

Lung Cancer and the Debrisoquine Metabolic Phenotype

Neil E. Caporaso,* Margaret A. Tucker, Robert N. Hoover, Richard B. Hayes, Linda W. Pickle, Haleem J. Issaq, Gary M. Muschik, Laureen Green-Gallo, Daina Buiivys, Seena Aisner, James H. Resau, Benjamin F. Trump, David Tollerud, Ainsley Weston, Curtis C. Harris

In a case-control study, we tested the hypothesis that the genetically determined ability to metabolize debrisoquine is related to risk of lung cancer. Overall, individuals who were extensive metabolizers of debrisoquine were at significantly greater risk of lung cancer than those who were poor or intermediate metabolizers (odds ratio = 6.1; 95% confidence interval = 2.2-17.1). In this study, case patients had lung cancer, and control subjects had either chronic obstructive pulmonary disease or cancers other than lung cancer. Results were adjusted for age, race, asbestos exposure, and smoking. Both black and white individuals who were extensive metabolizers of debrisoquine were at significantly increased risk after similar adjustment (for blacks, odds ratio = 4.5, 95% confidence interval = 1.1-18.1; for whites, odds ratio = 10.2, 95% confidence interval = 2.0-51.4). Significantly increased risk of lung cancer was also present for individuals who were extensive metabolizers when subjects with chronic obstructive pulmonary disease or other cancers were considered separately. These data confirm that the ability to metabolize debrisoquine is a major determinant of susceptibility to lung cancer. Evaluation of the marker in other case-control settings, further exploration of racial differences, and the prospective evaluation of this marker in subgroups at high risk of lung cancer are areas worthy of further study. [J Natl Cancer Inst 82:1264-1272, 1990]

While exposure to tobacco smoke is widely accepted as the major etiologic factor in lung cancer, differences in individual susceptibility have been inferred from the observation that only a minority of cigarette smokers have diagnoses of lung cancer (1,2). Variation in the ability to metabolize xenobiotics has been considered as a possible explanation for this phenomenon (3), and this hypothesis is consistent with family and twin studies (4-6), as well as cytogenetic and molecular investigations (7-12) that have indicated a role for genetic predisposition in lung cancer etiology.

The metabolism of the antihypertensive drug debrisoquine is under autosomal genetic control (13-15), and inheritance of the trait conferring ability to "extensively" metabolize the drug has been suggested as a host susceptibility factor for lung cancer (16,17). In an earlier study comparing patients with lung cancer and patients with chronic obstructive pulmonary disease, white, British cigarette smokers with the extensive debrisoquine-metab-

olizer phenotype were at higher risk of developing bronchial carcinoma than were intermediate and poor metabolizers of the drug. One concern in interpreting these data was the use of subjects with chronic obstructive pulmonary disease as a sole comparison group. Other studies considering this association have produced conflicting results (18,19) or have attracted criticism of the epidemiologic methods (20,21).

To examine the hypothesis of an association of risk of lung cancer with the ability to metabolize debrisoquine, we conducted a formal epidemiologic case-control study. We used two separate comparison groups and adjusted results for recognized risk factors for lung cancer. This work focuses on the assessment of genetically determined differences in the ability to metabolize debrisoquine as an approach to elucidating a genetic component of lung cancer susceptibility.

Subjects and Methods

Subjects

Patients with histologically confirmed lung cancer who had not yet received radiation or chemotherapy were identified at the University of Maryland Hospital and the Baltimore Veterans

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N. E. Caporaso, M. A. Tucker, R. N. Hoover, R. B. Hayes (Environmental Epidemiology Branch), A. Weston, C. C. Harris (Laboratory of Human Carcinogenesis, Division of Cancer Etiology), National Cancer Institute, Bethesda, Md.

L. W. Pickle, Vincent T. Lombardi Cancer Research Center, Georgetown University, Washington, DC.

H. J. Issaq, G. M. Muschik, Laboratory of Chemical Synthesis and Analysis, Frederick Cancer Research Center, National Cancer Institute, Frederick, Md.

L. Green-Gallo, D. Buiivys, S. Aisner, J. H. Resau, B. F. Trump, University of Maryland School of Medicine, Department of Pathology, and Veterans Administration Medical Center, Baltimore, Md.

D. Tollerud, University of Pittsburgh, Graduate School of Public Health, Pittsburgh, Pa.

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*Correspondence to: Neil E. Caporaso, M.D., Environmental Epidemiology Branch, Executive Plaza North, Rm. 439, National Institutes of Health, Bethesda, MD 20892.

Administration Hospital between July 1985 and March 1989. These patients constituted the case group. Histologic diagnoses of lung cancer were confirmed by pathology review.

Two groups of controls were selected. The first group consisted of patients with a clinical diagnosis of chronic obstructive pulmonary disease. They were identified in the pulmonary disease departments of the hospitals through the use of clinical criteria. All control subjects in this group also had a history of 40 or more pack-years (a unit of cigarette use equal to one pack a day for 1 yr) cigarette smoking and/or abnormalities in pulmonary function tests (forced expiratory volume in 1 sec that was <75% of the predicted normal volume and/or a ratio of forced expiratory volume in 1 sec to forced vital capacity that was <75%). The second group of controls was selected from individuals with a variety of other malignancies, including cancers of the colon, esophagus, stomach, breast, and melanoma. Individuals with bladder cancer were excluded because of the proposed association between bladder cancer and debrisoquine metabolism (22,23).

Subjects were accrued by study nurses, who reviewed rosters of potentially eligible individuals from the surgical, pulmonary, oncology, and medical outpatient clinics and inpatient wards of the University of Maryland Hospital and the Baltimore Veterans Administration Hospital. To determine eligibility, we gave short questionnaires to potential subjects. They were then screened for the following exclusion criteria: presence in the intensive care unit, blood pressure of less than 100/60 mmHg, an inability to take oral medications or to be interviewed, use of general anesthesia within the last 5 days, presence of severe renal disease (creatinine > 4.0 mg/100 mL) or severe liver disease (total bilirubin > 3 mg/100 mL, or serum glutamic-oxaloacetic transaminase or serum glutamic-pyruvic transaminase > 300 units/mL), previous diagnosis of separate primary malignancy other than basal cell carcinoma, and an inability or refusal to give informed consent.

Over a 3.5-year period, 1,240 subjects were screened at the two study hospitals; 719 potentially eligible subjects with the required diagnoses and no previous treatment were identified (table 1). Of these 719 subjects, 169 were not eligible because of potential medical hazards of the phenotyping procedure. Physician consent, informed consent from the subject, and coordination with the nursing staff were then sought for the remaining 550 subjects, and an attempt to schedule the patient for phenotyping was made. Because the protocol for debrisoquine phenotyping

required a detailed explanation, informed consent, an 8-hour urine collection following administration of the drug, phlebotomy, and administration of a questionnaire, the major source of exclusions at this stage (141 subjects) was inability to schedule protocol requirements into the period between diagnosis and treatment initiation. Other exclusions included physician refusal (126) and patient refusal (83). Of the final 200 subjects for whom data were collected, 12 were excluded because of use of the drug quinidine, which interferes with debrisoquine phenotyping, or because of missing laboratory data or histology. The excluded cases and controls resembled those that we analyzed with regard to gender, age, race, and hospital (data not shown).

Questionnaire and Medical Record Review

A structured interview of approximately 45 minutes was conducted in person by a trained interviewer/phlebotomist. Data were collected on sociodemographic and anthropomorphic characteristics, recent and remote tobacco use, personal medical history, usual and recent diet, current use of medications, family history of cancer, alcohol use, and histories of occupations and residence history. Medical records were reviewed to abstract selected information, including histologic diagnoses from pathology reports, results of clinical staging, medications administered, and results of routine clinical laboratory studies.

Debrisoquine Administration

Debrisoquine (Declinax, Hoffman-LaRoche) is an adrenergic blocker, antihypertensive drug used in Canada and Europe. Following an overnight fast, a 10-mg tracer dose of debrisoquine was administered orally. The initial urine sample was discarded, and urine was collected over the next 8 hours. The procedure used was the same as that used in earlier studies (16). A few subjects received debrisoquine in the evening, and urine was collected overnight in a protocol for phenotyping that has been shown to be equivalent to the original method (24). Nonessential medications were not given on the morning of debrisoquine administration; fluids and a light breakfast were permitted 1 hour after the drug was given.

We monitored all patients by recording blood pressure readings at the time of debrisoquine administration and then at 30-minute intervals. Because of the possibility of postural hypotension, readings were continued until two normal readings were recorded. No significant hypotensive or other adverse reactions were noted in the study.

Table 1. Exclusions from study by type and diagnosis

	Cases	Controls*		Total (%)
		COPD	Other cancer	
No. of subjects eligible for study	291	187	241	719 (100)
Excluded from study	195	132	204	531 (74)
1. Hazard with phenotyping	56	28	85	169 (23)
2. Refusal or schedule conflict	134	99	117	350 (49)
3. Insufficient data†	5	5	2	12 (2)
Included in study	96‡	55	37	188 (26)

* COPD = chronic obstructive pulmonary disease. Other cancer = cancer other than lung cancer.

† Laboratory data were missing for seven cases, and quinidine use was documented for five cases.

‡ Some data were missing for seven cases.

Laboratory Methods

The determination of debrisoquine and its chief metabolite 4-hydroxydebrisoquine in an aliquot of the urine was accomplished through the use of the method of Idle et al. (25) except for the addition of a water wash step for the toluene extract after derivitization. The addition of this step allowed the analysis to be performed on a capillary column without degrading column performance.

Analysis was performed on a capillary gas chromatograph (model 6000, Varian, Walnut Creek, Calif) fitted with a nickel-63 electron-capture detector and a splitting capillary injector with a split ratio of 12:1. The column used was a 30-m DB-1 fused-silica capillary column (J & W Scientific, Folsom, Calif) with an inside diameter of 0.32 mm and a film thickness of 0.25 μm . The carrier gas used was helium at a linear velocity of 24 cm/sec. The oven temperature was held at 150 $^{\circ}\text{C}$ for 1 minute. The injector and detector temperatures were set at 250 $^{\circ}\text{C}$ and 275 $^{\circ}\text{C}$, respectively. The sample size injected was 1 μL .

Quantitation was performed on a Chromatopac (model CR4A, Shimadzu Scientific Instruments, Columbia, Md). Internal standardization and single-point calibration were used. The retention times determined for debrisoquine, 4-hydroxydebrisoquine, and 7-methoxy-guanoxan (internal standard) were 8.0, 10.7, and 15.2 minutes, respectively.

Statistical Analysis

The debrisoquine metabolic phenotype was determined by calculating the metabolic ratio, which is the percent dose excreted as unchanged debrisoquine divided by the percent dose excreted as 4-hydroxydebrisoquine. The metabolic ratio classifies individuals into one of three categories: extensive metabolizers (EMs), intermediate metabolizers (IMs), or poor metabolizers (PMs) of debrisoquine. The method used for cutpoint determination is described elsewhere (26). Briefly, a mixture model was used to fit three normal distributions to the frequency distribution observed in controls. Cutpoints for the metabolic phenotype determination were derived from the intersections of the three theoretical distributions. Model parameters were estimated through the use

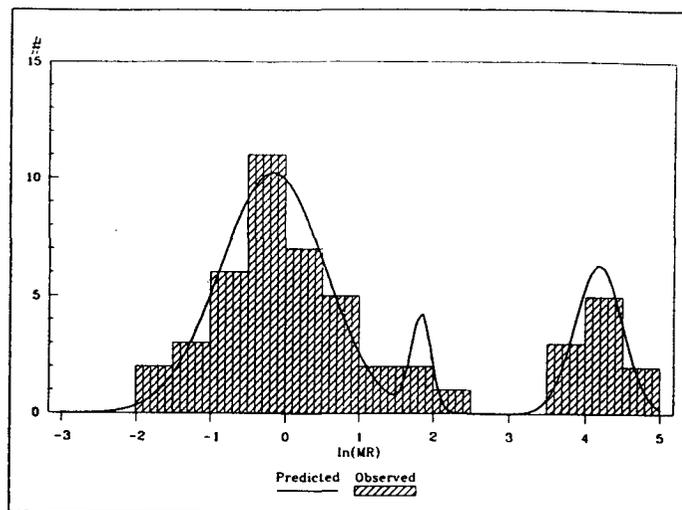


Figure 2. Distribution of $\ln(\text{metabolic ratio})$ predicted with a three-mix model and the corresponding histogram of observed frequency for white controls.

of an iterative maximum-likelihood procedure. The theoretical distributions for black (fig. 1) and white (fig. 2) control subjects were derived separately from those of the control subjects of each race. For blacks, the derived cutpoints were $\text{EM/IM} = 4.2$ and $\text{IM/PM} = 26.4$; for whites, the derived cutpoints were $\text{EM/IM} = 4.8$ and $\text{IM/PM} = 11.7$.

The distributions for blacks and whites resemble each other and resemble the distributions for other western populations (27). Specifically, the cutpoint values are similar, the EM and IM distributions overlap, and the PM distribution is distinct (see combined distribution, fig. 3). Despite the similarities observed in blacks and whites, ethnic variation in debrisoquine metabolism on a genetic basis is expected, and arbitrary a priori application of the cutpoints determined in one ethnic group to another has the potential to produce unacceptable misclassification bias (28-32).

We searched for, but failed to find, convincing evidence for multiplicative interactions between debrisoquine metabolism and other risk factors for lung cancer by looking for nonuniformity of

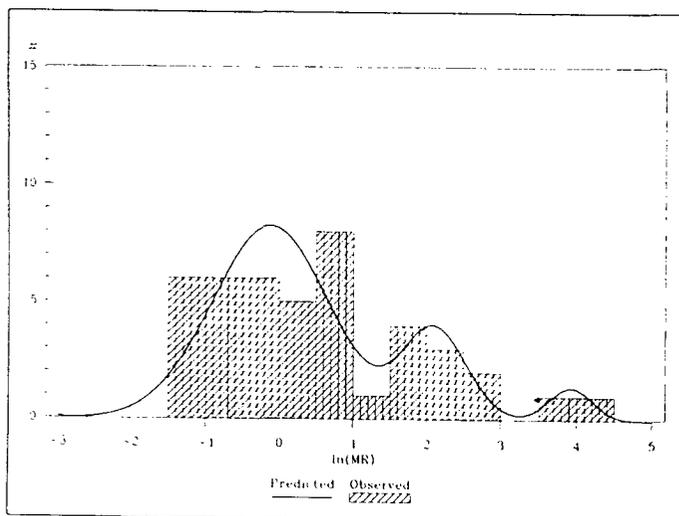


Figure 1. Distribution of $\ln(\text{metabolic ratio})$ predicted with a three-mix model and the corresponding histogram of observed frequency for black controls.

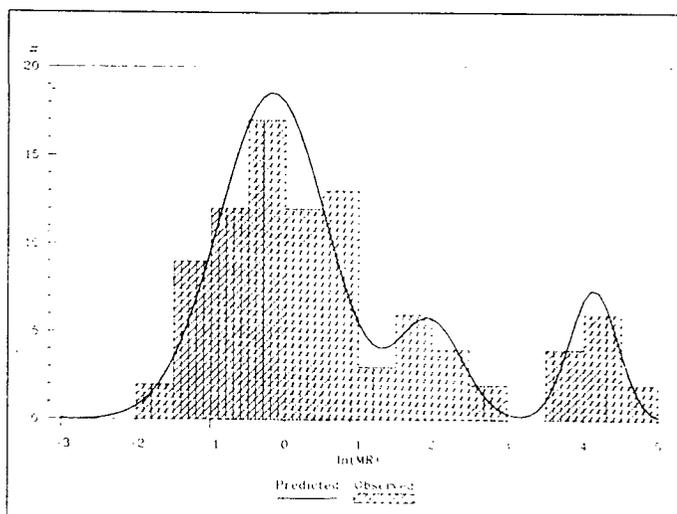


Figure 3. Distribution of $\ln(\text{metabolic ratio})$ predicted with a three-mix model and the corresponding histogram of observed frequency for all controls.

Table 2. Comparison of relative risk of lung cancer in subjects with extensive metabolism of debrisoquine vs. subjects with poor metabolism and intermediate metabolism

	EM		PM and IM		Relative risk		95% confidence interval
	No. of cases	No. of controls	No. of cases	No. of controls	Crude	Adjusted*	
Total	84	68	5	24	5.9	6.1	2.2-17.1
Men	81	56	5	22	6.4	6.2	2.2-17.6
Race							
Black	42	32	3	11	4.8	4.5	1.1-18.0
White	42	36	2	13	7.6	10.2	2.0-51.5

* Values were adjusted for age (<64, ≥64 yr), race (black or white), smoking (pack yr, < or > median), and history of occupational exposure to asbestos (yes or no).

effect in simple stratified analyses and by attempting to fit models with the appropriate interaction terms (33). We explored for confounding of the major effect by performing exclusion, simple stratified analysis, and multivariate (stepwise and logistic regression) analysis. The results achieved from each of these approaches were in good agreement; the results of the multivariate analysis are presented (table 2).

Simple means and Wilcoxon ranking statistics were determined for the analysis of continuous variables. For categorical variables, including the metabolic phenotype, relative risks were estimated by calculation of odds ratios and corresponding 95% confidence intervals. Adjustment of the results for the individual and combined effects of potentially confounding variables were accomplished with logistic regression performed with the SAS Proc Logist procedure. Categorical variables were created from continuous variables (e.g., pack-years for smoking history; age) for both stratified and multivariate analysis. The use of continuous variables in logistic regression and stepwise regression analyses yielded similar results to those presented here. All statistical analyses were performed with the SAS statistical analysis package (34).

Results

This analysis was conducted on 96 cases and 92 controls; 32 cases and 30 controls were from the University of Maryland Hospital, and 64 cases and 62 controls were from the Baltimore Veterans Administration Hospital. Of the 92 controls, 55 were patients with chronic obstructive pulmonary disease and 37 had cancers other than lung cancer. Table 3 illustrates the characteristics of the study groups with respect to several major risk factors for lung cancer. The groups were similar in age and race. Only three case patients and one control subject with chronic obstructive pulmonary disease were women, while 13 women were in the "other cancer" group. The mean for lifetime pack-years of cigarette smoking for the control group with chronic obstructive pulmonary disease was greater than that for the control group with "other cancer" (67 vs. 36 pack-yr), while the mean value for cases was intermediate (57 pack-yr). Thus, the combined control group resembled the case group.

The case patients were slightly more likely than the control subjects to have smoked in the past week. Approximately 40% of the subjects in the case group and in the control group with chronic obstructive pulmonary disease reported a history of

occupational exposure to asbestos, compared with 14% for the "other cancer" group. Half of the case patients with lung cancer and half of the control subjects with "other cancer" reported a family history of malignancy versus about one third of the control subjects with chronic obstructive pulmonary disease. A family history of a smoking-related malignancy was clearly excessive for the groups with lung cancer, compared with either control group. This was due almost exclusively to an excess of lung cancer (12% of cases vs. 5% for the control group with chronic obstructive pulmonary disease and 3% for the control group with "other cancer.").

A frequency histogram depicting the distribution of metabolic ratios for the cases of lung cancer and for the controls is presented in figure 4. The natural logarithm of the metabolic ratio (-0.03) was significantly lower for the cases than for the pooled controls (0.68) (Wilcoxon ranking statistics, $z = 2.28$; $P = .02$). These data confirm the visual impression of a difference in the distribution of the metabolic ratios between the cases and controls. When categorized into phenotypes, only one case was classified as PM, and four were classified as IMs. In contrast, 12 controls were classified as PMs and 12 as IMs. Thus, compared with a relative risk of 1.00 for PMs, the relative risk for IMs was 4.0 and that for EMs was 16.0 ($P_{\text{trend}} = 5.6 \times 10^{-5}$, two-tailed test).

Because of paucity of cases in the PM and IM categories, these two groups were combined to assess potential confounding and interaction. In the aggregate, the crude relative risk of lung cancer for EMs versus PMs/IMs was 5.9 (95% confidence interval = 2.1-16.3). This association was also controlled individually for the following variables: age, race, hospital, educational level, lifetime pack-years of cigarette smoking, history of exposure to occupational lung carcinogens, height, weight, recent smoking, family history of lung cancer, and clinical laboratory results of routine medical tests, including tests for liver and kidney function. Slight changes in the estimate of relative risk occurred with adjustment for age, race, pack-years of cigarette smoking, and asbestos exposure. Family history of lung cancer (i.e., number of first degree relatives with lung cancer) increased the risk estimates for debrisoquine EM slightly. However, this variable was withheld from the estimates presented here because the degree to which this variable subsumes risk due to the debrisoquine phenotype is indeterminate.

The adjusted estimate of the relative risk of lung cancer for EMs versus PMs and IMs was 6.1 (95% confidence interval = 2.2-17.1) (table 2). The adjusted risks were elevated for both

Table 3. Risk factors for lung cancer by diagnostic group*

Risk factor	Cases	Controls	
		Chronic obstructive pulmonary disease	Other cancer
No. of subjects	96†	55	37
Age, mean in yr	64.1	62.1	60.7
Sex			
Male	93	54	24
Female	3	1	13
%	97	98	65
Race			
Black	49	26	17
White	47	29	20
%	51	47	46
Lifetime pack-yr of cigarette smoking	57.0	66.9	36.4
Smoking last wk			
Yes	39	17	12
No	50	38	25
%	44	31	32
Asbestos exposure			
Yes	36	20	5
No	53	35	32
%	40	36	14
Family history‡			
Cancer			
Yes	43	20	17
No	46	35	20
%	48	36	46
Smoking-related cancer§			
Yes	19	4	7
No	70	51	30
%	21	7	4
Lung cancer			
Yes	11	3	1
No	78	52	36
%	12	5	3

*Unless otherwise indicated, values = No. of subjects. % = male, black, or "yes."

†Seven subjects with certain questionnaire data missing are excluded.

‡First-degree relatives.

§The following malignancies were classified as smoking-related: cancers of the lung, urinary bladder, nasopharynx or oropharynx, esophagus, stomach, and head and neck (including nose, mouth, throat, and lip).

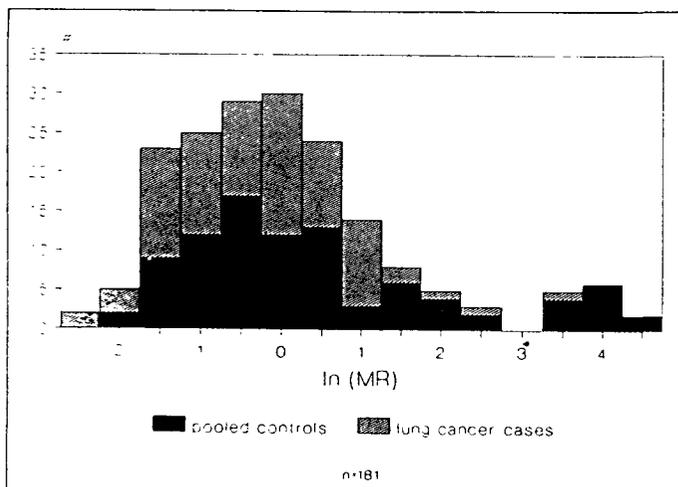


Figure 4. Distribution of $\ln(\text{metabolic ratio})$ for lung cancer cases (pattern) and pooled controls (solid).

racess, although they were somewhat higher for whites (10.2) than for blacks (4.5). When the analysis was restricted to males, the relative risk was 6.2. The distribution of phenotypes was similar in both control groups, thus yielding similar estimates of the relative risk when the cases were compared either with the chronic obstructive pulmonary disease control group (6.0; 95% confidence interval = 2.0–17.9) or with the "other cancer" control group (10.4; 95% confidence interval = 2.0–40.3).

The metabolic ratio for debrisoquine was also evaluated for the various subgroups of cases and controls (table 4). Among lung cancer patients, those with adenocarcinoma had a higher natural logarithm of the metabolic ratio (0.27) than those with the other histologies considered as a group (-0.17) (Wilcoxon ranking statistic, $z = 1.83$; $P = .06$). This difference fell just short of statistical significance. Among the controls, the natural logarithm of the metabolic ratio was similar for both the group with chronic obstructive pulmonary disease (0.59) and the group with "other cancer" (0.81) (Wilcoxon ranking statistic, $z = .73$; $P = .47$),

Table 4. Metabolic phenotype and natural logarithm of the debrisoquine metabolic ratio by diagnostic subgroupings*

Group	No. of patients	LN(MR) ± SE	No. in each metabolic phenotype		
			EM	IM	PM
<i>Cases</i>					
Lung cancer	89†	-0.03 ± 0.11	84	4	1
Squamous cell carcinoma	47	-0.21 ± 0.16	45	2	0
Small cell carcinoma	9	0.03 ± 0.42	8	1	0
Large cell carcinoma	4	-0.15 ± 0.18	4	0	0
Adenocarcinoma	29	0.27 ± 0.18	27	1	1
<i>Controls</i>					
Pooled controls	92	0.68 ± 0.11	68	12	12
COPD	55	0.59 ± 0.23	41	7	7
Other cancer‡	37	0.81 ± 0.27	27	5	5

*COPD = chronic obstructive pulmonary disease. Other cancer = cancer other than lung cancer.

†For lung cancer other than adenocarcinoma lung histologies: ln(MR) = -0.17 ± 0.14 SE.

‡Types of cancer (No. of controls) were colon (9), esophagus (9), breast (6), melanoma (5), gastric (3), prostate (3), gall-bladder (1), and liver (1).

and for blacks (0.50) and whites (0.83) as well (Wilcoxon ranking statistic, $z = 0.56$; $P = .58$).

Although the control group was small, it did provide some opportunity to search for other correlates of debrisoquine metabolism. As noted, the proportion of whites who were EMs was similar to that for blacks (73% and 74%, respectively). However, some evidence of a racial difference in the distribution of IMs and PMs was observed. Twenty percent of the white control subjects were PMs and 6% were IMs, while 5% of the black control subjects were PMs and 21% were IMs. Of the variety of other variables investigated (table 5), none were significantly related to the metabolic ratio, except age. Previous studies have not found an association of age with debrisoquine metabolism (14).

The study included mostly newly diagnosed cases; however, six cases with lung cancer and eight patients with "other cancer" were originally diagnosed more than 6 months prior to study accrual. Excluding these subjects, or adjusting data for them by

stratification or multivariate methods, did not alter the risk estimates.

Discussion

The original report by Ayesh et al. (16) of a substantial association between a genetically determined metabolic pathway and the risk of lung cancer was a clinical description of the association. Follow-up studies have been small (19), have reported inconclusive results (18), or have attracted criticism of the epidemiologic methods (20,21).

The study presented here utilized a formal epidemiologic research design. Attention was given to the populations from which the study subjects were obtained and to the selection of two distinct control groups. Particular attention was paid to the enumeration of the subjects who were excluded and description of criteria for exclusions and to an extensive assessment of, and control for, other variables that might confound the association with the metabolic phenotype. In this context, it is noteworthy that the overall association of risk (adjusted) of lung cancer with the metabolic phenotype (sixfold for EMs vs. IMs and PMs) was close to that which can be calculated from a roughly similar reanalysis of the data of Ayesh et al. (17) (relative risk = 6.2; 95% confidence interval = 3.3-11.9). The similarity in the ability to metabolize debrisoquine by the two control groups provides reassurance that the association is with lung cancer, and not chronic obstructive pulmonary disease, which was the single diagnostic category of controls used in the study by Ayesh et al. The finding that both black and white debrisoquine EMs have significantly elevated risk of lung cancer further enhances the credibility of a suggested genetic risk factor.

In an extensive evaluation of potential confounding factors, we found little evidence that any measure of cigarette smoking, either recent or remote as determined by questionnaire or by biochemical methods (urine cotinine), was related to debrisoquine metabolism. Indeed, if anything, control for cigarette smoking slightly increased the estimate of the relative risk. This finding corroborates other work and provides additional evidence against the hypothesis that recent smoking could induce the enzyme that metabolizes debrisoquine.

Table 5. Correlation coefficient (r) between various study parameters and debrisoquine metabolic ratio in controls*

Variable	r †	P value
Weight	.11	.30
Height	.11	.31
Quetelet's index	.11	.32
Age	.28	.008
FEV ₁	-.05	.73
FVC	.00	.99
FEV ₁ /FVC‡	-.25	.10
Alcohol (drinks/day)	.05	.66
Smoking		
Pack-year	.11	.30
Cigarettes/wk	.05	.65
Cigarettes/day‡	-.08	.66
Cotinine§ (ng/mL of urine)	-.07	.52

* r = Spearman correlation coefficient. Quetelet's index = weight (kg)/height² (cm). FEV₁ = forced expiratory volume in 1 sec. FVC = forced vital capacity.

†Available for 44 of 55 controls with chronic obstructive pulmonary disease.

‡This variable was considered only for subjects who were currently smoking (26 of 85 controls).

§This variable was available on 86 of 92 controls.

There was also little evidence that a variety of other variables were major confounding factors of the association of lung cancer and the debrisoquine metabolic ratio. These other variables included occupational exposures, body size, alcohol use, recent diet, socioeconomic status, and liver or renal function. The positive association of the debrisoquine metabolic ratio with age, which we observed only in the controls, is unlikely to reflect an actual impairment of metabolic capacity in the elderly, as such an impairment in the elderly has not been observed in other normal populations (14). A slight decline in oxidative metabolic capacity associated with age-related decrease in parenchymal liver function is one potential explanation. A more speculative possibility is that the PM phenotype becomes more prevalent in aging cigarette-smoking populations as the more susceptible EM subjects succumb to lung cancer. Such a possibility is consistent with both the lack of an age association in the cases and the slight overrepresentation of PMs in the controls in this study, compared with other western populations (27,35).

Two aspects of this study warrant attention in the interpretation of the findings. The first is the large number of exclusions that were necessary. Exclusions in the study fell into three broad categories: potential interference with phenotyping, medical exclusions, and time conflicts. The three major potential sources of interference with phenotyping were prior chemotherapy or radiation therapy, recent general anesthesia, and quinidine therapy (36,37). Studies suggest that chemotherapy has an insignificant influence on the metabolic ratio (18,38,39), and thus future investigations might elect to ease exclusions based on this criterion.

Individuals who had organ failure or who were in the intensive care unit fell into the second category of exclusions because of the potential for postural hypotension in PMs. The third category, time conflicts, has been discussed in the context of the demanding study protocol, which was a substantial source of exclusions. While only 26% of subjects potentially eligible were ultimately enrolled in the study, we feel that the major reasons for exclusion are unlikely to be associated with the genetic basis for the metabolic phenotype. Thus, they are unlikely to have resulted in any bias.

The second aspect to be considered is that because of the case-control design, the current study cannot address whether the association observed is a cause or an effect of tumor. Theoretically, the tumor, tumor products, and therapeutic or diagnostic methods could modulate the expression or measurement of the ability to metabolize debrisoquine (i.e., the phenotyping procedure). On the basis of work performed by our group and others, this hypothesis appears unlikely; however, a final resolution of this question will require either a reliable molecular test for the genotype or a prospective study. Misclassification in the assignment of the metabolic phenotypes should be minimized by the maximum-likelihood procedure used in this study. Even with this procedure, though, the possibility of misclassification cannot be completely eliminated in light of family studies that have shown obligate heterozygotes to be distributed in the EM and IM groups (40).

We used metabolic phenotypes in this study to allow a degree of control for ethnicity, to produce categorizations for epidemiologic inference, and to allow comparison with earlier studies that have relied on such categorizations. One comparison of the cases

and controls that bypassed the need for such categorization (i.e., a ranking test based on the metabolic ratios), confirmed a significant difference between the study groups. The anticipated future refinement of a restriction-fragment-length polymorphism marker for the metabolic phenotype will simplify epidemiologic studies involving the relationship between the ability to metabolize debrisoquine and risk of lung cancer. The present phenotyping procedure requiring drug administration followed by urine collection will be replaced by a simple blood test (41,42), and genotype assignment will presumably be unequivocal. Unfortunately, while work in the characterization of the CYP2D6 gene progresses (43), the currently available marker for a restriction-fragment-length polymorphism for phenotype determination is presently inadequate for field studies (44,45).

Most of the study subjects with cancer had newly diagnosed disease; we feel there is no possibility that EMs are overrepresented in the case group, because this metabolic phenotype is a "good prognosis" factor. Removing the study subjects with previously diagnosed disease from the analysis did not alter the results.

If this association between debrisoquine metabolism and risk of lung cancer is causal, then the biologic mechanism responsible will be of importance. Strong evidence exists that other specific P-450 isozymes activate known human carcinogens (46,47). Despite the demonstrated activity of the CYP2D6 gene in the metabolism of numerous drugs and xenobiotics (48), a role in the metabolism of a specific carcinogen remains to be demonstrated (49). Thus, it is possible that the debrisoquine gene is in linkage disequilibrium with another gene important in the carcinogenic process. Although EMs were labeled as high risk in this study, in the absence of convincing data regarding the mechanism, it is also appropriate to consider the underrepresentation of PMs in the lung cancer group to be a "protective" effect of this phenotype.

The data suggest that the ability to metabolize debrisoquine differs among individuals with different lung cancer histologies, as previously observed. The finding of a smaller risk associated with the histologic subtype that is less related to smoking (i.e., adenocarcinoma) is consistent with this histology being dependent on a carcinogen that is not metabolized by the debrisoquine isozyme.

Conclusions

This case-control study found that extensive metabolizers of debrisoquine were at a sixfold increased risk of lung cancer compared to poor and intermediate metabolizers. This "biochemical epidemiology" study included a number of features designed to increase the epidemiologic rigor: careful assessment and adjustment for potential risk factors of lung cancer; use of well-defined exclusions and study populations; consideration of the possibility of medication-related interference with phenotyping; use of a standardized questionnaire, two control groups, control for ethnic variation, and a maximum likelihood technique to minimize misclassification in the assignment of the metabolic phenotype categories; and finally, close collaboration between epidemiologists, clinicians, and laboratory scientists throughout the course of the study. Limitations of the study included the relatively high number of exclusions, the inherent inability of the metabolic ratio to perfectly assign the genotype, and inability of

the study design (case-control) to directly prove a causal relation between the debrisoquine metabolic phenotype and lung cancer. Consideration of the methodologic strengths and weaknesses of this study will be instructive for future collaborative studies involving other putative markers of cancer risk.

The results have implications for future work. The establishment of a genetic risk factor (or protective factor) for lung cancer would have major implications for studies in etiology and carcinogenesis. If a compound that undergoes an oxidation by the CYP2D6 enzyme could be identified in tobacco smoke, a completely new strategy for prevention could be devised. A fivefold to tenfold spectrum of risk in the population on the basis of a genetic factor, if verified, will be important from the perspective of risk assessment and public health. Screening strategies for lung cancer that are presently ineffective might prove efficacious if focused on high-risk groups. Ethical questions raised by the ability to assess this and other genetic markers of cancer risk will challenge health professionals, politicians, and the public to confront difficult questions.

Evaluation of this marker in other case-control settings, further exploration of racial differences, and the evaluation of this and other P-450 enzymes in prospective settings are areas worthy of further study.

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