Microsomal Epoxide Hydrolase Polymorphisms, Cigarette Smoking, and Risk of Colorectal Cancer: The Fukuoka Colorectal Cancer Study

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Microsomal epoxide hydrolase (EPHX1) plays an important role in the activation and detoxification of polycyclic aromatic hydrocarbons, carcinogens found in cigarette smoke. Polymorphisms in exon 3 (Y113H) and exon 4 (H139R) of the *EPHX1* have been associated with enzyme activity. We investigated the risk of colorectal cancer in relation to the *EPHX1* Y113H and H139R polymorphisms and assessed effect modifications of cigarette smoking and the other covariates. The interaction between the *EPHX1* polymorphisms and selected genetic polymorphisms was also examined. We used data from Fukuoka Colorectal Cancer Study, a community-based case–control study, including 685 cases and 778 controls. In-person interviews were conducted to assess lifestyle factors. The *EPHX1* Y113H and H139R polymorphisms were determined by the TaqMan assay and the polymerase chain reaction-restriction fragment length polymorphism, respectively. Neither of the two polymorphisms nor the imputed EPHX1 phenotype was associated with colorectal cancer risk. Cigarette smoking and alcohol intake showed no effect modification on the association with the *EPHX1* polymorphisms or the imputed EPHX1 phenotype. Increased risks of colorectal cancer associated with the 113Y allele and imputed EPHX1 phenotype were observed among individuals with high body mass index (BMI; $\geq$25.0 kg/m^2), but not among those with low BMI ($<$25.0 kg/m^2). The risk decreased with an increasing number of the 139R allele in the null genotypes of *GSTM1*/*GSTT1*. It is unlikely that the *EPHX1* polymorphisms play an important role in colorectal carcinogenesis. The observed interactions of the *EPHX1* polymorphisms with BMI and the *GSTM1*/*GSTT1* genotypes warrant further investigation.

Key words: microsomal epoxide hydrolase; polymorphism; cigarette smoking; colorectal cancer

INTRODUCTION

Colorectal cancer accounts for 10% of all cancers and is the third most common cancer in the world [1]. In Japan, the temporal trend showed a marked increase in the incidence of and mortality from colorectal cancer until 1990s [2], and the rates are currently among the highest in the world [1]. Risk for colorectal cancer is influenced by both environmental and genetic factors [3]. Several lifestyle factors such as physical inactivity, alcohol use, and high intake of red meat have been implicated in increased risk of colorectal cancer [4]. It has been a matter of controversy whether smoking is related to increased risk of colorectal cancer [5]. Smoking is consistently related to increased risk of colorectal adenomas [6], and a recent meta-analysis reported a small increase in the risk of colorectal cancer associated with long-term smoking although the findings are rather

Abbreviations: EPHX1, microsomal epoxide hydrolase 1; BMI, body mass index; GST, gluthathione *S*-transferase; OR, odds ratio; CI, confidence interval; CYP, cytochrome P-450; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism.

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Received 27 July 2011; Revised 6 February 2012; Accepted 14 February 2012

DOI 10.1002/mc.21897

Published online in Wiley Online Library (wileyonlinelibrary.com).

disparate [7]. While descriptive features of lung and colorectal cancers are not supportive of a causal role for smoking in colorectal carcinogenesis [8], it is possible that smoking may confer increased risk of colorectal cancer in genetically susceptible individuals in terms of the metabolism of carcinogens in tobacco smoke [9].

Microsomal epoxide hydrolase (EPHX1) is an enzyme involved in the metabolism of reactive epoxides including polycyclic aromatic hydrocarbons, carcinogens found in cigarette smoke [10]. The EPHX1 converts benzo(a)pyrene 7,8 epoxide to the less reactive and more water-soluble dihydrodiol, benzo[a]pyrene 7,8 diol [10]. Although this reaction is generally considered as a detoxification reaction, the less reactive dihydrodiol can be further activated into a highly reactive benzo(a)pyrene 7,8 dihydrodiol 9,10 epoxide [11]. Two functional polymorphisms are known in the *EPHX1* gene; one is the Y113H in exon 3 (rs 1051740), and the other is the H139R in exon 4 (rs 2234922) [12]. In vitro, the *EPHX1* 113H allele is associated with a 40% decrease in enzyme activity, and the 139R allele has an approximately 25% higher activity [12]. Individuals homogyzous or heterozygous for the 113H were shown to have decreased risks of lung cancer [13–15] and upper aerodigestive cancer [16]. Furthermore, high-activity phenotype imputed from the combined genotypes of the Y113H and H139R was associated with increased risks for cancers of the lung [13] and upper aerodigestive tract [16] among those with a high exposure to cigarette smoking. These findings suggest that the *EPHX1* polymorphisms may play a role in the development of tobacco-related cancers. The 113H allele was associated with an increased risk of bladder cancer [17], however.

Several studies have addressed the association of the *EPHX1* polymorphisms with colorectal cancer [18–23] and adenomas [23–28], reporting inconsistent findings. Individuals with the 113HH genotype had an increased risk of colorectal cancer in the earliest study [18] but a decreased risk in the subsequent study [19]. The other studies showed no measurable association of Y113H, H139R, or the imputed phenotype activity with colorectal cancer risk [20–23]. On the other hand, high-activity phenotype was associated with an increased risk of colorectal adenomas among smokers [24,25], whereas individuals homozygous for the 113H allele and those with the composite genotype representing very slow activity showed an increased risk of colorectal adenomas when they had a high exposure to smoking [28]. In the present study, we examined the risk of colorectal cancer in relation to the *EPHX1* Y113H and H139R polymorphisms and assessed the interaction between these polymorphisms and cigarette smoking in the Fukuoka Colorectal Cancer Study, a community-based case–control study in Japan. We also explored the effect modifications of alcohol intake and body mass index (BMI) and the interactions between the *EPHX1* polymorphisms and other genetic polymorphisms of the enzymes involved in tobacco carcinogens.

MATERIALS AND METHODS

Methodological issues of the survey in the Fukuoka Colorectal Cancer Study have been described previously [29]. The study was approved by the ethics committee of the Kyushu University Faculty of Medical Sciences and the collaborating hospitals except two; in the two hospitals, ethics committee was not available at the time of the survey, and the survey was done with an approval of each hospital director.

Subjects

Both cases and controls were residents of Fukuoka city or three adjacent areas. Cases consisted of consecutive patients with histologically confirmed incident colorectal cancer who were admitted to the two university hospitals and six affiliated hospitals for surgical treatment during the period of September 2000 to December 2003. Eligible cases were those aged 20–74 yr at the time of diagnosis and lived in the study area. They also had to be mentally competent to complete the interview. Exclusion criteria were patients who had history of partial or total removal of the colorectum, familial adenomatous polyposis, or inflammatory bowel disease. Of the 1,053 eligible cases, a total of 840 (80%) participated in the interview and 685 gave an informed consent for genotyping.

Controls were frequency matched with cases on sex and 10-yr age class using the same inclusion criteria as for the cases except they did not have a prior diagnosis of colorectal cancer. Exclusion criteria were the same as those for the cases. A total of 1,500 subjects were selected by a two-stage random sampling using residential registry and were invited to participate in the study by mail. Among them, 1,382 were found to be eligible; 833 (60%) participated in the survey, and 778 gave an informed consent for genotyping.

Data Collection

Lifestyle factors were ascertained by in-person interview using a uniform questionnaire. Cases were interviewed in the respective hospitals while controls were interviewed in the public community centers or collaborating clinics. The index date was defined as the date of the onset of symptoms or screening leading to the diagnosis for the cases and the date of interview for controls. BMI (kg/m^2) 10 yr earlier, which was estimated by reported height and weight, was used because the current body mass index was unrelated to colorectal cancer risk [30].

Body weight 10 yr earlier was not available for 2 cases and 10 controls and was substituted with the current body weight. Years of smoking and numbers of cigarettes smoked per day were ascertained for each decade of age if the subjects had ever smoked cigarettes daily for 1 yr or longer. Cigarette-yr until the beginning of the previous decade of age was determined by multiplying the number of cigarettes smoked per day by the years of smoking, and classified into 0, 1–399, 400–799, and ≥800 cigarette-yr. Information on alcohol consumption, type of job and non-occupational physical activity at the time of 5 yr prior to the index date was ascertained. Non-occupational physical activity was expressed as a sum of metabolic equivalents (MET) multiplied by hours of weekly participation in each activity [30].

Genotyping

DNA was extracted from the buffy coat by using a commercial kit (Qiagen GmbH, Hilden, Germany). The following genotyping procedures used 1 μl template DNA with a concentration of 10 ng/μl. Genotyping of the *EPHX1* Y113H polymorphism was carried out by the TaqMan assay (assay ID C_14938_30; Applied Biosystems, Inc., Foster City, CA), using the Stratagene Mx3000P Real-Time QPCR system (Agilent Technologies, Inc., Santa Clara, CA). The *EPHX1* H139R polymorphism was determined by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method as described elsewhere [31], using primers 5′-GGTGCCAGAGCCTGACCGTGC-3′ (sense) and 5′-ATGGAACCTCTAGCAGCCCCGTACC-3′ (anti-sense). The PCR product of 319 bp was digested with *Rsa*I, resulting in fragments of 297 and 22 bp for the 139H allele and fragments of 177, 122, and 22 bp for the 139R allele. The digestion products were separated on a 3% agarose gel (NuSieve, Lonza, Rockland, ME).

Statistical Methods

The EPHX1 activity phenotype was imputed on the basis of the number of putative high-activity alleles (113Y and 139R) in the combined genotype [13]. Associations of the *EPHX1* genotypes with colorectal cancer risk were examined in terms of odds ratio (OR) and 95% confidence interval (CI), which were obtained from logistic regression analysis. Statistical adjustment was made for 5-yr age class (starting with the lowest class of <50 yr), sex, residence area (Fukuoka City or the adjacent areas), and smoking (0, 1–399, 400–799, or ≥800 cigarettes-yr). The results did not change with additional adjustment for BMI 10 yr ago (<22.5, 22.5–24.9, 25.0–27.4, or ≥27.5 kg/m^2), alcohol intake (0, 0.1–0.9, 1.0–1.9, or ≥2.0 units/day), type of job (sedentary, moderate, or hard), non-occupational physical activity (0, 1–15.9, or ≥16 MET-h/wk), and parental history of colorectal cancer. Thus, we presented the ORs with adjustment for age, sex, residence area, and smoking.

Trend of the association was assessed with scores 0, 1, and 2 assigned to the three genotype categories. Effect modifications of smoking and the other covariates were tested by the Wald statistic for a product term of the ordinal variable for genotype and a dichotomous variable for smoking (<400 and ≥400 cigarette-yr) [32], alcohol intake (<2.0 and ≥2.0 unit) [33], and BMI (<25.0 and ≥25.0 kg/m^2) [30] with reference to the previous results. Previously, we reported the associations with *Cytochrome P450 (CYP) 1A1*, *Gluthathione S-transferase (GST) M1*, and *GSTT1* polymorphisms in relation to colorectal cancer risk in the same study subjects [34]. Since the EPHX1 is in the interplay with the CYP1A1 and GST in the metabolism of tobacco-related carcinogens [19], interactions between the *EPHX1* polymorphisms and these other polymorphisms (*CYP1A1*2A*, *CYP1A1*2C*, and the combination of the *GSTM1* and *GSTT1* genotypes) were also explored. The Hardy–Weinberg equilibrium was tested using Pearson's χ^2-test with 1 degree of freedom. A two-sided *P*-value <0.05 was considered as statistically significant. Statistical analyses were calculated using SAS version 9.2 (SAS Institute, Cary, NC).

RESULTS

Characteristics of cases and controls have been previously reported [33]. In brief, the mean age (SD) of the cases and controls were 60.2 (8.7) and 58.6 (10.7) yr, respectively ($P = 0.003$). Males numbered 426 (62%) in the case group and 490 (63%) in the control group. As compared with controls, cases were more likely to be heavy drinkers, had greater BMI 10 yr earlier, and had a higher frequency of family history of colorectal cancer. Cases and controls were not different with respect to residence area, smoking, type of job and non-occupational physical activity.

Genotype distribution of the controls was in Hardy–Weinberg equilibrium for both the *EPHX1* Y113H ($P = 0.35$) and H139R ($P = 0.41$). Frequencies of the *EPHX1* 113H allele were 0.42 in cases and 0.44 in controls, and frequencies of the *EPHX1* 139R allele were 0.16 in cases and 0.18 in controls. As compared with the *EPHX1* 113YY genotype, the *EPHX1* 113HH genotype was associated with a slightly decreased risk of colorectal cancer. The *EPHX1* 139R allele tended to be related to a decreased risk. These decreases in risk were far from the statistical significance, however. The imputed EPHX1 phenotype activity was unrelated to colorectal cancer (Table 1). Sex-specific analyses showed no difference in the association with the *EPHX1* Y113H, H139R polymorphisms, and the imputed EPHX1 phenotype activity between men and women ($P = 0.94$, 0.82, and 0.93, respectively). The associations did not differ in two age groups of <50 and ≥50 yr ($P = 0.29$ for Y113H, 0.70 for H139R, and 0.37 for the imputed phenotype). Furthermore, we

Table 1. The *EPHX1* Polymorphisms and Colorectal Cancer Risk

	N (%)			
Genotype	Cases, $n = 685$	Controls, $n = 778$	OR (95% CI)[a]	*P*-value
Y113H (exon 3)				
YY	228 (33.3)	239 (30.7)	1.00 (referent)	
YH	342 (49.9)	396 (50.9)	0.89 (0.70–1.12)	0.32
HH	115 (16.8)	143 (18.4)	0.81 (0.59–1.10)	0.18
P for trend			0.16	
H139R (exon 4)				
HH	485 (70.8)	525 (67.5)	1.00 (referent)	
HR	182 (26.6)	224 (28.8)	0.88 (0.70–1.11)	0.28
RR	18 (2.6)	29 (3.7)	0.70 (0.38–1.28)	0.25
P for trend			0.14	
Imputed phenotype[b]				
Low	367 (53.6)	414 (53.2)	1.00 (referent)	
Intermediate	223 (32.6)	246 (31.6)	1.06 (0.84–1.33)	0.64
High	95 (13.9)	118 (15.2)	0.92 (0.67–1.25)	0.59
P for trend			0.79	

[a]Adjusted for sex, age, residence area, and cigarette smoking.
[b]Imputed phenotype was based on the number of high-activity alleles (113Y and 139R): low, 0–1; intermediate, 2; and high, 3–4 [13].

repeated the analysis excluding 16 cases and 40 controls aged <40 yr because younger cases may have included more cases of familial colorectal cancer. The results were essentially the same as those described above; mean ages (SD) were 60.8 yr (8.4) in the cases and 60.1 yr (9.0) in the controls ($P = 0.12$).

The OR of colorectal cancer associated with the combined genotypes of the *EPHX1*, Y113H, and H139R are shown in Table 2. There were 56 samples with combinations of the *EPHX1* Y113H and H139R genotypes which were not captured by the imputed phenotype according to the Smith and Harrison's method [35]. Only one of those having the 113HH genotype was a variant homozygote of the 139RR genotype. As compared with the composite genotypes of 113HH and 139HH (very slow imputed phenotype activity) [35], the combined genotypes of the 113YY and 139HH (intermediate imputed phenotype activity) showed a statistically significant increase in the OR ($P = 0.04$). However, the interaction between the two polymorphisms was far from the statistical significance ($P = 0.23$; Table 2).

In the analysis stratified by smoking, neither of the polymorphisms nor the imputed EPHX1 phenotype was related to colorectal cancer risk in each stratum (Table 3). The results were the same when stratified into never-smoking and ever-smoking

Table 2. Adjusted ORs for the Combination of Genotypes of the *EPHX1* Y113H and H139R Polymorphisms

	H139R		
Y113H	HH	HR	RR
HH			
N[a]	94/123	20/20	1/0
OR (95% CI)[b]	1.00 (referent)	1.39 (0.70–2.75)	—
YH			
N[a]	253/271	84/115	5/10
OR (95% CI)[b]	1.26 (0.91–1.73)	0.99 (0.67–1.47)	0.71 (0.23–2.17)
YY			
N[a]	138/131	78/89	12/19
OR (95% CI)[b]	1.48 (1.02–2.13)[c]	1.17 (0.78–1.77)	0.88 (0.40–1.93)

[a]Number of cases/controls.
[b]Adjusted for sex, age, residence area, and cigarette smoking.
[c]$P = 0.04$.
Imputed phenotypes according to Smith and Harrison [35]: rapid, 113YY/139RR or 113YY/139HR; normal, 113YY/139HH or 113YH/139HR; slow, 113YH/139HH; and very slow, 113HH/139HH.

Table 3. Effect Modification of Cigarette Smoking on Colorectal Cancer Risk Associated With the *EPHX1* Polymorphisms

	Cigarette-yr					
	<400			≥400		
Genotype	*N*[a]	OR (95% CI)[b]	*P*-value	*N*[a]	OR (95% CI)[b]	*P*-value
Y113H (exon 3)						
YY	144/164	1.00 (referent)		84/75	1.40 (0.92–2.12)	0.11
YH	213/274	0.88 (0.66–1.18)	0.39	129/122	1.32 (0.91–1.91)	0.15
HH	59/89	0.77 (0.52–1.16)	0.21	56/54	1.24 (0.78–1.97)	0.36
P for trend		0.21			0.62	
P for interaction		0.67				
H139R (exon 4)						
HH	284/352	1.00 (referent)		201/173	1.55 (1.15–2.11)	0.005
HR	120/152	0.99 (0.74–1.32)	0.92	62/72	1.15 (0.77–1.73)	0.49
RR	12/23	0.62 (0.30–1.27)	0.19	6/6	1.38 (0.43–4.42)	0.59
P for trend		0.40			0.25	
P for interaction		0.56				
Imputed phenotype[c]						
Low (0–1)	209/278	1.00 (referent)		158/136	1.67 (1.19–2.33)	0.003
Intermediate (2)	146/166	1.18 (0.88–1.57)	0.27	77/80	1.40 (0.94–2.08)	0.10
High (3–4)	61/83	0.96 (0.66–1.41)	0.84	34/35	1.43 (0.84–2.43)	0.19
P for trend		0.83			0.46	
P for interaction		0.41				

[a]Number of cases/controls.
[b]Adjusted for sex, age, and residence area.
[c]Based on the number of 113Y and 139R alleles shown in parentheses.

groups (data not shown). Alcohol intake did not modify the associations with the *EPHX1* Y113H ($P = 0.36$), H139R ($P = 0.16$) or the imputed EPHX1 phenotype ($P = 0.06$). On the other hand, BMI showed statistically significant effect modifications on the associations with the Y113H and the imputed EPHX1 phenotype (Table 4). The 113Y allele (fast allele) and the imputed EPHX1 phenotype were positively associated with colorectal cancer risk among individuals with high BMI ($\geq$25.0 kg/m^2), but not among those with low BMI (<25.0 kg/m^2).

As regards the gene-gene interactions, there was a statistically significant interaction between H139R and the *GSTM1*/*GSTT1* genotypes (Table 5). The OR decreased progressively with an increasing number of the 139R allele (fast allele) in the null genotype of both *GSTM1* and *GSTT1*. Neither the *CYP1A1*2A* nor the *CYP1A1*2C* showed measurable interactions with the *EPHX1* polymorphisms (data not shown).

DISCUSSION

In this study, neither of the *EPHX1* Y113H and H139R polymorphisms nor the combination of these polymorphisms predicting EPHX1 activity was associated with the risk of colorectal cancer. The present findings are consistent with the results from four [20–23] of the six previous studies [18–23] that showed no association between either of the *EPHX1* polymorphisms and colorectal cancer. While cigarette smoking and alcohol intake showed no effect modification on the association with the *EPHX1* genotype, there were statistically significant effect modifications of BMI and the *GSTM1/GSTT1* null genotypes.

Two British case–control studies reported contradictory findings on the association between the *EPHX1* Y113H polymorphism and colorectal cancer. The *EPHX1* 113HH genotype was associated with a 3.8-fold increase in the risk of colorectal cancer in a small case–control study [18], but was associated with a 32% decrease in the risk in another larger study [19]. However, genotype distribution of the *EPHX1* Y113H polymorphism differed substantially and was not in Hardy–Weinberg equilibrium in these two studies. The 113HH genotype accounted for 21.8% of the controls in the latter study [19] and 6.4% of the controls in the former [18]. Deviation from the Hardy–Weinberg equilibrium was highly significant in the latter ($P < 0.001$) and marginally significant in the former ($P = 0.04$). The PCR-RFLP method used in the two studies may have caused an error in genotyping the Y113H polymorphism due to the presence of a synonymous polymorphism (AAG to AAA) at codon 119 [36]. The reverse primer in these studies contained this polymorphism, and

Table 4. Effect Modification of Body Mass Index on Colorectal Cancer Risk Associated With the *EPHX1* Polymorphisms

	Body mass index (kg/m^2)					
	<25.0			≥25.0		
Genotype	*N*[a]	OR (95% CI)[b]	*P*-value	No.[a]	OR (95% CI)[b]	*P*-value
Y113H (exon 3)						
YY	158/197	1.00 (referent)		70/42	2.17 (1.39–3.38)	0.001
YH	239/290	1.02 (0.77–1.34)	0.91	103/106	1.21 (0.85–1.72)	0.28
HH	90/105	1.03 (0.72–1.47)	0.87	25/38	0.80 (0.46–1.40)	0.44
P for trend		0.79			0.001	
P for interaction		0.004				
H139R (exon 4)						
HH	348/395	1.00 (referent)		137/130	1.20 (0.90–1.60)	0.21
HR	129/175	0.83 (0.63–1.09)	0.18	53/49	1.28 (0.84–1.95)	0.25
RR	10/22	0.53 (0.24–1.15)	0.11	8/7	1.38 (0.49–3.89)	0.54
P for trend		0.04			0.51	
P for interaction		0.18				
Imputed phenotype[c]						
Low (0–1)	270/306	1.00 (referent)		97/108	1.01(0.73–1.40)	0.94
Intermediate (2)	151/187	0.94 (0.71–1.23)	0.65	72/59	1.46 (0.99–2.16)	0.06
High (3–4)	66/99	0.75 (0.52–1.07)	0.11	29/19	1.87 (1.01–3.43)	0.05
P for trend		0.11			0.02	
P for interaction		0.01				

[a]Number of cases/controls.
[b]Adjusted for sex, age, residence area, and cigarette smoking.
[c]Based on the number of 113Y and 139R alleles shown in parentheses.

Table 5. Combinations of the *EPHX1* and *GST* Polymorphisms and Colorectal Cancer Risk

	GSTM1 + *GSTT1*					
	Non-null			Null		
Genotype	*N*[a]	OR (95% CI)[b]	*P*-value	*N*[a]	OR (95% CI)[b]	*P*-value
Y113H (exon 3)						
YY	167/182	1.00 (referent)		61/57	1.12 (0.74–1.72)	0.59
YH	254/297	0.91 (0.69–1.19)	0.49	88/99	0.93 (0.65–1.33)	0.68
HH	81/110	0.76 (0.53–1.09)	0.13	34/33	1.07 (0.63–1.82)	0.79
P for trend		0.13			0.73	
P for interaction		0.64				
H139R (exon 4)						
HH	357/416	1.00 (referent)		128/109	1.33 (0.99–1.79)	0.06
HR	131/161	0.95 (0.72–1.25)	0.72	51/63	0.92 (0.62–1.38)	0.69
RR	14/12	1.35 (0.61–2.97)	0.46	4/17	0.29 (0.10–0.89)	0.03
P for trend		0.86			0.003	
P for interaction		0.01				
Imputed phenotype[c]						
Low (0–1)	270/323	1.00 (referent)		97/91	1.26 (0.90–1.76)	0.17
Intermediate (2)	162/188	1.07 (0.82–1.40)	0.62	61/58	1.27 (0.85–1.89)	0.25
High (3–4)	70/78	1.09 (0.76–1.57)	0.64	25/40	0.74 (0.43–1.26)	0.27
P for trend		0.51			0.13	
P for interaction		0.11				

[a]Number of cases/controls.
[b]Adjusted for sex, age, residence area, and cigarette smoking.
[c]Based on the number of 113Y and 139R alleles shown in parentheses.

the heterozygous 113YH genotype may have been misclassified as the homozygous 113HH genotype due to failure of the 113Y allele to be amplified. The present study was based on the validated Taqman assay as used in the previous four studies [20–23] that showed no association between the Y113H polymorphism and colorectal cancer risk. Genotype distribution was in Hardy–Weinberg equilibrium in the four studies [20–23] and in the present study.

A somewhat decreased risk of colorectal cancer among those with the 139RR genotype in the present study is consistent with the finding in the Physicians' Health Study, in which the OR for 139HR and 139RR compared with 139HH were 0.68 (95% CI 0.47–0.97) and 0.94 (95% CI 0.38–2.32), respectively, the OR for 139HR and 139RR combined being 0.70 (75% CI 0.49–0.99). However, no such association was replicated in the Nurses' Health Study [20] and the other three [18,19,21].

It was found in the present study that 4% of the whole sample was not included in any of the four class categories of the imputed EPHX1 phenotype as classified by the Smith and Harrison's method [35]. Likewise, 5% sample had been unclassified in a study in Germany [37]. Ulrich et al. [28] also noted that several genotype combinations were not captured by this method.

Two studies addressed the interaction between the *EPHX1* polymorphisms and smoking on colorectal cancer risk to find no effect modification of smoking on the association with the *EPHX1* polymorphisms singly or in combination in the United States [20,22]. The present study also found no interaction between smoking and the *EPHX1* polymorphisms in a Japanese population. Previously, none has addressed the effect modifications of alcohol intake and BMI on colorectal cancer risk associated with the *EPHX1* polymorphisms. The present study showed that the associations of the *EPHX1* polymorphisms with colorectal cancer differed by BMI. High BMI is known to be related with increased risk of colorectal cancer [38], and was the case in the present study population [30]. The present findings are very intriguing, but we could not prepare a prompt explanation for an elevated risk associated with the 113Y allele (fast allele) among those with high BMI. Another interesting finding was a decreased risk associated with the 139R allele in individuals with the *GSTM1*/*GSTT1* null genotypes. The null genotypes of the *GSTM1* and *GSTT1* genes are associated with the loss of enzyme activity [39]. Given the dual role of the EPHX1 enzyme on the activation and detoxification of carcinogenic hydrocarbons [10], it is possible that the 139R allele (fast allele) is protective in the absence of the GSTM1/GSTT1 enzyme activity.

The use of community controls and the large number of subjects were strengths of the present study. The other strength was that the study subjects consisted of an ethnically homogenous population of Japanese, and concern over population stratification was negligible. There were several weaknesses to be discussed. The participation rate in terms of genotyping was rather low (65% in cases and 56% in controls). However, it is unlikely that participation had been affected by genetic polymorphisms under study. As reported previously [40], older persons (particularly among the controls) and women were less likely to give consent for genotyping, but there was no difference between those who gave consent and those who did not in terms of smoking, residence area, and alcohol intake.

In conclusion, neither of the *EPHX1* polymorphisms nor the imputed phenotype activity was associated with colorectal cancer risk. Cigarette smoking did not modify the associations with the *EPHX1* polymorphisms, nor did alcohol intake. The observed interactions with BMI and the *GSTM1/GSTT1* null genotypes remain to be consolidated in further studies.

ACKNOWLEDGMENTS

The authors acknowledge support from Emeritus Professors Keizo Sugimachi, Seiyo Ikeda, Takayuki Shirakusa, and Sumitaka Arima; and Drs. Motonori Saku, Yoichi Ikeda, Soichiro Maekawa, Kazuo Tanoue, Kinjiro Sumiyoshi, and Shoichiro Saito in conducting the survey of cases. The following physicians kindly supervised the survey of the controls at their clinics: Drs. Hideaki Baba, Tomonori Endo, Hiroshi Hara, Yoichiro Hirokata, Motohisa Ikeda, Masayoshi Ishibashi, Fumiaki Itoh, Yasuhiro Iwanaga, Hideki Kaku, Shoshi Kaku, Minori Kanazawa, Akira Kobayashi, Ryunosuke Kumashiro, Shinichi Matsumoto, Soukei Mioka, Umeji Miyakoda, Osamu Nakagaki, Nobuyoshi Nogawa, Nobuyoki Ogami, Toyoaki Okabayashi, Hironao Okabe, Nishiku Saku, Masafumi Tanaka, Masahiro Ueda, Bunichi Ushio, and KoheishoYasunaga. The authors are grateful to Ms. Hiroko Mizuta, Ms. Masumi Koga, and Ms. Kumiko Arie for their assistance. This work was supported by Scientific Support Programs for Cancer Research, Grant-in-Aid for Scientific Research on Innovative Areas, the Ministry of Education, Culture, Sports and Technology, Japan (221S0001).

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