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Original Article

Human RAD 17 Polymorphism at Codon 546 Is Associated with the Risk of Colorectal Cancer

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Human RAD17 acts as an activator of checkpoint signals in response to DNA damage. Here we evaluated the association of *hRAD17* Leu546Arg (rs1045051), a missense single nucleotide polymorphism, with the risk of colorectal cancer (CRC) in relation to smoking and alcohol consumption habits in 212 CRC patients and 1,142 cancer-free controls in a case-control study conducted in Japan. The results showed that the *hRAD17* Leu/Arg genotype compared to the Leu/Leu genotypes was significantly associated with the protective effect on CRC risk with the adjusted odds ratio (OR) of 0.68 [95% confidence interval (CI): 0.49 – 0.95, $p = 0.024$], and the males with the Arg/Arg genotype had a greater risk of CRC compared to those with the Leu/Leu and Leu/Arg genotypes (OR = 1.87, 95%CI 1.03 – 3.40, $p = 0.04$). In stratified studies, the protective effect of the Leu/Arg genotype on CRC risk was markedly higher in the light smokers (< 20 pack years) (OR = 0.61, 95%CI 0.40 – 0.94, $p = 0.024$) and the rectal cancer patients (OR = 0.49, 95%CI 0.31 – 0.78, $p = 0.003$). The risk of the Arg/Arg genotype was associated with heavy smoking (≥ 20 pack-years) (OR = 2.24, 95%CI 1.09 – 4.61, $p = 0.03$). These findings suggest that the genetic variant of *hRAD17* Leu546Arg polymorphism has a significant effect on CRC susceptibility in Japanese.

Key words: colorectal cancer, single nucleotide polymorphism, human RAD17, DNA damage

In Japan, colorectal cancer (CRC) has a high mortality rate for both males and females, and it is one of the most common malignancies: in 2011, it showed 4th (14.5%) incidence rate in males and 2nd (14.9%) in females [1]. In a 2004-2007 study, the distribution of clinical stages among CRC patients who underwent initial treatment showed that the proportions of stage I, II and III CRC cases were comparable. However, the 5-year relative survival rate of stage I CRC patients was high at over 97% for both males and females, and the 10-year relative survival rate was still high at over 94%. Therefore, a screening system to detect earlier stage of CRC is more important to decrease morbidity and mortality [1].

Epidemiological studies have revealed that most CRCs can be ascribed to dietary, environmental and lifestyle factors, and of these factors cigarette smoking and alcohol consumption significantly influence the risk of CRC development [2-7]. A number of epidemiological studies across the world have found that alcohol consumption has a more significant positive association with the risk of CRC in Japanese than in Western populations [8].

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Human *RAD17*, a human homologue of the yeast Schizosaccharomyces pombe cell-cycle checkpoint gene *RAD17*, plays an important role in cell-cycle arrest with CHK1 activation and DNA damage repair in response to DNA damage and incomplete DNA replication by the phosphorylation of hRAD17 protein by ATR [9-15]. Two significant regions in hRAD17 protein are the P-loop (also known as Walker A motif), which is critical for the nucleotide binding of ATPases, and the C terminus containing two SQ motifs (Ser635 and Ser645) that are strong targets for ATR kinase. For the hRAD17 function of assisting interaction with the RAD9-HUS1-RAD1 complex, the phosphorylation of both SQ sites is essential and is associated with the activation of the G2/M checkpoint [13-15].

hRAD17 is known to be overexpressed in CRC relative to normal colon [9]. The expression of hRAD17 in an S. pombe RAD17-deleted strain induced a reduction of yeast colony growth and slower progression through the cell cycle [16]. These findings suggest that hRAD17 is associated with the regulation of tumor growth. In addition to altered expression, genetic polymorphisms in genes related to DNA damage repair are known to be associates with the development of CRC [17]. We previously reported the association of the Arg/Arg genotype of *hRAD17* Leu546Arg (rs1045051) polymorphism with the risk of esophageal squamous cell carcinoma [18]. As *hRAD17* Leu546Arg polymorphism is located near the SQ motif, we speculated that the amino acid substitutions from leucine to arginine at codon 546 may have an effect on the ATR-dependent phosphorylation and that the deregulation may negatively or positively affect tumor development.

In the present study, we investigated the effect of *hRAD17* Leu546Arg polymorphism on the risk of CRC in relation to cigarette smoking and alcohol consumption habits in Japanese.

Materials and Methods

Study population. We retrospectively examined the cases of 212 Japanese patients with primary colorectal cancer whose tumors were operated on and histologically confirmed at Okayama University Hospital (Okayama, Japan) from 1994 to 2005. The clinical and histopathological classifications of the tumors were defined according to the criteria of the UICC Tumor-Node-Metastasis Classification of Malignant Tumors (TNM), 6th edition, 2002. There were two hereditary CRC cases (one case of hereditary non-polyposis colorectal cancer and one case of familial adenomatous polyposis) and one case of ulcerative colitis-associated colorectal cancer, and these cases were also included in the analysis.

The controls were 1,142 Japanese who consisted of two groups: 228 outpatients without cancer who visited the Kusaka Hospital at Okayama in 2005, and 914 healthy individuals who attended Junpukai Health Care Center at Okayama in 2009.

All patients and controls gave their written informed consent to have their information analyzed and published. The Bioethics Committee of Okayama University Medical School approved this study.

The age, gender, personal and family medical histories, and smoking and drinking history of the subjects were obtained from interviews and medical questionnaire records. The smoking status was assessed by pack-year equivalents ([cigarettes/day ÷ 20] × [smoking years]). The classifications of smoking status for the present study was as follows: non-smoker, <20 pack-years (light smokers), or ≥ 20 pack-years (heavy smokers). Alcohol consumption status was assessed as the daily ethanol intake using the method of calculating alcohol consumption (one serving of sake contains nearly 23 g ethanol, which is roughly the equivalent of two U.S. standard drinks, when one U.S. drink's contents = 14 g of ethanol) [5]. The recommended daily maximum amount of alcohol was based on the report regarding the limitation on daily ethanol intake issued by the Ministry of Health, Labor and Welfare (20 g/day; Healthy Japan 21, a national healthcare movement). The results of a prospective large cohort study showed that the risk of cancer mortality was lowest among Japanese drinkers with ethanol intake <23 g/day [5]. The subjects were classified as non-drinkers, <23 g/day (light drinkers), or ≥ 23 g/day (heavy drinkers).

Genotype analysis. Genomic DNA was extracted from peripheral blood lymphocytes or freshly frozen non-neoplastic colorectal mucosae by the standard procedures using proteinase K and phenol-chloroform. The genotyping of the polymorphisms including the *hRAD17* Leu546Arg (rs1045051) was performed by the SNaPshot method as described [16]. Briefly, the target region for genotyping was amplified by polymerase chain reaction (PCR), with a final reaction volume of 10 μl containing 10 ng of template DNA, 1.0 pmol of

each primer, 2.0 mM of each dNTP, 1 μl of 10×PCR buffer, and 0.25 units of Taq DNA polymerase (TaKaRa Bio, Kusatsu, Shiga, Japan).

Thermal cycling was performed with an initial denaturation at 94°C for 3 min, followed by 32 cycles at 94°C for 30 sec, 60°C for 30 sec and 72°C for 30 sec, and final extension at 72°C for 7 min. The PCR products were treated with 2.0 units of exonuclease I and shrimp alkaline phosphatase to remove the unreacted primers and dNTPs by incubating at 37°C for 90 min and at 75°C for 15 min. The first PCR primer set was designed as: sense 5'-CAGTATCGGGAAAATTGCC TGG-3' and anti-sense 5'-GGACAGTAGAGACTCCC CCT-3'.

Next, a single nucleotide primer extension reaction was performed using an ABI PRISM SNaPshot kit (Applied Biosystems, Foster City, CA, USA) with a final reaction volume of 10 μl containing 3 μl of the purified PCR products, 20 mM of $(NH_4)_2SO_4$, and 2 μl of SNaPshot Ready Reaction mix containing fluorescently labeled ddNTPs and DNA polymerase. The typing primer used for *hRAD17* Leu546Arg was 5'-TTTTTTTTTTTTTTTTTTTTTCAAGGTATGGC AATAGCTGAGTTTGG-3'. Thermal cycling was performed with an initial denaturation at 96°C for 3 min, followed by 30 cycles at 96°C for 10 sec, 50°C for 5 sec and 60°C for 30 sec.

After treatment with 1.0 unit of shrimp alkaline phosphatase to remove the unincorporated ddNTPs by incubating at 37°C for 90 min and at 75°C for 15 min, 8.5 μl of HI-Di formamide, 0.5 μl of Genescan 120 LIZ size standard (Applied Biosystems) and 1 μl of the reaction mixture were combined and denatured at 95°C for 5 min and placed at 4°C for 2 min. The products were electrophoresed with an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems) and analyzed by Gene Mapper Software SNaPshot Analysis (Applied Biosystems).

Statistical analysis. The allele frequency was calculated by direct counting. Deviation of the genotype frequency from Hardy-Weinberg equilibrium was analyzed by the exact test. The distribution of genotype frequencies between the cancer cases and controls were analyzed using Chi-square tests. The risk of colorectal cancer was estimated using odds ratio (ORs) and 95% confidence interval (CIs). Analyses were adjusted for age, smoking status and alcohol consumption status using a multivariate logistic regression model by comparing the genotypes between the cases and controls. *P*-values < 0.05 were considered significant. All analyses were performed with SPSS software (ver. 12.0, SPSS, Tokyo).

Results

The characteristics of the CRC patients and the controls are summarized in Table 1. There were significant differences in the age distribution and the daily alcohol consumption between the CRC patients and controls. The ratio of males to females and smoking habit did not show any significant difference. Among the control subjects recruited from the 2 groups in Okayama, we assessed the genotype distributions of *hRAD17* Leu546Arg polymorphism by Hardy-Weinberg equilibrium (HWE), and the *p*-value was 0.55 for the Kusaka Hospital outpatients without cancer and 0.79 for the healthy individuals who attended Junpukai Health Care Center (data not shown).

Table 2 shows the allele and genotype frequencies of the *hRAD17* Leu546Arg in the CRC patients and controls. The Leu/Arg genotype compared to the Leu/Leu genotype was significantly associated with the decreased risk of CRC (OR = 0.68, 95%CI 0.49 – 0.95, $p = 0.02$), whereas the allele frequencies between the patients and controls were not significantly different. Among the males, the Arg/Arg genotype compared to the Leu/Leu and the Leu/Arg genotypes in the recessive genetic model was associated with a significantly increased risk of CRC (OR = 1.87, 95%CI 1.03 – 3.40, $p = 0.04$). We did not find any association between the genotypes and the risk of CRC among the females.

Next, we examined the association of smoking and alcohol consumption history between the *hRAD17* Leu546Arg genotypes and the risk of CRC in the stratified analysis (Table 3). Among the light smokers, the Leu/Arg genotype compared to the Leu/Leu genotype was significantly associated with a decreased risk of CRC (OR = 0.61, 95%CI 0.40 – 0.94, $p = 0.02$). We also found a significant association of the Arg/Arg genotype compared to the Leu/Leu and the Leu/Arg genotype in the recessive genetic model with an increased risk (OR = 2.24, 95%CI 1.09 – 4.61, $p = 0.03$) among the heavy smokers. There was no significant association among the stratified alcohol drinkers.

We further performed a stratified analysis of combined smoking and alcohol consumption history on

Table 1 Characteristics of the CRC patients and healthy controls

	CRC patients *n* (%)	Controls *n* (%)	*p*-value
Total	212 (100.0)	1,142 (100.0)	
Males	124 (58.5)	676 (59.2)	0.85
Females	88 (41.5)	466 (40.8)	
Age (yrs)			
<50	20 (9.4)	255 (22.3)	<0.001
50–59	59 (27.8)	501 (43.9)	
60–69	61 (28.8)	261 (22.9)	
≥70	72 (34.0)	125 (10.9)	
Median	64	55	
Range	23–89	32–94	
Smoking			
Never	96 (46.2)	599 (52.5)	0.29
<20 pack-year	31 (14.9)	182 (15.9)	
≥20 pack-year	81 (38.9)	361 (31.6)	
Alcohol drinking*			
Never	107 (50.7)	534 (46.8)	<0.001
<23 g of ethanol/day	38 (18.0)	373 (32.7)	
≥23 g of ethanol/day	66 (31.3)	235 (20.6)	

*23 g of ethanol approximates one serving of sake, or two U.S. standard drinks.

Table 2 *hRAD17* genotype in the CRC patients and controls

Genotype	CRC patients *n* (%)	Controls *n* (%)	Adjusted OR† (95%CI)	*p*‡
Total:				
Leu/Leu	107 (50.5)	513 (44.9)		
Leu/Arg	77 (36.3)	510 (44.7)	0.68 (0.49–0.95)	0.024
Arg/Arg	28 (13.2)	119 (10.4)	1.16 (0.71–1.87)	0.56
Leu/Arg+Arg/Arg	105 (49.5)	629 (55.1)	0.77 (0.56–1.04)	0.09
Leu/Leu+Leu/Arg	184 (86.8)	1,023 (89.6)		
Arg/Arg	28 (13.2)	119 (10.4)	1.38 (0.87–2.18)	0.17
Allele frequencies:				
Leu	291 (68.6)	1,536 (67.3)		0.58
Arg	133 (31.4)	748 (32.7)		
Males:				
Leu/Leu	60 (48.4)	311 (46.0)		
Leu/Arg	46 (37.1)	306 (45.3)	0.71 (0.46–1.10)	0.12
Arg/Arg	18 (14.5)	59 (8.7)	1.60 (0.85–2.99)	0.14
Leu/Arg+Arg/Arg	64 (51.6)	365 (54.0)	0.85 (0.57–1.27)	0.42
Leu/Leu+Leu/Arg	106 (85.5)	617 (91.3)		
Arg/Arg	18 (14.5)	59 (8.7)	1.87 (1.03–3.40)	0.039
Females:				
Leu/Leu	47 (53.4)	202 (43.3)		
Leu/Arg	31 (35.2)	204 (43.8)	0.65 (0.39–1.08)	0.10
Arg/Arg	10 (11.4)	60 (12.9)	0.75 (0.35–1.63)	0.47
Leu/Arg+Arg/Arg	41 (46.6)	264 (56.7)	0.67 (0.42–1.08)	0.10
Leu/Leu+Leu/Arg	78 (88.6)	406 (87.1)		
Arg/Arg	10 (11.4)	60 (12.9)	0.92 (0.44–1.92)	0.83

†Adjusted for sex, age, smoking, and alcohol drinking.
‡*p*-values, difference in genotype frequencies between the CRC patients and controls.

Table 3 Association of *hRAD17* genotypes with smoking and alcohol consumption status

Genotype	CRC patients *n* (%)	Controls *n* (%)	Adjusted OR* (95%CI)	*p*
Smoking < 20 pack-yrs				
Leu/Leu	68 (54.8)	345 (44.1)		
Leu/Arg	43 (34.7)	349 (44.6)	0.61 (0.40–0.94)	0.024
Arg/Arg	13 (10.5)	88 (11.3)	0.78 (0.41–1.50)	0.45
Leu/Arg + Arg/Arg	56 (45.2)	437 (55.9)	0.65 (0.44–0.96)	0.029
Leu/Leu + Leu/Arg	111 (89.5)	694 (88.7)		
Arg/Arg	13 (10.5)	88 (11.3)	0.97 (0.51–1.82)	0.91
Smoking ≥ 20 pack-yrs				
Leu/Leu	38 (45.2)	168 (46.7)		
Leu/Arg	31 (36.9)	161 (44.7)	0.82 (0.47–1.42)	0.47
Arg/Arg	15 (17.9)	31 (8.6)	2.04 (0.95–4.38)	0.07
Leu/Arg + Arg/Arg	46 (54.8)	192 (53.3)	1.01 (0.61–1.69)	0.96
Leu/Leu + Leu/Arg	69 (82.1)	329 (91.4)		
Arg/Arg	15 (17.9)	31 (8.6)	2.24 (1.09–4.61)	0.028
Alcohol < 23 g of ethanol/day				
Leu/Leu	71 (49.0)	409 (45.1)		
Leu/Arg	54 (37.2)	398 (43.9)	0.75 (0.50–1.11)	0.14
Arg/Arg	20 (13.8)	100 (11.0)	1.17 (0.67–2.05)	0.57
Leu/Arg + Arg/Arg	74 (51.0)	498 (54.9)	0.83 (0.58–1.19)	0.32
Leu/Leu + Leu/Arg	125 (86.2)	807 (89.0)		
Arg/Arg	20 (13.8)	100 (11.0)	1.34 (0.79–2.28)	0.28
Alcohol ≥ 23 g of ethanol/day				
Leu/Leu	35 (53.0)	104 (44.3)		
Leu/Arg	23 (34.8)	112 (47.7)	0.54 (0.28–1.01)	0.06
Arg/Arg	8 (12.1)	19 (8.1)	1.05 (0.39–2.83)	0.92
Leu/Arg + Arg/Arg	31 (47.0)	131 (55.7)	0.61 (0.34–1.11)	0.11
Leu/Leu + Leu/Arg	58 (87.9)	216 (91.9)		
Arg/Arg	8 (12.1)	19 (8.1)	1.39 (0.54–3.62)	0.50

*Adjusted for sex, age, smoking, and alcohol drinking.

hRAD17 Leu546Arg genotype in the patients and controls (Table 4). Among the group of light smokers/heavy drinkers, the Leu/Arg genotype compared to the Leu/Leu genotype was significantly associated with a decreased risk of CRC (OR = 0.32, 95%CI 0.11 – 0.98, $p = 0.046$). The Arg/Arg genotype compared to the Leu/Leu and the Leu/Arg genotypes in the recessive model increased the risk among the heavy smokers/light drinkers (OR = 2.89, 95%CI 1.11 – 7.51, $p = 0.03$). No significant association was revealed between the risk of CRC and any genotypes within the group of heavy smokers/heavy drinkers, as well as the light smokers/light drinkers.

The results of the stratified analysis of smoking and alcohol consumption history on *hRAD17* Leu546Arg genotype among the males are summarized in Table 5. In only the group of heavy smokers, the Arg/Arg genotype compared to the Leu/Leu and the Leu/Arg genotypes in the recessive model showed a significant association with the risk of CRC (OR = 2.53, 95%CI 1.19 – 5.39, $p = 0.016$). Our stratified analysis of the combined effect of smoking and alcohol consumption history among the males showed that the Arg/Arg genotype compared to the Leu/Leu and the Leu/Arg genotypes also had a significant risk among the heavy smokers/light drinkers (OR = 3.10, 95%CI 1.13 – 8.48, $p = 0.028$) (Table 6).

As it is known that the left and right colon have different embryologic origins and consequently different molecular backgrounds, we analyzed the association of those cancer locations with the *hRAD17* Leu546Arg genotypes. In the separate evaluation of the left side of

Table 4 Association of *hRAD17* genotype with the combination of smoking and alcohol consumption status

Genotype	CRC patients *n* (%)	Controls *n* (%)	Adjusted OR* (95%CI)	*p*
Smoking < 20 pack-yrs, Alcohol < 23 g of ethanol/day				
Leu/Leu	56 (53.3)	304 (40.6)		
Leu/Arg	37 (35.2)	295 (53.5)	0.69 (0.44–1.09)	0.11
Arg/Arg	12 (11.4)	82 (5.9)	0.83 (0.42–1.64)	0.59
Leu/Arg + Arg/Arg	49 (46.7)	377 (59.4)	1.39 (0.91–2.13)	0.13
Leu/Leu + Leu/Arg	93 (88.6)	599 (94.1)		
Arg/Arg	12 (11.4)	82 (5.9)	0.98 (0.51–1.89)	0.94
Smoking < 20 pack-yrs, Alcohol ≥ 23 g of ethanol/day				
Leu/Leu	12 (63.2)	41 (46.5)		
Leu/Arg	6 (31.6)	54 (45.6)	0.32 (0.11–0.98)	0.046
Arg/Arg	1 (5.3)	6 (8.0)	0.56 (0.06–5.44)	0.62
Leu/Arg + Arg/Arg	7 (36.8)	60 (53.5)	0.34 (0.12–1.00)	0.049
Leu/Leu + Leu/Arg	18 (94.7)	95 (92.0)		
Arg/Arg	1 (5.3)	6 (8.0)	0.96 (0.10–8.90)	0.97
Smoking ≥ 20 pack-yrs, Alcohol < 23 g of ethanol/day				
Leu/Leu	15 (39.5)	105 (47.0)		
Leu/Arg	15 (39.5)	103 (43.3)	0.93 (0.42–2.06)	0.86
Arg/Arg	8 (21.1)	18 (9.7)	2.79 (0.99–7.84)	0.05
Leu/Arg + Arg/Arg	23 (60.5)	121 (53.0)	1.22 (0.59–2.51)	0.60
Leu/Leu + Leu/Arg	30 (78.9)	208 (90.3)		
Arg/Arg	8 (21.1)	18 (9.7)	2.89 (1.11–7.51)	0.030
Smoking ≥ 20 pack-yrs, Alcohol ≥ 23 g of ethanol/day				
Leu/Leu	23 (50.0)	63 (47.0)		
Leu/Arg	16 (34.8)	58 (43.3)	0.75 (0.34–1.66)	0.48
Arg/Arg	7 (15.2)	13 (9.7)	1.74 (0.53–5.76)	0.36
Leu/Arg + Arg/Arg	23 (50.0)	71 (53.0)	0.90 (0.43–1.87)	0.77
Leu/Leu+Leu/Arg	39 (84.8)	121 (90.3)		
Arg/Arg	7 (15.2)	13 (9.7)	1.99 (0.63–6.24)	0.24

*Adjusted for sex, age, smoking, and alcohol drinking.

the colon (the descending colon, sigmoid colon, and rectum) and the right side of the colon (the cecum, ascending colon, and transverse colon), the Leu/Arg genotype compared to the Leu/Leu genotype showed a significant difference solely in the left colon group (OR = 0.64, 95%CI 0.44 – 0.93, $p = 0.02$) (Table 7). We further investigated the association of the genotypes among separate groups of colon cancer and rectal cancer patients. Consistent with the results observed for the left and right colon, only the rectal cancer cases showed a significant association for the Leu/Arg genotype (OR = 0.49, 95%CI 0.31 – 0.78, $p = 0.003$), whereas there was no significant association in colon cancer (Table 8).

Discussion

In this case-control study, we examined the association between the codon 546 polymorphism causing the amino acid substitution of leucine to arginine, a missense single nucleotide polymorphism (SNP) in *hRAD17* gene, and the risk of CRC. We found that the *hRAD17* Leu546Arg variant was associated with the suppression or occurrence of CRC. To our knowledge, this is the first report demonstrating that the *hRAD17* codon 546 polymorphism is associated with the risk of CRC.

In a pooled meta-analysis based on cohort and case-control studies across the world, smoking was not shown to be associated with a significantly increased risk of CRC in Japanese [7], whereas alcohol consump-

Table 5 Association of *hRAD17* genotypes with smoking and alcohol consumption status in the males

Genotype	CRC patients *n* (%)	Controls *n* (%)	Adjusted OR* (95%CI)	*p*
Smoking < 20 pack-yrs				
Leu/Leu	23 (56.1)	149 (44.3)		
Leu/Arg	14 (34.1)	156 (46.4)	0.56 (0.28–1.14)	0.11
Arg/Arg	4 (9.8)	31 (9.2)	0.84 (0.27–2.61)	0.76
Leu/Arg + Arg/Arg	18 (43.9)	187 (55.7)	0.61 (0.31–1.17)	0.14
Leu/Leu + Leu/Arg	37 (90.2)	305 (90.8)		
Arg/Arg	4 (9.8)	31 (9.2)	1.08 (0.36–3.25)	0.89
Smoking ≥ 20 pack-yrs				
Leu/Leu	36 (45.6)	162 (47.6)		
Leu/Arg	29 (36.7)	150 (44.1)	0.83 (0.47–1.47)	0.53
Arg/Arg	14 (17.7)	28 (8.2)	2.32 (1.04–5.16)	0.039
Leu/Arg + Arg/Arg	43 (54.4)	178 (52.4)	1.05 (0.62–1.78)	0.84
Leu/Leu + Leu/Arg	65 (82.3)	312 (91.8)		
Arg/Arg	14 (17.7)	28 (8.2)	2.53 (1.19–5.39)	0.016
Alcohol < 23 g of ethanol/day				
Leu/Leu	27 (43.5)	213 (46.6)		
Leu/Arg	25 (40.3)	202 (44.2)	0.88 (0.48–1.61)	0.68
Arg/Arg	10 (16.1)	42 (9.2)	1.87 (0.83–4.20)	0.13
Leu/Arg + Arg/Arg	35 (56.5)	244 (53.4)	1.05 (0.61–1.82)	0.85
Leu/Leu + Leu/Arg	52 (83.9)	415 (90.8)		
Arg/Arg	10 (16.1)	42 (9.2)	1.98 (0.92–4.24)	0.08
Alcohol ≥ 23 g of ethanol/day				
Leu/Leu	32 (52.5)	98 (44.7)		
Leu/Arg	21 (34.4)	104 (47.5)	0.55 (0.29–1.07)	0.08
Arg/Arg	8 (13.1)	17 (7.8)	1.27 (0.46–3.48)	0.65
Leu/Arg+Arg/Arg	29 (47.5)	121 (55.3)	0.65 (0.35–1.21)	0.18
Leu/Leu+Leu/Arg	53 (86.9)	202 (92.2)		
Arg/Arg	8 (13.1)	17 (7.8)	1.66 (0.63–4.37)	0.31

*Adjusted for age, smoking, and alcohol drinking.

tion showed a significant positive association with the risk of CRC in Western populations [8]. In the present study, the consumption of more than 23 g of ethanol/day was significantly associated with the risk of CRC, but smoking status had no association with the risk of CRC. Since several studies have revealed that long-term heavy smoking is associated with a higher risk of CRC, it is speculated that smoking plays an important role in the initiation of CRC. and it is thought that the carcinogenic effects of smoking on CRC development require a long induction period. The proportion of long-term smokers probably affects the association between smoking and the risk of CRC [7, 19].

Comparing males and females, the patients and controls showed different lifestyle tendencies. The number of male CRC patients who were heavy smokers/heavy alcohol drinkers was the largest in the combined stratified analysis of smoking and alcohol consumption history, whereas the light smokers/light alcohol drinkers were the most numerous among the females in both the patient and control groups.

We reported that the Arg/Arg genotype of *hRAD17* Leu546Arg polymorphism compared to the Leu/Leu genotype and the Leu/Arg genotype in a recessive model was significantly associated with an increased risk of esophageal squamous carcinoma in the study's group of male light alcohol drinkers [18]. In the stratified analysis of CRC patients in the present study, alcohol consumption in the males did not show any association with the risk of *hRAD17* polymorphism. In the combined stratified analysis of smoking and alcohol consumption, we did not observe an association of

Table 6 Association of *hRAD17* genotype with combination of smoking and alcohol consumption status in males

Genotype	CRC patients *n* (%)	Controls *n* (%)	Adjusted OR* (95%CI)	*p*
Smoking<20 pack-yrs Alcohol<23 g of ethanol/day				
Leu/Leu	14 (51.9)	114 (46.0)		
Leu/Arg	10 (37.0)	109 (44.0)	0.76 (0.32–1.80)	0.54
Arg/Arg	3 (11.1)	25 (10.1)	0.98 (0.26–3.68)	0.98
Leu/Arg+Arg/Arg	13 (48.1)	134 (54.0)	0.80 (0.36–1.79)	0.59
Leu/Leu+Leu/Arg	24 (88.9)	223 (89.9)		
Arg/Arg	3 (11.1)	25 (10.1)	1.11 (0.31–3.95)	0.87
Smoking<20 pack-yrs Alcohol≥23 g of ethanol/day				
Leu/Leu	9 (64.3)	35 (39.8)		
Leu/Arg	4 (28.6)	47 (53.4)	0.29 (0.08–1.06)	0.06
Arg/Arg	1 (7.1)	6 (6.8)	0.57 (0.06–5.50)	0.63
Leu/Arg+Arg/Arg	5 (35.7)	53 (60.2)	0.33 (0.10–1.08)	0.07
Leu/Leu+Leu/Arg	13 (92.9)	82 (93.2)		
Arg/Arg	1 (7.1)	6 (6.8)	1.01 (0.11–9.12)	0.99
Smoking≥20 pack-yrs Alcohol<23 g of ethanol/day				
Leu/Leu	13 (39.4)	99 (47.4)		
Leu/Arg	13 (39.4)	93 (44.5)	0.97 (0.42–2.27)	0.95
Arg/Arg	7 (21.2)	17 (8.1)	3.05 (1.03–9.07)	0.045
Leu/Arg+Arg/Arg	20 (60.6)	110 (52.6)	1.29 (0.60–2.81)	0.51
Leu/Leu+Leu/Arg	26 (78.8)	192 (91.9)		
Arg/Arg	7 (21.2)	17 (8.1)	3.10 (1.13–8.48)	0.028
Smoking≥20 pack-yrs Alcohol≥23 g of ethanol/day				
Leu/Leu	23 (50.0)	63 (48.1)		
Leu/Arg	16 (34.8)	57 (43.5)	0.75 (0.34–1.66)	0.48
Arg/Arg	7 (15.2)	11 (8.4)	1.74 (0.53–5.76)	0.36
Leu/Arg+Arg/Arg	23 (50.0)	68 (51.9)	0.90 (0.43–1.87)	0.77
Leu/Leu+Leu/Arg	39 (84.8)	120 (91.6)		
Arg/Arg	7 (15.2)	11 (8.4)	1.99 (0.63–6.24)	0.24

*Adjusted for age, smoking, and alcohol drinking.

Table 7 Association of *hRAD17* genotype with right and left colon cancer

Genotype	CRC patients *n* (%)	Controls *n* (%)	Adjusted OR* (95%CI)	*p*
Right colon cancer:				
Leu/Leu	22 (44.9)	513 (44.9)		
Leu/Arg	20 (40.8)	510 (44.7)	0.75 (0.41–1.38)	0.36
Arg/Arg	7 (14.3)	119 (10.4)	1.37 (0.59–3.17)	0.46
Leu/Arg+Arg/Arg	27 (55.1)	629 (55.1)	0.87 (0.50–1.51)	0.61
Leu/Leu+Leu/Arg	42 (85.7)	1,023 (89.6)		
Arg/Arg	7 (14.3)	119 (10.4)	1.57 (0.71–3.46)	0.22
Left colon cancer:				
Leu/Leu	81 (51.9)	513 (44.9)		
Leu/Arg	55 (35.3)	510 (44.7)	0.64 (0.44–0.93)	0.02
Arg/Arg	20 (12.8)	119 (10.4)	1.07 (0.63–1.84)	0.80
Leu/Arg+Arg/Arg	75 (48.1)	629 (55.1)	0.72 (0.51–1.02)	0.06
Leu/Leu+Leu/Arg	136 (87.2)	1,023 (89.6)		
Arg/Arg	20 (12.8)	119 (10.4)	1.31 (0.78–2.19)	0.31

*Adjusted for sex, age, smoking, and alcohol drinking.

Table 8 Association of *hRAD17* genotype with colon cancer and rectal cancer

Genotype	CRC patients *n* (%)	Controls *n* (%)	Adjusted OR* (95%CI)	*p*
Colon Cancer:				
Leu/Leu	45 (43.7)	513 (44.9)		
Leu/Arg	44 (42.7)	510 (44.7)	0.91 (0.58–1.43)	0.69
Arg/Arg	14 (13.6)	119 (10.4)	1.41 (0.73–2.71)	0.30
Leu/Arg + Arg/Arg	58 (56.3)	629 (55.1)	1.00 (0.66–1.53)	0.99
Leu/Leu + Leu/Arg	89 (86.4)	1,023 (89.6)		
Arg/Arg	14 (13.6)	119 (10.4)	1.47 (0.80–2.73)	0.22
Rectal Cancer:				
Leu/Leu	60 (57.1)	513 (44.9)		
Leu/Arg	31 (29.5)	510 (44.7)	0.49 (0.31–0.78)	0.003
Arg/Arg	14 (13.3)	119 (10.4)	0.98 (0.52–1.86)	0.95
Leu/Arg + Arg/Arg	45 (42.9)	629 (55.1)	0.58 (0.38–0.89)	0.012
Leu/Leu + Leu/Arg	91 (86.7)	1,023 (89.6)		
Arg/Arg	14 (13.3)	119 (10.4)	1.32 (0.71–2.45)	0.38

*Adjusted for sex, age, smoking, and alcohol drinking.

increased CRC risk between *hRAD17* polymorphism and heavy smokers/heavy drinkers. However, the Arg/Arg genotype compared to the Leu/Leu genotype and the Leu/Arg genotype in the recessive model was associated with an increased risk of CRC among the heavy smokers/light drinkers. Our results demonstrated that in males, cigarette smoking rather than alcohol consumption has a strong association between *hRAD17* polymorphism and the risk of CRC, unlike the findings for esophageal squamous cell carcinoma. The larger proportion of our present patients who were heavy smokers compared to the controls is probably attributable to the cumulative effect of DNA damage induced by chronic cigarette smoke.

A pooled meta-analysis of cohort studies revealed that in females, alcohol intakes more than 23 g ethanol/day was also reported to be associated with a higher risk of CRC by [8]. However, in the present females-only stratified analysis of lifestyle habits, no significant association of increased or decreased risk of CRC with either ethanol intake or *hRAD17* polymorphism was observed (data not shown).

Concerning the location of the cancer, the left colon and the right colon are developed from different embryonic origins, and they show different physiology and molecular backgrounds [20,21]. Generally, the right (proximal) colon cancer responds more poorly to chemotherapy compared to the left (distal) colon. In our patient series, only the left (distal) colon cancer and rectal cancer cases showed a significant association with the *hRAD17* Leu/Arg genotype. The implications of this difference remain to be elucidated.

It was reported that the association of smoking with rectal cancer among Japanese is stronger than that with colon cancer [7]. Although the association of smoking with the *hRAD17* genotype in rectal cancer was examined in the present study, a significant finding was not observed.

There were several limitations including small sample size, some self-selection bias for control group and inadequate adjustment for various confounding factors in our case-control study. For the subsequent stratified analyses, the small sample size might limit the statistical power of our study. We could not analyze a more detailed stratification of the present cases with the combined association of sex, lifestyle habit and the primary area of colorectal cancer because of the small number of female heavy smokers and heavy drinkers. Nonetheless, we identified the association between *hRAD17* polymorphism and the increased- and decreased-risk groups of CRC patients.

In conclusion, we found that in a Japanese population, the variant allele of *hRAD17* is significantly associated with a decreased risk of CRC among light smokers and rectal cancer patients and with an increased risk of CRC among heavy smokers. Larger studies on the *hRAD17* polymorphisms are warranted to test our results, and the genotyping of *hRAD17* codon546 could eventually enable the identification of individuals at high risk for the development of CRC cohort, giving

these individuals the incentive to improve their lifestyle habits.

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