Carcinogenesis vol.22 no.4 pp.673–677, 2001

Cytochrome P4501A2 (CYP1A2) activity and lung cancer risk: a preliminary study among Chinese women in Singapore

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There is increasing evidence for the role of heterocyclic and other arylamines in carcinogenesis, including lung carcinogenesis. Chinese women have a high rate of lung cancer despite a low smoking prevalence, and studies in this population may provide useful information on risk factors other than smoking. Hepatic CYP1A2 and NAT2 are involved in the metabolism of carcinogenic arylamines, and NAT2 also catalyzes the detoxification pathway for these compounds. In this study, we examined the effect of CYP1A2 activity using a urinary caffeine metabolic ratio assay for 54 Chinese women with newly diagnosed lung cancer (including 28 adenocarcinomas) and 174 hospital controls. Among them, *NAT2* genotype was available for 47 cases and 98 controls. There was no effect of CYP1A2 activity on overall risk of lung cancer in the study population [odds ratio (OR) 0.8, 95% confidence interval (CI) 0.4–1.6, adjusted for age at diagnosis, smoking and cruciferous vegetable intake]. For adenocarcinomas, the OR was 1.5, 95% CI 0.6–3.4. After further adjustment for *NAT2* acetylator genotype, the OR for adenocarcinoma was 1.8 (95% CI 0.7–4.8). When the combined NAT2/CYP1A2 status was examined, women with slow NAT2 and rapid CYP1A2 activity were at highest risk (adjusted OR 6.9, 95% CI 1.3–37.6) relative to women with rapid NAT2 and slow CYP1A2 activity, for lung adenocarcinoma. While larger studies are needed to confirm or refute these results, they are consistent with a role for heterocyclic arylamines in lung carcinogenesis in this primarily non-smoking population.

Cytochrome P4501A2 (CYP1A2) is one of the cytochrome P450 superfamily of enzymes, and plays a major role in both drug and carcinogen metabolism. It is known to catalyze the *N*-oxidation of 4-aminobiphenyl, 2-naphthylamine, mycotoxins and several aromatic amines such as the heterocyclic aromatic amines (HAA) including PhIP, IQ and MeIQx (1,2). The enzyme is expressed primarily in the liver, and individual differences in activity are thought to be both genetically determined, as well as due to differences in exposure to inducing agents such as cigarette smoke (1,2). While the genetic basis for this polymorphism has yet to be established (3), phenotyping methods using caffeine metabolites in plasma, saliva, exhaled air and urine (2) have demonstrated utility as measures of CYP1A2 activity in epidemiological studies.

There has been interest in exposure to heterocyclic aromatic amines as a possible risk factor for cancer (4,5). These compounds, which are formed during the cooking of meat at high temperature, are potent mutagens and carcinogens (6,7). Epidemiological studies have provided some evidence for a positive association between intake of fried or well-done meat, and cancers of the colon/rectum (8,9), breast (10) and lung (11).

CYP1A2 and NAT2 are key enzymes in the metabolic activation of heterocyclic amines to their final DNA-binding forms (1,12). The initial activation step is believed to be *N*-oxidation by CYP1A2 in the liver, followed by O-acetylation by NAT2. Interestingly, NAT2 also catalyzes *N*-acetylation of heterocyclic amines in the liver, a step that competes with *N*-oxidation and acts as a detoxification pathway (13). The different roles of NAT2 are thought to explain why rapid acetylator status appears to confer higher risk of colon cancer, while slow acetylator status increases risk of bladder cancer (14). In a study on patients with colorectal neoplasia (polyps or cancer) (15), 57% of cases were rapid CYP1A2 metabolizers compared with 41% of healthy controls ($P = 0.03$). The combination of rapid NAT2/rapid CYP1A2 phenotype was found to accord the highest risk, particularly among subjects who indicated a preference for well-done meat; a finding which is consistent with the proposed pathway for heterocyclic amine carcinogenesis in the colon.

We used the urinary caffeine metabolic ratio (CMR) (16–18), which is well suited to population-based studies, as a test for CYP1A2 activity among participants of a case-control study on lung cancer conducted among female Chinese women in Singapore. This is a largely non-smoking population, but, similar to other Chinese populations worldwide, exhibits high rates of lung cancer, of which a large proportion are adenocarcinomas (19). The reasons for this are not fully understood, but we hypothesized that the effect of CYP1A2 activity would shed light on the possible role of heterocyclic amines in lung carcinogenesis in this population.

A total of 54 incident cases of lung cancer and 174 hospital controls, frequency-matched for age, were included in this study. This study was part of a larger project on lung cancer carried out between 1 April 1996 and 30 December 1998. Subjects recruited between July 1997 and December 1998 were eligible for the present study. All participants in this study were female, Chinese, Singapore residents and gave informed consent to caffeine dosing and the collection of an overnight urine sample as described below. They were similar to the overall larger study population in terms of country of

Abbreviations: PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; IQ, 2-amino-3-methylimidazo[4,5-*f*]quinoline; MeIQx, 2-amino,3,8-dimethylimidazo[4,5-*f*]quinoxaline; 1U, 1-methylurate; 1X, 1-methylxanthine; 17U, 1,7-dimethylurate; AFMU, 5-acetylamino-6-formylamino-3-methyluracil; AAMU, 5-acetylamino-6-amino-3-methyluracil .

Table I. Characteristics of study population[a]

	Controls (n = 174)	All lung cancers (n = 54)	Adenocarcinoma (n = 28)
Age (years; mean ± SD)	61.9 ± 12.1	62.3 ± 11.8	61.8 ± 11.6
Dialect group			
Hokkien (Fujian)	79 (45.4)	17 (31.5)	6 (21.4)
Teochew (Chaozhou)	37 (21.3)	13 (24.1)	7 (25.0)
Cantonese (Guangdong)	27 (15.5)	13 (24.1)	8 (28.6)
Other	31 (17.8)	11 (20.4)	7 (24.0)
Place of birth			
Singapore	115 (66.1)	37 (68.5)	18 (64.3)
Malaysia	28 (16.1)	5 (9.3)	4 (14.3)
China	31 (17.8)	12 (22.2)	6 (21.4)
Smoking status			
Lifetime non-smoker	154 (89.0)[b]	33 (61.1)	22 (78.6)
Ex-smoker	8 (4.6)	5 (9.3)	3 (10.7)
Current smoker	11 (6.4)	16 (29.6)	3 (10.7)
Daily number of cigarettes smoked (mean ± SD) among current smokers	11.5 ± 4.1	12.7 ± 7.1	–
Cruciferous vegetable intake (servings/week)			
<9	59 (33.9)	30 (55.6)	9 (32.1)
9–15	55 (31.6)	13 (24.1)	10 (35.7)
⩾16	60 (34.5)	11 (20.4)	9 (32.1)
NAT2 acetylator status[c]			
Slow acetylators	24 (24.5)	22 (46.8)	12 (48.0)
Rapid acetylators	74 (75.5)	25 (53.2)	13 (52.0)

[a]All subjects were female and ethnic Chinese.
[b]Smoking status was missing for one control subject. Number of cigarettes/day omitted for adenocarcinoma as only three cases were current smokers.
[c]A total of 98 controls and 47 cases had both NAT2 and CYP1A2 status determined.

birth, dialect group (indicating provincial origin in China) and smoking history.

Classification of histological subtype was by independent review carried out by two study pathologists in the majority (46 or 86.7%) of cases. Based on this review, 28 cases (60.9%) were classified as adenocarcinomas, 14 (30.4%) as squamous or small cell carcinomas and the remainder as large cell or other subtype. A further five (9.3%) cases had pathological confirmation but material was not available for independent review and subtyping. The remaining three (5.7%) cases were diagnosed based on radiological and clinical grounds. We report the results for all 54 cases combined, and for the subgroup of 28 adenocarcinomas classified by independent pathological review.

Demographic information, smoking history, number of cigarettes smoked daily and cruciferous vegetable intake data were obtained by face-to-face interview with a research nurse. The index of cruciferous vegetable intake elicited usual intake (number of weekly servings) over the past 3 years, of a total of nine common cruciferous vegetables available locally.

The protocol used for the caffeine test was that of Tang and Kalow (20). Briefly, subjects were asked to abstain from chocolate and acetaminophen for two days prior to the test date. They then consumed between one and two cups of coffee (60–90 mg caffeine, or 1–2 mg/kg) in the afternoon, and limited total coffee intake to not more than four cups that same day. Their pooled overnight urine specimen was collected the next morning by the research nurse. At this time, the nurse also ascertained adherence to the protocol and asked about medication consumed and cigarettes smoked in the past 24 h.

Urinary caffeine metabolites were measured by high performance liquid chromatography using the method of Tang and Kalow (20) with minor modifications. Briefly, 0.05 ml internal standard (1.2 mg *N*-acetyl-*p*-aminophenol in 10 ml 50% isopropanol water) and 3 ml dicloromethane/isopropanol (88:12, v/v) were added to 0.1 ml urine in a glass tube. The mixture was vortexed for 30 min and the organic phase separated and dried under nitrogen at 37°C for 30 min. The residue was dissolved in 0.25 ml HPLC mobile-phase solvent (1.3% isopropanol, 0.1% acetonitrile, 0.05% acetic acid) and 0.02 ml of the solution injected into an Ultrasphere IP ODS column (Beckman Instruments, CA). The caffeine and metabolites eluted at 1.5 ml/min and were detected by UV absorbance at 280 nm. Using standard samples, the retention times under these conditions of 1U, 1X, 17U and the internal standard were 4.87, 6.07, 11.54 and 7.9 min, respectively.

AFMU was determined separately after conversion to AAMU by the following method: 0.05 ml 0.25 N sodium hydroxide was added to 0.05 ml urine in a glass tube at a pH ⩾ 10. After 20 min, 0.05 ml 0.25 N HCl was added as a neutralizer. A 0.1 ml aliquot of the internal standard solution (10 mg benzyloxyurea dissolved in 10 ml water) was added, and a 0.02 ml aliquot of the mixture injected into the TSK-GEL G2000PW column (TosoHaas, Montgomeryville, PA). This was eluted with 0.1% acetic acid at a flow rate of 0.8 ml/min, and monitored by UV absorbance at 263 nm. The retention times of AAMU and the internal standard were 15.10 and 31.73 min, respectively.

The CYP1A2 index, or caffeine metabolic ratio (CMR) was calculated as the urinary molar ratio of (AAMU + 1X + 1U)/17U in accordance with Tang and Kalow (20).

Among the study subjects, 47 cases and 98 controls also donated a blood specimen for *N*-acetyltransferase 2 genotyping. The methods and results have been described elsewhere (21). Subjects homozygous or heterozygous for the wild-type *4 allele were classified as rapid acetylators and those homozygous for mutant (*5A, *6A or *7A) alleles as slow acetylators.

Differences in CYP1A2 metabolic activity were expressed

Table II. Odds ratios (OR) and 95% confidence interval (CI) for risk of lung cancer by cytochrome P450 1A2 phenotype

	Cases/controls	Crude OR (95% CI)	Adjusted OR[1] (95% CI)[a]	Adjusted OR[2] (95% CI)[b]
All subjects				
Low activity phenotype[c]	28/88	1.0	1.0	1.0
High activity phenotype	26/86	1.0 (0.5–1.8)	0.8 (0.4–1.5)	0.8 (0.4–1.6)
Adenocarcinoma				
Low activity phenotype	11/88	1.0	1.0	1.0
High activity phenotype	17/86	1.6 (0.7–3.6)	1.5 (0.7–3.4)	1.5 (0.6–3.4)
Non-smokers				
All cases				
Low activity phenotype	20/80	1.0	1.0	1.0
High activity phenotype	13/74	0.7 (0.3–1.5)	0.7 (0.3–1.5)	0.7 (0.3–1.5)
Adenocarcinoma				
Low activity phenotype	10/80	1.0	1.0	1.0
High activity phenotype	12/74	1.3 (0.5–3.2)	1.3 (0.5–3.2)	1.3 (0.5–3.2)

[a]Adjusted for age and smoking status (never, ex-, current).
[b]Adjusted in addition for cruciferous vegetable intake (in tertiles) and current number of cigarettes smoked per day (smokers only).
[c]Defined as molar ratio above ('high activity') or below ('low activity') the median value for controls.

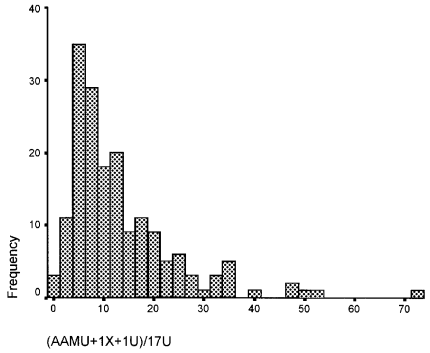

Fig. 1. Frequency distribution of urinary molar excretion ratios of (AAMU + IX + IU)/17U in 174 Chinese control subjects.

Table III. Odds ratio (OR) and 95% confidence interval (CI) for risk of lung adenocarcinoma by combined cytochrome P450 1A2 activity and NAT2 acetylator status

NAT2 acetylator status	CYP1A2	Cases/ controls	Crude OR (95% CI)	Adjusted OR (95% CI)[a]
Adenocarcinomas only				
Rapid	Low	4/39	1.0	1.0
Rapid	High	9/35	2.5 (0.7–8.9)	2.9 (0.8–10.9)
Slow	Low	5/12	4.1 (0.9–17.6)	3.9 (0.6–26.3)
Slow	High	7/12	5.7 (1.4–22.8)	6.9 (1.3–37.6)

[a]Adjusted for age, smoking status (further adjustment for number of cigarettes smoked did not alter risk estimates appreciably), and cruciferous vegetable intake (in tertiles).

as odds ratios (OR) with 95% confidence intervals (CI). Unconditional logistic regression analyses were used to obtain ORs adjusted for age (as a continuous variable), smoking history (never, ex-, current smoker), current number of cigarettes smoked per day (for smokers, with zero input for never and ex-smokers) and usual cruciferous vegetable intake. Analyses involving *NAT2* genotype as a covariate were confined to the subset that had both measurements done. All calculations were performed using the SPSS WIN v9.01 statistical package (SPSS, Chicago, IL).

The demographic and other relevant characteristics of the study population are given in Table I. The dialect group distribution of controls was similar to the general population, but among cases there tended to be slightly more Cantonese women. Slightly more cases (22.2%), compared with controls (17.8%), were migrants from China or Taiwan. The mean cruciferous vegetable intake was lower among cases than controls (10.5 $\pm$ 8.1 and 15.8 $\pm$ 12.7 standard servings a week, respectively). The 16 cases and 11 controls who were current smokers smoked an average of 12.7 and 11.5 cigarettes/day, respectively.

Among the 174 control patients included in the analysis, the distribution of CMR was unimodal and markedly skewed to the right, with a range of 0.4–71.8 and a median of 9.8 (Figure 1). A probit plot revealed the absence of distinct antimodes, which is consistent with Kalow and Tang (17) who used the same ratio of urinary metabolites. There was a 6.5-fold difference between the 90th and the 10th percentiles in the distribution. The average CMR of cases and controls was similar [geometric mean (95% CI) 9.2 (7.0–12.0) and 9.8 (8.7–11.1), respectively].

We divided the subjects into two groups (high and low activity) according to whether their CMR was above or below the median value for controls. This cut-off point was empirically chosen so as to obtain the most stable estimates, given the small sample size. From Table II, there was no increased risk associated with higher CYP1A2 activity for all cases combined, but a small increase was observed when adenocarcinomas alone were considered, although this was not statistically significant. The odds ratio (OR) and 95% confidence interval, adjusted for smoking status, current daily number of cigarettes and cruciferous vegetable intake was 1.5 (0.6–3.4) for all adenocarcinomas, and 1.3 (0.5–3.2) when analysis was confined to non-smokers.

Since NAT2 has been shown to be a key enzyme in the metabolism of carcinogenic arylamines, we examined risks adjusted for NAT2 acetylator status among the 145 subjects who also donated a blood specimen for genotyping. For all cases combined, high CYP1A2 activity did not increase risk (adjusted OR 0.9, 95% CI 0.4 – 1.9). Risk of adenocarcinoma, however, was higher among those with high CYP1A2 activity compared with those with the low activity phenotype, although the increase was not statistically significant (adjusted OR 1.8, 95% CI 0.7–4.8). We repeated the analyses with CMR values as continuous variables in the regression model. The risk of

lung adenocarcinomas increased by 3% for each unit increase in CMR (OR 1.03 95% CI 0.99–1.08) after adjusting for NAT2 status.

We previously showed that slow NAT2 acetylator status increases risk of lung cancer in this population (21). We further examined risk of lung adenocarcinoma by combined NAT2/CYP1A2 status ($n = 145$) and the results are shown in Table III. The risk of lung cancer was highest in subjects with slow NAT2/high CYP1A2 activity. This combination accorded a significantly increased risk of lung adenocarcinoma (OR 6.9, 95% C.I. 1.3–37.6) compared with the group at lowest risk (rapid NAT2/low CYP1A2). This estimate was adjusted for age, smoking status and cruciferous vegetable intake. Further adjustment of number of cigarettes smoked did not alter the estimates appreciably (OR 6.8, 95% CI 1.2–37.2).

In summary, Chinese women with higher CYP1A2 activity measured by the caffeine metabolic ratio may have a higher risk of lung adenocarcinoma, although this conclusion is limited by the small numbers in this study. Overall, a combination of slow NAT2 and high CYP1A2 activity accorded the highest risk (almost 7-fold relative to the lowest risk subgroup) of lung adenocarcinoma in this study population.

To our knowledge, this is the first study to examine the role of CYP1A2 phenotype in lung cancer, and in a primarily non-smoking population. Since the sample size is small, the results should be considered preliminary. Within the constraints of the small numbers of subjects and the other limitations discussed below, however, our data are consistent with a role for heterocyclic amines in the aetiology of lung adenocarcinoma among Chinese women.

The role of cytochrome P4501A2 activity in metabolism of xenobiotics and its *in vivo* effects have been well described, but there have been few studies on the relationship between activity of this enzyme and disease outcome (2). One of the difficulties faced in studies of this nature is disentangling the genetically-determined component of enzyme activity from the inducible. CYP1A2 is induced by smoking, polybrominated biphenyls, certain drugs (2), as well as components of the diet, namely cruciferous vegetables and pan-fried meat (22). We attempted to account for this by collecting information on current smoking, medication and cruciferous vegetable intake. Unfortunately, information on meat intake was not collected. None of our participants were on phenytoin, rifampicin or omeprazole, which are known to influence CYP1A2 activity, at the time of the test, although one control patient was on allopurinol. We collected information on exogenous hormone use from 168 (74%) consecutive participants. As expected from the age distribution, none were on oral contraceptives. Five controls reported being on hormone replacement therapy. Exclusion of these five subjects did not materially affect the risk estimates. Our results require verification by larger studies with better control for inducing agents, and a clearer picture may emerge with further understanding of the genetic basis for CYP1A2 activity.

The current investigation also faces limitations inherent in retrospective studies. It might be argued that persons with lung cancer could be receiving treatment which would affect liver function and consequently reduce liver enzyme activity. While we acknowledge this possibility, the effect on our results would be a conservative one, or a bias towards the null.

Our observation that higher CYP1A2 activity may influence risk of lung cancer is consistent with the known pathway of activation for heterocyclic amines in the liver (13,14). This enzyme is involved in the conversion of the pro-carcinogen to its ultimate electrophilic form. Arylamines undergo *N*-oxidation by hepatic CYP1A2 to *N*-hydroxy-arylamines. The *N*-hydroxy moiety is further activated by *O*-acetylation to form the unstable *N*-acetoxyarylamine, which undergoes spontaneous hydrolysis to arylnitrenium ions capable of binding to DNA (13,14,23). On the other hand, NAT2 catalyzes a detoxification pathway which competes with *N*-oxidation (13) in the liver. Our finding that subjects with slow NAT2/rapid CYP1A2 activity are at highest risk is consistent with this pathway; this combination would enhance the rate of activation of pro-carcinogens and also reduce the rate at which they are detoxified.

Our finding that NAT2 and CYP1A2 activities influence lung adenocarcinoma risk in this population suggest that heterocyclic arylamines may play an important role in the pathogenesis of this disease among these women. *In vivo* animal feeding studies (24,25), as well as identification of arylamine–DNA adducts in peripheral lung tissue (26), provide support for the lung as a susceptible site for arylamine-induced carcinogenesis, and the role of these ubiquitous compounds as causes of lung adenocarcinoma among Chinese women deserves further study. The environmental sources of heterocyclic amines are likely to be chiefly dietary in many populations. Further data on the contribution of dietary heterocyclic amines to risk in Chinese women, and the role of other, possibly ethnic-specific, sources of these compounds, may shed light on the reasons for their high incidence of lung cancer despite low smoking rates.

Acknowledgement

This project was supported by a research grant from the Singapore National Medical Research Council.

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Received June 6, 2000; revised November 16, 2000; accepted December 20, 2000