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L-myc Genotype is Associated with Different Susceptibility to Lung Cancer in Smokers

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We have shown that *L-myc* genotype is associated with the risk of esophageal cancer from smoking and heavy drinking. In this study, we have analyzed the relationship between the *L-myc* genotypes and lung cancer risk from smoking in 191 Japanese lung-cancer patients and 241 non-cancer controls. The odds ratios (ORs) were markedly higher in SS and LS genotypes than in LL genotype; age-sex-adjusted ORs were 3.19, 2.30 and 0.92, respectively. This result suggests that the *L-myc* polymorphism may affect the induction of lung cancer by smoking. The OR for smoking in SS-genotype patients diagnosed within 2 years was higher than that in other SS patients, suggesting that smoking-related lung cancer in SS genotype might exhibit a poorer prognosis.

Key words: Lung cancer—*L-myc* polymorphism—Risk factor—Smoking

The *L-myc* gene belonging to the *Myc* family was first isolated from a small cell lung cancer and shown to be located on chromosome 1p32.[1)] Genomic DNA within the *L-myc* locus shows an *Eco*RI restriction fragment length polymorphism (RFLP) defined by two alleles, giving 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, it was reported that lung cancer patients with s-allele exhibited a much higher incidence of metastases to the lymph nodes and other organs at the time of surgery.[5–7)] A poor prognosis was also observed in lung cancer patients with SS and LS genotypes.[5,6)] On the other hand, no differences were reported in prognosis or in metastases to the lymph nodes and other organs among the three genotypes in lung cancer patients.[8–10)] Therefore the relationship between lung cancer and the *L-myc* polymorphism remains to be established.

Recently we showed a clear association between the *L-myc* genotype and the risks from lifestyle factors, such as smoking and heavy drinking, in esophageal cancer.[11)] The risk of esophageal cancer from smoking and/or heavy drinking was increased in SS and LS genotypes. Since it is well known that smoking is a strong risk factor for lung cancer,[12,13)] and some reports have suggested that drinking might also be related with it,[14,15)] further studies on lung cancer are required to establish whether a different risk from smoking and/or heavy drinking is observed among each *L-myc* genotype. In the present study, we assessed the relationship between the *L-myc* genotype and lung cancer risk, after which the risk from smoking and heavy drinking was examined in each genotype.

Blood samples were collected in 1999 and 2000 from 191 lung cancer patients (cases) who were diagnosed from 1984 to 2000 at the Aichi Cancer Center Hospital and could visit the hospital at the time of collection. The total number of lung cancer patients diagnosed between 1984 and 2000 at the Aichi Cancer Center Hospital was 1405. Histological characteristics of these cancer patients and the cases in this study are shown in Table I. Since adenocarcinoma has become more common and squamous cell carcinoma less frequent at the Aichi Cancer Center Hospital, and half of the blood samples from cases were obtained less than 3 years after diagnosis, adenocarcinoma is more frequent in cases than in all patients. However, the overall distribution of cell types in cases and that in all lung cancer patients are not very different. Non-cancer controls were also collected in 1999 and 2000 from 241 subjects of a *Helicobacter pylori* eradication study and gastroscopy examinees. Subjects were first asked to provide written informed consent for genotyping study at various polymorphic loci. Then 7 ml of blood was collected and the questionnaire was distributed to subjects to elicit information about age, sex, smoking status and alcohol consumption at the time of diagnosis from lung cancer patients, and those at the present time from non-cancer controls. This study has been approved by the ethical committee of the

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Table I. Histological Characteristics in Total Lung Cancer Patients Diagnosed from 1984 to 2000 and Cases Enrolled in This Study

Cell type	All lung cancer patients (%)	Cases (%)[a)]
Adenocarcinoma	828 (58.9)	137 (71.7)
Squamous cell carcinoma	383 (27.3)	38 (19.9)
Large cell carcinoma	79 (5.6)	14 (7.3)
Small cell carcinoma	25 (1.8)	0 (0.0)
Others and unknown	90 (6.4)	2 (1.1)
Total	1405	191

a) Cases were enrolled into this study from among all lung cancer patients.

Table II. Age and Sex Distribution among Cases and Controls

	No. (%)	
	Cases	Controls
Age (yr.)		
−49	21 (11.0)	46 (19.1)
50−59	60 (31.4)	90 (37.3)
60−69	72 (37.7)	105 (43.6)
70−	38 (19.9)	0 (0.0)
Mean	61.0±9.7	56.8±7.9
Sex		
Male	114 (59.7)	118 (49.0)
Female	77 (40.3)	123 (51.0)
Total	191	241

Aichi Cancer Center for genetic polymorphism examinations.

For analyzing the risk from smoking, current and ex-smokers were included in the category. For analyzing the risk from heavy drinking, people who drank alcoholic beverages 5 days or more a week at a level of 50 ml ethanol or more per day were included in the category. Age and sex distributions in cases and controls are shown in Table II. Without age-sex-matching, all the controls were used for a case-control analysis, since this was demonstrated to be a better approach.[16)]

Isolation of genomic DNA from blood samples, genotyping of the *L-myc* locus, statistical analysis and a risk assessment for smoking and heavy drinking were performed according to our previous study.[11)] The 'logistic' procedure provided by STATA version 6.0 (STATA Corp., College Station, TX) was used for the calculations. Adjustments for the multiple comparisons were not conducted because this is an exploratory study.

The genotype distribution is summarized in Table III. The observed frequency of each genotype in cases and controls fitted the Hardy-Weinberg equilibrium (χ^2=0.066, P=0.968 and χ^2=1.64, P=0.441, respectively). The genotype distribution in cases was not significantly different from that in controls, although the s-allele was more frequent in the patients (Table III) (χ^2=3.24, P=0.198). The age-sex-adjusted odds ratios (ORs) for LS and SS genotypes were not significantly different from that for LL genotype (Table III). This suggests that there is no simple association between lung cancer susceptibility and *L-myc* genotypes.

We then analyzed risks from smoking or heavy drinking in each genotype group, as well as in all the subjects. The results are summarized in Table IV. The age-sex-adjusted OR for smoking was significantly high, and the age-sex-drinking status-adjusted OR for smoking was also significantly high. When subjects were divided according to *L-myc* genotypes, marked differences in the ORs for smoking among LL, LS and SS genotypes were observed, i.e., 0.92, 2.30 and 3.19, respectively. The ORs in LS and SS genotypes were about 2.5- and 3.5-fold higher than that in the LL genotype. However, the interaction term was not statistically significant (the age-sex-adjusted interaction was 2.12, 95% confidence interval (95%CI)=0.83−5.42) because of the insufficient number of patients. We separated smokers into two groups, light smokers who smoke less than 20 cigarettes a day and heavy smokers who smoke 21 cigarettes or more a day. The differences of smoking intensity were not significantly different among

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

	n	Genotypes (%)			Allele frequencies	
		LL	LS	SS	l	s
Patients	191	41 (21.5)	98 (51.3)	52 (27.2)	0.471	0.529
Healthy controls	241	59 (24.5)	134 (55.6)	48 (19.9)	0.523	0.477
Age-sex-adjusted OR		1.00	0.98	1.46		
(95%CI)			(0.60−1.60)	(0.82−2.61)		
P value			0.932	0.196		

95%CI, 95% confidence interval.

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

	Genotype	Category	Cases	Controls	OR[a)]	95%CI[a)]	*P* value[a)]	OR	95%CI	*P* value
Smoking	Total	never-	82	140	1.00					
		ever-	109	101	2.00	1.16–3.44	0.013	1.99[b)]	1.14–3.45[b)]	0.015[b)]
	LL	never-	19	28	1.00					
		ever-	22	31	0.92	0.32–2.68	0.883	0.94	0.32–2.74	0.907
	LS	never-	44	83	1.00					
		ever-	54	51	2.30	1.05–5.04	0.038	2.26	1.01–5.05	0.046
	SS	never-	19	29	1.00					
		ever-	33	19	3.19	0.92–11.06	0.067	3.24	0.91–11.58	0.070
Heavy drinking	Total	no-	163	215	1.00					
		drinker	28	26	1.21	0.65–2.25	0.558	1.04[c)]	0.55–1.97[c)]	0.908[c)]
	LL	no-	37	52	1.00					
		drinker	4	7	0.83	0.20–3.49	0.803	0.84	0.20–3.57	0.816
	LS	no-	84	121	1.00					
		drinker	14	13	1.33	0.54–3.26	0.539	1.09	0.43–2.74	0.860
	SS	no-	42	42	1.00					
		drinker	10	6	1.19	0.37–3.87	0.771	0.93	0.28–3.15	0.911

OR, odds ratio.
a) Age-sex-adjusted.
b) Age-sex- and drinking status-adjusted.
c) Age-sex- and smoking status-adjusted.

each genotype (data not shown). We also analyzed risks from smoking in each sex, since smoking habit is substantially different between sexes. The age-adjusted ORs in males showed a similar trend to those analyzed among all subjects, while those in females could not be evaluated because of the insufficient number of smokers (data not shown). These results suggest that marked differences in the ORs for smoking among genotypes may not be due to the differences of smoking intensity in each genotype, but to the differences of genotypes themselves.

The age-sex-adjusted OR for heavy drinking was not significant, nor was the age-sex- and smoking status-adjusted OR for heavy drinking. When subjects were divided according to the *L-myc* genotypes, the ORs for heavy drinking were similar among each genotype. These results suggest that the risk from smoking in lung cancer patients may be influenced by *L-myc* genotypes, but the risk from heavy drinking may not. To confirm this, a further study with a much larger number of subjects is essential.

In our previous study on esophageal cancer, marked differences according to the *L-myc* genotypes were observed in ORs for smoking and heavy drinking.[11)] Both smoking and alcohol consumption are well known risk factors for esophageal cancer. *L-myc* genotypes may modify their effects on carcinogenesis. Smoking is also known risk factor for lung cancer,[12, 13)] and ORs for smoking in SS and LS genotypes were also higher than that in LL genotype,

Table V. Age-sex-adjusted ORs and 95%CIs of Smoking in Each *L-myc* Genotype Less than 3 Years after Diagnosis and 3 Years or More after Diagnosis

Genotype		Less than 3 years after diagnosis				3 years or more after diagnosis				Total		
		Cases	OR	95%CI	*P* value	Cases	OR	95%CI	*P* value	OR	95%CI	*P* value
Total	never-	40	1.00			42	1.00			1.00		
	ever-	52	2.40	1.21–4.78	0.013	57	1.84	0.96–3.53	0.065	2.02	1.18–3.49	0.011
LL	never-	10	1.00			9	1.00			1.00		
	ever-	12	1.21	0.34–4.33	0.771	10	0.68	0.16–2.93	0.609	0.92	0.32–2.68	0.883
LS	never-	23	1.00			21	1.00			1.00		
	ever-	27	1.98	0.76–5.17	0.163	27	2.64	1.00–6.97	0.050	2.24	1.07–5.13	0.034
SS	never-	7	1.00			12	1.00			1.00		
	ever-	13	11.58	1.80–74.71	0.010	20	1.98	0.54–7.31	0.306	3.19	0.92–11.06	0.067

suggesting that *L-myc* genotype may affect the risk from smoking in a variety of smoking-related cancers. On the other hand, alcohol consumption is not a clear lung-cancer risk. Some studies showed a positive relationship between heavy consumption of alcohol and the risk of lung cancer,[14] but others did not.[17] In the present study, the risk of lung cancer from heavy drinking was not high, nor did *L-myc* genotypes affect that risk. These results suggest that the effect of the *L-myc* genotype on the risk for carcinogenesis might only emerge when the risk is significantly high.

Since blood samples of lung cancer patients were collected in 1999 or 2000 from patients diagnosed from 1984 to 2000, it is possible that many patients with a poor prognosis had died before 1999 and thus were excluded from this study. If prognoses differ among each genotype, the risk in each genotype of the *L-myc* gene may not be compared correctly.[18] For this reason, we recalculated the ORs for smoking after dividing the patients into two groups; one including patients less than 3 years after diagnosis and the other, 3 years or more after diagnosis. When ORs in each genotype and in all subjects were compared between the two groups, they were similar to each other except that the OR for smoking in SS genotype less than 3 years after diagnosis was much higher than that 3 years or more after diagnosis (Table V). The higher risk of patients less than 3 years after diagnosis over those 3 years or more after diagnosis may suggest a poorer prognosis of smoking-associated lung cancers in SS genotype. It is necessary to conduct a follow-up study on the possible relationship between the *L-myc* polymorphism and prognosis of smoking-related lung cancer.

When the genotype related with the risks from smoking and/or alcohol in carcinogenesis is established, personalized recommendations to quit smoking and/or to reduce alcohol intake will be possible with a large beneficial impact on a cancer prevention. We are now conducting case-control studies of the possible relationship between the *L-myc* polymorphism and risk factors among cancers related with smoking and/or drinking.

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