ORIGINAL ARTICLE

Single-nucleotide polymorphisms in the promoter of the *CDK5* gene and lung cancer risk in a Korean population

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Cyclin-dependent kinase 5 (CDK5), a proline-directed serine/threonine kinase, which was originally known for its regulatory role in neuronal activities, has recently been suggested to play a role in extraneuronal activities. For example, a recent study detected overexpression of the *CDK5* gene in non-small-cell lung cancer. Therefore, in order to explore the association of the *CDK5* gene with lung cancer risk in a Korean population, the genotypes of the *CDK5* promoter region were determined in 407 lung cancer patients and 402 normal participants. The result showed that the –904 G>A genotype affected susceptibility to lung cancer risk (odd ratios (OR)=1.53, 95% confidence interval (CI)=1.02–2.32). Furthermore, subsequent haplotype analysis of three single-nucleotide polymorphism (SNP) regions suggested that the A–G–C haplotype was associated with a higher overall risk of lung cancer (OR=1.59, 95% CI=1.16–2.18). These results suggest that *CDK5* promoter polymorphisms contribute to the genetic susceptibility to lung cancer in the Korean population.

Journal of Human Genetics (2009) 54, 298–303; doi:10.1038/jhg.2009.29; published online 3 April 2009

Keywords: cyclin-dependent kinase 5 (CDK5); lung cancer; single-nucleotide polymorphism

INTRODUCTION

Cyclin-dependent kinase 5 (CDK5), located in 7q36 chromosome, is a proline-directed serine/threonine kinase that was originally identified as a member of the CDK family, on the basis of sequence homology.^{1,2} It is activated by p35Nck5a, which is autophosphorylated into a complex with CDK5.³ Although it is classified as a CDK, CDK5 does not regulate cell cycle.⁴ CDK5 is recognized as an essential molecule in the brain, where it regulates several neuronal activities, including cytoskeletal remodeling and synaptic transmission. There is presently a substantial amount of data to indicate that CDK5 plays important roles in extraneuronal cells during the process of myogenesis, lens differentiation, spermatogenesis, insulin secretion, apoptosis of ovarian cells and hematopoietic cell differentiation.^{3,5–11}

Furthermore, CDK5 has been suggested to play roles in the regulation of motility of prostate cancer cells,¹² control of glioblastoma cell invasion,¹³ modulation of proliferation of medullary thyroid carcinoma cells¹⁴ and apoptotic control of leukemia cells.¹⁵ These potential roles of CDK5 activity in the carcinogenesis of each cell type seem to involve distinct target substrates.¹⁶

Frequent amplification and overexpression of the *CDK5* gene along with multiple epidermal growth factor receptor (EGFR)-family-signal-

ing pathway components, including SHC1, AKT1 and MYC, was recently discovered in non-small cell lung cancer (NSCLC).¹⁷ As it has been known earlier that CDK5/p35 activation by neuregulin subsequently activates PI3K and Akt through ErbB3 and ErbB2 receptors in cultured cortical neurons,¹⁸ the activation of *CDK5*, along with the other genes, may play a role in NSCLC tumorigenesis.

It has been reported that an individual's susceptibility to a disease such as lung cancer can vary depending on the presence of single-nucleotide polymorphisms (SNPs), an important class of genetic variation, in genes affecting cell proliferation or cell death.^{19–21} Moreover, SNPs in the coding regions or the regulatory regions of genes are more likely to cause functional differences than SNPs located elsewhere.¹⁹

Therefore, this study was undertaken to examine those possible relationship between three novel SNPs found in *CDK5* promoter region and the risk of lung cancer occurrence in Korean lung cancer patients.

MATERIALS AND METHODS

Study patients

From August 2001 to February 2007, blood samples were collected from successive series of 809 participants, including 407 lung cancer cases

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Received 17 October 2008; revised 8 January 2009; accepted 23 February 2009; published online 3 April 2009

Figure 1 The cyclin-dependent kinase 5 (*CDK5*) gene promoter region and genotyping. In this schematic, the translation initiation site for the *CDK5* protein is indicated as +1 (**a**). Polymorphism analysis of -904 (**b**), -270 (**c**) and -238 (**d**) loci in the *CDK5* gene by PCR, followed by specific restriction enzyme digestion. The left panel of each figure represents schematic presentation of the enzyme restriction pattern generated by polymorphisms at each locus. As indicated on the top of each lane on the right panels, representative samples with polymorphisms at -904, -270 and -238 are shown. Lane M was loaded with appropriate DNA markers.

and 402 controls. The lung cancer cases were recruited from patients at the Korea Genomic Research Center for Lung and Breast/Ovarian Cancer and Inha University Medical Center, and age- and gender- matched control individuals were selected from a pool of healthy volunteers at the Cardiovascular Genome Center, Genomic Research Center for Allergy and Respiratory Diseases and Keimyung University Dongsan Medical Center. All participants were given a structured questionnaire that elicited information on diet, smoking status, alcohol consumption, lifestyle and medical history. All study participants were ethnic Koreans, and their written

consent and institutional review board approval for the study protocol were obtained.

Screening and genotyping of *CDK5* promoter polymorphism

The genotype analysis was carried out by investigators blinded to all patient identifiers and information. Genomic DNA of all participants was isolated from blood samples using a Puregene blood DNA kit (Gentra, Minneapolis, MN, USA), according to the manufacturer's instructions. To identify SNPs in the

Table 1 Baseline characteristics of study participants

	Cases (n=407)	Controls (n=402)	P-value
Age (years)	60.78 ± 0.47	60.76 ± 0.54	0.808 ^a
Gender			
Male	303	297	0.854 ^b
Female	104	105	
Smoking status			
Non-smoker	107	162	<0.001 ^b
Smoker	289	147	
Drinking status			
Non-drinker	118	72	0.404 ^b
Drinker	195	139	
Stage			
1–3a	84	—	
3b–4	227	—	
Cell type			
Adenocarcinoma (AdC)	157	—	
Squamous cell carcinoma (SqC)	121	—	
Other carcinomas	107	—	

^aMann–Whitney test.^b χ^2 -test.

promoter of the *CDK5* gene, the genomic DNA of 24 randomly selected samples from the lung cancer patients was amplified for the 5' regulatory region (~3 kb) from the translation initiation site of the *CDK5* gene. PCR amplification was carried out in a PTC-225 Peltier Thermal cycler (MJ research Inc., Waltham, MA, USA) using AmpliTaqGold (Roche, Branchburg, NJ, USA). An Applied Biosystems 3700 DNA analyzer (Applied Biosystems, Foster City, CA, USA) was used for direct sequencing. Next, we focused on the three SNPs (–904, –270 and –238 locus) of the *CDK5* gene using the PCR-based restriction fragment length polymorphism (PCR-RFLP) method for further analyses of the three *CDK5* SNPs in 407 lung cancer cases and 402 controls. The designs of primers for RFLP were based on the GenBank sequence (NT_007914). The genotypes of the sample were amplified by PCR using 5'-CTAGGCAGAGCCATCACTTA-3' (forward) and 5'-GATTTTGTGGAG GGACACA-3' (reverse) primer pairs for –904 locus, and 5'-CTGGGAGCACAT AAAAGGAA-3' (forward) and 5'-CTTCTGGGGCTTGTAGTCCT-3' (reverse) primer pairs for –270 locus, and 5'-ACTTGAAGTCCAAGGTTTGC-3' (forward) and 5'-CTTCTGGGGCTTGTAGTCCT-3' (reverse) primer pairs for –238 locus. The PCR was carried out using 50 ng of genomic DNA, 1×Taq buffer, 10 μM each of primers, 200 μM 2'-deoxynucleoside 3'-triphosphate and 0.5 μl (2.5 U μl^{–1}) of Taq polymerase (Han Taq, Genenmed, Seoul, Korea) in a final volume of 20 μl. PCR amplification was carried out for 35 or 40 cycles with heat denaturing at a temperature of 94 °C for 1 min, annealing at 55 °C (–904 locus), 60 °C (–270 locus), 58 °C (–238 locus) for 30 s, primer extending at 72 °C for 30 s and a final step at 72 °C for 7 min. PCR products were verified by agarose gel electrophoresis. The PCR products were digested with restriction endonuclease to determine the genotype of each participant. DNA fragments were separated using 2–3% agarose gel and were detected by ethidium bromide staining. In the –904 locus, the A to G transition created an SfaNI (New England Biolabs, Beverly, MA, USA) restriction site. The PCR product containing the G allele was broken into two fragments, whereas the PCR product containing the A allele could not be cut by SfaNI (Figure 1b). In the same manner, the G to C transition in the –270 locus created a BsmFI (New England Biolabs) restriction site (Figure 1c), whereas the A to C

transition in the –238 locus created a HpaII (New England Biolabs) restriction site (Figure 1d). More than 10% of the DNA samples were randomly selected for genotype confirmation using the SNaPshot (PE Applied Biosystems, Warrington, UK) ddNTP primer extension method as described earlier,²² and the results were in complete concordance.

Statistical analysis

Allele frequencies, genotype frequencies and departures of the genotype distribution from Hardy–Weinberg equilibrium for each SNP were analyzed using the χ^2 -test or Fisher's exact test. Linkage disequilibrium (LD) was tested on pairwise combinations of SNPs using the absolute value of the standardized measure of LD, $|D'|$, calculated using the Haploview program version 3.2. A P-value of <0.05 was considered statistically significant. Genotype-specific risks were estimated as odd ratios (ORs) with associated 95% confidence intervals (CIs) by unconditional logistic regression and adjusted for age, gender and smoking status. In the codominant model, the common homozygous genotype in the controls was defined as the reference group; the rare homozygous and heterozygous genotypes were variant genotypes, and their effects were individually estimated by comparison with the reference. Alternatively, the rare homozygotes and the heterozygotes were combined to form the reference group and compared with the common homozygote in the dominant model. In the recessive model, only the rare homozygotes were defined as the variant genotype, whereas the other two genotypes were combined as the reference. Statistical analyses were carried out using the SAS system, version 9.1 (SAS Institute, Cary, NC, USA).

RESULTS

The study population consisted of 407 lung cancer patients and 402 controls (Table 1). All the clinical characteristics, except for smoking history, were not significantly different between the cases and controls. The initial screening of *CDK5* promoter region from the genomic DNA of 24 Korean lung cancer patients identified three polymorphisms: –904 G>A, –270 C>G and –238 A>C, and the minor allele frequencies of the study population were 0.260, 0.313 and 0.298, respectively. Further genotyping on the lung cancer and control populations showed that the genotype distributions of the three polymorphisms among all study populations were in Hardy–Weinberg equilibrium. Furthermore, the three polymorphisms showed strong LD in both the controls and the cases ($|D'| \geq 0.92$, $P < 0.0001$) (data not shown).

The distribution of the selected *CDK5* genotypes and the estimated associations between the *CDK5* functional SNPs and lung cancer risk are shown in Table 2. Among the distributions of the genotypes between the overall lung cancer cases and the controls, the –904 G>A genotype was shown to affect lung cancer risk. Patients with the A allele at the site had a significantly higher risk of lung cancer than those with only the G allele (dominant model; adjusted OR=1.53, 95% CI=1.02–2.32). Genotypes –270 C>G and –238 A>C were also shown to be somewhat related to the increase in risk of lung cancer; however, their CIs were too wide to be statistically significant (dominant model; –270 C>G OR=1.18, 95% CI=0.86–1.61; –237 A>C OR=1.20, 95% CI=0.88–1.65). In the analysis stratified by gender, smoking and drinking history, the effect of A genotype of –904 G>A on the lung cancer risk was found in females (OR=2.02, 95% CI=1.12–3.64) and non-smokers (OR=1.78, 95% CI=1.06–3.00) in the dominant model.

Subsequent haplotype analysis of the three *CDK5* SNP sites (–904 G>A, –270 C>G, –238 A>C) showed that the A–G–C haplotype was associated with a higher overall risk of lung cancer (OR=1.59, 95% CI=1.16–2.18) in the dominant model (Table 3). Further analysis stratified by gender, smoking and drinking history showed that the individuals carrying the A–G–C haplotype had significantly higher risks of lung cancer in subgroups, including females, non-smokers and

Table 2 Distribution of SNPs and their association with lung cancer risk in overall population and subpopulation stratified by gender, smoking and drinking status

Group	Loci	Genotype	Cases (%)	Controls (%)	Dominant OR (95% CI) ^a	Recessive OR (95% CI) ^a	Codominant OR (95% CI) ^a
Overall	-904 G>A	GG	183 (45.1)	225 (57.8)	1.52 (1.11–2.09) ^b	1.19 (0.71–2.00)	1.32 (1.04–1.67) ^b
		GA	179 (44.1)	126 (32.4)			
		AA	44 (10.8)	38 (9.8)			
	-270 C>G	CC	183 (45.2)	198 (50.6)	1.18 (0.86–1.61)	0.83 (0.51–1.34)	1.04 (0.83–1.31)
		CG	178 (44.0)	141 (36.1)			
		GG	44 (10.8)	52 (13.3)			
	-238 A>C	AA	182 (45.1)	200 (52.5)	1.20 (0.88–1.65)	0.87 (0.53–1.41)	1.07 (0.85–1.35)
		AC	178 (44.1)	135 (35.4)			
		CC	44 (10.8)	46 (12.1)			
Male	-904 G>A	GG	139 (46.0)	168 (59.2)	1.33 (0.91–1.97)	1.05 (0.57–1.94)	1.18 (0.89–1.58)
		GA	129 (42.2)	85 (29.9)			
		AA	34 (11.3)	31 (10.9)			
	-270 C>G	CC	140 (46.5)	148 (51.8)	1.03 (0.70–1.52)	0.72 (0.41–1.27)	0.94 (0.71–1.24)
		CG	127 (42.2)	96 (33.6)			
		GG	34 (11.3)	42 (14.7)			
	-238 A>C	AA	140 (46.5)	150 (54.4)	1.06 (0.72–1.56)	0.76 (0.43–1.36)	0.97 (0.73–1.28)
		AC	127 (42.2)	90 (32.6)			
		CC	34 (11.3)	36 (13.0)			
Female	-904 G>A	GG	44 (42.3)	57 (54.3)	2.02 (1.12–3.64) ^b	1.55 (0.55–4.38)	1.67 (1.05–2.66) ^b
		GA	50 (48.1)	41 (39.1)			
		AA	10 (9.6)	7 (6.7)			
	-270 C>G	CC	43 (41.4)	50 (47.6)	1.58 (0.88–2.86)	1.09 (0.42–2.82)	1.33 (0.85–2.07)
		CG	51 (49.0)	45 (42.9)			
		GG	10 (9.6)	10 (9.5)			
	-238 A>C	AA	42 (40.8)	50 (47.6)	1.60 (0.88–2.88)	1.09 (0.42–2.83)	1.33 (0.85–2.08)
		AC	51 (49.5)	45 (42.9)			
		CC	10 (9.7)	10 (9.5)			
Non-smoker	-904 G>A	GG	43 (40.2)	86 (53.1)	1.78 (1.06–3.00) ^b	1.13 (0.45–2.80)	1.45 (0.97–2.17)
		GA	54 (50.5)	63 (38.9)			
		AA	10 (9.4)	13 (8.0)			
	-270 C>G	CC	42 (39.3)	75 (46.6)	1.41 (0.84–2.38)	0.84 (0.35–1.99)	1.18 (0.79–1.75)
		CG	55 (51.4)	69 (42.9)			
		GG	10 (9.4)	17 (10.6)			
	-238 A>C	AA	41 (38.7)	76 (47.2)	1.45 (0.86–2.44)	0.87 (0.37–2.08)	1.21 (0.81–1.79)
		AC	55 (51.9)	69 (42.9)			
		CC	10 (9.4)	16 (9.9)			
Smoker	-904 G>A	GG	134 (46.5)	82 (56.2)	1.35 (0.90–2.05)	1.25 (0.65–2.44)	1.24 (0.91–1.68)
		GA	120 (41.7)	49 (33.6)			
		AA	34 (11.8)	15 (10.3)			
	-270 C>G	CC	136 (47.4)	74 (50.3)	0.99 (0.66–1.50)	0.82 (0.45–1.50)	0.95 (0.71–1.28)
		CG	117 (40.8)	52 (35.4)			
		GG	34 (11.9)	21 (14.3)			
	-238 A>C	AA	136 (47.4)	74 (50.1)	1.01 (0.67–1.52)	0.86 (0.47–1.59)	0.97 (0.72–1.30)
		AC	117 (40.1)	52 (35.6)			
		CC	34 (11.9)	20 (13.7)			
Non-drinker	-904 G>A	GG	46 (39.3)	37 (51.4)	1.88 (1.01–3.50) ^b	1.70 (0.59–4.85)	1.63 (1.00–2.64) ^b
		GA	58 (49.6)	29 (40.3)			
		AA	13 (11.1)	6 (8.3)			
	-270 C>G	CC	49 (42.4)	33 (45.8)	1.21 (0.65–2.25)	0.99 (0.38–2.57)	1.11 (0.70–1.76)
		CG	55 (47.4)	30 (41.7)			
		GG	12 (10.3)	9 (12.5)			
	-238 A>C	AA	48 (41.7)	33 (45.8)	1.22 (0.66–2.27)	0.99 (0.38–2.57)	1.11 (0.70–1.77)
		AC	55 (47.8)	30 (41.7)			
		CC	12 (10.4)	9 (12.5)			
Drinker	-904 G>A	GG	90 (46.2)	82 (59.4)	1.64 (1.03–2.62) ^b	1.71 (0.80–3.64)	1.46 (1.03–2.06) ^b
		GA	80 (41.0)	43 (31.2)			
		AA	25 (12.8)	13 (9.4)			
	-270 C>G	CC	90 (46.2)	70 (50.4)	1.05 (0.66–1.68)	0.92 (0.47–1.80)	1.00 (0.72–1.40)
		CG	80 (41.0)	49 (35.3)			
		GG	25 (12.8)	20 (14.4)			
	-238 A>C	AA	90 (46.2)	70 (50.7)	1.07 (0.67–1.70)	0.97 (0.49–1.91)	1.03 (0.74–1.43)
		AC	80 (41.0)	49 (35.5)			
		CC	25 (12.8)	19 (13.8)			

Abbreviations: CI, confidence interval; ORs, odds ratios; SNPs, single-nucleotide polymorphisms.

^aORs and 95% CI were calculated by logistic regression model and adjusted for age, gender and smoking status.

^b*P*<0.05.

Table 3 Distribution of haplotypes of selected SNPs of *CDK5* and their association with lung cancer risk in overall population and subpopulation stratified by gender, smoking and drinking status

Group	Haplotypes		Cases (%)	Controls (%)	Dominant	Recessive	Codominant
	(-904/-270/-237)				OR (95% CI) ^a	OR (95% CI) ^a	OR (95% CI) ^a
Overall	G-C-A	-/-	173 (42.8)	181 (49.1)			
		+/-	183 (45.3)	142 (38.5)	1.03 (0.64–1.67)	0.86 (0.62–1.18)	0.93 (0.74–1.17)
		+/+	48 (11.9)	46 (12.5)			
	A-G-C	-/-	40 (9.9)	37 (10.0)			
		+/-	174 (43.1)	112 (30.4)	1.59 (1.16–2.18) ^b	1.08 (0.63–1.84)	1.33 (1.04–1.69) ^b
		+/+	190 (47.0)	220 (59.6)			
Male	G-C-A	-/-	133 (44.2)	134 (50.8)			
		+/-	131 (43.5)	94 (35.6)	1.16 (0.66,2.05)	0.99 (0.67–1.46)	1.03 (0.78–1.36)
		+/+	37 (12.3)	36 (13.6)			
	A-G-C	-/-	31 (10.3)	30 (11.4)			
		+/-	125 (41.5)	74 (28.0)	1.40 (0.95–2.06)	0.94 (0.50–1.75)	1.19 (0.89–1.59)
		+/+	145 (48.2)	160 (60.6)			
Female	G-C-A	-/-	40 (38.8)	47 (44.8)			
		+/-	52 (50.5)	48 (45.7)	0.85 (0.33–2.15)	0.64 (0.35–1.16)	0.75 (0.48–1.17)
		+/+	11 (10.7)	10 (9.5)			
	A-G-C	-/-	9 (8.7)	7 (6.7)			
		+/-	49 (47.6)	38 (36.2)	2.09 (1.16–3.77) ^b	1.43 (0.50–4.13)	1.68 (1.06–2.68) ^b
		+/+	45 (43.7)	60 (57.1)			
Non-smoker	G-C-A	-/-	39 (36.8)	68 (42.5)			
		+/-	56 (52.8)	76 (47.5)	1.06 (0.45–2.47)	0.74 (0.44–1.26)	0.85 (0.57–1.27)
		+/+	11 (10.4)	16 (10.0)			
	A-G-C	-/-	9 (8.5)	12 (7.5)			
		+/-	53 (50.0)	56 (35.0)	1.94 (1.15–3.26) ^b	1.08 (0.42–2.78)	1.52 (1.01–2.29) ^b
		+/+	44 (41.5)	92 (57.5)			
Smoker	G-C-A	-/-	129 (45.0)	71 (48.6)			
		+/-	121 (42.2)	55 (37.7)	1.01 (0.55–1.85)	0.99 (0.65–1.50)	1.00 (0.74–1.34)
		+/+	37 (12.9)	20 (13.7)			
	A-G-C	-/-	31 (10.8)	15 (10.3)			
		+/-	116 (40.4)	46 (31.5)	1.36 (0.90–2.05)	1.08 (0.55–2.11)	1.20 (0.88–1.64)
		+/+	140 (48.8)	85 (58.2)			
Non-drinker	G-C-A	-/-	43 (37.4)	27 (37.5)			
		+/-	59 (51.3)	36 (50.0)	0.83 (0.32–2.19)	0.84 (0.43–1.61)	0.87 (0.53–1.41)
		+/+	13 (11.3)	9 (12.5)			
	A-G-C	-/-	12 (10.4)	6 (8.3)			
		+/-	54 (47.0)	23 (31.9)	2.48 (1.29–4.78) ^b	1.90 (0.63–5.78)	1.95 (1.17–3.25) ^b
		+/+	49 (42.6)	43 (59.7)			
Drinker	G-C-A	-/-	86 (44.1)	66 (47.8)			
		+/-	81 (41.5)	53 (38.4)	0.98 (0.50–1.94)	0.97 (0.59–1.59)	0.98 (0.70–1.38)
		+/+	28 (14.4)	19 (13.8)			
	A-G-C	-/-	22 (11.3)	13 (9.4)			
		+/-	79 (40.5)	39 (28.3)	1.61 (0.98–2.64)	1.05 (0.48–2.30)	1.30 (0.90–1.88)
		+/+	94 (48.2)	86 (62.3)			

Abbreviations: CDK5, cyclin-dependent kinase 5; CI, confidence interval; ORs, odds ratios; SNPs, single-nucleotide polymorphisms.

^aORs and 95% CI were calculated by logistic regression model and adjusted for age, gender and smoking status.^b*P* < 0.03.

non-drinkers (female: OR=2.09, 95% CI 1.16–3.77; non-smoker: OR=1.94, 95% CI=1.15–3.26; non-drinker: OR=2.48, 95% CI=1.29–4.78).

DISCUSSION

It is quite possible that DNA sequence variations in the *CDK5*, a gene that has recently been studied for its association with non-neuronal cell differentiation and apoptosis in many areas, may lead to alteration in the activity of CDK5, which can cause individual differences in lung cancer susceptibility. Indeed, a recent study on CDK5-mediated regulation of the PIKE-A-Akt pathway suggests an important clue to

the association of CDK5 with tumorigenesis, as the accumulation of phosphorylated PIKE-A (PI3-kinase enhancer), a recently identified GTP-binding protein shown to regulate the PI3K–Akt pathway and activation of Akt in the nucleus of glioblastoma cell, is a CDK5-dependent activity,²³ suggesting possible association of CDK5 with non-neuronal cell survival. This was further supported by another study that showed the overexpression of CDK5, along with the other downstream components of the EGFR-family signaling pathway, such as AKT1 and SHC1, as a direct result of gene amplification, specifically in lung cancer cells,¹⁷ signifying that the alteration of the *CDK5* gene may play a causal role in the development of lung cancer.

In this study, we evaluated the associations between three newly found SNPs (−904 G>A, −270 C>G and −238 A>C) in the promoter region of the *CDK5* gene and the risk for lung cancer. Of the three SNPs, −904 G>A showed a possibility to play an important role in carcinogenesis (overall OR=1.53, 95% CI=1.11–2.09), whereas the other two SNPs (−270 C>G and −238 A>C) showed some increase in the risk of lung cancer, although not adequately proven to be statistically significant.

Many studies showed that analysis of haplotypes has a greater power to detect the association with disease than an individual polymorphism. Moreover, haplotype analysis offers the advantages of not assuming that any of the genotyped polymorphisms is functional, and it allows for the possibility of an ungenotyped functional variant to be in LD with the genotyped polymorphisms.^{24–27} Therefore, we conducted a subsequent analysis on *CDK5* haplotypes consisting of −904 G>A, −270 C>G and −238 A>C polymorphisms, which are in strong LD. In the dominant haplotype analysis, the A–G–C haplotype of the three polymorphic sites was shown to be related to the higher risk of lung cancer (overall OR=1.59, 95% CI=1.16–2.18). Notably, our stratified analyses of *CDK5* showed that, when having the A–G–C haplotype, the risk of lung cancer increased significantly in females, non-smokers and non-drinkers (female: OR=2.09, 95% CI=1.16–3.77; non-smoker: OR=1.94, 95% CI=1.15–3.26; non-drinker: OR=2.48, 95% CI=1.29–4.78). To examine whether the A–G–C haplotype influences promoter activity of the *CDK5* gene, the *CDK5* promoter sequence connected to the luciferase reporter gene was transfected into NSCLC cell lines. However, we found no significant correlation (data not shown).

Tobacco smoking has well been established to play a critical role in the development of lung cancer; however, only a fraction of the smokers actually develops cancer. Moreover, the cause of increasing incidence of lung cancer in never-smokers has never been clearly explained.

This observation suggests that individual genetic variations may determine susceptibility to lung cancer. The present result on our study that shows a close association between lung cancer in Korean population and the polymorphisms in the promoter region of *CDK5* further validates this claim. The GWA database on various ethnicities has been reported to have no frequency data on −270 C>G polymorphism, whereas data on the −904 G>A and −238 A>C polymorphisms have been reported (HmapEU, 0.25 and 0.21; HmapCN, 0.26 and 0.26; HmapJP, 0.33 and 0.30; HmapNG, 0.43 and 0.20, respectively).

To the best of our knowledge, this is the first study to delineate the association between *CDK5* promoter polymorphisms and the risk of lung cancer, and to show that one of the *CDK5* polymorphisms, −904 G>A, and the A–G–C haplotype (−904 A/−270 G/−238 C) cause overall increase in the susceptibility of lung cancer. Moreover, subsequent stratified analyses showed statistically significant increases in the risk of lung cancer in females, non-smokers and non-drinkers who have the A–G–C haplotype of the three polymorphic sites. Therefore, the present result suggests an important association between the genetic polymorphisms of *CDK5* and lung cancer development, and the necessity for further functional studies on the relationship between the *CDK5* gene and lung cancer.

ACKNOWLEDGEMENTS

We thank Young Lim, Dong-Hoon Shin, Choon-Sik Park and Yangsoo Jang for sample collection and provision. This study was supported by a grant of the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (A010250).

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