

Effects of Meat Intake Frequency and Polymorphic Cytochrome P450 2A6 Activity on Individual Colorectal Tumour Risk in a Japanese Cohort

Hiroshi Yamazaki^{1*}, Masaki Fujieda², Makiko Shimizu¹, Akiko Shiotani³, Mie Shimabukuro⁴, Kanae Mure⁴, Tatsuya Takeshita⁴, Hideki Ishikawa^{5*}

¹Laboratory of Drug Metabolism and Pharmacokinetics, Showa Pharmaceutical University, Machida, Tokyo, Japan

²Faculty of Pharmaceutical Sciences, Toho University, Funabashi, Japan

³Otemae College of Nutrition and Confectionery, Chuo-ku, Osaka, Japan

⁴Department of Public Health, Wakayama Medical University, Wakayama, Japan

⁵Department of Molecular-Targeting Cancer Prevention, Kyoto Prefectural University, Sakyo-ku, Kyoto, Japan

Email: *hyamazak@ac.shoyaku.ac.jp.orcid.org, *cancer@gol.com

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Abstract

The relationships among meat consumption, smoking habits, and phenotypic cytochrome P450 2A6 variation with respect to colorectal cancer risk remain unclear. In this study, the relationships among colorectal tumour risk, meat consumption by questionnaire, and phenotypic P450 2A6 activity by genotyping in a case-control study (299 cases and 170 controls) were analyzed for never-smokers and ever-smokers. In never-smokers consuming ≥ 1 serving per day of total meat, a significant odds ratio of 4.42 (95% confidence interval, 1.29 - 15.2), adjusted by logistic regression for age and gender, was observed, compared with the group consuming ≤ 2 servings per week. Furthermore, in Japanese never-smokers, the susceptibility to colorectal tumours was dependent on the frequency of meat intake (trend test $p = 0.011$). In never-smokers who were P450 2A6 poor metabolizers and had a high frequency of meat intake, the apparent odds ratio was 3.57 (95% confidence interval, 0.30 - 42.2) compared with the P450 2A6 normal group with a low meat intake frequency. These results suggested that colorectal tumour risk was inversely associated with the phenotypic P450 2A6 activities in Japanese never-smokers with a high meat intake.

Keywords

Meat Consumption, Colon Cancer Risk, Susceptibility, Non-Smoking Subjects, CYP2A6

1. Introduction

The consumptions of red meat, processed meat, and high-fat products and smoking have been positively associated with colorectal tumour risk in previous epidemical studies [1] [2] [3] [4] [5]. Epidemiological and human biomonitoring studies indicate that heterocyclic amines, polycyclic hydrocarbons, and *N*-nitrosamines in meat-derived products may play important roles in colorectal carcinogenesis [6] [7].

Cigarette smoking has been consistently identified as a potential risk factor for colorectal tumours [2] [8]. Because polymorphic cytochrome P450 (P450) 2A6 enzymes mediate nicotine oxidation and the metabolic activation of several procarcinogens, including environmental and tobacco-related *N*-nitrosamines [9] [10], the involvement of P450 2A6 in cancer development has been postulated. However, there have been contradictory findings about the role of P450 2A6 in colorectal carcinogenesis. In a case-control study, subjects with medium or high P450 2A6 activities (as determined by the urinary caffeine test) were found to have an increased risk of colorectal cancer compared with subjects with low P450 2A6 activity [6]. In contrast, higher P450 2A6 activities were found not to be associated with the colorectal cancer risk in a different study [11]. We previously found that a high risk of colorectal tumours in Japanese subjects was associated with higher P450 2A6 activity and higher cumulative tobacco exposure [8]. Moreover, the susceptibility to colorectal tumours was dependent on the predicted P450 2A6 phenotype among Japanese ever-smokers, but not among never-smokers [8]. However, compared with the abundant data from Western countries [1] [2] [3] [4], evidence regarding meat consumption and colorectal cancer is limited in the Japanese population. Furthermore, the Japanese population is known to have a wide range of P450 phenotypes with different enzyme activities [12]. The relationships among meat consumption, smoking habits, and phenotypic P450 2A6 variation with respect to colorectal cancer risk remain unclear.

In the current study, we found that the susceptibility to colorectal tumours was dependent on the frequency of meat intake in Japanese never-smokers. Moreover, on reanalysis of our previous case-control study, the risk for colorectal tumours was found to be inversely dependent on the enzyme activity of the predicted P450 2A6 phenotype among Japanese never-smokers with a high meat intake.

2. Materials and Methods

2.1. Subjects

Study participants recruited from June 1993 to September 1997 at the Osaka Medical Center for Cancer and Cardiovascular Diseases [8] were reanalyzed in this study. The colorectal tumour cases included adenomas and adenocarcinomas ($n = 299$, 81% male) but excluded individuals with a history of intestinal or gastric resection, familial adenomatous polyposis, or advanced or invasive tu-

mours. Control subjects were healthy volunteers ($n = 170$, 79% male) who required hospitalization for a health check-up. The ages of the cases (56 years) and the controls (58 years) were defined as those at the time of the first hospital visit. Signed consent forms and completed questionnaires were collected from all subjects. The questionnaire covered smoking status (never-smoker, ex-smoker, or current smoker), the total duration of smoking, the average number of cigarettes smoked daily, and meat intake (≤ 2 serving per week, 1 serving per 2 days, or ≥ 1 serving per day) as shown in **Table 1**. The number of pack-years was calculated as a measure of cumulative cigarette smoking: one pack-year was defined as smoking 20 cigarettes daily for 1 year. Meat was defined as beef, pork, poultry, ham, and sausages. This study was approved by the ethics committees of Kyoto Medical University and Showa Pharmaceutical University.

2.2. Data Collection and Analysis

The genotyping of *P450 2A6*4* (whole-gene deletion), *2A6*7* (amino acid substitution), and *2A6*9* (upstream mutation) were performed using a previously described method [8] [12] [13]. Subjects were assigned to four groups based on their *P450 2A6* genotypes according to the definitions of Fujieda *et al.* [12]: the putative normal phenotypic group, *2A6*1/*1*; the intermediate group, *i.e.*, those heterozygous for the *P450 2A6*1* allele (*2A6*1/*4*, **7*, **9*); the slow group, *i.e.*, those heterozygous or homozygous for variant alleles except for those homozygous for the *P450 2A6*4* allele (*2A6*4*, **7*, **9/*7*, **9*); and the poor group, *P450 2A6*4/*4*. Associations between susceptibility to colorectal tumours and smoking history, meat intake, and phenotypic *P450 2A6* activity were assessed using odds ratios and 95% confidence intervals calculated from logistic regression models with adjustment for age and gender and/or smoking pack-years (in smokers). *p* values for trends were also calculated by assigning ordinal scores as continuous variables in the logistic regression models, with the ordinal scores 1 - 3 assigned to the three levels of meat intake or ordinal scores of 1 - 4 assigned to the normal *P450 2A6* phenotype and intermediate, slow, and poor metabolizer groups, respectively [8]. All tests of statistical significance were two-sided. A *p*

Table 1. The numbers of participants in this study.

Frequency of meat intake	Number of subjects			
	Never-smokers		Ever-smokers	
	Case	Control	Case	Control
Low frequency				
≤ 2 serving per week	34	49	108	48
Medium/high frequency				
1 serving per 2 days	37	30	80	25
≥ 1 serving per day	12	4	28	14

The colorectal tumour cases included adenomas and adenocarcinomas ($n = 299$, 81% male). Control subjects were healthy volunteers ($n = 170$, 79% male). The mean ages of the cases and controls were 56 and 58 years, respectively.

value of 0.05 was considered the threshold of significance. All statistical analyses were carried out by using the statistical software SAS, version 5.0 (SAS Institute, Inc., Cary, NC, USA).

3. Results

The frequency of meat intake was analyzed in a case-control study of colorectal tumours in a Japanese cohort divided into ever-smokers (*i.e.*, ex-smokers and current smokers) and never-smokers. For those with a total meat intake of ≥ 1 serving per day compared with ≤ 2 servings per week, the odds ratio for the presence of colorectal tumours adjusted by logistic regression for age, gender, and smoking pack-years was 1.48 (95% confidence interval, 0.78 - 2.81); however, this result was not statistically significant (data not shown). Because the highest risk of colorectal tumours was previously found in subjects with both high P450 2A6 activity and high cumulative tobacco exposure [8], separate analyses were performed for never-smokers and ever-smokers (Figure 1). In never-smokers with a high total meat intake of ≥ 1 serving per day (12 cases and 4 controls, Figure 1(a)) compared with ≤ 2 servings per week (34 cases and 49 controls), we observed a significant odds ratio of 4.42 (95% confidence interval, 1.29 - 15.2, Figure 1(c)) adjusted by logistic regression for age and gender. Furthermore, with ordinal scores of 1 - 3 assigned to the low (≤ 2 servings per week), medium (1 serving per 2 days), and high (≥ 1 serving per day) frequencies of meat intake, in Japanese never-smokers, the susceptibility to colorectal tumours was found to be dependent on the frequency of meat intake (trend test $p = 0.011$, Figure 1(c)). Moreover, in ever-smokers with low, medium, and high meat intake (Figure 1(b)), respectively, significant and similar odds ratios of 4.09 (95% confidence interval, 2.19 - 7.66), 5.48 (2.75 - 10.9), and 3.46 (1.50 - 7.98), adjusted by logistic regression for age, gender, and smoking pack-years, were observed

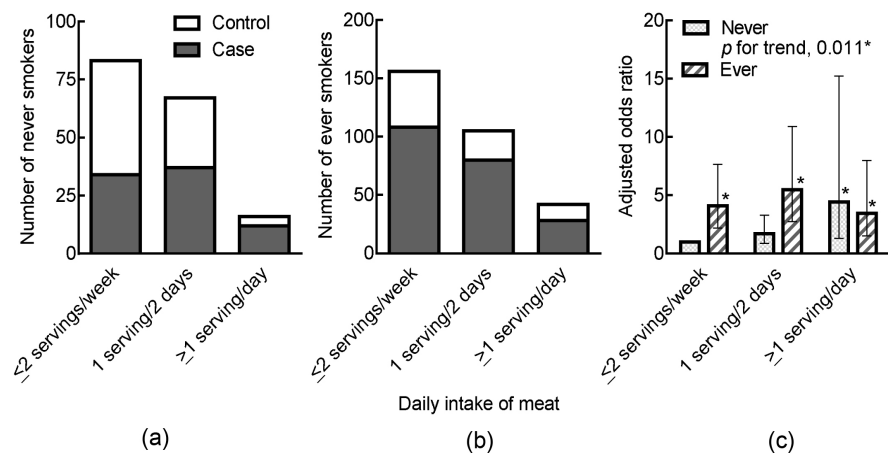


Figure 1. Frequency of meat intake and risk of colorectal tumours in never-smokers (a) and ever-smokers (b) in a case-control study. Odds ratios with 95% confidential interval values (c) adjusted for age and gender are shown compared with never-smokers with a low meat intake as the reference group. p values for trends (c) were calculated by assigning ordinal scores as continuous variables in the logistic regression models with the ordinal scores 1 - 3 assigned to the three frequencies of meat intake.

compared with never-smokers with low meat intake (**Figure 1(c)**).

Associations between susceptibility to colorectal tumours, frequency of meat intake, and putative P450 2A6 activity based on the P450 2A6 genotype were assessed in never-smokers (**Figure 2**). In the low meat intake group, no significant odds ratios or trends were evident for the different P450 2A6 genotypes. However, in the combined medium and high meat intake groups (**Figure 2(b)**), poor metabolizers (3 cases and 1 control) had an apparent odds ratio of 3.57 (95% confidence interval, 0.30 - 42.2, **Figure 2(c)**) adjusted by logistic regression for age and gender compared with the low meat intake group with the normal P450 2A6 phenotype (8 cases and 10 controls, **Figure 2(c)**). In Japanese never-smokers, the risk for colorectal tumours was suggested to be inversely associated with the predicted activities of the P450 2A6 phenotypes in the combined medium and high meat intake groups (trend test $p = 0.045$, **Figure 2(c)**).

4. Discussion

There is accumulating evidence that both diet and smoking are major environmental risk factors for colorectal tumours [1] [2] [3] [5]. The activities of polymorphic enzymes (glutathione *S*-transferase A1, sulfotransferase 1A1, and *N*-acetyltransferase 2) associated with the metabolism of heterocyclic amines and polycyclic hydrocarbons, were found to be possibly associated with colon cancer risk [3]. Furthermore, in a population-based prospective cohort study, the intake of red and processed meat reportedly increased the risk of colorectal or colon cancer among Japanese men, but not among Japanese women [5]. In the current study, the effects of the frequency of meat intake and smoking habits (acquired by questionnaires) were obtained by reanalysis of our previous case-control study of colorectal tumours [8]. The roles of phenotypic P450 2A6 activity were assessed, especially in subjects who had never smoked. In never-smokers, the

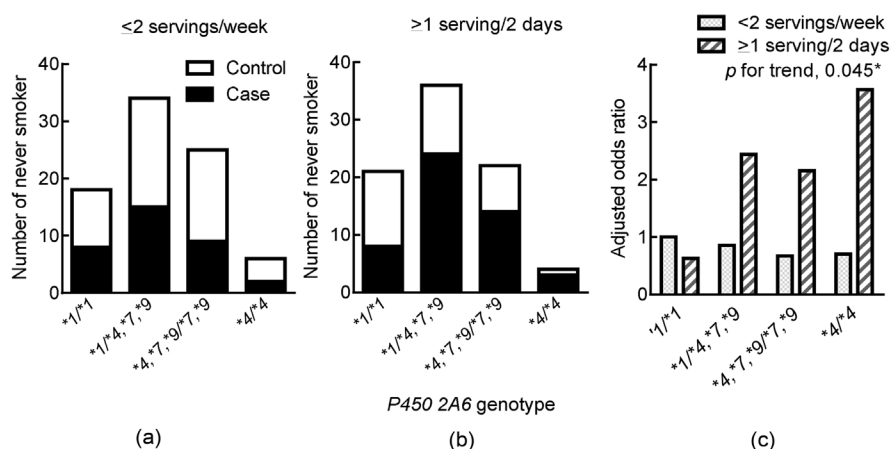


Figure 2. Effects of phenotypic P450 2A6 activity and low (a) and medium/high (b) frequency of meat intake on the risk of colorectal tumours in never-smokers in a case-control study. A low frequency of meat intake was defined as ≤ 2 servings per week and a high frequency of meat intake was defined as ≥ 1 serving per 2 days. Odds ratios (c) adjusted for age and gender are shown compared to never-smokers with a low intake of meat with normal P450 2A6 activity (*P450 2A6**1/*1 genotype) as the reference group.

high meat intake group had a significant adjusted odds ratio of 4.42 compared with the low meat intake group (**Figure 1**). The susceptibility to colorectal tumours was significantly dependent on the frequency of meat intake in Japanese never-smokers (**Figure 1**).

Subjects with the high P450 2A6 activity phenotype reportedly either have an increased risk [6] or no increased risk [5] of colorectal cancer. In Asian populations, such as the Japanese population, a relatively high frequency of P450 2A6 whole-gene deletion has been identified [12], which is different from that in Western populations. In the current study, never-smokers in the combined medium and high meat intake groups who were P450 2A6 poor metabolizers had an apparent adjusted odds ratio of 3.57 compared with low-meat-intake never-smokers with the normal P450 2A6 phenotype (**Figure 2**). In terms of the individual colorectal cancer risk of high-meat-intake never-smokers who were P450 2A6 poor metabolizers (**Figure 2**), our assessment suggested that dietary procarcinogens or undefined compounds may be predominantly deactivated by polymorphic P450 2A6 and, consequently, poor metabolizers would be at increased risk of colorectal cancer. To our knowledge, these causal factor(s) that are deactivated by P450 2A6 remain to be elucidated. We previously found the highest risk of colorectal tumours to be in Japanese subjects with high P450 2A6 activity and high cumulative tobacco exposure [8]. In contrast, here we suggest that the potential colorectal cancer risk in those with a medium or high meat intake is inversely associated with polymorphic P450 2A6 activity in a Japanese cohort, especially in those who have never smoked.

Our results are consistent with epidemiological studies of colorectal tumours that observed an increased risk with the frequency of meat intake. The inverse association of colorectal cancer risk with phenotypic P450 2A6 activity identified in the trend analysis ($p = 0.045$) of never smokers with high meat intake may somewhat attenuate the smoking risk of colorectal tumours in those with normal P450 2A6 activity in a Japanese cohort. Additional studies with detailed assessments of meat intake are needed to further evaluate the contributions of various components of meat intake, smoking habit, and other lifestyle factors to the risk of colorectal tumours in a cohort genotyped for P450 2A6. In conclusion, colorectal tumour risk was inversely associated with the phenotypic P450 2A6 activities in Japanese never-smokers with a high meat intake.

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Declaration of Interest

The authors report no declarations of interest.

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