RESEARCH COMMUNICATION

Different Risk Relations with Smoking for Non-small-cell lung Cancer: Comparison of *TP53* and *TP73* Genotypes

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Abstract

Background: The association between *TP53/TP73* gene polymorphisms and tobacco smoking was evaluated with regard to risk of non-small cell lung cancer (NSCLC). Methods: A case-control study with 192 histologically confirmed NSCLC cases and 241 non-cancer controls was conducted. Subjects were genotyped for *TP53* Arg72Pro and *TP73* G4C14 to A4T14 polymorphisms by PCR-based methods. Risk and interactions were assessed as odds ratios (ORs) and 95% confidence intervals (CIs). Results: The analyses according to *TP53* genotypes for the risk of tobacco smoking illustrated that risk with heavy smoking was much higher for subjects with the *TP53* ProPro genotype (OR: 16.4, 95% CI 1.77-151.7) as compared with those with *TP53* ArgArg/ArgPro (3.36, 1.69-6.68). Similar analyses for *TP73* genotypes did not show any differences for NSCLC risk. Conclusion: A risk relation of heavy smoking for the NSCLC is suggested with the *TP53* but not the *TP73* polymorphism.

Key Words: non-small-cell lung cancer - p53 - p73 - polymorphism - smoking - gene-environment interaction

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Introduction

Lung cancer has become the leading cause of cancer death for males in Japan (Tominaga and Oshima 1999), with smoking established as the main etiological agent. Genetic change in cell populations plays important roles in carcinogenesis leading to development of lung cancer and there is an expanding body of literature suggesting that host factors, including genetic polymorphisms, may explain individual differences in cancer risk.

The *TP53*, tumor suppressor gene, located on chromosome 17p13, is one of the most commonly mutated genes in all types of human cancers, including those occurring in the lung (Mitsudomi et al., 2000). In addition, several other members of the *TP53* family have been identified recently. For example, *TP73* on chromosome 1p36 shares considerable sequence homology with *TP53*. p73 can induce apoptosis in cancer cells and may act as a tumor suppressor overlapping in its functions with p53 (Davis and Dowdy 2001). It has been suggested that different levels of p73 in the cell might modulate the p53 response after DNA damage (Wang et al., 2001). Although there is evidence that loss of heterozygosity of *TP73* contributes to human cancers, the biological significance of *TP73* for neoplasia in the lung has yet to be established in detail.

To date, several polymorphisms have been reported for *TP53* and *TP73*. For example, the *TP53* codon 72 polymorphism in exon 4 produces a variant protein with a proline (Pro) in place of an arginine (Arg). This polymorphism is located in a proline-rich domain of *TP53*

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that has been shown to be required for the growth suppression activity of p53, forming part of an SH3-binding PXXP motif (Walker and Levine 1996). *TP53* variants cause differences in the ability to bind components of the transcriptional machinery, to activate transcription, to induce apoptosis, and to repress the transformation of primary cells (Thomas et al., 1999). Associations between this polymorphism and lung cancer susceptibility have been examined by several investigators (Weston et al., 1992, Kawajiri et al., 1993, Birgander et al., 1995, Jin et al., 1995, Murata et al., 1996, To-Figueras et al., 1996, Fan et al., 2000, Pierce et al., 2000, Liu et al., 2001). In most but not all cases, the Pro allele was found consistently to increase the risk of lung cancer (Weston et al., 1992, Kawajiri et al., 1993, Birgander et al., 1995, Jin et al., 1995, To-Figueras et al., 1996, Fan et al., 2000, Pierce et al., 2000, Liu et al., 2001), but the extent differed markedly with the study. Of interest is a possible interaction between harboring the Pro allele and the smoking habit reported by the group of Jin (Fan et al., 2000). They showed that risk of lung cancer in smokers may differ with the *TP53* genotype. Others showed interactions with the GSTM1 polymorphism that impacts on metabolism of tobacco related substances (To-Figueras et al., 1996, Liu et al., 2001). However, to our knowledge, there have been no case-control study of *TP73* polymorphisms with reference to lung cancers. Among *TP73* polymorphisms so far reported (Peters et al., 2001, Ryan et al., 2001), a dinucleotide polymorphism at positions 4 and 14 of exon2 (G4C14 to A4T14, designated in this paper simply as G4A) is regarded as functional, with possible formation of a stem-loop structure which influences *TP73* translation (Kaghad et al., 1997). Another group reported that *TP73* polymorphic transcripts are differently expressed between lung cancer and normal tissue, suggesting *TP73* may play an important role in lung carcinogenesis via different transcription by polymorphism (Mai et al., 1998). This polymorphism has been reported in Japanese (Nomoto et al., 1998), but neither genotype frequency nor association with lung cancer risk have been reported.

Recently, enhanced binding of mutant p53 to p73, which neutralizes p73-induced apoptosis, was reported for the Arg allele (Marin et al., 2000). The Arg allele in human cancers was found to be more prevalent in recessive *TP53* mutation cases than in transdominant *TP53* mutation cases (Tada et al., 2001). These findings indicate an interaction between functional *TP73* and *TP53* polymorphisms, and that functional polymorphisms of *TP73* may alter cancer risk independently or dependently with *TP53* polymorphisms. In the present study, we conducted a case-control study to examine the association between lung cancer susceptibility and *TP53* Arg72Pro and/or *TP73* linked G4C14-to-A4T14 at exon 2 polymorphisms, and potential interaction between these polymorphisms and the smoking habit.

Materials and Methods

This study was approved by the Institutional Review Board of Aichi Cancer Center. Recruitment was made from patients at Aichi Cancer Center who received histological confirmation of lung cancer between 1984 and 2000. A total of 192 patients (age range, 26-81 years; mean age 61.0 years; male, 59.8%) were recruited between September 1991 and August 2000 (Hamajima et al., 2001a, Ito et al., 2002, Kumimoto et al., 2002). Patients visiting the follow-up outpatient clinic of thoracic surgery were asked to participate in this study by doctors in charge (it was not counted how many outpatients were actually asked by doctors). Those assenting were enrolled after providing written informed consent by staff of the Division of Epidemiology and Prevention. With a few exceptions all agreed to participate. Information about stage was obtained from medical records. Histological types were adenocarcinoma (n=138, 71.9%), squamous cell carcinoma (n=38, 19.8%), large cell carcinoma (n=14, 7.3%) and others (n=2, 1.0%). About 50% of patients with lung cancer participated in this study within 2 years after their diagnosis. Clinical stages comprised mainly early stages (stage 1, n=124; stage 2, n=19; stage 3A, n=36, stage 3B, n = 6; stage 4, n=3). Controls were 241 non-cancer patients who underwent gastroscopy in 1999 at Aichi Cancer Center Hospital (age range, 39-69 years; mean age 56.8 years; male, 49.0%). Out of these, 97 individuals were under medication for 107 diseases; 23 for gastric/duodenal ulcers, another 23 for so-called gastritis, 16 for hypertension, 8 for pain including arthritis and lumbago, 7 for diabetes mellitus, 7 for hyperlipidemia, and 23 for other miscellaneous diseases. All patients and control subjects were Japanese. All participants provided written informed consent for genotyping and a 7 ml sample of peripheral blood and answered a self-administered questionnaire covering lifestyle varaibles. Smoking status was categorized into smokers, former smokers, current light-smokers (pack-years (PY) < 50), and current heavy-smokers (PY ≥ 50).

DNA was extracted from buffy-coat fraction, using a QIAamp DNA Mini Kit (Qiagen Inc., Valencia, CA, USA). Genotyping was conducted by polymerase chain reaction with confronting two-pair primers (PCR-CTPP) (Hamajima et al., 2000) as previously described in detail (Hamajima et al., 2002).

All statistical analyses were performed using STATA ver. 7 software with a Macintosh computer (STATA Corporation, College Station, TX). Accordance with the Hardy-Weinberg equilibrium, which indicates an absence of discrepancies between genotype and allele frequencies, was checked for the controls with the Fisher's exact test of probability. The genotype frequencies for all cases and each case group were compared with those for controls by a χ^2 test (2 x 3 tables) or the Fisher's exact test (2 x 2 tables). Unconditional logistic regression was applied to estimate the odds ratios (ORs) and 95% confidence intervals (CIs). Gene-gene interactions were examined with a case-only design (Yang et al., 1997). The magnitude of any interaction was expressed as the OR with a 95% CI. The statistical power for 241 controls and 192 cases was between 38 % and 88 % for an OR = 2 or 0.5 with a two-sided significance level of 0.05, the genotype proportions for the controls being between 5% and 20%.

Results

Since the cancer patients were prevalent cases, prognostic effects could have influenced the distributions. Accordingly, stratified subset analysis according to the intervals from diagnosis was performed (Hamajima et al., 2001b); there was no difference in the distribution between cases with a shorter interval (2 years or shorter) and cases with a longer interval (more than 2 years). The ORs for the TP53 ProPro genotype relative to *ArgArg/ArgPro* genotypes were 0.58 (0.28-1.17) and 0.74 (0.38-1.45) for the shorter and longer interval groups, respectively. For the *TP73* polymorphism, the ORs for the *TP73* AA genotype relative to the *GG* and*GA* genotypes were 1.24 (0.41-3.72) and 1.83 (0.68-4.96), respectively. These results indicated that the prognostic effects of two polymorphisms were limited in the present cases, even though the *TP73* and *TP53* polymorphisms could influence the prognosis in general.

The crude genotype frequencies of genotype for patients and control for each polymorphism are summarized in Table 1. The frequencies of genotypes of *TP53, ArgArg, ArgPro*, and *ProPro* were 35.6%, 51.8%, and 12.6% and that of *TP73, GG, GA*, and *AA* were 57.7%, 35.0% and 6.4%. The crude genotype frequencies in the lung cancer patients were similar to those of the controls. The frequencies for the controls were in Hardy-Weinberg equilibrium; p=0.228 for *TP53* Arg72Pro and p=0.215 for *TP73* G4A. There were no significant differences in the genotype frequencies for *TP73* G4A and TP53 Arg72Pro polymorphisms between controls and case by a χ^2 test for 2 x 3 tables. Genotype distributions according to histological subtypes or clinical stage were not found to show significant differences.

Since the genotypes of two polymorphisms were independently distributed among the controls (Fisher's exact test, p=0.216, OR=0.91), gene-gene interactions were examined by a case-only design (Birgander et al., 1995).

Table 1. Genotype Frequencies for *TP73* G4C14-to-A4T14 at Exon 2 and *TP53* Arg72Pro Polymorphisms.

Gene	Genotype	Controls, n (%)	Cases, n (%)	2 Test
TP53	*ArgArg*	90 (37.7%) [a]	68 (35.6%)[b]	
	ArgPro	106 (44.4%)[a]	99 (51.8%)[b]	2 = 3.37
	ProPro	43 (18.0%) [a]	24 (12.6%)[b]	p = 0.185
TP73	*GG*	130 (55.3%)[c]	109 (57.7%) [d]	
	GA	95 (40.4%)[c]	68 (36.0%) [d]	2 = 1.53
	AA	10 (4.3%)[c]	12 (6.4%) [d]	p = 0.466

Not genotyped by two independent PCRs: [a] for two samples, [b] for one sample, [c] for six samples, and [d] for three samples. Genotype distributions between cases and controls were examined by a 2 test.

The distribution for *TP73* G4A was independent of the *TP53* Arg72Pro genotype distribution for lung cancer patients. The OR for the interaction was 1.40 (0.29-6.81), showing no statistical significance.

We then examined the association of *TP53* genotype with lung cancer. Table 2 shows the crude ORs for sex, age, and smoking status according to *TP53* genotype (*ArgArg/ArgPro* or *ProPro*). As expected, lung cancer risk increased with increasing cumulative cigarette dose. The OR for heavy-smoking (current smoker with PY ≥ 50) was higher for persons with the ProPro genotype (16.4, 1.77-151.7) compared with for those with the *ArgArg/ArgPro* genotype (3.36, 1.69-6.68). However, this was not the case for the *TP73* genotype and smoking exposure (Table 3).

To examine the possible interaction between *TP53* and the smoking habit, subjects were divided into four groups: excluding heavy-smokers with the *ArgArg/ArgPro* genotype; subjects excluding heavy-smokers with the *ProPro* genotype; heavy smokers with the *ArgArg/ArgPro* genotype; and heavy-smokers with the *ProPro* genotype. Table 4 shows the ORs for the latter three groups relative to first group for

Table 2. Crude Odds Ratios for the Risk of Lung Cancer by *TP53* Arg72Pro Polymorphism According to Sex, Age and Smoking Characteristics.

	TP53 genotype			
	ArgArg / ArgPro		*ProPro*	
	Case/Control (n)	OR(95% CI)	Case/Control (n)	OR(95% CI)
Sex				
Male	100/ 92	1.00 (Reference)	14/24	1.00 (Reference)
Female	67/104	0.59 (0.39-0.90)	10/19	0.90 (0.33-2.48)
Age (years)				
<55	45/77	1.00 (Reference)	4/16	1.00 (Reference)
55-64	59/89	1.22 (0.74-2.00)	10/18	2.22 (0.58-8.49)
65-	63/36	2.99 (1.73-5.19)	10/9	4.44 (1/08-18.4)
Smoking status [c]				
Never	72/115	1.00 (Reference)	10/25	1.00 (Reference)
Former	36/37	1.43 (0.84-2.45)	5/5	2.73 (0.66-11.3)
Current (PY < 50[b])	27/29	1.07 (0.53-2.16)	3/12	0.78 (0.14-4.34)
Current (PY ≥ 50[b])	32/14	3.36 (1.69-6.68)	6/1	16.4 (1.77-151.7)

[a]One case and two controls were excluded because their genotypes were not defined. [b] PY indicates pack-years. One control was excluded because PY could not be calculated. [c] One control was additionally excluded from the analysis because of lack of PY information.

Table 3. Crude Odds Ratios for the Risk of Lung Cancer by the *TP73* Polymorphism

	TP73 genotype [a]			
	GG/GA		*AA*	
	Case/Control (n)	OR(95% CI)	Case/Control (n)	OR(95% CI)
Sex				
Male	104/105	1.00 (Reference)	8/8	1.00 (Reference)
Female	73/120	0.61 (0.41-0.91)	4/2	2.00 (0.28-14.2)
Age (years)				
<55	48/88	1.00 (Reference)	2/4	1.00 (Reference)
55-64	64/95	1.24 (0.77-1.98)	5/4	2.50 (0.29-21.4)
65-	65/42	2.84 (1.68-4.79)	5/2	5.00 (0.47-53.0)
Smoking status[b]				
Never	77/134	1.00 (Reference)	5/3	1.00(Reference)
Former	38/38	1.63 (0.97-2.74)	3/4	0.45 (0.06-3.57)
Current (PY < 50 [c])	29/38	1.03 (0.53-1.99)	0/2	NA [d]
Current (PY ≥ 50[c])	33/14	3.84 (1.95-7.57)	4/1	2.4 (0.18-32.9)

[a] Three cases and six controls were excluded because their genotypes were not defined. [b] One control was additionally excluded from analysis because of a lack of PY information. [c] PY indicates pack-years. One control was excluded because a PY could not be calculated. [d] NA indicates not available because of absence of cases in this category.

in total, adenocarcinoma, and squamous cell carcinoma. In every analysis, heavy-smokers with the ProPro genotype consistently showed the highest ORs, and this trend was particularly marked for squamous cell carcinomas with an OR of 28.4 (2.32-348.1).

Discussion

We found the following in this study: 1) genotype and allele distributions of *TP73* G4C14-A4T14 and *TP53* Arg72Pro polymorphisms did not differ between non-small cell lung cancer (NSCLC) cases and non-cancer controls among Japanese, indicating both polymorphisms themselves did not have appreciable importance for carcinogenesis of NSCLC when viewed independently; 2) the ORs for heavy smoking for the risk of NSCLC with each genotype of *TP73* polymorphism are not different, whereas the *ProPro* genotype of the TP53 polymorphism has an approximately five-times higher OR than that for the *ArgArg/ArgPro* genotype (16.4 vs. 3.36), indicating a gene-environment interaction with tobacco-smoking probable for the *TP53* but not the *TP73* polymorphism; 3) the hypothesized gene-gene interaction between two polymorphisms was not observed; and 4) the ORs for the *TP53 ProPro* or *TP73 AA* genotype among long term survivors (more than two years) and others were similar , indicating indirectly that the *TP53* and *TP73* polymorphisms did not have influence survival of NSCLC cases.

Contrary to the literature indicating an increased risk of lung cancer in those harboring the Pro allele, our result did not show the *TP53* genotype distribution to differ in total between cases and controls. This was also observed on subgroup analysis according to the histological subtype. Liu et al. reported the results of the largest case-control study in the United States (1,144 cases and 1,256 controls mainly from Caucasian) and those with the *TP53* Pro allele exhibited a slightly increased risk of adenocarcinoma (OR 1.36, 95%CI: 1.1-1.7) but not that of squamous cell carcinoma

Table 4. Age-sex Adjusted Odds Ratios for Combination of Smoking Status and *TP53* Genotype.

	TP53 genotype			
	ArgArg / ArgPro		*ProPro*	
	case/control(n)	aOR (95% CI)	case/control(n)	aOR (95% CI)
All cases vs. controls [a]				
Subjects excluding heavy smokers[*b]	135/181	1.0 (Reference)	18/42	0.50 (0.27-0.93)
Heavy smokers[*c]	31/14	2.46 (1.20-5.06)	6/1	8.39 (1.20-72.4)
Adenocarcinoma cases vs. controls				
Subjects excluding heavy smokers	105/181	1.0 (Reference)	16/42	0.57 (0.30-1.09)
Heavy smokers	14/14	1.53 (0.66-3.57)	2/1	4.77 (0.40-56.1)
Squamous cell carcinoma cases vs. controls				
Subjects excluding heavy smokers	17/181	1.0 (Reference)	2/42	0.43 (0.09-2.00)
Heavy smokers	16/14	5.98 (2.33-15.3)	3/1	28.4 (2.32-348.1)

[a] One case and three controls were excluded because of either the genotypes or smoking status were not defined.
[*b] They include never smoker, former smoker, and current smoker (PY < 50). [*c] Smokers with PY ≥ 50.

(OR 1.04, 0.8-1.4) (Liu et al., 2001). Although adenocarcinomas predominated in our cases, the observed risk trend was opposite in this study. There has been three studies conducted for Japanese populations (Kawajiri et al., 1993, Murata et al., 1996, Pierce et al., 2000), and two did not demonstrate the Pro allele to be a risky allele for adenocarcinoma. Considering the available information, although further evaluation is required, we can speculate that the effect of Pro allele on the risk of lung cancer may differ by ethnicity.

Observed genotype and allele frequencies for *TP73* polymorphism among the NSCLC cases were similar to those among controls as well as among esophagus, stomach, and colorectal cancer cases of our previous reports (Liu et al., 2001, Hamajima et al., 2002). The lack of appreciable differences between genotype frequencies for cases and controls might be interpreted as indicating no independent effects of this polymorphism. In addition, contrary to our expectation, no gene-gene interaction with *TP53* polymorphism was observed.

Concerning gene-environment interactions with heavy smoking, a clear difference was evident between *TP53* and *TP73* polymorphisms. For the former, the OR for heavy smoking was five-times higher for the ProPro genotype compared with the *ArgArg/ArgPro* genotype. Of interest was that the analyses according to histological subtype showed this trend to be more evident for squamous-cell carcinoma, which is widely considered to be more closely related with smoking compared with adenocarcinoma. Although detailed biological mechanisms of interaction remain to be clarified, these findings fit in with the idea of interaction between smoking and *TP53* polymorphism. On the other hand, *TP73* polymorphism did not show any kind of association even when focusing on the interaction.

In this study, outpatients without malignancies were adopted as controls, and genotype frequencies of the two polymorphisms were in accordance with the Hardy-Weinberg law of equilibrium, indicating that no selective mechanisms for a specific genotype of these polymorphisms existed among the controls. Although some controls were under medication for non-cancer disease, the absence of evidence for any association with the *TP53* or *TP73* polymorphisms supported their adoption. Since the obtained genotype frequencies were 4.3% for *TP73 AA* and 18% for *TP53 ProPro*, statistical power to detect an OR of 0.5 or 2 for 192 cases and 241 controls with a two-sided alpha error of 0.05 was approximately 43.9% for *TP73 AA* (genotype frequency 6.4%) and 71.6% for *TP53 ProPro* (genotype frequency 12.6%). Taken these statistical powers into consideration, our results at least allow conclusions regarding the independent risk of the *TP53 ProPro* genotype of the Arg72Pro polymorphism, while the results for the *TP73* polymorphism needed careful interpretation.

In conclusion, the present study revealed that the risk of lung cancer is not associated independently with either TP73 G4C14-A4T14 dinucleotide polymorphism at exon 2 or *TP53* Arg72Pro polymorphism. A gene-environment interaction with heavy smoking was not observed with the *TP73* polymorphism while one was evident with the *TP53* polymorphism. Heavy smokers with the *ProPro* genotype show a five times higher risk of NSCLC due to smoking compared with those with the *ArgArg/ArgPro* genotype. No gene-gene interaction between *TP53* and *TP73* polymorphisms was evident for the present subjects.

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