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Different Susceptibility of Each *L-myc* Genotype to Esophageal Cancer Risk Factors

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To understand the relationship between the *L-myc* genotypes and esophageal cancer risk, a polymerase chain reaction-based restriction fragment length polymorphism analysis was performed on 91 Japanese patients with esophageal cancer and 241 non-cancer outpatients. No significant difference in the distribution of genotypes was observed between patients and controls; 18.7% LL genotype, 56.0% LS and 25.3% SS among patients, and 24.5%, 55.6% and 19.9%, respectively, among controls. Frequency of the s-allele in patients (0.533) was slightly higher than in controls (0.477), but the difference was not statistically significant. However, the odds ratios (ORs) for smoking or heavy drinking were markedly higher in SS and LS genotypes than in LL genotype; age-sex-adjusted ORs for smoking was 7.57 in the SS genotype, 6.40 in the LS genotype and 1.77 in the LL genotype. Age-sex-adjusted ORs for heavy drinking were 19.78, 18.20 and 7.40, respectively. The age-sex-adjusted ORs for both factors combined were 12.77, 18.45 and 1.44, respectively. These results suggested that the *L-myc* polymorphism might modify the effects of lifestyle factors on esophageal cancer risk.

Key words: Esophageal cancer — *L-myc* polymorphism — Risk factor — Lifestyle

The *L-myc* gene, belonging to the *Myc* family, was first isolated from a small cell lung cancer and located on chromosome 1p32.[1)] Genomic DNA within the *L-myc* locus shows an *Eco*RI RFLP defined by two alleles with the 10 kb fragment (l-allele) and the 6.6 kb fragment (s-allele), which gives three genotypes, LL, LS and SS.[2)] This polymorphism can also be detected by *Eco*RI digestion of the PCR fragment including the polymorphic site.[3, 4)] Though no functional differences between l- and s-alleles are known, the association of s-allele with tumor susceptibility was reported in non-Hodgkin's lymphoma,[5)] gastric cancer,[6, 7)] hepatocellular carcinoma[8)] and sarcoma.[9)] It was also reported that lung cancer patients with s-allele exhibited a much higher incidence of metastasis.[10–12)] On the other hand, no associations were reported between the *L-myc* genotype and susceptibility to renal cancer,[13)] oral cancer,[14)] gastric cancer,[6, 15)] breast cancer,[6)] lung cancer[16, 17)] or bladder cancer.[18)] Therefore the relationship between cancer susceptibility and the *L-myc* polymorphism is not yet well established.

To date, only one study has been reported on the relationship between susceptibility to esophageal cancer and the *L-myc* polymorphism, showing that esophageal cancer risk was increased in the SS genotype.[19)] Since opposite results were reported for the relationship between the susceptibility to some types of cancer and the *L-myc* polymorphism as described above, further studies are also required on esophageal cancer. In the present study, we assessed the relationship between the *L-myc* genotype and esophageal cancer risk. The risk of lifestyle factors was also examined in each genotype, since some lifestyle factors such as smoking and drinking are well-known risk factors for esophageal cancer.[20, 21)]

MATERIALS AND METHODS

Subjects Blood samples were collected from 91 esophageal cancer patients diagnosed between 1984 and 2000 (cases) and 241 non-cancer volunteers among gastroscopy examinees (controls) at the Aichi Cancer Center Hospital. Effects of lifestyle factors were analyzed with questionnaires obtained from 91 cases and 241 controls. Age and sex distributions in cases and controls are shown in Table I. Consistent with a previous report,[21)] the majority of cases in our study were males. All the controls were used

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Abbreviations: OR, odds ratio; RFLP, restriction fragment length polymorphism; 95%CI, 95% confidence intervals.

for case-control analysis without age-sex-matching, since it was demonstrated to be a better approach.[22] Informed consent was obtained from all subjects in this study.

Analysis of *L-myc* genotype by PCR-RFLP Genomic DNA was isolated from peripheral blood cells using the QIAamp DNA Blood Mini Kit (QIAGEN Inc., Hilden, Germany). For specific amplification of intron 2 of the *L-myc* locus including the *Eco*RI polymorphic site, PCR was performed according to Shibuta *et al.*[7] with the upstream primer, 5′-ACGGCTGGTGGAGTGGTAGA-3′, and the downstream primer, 5′-AAGCTTGAGCCCCTTTGTCA-3′. The PCR was performed with 30 cycles of denaturation at 94°C for 20 s, annealing at 55°C for 20 s and extension at 72°C for 30 s. Amplified fragments were then digested with *Eco*RI and separated by electrophoresis on 2% agarose gel.

Table I. Sex and Age among Cases and Controls

	Cases No. (%)	Controls No. (%)
Sex		
male	76 (83.5)	118 (49.0)
female	15 (16.5)	123 (51.0)
total	91	241
Age (yr)		
−49	8 (8.7)	46 (19.1)
50−59	34 (37.4)	90 (37.3)
60−69	34 (37.4)	105 (43.6)
70−	15 (16.5)	0 (0.0)
mean	60.4±8.0	56.8±7.9

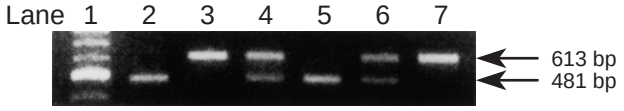

Fig. 1. PCR-RFLP analysis of the *L-myc* polymorphism. A representative example of agarose gel electrophoresis is shown here. Lane 1, a 100 bp-ladder marker; lanes 2 and 5, the homozygote for s-allele (481 bp); lanes 3 and 7, the homozygote for l-allele (613 bp); lanes 4 and 6, the heterozygote.

Questionnaire and data collection The questionnaire elicited information about subjects' age, sex, smoking status and alcohol consumption. For analyzing the risk of smoking, only current smokers were included in the category. For analyzing the risk of heavy drinking, people who drink alcohol 5 days or more a week and 50 ml ethanol or more per day were included in the category.

Statistical analysis and risk assessment for lifestyle For statistical analysis of genotype distribution, the χ^2 test was performed. The age (as a continuous variable)- and sex-adjusted ORs and 95%CI were estimated for smoking status and alcohol consumption, using an unconditional logistic regression model. The model was also applied to the estimation of age-sex-adjusted interaction terms of genotype with smoking and/or heavy drinking, i.e., the ratio of the ORs for the lifestyle factors by genotype. The 'logistic' procedure provided by STATA version 6.0 (STATA Corp., College Station, TX) was used for the calculations. Adjustments for multiple comparisons were not conducted because this is an exploratory study.

RESULTS

Distribution of *L-myc* genotype in cases and controls The *Eco*RI digestion of PCR products clearly revealed the l-allele band (613 bp) and the s-allele band (481 bp). Representative results are shown in Fig. 1, and the results are summarized in Table II. The observed frequency of each genotype in cases and controls fitted the Hardy-Weinberg equilibrium (χ^2=1.44, P=0.230 and χ^2=3.15, P=0.076, respectively). No statistically significant difference in the distribution of the genotypes was found between cases and controls (χ^2=1.86, degree of freedom=2, P=0.39). The age-sex-adjusted ORs for LS and SS genotypes were not significantly different from that for LL genotype.

ORs for smoking and heavy drinking according to *L-myc* genotype We analyzed the effects of smoking and/or heavy drinking in each genotype group, as well as the whole subjects. As summarized in Table III, the age-sex-adjusted ORs for smoking and/or heavy drinking were significantly high. Subgroup analyses according to the *L-myc* genotypes showed that the ORs in the SS and LS genotypes for smoking and/or heavy drinking were signifi-

Table II. Distribution of *L-myc* Genotypes and Allele Frequencies in Cases and Controls

	n	Genotypes (%)			Alleles frequencies	
		LL	LS	SS	l	s
Patients	91	17 (18.7)	51 (56.0)	23 (25.3)	0.467	0.533
Healthy controls	241	59 (24.5)	134 (55.6)	48 (19.9)	0.523	0.477
Age-sex-adjusted OR (95%CI)		1.00	1.18 (0.60−2.31)	1.76 (0.80−3.88)		

Table III. Age-sex-adjusted ORs and 95%CIs of Smoking and Heavy Drinking According to *L-myc* Genotype

	Genotype	Cases[a)]	Controls[a)]	OR[b)]	95%CI[b)]	*P*[b)]	OR	95%CI	*P*
Smoking	total	33/58	186/55	4.99	2.76–9.02	<0.001	4.22[c)]	2.15–8.30[c)]	<0.001[c)]
	LL	8/9	39/20	1.77	0.54–5.75	0.345	1.38	0.37–5.11	0.631
	LS	18/33	111/23	6.40	2.74–14.95	<0.001	7.49	2.65–21.16	<0.001
	SS	7/16	36/12	7.57	1.91–30.03	0.004	3.26	0.65–16.44	0.152
Heavy drinking	total	30/61	215/26	13.95	6.99–27.86	<0.001	12.26[d)]	5.97–25.19[d)]	<0.001[d)]
	LL	7/10	52/7	7.40	1.86–29.43	0.004	7.08	1.75–28.57	0.006
	LS	16/35	121/13	18.20	6.52–50.81	<0.001	20.40	6.52–63.81	<0.001
	SS	7/16	42/6	19.78	4.26–91.84	<0.001	13.17	2.64–65.71	0.002
Smoking and heavy drinking	total	51/40	226/15	9.19	4.42–19.12	<0.001			
	LL	13/4	53/6	1.44	0.33–6.34	0.630			
	LS	28/23	129/5	18.45	5.43–62.61	<0.001			
	SS	10/13	44/4	12.77	3.13–52.18	<0.001			

a) "Non-current smokers"/"current smokers" for smoking and "those who drink 50 ml or over ethanol and 5 days or more a week"/"the others" for heavy drinking.
b) Age-sex-adjusted.
c) Age-sex- and drinking status-adjusted.
d) Age-sex- and smoking status-adjusted.

cantly high, while the ORs in the LL genotype for smoking and for smoking and heavy drinking were not. Though not statistically significant, marked differences in the ORs between LL and LS/SS genotypes were observed for smoking or heavy drinking, especially for smoking, the OR in LS/SS genotypes being four times higher than that in the LL genotype; the age-sex-adjusted interaction was 2.56 (95%CI 0.98–6.66). Meanwhile, that for heavy drinking was 2.5 times higher than that in the LL genotype, and the corresponding interaction between LL and LS/SS genotypes was 1.80 (95%CI 0.58–5.58). When both smoking and heavy drinking were added to the logistic model, the ORs for smoking or heavy drinking were similar for the whole subjects and each genotype, except that the OR in the SS genotype for smoking lost significancy. The OR for both factors combined in LS/SS genotypes was 9–13 times higher than that in the LL genotype. The age-sex-adjusted interaction between LL and LS/SS genotypes was statistically significant (7.20, 95%CI 1.57–32.94).

DISCUSSION

Several studies were reported on the relationship between the *L-myc* polymorphism and susceptibility to a variety of cancers after the polymorphism was identified. Some of these studies showed a positive relationship between *L-myc* genotypes and susceptibility to some types of cancer,[7, 9, 19)] and metastasis,[6, 11, 12)] but other studies found no relationship between *L-myc* genotypes and susceptibility to the same and other types of cancer[6, 13–18, 23)] and metastasis.[6, 13, 15)] This suggests that further studies are required to clarify the relationship between *L-myc* genotypes and susceptibility to cancer.

In our results, the frequencies of the three genotypes in esophageal cancer patients and controls fitted the Hardy-Weinberg equilibrium. The genotype distribution in cases was not significantly different from that in controls, although the s-allele was more frequent in the patients. Shibuta *et al.*[19)] recently reported a significantly different distribution of the genotype and significantly more s-allele in esophageal cancer patients, though the OR for LS/SS genotypes relative to LL genotype was not significant (OR=2.90, 95%CI 0.54–6.54). The allele frequency in this study was not significant (χ^2=1.65, P=0.200), but both studies showed a similar role of the s-allele.

The age-sex-adjusted OR for smoking was 4.99 and the age-sex-drinking status-adjusted OR was 4.22, while the corresponding ORs for heavy drinking were 13.95 and 12.26, which is in good accordance with a previous report.[24)] When cases were divided into the *L-myc* genotypes, the ORs for smoking or heavy drinking were markedly high in the LS and SS genotypes, while the LL genotypes had relatively small ORs for smoking or heavy drinking, respectively (Table III). However, the interaction term was not significant because of the small number of patients. The OR for smoking and heavy drinking combined was 9.19. The OR was markedly high in the LS and SS genotypes, while the LL genotype had a relatively small OR. The interaction term was significant. To our knowledge, this is the first report on the interaction of the *L-myc* polymorphism with smoking and drinking. To confirm this result, a further study with a much larger sample of patients is essential.

Since alcohol and tobacco consumption differ greatly among local areas in Japan,[25, 26] it is possible that the proportion of smokers and/or heavy drinkers among the subjects was larger in the study by Shibuta *et al.*[19] than ours, though they did not mention the frequencies of smokers and heavy drinkers among their subjects. If so, populations with s-allele might exhibit a higher susceptibility to esophageal cancer than those without s-allele.

It is unclear why the risks of smoking and heavy drinking are different among the genotypes, since functional differences between l- and s-alleles have not been reported. Members of the *Myc* gene family are often activated in a variety of human tumors, such as the *L-myc* gene amplification in human small cell lung cancer,[1] the *N-myc* gene amplification in neuroblastoma,[27] and the *c-myc* gene overexpression in breast cancer cells.[28] Though the relationship between the level of *L-myc* expression and genotypes is not clear, it is possible that a different level of expression might be caused by each allele. Alternatively, each allele of the *L-myc* gene may be associated with polymorphism of an unknown gene located in the vicinity of the *L-myc* locus that influences susceptibility to esophageal cancer. Furthermore, alternative splicing of the *L-myc* transcript was reported, and could play a different role.[2, 29] It is also possible that each allele of the *L-myc* gene influences this alternative splicing. In any case, to elucidate the molecular mechanism of the relationship between each *L-myc* genotype and the risk associated with lifestyle factors, further molecular analysis of the *L-myc* gene function must be performed.

It would be of interest to know whether the differences in the ORs for smoking and/or heavy drinking according to the *L-myc* genotype are also observed in other smoking and/or heavy drinking related cancers, such as head and neck cancer and lung cancer. If the genotypes enhancing the effects of smoking and/or alcohol are identified, more individualized recommendations to quit smoking and/or to reduce alcohol intake will become possible with a beneficial impact on cancer prevention. We are now conducting case-control studies of the possible relationship between the *L-myc* polymorphism and risk factors among these cancers.

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