Association of Single Nucleotide Polymorphisms in *PIM-1* Gene with the Risk of Korean Lung Cancer

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Purpose: The expression of the *PIM-1* gene, which is a proto-oncogene that encodes a serine/threonine kinase, is associated with multiple cellular functions such as proliferation, differentiation, apoptosis and tumorigenesis. In particular, several studies have reported that the *PIM-1* gene is associated with the development of lymphoma, leukemia and prostate cancer. Therefore, this study was conducted to evaluate the association between the single nucleotide polymorphisms in the *PIM-1* gene and the risk of lung cancer occurrence in the Korean population.

Materials and Methods: To evaluate the role of the *PIM-1* gene in the development of lung cancer, the genotypes of the *PIM-1* gene were determined in 408 lung cancer patients and 410 normal subjects.

Results: We found that the T-C-T-C haplotypes of the *PIM-1* gene (−1196 T>C, IVS4 +55 T>C, IVS4 +1416 T>A and +3684 C>A) were associated with an increased risk of lung cancer [adjusted odds ratio (aOR): 3.98; 95% CI: 1.24~12.75, p-value: 0.020]. In particular, these haplotypes showed an increased risk of lung cancer in males (aOR: 5.67; 95% CI: 1.32~24.30, p-value: 0.019) and smokers (aOR: 7.82; 95% CI: 1.75~34.98, p-value: 0.007).

Conclusions: The present results suggest that the T-C-T-C haplotype of the *PIM-1* gene could influence the risk of developing smoking-related lung cancer in the Korean population. Additional functional studies with an larger sample sized analysis are warranted to reconfirm our findings. *(Cancer Res Treat. 2008;40:190-196)*

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Key Words: PIM-1, PIM, Single Nucleotide Polymorphism, SNP, Lung neoplasms, Oncogenes, Haplotypes

INTRODUCTION

There have been numerous previous researches and reports about proto-oncogenes, tumor suppressor genes and their roles in the development of cancer. *PIM-1* gene is a proto-oncogene that encodes a serine/threonine kinase, which belongs to the group of calcium/calmodulin-regulated kinases, and this *PIM-1* kinase controls such cellular functions as proliferation, differentiation, apoptosis and tumorigenesis through various signaling pathways. It has been verified that the *PIM-1* gene is related with several human cancers, including lymphoma, leukemia and prostate cancer (1-3). However, the relationship between the

PIM-1 gene and lung cancer has not yet been reported.

It is well known that various signaling pathways are related with the development of lung cancer. Several tyrosine kinase inhibitors and serine/threonine kinase inhibitors that block serine/threonine signaling pathways have been introduced as anti-neoplastic agents against lung cancer (4,5). Therefore, we attempted to characterize the association between the polymorphisms of the *PIM-1* gene that encodes serine/threonin kinase and the risk of developing lung cancer in a Korean population by using genotype and haplotype analysis.

Lung cancer has been the leading cause of cancer- related deaths in Korea, and its incidence continues to rise (6). Nevertheless, the prognosis of lung cancer has remained poor despite the innovations in diagnostic testing and surgical technique, and the development of new chemotherapeutic agents (7). The poor outcomes of lung cancer may partly be due to the heterogeneity of lung cancer histobiology (8) because different histological subtypes have distinctive etiologies and different responses to anticancer therapy, and these subtypes should be treated differently. Moreover, the recently introduced targeted agents show different responses according to the histologic subtype (5), and the efficiency of the treatment modalities for lung cancer depends on the time of diagnosis. Thus, there

is a great need for rapid and efficient methods to detect lung cancer at an early stage. For developing improved molecular biomarkers for early detection and to predict the response to chemotherapy, it is important to identify the genetic alterations that are specific to each subtype of lung cancer.

Single nucleotide polymorphisms (SNPs) are the most abundant form of genetic variation in the human genome, and these occur when a single nucleotide in the genome sequence is altered (9). Theoretically, the numbers of SNPs in human population have been estimated to be about 5 million SNPs for minor allele frequencies of at least 10%, and possibly as many as 11 million for minor allele frequencies of at least 1% (10). SNPs have an excellent potential to be used as biomarkers for diagnosing genetic diseases, including cancers, as compared to other less common polymorphisms and microsatellite markers.

This study was conducted to examine the *PIM-1* polymorphisms and their relation with the risk of lung cancer in a Korean population. To the best of our best knowledge, this is the first study to determine an association of the *PIM-1* polymorphisms with lung cancer in Korean lung cancer patients.

MATERIALS AND METHODS

1) The study subjects

Between August 2001 and June 2007, blood samples were collected from 818 subjects, and these included 408 lung cancer patients and 410 normal controls without cancer. The lung cancer patients were recruited from the patient pool at the Genomic Research Center for Lung and Breast/Ovarian Cancer and the Inha University Medical Center, and the control subjects were randomly selected from a pool of healthy volunteers who had visited the Cardiovascular Genome Center, the Genomic Research Center for Allergy and Respiratory Diseases and Keimyung University Dongsan Medical Center. Detailed information on the subjects' diet, their smoking status, drinking status, lifestyle and medical history were collected by trained interviewers with using a structured questionnaire. Out of the 408 cases, 397 smoking statuses, 311 drinking statuses, 313 stages and 385 cell types were available for the characteristic information, while 309 smoking statuses and 211 drinking statuses for the 410 controls were available for the characteristic information. All the study subjects provided written consent and they were all ethnic Koreans, and all the participating Institutional Review Boards approved the study protocol.

2) Preparation of genomic DNA and direct sequencing

Genomic DNA was prepared from peripheral blood samples with using a Puregene blood DNA kit (Gentra, Minneapolis, MN) and following the manufacture's protocol. For identification of the frequent polymorphism sites in the *PIM-1* gene in the Korean population, human genomic DNA was isolated for direct sequencing from the whole blood of 24 samples. We amplified the entire *PIM-1* gene at 6p21, and PCR amplifications were performed on a PTC-225 Peltier Thermal cycler (MJ Research Inc., Waltham, MA) with using AmpliTagGold (Roche, Branchburg, NJ); there were 35 cycles of 30 s at 95°C, 1 min at 64°C and 1 min at 72°C, followed by a single 10 min extension step at 72°C. The PCR products were purified using

a Montage PCR96 Cleanup kit (Millipore, Bedfore, MA) and they were eluted with 20 μl of nuclease free H$_2$O. DNA cycle sequencing was carried out using a BigDye Terminator V 3.1 Cycle Sequencing kit (PerkinElmer, Foster City, CA). Multi-screen SEQ 384 well filter plates were used for dye terminator removal, and the sequences were analyzed on an Applied Biosystems 3,700 DNA analyzer. All the SNPs and sequence alignments were analyzed with using Polyphred.

3) Genotyping

After direct sequencing of the *PIM-1* gene, we performed genotyping for the SNPs with a frequency greater than 5%. The genotypes of the sample were assayed using a single base primer extension assay and using a SNaPShot assay kit (ABI, Foster City, CA). Briefly, the genomic regions containing the SNPs of interest in the *PIM-1* gene were amplified by PCR. PCRs were performed using an initial denaturation step at 95°C for 10 min and 35 amplification cycles (30 s at 95°C, 1 min annealing at 63.9°C and 1 min at 72°C), followed by a single 7 min extension cycle at 72°C. The PCR products were subsequently purified by incubating them with 10 units of ExoI (USB, Cleveland, OH) and 1 unit of shrimp alkaline phosphatase (Roche, Indianapolis, IN) at 37°C for 1 h and at 72°C for 15 min. Extension reactions with 1 μl of purified PCR product, 0.15 pmol of genotyping primer and a SNaPshot Multiplex Ready Reaction Mix (Applied Biosystems, Foster City, CA) were carried out by repeating the following cycle 25 times: 96°C for 10 s, 50°C for 5 s and 60°C for 30 s. The extension products were incubated with 1 unit of shrimp alkaline phosphatase (Roche, Indianapolis, IN) at 37°C for 1 h and next at 72°C for 15 min. Nine microliters of deionized formamide was mixed with 1 μl of the purified extension product, and the mixture was electrophoresed on an ABI Prism 3,700 genetic analyzer (Applied Biosystems, Foster City, CA). The results were analyzed using GeneScan analysis, version 3.7 (Applied Biosystems, Foster City, CA).

4) Statistical analysis

The allele frequencies and genotype frequencies and the departures of the genotype distributions from Hardy-Weinberg equilibrium for each SNP were analyzed using the chi-square test or Fisher's exact test. A p-value of <0.05 was considered statistically significant. Linkage disequilibrium (LD) was tested on pairwise combinations of SNPs with using the absolute value of the standardized measure of linkage disequilibrium, D', as calculated by the Haploview program version 3.2 (11). The haplotypes and their frequencies were estimated by the Haploview program version 3.2. The genotype-specific risks were estimated as odds ratios (ORs) with associated 95% confidence intervals by unconditional logistic regression (SAS Institute, Cary, NC) and these were adjusted for age, gender and the smoking status.

RESULTS

A total of 818 patients were enrolled in the study. Table 1 shows the characteristics of the study subjects. The baseline characteristics were similar between the case group and control

Table 1. Baseline characteristics of the study subjects

Variable	Cases (n=408)	Controls (n=410)	p-value
Age (years)	60.83	60.75	0.896
Gender			
Male	305	305	
Female	103	105	0.905
Smoking status			
Non-smoker	107	162	
Smoker	290	147	<0.0001
Drinking status			
Non-drinker	118	72	
Drinker	193	139	0.374
Stage			
1-3a	87		
3b-4	226		
Cell type			
Adenocarcinomas	182		
Squamous-cell carcinomas	107		
Small cell carcinoma	67		
Other carcinomas*	29		

*Including the large cell carcinomas and mixed cell carcinomas or the undifferentiated carcinomas.

group; however, there were significant differences in the smoking status between groups. By direct sequencing of the *PIM-1* gene, we discovered 15 polymorphisms among the 24 samples from lung cancer patients. We focused on 4 SNPs in the *PIM-1* gene (−1196 T>C, IVS4 +55 T>C, IVS4 +1416 T>A and +3684 C>A), which showed more than 5% minor allele frequencies of 0.072, 0.285, 0.139 and 0.139, respectively. Fig. 1 shows the structure of the *PIM-1* gene and the linkage disequilibrium. The genotype frequencies of these SNPs in the patients and controls were in Hardy-Weinberg equilibrium.

The associations between the risk of lung cancer in Koreans and the polymorphisms of the *PIM-1* gene and the haplotypes were analyzed. The genotype distributions for the cancer and control groups and the estimated associations with the lung cancer risk, as stratified by gender and the smoking status, are shown in Table 2. No significant difference was found in the distributions of the genotypes for all the tested polymorphisms between the overall lung cancer cases and the controls. However, the subsequent haplotype analysis revealed that the T-C-T-C haplotype was associated with susceptibility to the risk of lung cancer (aOR: 3.98; 95% CI: 1.24~12.75, p-value: 0.020), according to the multiple logistic regression analysis (Table 3). The T-C-T-C haplotype was more significantly associated with the increased risk for lung cancer in males

A

B

C

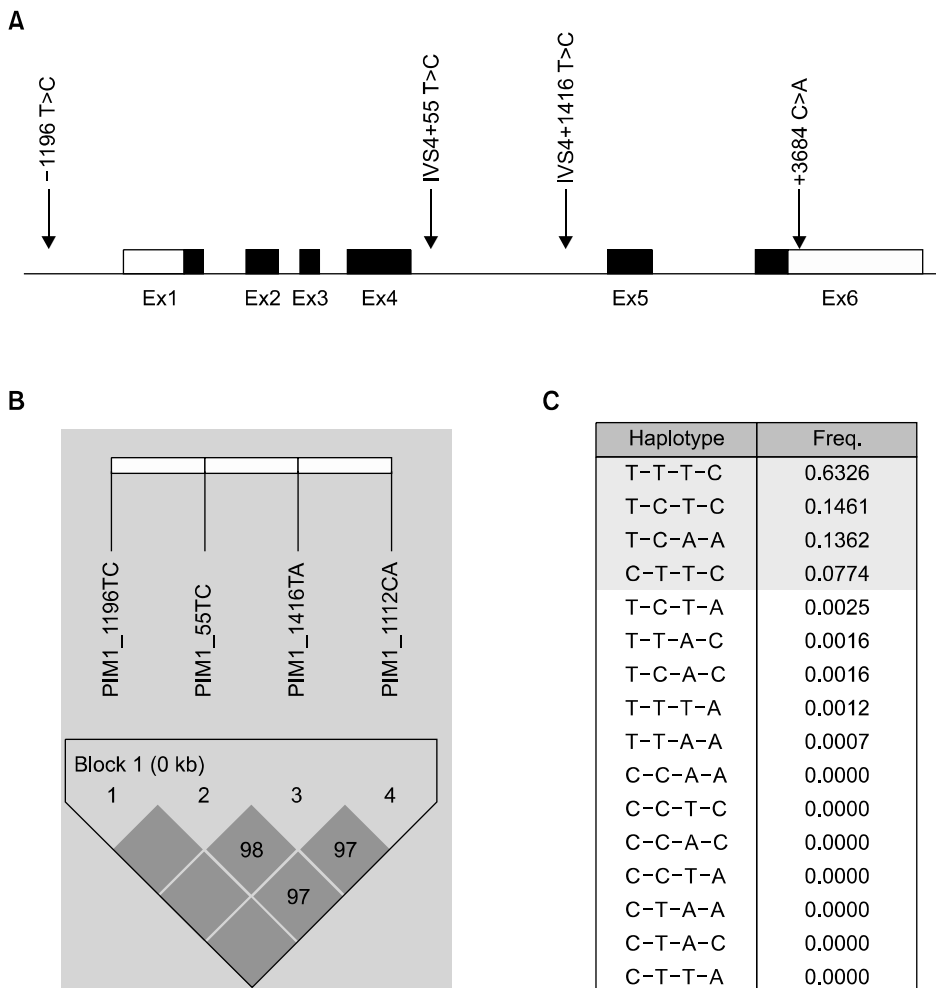| Haplotype	Freq.
T-T-T-C	0.6326
T-C-T-C	0.1461
T-C-A-A	0.1362
C-T-T-C	0.0774
T-C-T-A	0.0025
T-T-A-C	0.0016
T-C-A-C	0.0016
T-T-T-A	0.0012
T-T-A-A	0.0007
C-C-A-A	0.0000
C-C-T-C	0.0000
C-C-A-C	0.0000
C-C-T-A	0.0000
C-T-A-A	0.0000
C-T-A-C	0.0000
C-T-T-A	0.0000

Fig. 1. Gene map and haplotypes of the *PIM-1* gene. Coding exons are marked by the black blocks and the 5' and 3' UTR are marked by the white blocks. The first base of the translational site was denoted as nucleotide +1 (A). The LD blocks organization of the *PIM-1* gene (B). Haplotypes of the *PIM-1* gene (C).

Table 2. Distribution of the genotypes and their association with the risk of lung cancer in the overall population and with the subpopulations stratified according to gender and the smoking history

Group	Loci	Genotype	Cases (%)	Controls (%)	aOR (95% CI)*	p-value
Overall	−1196 T>C	TT	353 (86.5)	339 (84.3)	1 (Reference)	
		TC	51 (12.5)	60 (14.9)	0.65 (0.42~1.02)	0.061
		CC	4 (0.98)	3 (0.8)	4.94 (0.48~51.27)	0.181
	IVS4+55 T>C	TT	213 (53.1)	204 (52.0)	1 (Reference)	
		TC	153 (38.2)	147 (37.5)	0.88 (0.63~1.23)	0.456
		CC	35 (8.7)	41 (10.5)	0.85 (0.48~1.50)	0.573
	IVS4+1416 T>A	TT	292 (72.1)	308 (77.2)	1 (Reference)	
		TA	106 (26.2)	79 (19.8)	1.32 (0.91~1.91)	0.141
		AA	7 (1.7)	12 (3.0)	3.93 (0.45~34.37)	0.216
	+3684 C>A	CC	292 (71.7)	312 (77.0)	1 (Reference)	
		CA	105 (25.8)	86 (21.2)	1.32 (0.92~1.91)	0.136
		AA	10 (2.5)	7 (1.7)	2.13 (0.55~8.30)	0.275
Male	−1196 T>C	TT	261 (85.6)	252 (84.8)	1 (Reference)	
		TC	42 (13.7)	42 (14.1)	0.68 (0.40~1.16)	0.156
		CC	2 (0.7)	3 (1.0)	3.10 (0.17~8.16)	0.450
	IVS4+55 T>C	TT	164 (54.5)	158 (54.9)	1 (Reference)	
		TC	115 (38.2)	101 (35.1)	0.96 (0.64~1.45)	0.853
		CC	22 (7.3)	29 (10.1)	0.77 (0.37~1.59)	0.473
	IVS4+1416 T>A	TT	225 (73.8)	235 (79.1)	1 (Reference)	
		TA	76 (24.9)	50 (16.8)	1.45 (0.91~2.32)	0.120
		AA	4 (1.3)	12 (4.0)	1.65 (0.17~16.24)	0.668
	+3684 C>A	CC	224 (73.4)	237 (78.7)	1 (Reference)	
		CA	76 (24.9)	57 (18.9)	1.47 (0.92~2.34)	0.109
		AA	5 (1.6)	7 (2.3)	0.71 (0.16~3.25)	0.658
Female	−1196 T>C	TT	92 (89.3)	87 (82.9)	1 (Reference)	
		TC	9 (8.7)	18 (17.1)	0.45 (0.18~1.14)	0.092
		CC	2 (1.9)	0 (0.0)	−	0.987
	IVS4+55 T>C	TT	49 (49.0)	46 (44.2)	1 (Reference)	
		TC	38 (38.0)	46 (44.2)	0.83 (0.44~1.57)	0.571
		CC	13 (13.0)	12 (11.5)	1.24 (0.47~3.27)	0.665
	IVS4+1416 T>A	TT	67 (67.0)	73 (71.6)	1 (Reference)	
		TA	30 (30.0)	29 (28.4)	1.27 (0.64~2.30)	0.548
		AA	3 (3.0)	0 (0.0)	−	0.984
	+3684 C>A	CC	68 (66.7)	75 (72.1)	1 (Reference)	
		CA	29 (28.4)	29 (27.9)	1.20 (0.63~2.28)	0.580
		AA	5 (4.9)	0 (0.0)	−	0.978
Never	−1196 T>C	TT	97 (90.7)	132 (81.5)	1 (Reference)	
		TC	8 (7.5)	29 (17.9)	0.40 (0.17~0.93)	0.034
		CC	2 (1.9)	1 (0.6)	2.47 (0.21~28.83)	0.470
	IVS4+55 T>C	TT	49 (47.1)	76 (47.5)	1 (Reference)	
		TC	40 (38.5)	71 (44.4)	0.86 (0.49~1.49)	0.581
		CC	15 (14.4)	13 (8.1)	1.94 (0.80~4.70)	0.142
	IVS4+1416 T>A	TT	68 (64.8)	119 (74.8)	1 (Reference)	
		TA	35 (33.3)	40 (25.2)	1.44 (0.82~2.54)	0.209
		AA	2 (1.9)	0 (0.0)	−	0.987
	+3684 C>A	CC	68 (64.2)	121 (75.2)	1 (Reference)	
		CA	34 (32.1)	40 (24.8)	1.44 (0.81~2.53)	0.213
		AA	4 (3.8)	0 (0.0)	−	0.982
Ever	−1196 T>C	TT	247 (85.2)	124 (84.4)	1 (Reference)	
		TC	41 (14.1)	23 (15.7)	0.85 (0.48~1.49)	0.568
		CC	2 (0.69)	0 (0.0)	−	0.988
	IVS4+55 T>C	TT	156 (54.6)	73 (50.0)	1 (Reference)	
		TC	111 (38.8)	57 (39.0)	0.92 (0.59~1.42)	0.701
		CC	19 (6.6)	16 (11.0)	0.48 (0.23~1.03)	0.060
	IVS4+1416 T>A	TT	214 (74.1)	111 (77.6)	1 (Reference)	
		TA	71 (24.6)	31 (21.7)	1.26 (0.77~2.06)	0.367
		AA	4 (1.4)	1 (0.7)	1.85 (0.20~17.44)	0.591
	+3684 C>A	CC	214 (73.8)	112 (76.7)	1 (Reference)	
		CA	71 (24.5)	31 (21.2)	1.27 (0.77~2.08)	0.347
		AA	5 (1.7)	3 (2.1)	0.78 (0.18~3.41)	0.736

*aORs and confidence intervals (95% CIs) were calculated by unconditional logistic regression analysis and they were adjusted for age, gender and the smoking history.

Table 3. Association analysis of the haplotypes with the lung cancer risk

Group	Haplotype	Haplotype pairs	Cases (%)	Controls (%)	aOR (95% CI)*	p-value
Overall	ht1 T-T-T-C	−/−	175 (43.9)	148 (39.4)	1 (Reference)	
		+/−	167 (41.9)	165 (43.9)	0.79 (0.55~1.11)	0.175
		+/+	57 (14.3)	63 (16.8)	0.76 (0.47~1.23)	0.264
	ht2 T-C-T-C	−/−	5 (1.3)	13 (3.5)	1 (Reference)	
		+/−	89 (22.3)	106 (28.2)	2.30 (0.72~7.28)	0.158
		+/+	305 (76.4)	257 (68.4)	3.98 (1.24~12.75)	0.020
	ht3 T-C-A-A	−/−	7 (1.8)	4 (1.1)	1 (Reference)	
		+/−	106 (26.6)	81 (21.5)	0.30 (0.03~2.67)	0.279
		+/+	286 (71.7)	291 (77.4)	0.25 (0.03~2.18)	0.209
	ht4 C-T-T-C	−/−	4 (1.0)	3 (0.8)	1 (Reference)	
		+/−	50 (12.5)	59 (15.7)	0.07 (0.01~1.21)	0.067
		+/+	345 (86.5)	314 (83.5)	0.20 (0.02~2.11)	0.182
Gender						
Male	ht1 T-T-T-C	−/−	135 (44.9)	118 (42.9)	1 (Reference)	
		+/−	127 (42.2)	111 (40.4)	0.92 (0.60~1.41)	0.707
		+/+	39 (13.0)	46 (16.7)	0.75 (0.41~1.35)	0.333
	ht2 T-C-T-C	−/−	3 (1.0)	11 (4.0)	1 (Reference)	
		+/−	67 (22.3)	69 (25.1)	3.66 (0.89~14.99)	0.071
		+/+	231 (76.7)	195 (70.9)	5.67 (1.32~24.30)	0.019
	ht3 T-C-A-A	−/−	4 (1.3)	4 (1.5)	1 (Reference)	
		+/−	76 (25.3)	52 (18.9)	0.84 (0.08~9.01)	0.884
		+/+	221 (73.4)	219 (79.6)	0.59 (0.06~5.84)	0.655
	ht4 C-T-T-C	−/−	2 (0.7)	3 (1.1)	1 (Reference)	
		+/−	42 (14.0)	41 (14.9)	0.16 (0.01~6.67)	0.334
		+/+	257 (85.4)	231 (84.0)	0.33 (0.02~6.16)	0.455
Female	ht1 T-T-T-C	−/−	40 (40.8)	30 (29.7)	1 (Reference)	
		+/−	40 (40.8)	54 (53.5)	0.56 (0.28~1.09)	0.087
		+/+	18 (18.4)	17 (16.8)	0.94 (0.39~2.24)	0.889
	ht2 T-C-T-C	−/−	2 (2.1)	2 (2.0)	1 (Reference)	
		+/−	22 (22.5)	37 (36.6)	0.46 (0.06~3.85)	0.473
		+/+	74 (75.5)	62 (61.4)	0.91 (0.11~7.39)	0.927
	ht3 T-C-A-A	−/−	3 (3.1)	0 (0.0)	1 (Reference)	
		+/−	30 (30.6)	29 (28.7)	−	0.974
		+/+	65 (66.3)	72 (71.3)	−	0.984
	ht4 C-T-T-C	−/−	2 (0.0)	0 (0.0)	1 (Reference)	
		+/−	8 (0.1)	18 (17.8)	−	0.968
		+/+	88 (89.8)	83 (82.2)	−	0.987
Smoking history						
Never	ht1 T-T-T-C	−/−	43 (41.8)	52 (33.1)	1 (Reference)	
		+/−	39 (37.9)	83 (52.9)	0.54 (0.30~0.98)	0.042
		+/+	21 (20.4)	22 (14.0)	1.14 (0.54~2.42)	0.738
	ht2 T-C-T-C	−/−	2 (1.9)	3 (1.9)	1 (Reference)	
		+/−	25 (24.3)	51 (32.5)	0.56 (0.08~3.94)	0.560
		+/+	76 (73.8)	103 (65.6)	0.69 (0.10~4.87)	0.710
	ht3 T-C-A-A	−/−	2 (1.9)	0 (0.0)	1 (Reference)	
		+/−	35 (34.0)	40 (25.5)	−	0.980
		+/+	66 (64.1)	117 (74.5)	−	0.987
	ht4 C-T-T-C	−/−	2 (1.9)	1 (0.6)	1 (Reference)	
		+/−	7 (6.8)	28 (17.8)	0.12 (0.01~2.10)	0.146
		+/+	94 (91.3)	128 (81.5)	0.41 (0.04~4.76)	0.473
Ever	ht1 T-T-T-C	−/−	125 (43.9)	60 (42.0)	1 (Reference)	
		+/−	126 (44.2)	58 (40.6)	1.02 (0.65~1.60)	0.932
		+/+	34 (11.9)	25 (17.5)	0.60 (0.32~1.12)	0.111
	ht2 T-C-T-C	−/−	3 (1.1)	7 (4.9)	1 (Reference)	
		+/−	62 (21.8)	38 (26.6)	3.69 (0.89~15.24)	0.071
		+/+	220 (77.2)	98 (68.5)	7.82 (1.75~34.98)	0.007
	ht3 T-C-A-A	−/−	4 (1.4)	1 (0.7)	1 (Reference)	
		+/−	71 (24.9)	31 (21.7)	0.51 (0.05~5.33)	0.571
		+/+	210 (73.7)	111 (77.6)	0.53 (0.06~5.00)	0.579
	ht4 C-T-T-C	−/−	2 (0.7)	0 (0.0)	1 (Reference)	
		+/−	41 (14.4)	23 (0.2)	−	0.981
		+/+	242 (84.9)	120 (83.9)	−	0.988

*aORs and confidence intervals (95% CIs) were calculated by unconditional logistic regression analysis and they were adjusted for age, gender and the smoking history.

(aOR: 5.67; 95% CI: 1.32~24.30, p-value: 0.019) and smokers (aOR: 7.82; 95% CI: 1.75~34.98, p-value: 0.007).

DISCUSSION

In this study on 818 Korean lung cancer patients and controls, we found that four single nucleotide polymorphisms (−1196 T>C, IVS4 +55 T>C, IVS4 +1416 T>A and +3684 C> A) in the *PIM-1* gene were not significantly associated with an increased risk for lung cancer. However, the haplotype analysis showed that the T-C-T-C haplotype was significantly associated with an increased risk for lung cancer, and especially in males (aOR: 5.67; 95% CI: 1.32~24.30, p-value: 0.019) and smokers (aOR: 7.82; 95% CI: 1.75~34.98, p-value: 0.007). Although smoking itself plays a critical role in the development of lung cancer (12), only a fraction of smokers develop lung cancer. The reason for this discrepancy in the smoking population has not been clearly delineated. Nevertheless, it is highly possible that smoking plays an additional role to the genetic alterations in lung cancer patients. Although there has been no direct evidence to show that smoking induces DNA methylation, several studies have suggested that DNA hyper-methylation is associated with exposure to cigarette smoking (13,14). The studies on RASSF1A, which is a tumor suppre-ssion gene, indicated that the loss of heterozygosity and epigenetic inactivation of the RASSF1A promoter lead to down-regulation of gene expression, and this subsequently increases the risk of lung cancer (15). This suggests that the genetic constitution of individuals is of importance in determining the susceptibility of individuals to lung cancer (16). In our previous study on the RASSF1A promoter, similar results were also obtained for the smoking status (17). In the present study, we obtained further evidence to indicate that polymorphisms of the *PIM-1* proto-oncogene increased the susceptibility of lung car-cinogenesis due to smoking. However, an additional functional study of *PIM-1* kinase is needed regarding this subject.

Since the *PIM-1* gene was first recognized 20 years ago as a proviral insertion site of the Moloney murine leukemia virus (MoMuLV), many serial studies have established the *PIM-1* gene as a proto-oncogene and an important player in the pro-cess of malignant transformation (18,19). The overexpression and mutation of *PIM-1* kinase have been reported in various tumors of animal models, as well as in human tumors (18,19). The *PIM* family is composed of three highly homologous genes, *PIM-1*, *PIM-2* and *PIM-3*, and they are well conserved in vertebrates and they show high degrees of sequence and structural similarities (20). In animal models, *PIM-1* protein kinases have been shown to enhance the development of lymphoma, acute leukemia and prostate cancer (21). *PIM-2* overexpression is associated with chronic lymphocytic leukemia and non-Hodgkin's lymphoma (22), whereas *PIM-3* shows an aberrant expression in hepatocellular carcinoma (23). In humans, the protein levels of *PIM-1* have been shown to be elevated in lymphomas, leukemias and prostate cancer (1-3). Especially, the *PIM-1* gene has the potential to be a diagnostic and pro-gnostic marker for prostate cancer because microarray analysis (RT-PCR or immunohistochemical analysis) has revealed that the *PIM-1* gene was differentially expressed between benign

prostatic hyperplasia and prostate cancer (3).

The tumor suppressor genes and proto-oncogenes of lung cancer have been extensively studied, and this has revealed new information about their polymorphisms and hypermethylation (17,24,25). However, there has been no study on the role of the *PIM-1* gene in the development of lung cancer. To the best of our knowledge, this study is the first to address this issue. In the present study, we report on 4 SNPs of the *PIM-1* gene, and these SNPs were found for the first time in a Korean population. The polymorphism of −1196 T>C was the novel SNP, and the polymorphism of IVS4 +55 T>C was already known, but the frequencies of these SNPs have not been reported in the world's medical literature. The other 2 SNPs had not been reported in the Korean population. The observed frequencies of the minor alleles of IVS4 +1416 T>A and +3684 C>A in our study were 0.139 and 0.139, respectively. In the dbSNP database (www.ncbi.nlm.nih.gov/SNP), the Asian ethnic group's frequency of the minor allele of IVS4 +1416 T>A had been reported to be 0.628 (Han Chinese) and 0.489 (Japanese), but the frequency of the minor allele of +3684 C> A has not been previously reported for an Asian ethnic group.

The limitation of this study was the small size of the (−/−) haplotype pair group in the ht2 T-C-T-C haplotype, and this small size could lead to random error. However, we thought the results had significance because the above results were adjusted according to multiple variables and the results were derived from a relatively large-sized sample group.

CONCLUSION

This is the first study to identify the association between the *PIM-1* gene polymorphisms and the risk of lung cancer. In this study, we found that the T-C-T-C haplotype of the *PIM-1* gene (−1196 T>C, IVS4 +55 T>C, IVS4 +1416 T>A and +3684 C>A) could influence the risk of developing smoking-related lung cancer in the Korean population. Additional func-tional studies with larger sized study populations are needed in the future to reconfirm our findings.

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