Interaction between Cytochrome P-450 2E1 Polymorphisms and Environmental Factors with Risk of Esophageal and Stomach Cancers in Chinese[1]

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Abstract

Because cytochrome P-450 2E1 (*CYP2E1*) is involved in metabolic activation of environmental chemical carcinogens, gene polymorphisms that alter its functions may be associated with cancer susceptibility. However, previous studies have revealed disconcordant results with regard to cancer risk. To investigate gene-environment interactions with the *Rsa*I polymorphism of *CYP2E1* in terms of risk of esophageal and stomach cancers, we conducted a case-control study with 93 esophageal and 98 stomach cancer cases and 196 population-based controls in a high-endemic area for these cancers of China. We assayed genomic DNA samples for RFLPs in the *CYP2E1* by PCR amplification, followed by digestion with *Rsa*I, and collected information on environmental factors by a questionnaire approach. Odds ratios were estimated with an unconditional logistic model, after adjustment for potential confounding factors. The proportional distribution of the homozygous common *Rsa*I alleles did not differ between cancer cases of the esophagus (59.1%) and stomach (59.2%), and controls (61.7%). However, we found a significant positive interaction between the heterozygous and homozygous *Rsa*I rare alleles and ever-smoking in the odds ratio for stomach cancer ($P = 0.015$). The interaction between the gene polymorphism and dietary factors, such as garlic consumption, was not observed in both cancer cases. These results suggest that gene-environment interactions between the *CYP2E1* polymorphism and smoking may have the potential to alter the susceptibility for cancer development in the stomach.

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Introduction

CYP2E1, a member of the cytochrome P-450 superfamily, is involved in the metabolic activation of many low molecular weight compounds such as *N*-nitrosamines, aniline, vinyl chloride, and urethane (1, 2). *N*-Nitrosamines present in tobacco and diet are well-recognized as carcinogens involved in cancer development in various sites, including the esophagus and stomach (3, 4). Functional *CYP2E1* gene polymorphisms might therefore impact on susceptibility for cancers, for which a role is suspected for etiological agents such as *N*-nitrosamines.

RFLPs detected by *Rsa*I and *Pst*I in the 5′-flanking region show differences in transcriptional activation of the *CYP2E1* gene (5, 6), and a recent study demonstrated a positive association between the *Rsa*I polymorphism and the enzyme phenotype (7). The *Dra*I polymorphism is also associated with altered activity of CYP2E1, although *Dra*I is located in intron 6 and is not thought to affect transcription of the gene (8).

Activity of CYP2E1 is modulated by various determinants, such as obesity (9), fasting (9), and liver dysfunction (10), and induced by ethanol (11). In contrast, dietary isothiocyanates (12) and garlic (13, 14), as well as some drugs such as disulfiram (15) and chlormethiazole (16), inhibit its activity. A number of environmental factors may thus modify the cancer risk through altered CYP2E1 enzyme activity.

Previous studies have shown inconsistent findings on associations between the *CYP2E1* polymorphism and cancer risk. Six case-control studies demonstrated the common genotype or alleles to confer greater risk of oral (17), pharyngeal (17), esophageal (18, 19), liver (20), and lung (21, 22) cancers. On the other hand, increased risk of oral (23), nasopharyngeal (24), and liver (25) cancers was observed in rare genotype or allele carriers in three case-control studies. Furthermore, nine case-control studies failed to find a significant association between the *CYP2E1* polymorphisms and cancer risk of neoplasia of the oral cavity and pharynx (26), esophagus (27), stomach (28, 29), lung (30–33), and bladder (34). The reason for these inconsistent results is not clear, but one problem is a lack of sufficient investigation of gene-environment interactions, including dietary and smoking habits. We hypothesized that environmental factors alter the enzyme activity of CYP2E1 and therefore modify cancer susceptibility with the *CYP2E1* polymorphisms.

Huaian City, located in the northern part of Jiangsu Province, has relatively high incidence and mortality rates for both esophageal and stomach cancers, not only compared with other regions in the Province, but also in China as a whole. In the nationwide survey of 1990–1992, the age-adjusted mortality rates (per 100,000) for esophageal and stomach cancers in

Huaian City and Jiangsu Province were 122.4 *versus* 29.8, and 65.3 *versus* 32.7, respectively (35, 36). To investigate how environmental and genetic factors might be involved in cancer development, we have been conducting comparative epidemiological studies in high- and low-endemic areas for these neoplasms in Jiangsu Province. We have found that frequent consumption of pickled vegetables and residual gruel increased the risk of these two cancers, whereas garlic and raw vegetable consumption exerted the opposite effect in a high-endemic area near Huaian (37). Furthermore, risk reduction for esophageal cancer with frequent consumption of garlic was also observed in a low-endemic area (38), where the general population was found to consume garlic and raw vegetables much more frequently (36).

To investigate possible relations between the *CYP2E1 Rsa*I polymorphisms and environmental factors for the risk of esophageal and stomach cancers, we conducted a case-control study in Huaian, China.

Materials and Methods

Study Subjects. We recruited cases from patients 40–81 years of age who visited Huaian City Municipal Hospital from April 1999 to March 2000 and who were histopathologically diagnosed as having primary esophageal or stomach cancer at the time of surgery. We also recruited incident cases in the same period, using the data from the Regional Cancer Registry that had been conducted by the Public Health Center of Huaian City since 1987, because some of the patients did not visit this hospital. Physicians at the hospital asked eligible cases to participate in our study, and doctors or nurses interviewed cases and collected their blood samples from a peripheral vein after obtaining oral informed consent. Doctors of the public health center also visited the houses of eligible cases who were detected by the Regional Cancer Registry and performed interviews and blood collection at their houses in the same way. The patients with severe clinical symptoms were excluded as candidates for cases.

Population-based controls were recruited from healthy residents in the villages or towns where cases resided. Doctors of the public health center visited the local governmental office and randomly selected one control for each case, using the lists of residents after matching for sex, ethnicity, and age within 2 years of each case. Doctors of the public health center also asked eligible residents for their participation and performed interviews and blood collection in the same way. The collected blood samples were shipped to the public health center within a day, separated to buffy coat and plasma, and stored at −20°C.

Although the number of nonparticipants was not recorded, no patients and a few residents refused to participate; the response rates were 100% for cases and >90% for controls. Most of the cases (95%) were recruited from hospitals, because the quick shipment of collected blood samples of the patients in remote areas was difficult. Two patients with esophageal cancer were also excluded from cases after the matched controls were recruited, because collected buffy coat was inadequate for extracting DNA. The eligible subjects comprised 94 esophageal and 104 stomach cancer cases and 200 controls. We used all combined controls as common ones for esophageal and stomach cancer, respectively. The ethics committee of Hamamatsu University School of Medicine approved this study. The local government of Jiangsu Province and Huaian City gave permission for conducting the present study, although the Cancer Research Institute of Jiangsu Province had no ethics committee.

Environmental Factors. The items of our questionnaire covered smoking and drinking habits, tea consumption, and intake of 12 foods. Smokers were divided into never- and ever-smokers (current and former). Former smokers were defined as the persons who quit smoking 1 year or more before having diagnosis in cases and 1 year or more before the questionnaire survey in controls. Alcohol drinkers also were divided into two groups (≤5 times/week and >5 times/week) according to drinking frequency. Consumption of tea and selected foods was divided into two groups to give almost equal distributions of intake amount and frequency in controls. We asked cases to provide information about their lifestyles before the onset of disease.

DNA Extraction and Genotyping of CYP2E1. Whole blood was collected into EDTA-coated tubes and centrifuged for 15 min, and the buffy coat layer was isolated. Stored buffy coat was shipped to Cancer Research Institute of Jiangsu Province, and genomic DNA was extracted from 200 μl of buffy coat using a Qiagen QIAamp DNA Blood Mini kit (Qiagen Inc., Valencia, CA). The method for genotyping of CYP2E1 has been described previously (29). In brief, PCR was used to amplify the transcription regulation region of CYP2E1 that includes the *Rsa*I enzyme recognition site (5). The primers were 5′-CCAGTCGAGTCTACATTGTCA-3′ and 5′-TTCATTCT-GTCTTCTAACTGG-3′. The PCR product was subjected to *Rsa*I restriction enzyme digestion, and samples were then analyzed by electrophoresis in 5% polyacrylamide gels. There were three genotypes of CYP2E1 resulting from digestion with the restriction enzyme *Rsa*I: type A, the common homozygote *c1/c1*; type B, the heterozygote *c1/c2*; and type C, the rare homozygote *c2/c2*. Among 398 examined samples, visualized PCR products could not be obtained for 7 cases and 4 controls.

Statistical Analysis. Associations between the *Rsa*I polymorphism and esophageal and stomach cancer risk were estimated by OR,[3] using the unconditional logistic regression model (39). We calculated not only crude ORs but also adjusted ORs for age (continuous), sex, smoking and drinking habits, and consumption of meat, raw vegetables, pickled vegetables, and garlic to control for the effects of potential confounding factors, referred to from a previous study (37). To investigate gene-environment interactions, we calculated the ORs according to combinations of the *CYP2E1* genotypes and specific factors, with reference to the common *Rsa*I allele carriers who were the least exposed to each. The heterozygous and homozygous rare allele carriers were combined into a same group, because the number of the homozygous rare allele carriers was small. The OR and *P* values for interactions were calculated with the logistic regression model, using dummy variable multiplication of the gene factor (0, 1) by the environmental factor (0, 1). The procedure LOGISTIC from the statistical package SAS was used for the calculations (SAS Institute, Cary, NC). The probability of Hardy-Weinberg equilibrium was assessed by χ^2 test with the statistical package STATA (Stata Corporation, College Station, TX).

Results

Numbers of subjects were 55 male and 38 female cases with esophageal cancer, 75 male and 23 female cases with stomach cancer, and 131 male and 65 female controls (Table 1). The mean age of esophageal and stomach cancer cases and controls by sex did not differ (59.3 and 59.5 years *versus* 59.5 years in

[3] The abbreviations used are: OR, odds ratio; CI, confidence interval.

Table 1 Age distribution and smoking and drinking habits in esophageal and stomach cancer cases and controls by sex

	Males			Females		
	Controls (n = 131%)	Cases		Controls (n = 65%)	Cases	
		Esophagus (n = 55%)	Stomach (n = 75%)		Esophagus (n = 38%)	Stomach (n = 23%)
Age in years						
40–49	7.6	7.3	6.7	12.3	7.9	21.7
50–59	32.8	32.7	28.0	43.1	50.0	21.7
60–69	44.3	41.8	53.3	35.4	36.8	34.8
70–81	15.3	18.2	12.0	9.2	5.3	21.7
Total	100	100	100	100	100	100
Smoking status[a]						
Current	55.7	40.0	41.3	24.6	18.4	21.7
Former	9.9	41.8	42.7	12.3	31.6	21.7
Never	31.3	16.4	16.0	61.5	47.4	56.5
Drinking frequency						
≥5 times/week	16.0	32.7	18.7	1.5	0.0	0.0
2–4 times/week	19.1	18.2	25.3	3.1	0.0	13.0
1–4 times/month	29.8	12.7	20.0	4.6	0.0	0.0
<1 time/month	35.1	36.4	36.0	90.8	100	87.0

[a] Information on smoking status was not obtained in 4 male and 1 female controls and 1 male and 1 female cases with esophageal cancer.

Table 2 ORs for esophageal and stomach cancers according to the *Rsa*I polymorphism of CYP2E1

*Rsa*I polymorphism of *CYP2E1*[a]	No. (%) of		Crude OR (95% CI)	Adjusted[b] OR (95% CI)
	Cases	Controls		
Esophageal cancer				
A	55 (59.1)	121 (61.7)	1.00	1.00
B	31 (33.3)	62 (31.6)	1.10 (0.64–1.88)	1.13 (0.60–2.13)
C	7 (7.5)	13 (6.6)	1.19 (0.45–3.13)	1.23 (0.40–3.77)
A	55 (59.1)	121 (61.7)	1.00	1.00
B + C	38 (40.9)	75 (38.3)	1.12 (0.67–1.85)	1.15 (0.64–2.07)
Stomach cancer				
A	58 (59.2)	121 (61.7)	1.00	1.00
B	31 (31.6)	62 (31.6)	1.04 (0.61–1.78)	1.07 (0.59–1.96)
C	9 (9.2)	13 (6.6)	1.44 (0.58–3.57)	1.50 (0.54–4.18)
A	58 (59.2)	121 (61.7)	1.00	1.00
B + C	40 (40.8)	75 (38.3)	1.11 (0.68–1.83)	1.15 (0.66–2.01)

[a] A, *Rsa*I site present homozygote; B, heterozygote; C, *Rsa*I site absent homozygote.
[b] Adjusted for age (continuous), sex, smoking and drinking habits, and consumption of meat, pickled vegetables, raw vegetables, and garlic.

males; and 55.9 and 55.9 years and 56.8 years in females, respectively), although the age distribution slightly varied between cases and controls. The proportional distribution of former smokers was relatively higher in male cases with esophageal and stomach cancers and female cases with esophageal cancer than each control. Habitual drinking was observed less in female cases and controls than in males.

The distribution of the common homozygous *Rsa*I alleles (type A) did not differ between cases of cancer of the esophagus (59.1%) and stomach (59.2%), and controls (61.7%; Table 2). Increase or decrease in ORs for esophageal and stomach cancers with the heterozygous alleles (type B) or the homozygous rare alleles (type C) were not observed, with or without adjustment for age, sex, and potential confounding factors. The combined heterozygous and homozygous rare alleles were not associated with increased or decreased ORs. The allelic distribution of the *Rsa*I polymorphism for controls was in Hardy-Weinberg equilibrium (exact significance, $P = 0.703$).

Smoking and drinking habits were not associated with increased ORs for esophageal cancer in the homozygous common allele carriers but tended to be associated with elevated ORs in the heterozygous and homozygous rare allele carriers, when compared with referents of the homozygous common allele carriers who were never-smokers or drank less frequently, respectively (Table 3). The interaction between the heterozygous and homozygous rare alleles and ever-smoking was on the borderline of statistical significance ($P = 0.060$). The ORs for esophageal cancer tended to be lowered in cases who frequently consumed garlic in both genotype groups, but a significant gene-environment interaction was not observed. Frequent consumption of raw vegetables decreased the OR in the homozygous common allele carriers (OR, 0.42; 95% CI, 0.19–0.93).

Increased ORs for stomach cancer with smoking and drinking habits were not observed in either genotype group (Table 4). However, we observed a significant interaction between heterozygous and homozygous rare alleles and ever-smoking ($P = 0.015$). Frequent consumption of pickled vegetables elevated the ORs in both genotype groups (OR, 2.23; 95% CI, 1.09–5.54 in homozygous common allele carriers;

Table 3 Estimated ORs for esophageal cancer according to combination of selected environmental factors and the *Rsa*I polymorphism of *CYP2E1*

Environmental factors (Exposure+ *vs.* exposure−)	*Rsa*I polymorphism of *CYP2E1*[a]								Interaction *P*
	A				B + C				
	Exposure−		Exposure+		Exposure−		Exposure+		
	Case/Ctrl	OR[b]	Case/Ctrl	OR[b] (95% CI)	Case/Ctrl	OR[b] (95% CI)	Case/Ctrl	OR[b] (95% CI)	
Smoking and drinking habits									
Smoking (Current or former *vs.* never)	18/44	1.00	35/75	1.33 (0.58–3.03)	9/37	0.51 (0.18–1.46)	29/35	2.33 (0.98–5.54)	0.060
Drinking (≥5 times/week *vs.* less)	46/108	1.00	9/13	2.33 (0.82–6.58)	29/66	1.12 (0.59–2.14)	9/9	2.98 (0.91–9.80)	0.871
Tea consumption									
Tea (≥50 g/month *vs.* less)	40/72	1.00	15/49	0.74 (0.32–1.69)	29/51	1.18 (0.58–2.38)	9/24	0.73 (0.25–2.11)	0.796
Food consumption									
Meat (≥1 time/week *vs.* less)	21/44	1.00	34/77	1.04 (0.48–2.24)	12/24	1.00 (0.37–2.69)	26/50	1.29 (0.58–2.87)	0.728
Soybean (≥3 time/week *vs.* less)	36/91	1.00	19/29	2.23 (0.96–5.19)	25/51	1.43 (0.69–2.98)	12/23	1.33 (0.51–3.45)	0.191
Pickled vegetables (Frequently *vs.* less)	27/72	1.00	28/48	1.30 (0.62–2.73)	19/49	0.89 (0.40–2.00)	19/25	2.01 (0.85–4.77)	0.365
Raw vegetables (Occasionally or more *vs.* less)	39/56	1.00	15/62	0.42 (0.19–0.93)	20/39	0.72 (0.33–1.59)	17/34	0.88 (0.39–2.00)	0.078
Tomato (≥1 time/week *vs.* less)	40/97	1.00	13/24	1.94 (0.77–4.89)	27/52	0.38 (0.71–2.71)	11/22	1.28 (0.44–3.72)	0.30
Garlic (>1 time/week *vs.* less)	20/24	1.00	28/80	0.45 (0.20–1.04)	15/14	1.32 (0.48–3.63)	18/51	0.49 (0.20–1.17)	0.740

[a] A, *Rsa*I site present homozygote; B, heterozygote; C, *Rsa*I site absent homozygote. Ctrl, control.
[b] Adjusted for age (continuous), sex, smoking and drinking habits, and consumption of meat, pickled vegetables, raw vegetables, and garlic, except dependent variable.

Table 4 Estimated ORs for stomach cancer according to combination of selected environmental factors and the *Rsa*I polymorphism of *CYP2E1*

Environmental factors (Exposure+ *vs.* exposure−)	*Rsa*I polymorphism of *CYP2E1*[a]								Interaction *P*
	A				B + C				
	Exposure−		Exposure+		Exposure−		Exposure+		
	Case/Ctrl	OR[b]	Case/Ctrl	OR[b] (95% CI)	Case/Ctrl	OR[b] (95% CI)	Case/Ctrl	OR[b] (95% CI)	
Smoking and drinking habits									
Smoking (Current or former *vs.* never)	17/44	1.00	41/75	1.04 (0.48–2.28)	8/37	0.40 (0.14–1.14)	32/35	2.02 (0.88–4.66)	0.015
Drinking (≥5 times/week *vs.* less)	49/108	1.00	9/13	0.80 (0.29–2.20)	35/66	1.13 (0.62–2.06)	5/9	1.03 (0.27–3.86)	0.887
Tea consumption									
Tea (≥50 g/month *vs.* less)	38/72	1.00	20/49	0.68 (0.32–1.46)	27/51	1.06 (0.53–2.10)	13/24	0.88 (0.35–2.23)	0.739
Food consumption									
Meat (≥1 time/week *vs.* less)	19/44	1.00	38/77	1.50 (0.72–3.13)	17/24	2.11 (0.85–5.24)	22/50	1.18 (0.52–2.67)	0.098
Soybean (≥3 time/week *vs.* less)	43/91	1.00	15/29	1.03 (0.44–2.45)	28/51	1.26 (0.65–2.45)	11/23	0.93 (0.37–2.30)	0.605
Pickled vegetables (Frequently *vs.* less)	21/72	1.00	37/48	2.23 (1.09–4.54)	16/49	0.87 (0.38–1.99)	24/25	3.29 (1.46–7.41)	0.372
Raw vegetables (Occasionally or more *vs.* less)	26/56	1.00	32/62	1.34 (0.66–2.74)	18/39	0.96 (0.42–2.20)	22/34	1.80 (0.81–4.00)	0.559
Tomato (≥1 time/week *vs.* less)	48/97	1.00	10/24	1.30 (0.50–3.40)	29/52	1.21 (0.64–2.28)	10/22	1.17 (0.44–3.14)	0.678
Garlic (≤1 time/week *vs.* less)	12/24	1.00	44/80	0.99 (0.41–2.37)	6/14	0.78 (0.22–2.78)	29/51	1.25 (0.50–3.13)	0.507

[a] A, *Rsa*I site present homozygote; B, heterozygote; C, *Rsa*I site absent homozygote. Ctrl, control.
[b] Adjusted for age (continuous), sex, smoking and drinking habits, and consumption of meat, pickled vegetables, raw vegetables, and garlic, except dependent variable.

OR, 3.29; 95% CI, 1.46–7.41 in heterozygous and homozygous rare allele carriers), but no significant interactions with the polymorphism were evident ($P = 0.372$). Decreased ORs for stomach cancer with frequent consumption of garlic was not apparent, and its gene-environment interaction was not observed.

Among heterozygous and homozygous rare allele carriers, the ORs for esophageal cancer tended to be elevated according to the duration of smoking, without statistical significances (data not shown in table). ORs for esophageal and stomach cancer with current smoking for <30 years were 1.12 and 2.77, and those with current smoking for 30 years or more were 2.39 and 3.08, respectively, when compared with never smoking.

Discussion

The present study revealed no association between the *CYP2E1 Rsa*I polymorphism and overall risk of esophageal and stomach cancers. Previous investigations showed inconsistent findings. With regard to esophageal cancer, Lin *et al.* (18) and Tan *et al.* (19) reported a positive association between the common alleles of the *Rsa*I polymorphism and increased risk in a Chinese population. The proportional distribution of the *Rsa*I common genotype in their controls (44%) tended to be lower than those of other studies in Japanese (74, 64, and 52%) and Taiwanese (62 and 57%; Refs. 22–24, 27, 29), including ours (62%). However, Morita *et al.* (27) did not find any significant association between the *Rsa*I and *Pst*I polymorphisms and esophageal cancer risk in a Japanese population, although the OR for the rare alleles tended to be higher in male smokers. Two groups examined the relation between the *Rsa*I polymorphism and stomach cancer risk. Nishimoto *et al.* (29) found a higher prevalence of the rare genotype in Japanese Brazilian cases with diffuse type stomach cancer, but the overall OR was not elevated (28, 29). Other studies also demonstrated inconsistent results on links between the *Rsa*I, *Pst*I, and *Dra*I polymorphisms and risk of other sites of cancer (17, 20–26, 30–34).

The rare *Rsa*I allele may result in increased transcriptional activation of the *CYP2E1* gene (5, 6), with elevated expression levels of CYP2E1 mRNA and protein (6, 40). However, several studies demonstrated common genotype carriers to have the

higher CYP2E1 enzyme activity (7, 41). Differences in CYP2E1 activity by ethnicity and gender have also been reported, with females showing ~25% lower activity than males (9, 42). Japanese demonstrated 30–40% lower activity of CYP2E1 than Caucasians, even after taking into account differences in body size (43).

The reason for the inconsistent findings for the *CYP2E1* polymorphisms is unknown, but clearly the variation of the enzyme activity with ethnicity and gender could contribute to differences in influence on neoplasms. We here found a significant gene-environment interaction between the *CYP2E1* polymorphism and the smoking habit, ORs for esophageal and stomach cancers with the rare *Rsa*I alleles, suggesting increased susceptibility. Yu *et al.* (20) revealed a positive interaction between the common *Rsa*I polymorphism and smoking with regard to hepatocellular carcinomas, whereas Hildesheim *et al.* (24) found a positive interaction between the rare *Dra*I allele and smoking in nasopharyngeal cancer. Nishimoto *et al.* (29) reported the ORs for stomach cancer with the rare *Rsa*I polymorphism tended to be lower in nonsmokers and low meat consumers than in smokers and high meat consumers, respectively. In concordance with our results, Tan *et al.* (19) earlier suggested the rare *Rsa*I genotype and smoking to together increase the risk of esophageal cancer in a Chinese population. Biological mechanisms are not clear, but residual confounding would not be expected to be large, because confounding factors observed in our previous study (37) were also taken into account for adjustment.

Several studies have shown that garlic compounds can reduce the enzyme activity of CYP2E1 (13, 14), and garlic consumption decreases the risk of esophageal and stomach cancers (37, 38, 44). Unexpectedly, garlic consumption contributed little to altered cancer susceptibility with the *CYP2E1* polymorphism in the present study, although this might have been related to the relatively low intake in the population concerned. Under such conditions, there is potential for misclassification of consumption. Previous studies pointed to pickled vegetable consumption as a risk factor for esophageal and stomach cancers, possibly linked to nitrosamine compounds (3, 4). We also observed an increased OR for stomach cancer with frequent consumption of pickled vegetables, especially in the *Rsa*I rare allele carriers, although this latter was not statistically significant. The findings suggest pickled vegetable consumption to be an environmental factor more strongly involved in stomach cancer risk than the *CYP2E1* polymorphism.

One limitation of the present study was the relatively small number of subjects in subgroup analyses and the consequent reduction in the magnitude of statistical power, with increase in the potential for random error. Another possible problem that requires some discussion is selection bias for controls, these being recruited by local health staff, albeit from the general population with a high response rate. The prevalence of ever-smokers in the present controls was higher (58.7%) than those (47.0%) in the previous case-control study in Yangzhong near Huaian (37). This would tend to underestimate the ORs for smoking. The association between the *CYP2E1* polymorphism and environmental factors has the potential to produce another confounding element. Among the present controls, the ever-smoking rate (63.6%) in the common *Rsa*I allele carriers tended to be higher than that (48.6%) in the rare allele carriers, without statistical significance (data not shown).

In summary, the present study revealed a significant interaction between the *Rsa*I polymorphism and smoking in stomach cancer cases. The data provide support for our hypothesis that cancer susceptibility with the *CYP2E1* polymorphisms may be altered by background environmental factors. Because the sample size of the present study was small, the present finding should be confirmed with more number of subjects.

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