Analysis of Cytochrome P450 2E1 Genetic Polymorphisms in Relation to Human Lung Cancer

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Abstract

Human cancer risk assessment using molecular genetic techniques is a rapidly emerging field. Many studies suggest that both inherited and acquired genetic predispositions play an important role in carcinogenesis. Cytochrome P450 (CYP) 2E1 is involved in the metabolic activation of *N*-nitrosamines and other low molecular weight compounds. A recently described genetic polymorphism of CYP2E1 [*Dra*I restriction fragment length polymorphism (RFLP)] has been associated with an increased risk of lung cancer in Japanese. We have assessed the allelic frequency of three RFLPs (*Pst*I, *Rsa*I, and *Dra*I) in African-Americans (n = 109), Caucasian Americans (n = 153), and octogenarian Japanese (n = 42), and also in a United States case-control study of lung cancer (histologically confirmed lung cancer, n = 58; controls, n = 56; total, n = 114). The relationship of the CYP2E1 *Dra*I polymorphism to other CYP2E1 polymorphisms (*Pst*I and *Rsa*I RFLP) was examined. The allelic frequency of the *Dra*I C minor allele for all subjects was 0.09 in Caucasians, 0.09 in African-Americans, and 0.31 in Japanese. In the case-control study of lung cancer, no association of the CYP2E1 *Dra*I genotype with lung cancer was found (odds ratio, 1.57; 95% confidence interval, 0.59–4.18). Comparison after discordant CYP2E1 genotypes suggests the presence of different haplotypes in Americans and Japanese. These results indicate that the CYP2E1 *Dra*I RFLP is probably not a cancer risk factor in United States Caucasian or African-Americans, although statistical power is limited given the low frequency of the CYP2E1 *Dra*I C minor alleles.

Introduction

The application of molecular genetic techniques to human cancer risk assessment will likely emerge as a method of identifying subpopulations with different sensitivities to carcinogen exposure (1, 2). Specifically, the application of these techniques to the study of carcinogen activation, detoxification, and DNA repair has begun to elucidate a role for genetic predispositions (3). The capacity to metabolically activate or detoxify chemical carcinogens by CYP[2] enzymes (4) such as CYP2E1, CYP2D6, CYP1A1, CYP1A2, and CYP3A4, or by glutathione-*S*-transferase M1 and *N*-acetyltransferase can be assessed by phenotyping (4–8) or genotyping methods (9–11). Several reports suggest that some polymorphisms are related to lung cancer risk (9, 12–16).

A RFLP of CYP2E1 may be important in human carcinogenesis if it governs the metabolic activation of *N*-nitrosocompounds and other low molecular weight suspect carcinogens (17). One CYP2E1 RFLP located in intron 6, revealed by *Dra*I and identified as C (minor) alleles and D (common) alleles (18), has been associated with lung cancer in a Japanese case-control study (18). However, a mechanistic relationship of the RFLP to lung cancer remains unknown. There are two other CYP2E1 RFLPs, revealed by either *Rsa*I or *Pst*I (19), that are located in the transcription region of this gene and might have a biological effect. The CYP2E1 *Dra*I and *Rsa*I RFLPs were tested for lung cancer risk in Western populations (20,21). There was no association with either marker in Finnish persons (20) and for the latter in United States Caucasian and African-Americans (21). Similar data for the *Dra*I RFLP in Americans has not been reported.

Presented herein are the results from a United States case-control study designed to explore genetic risk factors for lung cancer. The CYP2E1 *Dra*I RFLP was studied in relation to lung cancer risk.

Materials and Methods

Subjects. Three separate groups were studied. The CYP2E1 *Dra*I polymorphism was studied in Americans from a Baltimore, Maryland, autopsy tissue donor program, Americans enrolled in a Baltimore Hospital case-control study of lung cancer, and octogenerian Japanese. The CYP2E1 *Pst*I and *Rsa*I RFLPs have been reported previously (21). The case-control study design has been described in more detail elsewhere (16). Briefly, cases (n = 58) were patients with histologically confirmed primary lung cancer. Two control groups were selected; one (n = 37) consisted of patients with COPD or a history of >40 pack-years of tobacco smoking (defined as average packs of cigarettes smoked/day

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[2] The abbreviations used are: CYP, cytochrome P450; RFLP, restriction fragment length polymorphism; OR, odds ratio; PCR, polymerase chain reaction; CI, confidence interval; COPD, chronic obstructive pulmonary disease.

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multiplied by the number of years consumed), and the other (n = 18) consisted of patients with cancer at anatomical sites other than lung or urinary bladder. The study design allowed for adjustment by age, gender, race, and tobacco usage. Tissues from the autopsy donor program (n = 148) were collected between 1985 and 1992. Autopsy cases were accrued from the medical examiners office, mostly from victims of sudden death (trauma, coronary artery disease, etc.). None were found to have cancer at autopsy. Japanese samples (n = 42) were obtained from residents of a nursing home in Tokyo whose ages ranged from 80 to 102. Most had chronic medical problems but only seven had cancer (two with lung cancer). These cancer cases were included in the analysis because this is representative of a Japanese octagenarian population. DNA from autopsy donors and Japanese were used to determine gene frequencies in these groups only and not to serve as a control group to lung cancer cases.

CYP2E1 Restriction Fragment Length Polymorphism Analysis. DNA (blood buffy coat for case-control study subjects and Japanese; lung tissue from autopsy donors) was isolated by phenol extraction methods as described previously (22). PCR was used to amplify intron 6 of CYP2E1 that includes the *Dra*I enzyme recognition site. Genomic DNA (0.1 μg) was amplified with primers flanking intron 6 of CYP2E1 and the *Dra*I RFLP (0.8 μM), position 7367–7387 (5′-TCGTCAGTTCCTGAAAGCAGG) and 8340–8361 (5′-GAGCTCTGATGCAAGTATCGCA) in buffer [10 mM Tris-HCl-50 mM KCl-2 mM $MgCl_2$ (pH 8.3)] with 2′-deoxynucleoside-3′-triphosphate (1.875 mM; Pharmacia, Piscataway, NJ) and Taq polymerase (2.5 units; Perkin Elmer Cetus, Norwalk, CT) in a total volume of 50 μl. An initial melting temperature of 94°C (4 min) was followed by 35 cycles of melting (94°C, 1 min), annealing (60°C, 1 min), and extension (72°C, 4 min). The reaction yielded a 995-base pair fragment. A portion of the PCR-amplified product (20 μl) was subjected to a *Dra*I restriction enzyme digestion (10 units; New England Biolabs, Inc., Beverly, MA) in buffer [50 mM potassium acetate-20 mM tris-acetate-10 mM magnesium acetate-1 mM dithiothreitol (pH 7.9)] at 37°C for 18 h. The samples were then analyzed by agarose gel electrophoresis (2.2%). Two *Dra*I restriction enzyme recognition sites exist in this amplified DNA sequence but only one is known to be polymorphic. The presence of the polymorphic *Dra*I restriction site yielded three fragments of 572, 302, and 121 base pairs (type D), while the absence of the polymorphic site was determined by the presence of 874-base pair and 121-base pair fragments (type C). The 121-base pair fragment band represents a constant *Dra*I restriction site.

Statistical Methods. Analyses were performed using the Statistical Analysis System (SAS Institute, Cary, NC). Fisher's exact tests or χ^2 tests were used as appropriate for the analysis of the categorical variables, genotypes and case status. The ORs were determined as an estimate of the relative risk. OR = AD/BC where A/C = odds of exposure in diseased individuals and B/D = odds of exposure in disease-free subjects. The OR gives the ratio of the odds of exposure in diseased compared to nondiseased. Mantel-Hanszel estimates of a summary odds ratio, adjusted for the effects of a stratification variable, were used as appropriate (23, 24). Logistics regression models for the case-control analysis were used to adjust for age, race, gender, smoking status, and other variables when sufficient numbers were available (23). Crude ORs were virtually unchanged by adjusting for these factors; therefore, only crude ORs are presented.

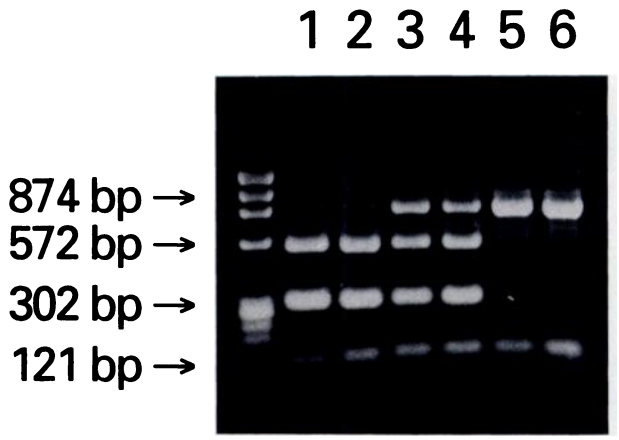

Fig. 1. DNA was amplified using the polymerase chain reaction and subjected to a *Dra*I restriction enzyme digestion. Samples were analyzed by agarose gel electrophoresis (2.2%). The three possible genotypes are shown: CC (*Lanes 1* and *2*); CD (*Lanes 3* and *4*); and DD (*Lanes 5* and *6*).

Table 1 Ethnic distributions of CYP2E1 *Dra*I restriction fragment length polymorphisms

Race	Source	Allelic Frequency		
		No.	D[a]	C
Caucasian[b]	Autopsy[c]	98	0.93(182)[d]	0.07(14)
	Lung Cancer[e]	27	0.85 (46)	0.15 (8)
	Controls[e]	28	0.91 (51)	0.09 (5)
	Pooled	153	0.91(279)	0.09(27)
African-Americans[b]	Autopsy[c]	50	0.87 (87)	0.13(13)
	Lung Cancer[e]	31	0.94 (58)	0.06 (4)
	Controls[e]	28	0.95 (53)	0.05 (3)
	Pooled	109	0.91(198)	0.09(20)
Japanese[f]		42	0.69 (58)	0.31(26)

[a] D, major allele: CYP23E1 *Dra*I restriction site present; C, minor allele: restriction site absent.
[b] Pooled Caucasian and African-American: $X^2 = 2.51$, dF = 1, $P > 0.05$; Japanese and Caucasian: $X^2 = 25.2$, dF = 2, $P < 0.05$; Japanese and African-American: $X^2 = 23.1$, dF = 2, $P < 0.05$.
[c] DNA samples obtained from autopsy tissue donor program.
[d] Parentheses indicate number of alleles.
[e] DNA samples obtained from case-control study of lung cancer.
[f] DNA samples obtained from Japanese octogenarians and older.

Table 2 Characteristics of lung cancer case-control study subject

	Cases	Controls		
		COPD	Other cancer[a]	Pooled
Subject number	58	38	18	56
Mean age (years)	64	61	63	62
Pack-years[b] (mean)	60	65	48	60
Gender (male/female)	56/2	37/1	14/4	41/15
Caucasians (%)	47	53	44	48

[a] Subjects with cancer at anatomical sites other than lung or urinary bladder.
[b] Average of cigarettes smoked/day multiplied by the number of years consumed (lifetime).

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Table 3 CYP2E1 *Dra*I restriction fragment length polymorphism case status *versus* genotype

Genotype	Lung cancer	Controls			Odds ratio
		COPD	Other cancer[a]	Pooled	
DD[b]	46 (79)[c]	33 (87)	15 (83)	48 (86)	1.00
CD	12 (21)	5 (13)	3 (17)	8 (14)	1.57 (0.59–4.18)[d]
CC[b]	0 (0)	0 (0)	0 (0)	0 (0)	—[e]

[a] Excluding urinary bladder cancer and lung.
[b] D, major allele: CYP2E1 *Dra*I restriction site present; C, minor allele: restriction site absent.
[c] Parenthesis indicate percentage.
[d] Parenthesis indicate 95% confidence interval.
[e] Not calculated.

Table 4 CYP2E1 *Dra*I genetic polymorphism, lifetime cigarette consumption, and case status

Case status	Pack-years[a]					
	<Median[b]			>Median[b]		
	DD[c]	CD	CC	DD	CD	CC
Case	24	5	0	17	5	0
Control	23	3	0	24	5	0
Odds ratio	1.0	1.60 (0.34–7.50)[d]		1.0	1.41 (0.35–5.71)[d]	

[a] Pack-years, average number of packs of cigarettes smoked/day multiplied by total number of years smoking during one's lifetime. Data available only for 107 subjects.
[b] Low or high smoker is defined by median pack-years (53).
[c] DD, major allele: CYP2E1 *Dra*I homozygote site present; CD, heterozygote; CC, minor allele: homozygote site absent.
[d] Parentheses indicate 95% confidence intervals.

Table 5 Distribution of CYP2E1 *Dra*I genotypes by lung cancer histology

Histology	DD	CD	CC	Odds ratio
Squamous cell	26	5	0	1.15 (0.34–3.91)[a]
Small cell	4	0	0	—[b]
Adenocarcinoma	15	4	0	1.60 (0.42–6.07)
Pooled controls	48	8	0	1.0 (referent)

[a] Parenthesis indicate 95% confidence interval.
[b] Not calculated.

Table 6 CYP2E1 genotypes in United States and Japanese populations[a]

		CYP2E1 *Dra*I		
		DD[b]	CD	CC
Americans				
*Pst*I	WW	138	24	0
	WM	6	5	0
	MM	0	0	0
*Rsa*I	WW	144	24	0
	WM	0	5	0
	MM	0	0	0
Japanese				
*Pst*I	WW	18	3	0
	WM	0	19	2
	MM	0	0	0
*Rsa*I	WW	18	3	0
	WM	0	19	2
	MM	0	0	0

[a] Data for all three CYP2E1 RFLPs available for 173 Americans and 42 Japanese.
[b] DD, major genotype: homozygous site present; CD, heterozygote; CC, minor genotype: homozygous site absent; WW, wild type homozygote; MM, mutant homozygote; WM, heterozygote.

Results

Samples subjected to PCR and *Dra*I enzymatic digestion revealed the expected fragment lengths, resulting in three possible patterns (Fig. 1). DNA from 148 nonmalignant lung tissues from noncancer donors obtained at autopsy and 156 buffy coat samples (114 from the United States lung cancer case-control study and 42 Japanese) were studied. The allelic distribution in Caucasians, African-Americans, and Japanese is presented in Table 1. The characteristics of subjects in the case-control study are listed in Table 2. The mean age of autopsy donors was 36.9 (SD, 15.6), 33% of whom were African-Americans *versus* Caucasian Americans. The mean age of the Japanese was 86.4. The frequencies for the CYP2E1 minor and common alleles, pooling autopsy lung and case-control study samples, were not statistically different in Caucasians and African-Americans ($\chi^2 = 2.51$, $P > 0.05$) but were different from the Japanese ($\chi^2 = 25.2$, $P < 0.05$ for Caucasians; $\chi^2 = 23.1$, $P < 0.05$ for African-Americans). The genotype frequency in the Japanese octogenarians was similar to that reported previously for younger populations (18). In all groups (by race and diagnosis), the allelic frequencies met Hardy-Weinberg equilibrium.

Cases and controls in the lung cancer study had similar mean ages, smoking history, and racial distribution (Table 2). The distribution of genotypes by cases and controls is presented in Table 3. No homozygous CC (minor genotype) individuals were found. There was no statistical difference in frequency by case status between DD (minor genotype) and CD genotypes (χ^2, 0.81; dF, 1; $P > 0.05$; odds ratio, 1.57; 95% CI, 0.59–4.18). Subset analysis by race still did not show an association of the CYP2E1 genotype and lung cancer. The odds ratio for the CD heterozygote genotype was 1.24 (95% CI, 0.25–6.14) in African-Americans and 1.94 (95% CI, 0.54–6.92) in Caucasians. When characterized by smoking status (defined as less or more than the median pack-years; 53 pack-years), there was still no association with lung cancer (Table 4). Subset analysis by histological diagnosis (Table 5), tumor stage, or grade was not associated with genotype (data not shown). The risk associated with the CYP2E1 genotype remained relatively unchanged after adjustment for age, race, tobacco use, and gender (OR, 1.93; 95% CI, 0.66–5.65).

The distribution of the *Dra*I CYP2E1 RFLP was compared to the CYP2E1 *Pst*I and *Rsa*I in the United States and Japanese samples (Table 6). Multiple haplotypes were observed, which indicates the presence of different haplotypes in Americans and Japanese, although it is not possible in

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this limited sample size to identify all possible haplotypes. Thus, the presence of the major or minor *Dra*I allele did not consistently predict the presence of a particular *Pst*I allele in Americans or Japanese.

Discussion

Herein we describe an analysis of a CYP2E1 *Dra*I RFLP in relation to lung cancer. The CYP2E1 RFLP was not related to lung cancer in this United States population, even when subjects were stratified by tobacco use or race.

The present study contrasts with a previous Japanese report (18) of lung cancer risk and the CYP2E1 *Dra*I RFLP. There are several possible explanations for this. According to the Japanese results, excess CC (minor) genotypes were found in controls but not in lung cancer. This could not be examined in American subjects, due to the absence of persons with the CC (minor) genotype. The power of our study to detect a significant excess is therefore limited and so these findings alone cannot exclude an association. Separately, the Japanese findings might represent a chance association in that when the Japanese data are analyzed by allelic frequency, the difference between cases and controls is not statistically significant. Lastly, there may be inherent differences in Japanese and Americans due to genetic heterogeneity. Specifically, the risk for the DD (common) genotype might only exist as a risk factor in conjunction with other genetic or environmental factors unique to Japanese. However, this study is consistent with other reports (20) and, taken together, strongly suggest that this polymorphism will not constitute an important risk factor for lung cancer in Caucasians or African-Americans.

While this study fails to find an association for the CYP2E1 *Dra*I genetic polymorphism and lung cancer, several factors exist that might account for a falsely negative result: (*a*) The low frequency of the CYP2E1 *Dra*I C minor allele limits the statistical power of the study as discussed above; (*b*) a majority of control subjects had COPD because the study was designed to test biomarkers that predict only why some smokers get lung cancer and others do not. If CYP2E1 was a risk factor for both lung cancer and COPD, then the control group would not be appropriately selected; and (*c*) cases and controls generally were heavy smokers so that the risk factor of smoking might significantly outweigh a lesser risk factor such as this genetic polymorphism, similar to that observed in Japanese with different CYP1A1 genotypes, smoking status, and lung cancer (12). In summary, the lack of an association of the CYP2E1 *Dra*I RFLP, located in intron 6, with lung cancer in an United States population is contrasted with the results of a previous Japanese study (18). Further studies in Japanese subjects are needed to clarify the role, if any, of the CYP2E1 RFLP in lung cancer development.

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