Cytochrome P450 2E1 Polymorphism in Gastric Cancer in Brazil: Case-Control Studies of Japanese Brazilians and Non-Japanese Brazilians[1]

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Abstract

Cytochrome P450 2E1 (*Cyp2E1*) is involved in the metabolic oxidation of carcinogenic nitroso compounds, including *N*-nitrosoamines. There is an *Rsa*I polymorphism in the transcriptional regulatory region of this gene, and *in vitro* evidence suggests that the variant type of this polymorphic site has higher transcriptional activity but less chlorzoxazone-metabolizing activity. Interindividual differences in the metabolic capacity of *Cyp2E1* are assumed to be associated with cancer susceptibility, but the results of the previous studies on the relation between *Cyp2E1 Rsa*I polymorphism and cancer susceptibility have been inconsistent.

Two case-control studies of gastric cancer in Japanese Brazilians (96 cases, 192 controls) and Brazilians not of Japanese ancestry (non-Japanese Brazilians; 236 cases, 236 controls) in São Paulo were designed to clarify the role of the *Cyp2E1 Rsa*I genotype in susceptibility to gastric cancer after considering multifactorial environmental influences. The subjects with variant *Rsa*I genotypes amounted to 47% (28 of 59) and 48% (64 of 133), respectively, of the Japanese cases and controls, and 6% (11 of 187) and 10% (19 of 192), respectively, of the non-Japanese cases and controls. As expected, a difference in the distributions of the two groups was observed. The odds ratio of the *Rsa*I variant genotype of *Cyp2E1* was 0.46 (95% confidence interval, 0.21–1.04) in the non-Japanese Brazilian population and 0.98 (95% confidence interval, 0.50–1.90) in the Japanese Brazilian population after adjusting for sex, age, tobacco use, and meat consumption. Additional adjustment for potential confounding factors did not change the odds ratio substantially. No significant interactions were observed between the polymorphism and environmental factors. In regard to the histological type of gastric cancer, the variant genotype was significantly more prevalent than the common genotype in Japanese subjects with diffuse type gastric cancer. Our study suggests that the *Cyp2E1 Rsa*I polymorphism is associated with a reduced risk of gastric cancer, although how the assumed increase in *Cyp2E1* expression produced by this polymorphism is related to a reduced risk of cancer remains unclear. The observations in this study are consistent with the recent observations of esophageal cancer in endemic areas of China.

Introduction

Searches and assessments of genetic risk markers for common, nongenetic human cancers should take into consideration environmental factors, such as smoking, diet, and other life-style factors. Gastric cancer has usually been considered as highly environmental disease by many epidemiological studies (1–3), although a familial clustering of gastric cancer has been documented (4), and familial gastric cancer attributable to a germ line mutation in E-cadherin has recently been discovered (5). Previous studies of dietary carcinogenesis of gastric cancer have shown that xenobiotics may also play a role (6, 7), *i.e.*, enzymes that activate procarcinogens in foods are present in the alimentary tract, and, thus, interindividual differences in these enzyme activities may be responsible, in some part, for the presumed interindividual difference in gastric cancer susceptibility.

In the complicated situations that exists among the genetic and environmental factors responsible for gastric carcinogenesis, *Cyp2E1*[3] is strongly suspected of candidates for gastric cancer susceptibility markers interacting with the environmental chemicals (8) and possibly generating DNA-damaging agent(s). *Cyp2E1* is naturally ethanol-inducible and is involved in the metabolic oxidation of carcinogenic nitroso compounds, including *N*-nitrosoamines. *N*-nitrosoamines are present in many different environmental factors, *e.g.*, tobacco smoke, and also form endogenously in the stomach (9, 10). Activated nitrosoamines have been related to the development of many

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[3] The abbreviations used are: *Cyp2E1*, cytochrome P450 2E1; OR, odds ratio; CI, confidence interval.

human common cancers including stomach cancer (11). Molecular cloning and the discovery of polymorphism in the regulatory region of the *Cyp2E1* gene has tempted many investigators to test the hypothesis that the *Cyp2E1* genotype is related to its metabolic power, and in turn, to individual susceptibility to human cancer. The results of previous studies, however, have not been consistent and ethnic difference has been reported (12). Reviewing the ambiguity in these previous case-control studies, we reasoned that it was probably attributable to the lack of consideration of environmental factors, especially dietary factors, in the case-control data sets, in studies of digestive tract cancers. Gastric cancer mortality among Japanese and descendants of Japanese living in São Paulo is as high as in residents of Japan, and it is >50% higher than that of non-Japanese Brazilians (13, 14). In this study, we included multifactorial environmental factors, such as dietary factors assessed by a food frequency questionnaire, in a genotyping study in the two hospital-based case-control studies of gastric cancer, composed of Japanese Brazilians and non-Japanese Brazilians living in São Paulo to clarify the role of the *Cyp2E1 Rsa*I genotype in susceptibility to gastric cancer. Although *Cyp2E1* is known to be responsible for metabolizing chemicals in the food, no studies have ever incorporated dietary factors in the assessment of the polymorphism of this gene.

Subjects and Methods

Subject Population. This hospital-based case-control study of gastric cancer was carried out in the city of São Paulo, Brazil, from June 1991 to June 1994. All of the eligible patients living in São Paulo, Japanese Brazilians, second-generation Japanese Bazilians and non-Japanese Brazilians, were interviewed by using a questionnaire that included personal, social, and demographic characteristics, personal and family medical history, tobacco and alcohol use, and frequency of consumption for about thirty common food items. Informed consent was obtained and blood samples were collected from all of the case-control matched pairs. The protocol was approved by the Institutional Review Board of each participating institution, and informed consent was obtained from each case-control study patient.

Cases. Potentially eligible case-controls were defined as Japanese Brazilian and non-Japanese Brazilian patients with newly diagnosed malignant neoplasms of the stomach at 13 collaborating hospitals. A total of 101 cases in Japanese Brazilians were selected, but 5 of them were excluded—2 cases because of a lack of histological confirmation and 3 because of gastritis—so that ultimately 96 cases (60 men and 36 women), ages 37 to 89 years old, were enrolled as participants in this study.

Another set of 250 non-Japanese-Brazilian patients were identified, but fourteen cases were excluded because of gastritis (3), ulcers (4), lack of histological records (5), or lymphoma (2). Thus, 236 patients were recruited in the non-Japanese-Brazilian group, 170 males and 66 females, ranging in age from 40 to 80 years old. There was no refusal to being interviewed or to providing blood samples among cases.

Controls. The controls were cancer-free patients or healthy volunteers matched for gender, age (within 5 years), ethnicity, and trimester of hospital admission. Control patients were preferentially selected in the same hospitals as the index cases. The controls for only one cooperating hospital, a specialized cancer institution, were selected from public hospitals in the vicinity. The control subjects were interviewed within 1 month of case assignment.

Two controls were matched for each Japanese-Brazilian case in the Japanese-Brazilian set in accordance with the eligibility criteria above. One hundred one inpatients, 11 outpatients, and 80 healthy volunteers were adopted as paired controls. The main reasons for hospital admission of the 106 Japanese patients were infectious disease (8), metabolic disease (1), neurological disease (4), cardiovascular disease (35), respiratory disease (14), gastric ulcer (1), gastritis (2), other digestive tract and abdominal diseases (14), urogenital disease (11), dermatological disease (1), musculoskeletal disease (8), trauma (6), and other (1). Among Japanese-and-their-descendants controls there were no refusals to being interviewed or to giving blood samples. Two hundred seventy non-Japanese-Brazilian individuals were eligible as controls; among them, none refused to be interviewed, but 20 (7%) did not agree to provide blood samples.

Two hundred thirty-six non-Japanese Brazilian-controls, comprising 235 inpatients and 1 outpatient, were eligible as matched controls. The main reason for hospital admission included infectious disease (10), metabolic disease (3), neurological disease (7), cardiovascular disease (67), respiratory disease (15), gastritis (3), other digestive and abdominal diseases (75), urinary disease (4), dermatological disease (1), musculoskeletal disease (17), trauma (31), and others (2).

The same questionnaire was used for cases and controls, Japanese Brazilians and non-Japanese Brazilians.

Sample Collection and DNA Extraction. A 10-ml sample of peripheral venous blood was collected in heparinized tubes. Buffy coat was removed by the personnel of in each hospital by centrifugation and stored at −80°C until analyzed. Genomic high-molecular-weight DNA was extracted with a DNA extraction kit (Wako, Osaka, Japan).

Polymorphism Analysis. *Rsa*I genotyping: A set of primers for detection of polymorphism in the regulatory region of *Cyp2E1* was designed as reported previously (15). Briefly, the primer set 5′-TTCATTCTGTCTTCTAACTGG-3′ and 5′-CCAGTCGAGTCGAGTCTACATTGTCA-3′ yielded a 412-bp fragment, which generated fragments with 360, 50, and 410 bp by digestion with *Rsa*I (Toyobo, Kyoto, Japan). *Rsa*I-site-homozygously-absent individuals have a single 412-bp band (*A* allele homozygous, *A/A*), whereas *Rsa*I-site homozygotes have a smaller-sized band (360 bp) in case 50-bp fragment has run out (homozygous type *C/C* genotype). Heterozygous cases should have two bands, 410-bp and 360-bp, after a 50-bp fragment has run out (genotype *C/A*).

For DNAs of low amplification quality, we used the primers 5′ GGTGCAGTGTTAGGTGCAGC-3′ and 5′-TTCATTCTGTCTTCTAACTGG- 3′ for secondary PCR, as reported previously (16, 17). The consistency of the genotype revealed by this alternative primer set was monitored in the 10 randomly selected DNA samples and was confirmed to be consistent (data, not shown). Genotypings was completed in 59 (61%) Japanese cases and 133 (69%) Japanese controls and in 189 (80%) Brazilian cases and 191 (81%) Brazilian controls. Demographic characteristics of the genotyped subjects were almost the same as the whole subjects.[4]

It was more difficult to completely control the quality of the buffy-coat samples, especially when the blood was collected at the local clinics far from the central hospitals. The reason for the low DNA quantity was mainly loss of nuclear buffy coat during separation from the serum and inadequate heparin usage. We performed our assays blindly to exclude the

[4] Details are available on request.

Table 1 Distribution of selected demographic characteristics and life-style factors

Categories	Japanese[a]		Brazilians[b]	
	Case $n = 96$	Control $n = 192$	Case $n = 236$	Control $n = 236$
Sex (male %)	63	63	72	72
Race (black %)	0	0	8	8
Age (mean ± SD)	65 ± 12	65 ± 12	59 ± 8	58 ± 8
Current smoker (%)	15	17	42	32
Daily meat consumer (%)	51	28	48	48
Daily alcohol drinker (%)	11	13	21	16

[a] Japanese and descendants living in São Paulo, Brazil.
[b] Non-Japanese Brazilians living in São Paulo, Brazil.

Table 2 Distribution of study subjects according to *Cyp2E1 Rsa* I genotype

Cyp2E1 Rsa I genotype[a]	Cases *n* (%)	Controls *n* (%)	OR[b] (95% CI)	OR[c,d] (95% CI)	OR[d,e] (95% CI)
JAPANESE[f]	59	133			
C/C	31 (53)	69 (52)	1.0	1.0	1.0
C/A	27 (46)	58 (44)	0.98 (0.50–1.90)	0.77 (0.36–1.62)	0.70 (0.30–1.62)
A/A	1 (2)	6 (5)			
BRAZILIANS[g]	189	191			
C/C	178 (94)	172 (90)	1.0	1.0	1.0
C/A	11 (6)	17 (9)	0.46 (0.21–1.04)	0.56 (0.25–1.27)	0.42 (0.17–1.02)
A/A	0 (0)	2 (1)			

[a] The polymorphism *Cyp2E1 Rsa* I corresponding to genotypes: *C/C* (homozygote for the common allele), *C/A* (heterozygote), and *A/A* (homozygote for the variant allele).
[b] Adjusted for age, sex, pack-years, and meat consumption by unconditional logistic regression.
[c] Crude OR by conditional logistic regression.
[d] Fifty-two matched pairs for Japanese and descendants case-control studies and 150 pairs for non-Japanese Brazilians were considered for conditional logistic regression.
[e] Adjusted for meat consumption and pack-years by conditional logistic regression.
[f] Japanese and descendants living in São Paulo, Brazil.
[g] Non-Japanese Brazilians living in São Paulo, Brazil.

possibility of any influence of the low-quality blood samples in which PCR was unsuccessful. The basic characteristics of the subjects whose samples were available for the polymorphism analysis were comparable with those of the parent group.

Statistical Analysis. To estimate the association between gastric cancer risk and *Cyp2E1 Rsa*I polymorphism, crude and adjusted ORs with 95% CIs were determined by using unconditional and conditional logistic regression models (18) performed by statistical software SAS (SAS Institute, Cary, NC).

Smoking Habit. We defined "nonsmokers" as persons who had never smoked at any time in their life, and "smokers" as persons who had smoked in the past or who were current smokers. Tobacco consumption was measured in pack-years as the cumulative exposure equivalent to packs-smoked-per-day times the number-of-years of smoking. We considered 20 commercially manufactured cigarettes (one pack), 4 hand-rolled black-tobacco cigarettes, 4 cigars, and 5 pipefuls of regular pipe tobacco to be equivalent (19).

Dietary Record. The frequency of intake of approximately 40 food items, including meat, vegetables, fruit, tea, and other foods was grouped into four categories: less than once a week, 1–2 times a week, 3–4 times a week, and almost every day. This is a simple food frequency questionnaire, and validation was not done.

Histological Classification of Stomach Cancer. Pathology reports and slides of the cases were obtained and reviewed independently and blindly by two pathologists (Iriya, Y., and Sugimura, H.). Histological classification of the stomach cancers was performed according to the Japanese classification system (20) and categorized into two types based on Lauren classification (21).

Results

Table 1 summarizes some demographic characteristics and life-style factors of the study subjects as a whole. Gender, race, age, and percentage of daily drinkers were almost the same in the cases and controls among both the Japanese and the non-Japanese Brazilians. In the Brazilian population, the percentage of current smokers was higher than that of the control group, but the percentage in the Japanese group was comparable with that of the control group. The frequency of meat consumption was higher in the Japanese population compared with that of the Japanese controls. The distributions of these exposure and demographic data were not different in the subjects that were not genotyped. On the basis of these observations, smoking and meat consumption were included in the cancer risk estimation for *Cyp2E1 Rsa*I genotypes. The relationships between gastric cancer and life-style factors or the consumption of common food items in these subjects has been assessed elsewhere.[5]

Table 2 shows the frequency distribution of *Cyp2E1 Rsa*I genotypes and estimated ORs for gastric cancer. There were very few patients in the homozygous variant allele (*A/A*) group, and, therefore, the comparison was made by combined prevalence of heterozygotes (*C/A*) and homozygotes (*A/A*) for the variant genotype group (*C/A* + *A/A*). The ORs for stomach cancer of grouped genotype (*C/A*+*A/A*) *versus* genotype *C/C*, a reference, are shown.

The significant difference in distribution between the two ethnicities is clear from Table 2. An unconditional logistic

[5] Hamada *et al.*, manuscript in preparation.

Table 3 Distribution of *Cyp2E1 Rsa* I genotype according to histological type of tumor

Categories[a]	Japanese		Brazilians	
	Intestinal *N* (%)	Diffuse *N* (%)	Intestinal *N* (%)	Diffuse *N* (%)
C/C	17 (74)	8 (33)	73 (94)	68 (96)
C/A + A/A	6 (26)	16 (67)	5 (6)	3 (4)
Total	23	24	78	71
	$\chi^2 = 7.77$, $P < 0.01$		$\chi^2 = 0.35$, $P = 0.55$	

[a] *C/C*, homozygote for the common allele; *C/A*, heterozygote; *A/A*, homozygote for the variant allele.

regression analysis, using the *C/C* type as a reference, disclosed that after adjusting for sex, age, and tobacco and meat consumption, the OR in Japanese Brazilians was 0.98 (95% CI, 0.50–1.90), and the OR in non-Japanese Brazilians was 0.46 (95% CI, 0.21–1.04). Because the genotyping of some DNA samples was not completed, we performed a conditional logistic regression analysis for the remaining matched pairs: 52 matched pairs for cases of Japanese Brazilians and their descendants and 150 pairs for non-Japanese Brazilians. The crude ORs by conditional logistic regression analysis were 0.77 (95% CI, 0.36–1.62) in the Japanese Brazilians and 0.60 (95% CI, 0.25–1.27) in the non-Japanese Brazilians. Reduced ORs were also observed after adjustment for tobacco and meat consumption alone: 0.7 (95% CI, 0.30–1.62) in the Japanese Brazilians and 0.47 (95% CI, 0.17–1.02) in the non-Japanese Brazilians (Table 2).

We did not exclude subjects with tobacco-related diseases from the control group in the original calculation. The inclusion of three Japanese Brazilians and seven non-Japanese-Brazilian controls with digestive and respiratory disease did not influence the results significantly (data not shown). No significant interaction was observed between smoking and meat consumption, and the *Cyp2E1* genotypes for both in Japanese Brazilians and non-Japanese Brazilians using an unconditional logistic regression analysis with interaction variables (smoking $\times$ genotype, meat consumption $\times$ genotype). In non-Japanese-Brazilian population, the ORs for nonsmokers (0.39; 95% CI, 0.12–1.26) and low meat consumers (0.37; 95% CI, 0.11–1.24) were lower than those for smokers (0.70; 95% CI, 0.22–2.23) and high meat consumers (0.86; 95% CI, 0.30–2.45). In the Japanese-Brazilian population, these trends were not observed. However, none of these observations were statistically significant.

In the gastric cancer patients as a whole (Japanese-Brazilian and non-Japanese-Brazilians cases), the cancers were histologically classified as the diffuse type or the intestinal type according to the Lauren Classification. The frequency of the *Cyp2E1 Rsa*I genotypes according to the histological subtypes are shown in Table 3. Ninety-five cases were classified as the diffuse type and 101 cases as the intestinal type; and, thus, 20 and 11% of them, respectively, were genotypes (*C/A + A/A*). Among the Japanese Brazilians, the prevalence of *C/A + A/A* was greater than for *C/C* in the diffuse type, whereas *C/C* was more dominant than the *C/A+A/A* genotypes in the intestinal type ($\chi^2 = 7.77$; $P < 0.01$), but no such tendency was observed in the non-Japanese Brazilians.

Discussion

We observed a borderline protective effect of the variant allele (*A*) of *Cyp2E1* against gastric cancer in the subjects of our case-control study, which included information on various lifestyle factors. Differences in the distribution of polymorphisms between these two ethnic groups were consistent with previous reports (12, 16, 22, 23). *Cyp2E1* is thought to be involved in the metabolism of various nitrosoamines, and cigarette smoke, and baked or grilled meat could contain several chemicals, including nitroso compound. Cigarette smoking and meat consumption were, therefore, included as risk factors in this study.

In these case-control studies, we originally used various models to obtain ORs and found that high meat consumption is a statistically significant risk for gastric cancer in Japanese Brazilians. The risk increased significantly, showing a dose-response pattern. In regard to the Brazilian data set, meat intake was not associated with gastric cancer risk. We have observed significantly increased risk as a result of tobacco consumption in the non-Japanese Brazilian, but no association of smoking with gastric cancer was detected in the Japanese Brazilians (Hamada *et al.*, manuscript in preparation).

Along these lines, we believe that our observation here described that OR of *C/A+A/A versus C/C* genotype including these factors in a multivariate analysis was less than unity is quite important. However, the estimated ORs in this study were not statistically significant and we could only obtain suggestive results. The sample size may have been too small to detect relatively small effects of susceptibility genes such as *Cyp2E1*. We estimated the numbers of cases required to detect an OR ratio of 0.5 at $P < 0.05$ by the power calculation described previously (18). Statistical power analysis showed that 138 Japanese Brazilians and 492 non-Japanese Brazilians were required to obtain a relative risk of 0.5, based on a 1:1 ratio. For further study, a much larger number of case-control pairs is required to detect such relatively small effects.

Cyp2E1 is an enzyme that catalyzes the activation of various nitrosamine and other low-molecular-weight carcinogens (22–25). Once the variant type (*A*) of *Cyp2E1* was demonstrated to have higher transcription activity (26, 27), studies aiming to demonstrate that allele-*A*-containing genotypes were associated with higher lung-cancer susceptibility followed, because nitroso compounds were known to be present in tobacco smoke [tobacco-specific nitrosamine: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)]. The *Pst*I polymorphism, which is linked with *Rsa*I polymorphism, has been shown to affect *Cyp2E1* transcription levels (27), and individuals who lack a "c2" allele (*PstI*-site-absent and *Rsa*I-site-present; we designate this allele "A" in this paper and the common allele "C") of the *Cyp2E1 Pst*I polymorphism have been reported to be at higher risk of developing lung cancer (27). The *Dra*I polymorphism, another polymorphism in the intron of this gene, has also been reported to be associated with an elevated lung cancer risk in a Japanese case-control study (28) and for men and ever-smokers in a African-American case-control study (29). However, there are considerable numbers of conflicting results on the *Rsa*I polymorphism and lung cancer. Watanabe *et al.* demonstrated different regulation and expression of the *Cyp2E1* gene attributable to the *Rsa*I polymorphism

(26) but failed to find a positive association with Japanese lung cancer (30). Hamada *et al.* rejected the hypothesis of the contribution of *Rsa*I genotype in a Brazilian lung cancer case-control study (16), and Ikawa *et al.* could not confirm the association with lung cancer in Japanese patients (31), although the statistical power of these studies was insufficient. Hirvonen *et al.* also reported a lack of sufficient numbers of variant *Rsa*I allele in a Finnish population and no association with lung cancer there (32).

Because *Cyp2E1* is responsible for catalyzing procarcinogens in food (22) as well as in tobacco smoke, digestive tract cancers are also a reasonable subject for evaluation of this genetic risk attributable to *Cyp2E1* polymorphism. Morita *et al.* found no correlations between *Rsa*I polymorphism and Japanese esophageal cancer (33), and Kato *et al.* (34) failed to detect any association between *Rsa*I genotype and stomach cancer. Hung *et al.* reported an association of variant *Rsa*I allele and oral cancer risk in the population that did not chew betel quid (35). By contrast, Lin *et al.* (36) reported a decreased risk of the *Rsa*I variant genotype for esophageal cancer in an endemic area of China. The conclusion of Lin *et al.* is contrary to the hypothesis by Hayashi *et al.*, a higher transcription level of a less prevalent genotype (27) but is consistent with the newly identified *in vitro* difference, that is, lower metabolic capacity for chlorzoxazone hydroxylation of the less prevalent allele (37). Le Marchand *et al.* also reported the protective contribution of homozygous *Rsa*I variant to lung cancer in a Hawaiian population (38). In any event, the genotype-phenotype relationship of this gene has been a matter of controversy. These two mechanistic bases are not mutually exclusive and well-designed epidemiological studies would identify which one is true for human carcinogenesis. Our observation is paradoxical in terms of transcription-activity difference between two genotypes. Metabolic activity itself may be more attributible to individual predisposition in epidemiological setting.

As far as we know, few studies have demonstrated the protective effect of the variant allele of *Cyp2E1* against susceptibility to any human cancer. Only the study conducted in Linxian, China (36) supports this hypothesis (37). Because the enzyme functions in the body, in which it interacts with various extrinsic chemicals, it seems quite reasonable to evaluate the previous studies from the standpoint of exposure risk factors. Lin *et al.* (36) reported that a *Cyp2E1* variant allele is apparently a protective allele for esophageal cancer and severe esophageal hyperplasia, a possible precancerous lesion in that area. Because that region is a well-known endemic area for esophageal cancer, the shared environmental exposure (food) may mask the more subtle individual difference in the other aspects of life-style. Actually the suspected procarcinogens known to induce esophageal cancer in rodents are in the everyday diet of the people there (39, 40).

Although we failed to obtain a statistically significant OR, a subgroup analysis of non-Japanese Brazilians suggested that the protective role of the *Cyp2E1 A* allele is more effective when exposure to the presumed carcinogens in the meat is minimal. In other words, the effects of the variant type may be overridden by higher environmental chemical exposure. However, similar results regarding meat consumption were not obtained in the Japanese-Brazilian population.

There have been few studies on genetic susceptibility to gastric cancer. Kato *et al.* (41) reported no association between the *Rsa*I genotype and gastric cancer in an age-matched case-control study. However, as mentioned previously, their study recruited outpatients in the same hospital, and the dietary record has not been included in the calculations. Therefore, we cannot compare our results directly with theirs.

The prevalence of variant genotypes was different for histological subtypes of gastric cancer. These differences have also been observed in our Japanese gastric cancer cases.[6] These findings are intriguing inasmuch as some investigators have suggested that the two histological subtypes of gastric cancer have a different etiology on the observation that the prevalence of intestinal gastric cancer is decreasing in Scandinavia (42).

Our study also suggests that the *Cyp2E1 Rsa*I variant genotype is associated with a reduced risk of upper gastrointestinal tract cancers and is the first case-control study of genetic markers for gastric cancer to include life-style-related information, such as diet. Recently Zheng *et al.* (43) succeeded in detecting a *NAT1* genotype contribution to breast cancer risk in subjects who were frequent meat consumers. Thus, our results also verify the strategical merit of this type of study design, that is, genotype assessment that incorporates environmental factors, to evaluate the genetic risk for common cancer.

A recent report (44) supports the finding that the rare *Cyp2E1* allele is associated with a decreased enzyme activity adding biological plausibility to the protective effect observed in this study. However, the relationship between the polymorphism and digestive tract cancers is still controversial. Because *Cyp2E1* is an inducible gene, past exposure must also be examined, and retrospective case-control studies have limitations in estimating the past dietary record. Prospective studies are needed to explore the relationship between *Cyp2E1* polymorphism and gastric cancer.

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