymorphisms
- CYP2E1 RsaI/PstI and DraI gene polymorphisms studied extensively with inconsistent results in describing association with lung cancer risk.
CYP2E1 RsaI/PstI Polymorphisms

- 2 base pair exchanges in the 5’ flanking region (RsaI and PstI restriction sites)
  - Complete linkage disequilibrium
  - Associated with increased transcription/enzyme activity
  - RsaI/PstI restriction site polymorphisms result in 3 genotypes
    - Predominant homozygous c1 allele (wild)
    - Heterozygous c1/c2
    - Rare homozygous c2 allele

- 26 studies examined with 4,436 lung cancer cases and 6,385 controls
<table>
<thead>
<tr>
<th>First author</th>
<th>Year</th>
<th>No. of cases</th>
<th>No. of controls</th>
<th>Source of controls</th>
<th>Ethnicity of subjects</th>
<th>OR(95% CI)</th>
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<td>67</td>
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<td>137</td>
<td>206</td>
<td>Population</td>
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<td>337</td>
<td>454</td>
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<td>2.61(0.63–10.75)</td>
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<td>Li</td>
<td>2000</td>
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<td>Population</td>
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<td>0.45(0.24–0.78)</td>
<td>0.67(0.15–2.92)</td>
<td>0.45(0.26–0.80)</td>
</tr>
<tr>
<td>Qu</td>
<td>1998</td>
<td>182</td>
<td>184</td>
<td>Hospital</td>
<td>Chinese</td>
<td>0.77(0.50–1.17)</td>
<td>2.16(0.54–8.58)</td>
<td>0.82(0.54–1.23)</td>
</tr>
<tr>
<td>Li</td>
<td>2004</td>
<td>217</td>
<td>200</td>
<td>Hospital</td>
<td>Chinese</td>
<td>1.00(0.66–1.50)</td>
<td>2.23(1.05–4.75)</td>
<td>1.15(0.78–1.70)</td>
</tr>
<tr>
<td>Quinones</td>
<td>2001</td>
<td>59</td>
<td>148</td>
<td>Hospital</td>
<td>Chinese</td>
<td>0.82(0.40–1.65)</td>
<td>0.33(0.02–6.54)</td>
<td>0.76(0.38–1.53)</td>
</tr>
<tr>
<td>Wang</td>
<td>1999</td>
<td>119</td>
<td>231</td>
<td>Hospital</td>
<td>Taiwanese</td>
<td>0.86(0.55–1.41)</td>
<td>0.1(0.01–0.84)</td>
<td>0.75(0.48–1.19)</td>
</tr>
<tr>
<td>Watanabe</td>
<td>1995</td>
<td>316</td>
<td>503</td>
<td>Population</td>
<td>Japanese</td>
<td>0.95(0.70–1.29)</td>
<td>1.28(0.60–2.72)</td>
<td>0.98(0.73–1.31)</td>
</tr>
<tr>
<td>Wang</td>
<td>2003</td>
<td>164</td>
<td>181</td>
<td>Hospital</td>
<td>Chinese</td>
<td>0.56(0.37–0.91)</td>
<td>0.05(0.00–0.79)</td>
<td>0.52(0.34–0.81)</td>
</tr>
<tr>
<td>Oyama</td>
<td>2003</td>
<td>126</td>
<td>612</td>
<td>Population</td>
<td>Japanese</td>
<td>0.73(0.47–1.14)</td>
<td>1.26(0.53–3.00)</td>
<td>0.79(0.53–1.20)</td>
</tr>
<tr>
<td>Persson</td>
<td>1993</td>
<td>184</td>
<td>202</td>
<td>Population</td>
<td>Swedish</td>
<td>0.44(0.19–1.02)</td>
<td>0.34(0.01–8.52)</td>
<td>0.41(0.18–0.96)</td>
</tr>
<tr>
<td>Persson</td>
<td>1999</td>
<td>76</td>
<td>113</td>
<td>Population</td>
<td>Chinese</td>
<td>0.78(0.42–1.43)</td>
<td>0.44(0.08–2.26)</td>
<td>0.74(0.41–1.33)</td>
</tr>
<tr>
<td>El-Zein</td>
<td>1997</td>
<td>52</td>
<td>48</td>
<td>Volunteer</td>
<td>Galveston–Houston area</td>
<td>3.58(0.70–18.16)</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>Gu</td>
<td>2007</td>
<td>279</td>
<td>684</td>
<td>Hospital and Volunteer</td>
<td>Chinese</td>
<td>Unknown</td>
<td>Unknown</td>
<td>0.96(0.72–1.27)</td>
</tr>
<tr>
<td>London</td>
<td>1996</td>
<td>341</td>
<td>706</td>
<td>Population</td>
<td>African–American and Caucasian</td>
<td>0.64(0.34–1.22)</td>
<td>Unknown</td>
<td>0.64(0.34–1.22)</td>
</tr>
<tr>
<td>Eom</td>
<td>2009</td>
<td>387</td>
<td>387</td>
<td>Hospital health</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Unknown</td>
<td>0.87(0.65–1.17)</td>
</tr>
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</table>
Summary of ORs and P values for Rsal/PstI association to lung cancer

<table>
<thead>
<tr>
<th>Genotype</th>
<th>OR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>All populations</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c1/c2 vs c1/c1</td>
<td>0.80 (0.72-0.89)</td>
<td>0.09</td>
</tr>
<tr>
<td>c2/c2 vs c1/c1</td>
<td>1.01 (0.65-1.55)</td>
<td>0.002</td>
</tr>
<tr>
<td>c1/c2 + c2/c2 vs c1/c1</td>
<td>0.82 (0.72-0.93)</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>Asian population only</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c1/c2 vs c1/c1</td>
<td>0.81 (0.69-0.96)</td>
<td>0.03</td>
</tr>
<tr>
<td>c2/c2 vs c1/c1</td>
<td>1.17 (0.75-1.82)</td>
<td>0.004</td>
</tr>
<tr>
<td>c1/c2 + c2/c2 vs c1/c1</td>
<td>0.86 (0.74-0.99)</td>
<td>0.01</td>
</tr>
</tbody>
</table>
CYP2E1 DraI Polymorphism

- T→A exchange within intron 6
  - Associated with increased transcription/enzyme activity
  - DraI restriction site polymorphism results in 3 genotypes
    - Predominant homozygous D allele (wild)
    - Heterozygous C/D
    - Rare homozygous C allele
- 13 studies examined with 1,666 lung cancer cases and 2,093 controls
<table>
<thead>
<tr>
<th>First author</th>
<th>Year</th>
<th>No. of cases</th>
<th>No. of controls</th>
<th>Source of controls</th>
<th>Ethnicity of subjects</th>
<th>CD</th>
<th>CC</th>
<th>CD + CC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kato</td>
<td>1994</td>
<td>58</td>
<td>38</td>
<td>Hospital</td>
<td>Japanese, Caucasian and African–American</td>
<td>1.72</td>
<td>0.55–5.36</td>
<td>1.72(0.55–5.36)</td>
</tr>
<tr>
<td>Le Marchand</td>
<td>1998</td>
<td>338</td>
<td>452</td>
<td>Population</td>
<td>Caucasian, Japanese and Hawaiians</td>
<td>0.98</td>
<td>0.71–1.35</td>
<td>0.86(0.63–1.16)</td>
</tr>
<tr>
<td>Wu</td>
<td>1998</td>
<td>126</td>
<td>193</td>
<td>Population</td>
<td>African–American and Mexican–American</td>
<td>0.47</td>
<td>0.25–0.88</td>
<td>0.46(0.25–0.84)</td>
</tr>
<tr>
<td>Uematsu</td>
<td>1992</td>
<td>74</td>
<td>73</td>
<td>Hospital</td>
<td>Japanese</td>
<td>1.79</td>
<td>0.89–3.60</td>
<td>1.28(0.67–2.46)</td>
</tr>
<tr>
<td>Liang</td>
<td>2004</td>
<td>152</td>
<td>152</td>
<td>Hospital</td>
<td>Chinese</td>
<td>0.84</td>
<td>0.53–1.35</td>
<td>0.85(0.54–1.34)</td>
</tr>
<tr>
<td>Qu</td>
<td>1998</td>
<td>174</td>
<td>178</td>
<td>Hospital</td>
<td>Chinese</td>
<td>0.85</td>
<td>0.55–1.32</td>
<td>0.89(0.58–1.35)</td>
</tr>
<tr>
<td>Quinones</td>
<td>2001</td>
<td>58</td>
<td>129</td>
<td>Hospital</td>
<td>Chilean</td>
<td>1.33</td>
<td>0.69–2.56</td>
<td>1.23(0.65–2.32)</td>
</tr>
<tr>
<td>Wang</td>
<td>1999</td>
<td>119</td>
<td>231</td>
<td>Hospital</td>
<td>Taiwanese</td>
<td>0.73</td>
<td>0.45–1.18</td>
<td>0.70(0.45–1.11)</td>
</tr>
<tr>
<td>Uematsu</td>
<td>1991</td>
<td>47</td>
<td>56</td>
<td>Unknown</td>
<td>Japanese</td>
<td>1.71</td>
<td>0.75–3.87</td>
<td>1.26(0.58–2.76)</td>
</tr>
<tr>
<td>Persson</td>
<td>1993</td>
<td>193</td>
<td>206</td>
<td>Population</td>
<td>Swedish</td>
<td>0.90</td>
<td>0.54–1.51</td>
<td>0.86(0.51–1.42)</td>
</tr>
<tr>
<td>Hirvonen</td>
<td>1993</td>
<td>101</td>
<td>121</td>
<td>Population</td>
<td>Finnish</td>
<td>0.66</td>
<td>0.32–1.35</td>
<td>0.72(0.36–1.44)</td>
</tr>
<tr>
<td>Persson</td>
<td>1999</td>
<td>76</td>
<td>112</td>
<td>Population</td>
<td>Chinese</td>
<td>0.64</td>
<td>0.34–1.20</td>
<td>0.69(0.38–1.24)</td>
</tr>
<tr>
<td>Li</td>
<td>2008</td>
<td>150</td>
<td>152</td>
<td>Hospital</td>
<td>Chinese</td>
<td>0.78</td>
<td>0.48–1.26</td>
<td>0.76(0.48–1.20)</td>
</tr>
</tbody>
</table>
Summary of ORs and P values for Dral association to lung cancer

<table>
<thead>
<tr>
<th>Genotype</th>
<th>OR (95%CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD vs DD</td>
<td>0.89 (0.76-1.02)</td>
<td>0.1</td>
</tr>
<tr>
<td>CC vs DD</td>
<td>0.58 (0.41-0.81)</td>
<td>0.002</td>
</tr>
<tr>
<td>CD + CC vs DD</td>
<td>0.84 (0.73-0.96)</td>
<td>0.01</td>
</tr>
</tbody>
</table>
Summary Points

- Nicotine is major addictive component in tobacco
- Biochemical dependence of nicotine mediated by desensitization and upregulation of nAChRs
- CYP2A6 polymorphisms causing decreased nicotine metabolism associated with decreased smoking/dependence
- Benzo(a)pyrene causes G to T mutation in KRAS proto-oncogene and p53 tumor suppressor genes, found in many lung cancers
- Cytochrome P450 polymorphisms cause increased metabolism of procarcinogens/carcinogens associated with lung cancer risk
  - CYP1A1 MstI associated with increased risk of lung cancer
  - CYP2E1 RsaI/PstI and DraI may be associated with decreased risk of lung cancer, more studies needed


http://www.who.int/tobacco/en/atlas2.pdf
http://archive.tobacco.org/History/Tobacco_History.html
http://rise.duke.edu/seek/pages/page.html?0105
http://www.iarc.fr
Restriction Fragment Length Polymorphisms

![Diagram showing restriction fragment length polymorphisms (RFLP) analysis. The MstI restriction sites are present in the normal sample, but a mutation destroys one restriction site in the disease sample. Digestion and separation by gel electrophoresis and Southern blotting are demonstrated, with the disease sample showing a difference in band patterns compared to the normal sample.]