# Inverse Relationship Between Obesity and Serum Prostate-Specific Antigen Level in Healthy Japanese Men: A Hospital-Based Cross-Sectional Survey, 2004-2006

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**OBJECTIVES** To confirm an inverse relationship between obesity and serum prostate-specific antigen (PSA) levels in Japanese men with a smaller body mass index (BMI) than white and African-American men.

**METHODS** We analyzed 5246 apparently healthy Japanese men aged >20 years who visited our medical center for a health checkup from January 2004 to December 2006. The men were divided into 6 groups by age decade, and the BMI was categorized into 5 groups. The body fat percentage (BFP) was also used and was grouped into quartiles. The Mantel-Haenszel $\chi^2$ test was used to check for trends in proportions of subjects with abnormal PSA values for each cutoff point (2.5 and 4.0 ng/mL) in these groups. The relationships between the PSA levels and BMI or BFP were examined using multivariate analysis.

**RESULTS** The median age, BMI, BFP, and PSA level was 46 years, 23.2 kg/m$^2$, 21.5%, and 0.78 ng/mL, respectively. The proportion of subjects with an abnormal PSA value increased significantly with age ($P$ for trend < .0001); however, no trends were found across the BMI or BFP categories. The geometric mean PSA level increased significantly with age ($P$ for linear trend < .0001) and decreased with BMI and BFP categories ($P$ for linear trend = .001 and $P$ for linear trend = .002, respectively).

**CONCLUSIONS** Our findings have demonstrated an inverse relationship between obesity and PSA levels even in Japanese men with a low prevalence of obesity, such as was previously reported for American men. Therefore, in prostate cancer screening, obesity, which can affect the accuracy of PSA testing, independent of race and ethnicity, should be taken into account. UROLOGY 72: 561–565, 2008. © 2008 Elsevier Inc.

The serum prostate-specific antigen (PSA) level has been widely used to screen for prostate cancer, although it is not specific for prostate cancer.[1] Because a high serum PSA level in apparently healthy men is the primary reason for prostate biopsy and subsequent prostate cancer diagnosis, it is clinically important to determine the biologic factors that affect the PSA levels. Some investigators have reported that the PSA level correlates positively with age and prostate volume, and they have suggested that age-adjusted PSA values and the PSA density can be used to more accurately screen for prostate cancer.[2,3] Other nonspecific factors can affect the serum PSA levels. For example, population-based[4-7] and prostate cancer case-based[8] studies have shown an inverse relationship between the body mass index (BMI) and the serum PSA value.

The World Health Organization 2000 report noted the rapid increase in the prevalence of obesity in Asian countries, including Japan and Korea, where obesity has traditionally been uncommon.[9] The BMI has gradually increased in middle-age Japanese men, and one third of them had a BMI value of ≥25 kg/m$^2$ in the National Nutrition Survey in 2003.[10] Additionally, about 30% of middle-age Japanese men appeared to have abdominal obesity as evaluated by BMI and waist circumference.[10]

The incidence of prostate cancer varies considerably worldwide, and it is greater in Europe and the United States than in Asian countries.[11] In Japan, the age-

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**Table 1.** Subject characteristics (n = 5246)

| Characteristic | Value | Minimum, 25th Percentile, Median, 75th Percentile, Maximum |
|---|---|---|
| Age (y) | 46.2 ± 11.5 | 20, 36, 46, 55, 90 |
| BMI (kg/m$^2$) | 23.4 ± 3.1 | 15.1, 21.4, 23.2, 25.3, 45.7 |
| BFP (%) | 21.6 ± 5.3 | 6.0, 18.1, 21.5, 24.9, 50.8 |
| PSA (ng/mL) | 1.11 ± 4.15 | 0.01, 0.55, 0.78, 1.16, 282.6 |
| PSA >2.5 ng/mL | 255 (4.9) | |
| PSA >4.0 ng/mL | 96 (1.8) | |
| PSA >10.0 ng/mL | 10 (0.2) | |
| Overweight (BMI ≥25 but <30 kg/m$^2$) | 1309 (25.0) | |
| Obese (BMI ≥30 kg/m$^2$) | 147 (2.8) | |

BMI = body mass index; BFP = body fat percentage; PSA = prostate-specific antigen.
Data presented as mean ± standard deviation or numbers, with percentages in parentheses.

standardized incidence of prostate cancer was 7.1 per 100 000 in 1975, but it increased steadily to 21.7 per 100 000 in 1999.[12]

These findings suggest that both obesity and prostate cancer are significant health concerns in Japan. However, almost all previous epidemiologic studies on the association between obesity and serum PSA level have been conducted in the United States, where obesity is very common.[4-8] To our knowledge, only 1 study, which involved 6005 Korean men, has targeted Asian men, who are relatively lean compared with white and African-American men. It failed to confirm an inverse relationship between BMI and PSA level in an analysis using a multivariate model.[13] Research findings involving Asian individuals could thus be informative.

Given the rapid increase in the prevalence of obesity and in the incidence of prostate cancer in Japan, it is important to clarify the relationships between obesity and PSA level. The relationship between BMI and PSA value in apparently healthy Japanese men, who, in general, have a lower BMI than do American men, was investigated. The body fat percentage (BFP) was also assessed in this study, because BMI does not adequately discriminate body fat content.[14]

## MATERIAL AND METHODS

### Study Population

The ethics committee of Chunichi Hospital approved all the procedures performed in this study. From January 2004 to December 2006, a total of 69 729 men visited Chunichi Hospital for a comprehensive health examination. Of the participants, 5395 men who had undergone serum PSA determination and anthropometric measurements, including height, weight, and BFP, were eligible for this study. All the data were collected retrospectively, with a waiver of informed consent, and analyzed anonymously. Non-Japanese men (n = 6), men <20 years of age (n = 4), men with a history of any malignancy (n = 86), and men who did not complete the questionnaire on medical history (n = 33) were excluded. Men with extreme or implausible (<15 or >50 kg/m$^2$) BMI values (n = 17) or who had a PSA value less than the lower limit of detection (≤0.001 ng/mL) (n = 3) were also excluded from the analyses. The remaining 5246 subjects, aged 20-90 years, were included in the present study. The men were divided into 6 subgroups by age: 20-29, 30-39, 40-49, 50-59, 60-69, and ≥70 years. The BMI was calculated as the weight in kilograms/height in meters squared. Because the recommended BMI cutoff points for the determination of overweight and obesity differ in Asian populations from those in Western populations,[15] the following categories were used: underweight (BMI <18.5 kg/m$^2$); lower range of normal weight (BMI 18.5-21.9 kg/m$^2$); upper range of normal weight (BMI 22.0-24.9 kg/m$^2$); overweight (BMI 25.0-29.9 kg/m$^2$); and obese (BMI ≥30.0 kg/m$^2$). The BFP, measured by a bioelectrical impedance analysis device (model BF-220, Tanita, Tokyo, Japan), was grouped into quartiles. The serum PSA levels were measured by a chemiluminescence enzyme immunoassay kit (Fujirebio, Tokyo, Japan). The standard threshold for an abnormal PSA level has not been established[16]; therefore, the data were examined using two cutoff points: 2.5 and 4.0 ng/mL.

### Statistical Analysis

The demographic variables were calculated and tabulated. The Mantel-Haenszel $\chi^2$ test was used to check for trends in proportions of subjects with abnormal PSA values for each cutoff point (2.5 and 4.0 ng/mL) for these variables. Spearman's rank correlation coefficients were used to detect correlations among age, BMI, BFP, and PSA. Pearson's correlation coefficients were also calculated, but these were similar to Spearman's coefficients; therefore Spearman's coefficients are given throughout this report. The relationships between PSA level and BMI or BFP were examined using multivariate linear regression analysis. Because the distribution of PSA levels was skewed, log-transformed PSA levels were used and subsequently back-transformed for interpretation of the results. All statistical analyses were performed using the Statistical Analysis System, version 8.0 (SAS Institute, Cary, NC).

## RESULTS

The mean ± standard deviation of age, BMI, and BFP was 46.2 ± 11.5 years, 23.4 ± 3.1 kg/m$^2$, and 21.6% ± 5.3%, respectively (Table 1). The mean ± standard deviation and median PSA value was 1.1 ± 4.2 ng/mL and 0.78 ng/mL, respectively (Table 1). Of the participants, 96 men (1.8%) had an abnormal PSA level >4.0 ng/mL. Using the PSA cutoff of 2.5 ng/mL, 255 men (4.9%) had an abnormal PSA level (Table 1). The number of overweight (BMI 25.0-29.9 kg/m$^2$) and obese (BMI ≥30 kg/m$^2$) men was 1309 (25.0%) and 147 (2.8%), respec-

**Table 2.** Proportions of subjects with abnormal PSA level by cutoff points according to age, BMI, and BFP

| Characteristic | Patients (n) | PSA >2.5 ng/mL | *P* Value* | PSA >4.0 ng/mL | *P* Value* |
|---|---|---|---|---|---|
| Total | 5246 | | | | |
| Age (y) | | | <.0001† | | <.0001† |
| 20-29 | 364 | 15 (4.12) | | 5 (1.37) | |
| 30-39 | 1375 | 27 (1.96) | | 7 (0.51) | |
| 40-49 | 1245 | 35 (2.81) | | 9 (0.72) | |
| 50-59 | 1548 | 103 (6.65) | | 40 (2.58) | |
| 60-69 | 648 | 62 (9.57) | | 29 (4.48) | |
| ≥70 | 66 | 13 (19.70) | | 6 (9.09) | |
| BMI (kg/m$^2$) | | | .62† | | .67† |
| <18.5 | 214 | 10 (4.67) | | 3 (1.40) | |
| 18.5-<22.0 | 1487 | 68 (4.57) | | 30 (2.02) | |
| 22-<25.0 | 2089 | 117 (5.60) | | 37 (1.77) | |
| 25-<30.0 | 1309 | 54 (4.13) | | 25 (1.91) | |
| ≥30 | 147 | 6 (4.08) | | 1 (0.68) | |
| BFP (%) | | | .12† | | .37† |
| <18.1 | 1303 | 63 (4.83) | | 24 (1.84) | |
| 18.1-<21.5 | 1303 | 86 (6.60) | | 34 (2.61) | |
| 21.5-<24.9 | 1319 | 64 (4.85) | | 22 (1.67) | |
| ≥24.9 | 1321 | 42 (3.18) | | 16 (1.21) | |

Abbreviations as in Table 1.
Data in parentheses are percentages.
* Mantel-Haenszel $\chi^2$ test.
† Crude data.
‡ Age-adjusted data.

tively (Table 1). The BMI and BFP correlated closely (Spearman's $r = 0.81$, $P < .0001$). A weak association was found between PSA level and age ($r = 0.14$, $P < .0001$). Spearman's coefficients between age and BMI or BFP ($r = 0.08$, $P < .0001$; and $r = -0.05$, $P < .001$, respectively) and age-adjusted coefficients between PSA and BMI or BFP ($r = -0.03$, $P = .02$ for both) were small. The proportions of subjects with abnormal PSA value using each cutoff point (2.5 and 4.0 ng/mL) increased significantly with age ($P$ for trend < .0001 for both). Although the groups with the greatest BMI and BFP had the lowest proportions of subjects with an abnormal PSA level for the two cutoff points, no significant trends were found between the proportions of subjects with an abnormal PSA level and BMI or BFP categories (Table 2). The geometric mean PSA level increased significantly with age ($P$ for linear trend < .0001; Table 3). In contrast, the age-adjusted geometric mean PSA value decreased significantly with the BMI and BFP categories ($P$ for linear trend = .001 and $P$ for linear trend = .002, respectively; Table 3).

**Table 3.** Geometric mean PSA levels with 95% CI according to age, BMI, and BFP

| Characteristic | Patients (n) | PSA (ng/mL)* | *P* Value for Linear Trend |
|---|---|---|---|
| Total (n) | 5246 | | |
| Age (y) | | | |
| 20-29 | 364 | 0.78 (0.73-0.83) | |
| 30-39 | 1375 | 0.75 (0.73-0.78) | |
| 40-49 | 1245 | 0.77 (0.74-0.80) | |
| 50-59 | 1548 | 0.88 (0.85-0.91) | |
| 60-69 | 648 | 0.99 (0.93-1.03) | |
| ≥70 | 66 | 1.33 (1.14-1.55) | <.0001† |
| BMI (kg/m$^2$) | | | |
| <18.5 | 214 | 0.84 (0.77-0.92) | |
| 18.5-<22.0 | 1487 | 0.84 (0.81-0.87) | |
| 22-<25.0 | 2089 | 0.83 (0.81-0.85) | |
| 25-<30.0 | 1309 | 0.81 (0.79-0.84) | |
| ≥30 | 147 | 0.67 (0.60-0.74) | .001† |
| BFP (%) | | | |
| <18.1 | 1303 | 0.84 (0.81-0.87) | |
| 18.1-<21.5 | 1303 | 0.85 (0.82-0.88) | |
| 21.5-<24.9 | 1319 | 0.82 (0.80-0.85) | |
| ≥24.9 | 1321 | 0.79 (0.77-0.82) | .002† |

Abbreviations as in Table 1.
Data in parentheses are 95% confidence intervals.
* Data were log-transformed and back-transformed.
† Crude data.
‡ Age-adjusted data.

## COMMENT

This study was conducted in apparently healthy Japanese men, who have a low prevalence of obesity. The geometric mean PSA level increased significantly with age ($P$ for linear trend < .0001) and decreased with BMI and BFP categories after adjustment for age ($P$ for linear trend = .001 and $P$ for linear trend = .002, respectively). The proportion of subjects with an abnormal PSA value for each cutoff point (2.5 and 4.0 ng/mL) increased significantly with age ($P$ for trend < .0001), but no significant trends were noted across BMI or BFP categories, independent of age adjustment.

An inverse association between BMI and PSA level has been previously reported in screened populations that included patients with prostate cancer,[5,7] in more selective healthy subjects, with biopsy-positive patients excluded,[4,6] and in a large population of patients with prostate cancer.[8] A greater BMI was associated with increased prostate cancer death,[17] greater Gleason scores at diagnosis,[18-20] and a worse prognosis after treat-

ment.[19,20] Some researchers have suggested that a lower PSA level in obese and overweight men might decrease the sensitivity of the screening test, leading to a delayed diagnosis with an unfavorable prognosis in the obese population.[6,7] Other researchers found that the inverse association was too weak to explain the delay in diagnosis of prostate cancer in obese men.[5] In the present study, the correlation coefficients between PSA and BMI or BFP were small; therefore, the inverse association might not be that important, biologically. Because obese men are more likely than men of normal weight to participate in screening, the associations between advanced stage, worse outcome, and obesity cannot be explained by disparities in screening of obese men for prostate cancer.[21]

Obesity is associated with lower testosterone and sex hormone-binding globulin levels and greater estrogen blood concentrations,[22] and these endocrine changes in obese men might affect the production of PSA.[23,24] Moreover, Banez et al.[8] demonstrated that a greater BMI is associated with more plasma volume and with lower serum PSA levels in patients with prostate cancer. Similarly, Werny et al.[5] found that total body water, an index of plasma volume, was associated with moderately lower PSA values in a population-based study. These results suggest that obesity-related plasma hemodilution could be a cause of the inverse association between obesity and PSA level. Because the plasma volume was estimated using body weight and height in the 2 studies,[5,8] more accurate estimates, such as hematocrit and lean body mass, are needed to evaluate the effects of hemodilution on the PSA level. Because hormone levels and plasma volume were not examined in the present study, the effects of these factors and their relationship to the mechanism of lower PSA levels in obese men were beyond the scope of this discussion.

Previous studies related to obesity and PSA levels have targeted white and African-American men whose mean BMI was $\geq$25 kg/m$^2$.[4-6,8] In 2003-2004, 39.7% of U.S. men ($\geq$20 years) were overweight (BMI $\geq$25 to $<$30 kg/m$^2$) and 31.1% were obese (BMI $\geq$30 kg/m$^2$).[25] In contrast, the mean BMI in the present study was 23.4 kg/m$^2$, and the proportion of overweight and obese men was only 25.0% and 2.8%, respectively. The proportion of obese men in the present study was less than one tenth that among American men. Our study also included more participants who were younger than in previous studies (mean age 46.2 years vs 56.7,[6] 62.4,[4] and 57.8-62.5 years[8] and median age 46 years vs 49-60 years[5,7]). It would appear that many Japanese clinicians have not sufficiently informed their patients about the risks and benefits of PSA measurement and/or that Japanese men are more likely to be concerned about prostate cancer, and thus repeatedly check their PSA level at their own expense starting at a younger age.

Alternative metrics such as PSA density, free-to-total PSA ratio, and age-specific reference ranges have been previously proposed to increase the accuracy of PSA testing.[2,26,27] Nam et al.[28] devised a clinical nomogram to assess individual risk of prostate cancer. They included PSA, digital rectal examination, age, ethnicity, family history, urinary voiding symptoms, and free-to-total PSA ratio as predictive variables in their nomogram, but they did not include obesity.[28] BMI and BFP could be included among the predictive variables in prostate cancer screening programs.

The limitations of our study were as follows. First, because we performed neither digital rectal examination nor biopsy, we could not exclude from our analysis patients with abnormal digital rectal examination or positive biopsy findings who might have had a high PSA level. However, a significant number of tumors are found even in men with a PSA level of $\leq$4.0 ng/mL, suggesting that latent tumors could still have been overlooked.[28,29] Moreover, because the present analysis did not include prostate volume measurements, the confounding effects of prostate volume could not be excluded. Second, total body adiposity was not measured. Because a greater BMI does not always indicate total body adiposity, the BFP was also examined in this study. The accuracy and reliability of the BFP measured by the bioelectrical impedance analysis device have been established in adults.[30] The BFP might have been expected to show a stronger correlation with the PSA level than the BMI; however, the degree of correlation of the PSA level with the BFP was the same as that with the BMI. Third, a relatively small number of subjects had an abnormal PSA level, even using a PSA cutoff of 2.5 ng/mL in the present study. This suggests that the prevalence of abnormal PSA values did not differ across BMI or BFP categories, independent of age adjustment. Larger numbers of subjects with borderline PSA levels are needed to correctly evaluate the influence of obesity on the PSA test results.

## CONCLUSIONS

Similar to previous analyses in Western countries,[4-8] inverse correlations between PSA level and BMI and BFP were found in Japanese men, who have a low prevalence of obesity. The results of the present study suggest that differences in race or body composition do not affect the inverse relationship between obesity and PSA level. In prostate cancer screening, the possibility that obesity, in addition to age and prostate volume, could affect the accuracy of PSA testing should be considered. However, it is still not known how biologically significant is the association between PSA and obesity. Additional prospective studies are warranted to clarify the relationships between the factors that could affect the accuracy of PSA testing and prostate cancer mortality in obese men.

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# *CYP1A1*, *GSTM1*, and *GSTT1* Polymorphisms, Smoking, and Lung Cancer Risk in a Pooled Analysis among Asian Populations

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## Abstract

**To evaluate the roles of *CYP1A1* polymorphisms [*Ile*$^{462}$*Val* and *T*$^{6235}$*C* (*MspI*)] and deletion of *GSTM1* and and *GSTT1* in lung cancer development in Asian populations, a pooled analysis was conducted on 13 existing studies included in Genetic Susceptibility to Environmental Carcinogenesis database. This pooled analysis included 1,971 cases and 2,130 controls. Lung cancer risk was estimated as odds ratios (OR) and 95% confidence intervals (95% CI) using unconditional logistic regression model adjusting for age, sex, and pack-year. The *CYP1A1* *$^{6235}$C* variant was associated with squamous cell lung cancer (TC versus *TT*: OR, 1.42; 95% CI, 0.96-2.09; *CC* versus *TT*: OR, 1.97; 95% CI, 1.26-3.07; $P_{trend}$ = 0.003). In haplotype analysis, *$^{462}$Val-$^{6235}$T* and *Ile-C* haplotypes were associated with lung cancer risk with reference to the *Ile-T* haplotype (OR, 3.41; 95% CI, 1.78-6.53 and OR, 1.39; 95% CI, 1.12-1.71, respectively). The *GSTM1*-null genotype increased squamous cell lung cancer risk (OR, 1.36; 95% CI, 1.05-1.77). When the interaction was evaluated with smoking, increasing trend of lung cancer risk as pack-year increased was stronger among those with the *CYP1A1* 6235 *TC/CC* genotype compared with those with *TT* genotype ($P_{interaction}$ = 0.001) and with the *GSTM1*-null genotype compared with the present type ($P_{interaction}$ = 0.08, when no genotype effect with no exposure was assumed). These results suggest that genetic polymorphisms in *CYP1A1* and *GSTM1* are associated with lung cancer risk in Asian populations. However, further investigation is warranted considering the relatively small sample size when subgroup analyses were done and the lack of environmental exposure data other than smoking.** (Cancer Epidemiol Biomarkers Prev 2008;17(5):1120–6)

## Introduction

Lung cancer mortality has increased rapidly during recent years in Asian countries. Cigarette smoking is the strongest established risk factor for lung cancer, but genetically determined variations in metabolism of tobacco-derived carcinogens may affect individual susceptibility to lung cancer. Cigarette smoke contains a variety of carcinogens, such as polycyclic aromatic hydrocarbons, *N*-nitrosoamines, and aromatic heterocyclic amines (1). These carcinogens undergo biotransformation by several enzymatic pathways, including P450s (CYP), glutathione *S*-transferase (GST), and *N*-acetyltransferase.

*CYP1A1* plays an important role in the metabolism of polycyclic aromatic hydrocarbons, including benzo(*a*)pyrene, as a phase I enzyme and two variants (i.e., *Ile*$^{462}$*Val* and *T*$^{6235}$C), which are potentially functional (2-4), have been evaluated as susceptibility factors for lung cancer by a number of investigators. An increased risk of lung cancer has been observed with the *$^{6235}$C* variant among smokers (5) and with *$^{462}$Val* among nonsmokers (6) in

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previous pooled analyses using the Genetic Susceptibility to Environmental Carcinogenesis (GSEC) database, whereas a separate meta-analysis did not find a significant association with lung cancer risk (7).

*GSTM1* catalyzes reactive electrophilic intermediates derived from cigarette smoking, such as benzo(*a*)pyrene-7,8-diol-9,10-epoxides (BPDE), to less reactive and more easily excreted glutathione conjugates (8). Deletion of *GSTM1* has most widely been evaluated for the association with lung cancer risk and a significant association was found in several studies. Although three meta-analyses concluded that the *GSTM1*-null genotype is associated with an increased lung cancer risk (9-11), a GSEC pooled analysis indicated that there is no strong evidence for increased risk of lung cancer among those with the *GSTM1*-null genotype (12). Another isoform of GST (GSTT1) is also involved in carcinogen detoxification and its deletion polymorphism has been suggested to be associated with lung cancer in several studies. In a recent GSEC pooled analysis, the association was not significant for either Asians or Caucasians and no interaction was observed between *GSTT1*-null genotype and smoking on lung cancer (13).

Pooled analyses based on the GSEC data suggest that the effects of these variants tend to differ according to ethnicity possibly because of differences in linkage disequilibrium and environmental exposures. Consequently, gene-environment or gene-gene interactions might differ by ethnic group. Thus, we focused on Asian populations and evaluated the potential role of four selected polymorphisms in the three aforementioned genes (*CYP1A1* $Ile^{462}Val$ and $T^{6235}C$, and null genotypes for *GSTM1* and *GSTT1*) in the development of lung cancer and its specific cell types.

## Materials and Methods

**Study Population.** Subjects were recruited from the International Collaborative Study on GSEC. The design of this collaborative project is explained in detail elsewhere (14). We obtained the original data of 15 case-control studies on genetic polymorphisms in *CYP1A1*, *GSTM1*, or *GSTT1* and risk of lung cancer conducted in Asian populations (15-30). Two studies were excluded due to a sample size of <10 subjects (29) or Caucasian ethnicity (Turkish; Table 1; ref. 30). The participation in GSEC was voluntary, and therefore, some relevant studies were not included in our analysis. The number of subjects included in this pooled analysis was 1,971 cases and 2,130 controls.

**Statistical Analysis.** All statistical procedures were conducted using Statistical Analysis System version 9.1.3 (SAS Institute) unless otherwise indicated. We estimated the study-specific odds ratios (OR) of lung cancer for each polymorphism using unconditional logistic regression. Results might vary slightly from those reported for some of the published studies because of differences in the inclusion criteria of cases and controls and in the statistical analyses. Heterogeneity among the studies was evaluated by means of the Cochrane Q test and publication bias was assessed by Begg's and Egger's test using STATA version 9. In the pooled analysis, lung cancer risk was estimated with the ORs and 95% confidence intervals (95% CI) by unconditional logistic regression, adjusting for age, sex, and pack-year.

In addition to conducting analyses of all lung cancer, we calculated cell type–specific ORs for the three most prevalent histologic subtypes of lung cancer: adenocarcinoma ($n$ = 905), squamous cell carcinoma ($n$ = 542), and small cell carcinoma ($n$ = 181). Subgroup analyses for other histologic subtypes were not conducted due to small numbers of cases.

Hardy-Weinberg equilibrium for each single nucleotide polymorphism of *CYP1A1* was tested among controls with a Pearson $\chi^2$ and linkage disequilibrium was assessed with $D'$ and $r^2$. Individual haplotypes for two *CYP1A1* polymorphisms ($Ile^{462}Val$ and $T^{6235}C$) were estimated by expectation-maximization method and the overall difference in haplotype frequency profiles between cases and controls was assessed using the likelihood ratio test. The subjects missing both polymorphisms were excluded in haplotype analysis. The program uses a weighting scheme based on expectation-maximization–derived haplotype frequency estimates. Thus, every haplotype is weighted by the probability of carrying each pair of haplotypes rather than assigning a most likely haplotype to an individual. Missing genotypes result in more low-probability haplotype pairs and

**Table 1. Selected characteristics of case-control studies pooled**

| Author | Ethnicity | Cases ($n$) | Controls ($n$) | Reference no. |
|---|---|---|---|---|
| Kihara et al. (1995) | Japanese | 179 | 259 | (15) |
| Ge et al. (1996) | Chinese | 98 (39)* | 27 (12) | (16) |
| Sugimura et al. (1998) | Japanese | 260 | 209 | (17) |
| Persson et al. (1999) | Chinese | 80 (35) | 123 (45) | (18) |
| Le Marchand et al. (1998) | Japanese | 112 (42) | 174 (50) | (19) |
| Kiyohara et al. (1998, 2000) | Japanese | 132 (49) | 84 | (20, 21) |
| Lan et al. (2000) | Chinese | 122 (43) | 122 (43) | (22) |
| Yin et al. (2001) | Chinese | 63 (9) | 62 (9) | (23) |
| Zhao et al. (2001) | Chinese | 233 (233) | 190 (190) | (24) |
| Sunaga et al. (2002) | Japanese | 198 | 152 | (25) |
| Wang et al. (2003) | Chinese | 112 (40) | 119 (40) | (26) |
| Lee et al. (2006) | Korean | 171 | 196 | (27) |
| Pisani et al. (2006) | Thai | 211 (71) | 413 (158) | (28) |
| Total | | 1,971 (635) | 2,130 (591) | |

NOTE: One study with <10 subjects [Dresler et al. (29)] and Caucasian subjects [Pinarbasi et al. (30)] was excluded.
*Number of female subjects.

**Table 2. Characteristics of subjects (1,971 cases and 2,130 controls)**

| | Cases, *n* (%) | Controls, *n* (%) | *P* | OR (95% CI)* |
|---|---|---|---|---|
| Age (y) | | | | |
| <50 | 219 (11.1) | 444 (20.9) | 0.0001 | |
| 50-59 | 501 (25.4) | 638 (30.0) | | |
| 60-69 | 718 (36.5) | 599 (28.2) | | |
| 70-79 | 447 (22.7) | 376 (17.7) | | |
| ≥80 | 85 (4.3) | 70 (3.3) | | |
| Mean (±SD) | 62.6 (±10.7) | 58.4 (±13.2) | 0.0001 | |
| Sex | | | | |
| Male | 1,336 (67.8) | 1,537 (72.2) | 0.002 | |
| Female | 635 (32.2) | 591 (27.8) | | |
| Smoking status | | | | |
| Never | 462 (24.9) | 764 (38.3) | 0.0001 | Reference |
| Ever | 1,396 (75.1) | 1,230 (61.7) | | 2.29 (1.94-2.70) |
| Missing | 113 | 136 | | |
| Pack-years in ever smokers | | | | |
| 0 < pack-year <35 | 468 (42.4) | 640 (64.6) | 0.0001 | 1.54 (1.28-1.36) |
| Pack-year ≥35 | 636 (57.6) | 351 (35.4) | | 4.36 (3.51-5.35) |
| Missing | 292 | 239 | | |
| Mean (±SD) | 66.8 (±146.5) | 49.4 (±107.9) | 0.002 | |
| Pathologic type | | | | |
| AD | 905 (50.2) | | | |
| SQ | 542 (30.1) | | | |
| SM | 181 (10.0) | | | |
| Other cell types | 174 (9.7) | | | |
| Missing | 169 | | | |

Abbreviations: AD, adenocarcinoma; SQ, squamous cell carcinoma; SM, small cell carcinoma.
*ORs were adjusted for age and sex.

each haplotype is weighted as such. An unconditional logistic regression model was used to estimate the effect of individual haplotypes by fitting an additive model, adjusting for sex, age, and pack-year.

Gene-smoking interactions (i.e., the modification of increasing pattern of lung cancer risk as the pack-year increases by different genotype) were evaluated by the significance of the coefficient of product term

**Table 3. *CYP1A1* genotypes and lung cancer risk by histologic types**

| | Controls, *n* (%) | All cases, *n* (%) | OR (95% CI)* | AD, *n* (%) | OR (95% CI)* | SQ, *n* (%) | OR (95% CI)* | SM, *n* (%) | OR (95% CI)* |
|---|---|---|---|---|---|---|---|---|---|
| *Ile$^{462}$Val* | *n* = 1,096 | *n* = 910 | | *n* = 337 | | *n* = 343 | | *n* = 121 | |
| *Ile/Ile* | 609 (55.6) | 502 (55.2) | Reference | 188 (55.8) | Reference | 180 (52.5) | Reference | 72 (59.5) | Reference |
| *Ile/Val* | 421 (38.4) | 329 (36.2) | 0.88 (0.71-1.08) | 117 (34.7) | 0.94 (0.69-1.27) | 132 (38.5) | 1.06 (0.78-1.45) | 41 (33.9) | 0.80 (0.50-1.28) |
| *Val/Val* | 66 (6.0) | 79 (8.7) | 1.06 (0.71-1.56) | 32 (9.5) | 1.53 (0.92-2.56) | 31 (9.0) | 1.01 (0.55-1.85) | 8 (6.6) | 0.60 (0.22-1.67) |
| $P_{trend}$ | | | 0.57 | | 0.37 | | 0.78 | | 0.21 |
| *Ile/Ile or Ile/Val* | 1,030 (94.0) | 831 (91.3) | Reference | 305 (90.5) | Reference | 312 (91.0) | Reference | 113 (92.4) | Reference |
| *Val/Val* | 66 (6.0) | 79 (8.7) | 1.14 (0.76-1.72) | 32 (9.5) | 1.57 (0.96-2.59) | 31 (9.0) | 1.14 (0.76-1.72) | 8 (6.6) | 0.65 (0.24-1.79) |
| *T$^{6235}$C (MspI)* | *n* = 953 | *n* = 729 | | *n* = 284 | | *n* = 261 | | *n* = 95 | |
| *TT* | 333 (34.9) | 241 (33.1) | Reference | 106 (37.3) | Reference | 75 (28.7) | Reference | 36 (37.9) | Reference |
| *TC* | 449 (47.1) | 341 (46.8) | 1.08 (0.84-1.39) | 125 (44.0) | 1.08 (0.84-1.39) | 120 (46.0) | 1.42 (0.96-2.09) | 45 (47.4) | 1.10 (0.65-1.86) |
| *CC* | 171 (17.9) | 147 (20.2) | 1.13 (0.82-1.56) | 53 (18.7) | 1.13 (0.82-1.56) | 66 (25.3) | 1.97 (1.26-3.07) | 14 (14.7) | 0.73 (0.36-1.51) |
| $P_{trend}$ | | | 0.43 | | 0.43 | | 0.003 | | 0.52 |
| *TC* or *CC* | 620 (65.1) | 488 (67.0) | 1.10 (0.86-1.39) | 178 (62.7) | 1.10 (0.86-1.39) | 186 (71.3) | 1.58 (1.10-2.27) | 50 (52.6) | 0.98 (0.60-1.62) |
| Haplotype† | *n* = 1,172 | *n* = 979 | | *n* = 361 | | *n* = 385 | | *n* = 123 | |
| | % | % | | % | | % | | % | |
| *Ile-T* | 56 | 52 | Reference | 55 | Reference | 49 | Reference | 57 | Reference |
| *Ile-C* | 19 | 21 | 1.39 (1.12-1.71) | 18 | 0.99 (0.73-1.34) | 24 | 2.10 (1.58-2.80) | 19 | 1.29 (0.83-2.01) |
| *Val-T* | 2 | 4 | 3.41 (1.78-6.53) | 4 | 4.84 (2.32-10.1) | 4 | 3.75 (1.70-8.27) | 1 | 0.37 (0.02-8.06) |
| *Val-C* | 23 | 23 | 0.96 (0.79-1.15) | 23 | 0.94 (0.73-1.12) | 24 | 1.06 (0.81-1.38) | 23 | 0.89 (0.60-1.31) |
| $P_{omnibus}$‡ | | | 0.0001 | | 0.0003 | | 0.0001 | | 0.40 |

*ORs were adjusted for age (<50, 50-59, 60-69, 70-79, and ≥80 y), sex, and pack-year.
†Subjects missing for both *CYP1A1 Ile$^{462}$Val* and *T$^{6235}$C (MspI)* data were excluded.
‡*P* value from the test of overall difference of haplotype distribution between cases and controls.

**Table 4. *GSTM1* and *GSTT1* genotypes and lung cancer risk by histologic types**

| | Controls, *n* (%) | All cases, *n* (%) | OR (95% CI)* | AD, *n* (%) | OR (95% CI)* | SQ, *n* (%) | OR (95% CI)* | SM, *n* (%) | OR (95% CI)* |
|---|---|---|---|---|---|---|---|---|---|
| *GSTM1* | *n* = 1,604 | *n* = 1,419 | | *n* = 760 | | *n* = 333 | | *n* = 169 | |
| Present | 713 (44.5) | 589 (41.5) | Reference | 332 (43.7) | Reference | 124 (37.2) | Reference | 59 (41.3) | Reference |
| Null | 891 (55.6) | 830 (58.5) | 1.11 (0.95-1.29) | 428 (56.3) | 0.99 (0.82-1.19) | 209 (62.8) | 1.36 (1.05-1.77) | 84 (58.7) | 1.27 (0.88-1.83) |
| *GSTT1* | *n* = 1,024 | *n* = 1,135 | | *n* = 579 | | *n* = 248 | | *n* = 71 | |
| Present | 538 (52.5) | 579 (51.0) | Reference | 300 (51.8) | Reference | 141 (56.9) | Reference | 25 (35.2) | Reference |
| Null | 486 (47.5) | 556 (49.0) | 1.02 (0.84-1.24) | 279 (48.2) | 1.00 (0.80-1.26) | 107 (43.2) | 0.87 (0.62-1.21) | 46 (64.8) | 1.36 (0.99-1.86) |

*ORs were adjusted for age (<50, 50-59, 60-69, 70-79, and ≥80 y), sex, and pack-year.

genotype*pack-year in the model. The test was equal to evaluate the difference of the slopes of two fitted lines stratified by categorized genotypes. Additionally, we tested the significance of the product term in the model without main effect term of genotype, which assumes that if there is no exposure to cigarette smoking, there is no difference in the risk of lung cancer between genotypes (27, 31). The assumption of no genotype effect when there is no smoking exposure was equal to common intercept assumption for two fitted lines by genotypes.

## Results

The distributions by age, sex, smoking status, and cell types of the 1,971 lung cancer cases and 2,130 controls are presented in Table 2. The mean age was 62.6 (±10.7 years) in cases and 58.4 (±13.2 years) in controls ($P$ = 0.0001). The proportion of ever smokers was much greater in cases (75.1%) than in controls (61.7%; $P$ = 0.0001). In terms of cell types, adenocarcinoma (50.2%) and squamous cell carcinoma (30.1%) were the most common.

Genotype frequencies of *CYP1A1* $Ile^{462}Val$ and $T^{6235}C$ were consistent with Hardy-Weinberg equilibrium in the control group ($P$ > 0.35) and the two polymorphisms were in moderate linkage disequilibrium ($D'$ = 0.86 and $r^2$ = 0.35). The variant allele frequencies of the three polymorphisms (*CYP1A1* $^{462}Val$, 0.25; $^{6235}C$, 0.42; and *GSTT1* null, 0.48) in the controls were higher compared with those of Caucasian or African populations (13, 32). The frequency of the *GSTM1* null (0.56) was similar to that of Caucasians but higher compared with Africans (32). The *CYP1A1* $^{6235}C$ variant was associated with squamous cell lung cancer (*TC* versus *TT*: OR, 1.42; 95% CI, 0.96-2.09; *CC* versus *TT*: OR, 1.97; 95% CI, 1.26-3.07; $P_{trend}$ = 0.003; Table 3). The *CYP1A1* $^{462}Val$ variant was moderately associated with adenocarcinoma (*Val/Val* versus *Ile/Ile* or *Ile/Val*: OR, 1.57; 95% CI, 0.96-2.59).

In haplotype analysis, $^{462}Val$-$^{6235}T$ and *Ile*-C haplotypes were associated with lung cancer risk with reference to the *Ile-T* haplotype (OR, 3.41; 95% CI, 1.78-6.53 and OR, 1.39; 95% CI, 1.12-1.71, respectively). An omnibus test showed that the distribution of the *CYP1A1* haplotypes was significantly different between all lung cancer cases and controls ($P$ = 0.0001). In subgroup analysis, the difference was also significant for adenocarcinoma ($P$ = 0.0003) and squamous cell carcinoma ($P$ = 0.0001) and not for small cell carcinoma ($P$ = 0.40).

The *GSTM1*-null genotype significantly increased squamous cell lung cancer risk (OR, 1.36; 95% CI, 1.05-1.77), and the *GSTT1*-null genotype was moderately associated only with small cell lung cancer risk (OR, 1.36; 95% CI, 0.99-1.86; Table 4). Analysis of combined genotypes did not reveal associations beyond what was apparent in the single polymorphism analyses (data not shown).

When the interaction was evaluated with smoking, increasing trend of lung cancer risk as pack-year increased was much stronger among those with the *CYP1A1* 6235 *TC/CC* genotype compared with those with *TT* genotype ($P_{interaction}$ = 0.001; Fig. 1). Although the association between smoking and lung cancer was stronger among those with the *GSTM1*-null genotype compared with the present type, it was only marginally significant with the assumption of no genotype effect in the absence of the smoking exposure ($P_{interaction}$ = 0.08). Significant interactive effect with smoking has not been observed for *GSTT1*.

There was no evidence of significant heterogeneity among studies or of publication bias for all four polymorphisms investigated in our study; we found only moderate heterogeneity for the effect of *CYP1A1* $^{462}Val/Val$ compared with *Ile/Ile* ($P$ = 0.08), and all Begg's and Egger's tests were not significant ($P \geq 0.2$ and 0.3, respectively).

## Discussion

Our results suggest that the *CYP1A1* polymorphisms ($Ile^{462}Val$ and $T^{6235}C$) and the *GSTM1*-null genotype are associated with lung cancer risk, especially for squamous cell carcinoma, in Asian populations. In addition, the association of smoking with lung cancer was significantly modified by the *CYP1A1* $T^{6235}C$ polymorphism in our study.

A significant interactive effect between the *CYP1A1* $^{6235}C$ allele and smoking is consistent with the results of previous pooled analysis that the stronger association between the $^{6235}C$ allele and lung cancer was found among ever smokers (5). The previous pooled analysis for the *GSTM1*-null genotype conducted by Benhamou et al. (12) found a nonsignificant elevated lung cancer risk among Asians, especially among heavy smoker (>40 pack-years). Likewise, our extended analysis with additional Asian populations also observed a moderate elevation of overall lung cancer risk by the *GSTM1* deletion and moderate interaction with smoking. On the

other hand, stronger effect of *CYP1A1* $^{462}$*Val* found in previous pooled analysis among nonsmokers (6) was not observed in Asian populations investigated in our study.

Le Marchand et al. (19) hypothesized that genetic susceptibility to polycyclic aromatic hydrocarbons (based on high-risk genotypes for *CYP1A1* and *GSTM1*) predominantly causes squamous cell carcinoma. In the multiethnic study conducted by Le Marchand et al. (19), *CYP1A1* $^{6235}$*C* allele was associated with a 3.1-fold risk of squamous cell carcinoma when combined with a *GSTM1* deletion. Decreasing trend of squamous cell carcinoma, relative to the increase in adenocarcinoma, associated with filter-tipped cigarettes in developed country indirectly supports this hypothesis (33). The increased risk of squamous cell carcinoma in relation with the *GSTM1*-null genotype observed in our study is consistent with the results of previous studies, including those of a meta-analysis (10, 19, 34, 35). The effect of the *CYP1A1* $^{6235}$*C* allele, especially when combined with a *GSTM1*-null genotype, also tended to be associated with a higher risk of squamous cell carcinoma among Asians (5); in our study, *CYP1A1 TC* or *CC* genotype was associated with significant elevation of squamous cell carcinoma risk compared with *TT* genotype (OR, 1.6) and Ile-C haplotype was significantly associated with squamous cell carcinoma risk (OR, 2.1).

BPDE is known to induce G:C to T:A transversion mutations in the hotspot codons of the *p53* tumor suppressor gene (36), which is found more frequent in squamous cell carcinoma than in adenocarcinoma (37). Cigarette smoke is also known to be causally related to BPDE-DNA adducts (38, 39), and BPDE-DNA adduct

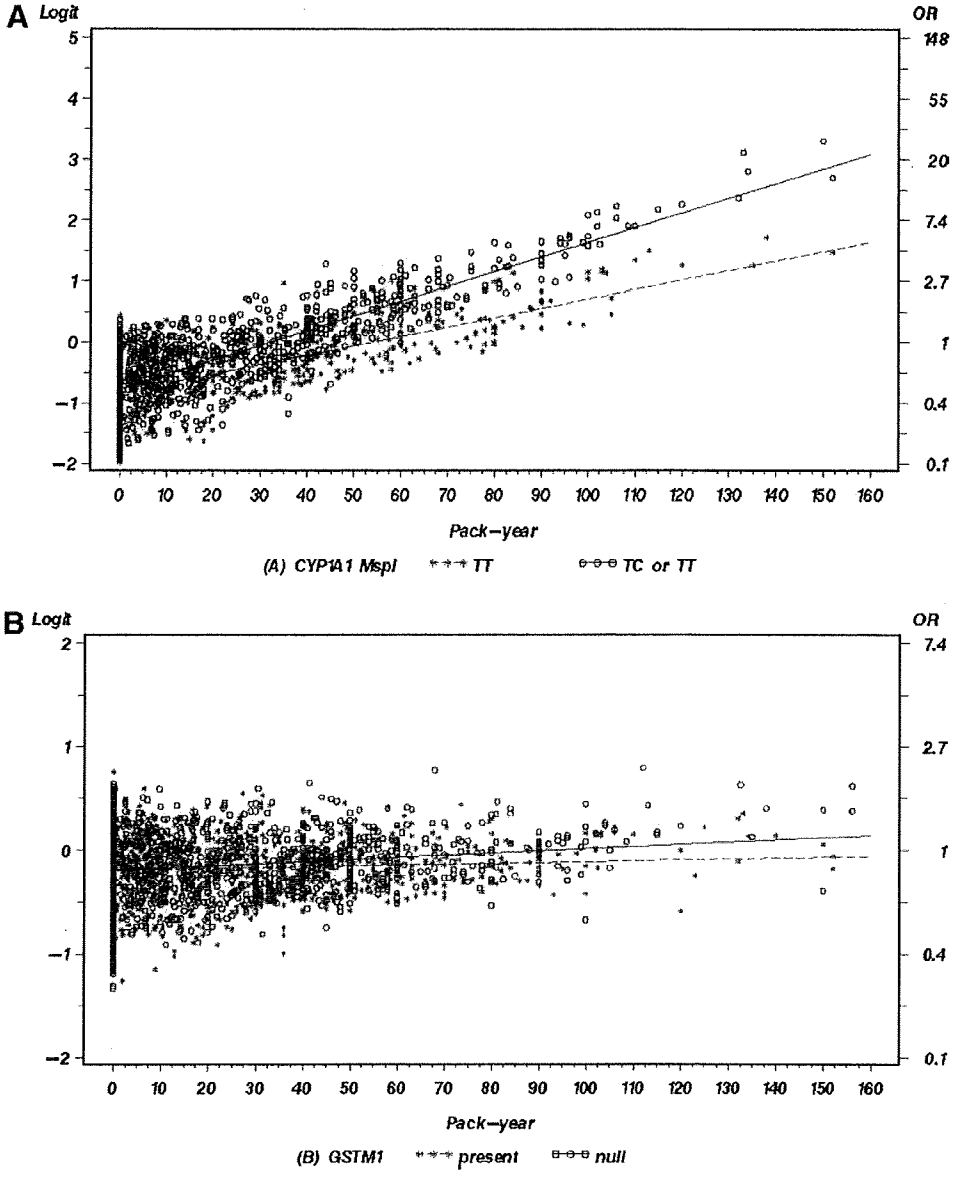


**Figure 1.** The smoking effect on lung cancer stratified by the *CYP1A1* $T^{6235}C$ *(MspI)* (**A**) and *GSTM1* null/present (**B**). When the interaction was evaluated with smoking, increasing trend of lung cancer risk as pack-year increased was stronger among those with the *CYP1A1* 6235 TC/CC genotype compared with those with TT genotype ($P_{interaction}$ = 0.001). Although the association between smoking and lung cancer was stronger among those with the *GSTM1*-null genotype compared with the present type, it was only marginally significant with the assumption of common intercept ($P_{interaction}$ = 0.08).

level is elevated in the lung parenchyma of smokers with *GSTM1*-null genotype (40). Moreover, the combined genotypes of *CYP1A1* $^{462}$*Val* and *GSTM1* null have been associated with increased adduct level in lung tissues of squamous cell carcinoma patients (41). Thus, it is speculated that our finding of an association between *GSTM1* and *CYP1A1* polymorphisms with the risk of squamous cell carcinoma is related to polycyclic aromatic hydrocarbon exposure derived from smoking because polycyclic aromatic hydrocarbons are primarily metabolized by *CYP1A1* and *GSTM1*. The greater effects observed among smokers also support this smoking-related etiology of squamous cell carcinoma in Asian population.

Our study is the largest pooled analysis conducted for Asian populations to evaluate the role of polymorphisms in carcinogen-metabolizing genes (i.e., *CYP1A1*, *GSTM1*, and *GSTT1*) in lung cancer development. We simultaneously evaluated the potential effect of four polymorphisms on lung cancer and the modification of those effects by smoking exposure reporting significant interaction between *CYP1A1* $^{6235}$*C* allele and smoking. Subtype-specific results in Asian population are also noteworthy.

However, our study has several limitations to be considered. First, not all published Asian studies were included in this study. However, there was no evidence of significant publication bias for this pooled analysis. In terms of heterogeneity, only marginally significant heterogeneity was found for *CYP1A1* $^{462}$*Val/Val* compared with *Ile/Ile* ($P$ = 0.08). We note that when the adjusted values were considered, the heterogeneity did not remain. Other limitation of our study may be the relatively small sample size in subgroup analyses. We found that the *GSTT1*-null genotype was marginally associated only with small cell lung cancer risk, whereas no association with lung cancer was observed for either Asians or Caucasians in the previous pooled analysis for *GSTT1*-null genotype (13). Although relatively higher variant allele frequencies, compared with other ethnic groups (13, 32), may compensate for the relative small sample size in terms of statistical power, we cannot exclude chance for the explanation of the significant association between the *GSTT1*-null genotype and small cell lung cancer risk, considering that only 71 cases were available. Sizable exclusion of subjects for missing data on smoking and pathologic subtypes also limits the conclusion from our results for interactive effects between the polymorphisms and smoking, and subtype-specific analysis. Thus, our findings need to be replicated in a larger study. Future study should also include the measurement of dietary factors, such as isothiocyanates, which are involved in the detoxification of tobacco-related carcinogens (42) and may have protective effects on lung cancer especially among smokers or those with *GST*-null genotypes, as observed in a Chinese population (24, 43).

In summary, the results of our study suggest that genetic polymorphism in *CYP1A1* and *GSTM1* plays a role in lung cancer susceptibility in Asian populations and that the effects are strongest for squamous cell carcinoma. Although our results are generally consistent with previous studies and are supported by epidemiologic and experimental observations, additional large studies are needed to help to elucidate the role of genetic polymorphisms in xenobiotic-metabolizing genes in lung cancer development. The interaction between environmental exposure other than smoking (e.g., indoor coal combustion) and these polymorphisms still remains to be evaluated.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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RESEARCH COMMUNICATION

# Serum Concentrations of Fatty Acids and Colorectal Adenoma Risk: A Case-Control Study in Japan

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## Abstract

**Background: Epidemiologic studies of n-3 fatty acids (FAs) and risk of colorectal cancer have generated inconsistent results, and relations with precursor colorectal adenomas (CRA) have not been evaluated in detail. We here focused on possible associations of serum FAs with CRA in the Japanese population. Methods: We conducted a case-control study of 203 asymptomatic CRA cases (148 men, 55 women) and 179 healthy controls (67 men, 112 women) during 1997-2003 in Nagoya, Japan. Baseline information was obtained using a lifestyle questionnaire and serum FA levels were measured by gas chromatography. Results: A non-significant inverse association with CRA was observed for eicosapentaenoic acid (EPA) among women. Moreover, the concentrations of docosahexaenoeic acid (DHA), a major component of n-3 highly-unsaturated FAs (HUFAs), were significantly lower in cases in both sexes. In addition, serum concentrations of total FAs, saturated FAs (SFAs) and mono-unsaturated FAs (MUFAs) had strong positive links with CRA risk. In contrast, arachidonic acid (AA) and DHA were inversely related, with 66% and 59% risk reduction, respectively. Ratios of SFAs/n-3 PUFAs and SFAs/n-3 HUFAs exhibited significant positive relations with CRA risk but there was no clear link with n-6 PUFAs/n-3 PUFAs. Conclusions: Our findings suggest a promoting influence of SFAs and MUFAs along with a protective effect of DHA on CRA risk. However, further research is needed to investigate the observed discrepancy with the generally accepted roles of the AA cascade in carcinogenesis.**

**Key Words:** Colorectal adenomas - fatty acids – biomarkers - serum concentrations

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## Introduction

Colorectal adenomas (CRA), assumed to be precursors of colorectal cancer (CRC) (Durno, 2007), are usually asymptomatic and are most commonly detected by chance in individuals undergoing colonoscopy or sigmoidoscopy for screening. They occur about a decade before clinical diagnosis of CRC. It has been hypothesized that dietary and lifestyle factors that influence CRC development may also affect the CRA risk (Jacobs et al., 2007; Miller et al., 2007). Of possible dietary factors, intake of fat and fatty acids (FAs) has long been a matter of interest.

Earlier studies focused on the relationships between risk and total fat and saturated FAs (Slattery et al., 1997; Nkondjock et al., 2003), but recently n-3 poly-unsaturated FAs (PUFAs) and n-3 highly-unsaturated FAs (HUFAs), including eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) derived from sea foods (Petrik et al., 2000; Fritsche, 2006; Stehr and Heller, 2006), have received increasing attention due to their potential roles in inflammatory processes, tumorigenesis, angiogenesis and cell proliferation (James et al., 2000; Larsson et al., 2004; Kimura, 2006). Results of animal and ecologic studies have suggested that n-3 PUFAs might reduce the risk of CRA/CRC (Caygill and Hill, 1995; Pietinen et al., 1999; Roynette et al., 2004). Moreover, clinical trials have found that n-3 PUFAs supplementation reduced cell proliferation (Anti et al., 1994; Tokudome et al., 2002; Cheng et al., 2003).

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However, the relationship between these FAs and CRC has been inconsistent in epidemiologic research. Some prospective (Pietinen et al., 1999; Terry et al., 2001) and case-control (Slattery et al., 1997; Busstra et al., 2003) studies reported no significant associations between n-3 PUFAs and risk of CRA/CRC, whereas one case-control study showed statistically significant inverse trends (Nkondjock et al., 2003).

Conversely, n-6 PUFAs are suggested to have a critical role in CRC promotion through cyclooxygenase (COX) and lipoxygenase pathways involved in the arachidonic acid (AA) cascade (Rao and Reddy, 1993; Poole et al., 2007). However, some epidemiologic studies indicated these FAs have no influences on colorectal carcinogenesis (Terry et al., 2001; Busstra et al., 2003; Theodoratou et al., 2007), while other reports noted the protective effects of n-6 PUFAs, especially AA, on CRA/CRC (Kojima et al., 2005; Kuriki et al., 2006).

As previous investigations of intake and serum concentrations of n-3 and n-6 PUFAs in relation to CRA/CRC risk have been inconclusive, we here focused on associations of serum FAs, especially n-3 PUFAs and n-3 HUFAs, with CRA, precursor lesions of CRC, to find potential risk factors in the Japanese population, which has the highest consumption of sea foods as a main source of n-3 PUFAs, especially n-3 HUFAs.

## Materials and Methods

*Study Subjects*

This case-control study was conducted as part of a research program on dietary and lifestyle factors related to the colorectal adenoma-carcinoma sequence in Nagoya, Japan.

Cases consisted of consecutive patients, aged from 35-75 years, with histologically-verified colorectal adenomatous polyp/polyps, who were admitted to Nagoya City University Hospital and agreed to participate in this study during 1997-2003. These subjects are part of a randomized control trial which has been continuously conducted since 1997 (Tokudome et al., 2002). The lesions included grade 3 adenomas with light/moderate atypia, grade 4 adenomas with severe atypia and grade 5 small adenocarcinomas (carcinoma *in situ*) according to the General Rules for Clinical and Pathological Studies on Cancers of the Colon, Rectum and Anus (Japanese Society for Cancer of Colon and Rectum, 1998).

Controls were selected from volunteer participants who had a negative faecal occult blood test for screening examinations for CRA and CRC, during the period of case collection at Naka Health Center, Nagoya. Subjects were 205 CRA cases and 181 controls with no history of familial adenomatous polyposis or hereditary non-polyposis colorectal cancer, bleeding diathesis or a past history of CRC, gastrectomy or cholecystectomy. Two patients, who had CRAs with a small *in situ* carcinoma, were included in the cases . Finally, after exclusion of 3 subjects (1 case, 2 controls) with extremely high serum FAs (more than mean ± 3SD), 203 CRA cases (148 men, 55 women) and 179 healthy controls (67 men, 112 women) were analyzed.

The Internal Review Board of the Nagoya City University Graduate School of Medical Sciences approved the research protocol, and all subjects provided written informed consent.

*Lifestyle questionnaire*

Detailed information on demographic characteristics, personal medical history, usual leisure-time physical activity/exercise, cigarette smoking and alcohol drinking, intake of supplements and history of CRA or CRC in first-degree relatives was collected by trained interviewers. Smokers were defined as those who had ever smoked cigarettes daily for at least 1 year, and smoking status was classified into 3 categories (never, past or current smoker). The consumption of five types of alcoholic beverages (beer, whisky, wine, sake and shochu) over the past years was categorized into never, past or current drinker. Also, history of hyperlipidemia includes cases under treatment or observation of hypercholesterolemia and/or hypertriglyceridemia.

*Measurements of serum FAs*

All participants were asked to provide 7 ml of overnight fasting venous blood, the sampling tubes being immediately placed in a 4°C refrigerator. Within 4 hours of blood collection, blood was separated into plasma, buffy coat (layer of white blood cells) and red blood cells (by centrifugation at 2,500 rpm for 15 min at 4°C, aliquoted into four tubes, and immediately stored at -80°C until analysis of FAs by gas chromatography as previously reported (Kuriki et al., 2002). The analyzers of FAs were completely blinded to information on study subjects. The precision of FA measurements in plasma intra- and inter assay coefficients of variation ranged from 1.8 to 4.8 and 2.5 to 7.2 %, respectively (Kuriki et al., 2003). Each plasma FA level was expressed as the absolute concentration (mg/dL).

*Selected FAs and grouping*

We measured the following 13 FAs: 14:0 (myristic acid); 16:0 (palmitic acid); 16:1n-7 (palmitoleic acid); 18:0 (stearic acid); 18:1n-9 (oleic acid); 18:2n-6 (linoleic acid, LA); 18:3n-6 (α-linolenic acid, GLA); 18:3n-3 (α-linolenic acid, ALA); 20:3n-6 (dihomo-α-linolenic acid, DGLA); 20:4n-6 (AA); 20:5n-3 (EPA); 22:5n-3 (DPA); 22:6n-3 (DHA).

We calculated mean compositions and concentrations of serum FAs and summarized the data into the following seven groups: SFAs (14:0 + 16:0 + 18:0); MUFAs (16:1n-7 + 18:1n-9); PUFAs (n-6 PUFAs + n-3 PUFAs); n-6 PUFAs (LA + GLA + DGLA + AA); n-3 PUFAs (ALA + n-3 HUFAs); and n-3 HUFAs (EPA + DPA + DHA). We also defined the ratios of specific FAs as follows: SFAs/n-6 PUFAs; SFAs/n-3 PUFAs; SFAs/n-3 HUFAs; n-6 PUFAs/n-3 PUFAs; n-6 PUFAs/n-3 HUFAs; AA/EPA; and AA/DHA.

*Statistical analysis*

We first compared the background characteristics between cases and controls using Student's *t*- test or analysis of variance for means, and the $x^2$ test or Cochran-Mantel-Haenszel $x^2$ test for proportions by sex.

**Table 1. Basic Characteristics of Colorectal Adenoma Cases and Controls by Sex**

| | Men | | Women | |
|---|---|---|---|---|
| | Cases (n= 148) | Controls (n= 67) | Cases (n=55) | Controls (n=112) |
| Age in years (Mean ± SD) | 59.5 ± 9.2 | 60.1 ± 10.8 | 60.1 ± 8.7 | 60.7 ± 9.1 |
| *p* value[1] | 0.503 | | 0.674 | |
| Body mass index (Mean ± SD) | 23.6 ± 3.0 | 23.3 ± 2.6 | 23.0 ± 3.6 | 22.6 ± 2.8 |
| *p* value | 0.641 | | 0.48 | |
| Family history of colorectal adenoma or cancer (%)[2] | 16 (10.7%) | 3 (4.8%) | 8 (14.5%) | 6 (5.6%) |
| *p* value | 0.05 | | 0.05 | |
| Smoking history (%) | | | | |
| Current smoker | 64 (43.3%) | 13 (19.4%) | 10 (18.2%) | 7 (6.3%) |
| Former smoker | 68 (45.9%) | 27 (40.3%) | 4 (7.3%) | 2 (1.8%) |
| Never smoker | 16 (10.8%) | 27 (40.3%) | 41 (74.5%) | 103 (91.9%) |
| *p* value | 0.001 | | 0.008 | |
| Alcohol drinking history (%) | | | | |
| Current drinker | 115 (77.7%) | 40 (59.8%) | 13 (23.7%) | 25 (22.3%) |
| Former drinker | 7 (4.7%) | 6 (8.9%) | 1 (1.8%) | 5 (4.5%) |
| Never drinker | 26 (17.6%) | 21 (31.3%) | 41 (74.5%) | 82 (73.2%) |
| *p* value | 0.015 | | 0.682 | |
| Leisure-time physical activity/ exercise (yes) (%) | 101 (68.2%) | 38 (56.7%) | 33 (54.5%) | 52 (46.4%) |
| *p* value | 0.09 | | 0.075 | |
| History of hyperlipidemia (%) [3] | 23 (15.6%) | 18 (26.9%) | 16 (29.1%) | 23 (20.5%) |
| *p* value | 0.052 | | 0.246 | |
| History of diabetes (%) | 6 (4.1%) | 3 (4.4%) | 1 (1.8%) | 5 (4.5%) |
| *p* value | 0.401 | | 0.322 | |

[1] Each *p* value is based on the Chi-square test for categorical and on the *t*-test for continuous variables . [2]Family history of colorectal adenomas or cancers in the first-degree relatives was considered. [3]History of hyperlipidemia includes cases under treatment or observation of hypercholesterolemia and/or hypertrigly ceridemia

Unconditional logistic regression models were used to calculate odds ratios (ORs) for the incidence of CRA for each FA. Blood levels of FAs were divided into quartiles based on the FA distributions in the controls. ORs were calculated for the second quartile (Q2), third quartile (Q3), and highest quartile (Q4) versus the lowest quartile (Q1). To test for linear trends in ORs over quartiles, we coded each quartile as 0, 1, 2 or 3 and incorporated these data into the logistic model as a single variable.

We adjusted for the following factors by including them in the logistic model: age; body mass index (weight (kg)/height (m)$^2$; <20, 20.0-24.9, or ≥25.0) calculated from reported height and weight; history of CRA or CRC in the first-degree relatives (yes or no); history of diabetes (yes or no); smoking status (never, past, or current); daily alcohol consumption (never, former, or current); vigorous exercise (yes or no) and season of data collection. In Japan, regular users of non-steroidal anti-inflammatory drugs are very few (Kuriki et al., 2006), and we did not include this factor in our model.

All statistical analyses were conducted using SPSS version 15, and $p$ <0.05 was considered statistically significant.

## Results

Table 1 shows the basic characteristics of subjects participating in the study by sex. There were no significant differences between cases and controls regarding age and body mass index in either sex, but smoking and drinking habits were more frequent in cases than controls. The cases were also more likely to have a family history of CRA or CRC in first-degree relatives. However, the two groups did not differ in variables for physical exercise and history of diabetes. A significantly lower proportion of history of hyperlipidemia was observed in cases than controls for males, but a non-significant inverse pattern was found for females.

Serum FA concentrations in case and control subjects are shown in Table 2. The multivariate adjusted mean values for serum SFAs, including myristic acid, palmitic acid and stearic acid in cases were significantly higher than in controls in both sexes. Similarly, serum MUFAs were higher among cases. For n-6 PUFAs, LA and GLA values in cases and controls were almost the same. Also, DGLA was significantly higher in cases, whereas the mean value for AA was lower. Among n-3 PUFAs, ALA compositions did not differ significantly between cases and controls for either sex. For EPA, the lower serum values in cases were not significantly different from those of controls for either sex. However, the concentrations of DHA, a major component of n-3 HUFAs, were lower in cases than controls, while values for DPA, a minor component, were higher. Ratios of SFAs/n-6 PUFAs, SFAs/n-3 PUFAs and SFAs/n-3 HUFAs in both males and females exhibited positive significant associations with CRA risk, but there were no obvious associations for n-6 PUFAs/n-3 PUFAs, n-6 PUFAs/n-3 HUFAs and AA/DHA.

When we conducted a stratified analysis in non-smoker women, all of the above-mentioned relations between CRA and FAs, including total FAs, SFAs, MUFAs, AA, EPA, DPA and DHA, remained alike (data not shown).Moreover, the compositions of total n-3 PUFAs, n-3 HUFAs, EPA, DHA and AA were significantly lower

**Table 2. Serum Fatty Acid Concentrations in Colorectal Adenoma Cases and Controls by Sex**

| Serum Fatty acids (mg/dL) [1,2] | Men | | | Women | | |
|---|---|---|---|---|---|---|
| | Cases (n= 148) | Controls (n= 67) | p-value | Cases (n=55) | Controls (n=112) | p-value |
| Total Fatty acids (FAs) | 350.42 (6.37) | 322.72 (6.22) | 0.01 | 315.98 (6.98) | 303.16 (3.89) | 0.05 |
| SFAs (Saturated FAs) | 116.13 (5.89) | 100.33 (6.49) | 0.001 | 100.05 (4.28) | 92.06 (4.57) | 0.01 |
| 14:0 (Myristic acid) | 3.12 (0.31) | 2.43 (0.34) | 0.005 | 2.68 (0.28) | 2.01 (0.30) | 0.002 |
| 16:0 (Palmitic acid) | 82.21 (4.24) | 73.05 (4.67) | 0.007 | 72.09 (3.21) | 67.08 (3.43) | 0.03 |
| 18:0 (Stearic acid) | 30.80 (2.87) | 24.87 (3.16) | 0.01 | 25.30 (1.05) | 22.98 (1.12) | 0.003 |
| MUFAs (Mono-unsaturated FAs) | 90.87 (5.43) | 78.39 (5.98) | 0.004 | 78.59 (3.91) | 73.34 (4.18) | 0.05 |
| 16:1n-7 (Palmitoleic acid) | 11.85 (0.92) | 8.82 (1.01) | 0.001 | 10.18 (0.71) | 7.81 (0.76) | 0.001 |
| 18:1n-9 (Oleic acid) | 79.01 (4.64) | 69.57 (5.11) | 0.01 | 68.41 (3.44) | 65.54 (3.67) | 0.252 |
| PUFAs (Poly-unsaturated FAs) | 144.11 (5.83) | 144.20 (6.43) | 0.693 | 137.36 (4.61) | 137.76 (4.93) | 0.988 |
| n-6 PUFAs (Poly-unsaturated FAs) | 113.07 (4.80) | 112.39 (5.29) | 0.859 | 109.29 (4.03) | 108.15 (4.31) | 0.697 |
| 18:2n-6 (Linoleic acid) | 87.69 (4.08) | 86.64 (4.49) | 0.747 | 86.64 (3.62) | 83.33 (3.86) | 0.21 |
| 18:3n-6 (α- linolenic acid) | 1.30 (0.14) | 1.17 (0.16) | 0.272 | 0.98 (0.11) | 0.92 (0.12) | 0.518 |
| 20:3n-6 (Dihomo-α- linolenic acid) | 4.59 (0.35) | 3.98 (0.38) | 0.03 | 4.32 (0.34) | 3.38 (0.37) | 0.001 |
| 20:4n-6 (Arachidonic acid) (AA) | 19.49 (1.04) | 20.60 (1.15) | 0.183 | 17.36 (0.88) | 20.52 (0.94) | 0.001 |
| n-3 PUFAs (Poly-unsaturated FAs) | 31.06 (2.19) | 31.81 (2.42) | 0.511 | 28.07 (2.07) | 29.61 (2.21) | 0.431 |
| 18:3n-3 (α-linolenic acid) | 3.06 (0.26) | 2.98 (0.29) | 0.696 | 2.84 (0.27) | 2.63 (0.29) | 0.278 |
| n-3 HUFAs (Highly-unsaturated FAs) | 28.08 (2.09) | 28.84 (2.30) | 0.527 | 25.23 (1.99) | 26.98 (2.12) | 0.183 |
| 20:5n-3 (Eicosapentaenoic acid) | 8.31 (0.95) | 8.37 (1.05) | 0.222 | 7.61 (0.91) | 8.24 (0.97) | 0.12 |
| 22:5n-3 (Docosapentaenoic acid) | 2.37 (0.29) | 1.95 (0.32) | 0.001 | 2.25 (0.25) | 1.65 (0.27) | 0.001 |
| 22:6n-3 (Docosahexaenoic acid) | 17.42 (1.16) | 18.52 (1.28) | 0.06 | 15.37 (1.14) | 17.09 (1.22) | 0.04 |
| SFA /n-6 PUFAs | 1.01 (0.02) | 0.89 (0.02) | 0.001 | 0.92 (0.02) | 0.85 (0.01) | 0.02 |
| SFA /n-3 PUFAs | 3.74 (0.13) | 3.18 (0.11) | 0.007 | 3.54 (0.20) | 3.11 (0.08) | 0.001 |
| SFA /n-3 HUFAs | 4.13 (0.15) | 3.78 (0.14) | 0.01 | 3.96 (1.89) | 3.41 (1.14) | 0.001 |
| n-6 PUFAs /n-3 PUFAs | 3.63 (0.28) | 3.54 (0.31) | 0.98 | 3.87 (0.39) | 3.65 (0.42) | 0.269 |
| n-6 PUFAs /n-3 HUFAs | 4.08 (0.35) | 3.89 (0.39) | 0.953 | 4.31 (0.48) | 4.01 (0.51) | 0.233 |
| AA/EPA | 2.39 (0.31) | 2.48 (0.34) | 0.33 | 2.31 (0.48) | 2.51 (0.52) | 0.278 |
| AA/DHA | 1.14 (0.09) | 1.12 (0.10) | 0.65 | 1.13 (0.12) | 1.21 (0.13) | 0.746 |

[1] Values are expressed as the mean (SE; standard error) absolute serum fatty acids (mg/dL) [2]Adjusted for age, body mass index, history of colorectal adenomas/cancers in first-degree relatives, history of diabetes, smoking, drinking, physical activity and season of data collection

in cases than controls, while values for SFAs and MUFAs were higher in the case group (data not shown).

As shown in Table 3, total FAs, SFAs and MUFAs were significantly associated with CRA risk. Compared with the lowest quartiles, multivariate adjusted ORs for the highest quartiles of total SFAs and MUFAs were 4.42 and 4.99 in males (95% CI, 1.52-12.87 and 1.56-15.95, $p$ for trend < 0.01) and 4.69 and 2.55 in females (95% CI, 1.45-15.14 and 0.84-7.71, $p$ for trend < 0.01), respectively. Also, ORs for associations between SFAs and MUFAs with CRA risk were higher in large adenomas (≥ 1 cm) than small type (data not shown).

Although there were no associations between total n-6 PUFAs, LA and GLA with CRA risk, AA was linked with a significant decrease in females, and with a similar non-significant trend among males. In contrast, increased risk was found with DGLA in both sexes (ORs, 2.65 and 3.05 for men and women, respectively) (data not shown). With n-3 HUFAs, an inverse dose-response relationship was evident for DHA among females, but not among men. This inverse association was more prominent in colon adenomas (data not shown). Also, non-significant associations between EPA and CRA risk were further noted, but with opposing directions in men and women.

## Discussion

In this study, serum concentrations of EPA were rather lower in cases than controls among women. However, the concentrations of DHA were significantly lower in cases in both sexes, suggesting a protective effect of DHA, a major component of n-3 HUFAs, on CRA. In addition, serum concentrations of total FAs, SFAs and MUFAs demonstrated strong positive associations with CRA risks. In contrast, AA and DHA were inversely related to CRA, with 66% and 59% risk reductions, respectively.

In our study, the values for total n-3 PUFAs and n-3 HUFAs were slightly lower in cases among women, even though dose-response relations with CRA risk were not statistically significant.

We also found a non-significant association of EPA with CRA among women, whereas men exhibited an opposite pattern, although the mean values of serum compositions of EPA were significantly lower in cases than controls. However, these observations are concordant with the findings of three case-control studies which were based on biomarkers of FAs in association with CRC, showing that the protective effects of EPA were not evident (Kojima et al., 2005; Kuriki et al., 2006; Hall et al., 2007). In this study, the relation between DPA and CRA risk was incompatible with other studies indicating no associations between this FA and CRC risk (Nkondjock et al., 2003; Kuriki et al., 2006; Hall et al., 2007). However, only one nested case-control study found an inverse association with CRC risk in men (Kojima et al., 2005). Thus, further studies are needed to evaluate the role of DPA, as a minor component of n-3 HUFAs, in carcinogenesis. In contrast, results of DHA are compatible with findings of the above-

**Table 3. Association Between Serum Fatty Acid Concentrations and Colorectal Adenoma Risk by Sex**

| | Men | | | Women | | |
|---|---|---|---|---|---|---|
| Serum fatty acids (mg/dL) | Value [1] | Cases/Controls | OR (95% CI) [2,3] | Value | Cases/Controls | OR (95% CI) |
| Total fatty acids (FAs) | <262.70 | 18/17 | 1:00 | <272.37 | 12/28 | 1:00 |
| | 262.70-306.40 | 35/17 | 2.36 (0.79-7.08) | 272.37-292.95 | 7/28 | 0.54 (0.14-2.02) |
| | 306.41-342.80 | 41/17 | 2.70 (0.93-7.87) | 292.96-324.37 | 16/28 | 1.54 (0.51-4.63) |
| | >342.80 | 52/16 | 3.58 (1.20-10.7) | >324.37 | 20/28 | 2.05 (0.73-5.78) |
| *p* for trend | | | 0.027 | | | 0.052 |
| SFAs (saturated FAs) | <78.40 | 19/17 | 1:00 | <78.90 | 6/29 | 1:00 |
| | 78.40-92.8 | 28/17 | 2.13 (0.69-6.51) | 78.90-88.50 | 10/27 | 1.93 (0.54-6.89) |
| | 92.81-105.40 | 38/17 | 2.78 (0.92-8.38) | 88.51-97.20 | 14/28 | 2.21 (0.60-8.12) |
| | >105.40 | 61/16 | 4.42 (1.52-12.87) | >97.20 | 25/28 | 4.69 (1.45-15.14) |
| *p* for trend | | | 0.007 | | | 0.009 |
| MUFAs* - | <59.50 | 14/17 | 1:00 | <59.95 | 9/28 | 1:00 |
| | 59.50-67.90 | 17/18 | 1.69 (0.49-5.83) | 59.95-66.05 | 5/28 | 0.49 (0.12-2.10) |
| | 67.91-86.10 | 55/16 | 4.48 (1.51-13.31) | 66.06-77.13 | 17/28 | 2.13 (0.69-6.58) |
| | >86.10 | 60/16 | 4.99 (1.56-15.95) | >77.13 | 24/28 | 2.55 (0.84-7.71) |
| *p* for trend | | | 0.002 | | | 0.015 |
| n-6 PUFAs* | <95.90 | 44/17 | 1:00 | <99.50 | 15/29 | 1:00 |
| | 95.90-108.30 | 30/17 | 0.95 (0.34-2.67) | 99.50-107.15 | 10/27 | 0.40 (0.12-1.40) |
| | 108.31-126.70 | 40/17 | 1.63 (0.57-4.63) | 107.16-121.15 | 15/28 | 0.79 (0.27-2.27) |
| | >126.70 | 32/16 | 0.67 (0.24-1.84) | >121.15 | 15/28 | 0.86 (0.29-2.58) |
| *p* for trend | | | 0.727 | | | 0.906 |
| 18:2n-6 (Linoleic acid) | <74.40 | 46/17 | 1:00 | <75.70 | 12/28 | 1:00 |
| | 74.40-83.00 | 17/17 | 0.69 (0.23-2.06) | 75.70-82.65 | 12/28 | 0.66 (0.21-2.13) |
| | 83.01-98.80 | 47/17 | 1.74 (0.64-4.71) | 82.66-93.87 | 8/28 | 0.56 (0.16-1.90) |
| | >98.80 | 36/16 | 0.86 (0.31-2.36) | >93.87 | 23/28 | 1.54 (0.54-4.36) |
| *p* for trend | | | 0.813 | | | 0.235 |
| 20:4n-6 (Arachidonic acid) | <17.40 | 67/19 | 1:00 | <18.05 | 30/28 | 1:00 |
| | 17.40-19.90 | 24/15 | 0.60 (0.21-1.68) | 18.05-20.50 | 16/29 | 0.49 (0.19-1.24) |
| | 19.91-22.50 | 28/18 | 0.58 (0.21-1.60) | 20.51-22.38 | 5/27 | 0.11 (0.28-0.45) |
| | >22.50 | 27/15 | 0.52 (0.19-1.42) | >22.38 | 4/28 | 0.11 (0.03-0.43) |
| *p* for trend | | | 0.104 | | | 0.001 |
| n-3 PUFAs* | <23.90 | 44/17 | 1:00 | <24.53 | 19/28 | 1:00 |
| | 23.90-29.10 | 41/17 | 0.97 (0.36-2.63) | 24.53-27.75 | 10/28 | 0.47 (0.15-1.40) |
| | 29.11-32.80 | 21/18 | 0.47 (0.16-1.41) | 27.76-35.78 | 16/28 | 0.58 (0.21-1.67) |
| | >32.80 | 40/15 | 0.81 (0.35-2.73) | >35.78 | 10/28 | 0.71 (0.23-2.14) |
| *p* for trend | | | 0.858 | | | 0.487 |
| 18:3n-3 (α linolenic acid) | <2.10 | 50/20 | 1:00 | <1.93 | 14/28 | 1:00 |
| | 2.10-2.60 | 29/17 | 0.82 (0.29-2.34) | 1.93-2.45 | 13/28 | 1.05 (0.35-3.17) |
| | 2.61-3.20 | 20/14 | 0.59 (0.20-1.76) | 2.46-2.90 | 8/31 | 0.43 (0.13-1.41) |
| | >3.20 | 47/16 | 1.12 (0.43-2.90) | >2.90 | 20/25 | 1.75 (0.64-4.83) |
| *p* for trend | | | 0.675 | | | 0.201 |
| n-3 HUFAs * | <21.80 | 50/18 | 1:00 | <21.32 | 18/28 | 1:00 |
| | 21.80-26.50 | 35/16 | 0.89 (0.32-2.43) | 21.32-25.20 | 13/29 | 0.58 (0.21-1.65) |
| | 26.51-29.50 | 21/17 | 0.54 (0.19-1.56) | 25.21-32.77 | 15/27 | 0.61 (0.21-1.78) |
| | >29.50 | 40/16 | 0.86 (0.28-2.21) | >32.77 | 9/28 | 0.54 (0.19-1.54) |
| *p* for trend | | | 0.94 | | | 0.429 |
| 20:5n-3 EPA* | <5.70 | 42/19 | 1:00 | <5.50 | 18/29 | 1:00 |
| | 5.70-7.80 | 36/15 | 1.14 (0.42-3.13) | 5.50-8.20 | 17/30 | 0.65 (0.23-1.86) |
| | 7.81-9.50 | 24/17 | 0.95 (0.31-2.87) | 8.21-10.48 | 7/25 | 0.29 (0.08-0.99) |
| | >9.50 | 44/16 | 1.22 (0.48-3.54) | >10.48 | 13/28 | 0.62 (0.22-1.80) |
| *p* for trend | | | 0.167 | | | 0.373 |
| 22:5n-3 DHA* | <1.10 | 7/18 | 1:00 | <1.00 | 3/32 | 1:00 |
| | 1.10-1.40 | 12/18 | 2.56 (0.63-10.41) | 1.00-1.30 | 4/29 | 1.79 (0.27-11.90) |
| | 1.41-1.70 | 25/16 | 7.96 (1.89-33.45) | 1.31-1.80 | 11/25 | 9.52 (1.62-55.56) |
| | >1.70 | 102/15 | 23.12 (6.73-73.21) | >1.80 | 37/26 | 16.32 (3.35-69.85) |
| *p* for trend | | | 0.001 | | | 0.001 |
| 22:6n-3DHA* | <14.20 | 67/17 | 1:00 | <13.83 | 21/28 | 1:00 |
| | 14.20-16.10 | 25/17 | 0.39 (0.14-1.09) | 13.83-16.35 | 15/28 | 0.83 (0.30- 2.24) |
| | 16.11-19.20 | 24/17 | 0.33 (0.12-0.95) | 16.36-20.15 | 12/28 | 0.52 (0.18-1.50) |
| | >19.20 | 30/16 | 0.40 (0.15-1.09) | >20.15 | 7/28 | 0.43 (0.14-1.38) |
| *p* for trend | | | 0.055 | | | 0.036 |
| n-6 PUFAs/ n-3 PUFAs | <3.30 | 42/21 | 1:00 | <3.00 | 10/30 | 1:00 |
| | 3.30-4.00 | 36/15 | 1.01 (0.37-2.72) | 3.00-3.70 | 10/27 | 0.63 (0.19-2.11) |
| | 4.01-4.80 | 31/17 | 1.58 (0.57-4.42) | 3.71-4.75 | 17/27 | 1.30 (0.43-3.91) |
| | >4.80 | 37/14 | 1.06 (0.37-3.06) | >4.75 | 18/28 | 1.63 (0.54-4.99) |
| *p* for trend | | | 0.656 | | | 0.249 |

[1]Values are expressed as absolute serum fatty acids (mg/dL) as quartile categories. [2]OR, Odds ratio; CI, confidence interval. [3] Adjusted for age, BMI, history of colorectal adenoma/cancer in first-degree relatives, history of diabetes, smoking, drinking, physical activity and season of data collection. #MUFAs (Mono-unsaturated FAs), PUFAs(Poly-unsaturated FAs), HUFAs(Highly-unsaturated FAs), (EPA)(Eicosapentaenoic acid), (DPA)(Docosapentaenoic acid), DHA (Docosahexaenoic acid)

mentioned studies (Kojima et al., 2005; Kuriki et al., 2006), which supported the preventive role of DHA in CRC carcinogenesis. The tumor growth suppressing mechanisms of n-3 HUFAs are thought to be due to generation of eicosanoid mediators with biological activity (Jones et al., 2003), modulation of signal transduction and gene expression with subsequent induction of apoptosis (Kubota et al., 1998; Cheng et al., 2003; Gutt et al., 2007), modulation of insulin sensitivity (Larsson et al., 2004; Kuriki et al., 2007), proteasomal regulation of beta-catenin levels and alteration expression of T-cell factor beta-catenin target genes (Calviello et al., 2007), production of free radicals and reactive oxygen species (Bartsch et al., 1999; Stoll, 2002) and changes in estrogen metabolism (Larsson et al., 2004).

Also, the serum AA value was associated with reduced CRA risk in women, although not men, in line with the reverse associations reported in two case-control studies in Japan in which the erythrocyte and serum compositions of FAs were measured (Kojima et al., 2005; Kuriki et al., 2006). Another study in the US also indicated blood levels of AA to be lower in cancer cases than controls (Hall et al., 2007). Assays of AA in previous reports from the United Kingdom, Spain, and Russia similarly pointed to preventive roles of AA in those cases in breast cancer (Neoptolemos et al., 1988; Zaridze et al., 1990; Baro et al., 1998). The observations contrasted, however, with the findings from some animal and other epidemiologic studies, showing elevated levels of AA to be associated with increased risk of carcinogenesis (Neoptolemos et al., 1991; Pala et al., 2001; Nkondjock et al., 2003) by altering membrane phospholipid turnover, releasing membrane AA from phospholipid, and affecting prostaglandin synthesis via COX enzymes (Rao and Reddy, 1993). Although excessive intake of AA is considered to be involved in cell inflammation, proliferation and may impact carcinogenesis (Neoptolemos et al., 1991; Nkondjock et al., 2003), intake by Japanese of AA from meat, animal fat, chicken eggs, as main sources of AA, seems to be lower than in Western populations, and thus would not induce harmful effects (Moore et al., 2005).

ALA, a plant-derived n-3 PUFA and a precursor of EPA and DHA, did not demonstrate any link with CRA in the present study. This finding is compatible with previous epidemiologic studies in Japan and other countries (Slattery et al., 1997; Pietinen et al., 1999; Terry et al., 2001; Kuriki et al., 2006). Also, another Japanese study (Kojima et al., 2005), which measured serum FA levels, reported significant inverse and non-significant positive associations for ALA in relation to CRC risk in men and women, respectively. Mechanistic studies suggest that ALA might protect against carcinogenesis by decreasing prostaglandin production, suppressing COX-2 induction and proliferation in the colorectal mucosa (Brouwer et al., 2004).

Our observed significant positive associations between CRA risk and concentrations of SFAs, including myristic acid, palmitic acid and stearic acid, are consistent with previous investigations (Slattery et al., 1997; Kuriki et al., 2006), in line with SFA roles in cell signaling, insulin resistance pathway and regulation of membrane lipid fluidity and "gate-keeping" ability in colonic cells (Bruce et al., 2000; Zhou et al., 2000). Similarly, palmitoleic acid and total MUFAs were dose-dependently associated with increased risks in both sexes. Although a few studies have examined the relationship between MUFAs and CRA, they have not been conclusive. This is because MUFAs, especially oleic acid, have different mechanisms of action on carcinogenesis including an effect on hormonal status, modification of cell membrane structure and function, cell signaling transduction pathway and gene expression, and even modulate the function of the immune system (Escrich et al., 2007). However, our findings may support the hypothesis that MUFAs promote tumor growth (Schloss et al., 1997; Kojima et al., 2005) by increasing essential FA incorporation into cell membranes and also by interacting with the AA-cascade (Schmeits et al., 1999).

The inverse associations of EPA, DHA and AA with CRA risk were clearly more prominent in women than in men, suggesting a gender-CRA interaction, as supported by previous epidemiologic and experimental studies (Panis et al., 1990; Nkondjock et al., 2003). Genetic and hormonal factors, lifestyle and dietary habits, and disease are all thought to influence the FA metabolism. In addition, it has been suggested that female sex hormones are related to the sex-specific differences because they affect bowel transit time (Triadafilopoulos et al., 1998), bacterial fermentation in the colon, and particularly bile acid production (Potter, 1995; Grodstein et al., 1999).

Potential limitations that might affect interpretation of our results should be noted. First, although we used FA concentrations as biomarkers, several alternative methods are available for biologic assessment of fat intake. Levels in adipose tissues and erythrocyte membranes reflect long- and medium-term FA intake, respectively, whereas serum values reflect rather short-term (a week to several weeks) intake (Arab and Akbar, 2002). Second, we were unable to entirely exclude the possibility that there are subjects with false-negative faecal occult blood test. Third, the sample size was relatively small; therefore, we could not assess the CRA risk separately in terms of size, site, and grade, which are naturally important in evaluating the risk in relation to the adenoma-carcinoma sequence (Kimura et al., 2007). Fourth, the control group in this study was selected from health-conscious participants voluntarily undergoing screening examinations for CRC. Since they clearly paid especial attention to their health, they might not be completely representative of a Japanese general population.

In conclusion, we would like to stress that total FAs, especially SFAs and MUFAs, as important risk factors for CRA, should be restricted and replaced with n-3 HUFAs for the prevention of CRA/CRC. However, the protective effects of AA seem somewhat incompatible with the literature, and future studies should focus on generating a better understanding of the role played by AA cascade in the adenoma-carcinoma sequence in the colorectum.

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