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Original Article

Genetic polymorphism at codon 546 of the human RAD17 contributes to the risk for esophageal squamous cell carcinoma

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Abstract: Human *RAD17*, a human homolog of the *Schizosaccharomyces pombe* cell cycle checkpoint gene *RAD17*, plays a significant role in activating checkpoint signals in response to DNA damage. We evaluated the association of *hRAD17* Leu546Arg (rs1045051), a missense single nucleotide polymorphism, with the risk of esophageal squamous cell carcinoma in relation to smoking and alcohol consumption history in 154 esophageal squamous cell carcinoma male patients and 695 cancer-free male controls by a case-control study conducted in Japan. The results showed that the *hRAD17* Arg/Arg genotype compared to the Leu/Leu and Leu/Arg genotypes was significantly associated with the risk of the esophageal squamous cell carcinoma with an adjusted odds ratios of 2.22 (95% CI: 1.19-4.16 *P*=0.013). In stratified studies, the risk of esophageal squamous cell carcinoma was markedly higher in light drinkers (less than 23 g ethanol/day) with the Arg/Arg genotype than in heavy drinkers (excess of 23 g ethanol/day) with the Arg/Arg genotype (OR=2.83, 95% CI: 1.05-7.61, *P*=0.04). We concluded that the genetic variant of *hRAD17* Leu546Arg polymorphism exerts a significant effect on esophageal squamous cell carcinoma risk among Japanese men.

Keywords: Esophageal squamous cell carcinoma, single nucleotide polymorphism, human RAD17, DNA damage

Introduction

Esophageal cancer is one of the common causes of cancer death in Japan. The age-adjusted mortality rate of male esophageal cancer deaths was about eight times greater than that of female esophageal cancer deaths. For both male and female esophageal cancer patients, the proportion of stage I at diagnosis is usually small (nearly 25%) and the 5 year relative survival rate for stage I is almost 80% [1]. Therefore, early detection is important for effective therapeutic intervention for patients with esophageal cancer. Squamous cell carcinoma and adenocarcinoma are the two main histological subtypes of esophageal cancer, and more than 90% of esophageal cancer patients are diagnosed with squamous cell carcinoma in Japan [2].

Various risk factors have been associated with development of esophageal squamous cell carcinoma (ESCC). Cigarette smoking and alcohol drinking are the two major behavioral traits known to significantly increase the risk of ESCC [3-5]. On the other hand, higher intake of fruit and vegetable has been shown to have protective effect on the development of ESCC [6].

Genetic factors also play an important role in the development of esophageal squamous cell carcinoma. Polymorphisms in various cancer-associated genes including those involved in DNA damage repair have been associated with the risk of developing esophageal cancer [7]. Variant alleles of genes involved in metabolism of alcohol and carcinogens, such as, *ALDH2*1*2* and *CYP1A1* show significant correlations with the risk of esophageal cancer [8-11].

DNA damage induced by normal endogenous metabolic processes or environmental carcinogens can lead to gene mutations and genomic instability, which are expected to be accelerated in cells with reduced DNA repair capacity, also associated with increased risk of cancer. Due to similar underlying reasons, polymorphism in DNA repair genes contributing variations in DNA repair capacity also may be associated with the risk of developing ESCC [7, 12].

hRAD17, a human homologue of the *Schizosaccharomyces pombe* cell cycle checkpoint gene *RAD17*, is one of the important key regulators involved in activating checkpoint signals in response to DNA damage and incomplete DNA replication [13, 14]. The gene is located at chromosome 5q13.2, a locus frequently deleted in human cancers [15]. The signal transduction of DNA damage checkpoints engages multiple damage sensor proteins. Two sensor proteins recognize DNA damage and activate checkpoint signals, a replication factor C (RFC) like clamp loader complex formed by hRAD17 and four replication factor C subunits and a proliferating cell nuclear antigen (PCNA)-like sliding clamp heterotrimeric complex formed by RAD9, RAD1 and HUS1 (the 9-1-1 complex) [16, 17]. The 9-1-1 complex, loaded by the RAD17-containing clamp loader, preferentially binds at sites of DNA damage and facilitates ATR-mediated phosphorylation and activation of Chk1 regulating S-shape progression, G2/M arrest and replication fork repair [17-19].

In this paper, we report the risk of ESCC associated with a genetic polymorphism of the *hRAD17* gene among Japanese men.

Materials and methods

Study population

A hundred and fifty-four Japanese male patients with primary ESCC were surgically treated and histologically confirmed at Okayama University Hospital (Okayama, Japan) between 1992 and 2009. The clinical and histopathological classification of the tumors was defined according to the criteria of the UICC Tumor-Node-Metastasis Classification of Malignant Tumors (TNM), 6th edition, 2002. As the controls, 695 Japanese males were enrolled and consisted of two groups. 140 outpatients without cancer were recruited from Kusaka Hospital at Okayama in 2005 and 555 healthy individuals from Junpukai Health Care Center at Okayama in 2009. All patients and controls gave written informed consent. The Bioethics Committee of Okayama University Medical School approved this study.

The age, gender, personal and family medical history, smoking and alcohol drinking of the subjects were obtained from interviews and medical records. Smoking status was evaluated by pack-year equivalents ([cigarettes/day ÷ 20] × [smoking years]) and classified as non-smoker, <20 pack-years (light smokers), or ≥20 pack-years (heavy smokers). Alcohol drinking was assessed by daily ethanol intake using the method of calculating alcohol consumption (One serving of sake contains nearly 23 g ethanol which approximates 2 US standard drinks, 1 US drink contains 14 g of ethanol) [5, 8]. The recommended daily maximum amount of alcohol was based on the report of the limitation on daily ethanol intake by the Ministry of Health, Labor and Welfare (20 g/day; "Health Japan 21", a national health promotion movement) and the result of the prospective large cohort study showed the risk of cancer mortality was lowest among Japanese drinkers with ethanol intake less than 23 g/day [5]. The subjects were classified as non-drinker, <23 g/day (light drinkers), or ≥23 g/day (heavy drinkers).

Genotype analysis

Genomic DNA was extracted from peripheral blood lymphocytes by the standard procedures using proteinase K and phenol-chloroform.

Genotypes on the polymorphisms including the *hRAD17* Leu546Arg (rs1045051) were analyzed by SNaPshot methods. Target region were amplified for genotyping by the multiplex PCR.

The PCR was performed with a final reaction volume of 10 μl containing 10 ng template DNA, 1.6-8.0 pmol of each primer, 2.0 mM of each dNTP, 10 × PCR buffer, 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

Table 1. Basic characteristics of cases and controls

	Cases		Controls		*P*
	n	(%)	*n*	(%)	
Total	154		695		
Age (years)					0.005
<50	8	(5.2)	45	(6.5)	
50-59	40	(26.0)	368	(52.9)	
60-69	61	(39.6)	206	(29.6)	
≥70	45	(29.2)	76	(10.9)	
Median	64		57		
Range	41-86		36-87		
Smoking (pack-years)					<0.001
0	12	(7.8)	196	(28.2)	
<20	15	(9.7)	152	(21.9)	
≥20	127	(82.5)	347	(49.9)	
Alcohol drinking[a] (g of ethanol/day)					<0.001
0	5	(3.2)	196	(28.2)	
<23	22	(14.3)	272	(39.1)	
≥23	127	(82.5)	227	(32.7)	

[a]23 g of ethanol=nearly one serving of sake.

primers and dNTPs by incubating at 37°C for 90 min and at 75°C for 15 min. First PCR primer set was designed as: sense 5'-CAGTATCGGGAAAATTGCCTGG-3' and anti-sense 5'-GGACAGTAGAGACTCCCCCT-3'.

Single nucleotide primer extension reaction was performed using 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 $(NH_4)_2SO_4$, 2 μl SNaPshot Ready Reaction mix containing fluorescently labeled ddNTPs and DNA polymerase. The typing primer used for *hRAD17* Leu546Arg was 5'-TTTTTTTTTTTTTTTTTTTTTCAAGGTATGGCAATAGCTGAGTTTGG-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 ABI PRISM 3100 Genetic Analyzer (Applied Biosystems) and analyzed by Gene Mapper Software SNaPshot Analysis (Applied Biosystems).

Statistics

The allele frequency was calculated by direct counting. Deviation of the genotype frequency from Hardy-Weinberg equilibrium was analyzed by the exact test.

Chi square tests were used to examine the distribution between cancer patients and controls. The risk of ESCC was estimated by odds ratio (OR) and 95% confidence interval (CI). Odds Ratio (OR) and 95% confidence interval (CI) were adjusted for age, smoking and alcohol consumption status using multivariate logistic regression model by comparing the genotypes between patients and controls. *P* values less than 0.05 were considered statically significant.

All analyses were performed with SPSS software (version 12.0, SPSS Inc., Tokyo, Japan).

Results

Table 1 shows that there were significant differences in the distribution of age, smoking and alcohol consumption habits between controls and ESCC patients. Most of the patients were heavy smokers or drinkers. The never smokers and drinkers of the controls were much larger than esophageal carcinoma patients. The control subjects were consisted from two sources, therefore, we assessed the genotype distributions of polymorphism in the control subjects using the Hardy-Weinberg equilibrium. The *P* values for the Hardy-Weinberg equilibrium of *hRAD17* Leu546Arg were 0.16 and 0.50, for these two data-sets, respectively. Excess deviation from the equilibrium in these control populations was not found.

The allele and genotype frequencies of *hRAD17* Leu546Arg among patients and control subjects are shown in **Table 2**. The Arg/Arg genotype compared to the Leu/Leu genotype and

Table 2. Human *RAD17* genotype in cases and controls

Gene	Genotype	Cases		Controls		Adjusted OR[a] (95% CI)		*P*[b]
		n	(%)	*n*	(%)			
RAD17 Leu546Arg								
	Leu/Leu	74	(48.1)	322	(46.3)		(Reference)	
	Leu/Arg	57	(37.0)	312	(44.9)	0.67	(0.43-1.05)	0.079
	Arg/Arg	23	(14.9)	61	(8.8)	1.85	(0.96-3.57)	0.067
	Leu/Leu + Leu/Arg	131	(85.1)	634	(91.2)		(Reference)	
	Arg/Arg	23	(14.9)	61	(8.8)	2.22	(1.19-4.16)	0.013
Allele frequencies								
	Leu	205	(66.6)	956	(68.8)			0.233
	Arg	103	(33.4)	434	(31.2)			

[a]Adjusted for age, smoking, and alcohol drinking. [b]*P* values, difference in genotype frequencies between cases and controls.

Table 3. Effect of tobacco and alcohol consumption on *hRAD17* genotype in cases and controls

	RAD17 Leu546Arg	Cases		Controls		Adjusted OR[a] (95% CI)		*P*
		n	(%)	*n*	(%)			
Smoking (pack-years)								
<20	Leu/Leu	12	(44.4)	155	(44.5)		(Reference)	
	Leu/Arg	10	(37.0)	161	(46.3)	0.67	(0.27-1.65)	0.379
	Arg/Arg	5	(18.5)	32	(9.2)	2.12	(0.67-6.73)	0.202
	Leu/Leu + Leu/Arg	22	(81.5)	316	(90.8)		(Reference)	
	Arg/Arg	5	(18.5)	32	(9.2)	2.58	(0.87-7.60)	0.087
≥20	Leu/Leu	62	(48.8)	167	(48.1)		(Reference)	
	Leu/Arg	47	(37.0)	151	(43.5)	0.71	(0.42-1.20)	0.198
	Arg/Arg	18	(14.2)	29	(8.4)	1.78	(0.82-3.87)	0.144
	Leu/Leu + Leu/Arg	109	(85.8)	318	(91.6)		(Reference)	
	Arg/Arg	18	(14.2)	29	(8.4)	2.07	(0.98-4.35)	0.055
Alcohol drinking (g of ethanol/day)								
<23	Leu/Leu	13	(48.1)	219	(46.8)		(Reference)	
	Leu/Arg	8	(29.6)	205	(43.8)	0.63	(0.25-1.58)	0.323
	Arg/Arg	6	(22.2)	44	(9.4)	2.32	(0.81-6.63)	0.117
	Leu/Leu + Leu/Arg	21	(77.8)	424	(90.6)		(Reference)	
	Arg/Arg	6	(22.2)	44	(9.4)	2.83	(1.05-7.61)	0.039
≥23	Leu/Leu	61	(48.0)	103	(45.4)		(Reference)	
	Leu/Arg	49	(38.6)	107	(47.1)	0.67	(0.39-1.14)	0.136
	Arg/Arg	17	(13.4)	17	(7.5)	1.64	(0.72-3.74)	0.242
	Leu/Leu + Leu/Arg	110	(86.6)	210	(92.5)		(Reference)	
	Arg/Arg	17	(13.4)	17	(7.5)	1.97	(0.90-4.34)	0.091

[a]Adjusted for age, smoking, and drinking status.

the Leu/Arg genotype at the *hRad17* Leu546Arg polymorphism in recessive genetic model was associated with increased risk of ESCC (OR=2.22, 95% CI: 1.19-4.16, p=0.01). The association of the Arg/Arg genotype compared to the Leu/Leu genotype in additive genetic model with the risk of ESCC was not found (OR=1.85, 95% CI: 0.96-3.57, p=0.07). The difference of the allele frequencies between the cases and controls was not statistically significant.

Next, we investigated the association between the *hRAD17* Leu546Arg genotypes and the risk of ESCC with stratification according to the history of smoking and alcohol consump-

Table 4. Combined effects of tobacco and alcohol consumption on *hRAD17* genotype in cases and controls

	RAD17 Leu546Arg	Cases		Controls		Adjusted OR[a] (95% CI)		*P*
		n	(%)	*n*	(%)			
Alcohol drinking (g of ethanol/day) <23								
Smoking (pack-years)								
<20	Leu/Leu	5	(55.6)	119	(46.3)		(Reference)	
	Leu/Arg	2	(22.2)	112	(43.6)	0.43	(0.08-2.26)	0.315
	Arg/Arg	2	(22.2)	26	(10.1)	2.09	(0.37-11.7)	0.402
	Leu/Leu + Leu/Arg	7	(77.8)	231	(89.9)		(Reference)	
	Arg/Arg	2	(22.2)	26	(10.1)	2.89	(0.55-15.1)	0.208
≥20	Leu/Leu	8	(44.4)	100	(47.4)		(Reference)	
	Leu/Arg	6	(33.3)	93	(44.1)	0.76	(0.25-2.32)	0.629
	Arg/Arg	4	(22.2)	18	(8.5)	2.45	(0.65-9.3)	0.188
	Leu/Leu + Leu/Arg	14	(77.8)	193	(91.5)		(Reference)	
	Arg/Arg	4	(22.2)	18	(8.5)	2.78	(0.80-9.61)	0.107
Alcohol drinking (g of ethanol/day) ≥23								
Smoking (pack-years)								
<20	Leu/Leu	7	(38.9)	36	(39.6)		(Reference)	
	Leu/Arg	8	(44.4)	49	(53.8)	0.60	(0.18-2.00)	0.405
	Arg/Arg	3	(16.7)	6	(6.6)	1.78	(0.32-9.90)	0.508
	Leu/Leu + Leu/Arg	15	(83.3)	85	(93.4)		(Reference)	
	Arg/Arg	3	(16.7)	6	(6.6)	2.38	(0.49-11.60)	0.282
≥20	Leu/Leu	54	(49.5)	67	(49.3)		(Reference)	
	Leu/Arg	41	(37.6)	58	(42.6)	0.69	(0.38-1.24)	0.213
	Arg/Arg	14	(12.9)	11	(8.1)	1.58	(0.61-4.04)	0.341
	Leu/Leu + Leu/Arg	95	(87.2)	125	(91.9)		(Reference)	
	Arg/Arg	14	(12.8)	11	(8.1)	1.85	(0.75-4.58)	0.181

[a]Adjusted for age, smoking, and drinking status.

tion (**Table 3**). Among light drinkers, including non-drinkers and rare-drinkers, the Arg/Arg genotype compared to the Leu/Leu genotype and the Leu/Arg genotype in recessive model was significantly associated with increased risk of ESCC (OR=2.83, 95% CI: 1.05-7.61, p=0.04). However, the association between the Arg/Arg genotype among heavy drinkers with the risk of ESCC was not found. There was no significant association between the Arg/Arg genotype and the risk of ESCC in light or heavy smokers.

We also examined the combined effects of smoking and alcohol consumption on the genotype distribution of *hRAD17* Leu546Arg polymorphism (**Table 4**). Among ESCC male patients, the combined group of heavy smokers and heavy drinkers outnumbered the other combined group. Thus, the sample size of the groups, consisting of heavy drinkers and light smokers, of light drinkers and light smokers, or of light drinkers and heavy smokers, were small among the patients. In contrast to the patients, the control subjects of light smokers and light drinkers were much larger. The result showed no statistically significant association between ESCC risk and the Arg/Arg genotype with all combinations of smoking and alcohol consumption.

In addition to ESCC, we also examined the association between the *hRAD17* Leu546Arg genotypes and the risk of various cancers including head and neck squamous cell carcinoma (HNSCC), gastric adenocarcinoma (GC), lung adenocarcinoma (LAD), and lung squamous cell carcinoma (LSQ) (**Table 5**). Cancer patients were diagnosed and treated at Okayama University hospital (Okayama, Japan) and gave written informed consent. The Bioethics Committee of Okayama University Medical School approved this study. Gastric

Table 5. The association between RAD17 Leu546Arg genotype and GC, HNSCC, LSQ and LAD risks

Gene	Genotype	Cases		Controls		Adjusted OR[a] (95% CI)		*P*
		n	(%)	*n*	(%)			
Gastric cancer (GC)								
	Leu/Leu	35	(47.9)	322	(46.3)		(Reference)	
	Leu/Arg	31	(42.5)	312	(44.9)	0.80	(0.46-1.39)	0.422
	Arg/Arg	7	(9.6)	61	(8.8)	1.00	(0.37-2.65)	0.994
	Leu/Leu + Leu/Arg	66	(90.4)	634	(91.2)		(Reference)	
	Arg/Arg	7	(9.6)	61	(8.8)	1.11	(0.44-2.85)	0.822
Allele frequencies								
	Leu	101	(69.2)	956	(68.8)			
	Arg	45	(30.8)	434	(31.2)	0.91	(0.61-1.37)	0.658
Head and neck squamous cell carcinoma (HNSCC)								
	Leu/Leu	41	(55.4)	322	(46.3)		(Reference)	
	Leu/Arg	27	(36.5)	312	(44.9)	0.71	(0.41-1.21)	0.207
	Arg/Arg	6	(8.1)	61	(8.8)	0.73	(0.27-1.97)	0.534
	Leu/Leu + Leu/Arg	68	(91.9)	634	(91.2)		(Reference)	
	Arg/Arg	6	(8.1)	61	(8.8)	0.86	(0.33-2.25)	0.754
Allele frequencies								
	Leu	109	(73.6)	956	(68.8)			
	Arg	39	(26.4)	434	(31.2)	0.79	(0.53-1.19)	0.261
Lung cancer squamous cell carcinoma (LSQ)								
	Leu/Leu	59	(47.2)	322	(46.3)		(Reference)	
	Leu/Arg	50	(40.0)	312	(44.9)	0.92	(0.58-1.48)	0.745
	Arg/Arg	16	(12.8)	61	(8.8)	1.38	(0.66-2.89)	0.399
	Leu/Leu + Leu/Arg	109	(87.2)	634	(91.2)		(Reference)	
	Arg/Arg	16	(12.8)	61	(8.8)	1.43	(0.70-2.90)	0.324
Allele frequencies								
	Leu	168	(67.2)	956	(68.8)			
	Arg	82	(32.8)	434	(31.2)	1.08	(0.77-1.52)	0.648
Lung cancer adeno carcinoma (LAD)								
	Leu/Leu	97	(46.0)	322	(46.3)		(Reference)	
	Leu/Arg	92	(43.6)	312	(44.9)	0.99	(0.69-1.42)	0.942
	Arg/Arg	22	(10.4)	61	(8.8)	1.19	(0.65-2.19)	0.567
	Leu/Leu + Leu/Arg	189	(89.6)	634	(91.2)		(Reference)	
	Arg/Arg	22	(10.4)	61	(8.8)	1.20	(0.67-2.15)	0.536
Allele frequencies								
	Leu	286	(67.8)	956	(68.8)			
	Arg	136	(32.2)	434	(31.2)	1.05	(0.81-1.36)	0.719

[a]LSQ, LAD and HNSCC were adjusted for age and smoking status. GC was adjusted for age, smoking, and drinking status.

cancer cohort was adjusted for age, smoking habit and alcohol consumption. Head and neck squamous carcinoma and lung cancers were adjusted for age and smoking habit because no alcohol consumption data was available. There was no significant association between *hRAD17* Leu546Arg polymorphism and these other cancers examined.

Discussion

In this case-control study, we analyzed association between the codon 564 polymorphism causing amino-acid substitution of leucine to arginine, a missense SNP in *hRAD17* gene, and ESCC risk. We found that the *hRAD17* Leu564Arg variant was associated with the occurrence of esophageal squamous cell cancer in the recessive genetic model. To our knowledge, this is the first report describing that the *hRad17* polymorphism has an association with the risk of ESCC.

Human *RAD17* is a human homologue of *Schizosaccharomyces pombe* cell cycle checkpoint gene *RAD17* [14]. The protein encoded by *hRAD17* gene is required for cell cycle arrest and DNA damage repair in response to DNA

damage and incomplete DNA replication. The phosphorylation of hRAD17 protein by ATR is required for cell cycle G2 phase arrest with CHK1 activation induced by DNA damage [18, 19].

HRAD17 protein has two important regions, P loop (Walker A motif) which is critical for nucleotide binding of ATPases and C terminus containing two SQ motifs (Ser635 and Ser645) that are strong targets for ATP kinase. The phosphorylation on both SQ sites is essential for hRAD17 function facilitating interaction with the RAD9-HUS1-RAD1 complex and is implicated in the activation of the G2/M checkpoint [18, 19].

Replication factor C is a clamp loader playing important roles in DNA metabolism by loading the clamps in response to DNA damage. HRAD17 forms a replication factor C like clamp loader complex with the four small RFC subunits and loads the RAD9-HUS1-RAD1 clamp complex, a PCNA-like sliding clamp, onto damaged DNA site in an ATP-dependent activation process. The RAD9-HUS1-RAD1 complex facilitates ATR mediated phosphorylation and activation of CHK1 which regulates S-shape progression, G2/M arrest, and replication fork repair [16]. The hRAD17 codon 546 polymorphism is located near SQ motif phosphorylated by ATR. The amino acid substitutions from leucine to arginine at codon 546 may have effect on the ATR-dependent phosphorylation at SQ site in response to DNA damage.

A recent study showed that loss of hRAD17 expression occurred frequently in head and neck squamous cell carcinoma [20]. The expression of *hRAD17* mRNA in tumor tissue decreased compared with normal tissue of head and neck squamous cell carcinoma patients. Although low expression of hRAD17 in ESCC has not been reported, a strong correlation between excess smoking and alcohol drinking and head and neck squamous cell carcinoma has been found, as is the case for ESCC. Therefore, it is likely that down regulation of hRAD17 may also be contributed to the development of ESCC.

The data showed that Arg/Arg homozygosity has significant association with the risk of ESCC among male patients compared to Leu/Leu homozygosity and Leu/Arg heterozygosity among light drinkers. Previous studies observed that excessive alcohol drinking increased the risk of ESCC among Japanese men who had a deficient phenotype for aldehyde dehydrogenase-2 (ALDH2) [8-10]. However, we could not find the association between the *hRAD17* Leu546Arg polymorphism and the risk of ESCC among male patients with history of heavy drinking. Our result also indicated that there was no significant association between the *hRAD17* Leu546Arg polymorphism and the risk of other cancers including head and neck squamous cell carcinoma, gastric cancer, lung squamous cell carcinoma and lung adenocarcinoma.

Recently, RAD17 homolog isofom1 has been reported to be down-regulated in ethanol-treated human embryonic carcinoma cell-derived embryoid bodies [21]. Chronic and heavy exposure of esophageal squamous cell to alcohol may lead to down-regulated *hRAD17* gene transcription. Consequently, allele independent transcriptional downregulation of the gene rather than the genetic variant *hRAD17* Leu546Arg may be associated with the risk of ESCC among heavy drinkers.

The expression level of hRAD17 is different for each type of cancer, lower expression in head and neck squamous cell carcinoma [20] and overexpression in colon carcinoma, non-small cell lung carcinoma and breast cancer [15, 22-24]. In fission yeast, the reduction of yeast colony growth and slower progression through cell cycle were observed by expression of hRAD17 in a *S. pombe rad17* deleted strain [25]. These reports suggest that hRAD17 plays an important role in regulation of tumor growth.

Our case-control study has several limitations including small sample size and inadequate adjustment for various confounding factors. The small sample size may limit the statistical power of our study, particularly for the subgroup analysis. The number of female patients with ESCC is smaller than male patients, so we could not investigate the effect of *hRAD17* polymorphism among female ESCC patients. The difference in smoking and alcohol drinking status between ESCC male patients and cancer-free controls was statistically significant. Therefore, we could not perform more detailed stratification of patients of non-smoker and non-drinker. Although our study has these limi-

tations, we identified the association between *hRAD17* polymorphism and the high risk group of ESCC.

In conclusion, we found that homozygosity of a variant allele of *hRAD17* is significantly associated with ESCC risk in Japanese men, in particular, among moderate alcohol drinkers. Larger studies on the *hRAD17* polymorphisms are warranted to confirm our results.

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Disclosure of conflict of interest

None.

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