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Cigarette smoking, *TP53* Arg72Pro, *TP53BP1* Asp353Glu and the risk of lung cancer in a Japanese population

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Abstract. The tumor suppressor protein p53 (TP53) plays a central role in directing cellular responses to DNA damage. Tumor protein 53-binding protein 1 (TP53BP1) binds to TP53 and has a potential role in DNA damage responses. DNA damage-dependent interaction between TP53 and TP53BP1 may contribute to lung cancer risk. We aimed to assess whether or not *TP53* and *TP53BP1* genetic polymorphisms modulate lung cancer susceptibility in a Japanese population. We investigated the relationship of the *TP53* Arg72Pro and *TP53BP1* Asp353Glu polymorphisms to lung cancer risk with special reference to polymorphism-polymorphism and polymorphism-smoking interactions among 462 lung cancer cases and 379 controls. The Glu/Glu genotype of *TP53BP1* Asp353Glu polymorphism was associated with a decreased risk of lung cancer [odds ratio (OR) = 0.46, 95% confidence interval (CI) = 0.29-0.74]. There was no polymorphism-smoking interaction. A combination of the Pro allele carriage of the *TP53* Arg72Pro polymorphism and the Glu/Glu genotype of the *TP53BP1* Asp353Glu polymorphism was associated with a decreased risk of lung cancer (OR=0.38, 95% CI=0.17-0.83). The multiplicative interaction measure was statistically significant (OR for interaction = 2.93, 95% CI=1.24-6.93). The relative excess risk due to interaction and attributable proportion due to interaction were 0.74 (95% CI=0.38-1.20) and 0.63 (95% CI=0.05-1.21), respectively. Both the additive interaction measures were not equal to zero, suggesting that the existence of a biological interaction. Our findings indicate the possible association of the Glu allele of the *TP53BP1* Asp353Glu polymorphism with lower risk of lung cancer especially among the Pro allele carriers of the *TP53* Arg72Pro polymorphism.

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Introduction

As the tumor suppressor protein p53, TP53, is a principal mediator of multiple cellular functions, such as gene transcription, DNA synthesis and repair, cell cycle regulation, cell senescence and apoptosis, the TP53 protein, is referred to as 'the guardian of the genome'. Somatic mutations inactivating the *TP53* gene are found in at least half of all human tumors (1), suggesting that loss of TP53 function plays an important role in carcinogenesis. Besides the acquired mutations, functional polymorphisms in *TP53* are among the most frequently studied cancer predisposing factors. These mutations may either be acquired or occur naturally in the form of common genetic variants. The list of polymorphisms (SNP) along the *TP53* gene has considerably grown in recent years, because of large-scale genome sequencing (2). A common SNP results in a non-conservative change of an arginine to a proline at codon 72 (Arg72Pro) that results in a structural change of the protein giving rise to variants of distinct electrophoretic mobility. Residue 72 may affect the structure of the putative SH3-binding domain in the TP53 protein. Several functional differences have been reported between the Arg72 and Pro72 alleles (3). Arg72 was shown to be more efficient in inducing apoptosis, a property that correlated with a greater capacity to interact with MDM2 which facilitate nuclear export and mitochondrial localization (3). In contrast, the Pro72 variant was found to be more efficient in inducing cell-cycle arrest (4) and DNA repair (5). Other differences include the ability to bind components of the transcriptional machinery, to activate transcription, and to repress the transformation of primary cells (6). The Arg72Pro SNP has been extensively studied for its association with lung cancer risk, although the findings have been inconclusive.

The TP53-binding protein 1 (TP53BP1) interacts with the DNA-binding core domain of the TP53, enhancing TP53-mediated transcriptional activation (7,8). The TP53BP1 contains two BRCA1 C-terminal (BRCT) domains (9), which are homologous to those found in the breast cancer protein, BRCA1. The BRCT domain is characterized by hydrophobic clusters of amino acids that are thought to stabilize the three-

dimensional structure of the protein. The domain is essential for DNA repair (10-14) and binding to the central domain of the TP53 (15,16), cell cycle control (17), regulation of gene expression (18) and tumor suppressor functions (19).

Several genetic polymorphisms were recently identified in the coding and promoter regions of *TP53BP1* (20). Although there are no published studies on the functional relevance of the *TP53BP1* SNPs, the SNPs may play an important role in the etiology of lung cancer because of a direct role of TP53BP1 in the cellular response to DNA damage. Three case-control studies, two of breast cancer (21,22) and one of squamous cell carcinoma of head and neck (SCCHN) (23), found no association between the variant genotype of the *TP53BP1* Asp353Glu SNP and cancer risk. Although there has been no apparent association between the *TP53BP1* Asp353Glu SNP and cancer risk, it is biologically plausible that the effects of TP53BP1 on cancer risk might depend on the status of TP53. It has been suggested that a gene-gene interaction between *TP53BP1* and *TP53* may alter cancer risk (21,23). We hypothesized that interactions between *TP53BP1* and *TP53* genes may jointly contribute to lung cancer as well as SCCHN cancer risk. To test this hypothesis, we investigated the relationship of the *TP53* Arg72Pro and *TP53BP1* Asp353Glu polymorphisms to lung cancer risk with special reference to polymorphism-polymorphism and polymorphism-smoking interactions among 462 lung cancer cases and 379 controls in a Japanese population.

Materials and methods

Study subjects and data collection. Subjects with histologically confirmed primary lung cancer were recruited from 1996 to 2008 at the Kyushu University Hospital (Research Institute for Diseases of the Chest, Kyushu University) and its collaborating hospitals. Three hundred and seventy-nine potential controls with no prior history of cancer were recruited on a voluntary basis from the Fukuoka Prefectural Government and the Kyushu University during the period from 1996 to 2008. All subjects were unrelated ethnic Japanese. Information on smoking, years of education and environmental tobacco exposure (ETS from spouse) was gathered from both patients and controls. The study protocol was approved by our institutional review board, and all participants provided written informed consent.

Genetic analyses. Genomic DNA was extracted from blood samples. Genotyping was conducted with blinding to case/control status. The genotyping of the *TP53BP1* Asp353Glu (rs560191) SNP was done with Taqman assay (genotyping protocols supplied centrally by IARC because of this SNP genotyping is part of an IARC-oriented international collaborative study on lung cancer) while the *TP53* Arg72Pro (rs1042522) genotypes were evaluated independently of the IARC-oriented international collaborative study on lung cancer using the PCR-restriction fragment length polymorphism (RFLP) method described by Wu *et al* (24) with some modifications. Generally, concordance rate between PCR-RFLP genotyping and the real-time PCR assay is considered to be high (25). For quality control, both assays were repeated on a random 5% of all samples, and the replicates were 100% concordant.

Statistical analysis. Comparisons of means, proportions and medians were based on Unpaired t-test, χ^2 test and Wilcoxon rank-sum test, respectively. The distribution of the *TP53* Arg72Pro or *TP53BP1* Asp353Glu genotypes in controls was compared with that expected from Hardy-Weinberg equilibrium (HWE) by the χ^2 (Pearson) test. Unconditional logistic regression was used to compute the odds ratios (ORs) and their 95% confidence intervals (CIs), with adjustments for several covariates found to be associated with risk (age, sex, smoking status and education). Subjects were considered current-smokers if they had smoked or stopped smoking less than one year before either the date of diagnosis (lung cancer patients) or the date of completion of the questionnaires (controls). Never-smokers were defined as those who had never smoked in their lifetime. Former-smokers were those who had stopped smoking one or more years before either the date of diagnosis of lung cancer (lung cancer patients) or the date of completion of the questionnaires (controls). To test for biological interactions between the polymorphism and smoking, we entered interaction terms (statistical interaction) reflecting the product of polymorphism-smoking status or polymorphism-polymorphism into the logistic models. In a logistic regression model, interaction is a departure from multiplicativity. Rothman has argued that interaction estimated as a departure from additivity better reflects biologic interaction (26). Three measures for biologic interaction as departure from additivity, namely the relative excess risk due to interaction (RERI, also referred to as interaction contrast ratio), attributable proportion due to interaction (AP) and synergy index (SI), were calculated by the method described by Andersson *et al* (27). The RERI is the excess risk due to interaction relative to the risk without exposure (smoking or 'at-risk' allele/genotype). AP refers to the attributable proportion of disease that is due to interaction among individuals with both exposures. SI is the excess risk from exposure (to both exposures) when there is interaction relative to the risk from exposure (to both exposures) without interaction. Biological interaction was absent if RERI and AP are equal to zero and SI and the multiplicative interaction term are equal to one.

All statistical analyses were performed using the computer program Stata Version 10.1 (Stata Corp., College Station, TX). All P-values were two-sided, with those <0.05 considered statistically significant.

Results

The distributions of selected characteristics among subjects are summarized in Table I. Our analysis included 462 lung cancer patients (242 with adenocarcinoma, 131 with squamous cell carcinoma, 69 with small cell carcinoma and 20 with large cell carcinoma). There were significant differences between cases and controls in terms of age, sex ratio, smoking status, pack-years of smoking and years of education.

As shown in Table II, the frequencies of Pro/Pro (ancestral based on National Center for Biotechnology Information SNP database), Pro/Arg and Arg/Arg genotypes of the *TP53* Arg72Pro SNP were 13.4, 42.0 and 44.6% in cases and 11.1, 46.2 and 42.7% in controls, respectively. Genotype distribution was consistent with HWE among controls. The distribution

elected characteristics of lung cancer cases and controls.

Characteristics	Cases (n=462)	Controls (n=379)	P-value[a]
Age (year), median (IQR)	68 (62-73)	58 (48-65)	<0.0001
Male, n (%)	287 (62.1)	283 (74.7)	0.0001
Smoking status, n (%)			<0.0001
Current-smoker	198 (42.9)	129 (34.0)	
Former-smoker	111 (24.0)	41 (10.8)	
Never-smoker	153 (33.1)	209 (55.2)	
Pack-years, median (IQR)	38 (0-58)	0 (0-34)	0.0005
Exposure to environmental tobacco smoke among non-smokers, n (%)	99 (64.7)	135 (64.6)	0.98
Education, median (IQR)	12 (12-16)	16 (12-16)	<0.0001
Histology, n (%)			
Adenocarcinoma	242 (52.4)		
Squamous cell carcinoma	131 (28.4)		
Small cell carcinoma	69 (14.9)		
Large cell carcinoma	20 (4.3)		

IQR, interquartile range.

Table II. Association between *TP53* Arg72Pro and *TP53BP1* Asp353Glu polymorphisms and risk of lung cancer.

	No. (%) of		OR (95% CI)			
	Cases	Controls	Crude	P-value	Adjusted[a]	P-value
TP53 Arg72Pro						
Pro/Pro (ancestral[b])	62 (13.4)	42 (11.1)	1.0 (ref.)		1.0 (ref.)	
Pro/Arg	194 (42.0)	175 (46.2)	0.75 (0.48-1.17)	0.20	0.72 (0.44-1.20)	0.21
Arg/Arg	206 (44.6)	162 (42.7)	0.86 (0.55-1.34)	0.51	0.84 (0.50-1.39)	0.49
	P=0.38[c]	P=0.72[d]	P-trend = 0.92		P-trend = 0.88	
Prevalence of Arg allele	0.656	0.658				
	P=0.92[c]					
TP53BP1 Asp353Glu						
Asp/Asp (ancestral)	174 (37.7)	110 (29.0)	1.0 (ref.)		1.0 (ref.)	
Asp/Glu	231 (50.0)	188 (49.6)	0.78 (0.57-1.06)	0.11	0.83 (0.59-1.18)	0.30
Glu/Glu	57 (12.3)	81 (21.3)	0.44 (0.29-0.67)	0.0001	0.46 (0.29-0.74)	0.001
	P=0.001[c]	P=0.97[d]				
Prevalence of Asp allele	0.627	0.538	P-trend = 0.0002		P-trend = 0.002	
	P=0.0003[c]					

[a]Adjusted for age, sex, education and smoking status. [b]Defined by National Center for Biotechnology Information SNP database. [c]P-value for χ^2 test. [d]P-value for Hardy-Weinberg equilibrium test among controls.

of the Asp/Asp (ancestral), Asp/Glu and Glu/Glu genotypes of the *TP53BP1* Asp353Glu SNP were 37.7, 50.0 and 12.3% in cases and 29.0, 49.6 and 21.3% in controls, respectively. This SNP was also in HWE among controls. Genotypic distri-

Table III. Interaction of *TP53* Arg72Pro polymorphism and cigarette smoking.

TP53 Arg72Pro	Smoking[a]	Cases/Controls	OR (95% CI) Crude	P-value	Adjusted[b]	P-value
Arg/Arg	Never	72/93	1.0		1.0	
Pro/Arg + Pro/Pro	Never	81/116	0.90 (0.59-1.37)	0.63	0.91 (0.57-1.45)	0.70
Arg/Arg	Ever	134/69	2.51 (1.64-3.83)	<0.0001	3.02 (1.87-4.86)	<0.0001
Pro/Arg + Pro/Pro	Ever	175/101	2.24 (1.51-3.32)	<0.0001	2.83 (1.82-4.42)	<0.0001
Multiplicative interaction measure			0.99 (0.56-1.74)	0.97	1.03 (0.55-1.93)	0.93
Additive interaction measure						
Relative excess risk due to interaction			-0.17 (-1.19-0.84)	0.74	-0.09 (-1.43-1.24)	0.89
Attributable proportion due to interaction			-0.08 (-0.53-0.38)	0.73	-0.03 (-0.51-0.44)	0.90
Synergy index			0.88 (0.42-1.82)	0.73	0.95 (0.47-1.91)	0.89

[a]Current- and former-smokers were combined (ever-smokers). [b]Adjusted for age, sex and education.

Table IV. Interaction of *TP53BP1* Asp353Glu polymorphism and cigarette smoking.

TP53BP1 Arg72Pro	Smoking[a]	Cases/Controls	OR (95% CI) Crude	P-value	Adjusted[b]	P-value
Glu/Glu	Never	20/46	1.0		1.0	
Asp/Glu + Asp/Asp	Never	133/163	1.88 (1.06-3.33)	0.03	1.96 (1.05-3.67)	0.04
Glu/Glu	Ever	37/35	2.43 (1.21-4.89)	0.01	3.08 (1.43-6.68)	0.004
Asp/Glu + Asp/Asp	Ever	272/135	4.63 (2.64-8.15)	<0.0001	5.92 (3.17-11.05)	<0.0001
Multiplicative interaction measure			1.02 (0.47-2.18)	0.97	0.98 (0.42-2.27)	0.96
Additive interaction measure						
Relative excess risk due to interaction			1.33 (-0.22-2.87)	0.09	1.87 (-0.36-4.10)	0.10
Attributable proportion due to interaction			0.29 (-0.03-0.61)	0.08	0.32 (-0.02-0.65)	0.06
Synergy index			1.57 (0.81-3.05)	0.18	1.61 (0.83-3.15)	0.16

[a]Current- and former-smokers were combined (ever-smokers). [b]Adjusted for age, sex and education.

butions of the *TP53BP1* Asp353Glu SNP were significantly different between cases and controls (P=0.0003). The *TP53* Arg72Pro SNP was not associated with lung cancer risk while the Glu/Glu genotype of the *TP53BP1* Asp353Glu SNP was significantly associated with a decreased risk of lung cancer, as compared with the Asp/Asp genotype (adjusted OR=0.46, 95% CI=0.29-0.74). Based on these results, we designated the allele that is presumed to increase the risk of lung cancer as the 'at-risk' allele. Subjects with at least one 'at-risk' allele were bundled in one group for subsequent analysis.

Table III shows the modifying effect of the *TP53* Arg72Pro genotypes on the association of smoking with lung cancer risk. To achieve adequate statistical power, current- and former-smokers were combined (ever-smokers). Ever-smoking was associated with an increased risk of lung cancer (adjusted OR=3.17; 95% CI=2.28-4.39) (data not shown). Individuals with at least one 'at-risk' Pro allele (adjusted OR=2.83, 95%

Interaction of *TP53* Arg72Pro and *TP53BP1* Asp353Glu polymorphisms and risk of lung cancer.

TP53 Arg72Pro	*TP53BP1* Asp353Glu	OR (95% CI) Cases/Controls	Crude	P-value	Adjusted[a]	P-value
Arg/Arg	Glu/Glu	32/29	1.0		1.0	
Pro/Arg + Pro/Pro	Glu/Glu	25/52	0.44 (0.22-0.87)	0.02	0.38 (0.17-0.83)	0.02
Arg/Arg	Asp/Glu + Asp/Asp	174/133	1.19 (0.68-2.06)	0.55	1.06 (0.56-2.00)	0.86
Pro/Arg + Pro/Pro	Asp/Glu + Asp/Asp	231/165	1.27 (0.74-2.19)	0.39	1.18 (0.63-2.21)	0.61
Multiplicative interaction measure			2.46 (1.15-5.23)	0.02	2.93 (1.24-6.93)	0.01
Additive interaction measure						
Relative excess risk due to interaction			0.65 (0.19-1.11)	0.006	0.74 (0.28-1.20)	0.002
Attributable proportion due to interaction			0.51 (0.02-1.00)	0.04	0.63 (0.05-1.21)	0.03
Synergy index			-0.71[b]		-0.32[b]	

[a]Adjusted for age, sex, education and smoking status. [b]As the SI measures were minus figures, their confidence intervals were not calculable.

CI=1.82-4.42) presented an equal risk of lung cancer to those with the Arg/Arg genotype (adjusted OR=3.02, 95% CI= 1.87-4.86) in ever-smokers, relative to never-smokers with the Arg/Arg genotype (reference). The multiplicative interaction between the *TP53* Arg72Pro SNP and smoking was far from significant. For assessment of additive interaction, adjusted measures (95% CI) of RERI, AP and SI were -0.09 (-1.43-1.24), -0.03 (-0.51-0.44) and 0.95 (0.47-1.91), respectively. These values suggested that there were no significant biologic (additive) interactions.

Table IV shows the modifying effect of the *TP53BP1* Asp353Glu genotypes on the association of smoking with lung cancer risk. Individuals with at least one 'at-risk' allele (adjusted OR=5.92, 95% CI=3.17-11.05) had a higher risk of lung cancer than those with the Glu/Glu genotype (adjusted OR=3.08, 95% CI=1.43-6.68) in ever-smokers, relative to never-smokers with the Glu/Glu genotype (reference). Never-smokers with at least one 'at-risk' allele (adjusted OR=1.96, 95% CI=1.05-3.67) presented a higher risk of lung cancer than those with the Glu/Glu genotype. However, there were no biological interactions (additive or multiplicative) between the *TP53BP1* Arg72Pro SNP and smoking.

We examined whether the *TP53* Arg72Pro genotypes had differential effects depending on the *TP53BP1* Asp353Glu genotypes (Table V). Unexpectedly, the adjusted OR for subjects with the Pro/Arg and Pro/Pro genotypes of the *TP53* Arg72Pro SNP combined and the Glu/Glu genotype of the *TP53BP1* Asp353Glu SNP compared with those with the Arg/Arg genotype of the *TP53* Arg72Pro SNP and Asp/Glu and Glu/Glu genotypes of the *TP53BP1* Asp353Glu SNP combined was 0.38 (95% CI=0.17-0.83). The multiplicative interaction measure was statistically significant (OR for interaction = 2.93, 95% CI=1.24-6.93). The adjusted AP due to interaction between the 2 SNPs was estimated to be 0.63 (95% CI=0.05-1.21), indicating that 63% of the excess risk for lung cancer in Pro allele carriers of the *TP53* Arg72Pro SNP with Asp allele carriers of the *TP53BP1* Asp353Glu SNP was due to additive interaction. The adjusted RERI of 0.74 (95% CI=0.38-1.20) indicates that the OR for lung cancer is 0.74 higher than expected based on the addition of the two SNPs. Both measures were not equal to zero, suggesting that the existence of a significant biological interaction. As the crude and adjusted SI measures were minus figures, their confidence intervals were not calculable.

Discussion

Since advances in molecular biology have allowed many allelic variants to be characterized at the molecular level, specific nucleotide changes have been identified as the basis for altered protein structure and/or function. Therefore, an individual with a high risk genotype can be determined easily. The *TP53* Arg72Pro and *TP53BP1* Asp353Glu genotypes were determined in 462 cases of lung cancer and 379 controls. The frequency of the Arg allele of the *TP53* Arg72Pro SNP was 0.658 in controls (Table II). Sharp ethnic differences in the allele frequencies of the *TP53* Arg72Pro SNP have been observed. In the Northern hemisphere, the Pro allele shows a North-South gradient, from 0.17 in Swedish Saamis to 0.63 in African Blacks (28). In Western Europe, North America, Central and South America and Japan, the most common allele is the Arg allele, with frequencies ranging from 0.60 to 0.83. However, less than 0.40 of the frequencies of the Arg allele have been observed in African-Americans (29,30) and in Chinese (31,32). Therefore, the frequency of the Arg allele in our controls was comparable with that in Japanese reported by Beckman *et al* (28).

As for the *TP53BP1* Asp353Glu SNP, the prevalence of the Asp allele in controls was 0.538 (Table II). Available information on the prevalence of the *TP53BP1* Asp353Glu

genotypes was very limited. The frequency of the Asp allele was found to be 0.287 in non-Hispanic whites (23), 0.437 in Han Chinese (21) and 0.311 in Germans (22). Based on the International HapMap project data (33), the prevalence of the Asp allele has been found to be 0.308 in Europeans, 0.511 in Japanese and 0.444 in Han Chinese. Therefore, the frequency of the Asp allele in our study was comparable with the International HapMap project data.

In this study, the *TP53* Arg72Pro SNP was not associated with lung cancer risk. The *TP53* gene encodes a key cellular component that helps maintain genomic stability by arresting the cell cycle long enough to allow DNA repair and/or induce apoptosis (34,35). Several studies have indicated that patients with the Pro/Pro genotype were diagnosed at an earlier median age of onset. The median age varied from 6 years earlier for SCCHN to 13 years earlier for non-polyposis colorectal cancer, and between 10 and 11 years earlier for oral cancer (36-38). These data are consistent with the hypothesis that the Arg allele, which has greater apoptotic ability, consequently possesses enhanced tumor suppression function. To investigate the impact of the *TP53* Arg72Pro SNP on tumor development, molecular epidemiological studies were conducted intensively for almost all major cancer types, including cervical (39,40), lung (24,41), colorectal (42), gastric (43), bladder (44) and breast cancer (45,46). However, the results from these association studies remain inconsistent. Further studies of the *TP53* Arg72Pro polymorphism should be based on sample sizes commensurate with the detection of small genotypic risks.

The Glu/Glu genotype of the *TP53BP1* Asp353Glu SNP was significantly associated with a 54% decrease in lung cancer risk in Japanese. To date, only three case-control studies have investigated the role of *TP53BP1* SNPs in cancer susceptibility (21-23). A Chinese study (404 breast cancer cases and 472 controls) has found no significant main effect of the *TP53BP1* T-885G, Asp353Glu (1059C→G), or Gln1136Lys (3406A→C) SNP or haplotype (except for GGC) on breast cancer risk but the *TP53BP1* T-885G, Asp353Glu and Gln1136Lys SNPs were significantly associated with elevated risk of progesterone receptor negative breast cancer and the T-885G and Gln1136Lys with estrogen receptor negative breast cancer (21). A relatively large German study (353 breast cancer patients and 960 controls) found no overall association between four SNPs (*TP53BP1* Asp353Glu, Gly412Ser, Gln1136Lys and 1347_1352delTATCCC) and breast cancer risk (21,22). An American study (818 SCCHN cases and 821 controls) has also found that the *TP53BP1* T-885G, Asp353Glu and Gln1136Lys SNPs did not individually affect the risk of SCCHN (23). In a large scale genome-wide association study among UK Caucasians (47), the *TP53BP1* Asp353Glu SNP showed a significant allelic association with lung cancer. Testing replication in different populations is an important step. Additional studies are warranted to corroborate the association among Japanese suggested in the present study.

Because smoking is an established cause of lung cancer, we therefore evaluated whether an interaction existed between the *TP53* Arg72Pro or TP53BP1 Asp353Glu SNP and smoking (Tables III and IV). To the best of our knowledge, no studies on the *TP53BP1* Asp353Glu SNP and smoking interaction with risk of lung cancer have been previously reported. No interactions (additive and multiplicative) of smoking and the *TP53* Arg72Pro or *TP53BP1* Asp353Glu SNP with lung cancer were observed in this study. Some studies have found no evidence for any interaction between the *TP53* Arg72Pro polymorphism and smoking (48-50) while other studies indicated that the interaction was probable (51,52). However, these studies did not indicate P-values for the interaction measures (additive or multiplicative) (51,52).

Risk alleles of the polymorphisms might confer different susceptibility to different histological types of lung cancer and to different pathogenic mechanisms. The distributions of the *TP53* Arg72Pro genotypes or the *TP53BP1* Asp353Glu genotypes did not differ among the different histological subtypes in our study (data not shown). No evidence of significant heterogeneity of ORs for the *TP53* Arg72Pro SNP among major histologic types of lung cancer has been found in a meta-analysis based on over 3000 cases and controls (41). The different effects of the risk genotype related to smoking in different subtypes, which might reflect the different etiologies of the different subtypes, have been suggested (53,54). Stratification by histological type did not reveal risk modification by the *TP53* Arg72Pro SNP or the *TP53BP1* Asp353Glu SNP in the association of smoking and lung cancer (data not shown). As for the interaction between the *TP53* Arg72Pro SNP and smoking in different histologic types, the difference in results might be due to the fact that our study had less statistical power. Additional studies are needed to clarify the polymorphism-smoking interaction in different subtypes.

As TP53 and P53BP1 work together in the DNA damage-signaling pathway, we simultaneously evaluated the potential interaction between these two genetic polymorphisms. As shown in Table V, we did observe a significant interaction between *TP53* Arg72Pro and *TP53BP1* Asp353Glu SNPs in relationship to lung cancer risk. The adjusted OR for subjects with at least one Pro allele of the *TP53* Arg72Pro SNP and the Glu/Glu genotype of the *TP53BP1* Asp353Glu SNP compared with those with the Arg/Arg genotype and at least one Glu allele was 0.38 (95% CI=0.17-0.83). Unexpectedly, a significant multiplicative polymorphism-polymorphism interaction was observed (OR for interaction = 2.93, 95% CI=1.24-6.93). The finding of an interaction in the same direction was reported in the SCCHN cancer study (23). The Arg allele of the *TP53* Arg72ProSNP is more efficient in inducing apoptosis while the Pro allele induces more G_1 arrest and is better at activation of TP53-dependent DNA repair (3,55,56). Depending on the severity of the damage, cells may either choose to arrest the cell cycle until the damage is repaired, or if the damage is irreparable, proceed to apoptosis (57,58). As the balance between cell cycle arrest/DNA repair and apoptosis is adequate to allow error-free repair or apoptotic removal of a heavily damaged genome, our study population might not be heavily exposed to exogenous carcinogens. If subjects are not heavily exposed to exogenous carcinogens then their DNA will not be heavily damaged, and this balance would be shifted to cell cycle arrest/DNA repair. In such a case, the Pro allele may be protective toward lung cancer risk. Thus, the protective effect of the Glu/Glu genotype of the TP53BP1 Asp353Glu may be more pronounced among carriers with the Pro allele of the TP53 Arg72Pro polymorphism. Therefore, it is likely that the

g72Pro and the *TP53BP1* Asp353Glu alleles may their protein interaction, a hypothesis consistent with a protective effect observed in this study.

An interaction in a different direction was found in a breast cancer study (21), however. Nucleotide excision repair (NER) plays a critical role in protecting the cell genome from insults of cancer-causing agents, such as smoking-related bulky adducts induced by benzo[α]pyrene (59,60). The polymorphisms in NER gene excision repair cross complementing group 2 (ERCC2) have been analyzed extensively for their potential ability to increase lung cancer risk. Although several meta-analyses and pooled analyses of candidate genetic polymorphisms reported that an SNP in one gene might substantially alter lung cancer risk, no candidate genetic variants other than the *ERCC2* Lys751Gln SNP emerged from the DNA repair gene polymorphisms (61). Lung cancer and breast cancer likely involve fundamentally different pathways of DNA repair. Indeed, the NER pathway seems to be more relevant in the etiology of lung cancer (61,62), whereas DNA double-strand break repair, as suggested by the roles of BRCA1 and BRCA2 (63), seems to be more relevant in the etiology of breast cancer (64). Obviously, the biological evidence for this gene-gene interaction needs further in-depth investigation.

Our study design might have several limitations. First, controls were younger than cases. After adjustment for age in logistic regression analysis, however, the differences between the crude and adjusted ORs were small, indicating that age did not have a strong influence on the risk estimates of the polymorphisms. Second, the moderate sample size limited the statistical power of our study and large well-designed studies are warranted to confirm our findings, particularly the polymorphism-polymorphism and polymorphism-environment interactions. Third, we only genotyped the *TP53* Arg72Pro and *TP53BP1* Asp353Glu SNPs and did not analyze other potentially functional SNPs, such as the *TP53* PIN3 (16-bp insertion/deletion), *TP53 Msp* I, *TP53BP1* Gln1136Lys and *TP53BP1* T-885G SNPs. The effects of polymorphisms are best represented by their haplotypes. It can be anticipated that in future association studies of lung cancer, the development of new approaches will facilitate the evaluation of haplotypic effects, either for selected polymorphisms physically close to each other or for multiple genes within the same causal pathway. Further studies with multiple SNPs of the genes in the same pathway may provide more valuable information in terms of the polymorphism-polymorphism interactions. Finally, our study lacked the related phenotypic and functional assays, which limited our inquiry into the functional consequence of the SNPs. However, such association studies with significant findings may lead to further functional studies that will elucidate the underlying mechanisms of lung cancer development associated with these genetic variants.

Our study demonstrated, for the first time, that the *TP53BP1* Asp353Glu SNP affects the risk of lung cancer. However, our data suggest that there was no smoking-polymorphism interaction while a polymorphism-polymorphism interaction (both multiplicative and additive) between *TP53BP1* and *TP53* SNPs was suggested. More studies of biochemical functions and interactions and of epidemiological associations are necessary to elucidate further the impact of polymorphisms in a cell cycle regulatory gene on lung cancer risk.

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