

Null Results in Brief

Lack of Associations among Cancer and Albumin Adducts, ras p21 Oncoprotein Levels, and CYP1A1, CYP2D6, NAT1, and NAT2 in a Nested Case-Control Study of Lung Cancer within the Physicians' Health Study

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Introduction

We conducted a "nested" case-control study within the Physicians' Health Study, a randomized trial of aspirin and BC at relatively modest doses in which baseline (enrollment/untreated) blood samples from incident cases and matched controls were selected from the bank of >14,000 stored "enrollment" samples (1). The goal was to test the hypothesis that biomarkers in blood of "healthy" individuals can indicate their susceptibility to lung cancer or detect early disease that would manifest itself years or decades later (1). The biomarkers were selected based on prior data implicating them in lung cancer and included polycyclic aromatic hydrocarbon (PAH)-DNA adducts, PAH-albumin adducts, ras p21, and polymorphisms in *GSTM1*, *GSTP1*, *NAT1*, *NAT2*, *CYP1A1*, and *CYP2D6* genes.

There was a priori evidence that PAH-albumin adducts and ras p21 of the biomarkers were biologically relevant, but their predictive ability had not been evaluated in a prospective design. For example, studies have shown that certain protein adducts, including PAH-albumin adducts, are correlated with the corresponding DNA adducts and might serve as feasible surrogates (2). However, the half-lives of the two biomarkers vary, and, unlike DNA adducts, protein adducts do not reflect interindividual variability in DNA repair. The ras oncogene has been shown to be activated by mutagens and clastogens. Moreover, ras mutation and/or overexpression has been directly implicated in the development of lung tumors (3-9). We also examined the contribution of polymorphisms in genes controlling metabolic activation and detoxification pathways for carcinogens that mediate the initial steps in environmental carcinogenesis (10). Genetic polymorphisms in the selected genes have been previously implicated in at least one study of lung cancer, although results have not been consistent (11-13).

In this nested case-control study, our prior analysis showed that PAH/aromatic-DNA adducts were significant predictors of lung cancer among smokers but not former or non smokers, and the combined *GSTM1* null/*GSTP1* Val genotype was significantly associated with lung cancer before and after adjusting for PAH-DNA adducts (1, 14). Here, we report on the relationship between lung cancer risk and PAH-albumin adducts, the ras p21 oncoprotein, and polymorphisms in the *CYP1A1*, *CYP2D6*, *NAT1*, and *NAT2* genes. Although not part of our original hypotheses, we also report on the association between the polymorphisms and the other biomarkers (PAH-albumin adducts and the ras p21 oncoprotein).

Materials and Methods

The Physicians' Health Study involved 22,071 U.S. male physicians, nearly all of whom were Caucasian (15). The subjects were asked to donate a blood sample at initial enrollment, and, roughly, 75% complied. Two controls (with no prior diagnosis of cancer) were matched to each incident case at the time of diagnosis. Cases were matched to controls on age (± 1 year), length of follow-up (+6 months), and smoking status at baseline (never used tobacco regularly, former cigarette smoker, current cigarette smoker of 1-19, 20-39, or ≥ 40 cigarettes per day), and pipe and cigar smoker (current or past).

PAH-albumin adducts were analyzed using a monoclonal antibody (8E11) as described (16). Overexpression and mutations of the ras p21 oncoprotein in plasma were analyzed by Western blotting as described (17). Study samples were scored as having detectable levels of ras if a peak seemed at the same relative position as either the normal ras p21 protein or the val 12 mutant protein standards in the control lanes. Although the primary emphasis in the analysis was on the prevalence of detectable ras, semiquantitative data on the intensity with which the bands were stained were also collected. The intensities of the pixels under the peaks were summed and reported as a score in integrated pixel units (17). DNA was extracted from blood leukocytes, and individual genotypes were analyzed by PCR-RFLP. The individual genotypes included *CYP1A1* ("MspI," T3801C, "M1"; A2455G, "M2," or Ile⁴⁶²Val; ref. 18), *CYP2D6* (A2549del, "A"; G1846A, "B" allele; ref. 19), *NAT1* (1065del9, "NAT1*11"; T1088A, "NAT1*10"; C1095A; ref. 20), and *NAT2* [*NAT2*4* (WT), *NAT2*5* (T341C, C481T, and A803G), *NAT2*6* (G590A), *NAT2*7* (G857A), and *NAT2*14* (G191A); ref. 21].

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In statistical analysis, PAH-albumin adducts (fmol/ μ g) and overexpression of ras oncoprotein (integrated pixel units) were treated as continuous variables or as binary variables (detectable/nondetectable and positive/negative). Polymorphisms in *CYP1A1*, *CYP2D6*, *NAT1*, and *NAT2* were treated in conditional logistic regression models as binary variables (present versus absent). Because the biomarkers were analyzed in baseline blood samples, they could not have been confounded by treatment. However, as in our prior reports (1, 14), to be conservative, we analyzed biomarker-lung cancer relationships with and without including treatment in the models. Analyses also distinguished between the major histologic groups of lung cancer (small cell lung carcinoma and non-small cell lung carcinoma). Our a priori power to detect hypothesized differences between cases and controls in albumin adducts and ras oncoproteins was >90%. Due to our small sample size, our power was limited to detect associations between genotypes and lung cancer. For example, the estimates of power of this study to detect odds ratios of 2.0 or 2.5 for *CYP1A1 MspI* were 34% and 45%, respectively. The corresponding power estimates for *CYP2D6B* were 70% and >80%; for *NAT1*, were 65% and >80%; and for *NAT2*, were 62% and >80%, respectively.

In secondary analyses, not part of our original hypotheses, we tested the effect of genotypes on adducts and ras p21. For PAH-albumin adducts, we selected *CYP1A1* because it is known to be involved in PAH metabolism. For ras p21, we tested all of the genotypes because ras is a non-chemical-specific biomarker.

Results and Discussion

Table 1 shows the number of subjects for each biomarker (ranges included) and genotype. Table 2 provides the results of conditional logistic regression for the selected biomarkers. The footnotes explain the risk ratio comparison for each biomarker. As shown in Table 2, PAH-albumin adducts and ras p21 oncoprotein were not significant predictors of lung cancer before or after controlling for treatment assignment, nor were *CYP1A1*, *CYP2D6*, *NAT1*, and *NAT2* genotypes significantly associated with lung cancer (Table 2). The same lack of association was seen when separate analyses were conducted within each of the major histologic groups (small cell lung carcinoma versus non-small cell lung carcinoma; data not shown). The results for PAH-albumin and ras p21 suggest that these biomarkers are not early predictors of risk. As noted, the analyses of the relationship between polymorphisms and lung cancer risk have limited statistical power and should be considered exploratory only.

We did not observe an effect of *CYP1A1* on PAH-albumin levels. Ras p21 was not associated with any of the genotypes.

Table 1. Number of subjects for each biomarker (ranges included) and genotype

Variable	n
PAH-albumin (range)	249 (0.15-4.79)
ras p21 (range)	254 (0-464.77)
<i>CYP1A1 MspI</i>	258
<i>CYP1A1 462</i>	258
<i>CYP2D6A</i>	257
<i>CYP2D6B</i>	257
<i>NAT1</i> *	257
<i>NAT2</i> [†]	253

*The risk ratio comparison for *NAT1* is based on any *NAT1*10* allele versus all other genotypes.

[†]The risk ratio comparison for *NAT2* is based on slow acetylator genotypes versus rapid genotypes.

Table 2. Results of conditional logistic regression for the selected biomarkers

Variable	Risk ratio	P	95% Confidence limits	
			Lower	Upper
PAH-albumin (Ln)*	0.92	0.71	0.61	1.41
ras p21 (Ln) [†]	0.96	0.52	0.85	1.09
<i>CYP1A1 MspI</i> [‡]	0.63	0.20	0.32	1.26
<i>CYP1A1 Ile462Val</i> [§]	0.27	0.09	0.06	1.24
<i>CYP2D6A</i>	0.50	0.40	0.10	2.48
<i>CYP2D6B</i> [¶]	0.94	0.82	0.56	1.59
<i>NAT1*10</i> [‡]	0.87	0.61	0.50	1.50
<i>NAT2 slow</i> **	0.76	0.32	0.45	1.30
<i>CYP2D6A, CYP2D6B</i> ^{††}	1.07	0.92	0.30	3.76

NOTE: This model did not include β -carotene assignment. Results are not materially altered by inclusion of treatment.

*The risk ratio comparison for PAH-albumin (Ln) is based on one log unit of PAH-albumin.

[†] The risk ratio comparison for ras p21 (Ln) is based on one log unit of ras p21.

[‡] The risk ratio comparison for any *CYP1A1 MspI* allele (heterozygous or homozygous *MspI* genotypes) versus *non-MspI* homozygous genotype.

[§] The risk ratio comparison for any *CYP1A1 462 Val* allele (heterozygous or homozygous) versus 462 Ile/Ile genotype.

^{||} The risk ratio comparison for *CYP2D6A* is based on any A allele versus wild type.

[¶] The risk ratio comparison for *CYP2D6B* is based on any B allele versus wild type.

**Risk ratio comparison for *NAT2* is based on slow acetylator genotypes versus rapid genotypes (21).

^{††} The risk ratio comparison for *CYP2D6* is based on slow metabolism genotypes versus rapid genotypes (19).

We note that tissue blocks are available through the hospitals where surgery was done.

Several investigators have recently reported associations between certain of these genotypes and risk of developing lung cancer, whereas others have not (12, 13, 22). Results have varied by ethnicity, gender, smoking status, age of onset, of lung cancer, histologic type, and sample size. The finding that PAH-albumin adducts were not predictive, unlike PAH-DNA adducts (1, 23), is biologically plausible, given that DNA, not protein, is the critical target in carcinogenicity of PAHs. No other studies have linked ras p21 overexpression to lung cancer risk. However, a relationship between ras p21 overexpression and survival has been seen for non-small cell lung carcinoma (24). In summary, this nested case-control study suggests that PAH-DNA albumin adducts and ras p21 overexpression in blood are not predictive of lung cancer in male Caucasians.

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