# Nicotine Metabolism in Three Ethnic/Racial Groups with Different Risks of Lung Cancer

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## Abstract

**Previously, we documented that smoking-associated lung cancer risk is greater in Hawaiians and lower in Japanese compared with Whites. Nicotine metabolism by cytochrome *P*450 2A6 (CYP2A6) varies across ethnicity/race and is hypothesized to affect smoking behavior. We investigated whether higher CYP2A6 activity results in the smoker extracting more nicotine (adjusting for cigarettes per day) and being exposed to higher levels of tobacco-specific nitrosamine [4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)] and pyrene, a representative polycyclic aromatic hydrocarbon. We conducted a cross-sectional study of 585 smokers among the three main ethnic/racial groups in Hawaii and examined whether differences in CYP2A6 activity correlate with the ethnic/racial differences in lung cancer risk. We assessed CYP2A6 activity by nicotine metabolite ratio (total *trans*-3-hydroxycotinine/total cotinine) and caffeine metabolite ratio (1,7-dimethyl uric acid/1,7-dimethylxanthine) in 12 h urine. We also measured urinary nicotine equivalents (sum of nicotine, cotinine, and *trans*-3-hydroxycotinine and their respective glucuronides), a marker of nicotine dose, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol and its glucuronide, markers of NNK exposure, and 1-hydroxypyrene, a marker of pyrene exposure. The nicotine metabolite ratio was higher in Whites than in Japanese and intermediate in Hawaiians (*P* values < 0.05). Cigarettes per day–adjusted nicotine equivalents were lower in Japanese compared with Hawaiians or Whites (*P* = 0.005 and *P* < 0.0001, respectively) and greater in men than women (*P* < 0.0001). Nicotine equivalents and total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol increased with CYP2A6 activity, indicating that smokers with greater nicotine metabolism smoke more extensively and have a higher internal NNK dose. The particularly low nicotine metabolism of Japanese smokers may contribute to their previously described decreased lung cancer risk.** (Cancer Epidemiol Biomarkers Prev 2008;17(12):3526–35)

## Introduction

The lung cancer risk due to cigarette smoking is known to differ across U.S. ethnic/racial groups and, possibly, between the sexes even after taking into account smoking dose and duration. Specifically, compared with Whites, African American and native Hawaiian smokers have been shown to be at a greater risk of developing lung cancer, whereas Latino and Japanese American smokers are less likely to develop the disease (1, 2). Female smokers have also been suggested to have a higher lung cancer risk than do men (3), although not all studies are in agreement on the gender differences in lung cancer risk. The mechanisms underlying these racial/ethnic and gender differences likely involve both genetic and behavioral factors.

Metabolism is the primary route of elimination of nicotine from the circulation. The cytochrome *P*450 2A6 enzyme (CYP2A6) metabolizes up to 80% of nicotine into cotinine via C-oxidation (4). Cotinine is further metabolized to *trans*-3-hydroxycotinine (3-HC) by the same enzyme. Differences in the rate of nicotine metabolism could contribute to interindividual, gender, and ethnic/racial variation in smoking behavior (5-8) and, as a consequence, lung cancer risk (9-13). To achieve the desired psychopharmacologic effects of nicotine, smokers adjust their cigarette consumption to maintain particular levels of nicotine in the circulation (14). A slower nicotine metabolism rate may result in a person needing to smoke less extensively [smoke fewer cigarettes per day (CPD) or extract lower nicotine dose per cigarette] to reach the same plasma nicotine level as someone who metabolizes nicotine more quickly (15). The molar sum of nicotine, cotinine, 3-HC, and their respective glucuronides has been used as a measure of total nicotine exposure and is referred to as nicotine equivalents (16).

Genotyping is one common method of assessing CYP2A6 enzymatic activity, as several reduced activity polymorphisms have been identified, particularly in Asians (17). However, it has been shown that, even among individuals with the wild-type genotype, there is variation in CYP2A6 activity (18). Phenotyping by measuring the ratio of nicotine metabolite over the parent compound has been suggested to be a convenient and accurate probe of CYP2A6 activity (18). CYP2A6 is also the primary enzyme responsible for the conversion

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**Table 1. Main characteristics of study participants by sex and race ($n$ = 585)**

| | Native Hawaiian, median (interquartile range) | White, median (interquartile range) | Japanese American, median (interquartile range) |
|---|---|---|---|
| Females | | | |
| $n$ (%) | 99 (51.6) | 99 (50.0) | 97 (49.7) |
| Age (y) | 60 (57-66) | 61 (57-66) | 61 (58-67) |
| CPD | 20.0 (12.0-16.0)* | 20.0 (18.7-30.0) | 16.0 (13.0-20.0)* |
| Smoking duration (y) † | 42.0 (37.0-46.0) | 43.0 (39.0-47.0) | 41.5 (38.0-46.0) |
| BMI (kg/m$^2$) | 28.2 (24.0-31.5)*, ‡ | 24.1 (21.2-29.0) | 24.9 (21.4-27.6) |
| Total urine volume (mL) | 739.0 (499.0-1,059.0) | 874.0 (444.0-1,160.0) | 824.0 (529.0-1,133.0) |
| Fruits intake (g/kcal/d) | 44.0 (2.35-168.2)*, ‡ | 117.4 (64.6-256.7) | 162.7 (41.4-264.9) |
| Vegetables intake (g/kcal/d) | 186.7 (101.4-247.6) ‡ | 241.3 (118.0-358.0) | 207.4 (152.2-281.6) |
| Caffeine intake (mg/kcal/d) | 196.2 (86.5-304.3) | 224.6 (137.9-348.9) | 221.5 (120.7-334.7) |
| Alcohol intake (g/kcal/d) | 0.28 (0.01-0.71) | 0.45 (0.16-24.0) | 0.31 (0.07-0.54) |
| Males | | | |
| $n$ (%) | 93 (48.4) | 99 (50.0) | 98 (50.3) |
| Age (y) | 59 (49-65)* | 61 (58-66) | 61 (57-66) |
| CPD | 20.0 (18.0-25.0)* | 25.0 (20.0-40.0) | 20.0 (20.0-25.0) † |
| Smoking duration (y)* | 41.0 (32.0-47.0) †, ‡ | 46.0 (41.0-50.0) | 45.0 (40.0-49.0) |
| BMI (kg/m$^2$) | 28.3 (24.2-33.2)*, ‡ | 26.4 (23.8-30.1) | 26.0 (23.1-28.9) |
| Total urine volume (mL) | 774.0 (534.0-1,114.0)*, ‡ | 914.0 (674.0-1,394.0) | 998.0 (674.0-1,454.0) |
| Fruits intake (g/kcal/d) | 45.9 (0.21-133.0)* | 90.3 (4.76-208.1) | 53.8 (0.29-151.6) † |
| Vegetables intake (g/kcal/d) | 169.2 (100.7-235.3) | 160.7 (93.7-237.5) | 170.3 (127.5-257.6) |
| Caffeine intake (mg/kcal/d) | 171.7 (69.6-299.5)*, ‡ | 292.8 (171.7-398.5) | 241.0 (181.5-401.0) |
| Alcohol intake (g/kcal/d) | 0.01 (0.00-1.59)*, ‡ | 0.94 (0.00-39.1) | 0.20 (0.00-35.8) |

NOTE: Values are medians and interquartile ranges, unless otherwise indicated. The number of CPD and dietary intake are estimated by averaging over the 3 d preceding the 12 h urine collection.

*$P$ value for comparison with Whites is <0.05 (except for comparison of total fruits intake among men between native Hawaiians and Whites and between Japanese Americans and Whites, where $P$ = 0.06).

†Smoking duration is sum of years using filtered cigarettes, nonfiltered cigarettes, cigars, pipes, and chewing tobacco.

‡$P$ value for comparison with Japanese Americans is <0.05 (except for comparison of total vegetable intake among females, where $P$ = 0.06).

of the caffeine metabolite 1,7-dimethylxanthine to 1,7-dimethyl uric acid, and the ratio of these two caffeine metabolites measured in the urine has been used as another index of CYP2A6 activity (19).

We asked whether a high CYP2A6 activity, measured by either the nicotine metabolite ratio or the caffeine metabolite ratio, affects smoking behavior in a way that could result in a greater level of nicotine intake, adjusted for CPD, and thereby in an increased exposure to tobacco smoke carcinogens, possibly contributing to the unexplained differences in lung cancer risk that we observed among Japanese American, White, and native Hawaiian smokers. Because other factors may also affect how extensively people smoke their cigarettes, we assessed whether nicotine equivalents varied by age, gender, body mass index (BMI), and diet. We also measured urinary metabolites as markers of internal dose of the tobacco-specific lung carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and pyrene, a representative polycyclic aromatic hydrocarbon [PAH; the sum of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and its glucuronide (NNAL-Gluc) and 1-hydroxypyrene (1-OHP), respectively] to assess whether greater nicotine equivalents adjusted for CPD was associated with higher exposure to these two classes of tobacco smoke carcinogens.

## Materials and Methods

**Study Population.** Between 1993 and 1996, the Multiethnic Cohort Study was established in Hawaii and Los Angeles to test hypotheses related to diet and cancer. The Multiethnic Cohort Study consists of >215,000 men and women, drawing from five racial/ethnic populations: African Americans, Japanese Americans, Latinos, native Hawaiians, and Whites (20). For the present cross-sectional study, two main sources of participants were used. The majority (88.1%) were randomly selected among all Multiethnic Cohort Study Oahu cohort members who reported on their baseline questionnaire that they smoked at least 10 CPD and had no previous history of cancer and both parents were of Japanese or Caucasian ethnicity or of any amount of Hawaiian ancestry. Another source of subjects (11.9%) was the control groups for completed population-based case-control studies of various cancer types, conducted in Hawaii (21, 22). The same inclusion/exclusion criteria as for the Multiethnic Cohort Study were used to recontact the participants of these studies. The overall target sample size was 100 in each sex and ethnic group. The study was approved by the University of Hawaii Committee on Human Subjects and all participants signed a consent form.

A total of 596 participants completed all aspects of the study. Eight participants were excluded for reporting to smoke <10 CPD during data collection, and 3 were excluded for missing BMI. Thus, 585 subjects were used in the final data analysis (see Table 1).

**Data Collection.** Interviews were conducted at home. The initial interview was to explain the study and to obtain a history of lifetime tobacco and alcohol use and lung cancer-related occupational exposures as well as a food frequency questionnaire. At that time, the interviewer also provided instructions on how to keep a 3-day food record and a diary of all medications and dietary supplements taken as well as how to conduct the 12 h (overnight) urine collection and the caffeine test. The food, medication, and supplement records were kept

during the 3 days preceding the blood draw and 12 h urine collection. The day before the second appointment, the overnight urine collection started between 5:00 and 9:00 p.m. (depending on subject) and included all urine passed during the night and the first morning urine to cover a period of 12 h. The urine was kept on ice in a cooler until pickup the following morning. For the caffeine test, the subjects were instructed to consume two cups of coffee (Maxwell House instant coffee; ~100 mg caffeine) on rising (after 12 h collection), maintain fasting for another 2 h, abstain from other caffeine consumption, and collect their urine during the fifth hour after caffeine dosage. At this second home visit, the interviewer/phlebotomist administered a short questionnaire (including tobacco use during the previous 3 days), measured weight and height, obtained the 12 h and caffeine test urine samples, and collected the blood sample. The biospecimens were kept on ice until processing, which occurred within 4 h. Samples were stored at −80°C until analysis.

**Laboratory Analysis.** Analysis of total urinary nicotine, cotinine, and 3-HC concentration was done by gas chromatography/mass spectrometry. To assay urine for total nicotine (free + nicotine *N*-glucuronide) and total cotinine (free + cotinine *N*-glucuronide) concentration, samples were treated with base to cleave the glucuronide conjugates, and the nicotine and cotinine were quantified by gas chromatography/mass spectrometry analysis as described previously (23). Total 3-HC (3-HC + its glucuronide) was analyzed by the sample first being treated with β-glucuronidase and then analyzing 3-HC by gas chromatography/mass spectrometry as described previously (24). The sum of nicotine, cotinine, 3-HC, and the respective glucuronides accounts for ~81% of nicotine and its metabolites (25). Based on 65 blind duplicate pairs analyzed with the study samples for nicotine and cotinine and 6 pairs for 3-HC, the intraclass correlation coefficient for total nicotine, total cotinine, and total 3-HC was 0.98, 0.96, and 0.62, respectively.

NNAL and NNAL-Gluc were determined as described with slight modifications (26). 1-OHP was determined as described previously with modifications (27). In brief, urine mixed with triethylamine buffer (pH 7) was incubated overnight with arylsulfatase and glucuronidase and using coumarin 138 (7-dimethylaminocyclopenta[*c*]coumarin; Sigma) as internal standard. 1-OHP was partitioned into diethyl ether and analyzed by high-performance liquid chromatography with a Supelcosil LC-PAH column (100 × 4.6 mm; 3 μm; Supelco) applying a linear water/methanol gradient and fluorescence monitoring (344 nm for excitation and 380 nm for emission). Interassay coefficients of variation ranged from 3% to 4%.

CYP2A6 is responsible for 8-hydroxylation of 1,7-dimethylxanthine to form 1,7-dimethyl uric acid. The ratio of these urinary metabolites of caffeine (1,7-dimethyl uric acid/1,7-dimethylxanthine) is used as a measure of CYP2A6 activity and phenotype (28). 1,7-Dimethylxanthine and 1,7-dimethyl uric acid were

**Table 2. Geometric means (95% confidence limits) for urinary total nicotine, total cotinine, total 3-HC, nicotine equivalents, nicotine metabolite ratio, and caffeine metabolite ratio**

| | Native Hawaiian | | White | | Japanese American | |
|---|---|---|---|---|---|---|
| | *n* | Mean (95% CI) | *n* | Mean (95% CI) | *n* | Mean (95% CI) |
| Females | | | | | | |
| Total nicotine* | 99 | 8.18 (6.99-9.58) | 99 | 6.86 (5.84-8.04) | 97 | 7.95 (6.76-9.34) † |
| Total cotinine* | | 12.4 (10.8-14.1) ‡ | | 12.4 (10.9-14.2) | | 9.50 (8.30-10.9) † |
| Total 3-HC* | | 8.29 (6.64-10.4) †,‡ | | 12.4 (9.91-15.5) | | 4.58 (3.65-5.75) † |
| Nicotine equivalents* | | 32.6 (28.8-36.9) ‡ | | 37.5 (33.2-42.4) | | 25.4 (22.4-28.7) † |
| Nicotine equivalents adjusted for CPD* | | 32.6 (28.9-36.7) ‡ | | 35.8 (31.7-40.4) | | 26.7 (23.6-30.1) † |
| Nicotine metabolite ratio* | | 0.67 (0.55-0.81) †,‡ | | 1.00 (0.82-1.22) | | 0.48 (0.40-0.59) † |
| Caffeine metabolite ratio* | | 1.72 (1.49-1.98) ‡ | | 1.79 (1.55-2.06) | | 1.04 (0.90-1.20) † |
| Males | | | | | | |
| Total Nicotine | 93 | 9.54 (7.88-11.6) | 99 | 9.99 (8.29-12.0) | 98 | 9.89 (8.26 -11.9) † |
| Total cotinine | | 13.9 (11.8-16.3) ‡ | | 16.8 (14.3-19.6) | | 11.4 (9.79-13.3) † |
| Total 3-HC | | 9.76 (7.77-12.3) †,‡ | | 15.2 (12.2-19.0) | | 5.92 (4.78-7.35) † |
| Nicotine equivalents | | 37.0 (31.7-43.0) † | | 47.2 (40.9-54.5) | | 31.3 (27.1-36.2) † |
| Nicotine equivalents adjusted for CPD | | 37.8 (32.4-43.9) | | 45.2 (39.1-52.5) | | 32.0 (27.7-37.0) † |
| Nicotine metabolite ratio | | 0.70 (0.59-0.84) ‡ | | 0.91 (0.76-1.08) | | 0.52 (0.44-0.62) † |
| Caffeine metabolite ratio | | 1.35 (1.17-1.56) ‡ | | 1.35 (1.26-1.67) | | 1.01 (0.88-1.16) † |
| All§ | | | | | | |
| Total nicotine | 192 | 8.95 (7.92-10.1) | 198 | 8.21 (7.27-9.27) | 195 | 8.84 (7.83-9.97) † |
| Total cotinine | | 13.3 (12.0 -14.7) ‡ | | 14.3 (12.9-15.8) | | 10.4 (9.37-11.5) † |
| Total 3-HC | | 8.29 (6.64-10.4) †,‡ | | 12.4 (9.91-15.5) | | 4.58 (3.65-5.75) † |
| Nicotine equivalents | | 35.0 (31.8-38.5) †,‡ | | 41.9 (38.2-46.1) | | 28.1 (25.6-30.9) † |
| Nicotine equivalents adjusted for CPD | | 35.2 (32.2-38.9) ‡ | | 40.0 (36.4-43.9) | | 29.2 (26.5-32.0) † |
| Nicotine metabolite ratio | | 0.68 (0.60-0.78) †,‡ | | 0.95 (0.84-1.09) | | 0.50 (0.44-0.57) † |
| Caffeine metabolite ratio | | 1.53 (1.38-1.70) ‡ | | 1.60 (1.44-1.77) | | 1.03 (0.93-1.14) † |

NOTE: Means are adjusted for age, number of CPD (except for nicotine equivalents model), and 12 h urine volume (except caffeine metabolite ratio, adjusted for caffeine test urine volume) by multiple covariance analysis.

*Total nicotine (free + nicotine *N*-glucuronide), total cotinine (free + cotinine *N*-glucuronide), total 3-HC (free + 3-HC *N*-glucuronide), and nicotine equivalents are expressed in nmol/mL. The ratios are unitless.

† *P* value for comparison with Whites is <0.05 (except for nicotine metabolite ratio in native Hawaiian men, where $P = 0.06$).

‡ *P* value for comparison with Japanese Americans is <0.05.

§ Further adjusted for sex.

**Table 3. Demographic and lifestyle determinants of nicotine equivalents and CYP2A6 activity ($n$ = 585)**

| Dependent variable | $R^2$ (%) | Independent variables | Regression coefficient | $P$ |
|---|---|---|---|---|
| Nicotine equivalents* | 32.9 | Age (y) | −0.041 | 0.26 |
| | | Native Hawaiian vs White | −0.067 | 0.12 |
| | | Japanese American vs White | −0.193 | <0.0001 |
| | | Male vs female | 0.143 | 0.0001 |
| | | CPD | 0.157 | <0.0001 |
| | | BMI (kg/m$^2$) | 0.017 | 0.65 |
| | | Urine volume (mL) | −0.510 | <0.0001 |
| | | Total fruits (g/kcal/d) | −0.023 | 0.52 |
| | | Total vegetables (g/kcal/d) | −0.025 | 0.50 |
| | | Soy (g/kcal/d) | 0.028 | 0.45 |
| | | Caffeine (mg/kcal/d) | 0.088 | 0.01 |
| | | Alcohol (g/kcal/d) | 0.022 | 0.58 |
| Nicotine metabolite ratio † | 14.4 | Age (y) | −0.030 | 0.47 |
| | | Native Hawaiian vs White | −0.121 | 0.01 |
| | | Japanese American vs White | −0.321 | <0.0001 |
| | | Male vs female | −0.002 | 0.96 |
| | | CPD | 0.116 | 0.01 |
| | | BMI (kg/m$^2$) | −0.142 | 0.001 |
| | | Urine volume (mL) | −0.153 | 0.0003 |
| | | Total fruits (g/kcal/d) | −0.035 | 0.38 |
| | | Total vegetables (g/kcal/d) | 0.030 | 0.47 |
| | | Soy (g/kcal/d) | 0.032 | 0.45 |
| | | Caffeine (mg/kcal/d) | 0.0003 | 0.99 |
| | | Alcohol (g/kcal/d) | 0.102 | 0.02 |
| Caffeine metabolite ratio ‡ | 12.2 | Age (y) | 0.113 | 0.01 |
| | | Native Hawaiian vs White | −0.096 | 0.05 |
| | | Japanese American vs White | −0.284 | <0.0001 |
| | | Male vs female | −0.128 | 0.002 |
| | | CPD | 0.055 | 0.19 |
| | | BMI (kg/m$^2$) | 0.104 | 0.02 |
| | | Urine volume (mL) | −0.096 | 0.03 |
| | | Total fruits (g/kcal/d) | −0.123 | 0.003 |
| | | Total vegetables (g/kcal/d) | −0.048 | 0.25 |
| | | Soy (g/kcal/d) | 0.064 | 0.14 |
| | | Caffeine (mg/kcal/d) | −0.005 | 0.91 |
| | | Alcohol (g/kcal/d) | 0.008 | 0.86 |

NOTE: Data are for three separate multiple linear regression models.
*Nicotine equivalents estimated by the sum of urinary levels of total nicotine, total cotinine, and total 3-HC expressed as nmol/mL.
†Nicotine metabolite ratio is the ratio of total 3-HC to total cotinine measured in a 12 h urine sample.
‡Caffeine metabolite ratio is the ratio of 1,7-dimethyl uric acid to 1,7-dimethylxanthine measured 5 h after caffeine administration.

determined, as described previously (29), in a modification of the method by Butler et al. (30), by partitioning from urine mixed with ammonium sulfate and acetaminophen as internal standard into chloroform/isopropanol (19:1, v/v) followed by high-performance liquid chromatography analysis with a Supelcosil LC18 column (250 × 4.6 mm; 5μm), a linear methanol/0.09% aqueous acetic acid gradient, and monitoring at 280 nm. Interday coefficients of variation ranged from 6% to 12% depending on the analyte concentration.

**Data Analysis.** Food group intakes were calculated by summing the intakes of the appropriate foods for each group as reported on the 3-day food record. Food group intakes were then adjusted for average daily calorie consumption and expressed as g/kcal/d or mg/kcal/d. Urinary metabolites were expressed in mol/mL urine. The data analysis was conducted using SAS 9.0 software (SAS).

Because the dependent variables were not normally distributed, a Box-Cox transformation test was done for each model to identify the most appropriate transformation. All models were log-transformed. Values presented in the tables are back-transformed to their natural scale for ease of interpretation. To determine differences between groups, least-square means were computed for each racial/ethnic group (total sample and by gender subgroup) using the general linear model procedure. Ninety-five percent confidence limits were computed for the means, as were $P$ values for differences for each group comparison.

Multivariate linear regression models were used to predict levels of total nicotine, total cotinine, total 3HC, nicotine equivalents, the nicotine metabolite ratio, and the caffeine metabolite ratio. Age, race (with White as the reference), sex (with female as the reference), 12 h urine volume, number of CPD during the previous 3 days, BMI, and dietary intakes of all fruits, all vegetables (or specifically cruciferous vegetables), soy, caffeine, alcohol, and processed meats were used as independent variables. Cruciferous vegetables and processed meats were not significant in any of the models and therefore were excluded from further analyses. The cumulative $R^2$ value was used to assess the percentage of variation of the dependent variable accounted for by the independent variables.

A general linear model was also used to predict levels of nicotine equivalents for each CYP2A6 activity group (categorized as less than or greater than the median or by tertiles) as assessed, successively, by the urinary nicotine

metabolite ratio and caffeine metabolite ratio and adjusting for age only (and sex and race, when appropriate) and then further for additional covariates (CPD and calorie-adjusted caffeine intake). Tests of interaction were conducted between nicotine metabolite ratio and, sequentially, age, BMI, and CPD to identify modifying effects. The interaction terms were not statistically significant and therefore were not included in the final models. The Spearman nonparametric correlation coefficient was used to assess the correlation between continuous variables.

## Results

The main characteristics of the participants are provided in Table 1. Native Hawaiian women had a greater BMI, smoked fewer CPD, and ate fewer total fruits compared with White women ($P$ = 0.003, 0.01, and 0.01, respectively). Japanese American women smoked fewer CPD than White women ($P$ < 0.0001) and had a lower BMI and greater total fruits intake compared with native Hawaiian women ($P$ = 0.003 and 0.0004, respectively). Within men, compared with Whites, native Hawaiians were younger, had a greater BMI, smoked fewer CPD and had been smoking for fewer years, and had lower total intake of caffeine and alcohol (all $P$ values < 0.03). Compared with Japanese American men, native Hawaiian men had a greater BMI, had been smoking for fewer years, and had a lower intake of caffeine and alcohol (all $P$ values < 0.04). Japanese American men smoked fewer CPD and had a lower total fruits intake than White men ($P$ = 0.0004 and 0.06, respectively).

The geometric means for urinary levels of nicotine and its metabolites adjusted for age, sex, race, and 12 h urine volume are shown in Table 2. The effect of further adjusting mean nicotine equivalents for CPD is also shown. Mean total nicotine, total cotinine, and nicotine equivalents (with and without CPD adjustment) were significantly higher in males than in females (all $P$ values < 0.0001). Urinary levels of total 3-HC were significantly different across ethnic groups, with the mean highest in Whites followed by native Hawaiians and lowest in Japanese Americans (all $P$ values < 0.001). Mean total cotinine was lower in Japanese Americans than in native Hawaiians or Whites ($P$ = 0.001 and $P$ < 0.0001, respectively). Nicotine equivalents (with and without CPD adjustment) followed this same ethnic/racial pattern (with CPD adjustment: $P$ = 0.005 and $P$ < 0.0001, respectively). Within each sex, the racial/ethnic comparisons of nicotine and its metabolites are similar to those of the total sample. When nicotine equivalents are adjusted for CPD, these values represent the total amount of nicotine absorbed and are used as a measure of smoking intensity.

As also shown in Table 2, mean CYP2A6 activity (measured by either the nicotine metabolite ratio or the caffeine metabolite ratio) was significantly lower in Japanese Americans than in native Hawaiians ($P$ = 0.001 and $P$ < 0.0001, respectively) or Whites ($P$ values < 0.0001). The nicotine metabolite ratio, but not the caffeine metabolite ratio, was also significantly lower in native Hawaiians compared with Whites ($P$ = 0.001). Although no gender difference was observed for the nicotine metabolite ratio, the mean caffeine metabolite

**Table 4. Geometric mean for nicotine equivalents (95% confidence limits) by CYP2A6 activity level**

| | Nicotine metabolite ratio* | | | | | Caffeine metabolite ratio* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | ≤ Median | | > Median | | $P$ † | ≤ Median | | > Median | | $P$ † |
| | $n$ | Mean (95% CI) | $n$ | Mean (95% CI) | | $n$ | Mean (95% CI) | $n$ | Mean (95% CI) | |
| Age-adjusted and urine volume-adjusted means | | | | | | | | | | |
| Female | | | | | | | | | | |
| All ‡ | 142 | 28.2 (25.4-31.2) | 153 | 34.7 (31.5-38.4) | 0.005 | 138 | 29.3 (26.4-32.6) | 157 | 33.4 (30.3-36.9) | 0.08 |
| Native Hawaiian | 45 | 28.6 (23.0-35.6) | 54 | 37.7 (31.0-45.8) | 0.06 | 42 | 32.0 (25.5-40.2) | 57 | 34.3 (28.3-41.7) | 0.65 |
| White | 33 | 33.5 (27.3-41.0) | 66 | 38.7 (33.5-44.8) | 0.25 | 36 | 32.1 (26.3-39.3) | 63 | 39.8 (34.3-46.3) | 0.10 |
| Japanese American | 64 | 23.7 (20.9-26.8) | 33 | 28.4 (24.0-33.7) | 0.09 | 60 | 23.5 (20.8-26.7) | 37 | 28.2 (24.0-33.2) | 0.08 |
| Male | | | | | | | | | | |
| All ‡ | 151 | 36.8 (32.8-41.4) | 139 | 39.2 (34.7-44.3) | 0.48 | 154 | 32.7 (29.2-36.6) | 136 | 45.0 (39.9-50.8) | 0.0002 |
| Native Hawaiian | 49 | 38.7 (30.9-48.5) | 44 | 46.1 (36.3-58.4) | 0.30 | 50 | 34.1 (27.5-42.2) | 43 | 53.7 (42.6-67.7) | 0.01 |
| White | 39 | 40.0 (32.7-48.9) | 60 | 47.0 (40.5-54.5) | 0.20 | 38 | 41.2 (34.0-50.0) | 61 | 46.5 (39.9-54.1) | 0.34 |
| Japanese American | 63 | 31.3 (26.3-37.4) | 35 | 25.8 (19.9-33.5) | 0.22 | 66 | 25.9 (21.8-30.8) | 32 | 38.6 (30.1-49.6) | 0.01 |
| Multivariate-adjusted means§ | | | | | | | | | | |
| Female | | | | | | | | | | |
| All ‡ | | 28.4 (25.8-31.4) | | 34.4 (31.3-37.9) | 0.01 | | 29.9 (27.1-33.0) | | 32.8 (29.8-36.1) | 0.20 |
| Native Hawaiian | | 28.4 (23.0-35.0) | | 37.9 (31.5-45.7) | 0.04 | | 32.7 (26.3-40.7) | | 33.9 (28.1-40.8) | 0.81 |
| White | | 33.7 (27.8-41.0) | | 38.6 (33.5-44.4) | 0.27 | | 33.0 (27.1-40.0) | | 39.3 (34.0-45.4) | 0.16 |
| Japanese American | | 23.9 (21.2-27.0) | | 27.9 (23.6-32.9) | 0.15 | | 23.9 (21.1-27.1) | | 27.5 (23.5-32.3) | 0.17 |
| Male | | | | | | | | | | |
| All ‡ | | 36.9 (32.8-41.5) | | 39.2 (34.6-44.3) | 0.51 | | 32.9 (29.4-36.8) | | 44.7 (39.7-50.5) | 0.0004 |
| Native Hawaiian | | 39.1 (31.2-49.1) | | 45.5 (35.8-57.9) | 0.37 | | 34.2 (27.5-42.7) | | 53.4 (42.1-67.8) | 0.01 |
| White | | 40.5 (32.9-49.8) | | 46.7 (40.1-54.3) | 0.29 | | 40.8 (33.5-49.7) | | 46.8 (40.1-54.5) | 0.29 |
| Japanese American | | 31.1 (26.1-37.0) | | 26.4 (20.3-34.2) | 0.31 | | 26.4 (22.2-31.4) | | 37.2 (28.8-47.9) | 0.03 |

NOTE: Nicotine equivalents is the sum of urinary total nicotine, total cotinine, and total 3-HC (nmol/mL).
*Medians were based on all participants, and median values are 0.73 and 1.27 for nicotine metabolite ratio and caffeine metabolite ratio, respectively.
†$P$ values were obtained in a model comparing nicotine equivalents as a continuous variable (with the independent variables described in the table) between low and high nicotine metabolite ratio or caffeine metabolite ratio for each subgroup analysis.
‡Further adjusted for race.
§Further adjusted for CPD and calorie-adjusted caffeine intake by multiple covariance analysis.

**Table 5. Geometric means (95% confidence limits) for total NNAL and 1-OHP by nicotine metabolite ratio level**

| | Nicotine metabolite ratio* | | | | | | $P_{trend}$ † |
|---|---|---|---|---|---|---|---|
| | Bottom tertile | | Middle tertile | | Top tertile | | |
| | *n* | Mean (95% CI) | *n* | Mean (95% CI) | *n* | Mean (95% CI) | |
| Total NNAL (pmol/mL) | | | | | | | |
| All* | 193 | 0.70 (0.64-0.76) | 192 | 0.79 (0.72-0.85) | 193 | 0.80 (0.73-0.87) | 0.03 |
| Native Hawaiian | 65 | 0.61 (0.53-0.71) | 62 | 0.72 (0.62-0.84) | 61 | 0.69 (0.59-0.81) | 0.17 |
| White | 34 | 0.95 (0.78-1.16) | 72 | 0.90 (0.79-1.03) | 91 | 0.99 (0.88-1.12) | 0.61 |
| Japanese American | 94 | 0.63 (0.56-0.71) | 58 | 0.73 (0.63-0.86) | 41 | 0.74 (0.62-0.89) | 0.16 |
| 1-OHP (pmol/mL) | | | | | | | |
| All* | 192 | 0.40 (0.31-0.51) | 188 | 0.34 (0.26-0.43) | 190 | 0.50 (0.39-0.65) | 0.47 |
| Native Hawaiian | 65 | 0.37 (0.23-0.59) | 60 | 0.35 (0.22-0.56) | 60 | 0.50 (0.31-0.81) | 0.65 |
| White | 34 | 0.40 (0.23-0.68) | 71 | 0.31 (0.22-0.45) | 90 | 0.52 (0.37-0.71) | 0.58 |
| Japanese American | 93 | 0.42 (0.30-0.60) | 57 | 0.35 (0.22-0.54) | 40 | 0.52 (0.31-0.88) | 0.54 |

NOTE: Mean total NNAL (NNAL + NNAL-Gluc) and mean 1-OHP were adjusted for age, sex, CPD, and 12 h urine volume by multiple covariance analysis. Tertile categorization for nicotine metabolite ratio was based on all participants. First tertile: ≤0.484; second tertile: 0.484 to 1.10; and third tertile: >1.10.

*$P$ values for trend were obtained by treating nicotine metabolite ratio as a continuous variable.

†Further adjusted for race.

ratio was significantly higher in women than in men ($P$ = 0.04).

We performed a multivariate regression to determine the associations of age, race, sex, CPD, BMI, total urine volume, and calorie-adjusted intakes of fruits, vegetables, soy, caffeine, and alcohol with, successively, nicotine equivalents, the nicotine metabolite ratio, and the caffeine metabolite ratio (Table 3). The variables explained 32.9% of the variation in nicotine equivalents. CPD, male sex, and caffeine intake were associated with an increase in nicotine equivalents, whereas Japanese race/ethnicity (compared with Whites) and 12 h urine volume were associated with a decrease in nicotine equivalents.

The same model for the nicotine metabolite ratio explained 14.4% of the variation (Table 3). CPD and alcohol consumption were directly associated and Hawaiian and Japanese ethnicities, BMI, and 12 h urine volume were inversely associated with the nicotine metabolite ratio. Similarly, 12.2% of the variation in caffeine metabolite ratio was explained by the model. Age and BMI were directly associated and native Hawaiian and Japanese ethnicity/race, male sex, 12 h urine volume, and total fruits intake were inversely associated with the caffeine ratio. Both ratios (and nicotine equivalents) were particularly strongly inversely associated with being of Japanese ancestry.

To determine if CYP2A6 activity (measured by either the nicotine metabolite ratio or the caffeine metabolite ratio) was associated with a higher amount of exposure to nicotine equivalents, we first compared the age-adjusted, race-adjusted, and urine volume-adjusted mean nicotine equivalents by CYP2A6 activity (activity groups were divided at the median based on the distribution for the total population) in each sex (Table 4). In females, the mean nicotine equivalents was significantly greater in the high, compared with the low, CYP2A6 activity group when assessed by the nicotine metabolite ratio, as is borderline significant when assessed by the caffeine metabolite ratio. This pattern was observed in each race/ethnic group, although none of the differences reach statistical significance (see Table 4). In males, the mean nicotine equivalents was significantly higher in the high CYP2A6 activity group, compared with the low activity group, when measured by the caffeine metabolite ratio, overall ($P$ = 0.0002) and in Hawaiians and Japanese ($P$ = 0.01), but not when measured by the nicotine metabolite ratio ($P$ = 0.48), although the increasing trends were mostly consistent. As shown in the lower half of Table 4, very similar results were obtained after further adjusting the means for CPD and the other variables associated with nicotine equivalents in Table 3.

We further explored the difference in nicotine equivalents in women by CYP2A6 activity (measured by nicotine metabolite ratio) in stratified analyses, dividing, successively, the female subjects at the median age (60 years), BMI (25.4 kg/m$^2$), and CPD (20.0 CPD). We found that nicotine equivalents were significantly greater in the high versus low CYP2A6 activity group among the older and high BMI categories ($P$ = 0.01 and 0.005, respectively) as well as among women who smoke <20 CPD ($P$ = 0.02) but not in female in the low age and BMI categories ($P$ = 0.20 and 0.55, respectively) and female smokers of >20 CPD ($P$ = 0.20). For all ages, BMI, and smoking levels among men, the difference in nicotine equivalents by CYP2A6 activity group was nonstatistically significant. None of the interaction tests for the variables above were statistically significant.

To determine whether smokers with greater nicotine metabolism were also exposed to a greater amount of carcinogens after adjusting for CPD, we compared mean urinary 1-OHP and total NNAL (sum of NNAL and NNAL-Gluc) by tertile of nicotine metabolite ratio level (Table 5). The model with total NNAL as the dependent variable was adjusted for age, sex, and race (where appropriate), CPD, and 12 h urine volume, as these variables were associated with total NNAL in our study. Likewise, the model with 1-OHP as the dependent variable was also adjusted for age, race, sex, CPD, and 12 h urine volume. Mean total NNAL was significantly greater with increasing CYP2A6 activity among all subjects ($P_{trend}$ = 0.03). Mean 1-OHP also shows an overall positive trend by CYP2A6 activity, but this trend

did not reach statistical significance. The race-specific analyses show similar patterns, but none of the trends were statistically significant. We also re-ran the model for total NNAL by tertile of nicotine metabolite ratio with values expressed as absolute amount (in nmol), instead of adjusting for urine volume, and the conclusions were similar.

We also compared NNK and 1-OHP exposure by nicotine equivalents level to assess whether smokers who smoke more intensively are exposed to higher levels of these two tobacco smoke carcinogens. As was expected, total NNAL and 1-OHP increased with nicotine equivalents in both sexes (after adjusting for CPD and other covariates; within each sex, $P_{trend} < 0.0001$; data not shown). The sum of NNAL and NNAL-Gluc was significantly correlated with nicotine equivalents in women and men (Spearman's correlation coefficient = 0.66 and 0.68, respectively). However, the corresponding correlations for CPD were weaker (corresponding correlation coefficients = 0.21 and 0.26). 1-OHP was moderately correlated with nicotine equivalents in women and men (corresponding correlation coefficient = 0.41 and 0.50), but the corresponding correlations for CPD were low (corresponding correlation coefficient = 0.01 and 0.0003). These results suggest that total NNAL is a better surrogate for total nicotine exposure than 1-OHP. This is consistent with findings by Jacob et al. (31), suggesting that excretion of fluorene metabolites is a better indicator than 1-OHP of smoking status.

## Discussion

We measured urinary nicotine and its metabolites and the extent of nicotine metabolism in a large sample of native Hawaiian, Japanese, and White smokers in Hawaii. Our hypothesis was that ethnic/racial differences in nicotine metabolism may affect smoking behavior in a way that could result in greater carcinogen exposure, adjusted for average daily cigarette consumption, and thereby may help explain the ethnic/racial differences in lung cancer risk that we have documented in this population (1, 2). Indeed, case-control and prospective data in Hawaii have shown that native Hawaiian smokers have a greater risk, and Japanese American smokers have a lower risk, compared with White smokers, even after taking into account smoking dose (specifically, CPD) and duration and other potential confounders (1, 2). Sobue et al. (32) also observed in a prospective study that the magnitudes of the smoking relative risks for different histologic types of lung cancer were substantially lower in Japan compared with those reported for the United States or Europe, particularly for adenocarcinoma. We were also interested in comparing nicotine equivalents, adjusted for CPD, across ethnic groups, as other population-specific factors may affect how extensively people smoke and, consequently, their exposure to tobacco carcinogens. To our knowledge, there are no previous studies assessing nicotine equivalents (especially including the glucuronidated metabolites) in these three ethnic/racial populations or the nicotine metabolite ratio in native Hawaiians.

In this study, we used two urinary phenotypic markers of CYP2A6 activity, the nicotine metabolite ratio and the caffeine metabolite ratio. We gave preference to the nicotine metabolite ratio, because we were specifically interested in the ability to metabolize nicotine, not caffeine; however, we sought to examine the internal consistency of the findings by having a second independent measure of CYP2A6 activity. The correlation between the two ratios was moderate but statistically significant (Spearman's correlation coefficient = 0.29; $P < 0.0001$). Previous studies have used the ratio of 3-HC/cotinine measured in plasma (8, 18, 33, 34), saliva (10, 18), or urine (15, 35) to assess ability to metabolize nicotine. The use of this ratio is based on the premise that the metabolism of cotinine to 3-HC is a measure of CYP2A6 activity (18). In all of these studies except one (35), the 3-HC/cotinine ratio does not include the glucuronide conjugates of these metabolites. The nicotine metabolite ratio used in the present study is total 3-HC/total cotinine. We believe that in urine this ratio may be a better measure of CYP2A6-catalyzed cotinine metabolism than is the free 3-HC/free cotinine ratio. More than half the cotinine excreted is excreted as its glucuronide conjugate (36, 37). Therefore, total urinary cotinine may better reflect the amount of cotinine available for CYP2A6 catalyzed metabolism. All the excreted 3-HC, glucuronidated or not, is the product of this metabolism and therefore is included in the numerator.

In the White female and male smokers in our study, the unadjusted mean value of the total 3-HC/total cotinine ratio was 1.51 and 1.19, respectively (Supplementary Table), whereas in the study by Kandel et al. (15) the mean 3-HC/cotinine ratio was 5.48. The large difference between these two values may in part be explained by the use of total cotinine in the denominator. Also, the previous studies used spot urine collected at time of interview (15, 35). In contrast, our urine specimens were collected in a standardized manner over a 12 h period. Our measurements, therefore, integrate tobacco exposure and metabolism over a longer period and thus are likely to be more stable. Nevertheless, in either study, the nicotine metabolite ratio in Asians (Japanese in our study) was 40% to 50% lower than in Whites. This was true in our study whether adjusted for CPD or not (Table 2; Supplementary Table).

We found that urinary levels of total cotinine, total 3-HC, and nicotine equivalents, adjusted for CPD, were higher in men than in women; these metabolites were also significantly lower in Japanese Americans compared with native Hawaiians and in turn lower in the latter group than in Whites. Particularly striking was the markedly lower levels of nicotine equivalents (even after adjusting for CPD), nicotine metabolite ratio, and caffeine metabolite ratio in Japanese American smokers, a pattern that is consistent with their lower lung cancer risk. Because native Hawaiians have a higher lung cancer risk than Whites, however, the ethnic/racial differences in nicotine equivalents per cigarette dose and in CYP2A6 activity do not parallel the known differences in risk of the disease. A lower nicotine metabolism (measured by 3-HC/cotinine in saliva) has already been reported for Polynesians, compared with Whites, in New Zealand, although these findings were based on 6 Maori and 6 European female smokers (10).

Kandel et al. (15) also found no correlation between nicotine metabolism and the previously documented

lung cancer risk across the populations studied. In their data, the ratio of urinary 3-HC to cotinine was higher in Whites and Hispanics than in African Americans and Asians. However, compared with Whites, we have shown that the lung cancer risk due to smoking is greater in African Americans and lower in Hispanics and Japanese Americans (2). In another study using the ratio of plasma cotinine to nicotine as the nicotine metabolite ratio, no ethnic difference was found among Whites, Blacks, and Koreans (34). However, a significantly lower metabolic ratio was observed in Japanese subjects compared with the other populations. Taken together, the data suggest that Whites consistently have higher nicotine metabolite ratios (no matter how nicotine metabolism is measured) than do Asians, particularly Japanese. This finding is supported by genotyping studies that have identified low-activity CYP2A6 alleles, the frequencies of which are much higher in Asians than in Whites (34).

Some previous studies have reported ethnic/racial differences in cotinine levels, particularly higher levels among African Americans smokers. Specifically, serum cotinine was reported to be higher in African American smokers than in White (6) or Mexican American (5) smokers. This has led to the suggestion that the observed increased cancer risk in this population is due to an increased dose of tobacco smoke. Pérez-Stable et al. (38) found that serum cotinine concentration per CPD was higher among African American than White smokers and attributed this result to a lower overall clearance of cotinine and a higher intake of nicotine per cigarette (which is metabolized to its proximate metabolite, cotinine) in African Americans. Similarly, in the study by Kandel et al. of 900 daily smokers ages 18 to 26 years and averaging 12 CPD, nicotine exposure per cigarette (measured by mean urinary cotinine per cigarette) was found to be greatest in African Americans, lowest in Whites, and intermediate in Asians and Hispanics (15). This is in contrast to our study in which total nicotine equivalents were significantly lower in Japanese. These dissimilar findings may be explained by the younger population studied by Kandel et al. (15), the lower number of CPD for their participants, and their different measure of nicotine equivalents per cigarette (urinary free cotinine versus the sum of urinary free and conjugated nicotine, cotinine, and 3-HC in our study).

There was no significant difference in the nicotine metabolite ratio by sex in our study. This was surprising in the context of previous studies, most of which report nicotine metabolism to be higher in women than in men (15, 33, 34, 35). Estrogens have been shown to have a stimulatory effect on nicotine metabolism by both inducing CYP2A6 and accelerating glucuronide conjugation (39-41). Interestingly, Benowitz et al. (40) found no significant difference in nicotine metabolism between men and perimenopausal or postmenopausal women. Because the median age for women in our study was 60 years, the absence of a gender difference in the ratio could be because the great majority of our female subjects were postmenopausal. Although there was no gender difference in mean nicotine metabolite ratio in our study, nicotine equivalents adjusted for CPD was significantly higher in men than in women. In an ethnic-specific analysis, this trend was consistent across groups but did not reach statistical significance.

Women with a high nicotine (or caffeine) metabolite ratio had a statistically significantly elevated amount of nicotine equivalents with CPD adjustment compared with women with a low nicotine metabolite ratio. In men, this comparison was not significant according to the nicotine metabolite ratio but was significant when measured by the caffeine metabolite ratio. This suggests that, at least in women, a faster nicotine metabolism could result in exposure to a higher level of nicotine equivalents and therefore to higher internal doses of tobacco carcinogens, possibly leading to an elevated risk of lung cancer, compared with women with a slow nicotine metabolism. This is supported by the fact that we observed a statistically significant relationship between the nicotine metabolite ratio and our biomarker of NNK internal dose. The association between nicotine metabolite ratio and 1-OHP was not significant but displayed a positive relationship. Although not completely consistent across sexes and metabolite ratios, overall, these data suggest that smokers with high a CYP2A6 activity have a greater nicotine exposure and are exposed to a greater internal dose of tobacco smoke carcinogens, especially NNK. An association between total NNAL exposure and lung cancer incidence in smokers has recently been reported (42).

We had also hypothesized that differences in diet between gender and racial groups may also contribute to differences in CYP2A6 activity. Numerous studies suggest an inverse association between vegetable and fruit consumption and lung cancer risk (43-45). An intervention study among Jordanian nonsmokers showed an increase in CYP2A6 activity (measured by the caffeine metabolite ratio) with broccoli consumption (46). We found that total fruits intake was negatively associated with the caffeine metabolite ratio but not with the nicotine metabolite ratio. There was no association between vegetable intake or, specifically, cruciferous vegetable intake and CYP2A6 activity. Well-controlled feeding studies are needed to determine whether fruits or vegetables affect CYP2A6 activity. Surprisingly, we also found an inverse association between nicotine equivalents adjusted for CPD and caffeine intake in this study. This may be due to chance, as we do not see any possible explanation for such a relationship.

Age and BMI were also associated with CYP2A6 activity in our study, but these results were not consistent across the two metabolite ratios. Age was directly associated only with the caffeine metabolite ratio. Johnstone et al. (33) also reported a direct association ($P = 0.04$) between the ratio of plasma 3-HC/cotinine and age. In contrast, an earlier study by Molander et al. (47) suggested that nicotine metabolism decreases with age. Unexpectedly, BMI was associated in opposite direction with our two markers of CYP2A6 activity. We found the nicotine metabolite ratio to be associated with number of CPD; however, previous studies were inconsistent in showing this relationship (15, 35).

Exploring the ethnic/racial and gender differences observed for smoking behavior, nicotine metabolism

and lung cancer risk might provide important insight into the pathogenesis of this neoplasm. A better understanding of nicotine metabolism may also lead to more individualized and effective smoking cessation programs. Our study suggests that a high nicotine metabolism results in a greater nicotine exposure after adjustment for cigarette consumption and, consequently, a greater exposure to tobacco carcinogens, such as NNK and polycyclic aromatic hydrocarbons. It is important to note, however, that we sought to assess exposure to only two smoking-related carcinogens; yet, >40 established carcinogens are found in cigarette smoke (48). However, it seems reasonable to assume that exposure to all, or most, carcinogens would also be increased. Finally, although the ethnic/racial differences in nicotine metabolism and nicotine equivalents per cigarette dose do not correlate directly with those for lung cancer risk in our three populations, the results for Japanese American smokers suggest that lower nicotine metabolism and exposure may contribute to their decreased lung cancer risk.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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# Nicotine Metabolism in Three Ethnic/Racial Groups with Different Risks of Lung Cancer

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