

Original Article

Association Between MicroRNA196a2 rs11614913 Genotypes and the Risk of Non-Small Cell Lung Cancer in Korean Population

Young-Seoub Hong¹, Ho-Jin Kang¹, Jong-Young Kwak², Byung Lae Park³,
Chang-Hun You¹, Yu-Mi Kim¹, Heon Kim⁴

¹Department of Preventive Medicine; ²Medical Research Center for Cancer Molecular Therapy, Dong-A University College of Medicine, Busan; ³Department of Genetic Epidemiology, SNP Genetics Inc., Seoul;
⁴Department of Preventive Medicine, Chungbuk National University College of Medicine, Cheongju, Korea

Objectives: The microRNA (miRNA) miR-196a2 may play an important role in lung cancer development and survival by altering binding activity of target mRNA. In this study, we evaluated their associations with the susceptibility of non-small cell lung cancers (NSCLC) by case-control study in a Korean population.

Methods: We performed genotyping analyses for miR-196a2 rs11614913 T/C at miRNA regions in a case-control study using blood samples of 406 NSCLC patient and 428 cancer-free control groups.

Results: The total C allele frequencies for miR-196a2 were 48.8% for the patients and 45.6% for the controls; and the genotype frequencies of TT, TC, and CC were 23.7%, 55.2%, and 21.1% for the patients and 31.1%, 46.35%, and 22.4% for the controls ($p < 0.05$). Participants who possess TC/CC genotypes showed high risk for NSCLC compared to those possessed TT genotypes (OR, 1.42; 95% CI, 1.03 to 1.96). The association was persisted in 60 and older age group, male, smokers, those without family history for cancer. However, no significant association of CC genotypes in recessive genetic model was observed.

Conclusions: In conclusion, this case-control study provides evidence that miR-196a2 rs11614913 C/T polymorphisms are associated with a significantly increased risk of NSCLC in a dominant model, indicating that common genetic polymorphisms in miR-196a2 rs11614913 are associated with NSCLC. The association of miR196a2 rs11614913 polymorphisms and NSCLC risk require confirmation through additional larger studies.

Key words: MicroRNA, Non-small cell lung cancer, Polymorphism
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INTRODUCTION

MicroRNAs (miRNAs) are 21 to 24 nucleotide small non-coding RNA gene products that play important roles in the regulation of eukaryotic gene expression by base pairing with target mRNAs at the 3'-untranslated region; they can lead to mRNA cleavage or translational repression [1-3]. It has been suggested that miRNAs are involved in various biological processes, including cell proliferation, cell death, stress resistance, and fat metabolism [4]. Moreover, several recent reports have shown that miRNAs participate in human tumorigenesis as tumor suppressors or oncogenes [5-7]. For example, miRNA let-7, which targets the oncogene Ras, is downregulated in lung cancer [8], whereas the miR-17-92 cluster at 13q31.3 is reportedly overexpressed in lung

cancer [9]. Single nucleotide polymorphisms (SNPs) or mutations in an miRNA sequence may alter miRNA expression or maturation. Expression of miRNA could have important consequences on the expression of the various protein coding oncogenes and tumor suppressors involved in malignant transformation. Furthermore, the mechanism through miRNA expression can be caused by genomic amplification [10], genomic deletion [11], epigenetic alteration [12], and retroviral insertion mutagenesis [13,14].

Recently, several miRNAs were used to screen for common SNPs and the screening identified four SNPs (rs2910164, rs2292832, rs11614913, and rs3746444) at the pre-miRNA regions of miR-146a, miR-149, miR-196a2, and miR-499, respectively. The rs11614913 SNP in miR-196a2 was associated with a shortened survival

time of non-small cell lung cancer (NSCLC) by altering the expression of mature miR-196a and the binding activity of target mRNA [15]. In addition, the miR-196a2 may play an important role in lung cancer development and survival by influencing the expression and maturation of miRNAs [16].

In this study, we hypothesize that this functional SNP, rs11614913 T/C in miR-196a2, is also associated with lung cancer susceptibility in a Korean population. We performed genotyping analyses of miR-196a2 rs11614913 T/C at miRNA regions and evaluated their associations with the susceptibility of NSCLC in a case-control study of 406 NSCLC patients and 428 cancer-free controls in a Korean population.

MATERIALS AND METHODS

I. Study Subjects

The subjects of this study were members of a hospital-based study population: they included 406 patients with lung cancer and 428 cancer-free control subjects. Our study was approved by the Institutional Review Board of Chungbuk University and Dong-A University, and written informed consent was obtained from all participants or their representatives. All patients were histopathologically diagnosed as having NSCLC and were prospectively recruited into an ongoing study of lung cancer molecular epidemiology that started in 2003. Healthy volunteers for controls were recruited from among the residents of Busan city by receive health checkups for diseases of adult. They had no reported current or past history of disorders. The age and sex distributions were not significantly different among the subjects in the NSCLC group and the control group. The mean age was 67.3 ± 10.2 for patients and 63.2 ± 10.2 for control subjects. All subjects were interviewed in accordance with a structured questionnaire to obtain information on demographic data, including age, gender, and residence. After the interview, a onetime sample of approximately 3 to 5 mL of venous blood was collected from each participant.

II. DNA Extraction and Genotyping

Blood samples were collected in tubes that contained EDTA. Genomic DNA was extracted from white blood cell fractions by means of a Qiagen blood kit (Qiagen, Chatsworth, CA, USA). Genotyping was performed with

Table 1. Distribution of selected variables between non-small cell lung cancer patient and control subjects

Variables	n (%)		p-value
	Patients n=406	Controls n=428	
Age (y) (mean±SD)	67.3 ± 10.2	63.2 ± 10.2	0.38
Age (y)			
≥ 60	317 (78.1)	278 (64.9)	0.07
< 60	89 (21.9)	150 (35.1)	
Gender			
Male	323 (79.6)	336 (78.5)	0.71
Female	83 (20.4)	92 (21.5)	
Smoking status			
Smokers	346 (85.2)	284 (66.4)	<0.01
Non-smokers	60 (14.8)	144 (33.6)	
Family history of cancer			
No	323 (79.6)	313 (73.1)	0.08
Yes	83 (20.4)	115 (26.9)	

SD: standard deviation.

p-value was revealed by two-sides Chi-square test.

a polymerase chain reaction (PCR). For genotyping of the polymorphic sites, the amplifying primers and probes were designed for TaqMan (Assay-on-demand ID; C_31185852_10). A primer (Applied Biosystems, Foster City, CA, USA) was used to design the PCR primers and the MGB TaqMan probes. The miR-196a2 specific primers were forward, 5'-CCAGGCTGGTCTCGAACTC-3', and reverse, 5'-CTGAATAAATGAGTTCTGCAAAA-CAGGTT-3'. One allelic probe was labeled at the 5' end with the FAM dye (5'-CAAGTGATGCACCCAC-3') and at the other end with fluorescent VIC dye (5'-CCTCAAGTGATTCACCCAC-3'). The PCRs were run on a TaqMan Universal Master mix without UNG (Applied Biosystems, Foster City, CA, USA) but with a PCR primer concentration of 900 nM and a TaqMan MGB-probe concentration of 200 nM. The reactions were carried out in a 384-well format in a total reaction volume of 5 uL with 20 ng of the genomic DNA. The plates then were placed in a thermal cycler (PE 9700, Applied Biosystems, Foster City, CA, USA) and heated to 50°C for 2 minutes and 95°C for 10 minutes; this step was followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. Ten percent of the samples were randomly selected for repeated genotyping for rs11614913 T/C polymorphism, and the results were 100% concordant. The TaqMan assay plates were then transferred to a Prism 7900HT instrument (Applied Biosystems, Foster City, CA, USA), which we used to measure the fluorescence intensity in each well of the plate. The fluorescence data files from each plate were analyzed with automated software (SDS 2.1).

Table 2. miR-196a allelic and genotype frequencies between non-small cell lung cancer patient and control subjects and their association with non-small cell lung cancer risks

Variables	n (%)			OR (95% CI)	p-value
	Genotypes	Patients (n=406)	Controls (n=428)		
Total ¹	T allele	416 (51.2)	466 (54.4)	1.00	0.36
	C allele	396 (48.8)	390 (45.6)	1.10 (0.90 - 1.35)	
	TT	96 (23.7)	134 (31.3)	1.00	
	TC	224 (55.2)	198 (46.3)	1.54 (1.10 - 2.17)	
	CC	86 (21.1)	96 (22.4)	1.17 (0.77 - 1.76)	
	TC/CC	310 (76.4)	294 (68.7)	1.42 (1.03 - 1.96)	
	CC vs TC+TT	86 (21.2)	96 (22.4)	0.08 (0.62 - 1.25)	
Age (y)					
≥60	T allele	336 (51.7)	311 (53.8)	1.00	0.46
	C allele	314 (48.3)	267 (46.2)	1.09 (0.87 - 1.36)	
	TT	81 (24.9)	93 (32.2)	1.00	
	TC	174 (53.5)	125 (43.3)	1.60 (1.10 - 2.33)	
	CC	70 (21.5)	71 (24.6)	1.13 (0.73 - 1.77)	
	TC/CC	224 (75.1)	196 (67.8)	1.43 (1.01 - 2.03)	
	CC vs TC+TT	70 (21.5)	71 (24.6)	0.84 (0.58 - 1.23)	
<60	T allele	80 (49.4)	155 (55.8)	1.00	0.2
	C allele	82 (50.6)	123 (44.2)	1.29 (0.88 - 1.90)	
	TT	15 (18.5)	41 (29.5)	1.00	
	TC	50 (61.7)	73 (52.5)	1.87 (0.94 - 3.74)	
	CC	16 (19.8)	25 (18.0)	1.75 (0.74 - 4.14)	
	TC/CC	66 (81.5)	98 (70.5)	1.84 (0.94 - 3.59)	
	CC vs TC+TT	16 (19.8)	25 (18.0)	1.12 (0.56 - 2.25)	
Sex					
Male	T allele	330 (51.1)	368 (54.8)	1.00	0.18
	C allele	316 (48.9)	304 (45.2)	1.16 (0.93 - 1.44)	
	TT	77 (23.9)	109 (32.4)	1.00	
	TC	176 (54.5)	150 (44.6)	1.66 (1.15 - 2.39)	
	CC	70 (21.7)	77 (22.9)	1.29 (0.83 - 1.99)	
	TC/CC	246 (76.2)	227 (67.6)	1.53 (1.09 - 2.16)	
	CC vs TC+TT	70 (21.7)	77 (22.9)	0.93 (0.64 - 1.34)	
Female	T allele	86 (51.8)	98 (53.3)	1.00	0.79
	C allele	80 (48.2)	86 (46.7)	1.06 (0.70 - 1.61)	
	TT	19 (22.9)	25 (27.2)	1.00	
	TC	48 (57.8)	48 (52.2)	1.32 (0.64 - 2.70)	
	CC	16 (19.3)	19 (20.7)	1.11 (0.45 - 2.71)	
	TC/CC	64 (77.1)	67 (72.8)	1.26 (0.63 - 2.50)	
	CC vs TC+TT	16 (19.3)	19 (20.7)	0.92 (0.44 - 1.93)	

OR: odds ratio, CI: confidence interval.

p-value was revealed by two-sides Chi-square test.

¹Adjusted for age, sex, and smoking status.

III. Statistical Analysis

A Chi-square test was used to evaluate differences between the cases and controls in the frequency of the selected demographic variables, including smoking status, family history of cancer, and the genotype distributions of the miR-196a T/C polymorphism. All data were computed by binary logistic regression in the program SPSS version 11.0 (SPSS Inc., Chicago, IL, USA). The associations between the miR-196a T/C polymorphism and the lung cancer risk were estimated by computing the odds ratios (OR,) and their 95% confident intervals (CIs) from both univariate and multivariate logistic regression analyses.

RESULTS

The baseline characteristics of the 406 NSCLC patients and 428 control subjects included in the analysis are summarized in Table 1. The distribution of age, sex, and family history of cancer among NSCLC patients and controls were comparable. As expected, the smoking status has a statistically significant difference in the risk factor for lung cancer in our study. About 85.2% of the patients were smokers, which is significantly higher than that of the controls ($p < 0.01$).

Table 2 shows the allele frequencies and genotype distributions of miR-196a2 rs11614913 in the patients and controls. The observed frequencies for this

Table 2. Continued

Variables	n (%)			OR (95% CI)	p-value	
	Genotypes	Patients (n=406)	Controls (n=428)			
Smoking status						
Smokers	T allele	351 (50.7)	307 (54.1)	1.00	0.24	
	C allele	341 (49.3)	261 (46.0)	1.14 (0.91 - 1.43)		
	TT	80 (23.1)	88 (31.0)	1.00	<0.05	
	TC	191 (55.2)	131 (46.1)	1.60 (1.10 - 2.33)		
	CC	75 (21.7)	65 (22.9)	1.27 (0.81 - 1.99)		
	TC/CC	266 (76.9)	196 (69.0)	1.49 (1.05 - 2.13)	<0.05	
Non-smokers	CC vs TC+TT	75 (21.7)	65 (22.9)	0.93 (0.64 - 1.36)	0.72	
	T allele	65 (54.2)	159 (55.2)	1.00	0.85	
	C allele	55 (45.8)	129 (44.8)	1.04 (0.68 - 1.60)		
	TT	16 (26.7)	46 (31.9)	1.00	0.55	
	TC	33 (55.0)	67 (46.5)	1.42 (0.70 - 2.87)		
	CC	11 (18.3)	31 (21.5)	1.02 (0.42 - 2.49)		
Family history of cancer	TC/CC	44 (73.3)	98 (68.6)	1.29 (0.66 - 2.52)	0.46	
	CC vs TC+TT	11 (18.3)	31 (21.5)	0.82 (0.38 - 1.76)	0.61	
	No					
	T allele	327 (50.6)	344 (55.0)	1.00	0.12	
	C allele	319 (49.4)	282 (45.0)	1.19 (0.95 - 1.48)		
	TT	75 (23.2)	100 (32.0)	1.00	0.03	
Yes	TC	177 (54.8)	144 (46.0)	1.64 (1.13 - 2.38)		
	CC	71 (22.0)	69 (22.0)	1.37 (0.88 - 2.14)		
	TC/CC	248 (76.8)	213 (68.1)	1.55 (1.09 - 2.21)	<0.01	
	CC vs TC+TT	71 (22.0)	69 (22.0)	1.00 (0.68 - 1.45)	0.98	
	T allele	89 (53.6)	122 (53.0)	1.00	0.91	
	C allele	77 (46.4)	108 (47.0)	0.98 (0.66 - 1.46)		
Family history of cancer	TT	21 (25.3)	34 (29.6)	1.00	0.4	
	TC	47 (56.6)	54 (47.0)	1.41 (0.72 - 2.75)		
	CC	15 (18.1)	27 (23.5)	0.90 (0.39 - 2.07)		
	TC/CC	61 (74.7)	81 (70.4)	1.24 (0.66 - 2.34)	0.51	
	CC vs TC+TT	15 (18.0)	22 (23.5)	0.72 (0.35 - 1.46)	0.36	

OR: odds ratio, CI: confidence interval.

p-value was revealed by two-sides Chi-square test.

'Adjusted for age, sex, and smoking status.

polymorphism were in agreement with the frequencies expected under the Hardy-Weinberg equilibrium in the patients and controls ($p = 0.66$). As shown in Table 2, the total allele frequencies for miR-196a C were 48.8% for the patients and 45.6% for the controls. The total genotype frequencies for TT, TC, and CC were 23.7%, 55.2%, and 21.1% for the patients and 31.1%, 46.3%, and 22.4% for the controls ($p < 0.05$). Significant associations were observed for the miR-196a2 rs11614913 CT/TT genotypes in a dominant model. The logistic regression analyses reveal that after adjusting for age, sex, and smoking status in a comparison with wild-type carriers there appears to be an association between a significantly increased NSCLC risk and variant genotypes of the miR-196a2 rs11614913 CT/TT genotypes ($p < 0.05$, OR, 1.42; 95% CI, 1.03 to 1.96). However, in the recessive genetic model, we found that the miR-196a2 rs11614913 variant homozygote CC was not significantly associated with the risk of patients in comparison with wild-type homozygote TT and heterozygote CT ($p = 0.48$, OR, 0.82; 95% CI, 0.62 to

1.25). Additionally, we analyzed the association between miR-196a2 rs11614913 genotypes and the NSCLC risk in the subgroups of age, sex, smoking status, and family history of cancer. A significant association was observed between the miR-196a2 rs11614913 (CT/TT) genotype and the NSCLC risk in the subgroups of those who were aged over 60 years ($p < 0.05$, OR, 1.43; 95% CI, 1.01 to 2.03), male ($p < 0.01$, OR, 1.53; 95% CI, 1.09 to 2.16), smokers ($p < 0.05$, OR, 1.49; 95% CI, 1.05 to 2.13), and those without family history of cancer ($p < 0.01$, OR, 1.55; 95% CI, 1.09 to 2.21).

DISCUSSION

miRNA profiling studies have revealed that many miRNAs are up-regulated or down-regulated in different types of human cancers and that most of them are down-regulated [5]. The expression of miR-196a2 reportedly correlates with multiple kinds of malignant tumor.

Accordingly, the functional variant rs11614913 is a candidate biomarker that may influence the tumor risk [16]. Thus, in our present study, we first evaluated the case-control studies in a Korean population with regard to whether the miR-196a2 rs11614913 T/C polymorphism is associated with risk factors in NSCLC lung cancer patients.

miRNAs are an evolutionarily-conserved and abundant class of small silencing RNAs [3,17]; and they are expressed in limited developmental stages or in specific tissues or cells, which suggests that they may be involved in the cell differentiation and maintenance of the properties of different cells [5,6]. In addition, they are involved in orchestrating the growth, development, function, and stress responses of various organs [18]. Although miRNA studies are generally focused on the cancer field, miRNAs also appear to play a key role as regulators of the growth, development, function, and stress responsiveness in the cardiac field [19-22]. Recently, a few studies have examined the association between cancer risk and the miR196a2 rs11614913 C/T polymorphism on human cancers and the CC genotype of this polymorphism could be related to decreasing survival in lung cancer [15]. In addition, the has-mir-196a2 TC and TT were associated significantly with a decreased risk of breast cancer [23], and the association with congenital heart disease could be established by altering the expression of mature miR-196a and the binding activity of the target mRNA [24]. This SNP was also shown to be associated with lung cancer susceptibility; that is, a statistically significant increase in the expression of the mature miR-196a is not preceded by changes in the levels of lung cancer tissue [16]. Interestingly, the miR-196a2 rs11614913 CC genotype poses a more frequent increase in risk for cancer patients. Therefore, the miR-196a2 rs11614913 CC genotype seems to be an important factor in lung cancer development and survival. In another study involving 1009 patients with breast cancer and 1093 healthy Chinese controls, the hsa-mir-196a2 CC and CC/CT genotypes are associated with a significantly increased risk of breast cancer [25]. In addition, mir-196a2 rs11614913 C/T polymorphisms are associated with a significantly increased risk of dilated cardiomyopathy in a dominant model [26]. In our present study, the result was similar to the corresponding genotype frequencies observed [16] and significant associations were observed for the miR-196a2 rs11614913 CT/CC genotypes with increased risk of NSCLC in a dominant model. In addition, our results reveal that after adjusting for age, sex, smoking status and without family history of cancer

in a comparison with wild-type carriers there appears to be an association between a significantly increased risk of NSCLC and variant genotypes of the miR-196a2 rs11614913 CT/CC genotypes. Therefore, miR-196a2 rs11614913 may influence role regulation processes in Korean NSCLC patients. However, we are not observed association between the homozygous miR-196a2 rs11614913 CC polymorphism and the risk of NSCLC lung cancer patients in the recessive genetic model; nor did we find any significant associations among different stratified subgroups. Further study is needed comprehensive approach for all the genes of a Korean population with a sufficient number of subjects.

Our study had the power of >82% in each gene to detect an OR of ≥ 1.5 (assuming dominant effect, with minor allele frequency of 0.1 and $\alpha = 0.05$).

In conclusion, this case-control study provides evidence that miR-196a2 rs11614913 C/T polymorphisms are associated with a significantly increased risk of NSCLC in a dominant model, indicating that common genetic polymorphisms in miR-196a2 rs11614913 are associated with NSCLC. Our knowledge of the effects of miR196a2 rs11614913 polymorphisms on the risk of NSCLC requires confirmation by additional larger studies.

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CONFLICT OF INTEREST

The authors have no conflicts of interest with the material presented in this paper.

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