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Combinations of Cytochrome P-450 Genotypes and Risk of Early-onset Lung Cancer in Caucasians and African Americans: A Population-based Study

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Abstract

Polymorphisms in *CYP1A1* and *CYP1B1* genes in humans are associated with reduction of enzymatic activity towards several substrates, including those found in tobacco smoke. To investigate the potential role these polymorphisms have as modulators of early-onset lung cancer risk, a population-based case-control study involving early-onset lung cancer cases was performed. Biological samples were available for 383 individuals diagnosed prior to 50 years of age identified from the metropolitan Detroit Surveillance, Epidemiology and End Results (SEER) program and 449 age, race and sex-matched controls ascertained through random digit dialing. Genotype frequencies varied significantly by race for *CYP1A1* exon 7 Ile⁴⁶²Val and *CYP1B1* Leu⁴³²Val genotypes, so all analyses were stratified by race. No association was seen between lung cancer risk and polymorphisms in *CYP1A1* MspI or *CYP1B1* Leu⁴³²Val for Caucasians or African Americans, after adjusting for age at diagnosis, sex, pack years of smoking and family history of lung cancer. In Caucasians, those with the Ile/Val genotype at *CYP1A1* exon 7 locus were at decreased risk of having lung cancer compared to those with the Ile/Ile genotype, after adjusting for age at diagnosis, sex, pack years of smoking and family history of cancer (OR=0.41 95% CI 0.19–0.90). These results were not replicated among the African American population, nor were they modified by amount of smoking.

Keywords

lung cancer; cytochrome p-450 polymorphisms; African Americans; Caucasians; smoking

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Conflict of interest statement

None declared.

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INTRODUCTION

Lung cancer is the leading cause of cancer-related deaths world-wide [1]. There is much interest in determining individual risk associated with lung cancer development. Several metabolic enzymes are currently under investigation for their possible role in lung cancer susceptibility, including members of the cytochrome P450 (CYP) superfamily. As Phase I enzymes, these enzymes catalyze one of the first steps in the metabolism of carcinogens. This oxidizing step often creates more reactive intermediates that are capable of binding with DNA and causing genetic mutations [2]. Two CYP genes are of particular interest, *CYP1A1* and *CYP1B1*. In the lung, it appears that *CYP1B1* has higher levels of basal mRNA and protein expression than *CYP1A1* [3], but that *CYP1A1* is more highly inducible [4].

We examined two polymorphisms in *CYP1A1*, an *MspI* cut site in an intron (*CYP1A1 MspI*) and an isoleucine to valine change in the heme binding region of exon 7 (*CYP1A1 Ile⁴⁶²Val*). We also examined an amino acid change in *CYP1B1* from leucine to valine at codon 432 (*CYP1B1 Leu⁴³²Val*), which has been associated with elevated levels of DNA adducts within white blood cells [5]. This study utilizes one of the largest collections of population-based, early-onset lung cancer patients to evaluate the connection between three *CYP* polymorphisms and lung cancer risk.

MATERIALS AND METHODS

Population Sample

Eligible early-onset cases were identified through the metropolitan Detroit Cancer Surveillance System (MDCSS), a participant in the National Cancer Institute's Surveillance, Epidemiology and End Results (SEER) program. Cases were defined as those with a primary neoplasm of the lung or bronchus, less than 50 years of age, and residing in the Wayne, Macomb and Oakland counties of Michigan at the time of diagnosis. Data collection has taken place continuously since September 15, 1990. Cases less than 45 were over sampled in the early years of the study. Population-based controls were ascertained concurrently via random digit dialing, frequency matched to cases by race, sex, 5-year age group and county of residence. Of the 1162 early-onset cases identified over this 13 year period, interviews were completed for 702 cases (60.4%). Over 98% of eligible controls contacted through random digit dialing agreed to participate in the study (n=822); however, eligibility was difficult to determine because of refusal to complete the screening questionnaire. In a sample of contacts from 2000–2002, of 2,748 randomly dialed numbers that were answered by a member of the household, 49.3% were ineligible due to age, 37.2% refused to answer the screening questionnaire, 12.4% participated in the study, and only 1.1% were eligible but declined to participate in the study.

This protocol was approved by the local institutional review board. Informed consent was obtained from all subjects prior to initiation of the interview and again prior to biospecimen collection. Trained interviewers conducted structured telephone interviews to collect demographic information, health history, cigarette use and family history of lung cancer in a first degree relative.

Biological samples with CYP genotyping results were available for 383 interviewed cases and 449 interviewed controls. Due to the rapidly fatal nature of lung cancer, it was necessary to collect 139 paraffin-embedded tumor blocks to obtain DNA from deceased cases. The remainder of DNA samples in case subjects was extracted from either venous blood (42.8%) or buccal cells (20.9%). While the blocks were dissected to obtain normal tissue for DNA extraction, the use of these blocks could lead to bias if the tissue from the blocks contained deletions or other mutations. When the genotype frequencies by tissue type (blood, buccal or block) were examined using Fisher's exact test, there were no differences in genotyping results

by tissue type for *CYP1A1 MspI* (p-value=0.22), *CYP1A1 exon 7* (p-value=0.15) or *CYP1B1 Leu⁴³²Val* (p-value=0.55). Any bias from the use of DNA from tumor blocks is minimal. All DNA from control subjects was collected via blood (67.7%) or buccal cells (32.3%).

Genotyping

DNA was isolated from whole blood with the Genomic DNA Purification System (Gentra Systems, Minneapolis, MN), buccal swabs with the BuccalAmp™ DNA Extraction Kit (Epicentre Technologies, Madison, WI), or paraffin embedded tissue with the QIAamp DNA Mini Kit (Qiagen, Valencia, CA) following the manufacturers' protocols. When multiple biospecimens were obtained from a study participant, DNA extracted from blood was used preferentially, followed by DNA extracted from buccal swabs and DNA extracted from normal tissue within paraffin blocks.

DNA isolated from buccal cells or paraffin embedded tissue was pre-amplified using a nested PCR strategy. Pre-amplification (outer amplification) was carried out in a 25 µl reaction containing 2.5 mmol/l MgCl₂, 0.5 µmol/l of the gene specific outer forward (-OF) and reverse (-OR) primers, 1.25 U AmpliTaq Gold polymerase, and 200 µmol/l of dATP, dCTP, dGTP, and dTTP. The outer amplification mixture was denatured at 95°C for 10 minutes and amplification was achieved by 15 cycles of 94 °C for 30 seconds, 63°C for 30 seconds, and 72°C for 1 minute, followed by a final extension at 72°C for 10 minutes. The outer amplification was performed on a Mastercycler® Gradient thermocycler (Eppendorf, Westbury, NY). Laboratory techniques and primers are described in greater detail in Wenzlaff et al. [6].

Genotypes for both *CYP1A1* polymorphisms were ascertained using restriction fragment length polymorphism (RFLP) protocols as described by Drakoulis et al. [7]. In both instances, products of the enzyme digests were run out on an 8% polyacrylamide gel and visualized by ethidium bromide staining. Sequencing verified a random sample of 5% of the subjects.

CYP1B1 Leu⁴³²Val genotyping was performed using a 5'-nuclease assay (TaqMan, Applied Biosystems) for all subjects. For the TaqMan assay, DNA isolated from buccal cells or from paraffin blocks was pre-amplified in an outer PCR reaction for added sensitivity. The outer reaction was carried out in a 25 µl reaction volume consisting of 2.5 mmol/L MgCl₂, 0.5 µmol/L of each of the forward (5'-CACTGCCAACACCTCTGTCTTG-3') and reverse (5'-AAGAATCGAGCTGGATCAAAGTTC-3') primers, 1.25 U AmpliTaq Gold polymerase, and 200 µmol/L each of dATP, dCTP, dGTP, and dTTP. The amplification mixture was denatured at 95°C for 10 min followed by 15 cycles of 94°C for 30 sec, 60°C for 30 sec and 72°C for 30 sec, and a final extension at 72°C for 10 min using an Eppendorf Master Cycler Gradient thermocycler (Eppendorf, Westbury, NY). Oligonucleotide primers and probes were designed using Primer Express software (Applied Biosystems). Standard TaqMan conditions using 1X Universal PCR mix (Applied Biosystems), either 25 ng DNA or 2 µl of an outer amplification reaction (DNA isolated from either buccal cells or paraffin blocks), forward (5'-GGTACCACATTCCCAAGGA-3') and reverse (5'-AAGAATCGAGCTGGATCAAAGTTC-3') primers, and leucine (6FAM-ATGACCCACTGAAGTG-MGBNFQ) and valine (VIC-ATGACCCAGTGAAGTG-MGBNFQ) probes. An Applied Biosystems 7900 was used for the amplification and detection of products. For quality control, 5% of the products were sequenced and 10% were directly repeated.

Statistical Analysis

Distributions of demographic variables were compared between cases and controls using chi-squared tests for categorical variables and t-tests for continuous variables. Unconditional logistic regression models were used to estimate odds ratios (OR) and 95% confidence intervals

(CI) to measure the association between lung cancer and genotype. All analyses presented in the tables were stratified by race. Potential confounders, including sex, age at diagnosis or interview (continuous), family history of lung cancer in a first degree relative (dichotomous) and pack years of cigarette smoking (continuous) were included in the final models. Subjects were categorized as “ever” smokers if they reported smoking at least 100 cigarettes in their lifetime. Pack years were calculated using the reported number of cigarettes smoked per day and the number of years smoked. Light smokers were those who smoked less than or equal to the mean number of pack years among smoking controls in each racial group (18 or fewer pack years of smoking in Caucasians, 14 or fewer in African Americans); heavy smokers were those who smoked more than the mean number of pack years (19 or greater pack years of smoking in Caucasians, 15 or greater in African Americans). *CYP1A1 MspI CC or CT genotypes*, *CYP1A1 exon 7 Ile/Val* genotype (there were no individuals with *Val/Val* genotype) and *CYP1B1 Leu⁴³²Val Leu/Val and Val/Val* genotypes were considered to be risk genotypes for lung cancer development in this analysis. To evaluate risk associated with combinations of genotypes, individuals were categorized as having 0, 1, or 2 of the risk genotypes, with those in the 0 category used as the reference group.

Results

Participant characteristics are presented in Table I. Approximately 29% of the cases and controls were African American. Mean ages of the cases and the controls were 42.5 and 41.4, respectively (p -value= <0.0001). The majority of cases reported ever smoking (91.9%), while controls were less likely to report being ever smokers (55.2%) (p -value= <0.0001). Cases were more likely to report having at least one first-degree relative with lung cancer compared to controls ($p=0.002$). Adenocarcinoma was the most common cell type diagnosed in this young onset population (49.9%), followed by small cell carcinoma, squamous cell carcinoma, and large cell carcinoma (13.3%, 12.3% and 9.4%, respectively).

Table II presents the distribution of genotypes by race and case-control status. Statistically significant differences by race in *CYP1A1 exon 7 Ile⁴⁶²Val* genotype and *CYP1B1 Leu⁴³²Val* genotype distribution were identified in the control population, (χ^2 p -value=0.009 and χ^2 p -value <0.0001 , respectively). Therefore, all further analyses were stratified by race. Caucasian and African American control genotype frequencies were in Hardy-Weinberg equilibrium for *CYP1A1 MspI*, *CYP1A1 exon 7 Ile⁴⁶²Val* and *CYP1B1 Leu⁴³²Val* (data not shown). In Caucasians, there were no statistically significant differences between cases and controls for genotypes at *CYP1A1 MspI* (p -value=0.84), *CYP1A1 exon 7 Ile⁴⁶²Val* (p -value=0.06) or *CYP1B1 Leu⁴³²Val* (p -value=0.42). In African Americans, there were no statistically significant differences between cases and controls for genotypes at *CYP1A1 MspI* (p -value=0.72), *CYP1A1 exon 7 Ile⁴⁶²Val* (p -value=0.29) or *CYP1B1 Leu⁴³²Val* (p -value=0.36).

Odds ratios and 95% confidence intervals are presented, by race, in Table III. Caucasian individuals who carried the Ile/Val genotype at *CYP1A1 exon 7* were at decreased risk of lung cancer compared to those who carried the Ile/Ile genotype at this locus, after adjusting for age, sex, pack years of smoking and family history of lung cancer (OR=0.41 95% CI 0.19–0.90). Additionally, for white individuals who carried both the Ile/Val genotype at *CYP1A1 exon 7* and at least one Val allele at *CYP1B1 Leu⁴³²Val*, the risk of lung cancer was further reduced (OR=0.31 95% CI 0.10–0.97). No other significant associations were seen in Caucasian or African American individuals. All analyses were also stratified by amount smoked, i.e. light or heavy smoker. No association between the CYP genes and risk of lung cancer was seen after stratifying by smoking (data not shown).

Discussion

Few studies have focused on genetic polymorphisms associated with risk of early-onset lung cancer. To the author's knowledge, the present study is the largest sample of early-onset lung cancer cases in the current literature. Other published studies of early-onset lung cancer include a pooled analysis of Caucasians under age 45 years [8] and a subset of cases under age 55 from a large hospital-based study of Caucasians [9]. An early-onset population is particularly appropriate for studies of genetic susceptibility, but lung cancer prior to age 60 is rare, making such studies difficult. A greater effect of metabolic polymorphisms at lower levels of tobacco exposure (as would be seen in a younger population) has been reported in lung cancer, particularly for cytochrome P-450 1A1 (CYP1A1) polymorphisms [10–12].

CYP1A1

The *CYP1A1 MspI* and *CYP1A1 exon 7 Ile⁴⁶²Val* polymorphisms are rare in both Caucasians and African Americans. It has been suggested that these polymorphisms are in linkage disequilibrium in Caucasians, but not in African Americans [13]. Additionally, a third polymorphism in *CYP1A1* that is unique to African Americans has also been identified. This polymorphism, located in intron 7 at base number 5996, results in a single A-T to G-C transition and does not appear to be linked to the other polymorphisms in *CYP1A1* [14]. Evidence suggests that this polymorphism is not significantly associated with lung cancer in African Americans [15–17] and this polymorphism was not examined in our African American population. Studies in African Americans have reported no associations between *CYP1A1 MspI* and *CYP1A1 exon 7 Ile⁴⁶²Val* polymorphisms and risk of lung cancer [12,17] but findings in Caucasian populations have been mixed for both polymorphisms [18–20].

CYP1A1 *MspI*

In our population, we found no increased risk of lung cancer associated with carrying the C/T or C/C variants, regardless of race or personal smoking history. Likewise, a meta-analysis by Houlston examined 16 studies of *CYP1A1 MspI* polymorphisms published between 1990 and 1998 and reported no statistically significant association with either the combined variant (C/T and C/C) or homozygous variant (C/C) [20]. However, a later pooled analysis of 1,759 Caucasian cases and 2,179 Caucasian controls included in the Genetic Susceptibility to Environmental Carcinogenesis (GSEC) database reported an association between homozygous carriers (C/C) and lung cancer (OR=2.36 95% CI 1.16–4.81), after adjusting for age and gender [19]. However, once these models were stratified by smoking level, no association was identified between lung cancer and the combined variant (C/T and C/C) or homozygous variant (C/C) in Caucasian or Asian individuals [19]. A final pooled analysis from the GSEC database that included only individuals diagnosed below the age of 45 years reported increased risk of lung cancer in those who carried the homozygous (C/C) genotype at *CYP1A1 MspI* (OR=5.7 95% CI 1.2–28.0) after adjusting for age, gender, smoking status, and study [8]. It should be noted that these findings were based on 109 cases and 423 controls, of which only 9 individuals carried the C/C genotype. While an early functional study suggested that *CYP1A1 MspI* variant genotypes result in increased ethoxyresorufin-O-deethylase (EROD) activity [21], later studies suggest that differing expression levels are due to polymorphisms in *CYP1A1 exon 7 Ile⁴⁶²Val* [22,23], and may also be influenced by smoking status [24] and gender [25,26].

CYP1A1 exon 7 *Ile⁴⁶²Val*

In our population, we report decreased risk of lung cancer for individuals carrying the *CYP1A1 exon 7 Ile/Val* genotype compared to those with the *Ile/Ile* genotype (OR=0.41 95% CI 0.19–0.90). This is contrary to the majority of other studies, which report either no association, or an increased risk associated with the *Ile/Val* or *Val/Val* genotype. A large, single institution study of Caucasians that examined 1,050 cases of primary non-small cell lung cancers from

1980–2003 and 581 controls (either COPD cases or healthy smokers) reported increased risk of lung cancer in carriers of *CYP1A1* exon 7 Ile/Val or Val/Val genotypes (OR=1.85 95% CI 1.20–2.85) after adjusting for age, sex and pack years [10]. Lung cancer risk associated with these genotypes was even greater for women, lighter smokers (less than 46 pack years), those less than age 63 at diagnosis, and those with adenocarcinoma of the lung [10]. A pooled analysis from the GSEC database found no association between *CYP1A1* exon 7 Ile⁴⁶²Val polymorphisms in Caucasians and Asians, but did identify significant interactions between smoking status (ever/never) and carriers of Ile/Val or Val/Val genotypes, after adjusting for age, sex, race and study, with risks being greatest for never smokers [18]. A pooled analysis of early-onset cases reported no association with *CYP1A1* exon 7 Ile⁴⁶²Val polymorphisms (OR=1.6 95% CI 0.9–3.1 for Ile/Val carriers) after adjustment for age, gender, smoking status and study number [8]. Contradictory findings are not uncommon in studies of candidate genes of low minor allele frequencies, as discussed in more detail below.

CYP1B1

The *CYP1B1* Leu⁴³²Val polymorphism is of particular interest because studies have demonstrated that the CYP1B1 enzyme is expressed in the lung [3,27] and appears in significantly higher levels within the peripheral leukocytes of lung cancer patients versus controls [28]. Although *CYP1B1* is a plausible candidate for contributing to lung carcinogenesis, there are few epidemiologic studies that have evaluated a possible association between polymorphisms within this gene and lung cancer. Watanabe et al. reported in 2000 that *CYP1B1* Leu⁴³²Val genotype frequencies did not differ significantly between lung cancer cases and healthy controls [29]. Results from Wu et al. concurred that *CYP1B1* Leu⁴³²Val was not associated with lung cancer (OR=1.41, 95% CI 0.49–4.00), although only 42 lung cancer cases were included in this analysis [28]. While these two studies focused on Asian populations, a recent Danish case-cohort study also reported no association between *CYP1B1* Leu⁴³²Val and lung cancer susceptibility [30]. In contrast, we recently evaluated *CYP1B1* Leu⁴³²Val in a population of never smokers and found that never-smoking Caucasians with the Leu/Val genotype had a 2.7-fold increased risk of lung cancer (95% CI 1.47–5.01) and those with Val/Val genotypes had an over three-fold increased risk of lung cancer (OR=3.16, 95% CI 1.57–6.35) compared to those with the Leu/Leu genotype after adjusting for age, sex, and years of household environmental tobacco smoke exposure [6]. However, these results were not replicated in African American nonsmokers, or in our early-onset population. CYP1B1 is also thought to mediate the hydroxylation of 17β-estradiol. The Val allele at Leu⁴³²Val has higher catalytic efficiency for the 4-hydroxylation of estradiol compared to Leu [31]. It is possible that *CYP1B1* is involved in lung carcinogenesis through the estrogenic activity of the 4-OH-E2. This pathway may be of more importance in nonsmokers, which may explain why an association between *CYP1B1* Leu⁴³²Val and lung cancer was identified in nonsmokers but not in smoking populations.

CYP1A1 exon 7 Ile⁴⁶²Val and CYP1B1

Risks associated with *CYP1A1* exon 7 Ile⁴⁶²Val and *CYP1B1* Leu⁴³²Val genotypes in combination were driven by the significant reduction in risk associated with the *CYP1A1* Ile⁴⁶²Val genotype. Other than our recent study of these polymorphisms in nonsmokers [6], there are no published manuscripts describing risk of lung cancer associated with jointly carrying *CYP1A1* and *CYP1B1* variant genotypes. While evaluating combinations of risk genotypes within a given pathway makes biologic sense and is necessary to move the field forward, combining risk genotypes for analysis exacerbates the limits of these studies.

Summary

Candidate gene studies using unrelated controls are currently the most widely used study design in lung cancer epidemiology to elucidate genetic susceptibility. However, these types of studies have limitations. Polymorphisms found at low frequencies in the population require a large sample size in order to detect an association. Many studies do not have adequate power to detect small effects, especially once individuals with missing data are excluded, or if the data are stratified to create more homogenous groups. In our population, the minimum detectable odds ratios for our Caucasian population was 4.0 for *CYP1A1 MspI*, 1.9 for *CYP1A1 exon 7*, and 1.6 for *CYP1B1* with 80% power and $\alpha=0.05$ (for protective effects, the minimum detectable ORs were 0.02, 0.4 and 0.5, respectively) [32]. These minimum ORs were even greater for the African American population. Next, spurious associations due to population stratification can occur. In our study, this is minimized by conducting all analyses stratified by race. Additionally, lack of replication of study findings across different populations is common. This may be due to genotyping errors, different control populations (i.e., hospital versus population), allele frequency variations in the populations studied, or some combination of these factors. There may also be true differences in the populations that account for the lack of consistency seen between these studies.

Our study had several important strengths. The focus on early-onset disease and the African American population, where genetic susceptibility might be greater, is unique in the current *CYP* literature. Our ability to examine and adjust for known confounders, collected through structured interviews, is an additional strength of this study. By stratifying on self-reported race, we minimized the possibility that our findings were distorted by population stratification. In addition, population-based incident cases were identified quickly after diagnosis, which limits survival bias. This may prove to be especially important if these genes play a role in response to treatment and subsequent survival.

The study also had several limitations that should be considered. First, although this is one of the largest single institution studies of early-onset lung cancer, we did not have the power to stratify on histology or other variables that may influence the relationship between these *CYPs* and lung cancer development. Additionally, we did not identify any individuals with the *CYP1A1 exon 7 Val/Val* homozygous genotype. While this is not unexpected since this genotype tends to be rare (<1%) in both Caucasian and African American populations [33], it is unclear whether carrying one Val allele at this site would result in a significant change in gene function. This may also be due to our choice of primers for *CYP1A1 exon 7*. There is a second polymorphism in *CYP1A1*, *Thr⁴⁶¹Asn*, which lies two bases upstream from the polymorphism described here (*CYP1A1 Ile⁴⁶²Val*). It is possible that if the second SNP were present, the allele specific primers used in this analysis may not bind efficiently, resulting in allele dropout and ultimately misclassification of genotype. Like *CYP1A1 Ile⁴⁶²Val*, the *Thr⁴⁶¹Asn* allele frequency in Caucasian populations ranges from 3–11% [34,35]. The two polymorphisms have not been shown to be in linkage disequilibrium [35]. The *CYP1A1 Thr⁴⁶¹Asn* SNP has not been associated with lung cancer [35] or breast cancer [34,36] in case-control studies. After a review of sequence data from 130 individuals, we found 5 instances (3.3%) where the second SNP was heterozygous; however, in each case the *Ile⁴⁶²Val* locus was homozygous and the call was not affected by the other SNP. If this SNP were present as a homozygous variant in our population, we may have misclassified those carrying the *Ile/Val* and *Val/Val* genotypes as *Ile/Ile*. We did not identify any homozygous variants in the 130 individuals with sequencing data. Thus, it is unlikely that this genotype is present in the population at a high enough frequency to alter our findings. However, it should be noted that our findings were sometimes based on small sample sizes, and thus should be interpreted with caution.

Our results suggest that *CYP1A1* exon 7 Ile⁴⁶²Val genotype may be associated with decreased risk of lung cancer in this early-onset population. It is difficult to interpret these findings in light of new functional studies that suggest CYP1A1 levels in the lung may be modulated by gender and smoking status. Additionally, the dual activities of *CYP1A1* and *CYP1B1* as both carcinogen and estrogen metabolizing enzymes adds to the complexity of assessing the association between lung cancer and these polymorphisms. Recent data demonstrating the expression of ER- β in the lung suggests that risk associated with these polymorphisms may vary by tumor ER- β positivity or other hormonal influences [37,38].

References

1. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin* 2005;55:74–108. [PubMed: 15761078]
2. Spivack SD, Fasco MJ, Walker VE, Kaminsky LS. The molecular epidemiology of lung cancer. *Crit Rev Toxicol* 1997;27:319–365. [PubMed: 9263643]
3. Spivack SD, Hurteau GJ, Reilly AA, Aldous KM, Ding X, Kaminsky LS. CYP1B1 expression in human lung. *Drug Metab Dispos* 2001;29:916–922. [PubMed: 11353763]
4. Willey JC, Coy EL, Frampton MW, Torres A, Apostolakis MJ, Hoehn G, Schuermann WH, Thilly WG, Olson DE, Hammersley JR, Crespi CL, Utell MJ. Quantitative RT-PCR measurement of cytochromes p450 1A1, 1B1, and 2B7, microsomal epoxide hydrolase, and NADPH oxidoreductase expression in lung cells of smokers and nonsmokers. *Am J Respir Cell Mol Biol* 1997;17:114–124. [PubMed: 9224217]
5. Schoket B, Papp G, Levay K, Mrackova G, Kadlubar FF, Vincze I. Impact of metabolic genotypes on levels of biomarkers of genotoxic exposure. *Mutat Res* 2001;482:57–69. [PubMed: 11535249]
6. Wenzlaff AS, Cote ML, Bock CH, Land SJ, Santer SK, Schwartz DR, Schwartz AG. CYP1A1 and CYP1B1 polymorphisms and risk of lung cancer among never smokers: a population-based study. *Carcinogenesis* 2005;26:2207–2212. [PubMed: 16051642]
7. Drakoulis N, Cascorbi I, Brockmoller J, Gross CR, Roots I. Polymorphisms in the human CYP1A1 gene as susceptibility factors for lung cancer: exon-7 mutation (4889 A to G), and a T to C mutation in the 3'-flanking region. *Clin Invest* 1994;72:240–248.
8. Taioli E, Gaspari L, Benhamou S, Boffetta P, Brockmoller J, Butkiewicz D, Cascorbi I, Clapper ML, Dolzan V, Haugen A, Hirvonen A, Husgafvel-Pursiainen K, Kalina I, Kremers P, Le Marchand L, London S, Rannug A, Romkes M, Schoket B, Seidegard J, Strange RC, Stucker I, To-Figueras J, Garte S. Polymorphisms in CYP1A1, GSTM1, GSTT1 and lung cancer below the age of 45 years. *Int J Epidemiol* 2003;32:60–63. [PubMed: 12690010]
9. Miller DP, Liu G, De Vivo I, Lynch TJ, Wain JC, Su L, Christiani DC. Combinations of the variant genotypes of GSTP1, GSTM1, and p53 are associated with an increased lung cancer risk. *Cancer Res* 2002;62:2819–2823. [PubMed: 12019159]
10. Larsen JE, Colosimo ML, Yang IA, Bowman R, Zimmerman PV, Fong KM. Risk of non-small cell lung cancer and the cytochrome P4501A1 Ile462Val polymorphism. *Cancer Causes Control* 2005;16:579–585. [PubMed: 15986113]
11. Nakachi K, Imai K, Hayashi S, Kawajiri K. Polymorphisms of the CYP1A1 and glutathione S-transferase genes associated with susceptibility to lung cancer in relation to cigarette dose in a Japanese population. *Cancer Res* 1993;53:2994–2999. [PubMed: 8319207]
12. Ishibe N, Wiencke JK, Zuo ZF, McMillan A, Spitz M, Kelsey KT. Susceptibility to lung cancer in light smokers associated with CYP1A1 polymorphisms in Mexican- and African-Americans. *Cancer Epidemiol Biomarkers Prev* 1997;6:1075–1080. [PubMed: 9419406]
13. Taioli E, Crofts F, Trachman J, Bayo S, Toniolo P, Garte SJ. Radical differences in CYP1A1 genotype and function. *Toxicol Lett* 1995;77:357–362. [PubMed: 7618162]
14. Crofts F, Cosma GN, Currie D, Taioli E, Toniolo P, Garte SJ. A novel CYP1A1 gene polymorphism in African-Americans. *Carcinogenesis* 1993;14:1729–1731. [PubMed: 8104732]
15. Kelsey KT, Wiencke JK, Spitz MR. A race-specific genetic polymorphism in the CYP1A1 gene is not associated with lung cancer in African Americans. *Carcinogenesis* 1994;15:1121–1124. [PubMed: 8020143]

16. London SJ, Daly AK, Fairbrother KS, Holmes C, Carpenter CL, Navidi WC, Idle JR. Lung cancer risk in African-Americans in relation to a race-specific CYP1A1 polymorphism. *Cancer Res* 1995;55:6035–6037. [PubMed: 8521388]
17. Taioli E, Ford J, Trachman J, Li Y, Demopoulos R, Garte S. Lung cancer risk and CYP1A1 genotype in African Americans. *Carcinogenesis* 1998;19:813–817. [PubMed: 9635868]
18. Le Marchand L, Guo C, Benhamou S, Bouchardy C, Cascorbi I, Clapper ML, Garte S, Haugen A, Ingelman-Sundberg M, Kihara M, Rannug A, Ryberg D, Stucker I, Sugimura H, Taioli E. Pooled analysis of the CYP1A1 exon 7 polymorphism and lung cancer (United States). *Cancer Causes Control* 2003;14:339–346. [PubMed: 12846365]
19. Vineis P, Veglia F, Benhamou S, Butkiewicz D, Cascorbi I, Clapper ML, Dolzan V, Haugen A, Hirvonen A, Ingelman-Sundberg M, Kihara M, Kiyohara C, Kremers P, Le Marchand L, Ohshima S, Pastorelli R, Rannug A, Romkes M, Schoket B, Shields P, Strange RC, Stucker I, Sugimura H, Garte S, Gaspari L, Taioli E. CYP1A1 T3801 C polymorphism and lung cancer: a pooled analysis of 2451 cases and 3358 controls. *Int J Cancer* 2003;104:650–657. [PubMed: 12594823]
20. Houlston RS. CYP1A1 polymorphisms and lung cancer risk: a meta-analysis. *Pharmacogenetics* 2000;10:105–114. [PubMed: 10761998]
21. Landi MT, Bertazzi PA, Shields PG, Clark G, Lucier GW, Garte SJ, Cosma G, Caporaso NE. Association between CYP1A1 genotype, mRNA expression and enzymatic activity in humans. *Pharmacogenetics* 1994;4:242–246. [PubMed: 7894496]
22. Cosma G, Crofts F, Taioli E, Toniolo P, Garte S. Relationship between genotype and function of the human CYP1A1 gene. *J Toxicol Environ Health* 1993;40:309–316. [PubMed: 7901425]
23. Crofts F, Taioli E, Trachman J, Cosma GN, Currie D, Toniolo P, Garte SJ. Functional significance of different human CYP1A1 genotypes. *Carcinogenesis* 1994;15:2961–2963. [PubMed: 8001264]
24. Kim JH, Sherman ME, Curriero FC, Guengerich FP, Strickland PT, Sutter TR. Expression of cytochromes P450 1A1 and 1B1 in human lung from smokers, non-smokers, and ex-smokers. *Toxicol Appl Pharmacol* 2004;199:210–219. [PubMed: 15364538]
25. Dresler CM, Fratelli C, Babb J, Everley L, Evans AA, Clapper ML. Gender differences in genetic susceptibility for lung cancer. *Lung Cancer* 2000;30:153–160. [PubMed: 11137199]
26. Mollerup S, Berge G, Baera R, Skaug V, Hewer A, Phillips DH, Stangeland L, Haugen A. Sex differences in risk of lung cancer: Expression of genes in the PAH bioactivation pathway in relation to smoking and bulky DNA adducts. *Int J Cancer*. 2006
27. Shimada T, Watanabe J, Kawajiri K, Sutter TR, Guengerich FP, Gillam EM, Inoue K. Catalytic properties of polymorphic human cytochrome P450 1B1 variants. *Carcinogenesis* 1999;20:1607–1613. [PubMed: 10426814]
28. Wu MF, Wu WJ, Chang GC, Chen CY, Hu SW, Tsai WT, Lee H, Lin P. Increased expression of cytochrome P4501B1 in peripheral leukocytes from lung cancer patients. *Toxicol Lett* 2004;150:211–219. [PubMed: 15093676]
29. Watanabe J, Shimada T, Gillam EM, Ikuta T, Suemasu K, Higashi Y, Gotoh O, Kawajiri K. Association of CYP1B1 genetic polymorphism with incidence to breast and lung cancer. *Pharmacogenetics* 2000;10:25–33. [PubMed: 10739169]
30. Sorensen M, Autrup H, Tjonneland A, Overvad K, Raaschou-Nielsen O. Genetic polymorphisms in CYP1B1, GSTA1, NQO1 and NAT2 and the risk of lung cancer. *Cancer Lett* 2005;221:185–190. [PubMed: 15808404]
31. Hanna IH, Dawling S, Roodi N, Guengerich FP, Parl FF. Cytochrome P450 1B1 (CYP1B1) pharmacogenetics: association of polymorphisms with functional differences in estrogen hydroxylation activity. *Cancer Res* 2000;60:3440–3444. [PubMed: 10910054]
32. Schlesselman, J. *Case Control Studies: Design, Conduct, Analysis*. Oxford University Press; 1982.
33. Garte S, Gaspari L, Alexandrie AK, Ambrosone C, Autrup H, Autrup JL, Baranova H, Bathum L, Benhamou S, Boffetta P, Bouchardy C, Breskvar K, Brockmoller J, Cascorbi I, Clapper ML, Coutelle C, Daly A, Dell’Omo M, Dolzan V, Dresler CM, Fryer A, Haugen A, Hein DW, Hildesheim A, Hirvonen A, Hsieh LL, Ingelman-Sundberg M, Kalina I, Kang D, Kihara M, Kiyohara C, Kremers P, Lazarus P, Le Marchand L, Lechner MC, van Lieshout EM, London S, Manni JJ, Maugard CM, Morita S, Nazar-Stewart V, Noda K, Oda Y, Parl FF, Pastorelli R, Persson I, Peters WH, Rannug A, Rebbeck T, Risch A, Roelandt L, Romkes M, Ryberg D, Salagovic J, Schoket B, Seidegard J, Shields

- PG, Sim E, Sinnet D, Strange RC, Stucker I, Sugimura H, To-Figueras J, Vineis P, Yu MC, Taioli E. Metabolic gene polymorphism frequencies in control populations. *Cancer Epidemiol Biomarkers Prev* 2001;10:1239–1248. [PubMed: 11751440]
34. Basham VM, Pharoah PD, Healey CS, Luben RN, Day NE, Easton DF, Ponder BA, Dunning AM. Polymorphisms in CYP1A1 and smoking: no association with breast cancer risk. *Carcinogenesis* 2001;22:1797–1800. [PubMed: 11698341]
35. Cascorbi I, Brockmoller J, Roots I. A C4887A polymorphism in exon 7 of human CYP1A1: population frequency, mutation linkages, and impact on lung cancer susceptibility. *Cancer Res* 1996;56:4965–4969. [PubMed: 8895751]
36. Masson LF, Sharp L, Cotton SC, Little J. Cytochrome P-450 1A1 gene polymorphisms and risk of breast cancer: a HuGE review. *Am J Epidemiol* 2005;161:901–915. [PubMed: 15870154]
37. Schwartz AG, Prysak GM, Murphy V, Lonardo F, Pass H, Schwartz J, Brooks S. Nuclear estrogen receptor beta in lung cancer: expression and survival differences by sex. *Clin Cancer Res* 2005;11:7280–7287. [PubMed: 16243798]
38. Stabile LP, Davis AL, Gubish CT, Hopkins TM, Luketich JD, Christie N, Finkelstein S, Siegfried JM. Human non-small cell lung tumors and cells derived from normal lung express both estrogen receptor alpha and beta and show biological responses to estrogen. *Cancer Res* 2002;62:2141–2150. [PubMed: 11929836]

Table 1

Participant characteristics by race and lung cancer status

	Cases N=383	Controls N=449	Cases N=272	Controls N=323	Cases N=111	Controls N=126
<i>Race</i>						
Caucasian	272 (71.0%)	323 (71.9%)				
African American	111 (29.0%)	126 (28.1%)				
<i>p-value</i>		0.77				
<i>Sex</i>						
Male	190 (49.6%)	199 (44.3%)	142 (52.2%)	156 (48.3%)	48 (43.2%)	43 (34.1%)
Female	193 (50.4%)	250 (55.7%)	130 (47.8%)	167 (51.7%)	63 (56.8%)	83 (65.9%)
<i>p-value</i>		0.13		0.34		0.15
<i>Mean Age (years)</i>	42.5	41.4	42.8	41.2	41.8	42.0
<i>p-value</i>		<0.0001		<0.0001		<0.0001
<i>Smoking Status</i>						
Never smoker	31 (8.1%)	201 (44.8%)	23 (8.5%)	149 (46.1%)	8 (7.2%)	52 (41.3%)
Ever smoker	352 (91.9%)	248 (55.2%)	249 (91.5%)	174 (53.9%)	103 (92.8%)	74 (58.7%)
<i>p-value</i>		<0.0001		<0.0001		<0.0001
<i>Family History of Lung Cancer in a First Degree Relative</i>						
Yes	68 (17.7%)	46 (10.2%)	45 (16.5%)	33 (10.2%)	23 (20.7%)	13 (10.3%)
No	315 (82.3%)	403 (89.8%)	227 (83.5%)	290 (89.8%)	88 (79.3%)	113 (89.7%)
<i>p-value</i>		0.002		0.02		0.03
<i>Histology</i>						
Squamous Cell Carcinoma	47 (12.3%)		35 (12.9%)		12 (10.7%)	
Small Cell Carcinoma	51 (13.3%)		42 (15.4%)		9 (7.8%)	
Adenocarcinoma	191 (49.9%)		129 (47.4%)		62 (57.3%)	
Large Cell Carcinoma	36 (9.4%)		25 (9.2%)		11 (8.7%)	
Non Small Cell, NOS	16 (4.2%)		11 (4.1%)		5 (2.9%)	
Other	42 (10.9%)		30 (11.0%)		12 (12.6%)	

Table II
Distribution of CYP1A1 and CYP1B1 genotypes by race and lung cancer status

	Total		Caucasian		African American	
	Cases N=383	Controls N=449	Cases N=272	Controls N=323	Cases N=111	Controls N=126
<i>CYP1A1</i> MspI ^a (M1)						
TT	269 (76.0%)	339 (77.0%)	203 (79.3%)	252 (79.5%)	66 (67.3%)	87 (70.7%)
CT	80 (22.6%)	95 (21.6%)	51 (19.9%)	61 (19.2%)	29 (29.6%)	34 (27.6%)
CC	5 (1.4%)	6 (1.4%)	2 (0.8%)	4 (1.3%)	3 (3.1%)	2 (1.6%)
<i>p</i> -value		0.94		0.84		0.72
<i>CYP1A1</i> Ile ⁴⁶² Val ^b (exon 7)						
Ile/Ile	326 (94.5%)	400 (92.2%)	234 (94.4%)	281 (90.1%)	92 (94.9%)	119 (97.5%)
Ile/Val	19 (5.5%)	34 (7.8%)	14 (5.6%)	31 (9.9%)	5 (5.1%)	3 (2.5%)
<i>p</i> -value		0.20		0.06		0.29
<i>CYP1B1</i> Leu ⁴³² Val ^c						
Leu/Leu	94 (26.5%)	124 (27.6%)	87 (34.3%)	115 (35.6%)	7 (7.0%)	9 (7.1%)
Leu/Val	160 (45.2%)	199 (44.3%)	127 (50.0%)	146 (45.2%)	33 (33.0%)	53 (42.1%)
Val/Val	100 (28.3%)	126 (28.1%)	40 (15.7%)	62 (19.2%)	60 (60.0%)	64 (50.8%)
<i>p</i> -value		0.94		0.42		0.36

^a 16 Caucasian cases, 6 Caucasian controls, 13 African American cases and 3 African American controls missing genotype results

^b 24 Caucasian cases, 11 Caucasian controls, 14 African American cases and 4 African American controls missing genotype results

^c 18 Caucasian cases and 11 African American cases missing genotype results

Table III

Odds ratios for risk of early onset lung cancer and *CYP1A1* and *CYP1B1* genotypes by race, adjusted for age, sex, pack years of smoking and family history of lung cancer

	Caucasian			African American		
	N _{Case}	N _{Control}	OR(95% CI)	N _{Case}	N _{Control}	OR (95% CI)
<i>CYP1A1</i> MspI (M1)	272	323		111	126	
TT	203	252	1.00	66	87	1.00
CT	51	61	1.10 (0.68–1.80)	29	34	1.12 (0.56–2.22)
CC	2	4	0.39 (0.05–3.30)	3	2	1.29 (0.16–10.59)
CC or CT	53	65	1.05 (0.65–1.71)	31	36	1.13 (0.58–2.20)
<i>CYP1A1</i> Ile462Val (exon 7)						
Ile/Ile	234	281	1.00	92	119	1.00
Ile/Val	14	31	0.41 (0.19–0.90)	5	3	2.00 (0.38–10.43)
<i>CYP1B1</i> Leu432Val						
Leu/Leu	87	115	1.00	7	9	1.00
Leu/Val	127	146	1.07 (0.69–1.65)	33	53	0.75 (0.21–2.65)
Val/Val	40	62	0.67 (0.37–1.20)	60	64	1.11 (0.33–3.80)
L/L vs. L/V or V/V	167	208	0.95 (0.63–1.43)	93	117	0.95 (0.29–3.16)
<i>CYP1A1</i> M1 and <i>CYP1A1</i> exon 7						
0	193	242	1.00	64	84	1.00
1	41	38	1.43 (0.81–2.50)	28	32	1.10 (0.54–2.25)
2	12	27	0.44 (0.19–1.04)	3	3	1.39 (0.22–8.87)
<i>CYP1A1</i> M1 and <i>CYP1B1</i>						
0	71	86	1.00	6	7	1.00
1	144	190	0.88 (0.56–1.39)	61	82	0.74 (0.19–2.88)
2	37	41	0.98 (0.51–1.89)	31	34	0.89 (0.22–3.69)
<i>CYP1A1</i> exon 7 and <i>CYP1B1</i>						
0	80	96	1.00	6	8	1.00
1	157	200	0.90 (0.58–1.39)	86	111	1.01 (0.27–3.81)
2	8	16	0.31 (0.10–0.97)	5	3	2.03 (0.25–15.99)

Testing for joint effects by the following scheme: 0=no risk genotypes, risk defined as CC or CT genotype at *CYP1A1* M1, Ile/Val at *CYP1A1* exon 7, and Ile/Val or Val/Val at *CYP1B1*. 1 =one risk polymorphism is present. 2 =two risk polymorphisms are present.