Association between microRNA-146a, -499a and -196a-2 SNPs and non-small cell lung cancer: a case-control study involving 2,249 subjects

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Abstract: MicroRNA (miR) acts as a negative regulator of gene expression. Many literatures have suggested that miRs may be involved in the process of cell proliferation, inflammation, oxidative stress, energy metabolism and epithelial mesenchymal transition. Thus, miRs may be implicated in the occurrence of non-small cell lung cancer (NSCLC). In the current investigation, we included 2,249 subjects (1,193 NSCLC patients and 1,056 controls) and designed a study to identify the relationship of miR-146a rs2910164 C/G, -499a rs3746444 A/G, and -196a-2 rs11614913 T/C with the risk of NSCLC. The risk factors (e.g., body mass index, sex, smoking, drinking and age), was used to adjust the odds ratios and 95% confidence intervals. After conducting a power value assessment, we did not confirm that the miR-SNPs genotypic distributions were different in NSCLC cases and controls. However, the association of miR-196a-2 rs11614913 with a decrease risk of NSCLC was identified in the female subgroup (adjusted $P=0.005$, power=0.809 for TC vs. TT, and adjusted $P=0.004$, power=0.849 for CC/TC vs. TT). In addition, gene-gene interaction analysis showed that rs11614913 TC/rs3746444 AA and rs11614913 CC/rs3746444 AA could also reduce the susceptibility to NSCLC (rs11614913 TC/rs3746444 AA vs. rs11614913 TT/rs3746444 AA, $P=0.001$, power=0.912 and rs11614913 CC/rs3746444 AA vs. rs11614913 TT/rs3746444 AA, $P=0.003$, power=0.836). In conclusion, in overall comparisons, we did not confirm that the rs2910164, rs3746444, and rs11614913 SNPs genotypic distributions were different in NSCLC cases and controls. However, this case-control study demonstrates that miR-196a-2 rs11614913 may be a protective factor for the development of NSCLC among female patients.
Introduction

Lung cancer (LC) caused about 11.6% of all new cancer cases and 18.4% of all cancer-related deaths worldwide [1]. In China, 733.3 thousand new LC patients and 610.2 thousand LC-related deaths were assessed to occur in 2015 [2]. The etiology of LC was unclear. It is reported that a number of genetic and environmental risk factors may cause the development of LC [3, 4, 5]. Non-small cell lung cancer (NSCLC) is the most common type of LC. The individual’s hereditary factor may be implicated in the occurrence of NSCLC.

MicroRNA (miR), a small non-coding RNA, acts as a negative regulator of gene expression. In the nucleus, the Drosha/DiGeorge syndrome critical region 8 complex cleaves pri-miRNAs [6]. Then, in the cytoplasm, Dicer crops these formed pre-miRNAs [7]. Finally, they are incorporated into the Argonaute-containing RNA-induced silencing complexes [8]. Mature miR is composed of about 22 nucleic acids, which is generated from primary miRs and further changed to mature miRs in cytoplasm. The target mRNAs located in 3’-untranslated regions (3’-UTRs). Matured miRs can recognize the 3’-UTRs of mRNA and bind to them, and then result in a weakened expression of target genes. The mechanism of the process is hybridization of seed sequences of matured miRs with 3’-UTRs. An individual miR can bind to masses of targets, and regulate a number of pathways. Many investigations have suggested that miRs may be involved in the process of cell proliferation, inflammation, oxidative stress, energy metabolism and epithelial mesenchymal transition (EMT) [9, 10, 11, 12, 13, 14, 15, 16]. Of late, some previous investigations have indicated that miRs have been implicated in the occurrence of NSCLC [17, 18]. There are single nucleotide polymorphisms (SNPs) in certain miRs. These SNPs might influence the generation process of miRs or alter target recognition/hybridization. Thus, miR polymorphisms may be implicated in the occurrence and/or progress of cancer [19, 20, 21, 22, 23, 24, 25].

Park et al. reported that miR-146a could restrain EMT progression in NSCLC by repressing the expression of insulin receptor substrate-2 [14]. It was found that miR-146a inhibited migratory capacity, downstream signaling of epidermal growth factor receptor and NSCLC cell growth; however, it could promote the apoptosis process of NSCLC cell lines [13]. Xiong et al. reported that miR-146a rs2910164 C>G locus could affect its maturation in peripheral blood mononuclear cells [26]. A recent study reported that G allele of rs2910164 might increase miR-146a level [27]. A previous study suggested that rs2910164 locus might influence the toxicity in LC chemotherapy [28]. Several reports indicated that rs2910164 polymorphism in miR-146a could decrease the risk to LC [29, 30]. However, other case-control studies suggested that rs2910164 might not influence the occurrence of LC [31, 32]. These controversial observations may be due to the limited sample sizes. Here, we explored the role of miR-146a rs2910164 SNP with the development of NSCLC and a potential interaction of this SNP with risk factors to identify whether this locus could be used as a biomarker for susceptibility to NSCLC in Chinese populations.

Rs11614913 T>C was widely explored in malignancy as a candidate locus of miR-196a-2 [33, 34]. Hu et al. reported that the rs11614913 T→C variant in miR-196a-2 could affect the binding ability of mature hsa-mir-196a-2-3p binding with its target mRNA [35]. Recently, this polymorphism was thought to alter LC cases’ sensitivity to platinum-based chemotherapy [23]. A functional study highlighted that rs11614913 might be involved in the development of LC through altering the secondary structure and the expression of miR-196a-2 [36]. Thus, rs11614913
polymorphism might be implicated in carcinogenesis of LC and could affect an individual’s susceptibility of LC. Indeed, several case-control studies have investigated the role of rs11614913 in the occurrence of LC [23, 36]. However, the observations were conflicting, even in a same ethnicity. For example, some recent studies indicated a significant relationship between miR-196a-2 rs11614913 and the development of LC [36, 37, 38], whereas others did not confirm the potential correlation [23, 32].

A previous investigation reported that miR-499a rs3746444 SNP could affect the process of miR-499-5p maturation and the role of antiapoptosis [39]. The relationship between miR-499a rs3746444 A>G and the susceptibility and progress of LC has been explored. Ge et al. reported that miR-499a rs3746444 AA genotype could inhibit the expression of miR-499a gene and CD200 [40]. And then this SNP could influence the survival of NSCLC cases. Several studies have focused on the role of miR-499a rs3746444 in the development of LC [40, 41]. However, recent meta-analyses have reported contradictory findings [42, 43, 44]. Thus, the correlation of miR-499a rs3746444 with the development of LC was more inconsistent.

In the current investigation, we designed a larger sample size study to identify the correlation of rs3746444, rs2910164 and rs11614913 with the occurrence of NSCLC.

Materials and methods

Study population and ethical approval

Each participant donated a peripheral blood sample. NSCLC cases in the current investigation were recruited from the Zhenjiang Medical College of Nanjing Medical University (Jiangsu Province, China) and the Union Medical College of Fujian Medical University (Fujian Province, China) between January 2014 and June 2018. All NSCLC cases were diagnosed via histopathological examination. In this study, the selection criteria were defined as the following: (1) Chinese Han populations, (2) sporadic cases and (3) without any history of other cancer. And the exclusion criteria were summarized as: (1) a patient who has an autoimmune disease, (2) NSCLC patients who have treated with chemoradiotherapy and/or targeted therapy, (3) NSCLC recurrent cases and (4) heterochronous NSCLC. In total, 1,193 NSCLC cases were enrolled. At the same time, 1,056 participants without a history of cancer were included as controls in the Medical Colleges mentioned above. The data of demographics and potential risk factors were collected by a pre-structured questionnaire. During the recruitment, each participant signed a written informed consent. This study was approved by the Ethics Review Committee of Fujian Union Hospital (2018KY023).

Isolation of DNA and genotyping

Using DNA Isolation Kit (Promega, Madison, USA), we extracted genomic DNA. The obtained DNA was kept at -80°C. The quality of DNA sample was assessed by Nanodrop ND-1000 UV. A custom-SNPscan™ Kit (Genesky Biotechnologies Inc., Shanghai, China) was used to analyze the genotypes. Briefly, no less than 120 ng DNA sample was used to conduct a double ligation and multiplex fluorescence polymerase chain reaction (PCR). ABI-3730XL sequencer (PE Applied Biosystems, Foster City, CA, USA) was used to detect the PCR products. The obtained raw data were analyzed by harnessing GeneMapper 4.1 (AppliedBiosystems, USA). To conduct a quality control, ninety samples were randomly chosen and
repeated genotyped in the same PCR method. The results indicated that 100% concordant results were observed.

**Statistical analysis**

Hardy–Weinberg equilibrium (HWE) (https://ihg.gsf.de/cgi-bin/hw/hwa1.pl) [45] and SAS 9.4 (SAS Institute, Cary, North Carolina) softwares were harnessed to analyze HWE and genetic data. Odds ratios (ORs) and 95% confidence intervals (CIs) were used to estimate the relationship of rs2910164, rs11614913 and rs3746444 with the risk of NSCLC. We also calculated adjusted ORs and 95% CIs using logistic regression analyses. In the current study, five risk factors [e.g., body mass index (BMI), smoking, drinking, age and gender] were included. Power Calculator (http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/PowerSampleSize) was used to calculate the power of sample size [19, 46]. We also used the false-positive report probability (FPRP) to evaluate the findings [47].

**Results**

**Characteristics of the study population**

In the current study, 1,193 cases with NSCLC (mean ± SD age, 58.92±10.44 years) and 1,056 controls (mean ± SD age, 59.36±9.19 years) were collected (Table 1). In NSCLC group, 642 males and 551 females were included. While in controls, there were 586 males and 470 females. The age and gender were well-mathed \((P=0.960\) and 0.425, respectively). The distribution of smoking, drinking and BMI were different between two groups \((All \ P<0.001\). Raw data of genotypes and characteristics were summarized in Table S1.

**Information of rs3746444, rs2910164 and rs11614913 SNPs**

The successful ratio of genotyping was more than 99.00%. Table 2 has summarized some vital information for rs2910164, rs11614913 and rs3746444. In controls, these included miR-SNPs genotype distributions met HWE \((P>0.05)\). Table S1 summarized the detailed information and genotypes for each individual.

**Rs3746444, rs2910164 and rs11614913 SNPs and NSCLC susceptibility**

The number of miR-146a rs2910164 allele and genotype in NSCLC cases and controls is summarized in Table 3. In this case-control study, for overall comparisons, we identified that the miR-146a genotype frequency was not significantly different among the two groups. As well, we also found that the miR-499a rs3746444 genotypic distribution was not different in NSCLC cases and controls.

Table 3 listes the miR-196a-2 rs11614913 genotype distribution in NSCLC cases and controls. It was notable that there was statistically significant in comparison of rs11614913 genotypes in three genetic models among NSCLC cases and controls. The decreased genotype frequencies of rs11614913 TC, CC and TC/CC were found in NSCLC patients. In relation to rs11614913 TT, individuals carrying rs11614913 TC genotypes had a decreased 21% susceptibility to the occurrence of NSCLC \((P=0.014)\), Table 4. Additionally, compared to rs11614913 TT, rs11614913 CC and TC/CC genotypes were also protective factors for the occurrence of NSCLC \((CC \ vs. \ TT: P=0.027\) and TC/CC vs. TT: \(P=0.007)\), Table 4). When we adjusted for risk factors, the decreased susceptibility for the occurrence of NSCLC was not changed (Table 4).

**MiR-SNPs and NSCLC susceptibility in different type of pathology**
Table S2 and Table S3 summarized the detailed information and genotypes for squamous cell carcinoma (SCC) and non-SCC cases, respectively. When we conducted a subgroup analysis by type of pathology, for rs11614913 SNP, the decreased susceptibility for the occurrence of NSCLC was also found in non-SCC subgroup (TC vs. TT: adjusted $P=0.026$ and TC/CC vs. TT: adjusted $P=0.015$, Table 4). For rs2910164 and rs3746444 polymorphisms, no significant association between these SNPs and NSCLC risk was found (Table 4).

**Stratification analysis of miR-SNPs and NSCLC susceptibility**

(a) **miR-146a rs2910164 C>G locus**

When we conducted stratification analyses by risk factors, an increased risk for the occurrence of NSCLC was identified in never drinking subgroup (CG vs. CC: adjusted $P=0.043$ and GG/CG vs. CC: adjusted $P=0.028$, Table 5).

(b) **MiR-499a rs3746444 A>G locus**

Table 6 listed the findings of stratification analyses for rs3746444 polymorphism. We identified that rs3746444 polymorphism elevated the susceptibility of NSCLC (never smoking subgroup: adjusted $P=0.035$ for GG vs. AA genetic model and adjusted $P=0.049$ for GG vs. AA/AG genetic model; never drinking subgroup: adjusted $P=0.032$ for GG vs. AA genetic model, adjusted $P=0.035$ for GG/AG vs. AA genetic model and adjusted $P=0.047$ for GG vs. AA/AG genetic model; BMI $<24$ (kg/m$^2$) subgroup: adjusted $P=0.042$ for AG vs. AA genetic model and adjusted $P=0.034$ for GG vs. AA/AG genetic model and never BMI $\geq 24$ (kg/m$^2$) subgroup: adjusted $P=0.046$ for GG vs. AA/AG genetic model).

(c) **miR-196a-2 rs11614913 T>C locus**

For miR-196a-2 rs11614913, significant difference in frequency of its genotype was found between NSCLC cases and controls. We identified that rs11614913 polymorphism may be a protective factor for the occurrence of NSCLC (female subgroup: adjusted $P=0.005$ for TC vs. TT genetic model, adjusted $P=0.038$ for CC vs. TT genetic model and adjusted $P=0.004$ for CC/TC vs. TT genetic model; never smoking subgroup: adjusted $P=0.038$ for CC vs. TT genetic model and adjusted $P=0.049$ for CC/TC vs. TT genetic model; never drinking subgroup: adjusted $P=0.024$ for TC vs. TT genetic model, adjusted $P=0.018$ for CC vs. TT genetic model and adjusted $P=0.009$ for CC/TC vs. TT genetic model, Table 7).

**Gene–gene interaction analysis**

We also conducted miR-SNPs combined analysis for three included SNPs. Three potential types (rs11614913/rs2910164, rs11614913/rs3746444, rs2910164/rs3746444 and rs11614913/rs2910164/rs3746444) were combined to explore the gene–gene interaction and their roles on the occurrence of NSCLC.

In analysis of rs11614913/rs2910164 loci combination, we used rs11614913 TT/rs2910164 CC as reference. It was notable that the rs11614913 CC/rs2910164 CC combination was a protective factor for the development of NSCLC ($P=0.010$, Table 8). In another analysis of rs11614913/rs3746444 loci combination, compared to rs11614913 TT/rs3746444 AA, frequency of rs11614913 TC/rs3746444 AA was lower in NSCLC patients 32.54% (384/1080) than in controls 37.70% (397/1053).
When rs11614913 TT/rs3746444 AA was used as a reference, frequency of rs11614913 CC/rs3746444 AA was also lower in NSCLC patients 12.46% (147/1080) than in controls 15.19% (160/1053). When rs11614913 TT/rs2910164 CC/rs3746444 AA was used as a reference, TC/CC/AA, TC/GG/AA and CC/CC/AA genotype combinations might decreased the risk of NSCLC (Table 8).

**Study power (α= 0.05) and FPRP method**

For overall comparisons, these miR-SNPs did not confer a risk to NSCLC. Each power value for overall positive report was less than 0.8 (data not shown). For the comparison of miR-SNPs and NSCLC susceptibility in different type of pathology, we also could not confirm the positive report (data not shown). In stratification analysis of miR-SNPs with NSCLC susceptibility, we only confirmed that rs11614913 polymorphism could be a protective factor for the occurrence of NSCLC in the female subgroup (the power values were 0.809 in TC vs. TT and 0.848 in CC/TC vs. TT). In these miR-SNPs combination analysis, compared to rs11614913 TT/3746444 AA, rs11614913 TC/3746444 AA and rs11614913 CC/rs3746444 AA could decrease the susceptibility of NSCLC (power value: 0.912 and 0.836, respectively). Other power values less than 0.8 were not shown. After assessing power value and FPRP, we highlighted that miR-196a-2 rs11614913 decreased the risk to NSCLC in the female subgroup. As well, gene-gene interaction analysis showed that rs11614913 TC/3746444 AA and rs11614913 CC/rs3746444 AA could also reduce the susceptibility to NSCLC.

**Discussion**

LC is a common malignancy with 18.4% of overall cancer-related deaths worldwide [1]. The etiology of LC is not well-known. NSCLC is the most common subtype of LC. MiR is a negative regulator of gene expression. It may involve in the development of cancer. Some investigations have focused on the role of miRs on the occurrence and survival of NSCLC [40, 48, 49]. The individual’s hereditary factor may be implicated in the occurrence of NSCLC. In this investigation, we designed a study to identify the correlation of miR-SNPs (rs3746444, rs2910164 and rs11614913) with the risk of NSCLC in Chinese populations. We highlighted that rs11614913, in the female subgroup, could decrease the risk to NSCLC. As well, gene-gene interaction analysis showed that rs11614913 TC/3746444 AA and rs11614913 CC/rs3746444 AA could also reduce the susceptibility to NSCLC.

Rs11614913 locates on the 3p strand region of mature miR-196a-2 [50]. Thus, this locus could participate in the process of pre-miR maturation and affect the combination of miR-196a-2 with target genes [51]. Hu et al. reported that that the T>C variant in rs11614913 locus could alter the ability of mature hsa-mir-196a-2-3p binding to its target mRNA [35]. Therefore, this SNP could be used as an important biomarker for NSCLC prognosis [35]. A previous study has suggested that annexin A1 (ANXA1), a regulator of inflammation, could be regulated by miR-196a-2 [52]. A bioinformatics analysis suggested that the expression of ANXA1 could influence the survival of NSCLC cases [53]. Additionally, knockdown of ANXA1 could inhibit the invasion, migration and proliferation of NSCLC cells. Thus, miR-196a-2 could be implicated in the occurrence of cancer. Fang et al. reported that variants of rs11614913 could alter the response of LC case to platinum-based chemotherapy [23]. Toraih et al. found that individuals carrying the rs11614913 C allele might be a protective factor of LC, which was associated with miR-196a-2 low-expression in
A recent investigation indicated that the polymorphism of rs11614913, through influencing the level of miR-196a-2 and secondary structure, conferred risk to LC in females [36]. In the current investigation, we found that the miR-196a-2 rs11614913 could reduce the susceptibility to NSCLC in female. In view of these investigations mentioned above, we might conclude that rs11614913 C allele could be a protective factor to the occurrence of NSCLC though altering the level of miR-196a-2 and secondary structure. It is well known that smoking is a major risk for LC. However, in this study, we did not find the interaction of tobacco using and rs11614913 SNP with the development of NSCLC. In the future, these conclusions should be confirmed by further studies.

Several literatures have focused on the relationship between gene-gene interaction and the occurrence of human diseases [55, 56, 57]. In this study, we analyzed the combined effect of these miR-SNPs. Gene-gene interaction analyses showed that rs11614913 TC/3746444 AA and rs11614913 CC/rs3746444 AA could also decrease the susceptibility of NSCLC, which suggested that rs11614913 C allele could inhibit the development of NSCLC. We first confirmed that rs11614913 TC/3746444 AA and rs11614913 CC/rs3746444 AA combinations could decrease the risk of NSCLC. However, this combination did not influence the risk of cervical cancer [56]. Therefore, the effect of rs11614913 TC/3746444 AA combination could be different in different cancer. In the future, the possible correlation is needed to verify in other studies.

Several limitations, in this investigation, should be pointed out. Firstly, some vital data were unknown; thus, a more extensively stratified analysis for other risk factors (e.g., vegetable and fruit intake, air pollution, lifestyle and occupational exposure) could not be done. Second, due to the hospital-based study, bias might have happened in our analysis. Third, the number of participants in this study was moderate. Last, we only included three miR-SNPs in this study, and other important miR-SNPs should not be ignored.

In conclusion, this study highlights that miR-196a-2 rs11614913 decreases the risk to NSCLC among female subgroup. Additionally, combined gene-gene analyses suggest that rs11614913 TC/3746444 AA and rs11614913 CC/rs3746444 AA are protective factors for the development of NSCLC. More investigations are needed to validate the potential effect of these miR-SNPs in NSCLC. And more functional studies should also be done.

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Author contribution statement:
Haiyong Gu and Qingfeng Zheng designed the study. Hao Qiu, Zhiqiang Xie, Weifeng Tang, Chao Liu and Yafeng Wang performed the experiments. Hao Qiu and Zhiqiang Xie analyzed the data. Hao Qiu drafted the manuscript and Haiyong Gu revised the manuscript.

Data Availability Statement: Full data are available via an online supplementary material. Raw data of genotypes and characteristics were summarized in Table S1. Table S2 and Table S3 summarized the detailed information and genotypes for squamous cell carcinoma (SCC) and non-SCC cases, respectively.
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Table 1 Distribution of selected demographic variables and risk factors in NSCLC cases and controls

| Variable          | NSCLC Cases (n=1,193) | Controls (n=1,056) | P   |
|-------------------|-----------------------|--------------------|-----|
|                   | n                     | %                  | n   | %  |
| Age (years)       | 58.92±10.44           | 59.36±9.19        | 0.293|
| <59               | 535                   | 44.84             | 452 | 42.80|
| ≥59               | 658                   | 55.16             | 604 | 57.20|
| Sex               |                       |                   | 0.425|
| Male              | 642                   | 53.81             | 586 | 55.65|
| Female            | 551                   | 46.19             | 470 | 44.35|
| Smoking status    |                       |                   | <0.001|
| Never             | 757                   | 63.45             | 857 | 81.16|
| Ever              | 436                   | 36.55             | 199 | 18.84|
| Alcohol use       |                       |                   | <0.001|
| Never             | 946                   | 79.30             | 967 | 91.83|
| Ever              | 247                   | 20.70             | 89  | 8.17 |
| BMI (kg/m²)       |                       |                   | <0.001|
| <24               | 801                   | 67.14             | 571 | 54.07|
| ≥24               | 392                   | 32.86             | 485 | 45.93|
| Type of NSCLC     |                       |                   |     |
| SCC               | 182                   | 15.26             |     |
| Non-SCC           | 1,011                 | 84.74             |     |
| Stage             |                       |                   |     |
| I                 | 703                   | 58.93             |     |
| II                | 87                    | 7.29               |     |
| III               | 222                   | 18.61             |     |
| IV                | 181                   | 15.17             |     |
| Lymph node status |                       |                   |     |
| Positive          | 381                   | 31.94             |     |
| Negative          | 812                   | 68.06             |     |

*Two-sided χ² test and student t test;

Bold values are statistically significant (P<0.05).

BMI: body mass index;
NSCLC: non-small-cell lung cancer

SCC: squamous cell carcinoma
| Genotyped SNPs | miR-146a rs2910164 C>G | miR-196a-2 rs11614913 T>C | miR-499a rs3746444 A>G |
|----------------|------------------------|--------------------------|------------------------|
| Chromosome     | 5                      | 12                       | 20                      |
| Function       | nc-transcript-variant  | nc-transcript-variant    | nc-transcript-variant  |
| Chr Pos (NCBI Build 38) | 160485411            | 53991815                 | 3499048                 |
| MAF\(a\) for Chinese in database | 0.35                  | 0.34                     | 0.15                    |
| MAF in our controls (n = 1,056) | 0.36                  | 0.46                     | 0.15                    |
| \(P\) value for HWE\(^b\) test in our controls | 0.217                | 0.208                    | 0.898                   |
| Genotyping method | SNPscan              | SNPscan                  | SNPscan                |
| % Genotyping value | 99.47%               | 99.47%                   | 99.29%                  |

\(^a\) MAF: minor allele frequency;

\(^b\) HWE: Hardy–Weinberg equilibrium
**Table 3** The frequencies of *miR-146a* rs2910164 C>G, *miR-196a-2* rs11614913 T>C and *miR-499a* rs3746444 A>G polymorphisms in CAD patients and controls

| Genotype                  | Overall NSCLC cases (n=1,193) | SCC cases (n=182) | Non-SCC cases (n=1,011) | Controls (n=1,056) |
|---------------------------|-------------------------------|------------------|------------------------|-------------------|
|                           | n | %       | n | %       | n | %       | n | %       |
| *miR-146a* rs2910164 C>G  |    |         |    |         |    |         |    |         |
| CC                        | 460 | 38.85  | 68 | 37.57  | 392 | 39.08  | 440 | 41.79  |
| CG                        | 555 | 46.88  | 91 | 50.28  | 464 | 46.26  | 467 | 44.35  |
| GG                        | 169 | 14.27  | 22 | 12.15  | 147 | 14.66  | 146 | 13.87  |
| G allele                  | 893 | 37.71  | 135 | 37.29  | 758 | 37.79  | 759 | 36.04  |
| *miR-499a* rs3746444 A>G  |    |         |    |         |    |         |    |         |
| AA                        | 814 | 68.98  | 128 | 71.11  | 686 | 68.60  | 757 | 71.89  |
| AG                        | 330 | 27.97  | 47 | 26.11  | 283 | 28.30  | 271 | 25.74  |
| GG                        | 36  | 3.05   | 5  | 2.78   | 31  | 3.10   | 25  | 2.37   |
| G allele                  | 402 | 17.03  | 57 | 15.83  | 345 | 17.25  | 321 | 15.24  |
| *miR-196a-2* rs11614913 T>C |    |         |    |         |    |         |    |         |
| TT                        | 392 | 33.11  | 59 | 32.60  | 333 | 33.20  | 293 | 27.83  |
| TC                        | 572 | 48.31  | 90 | 49.72  | 482 | 48.06  | 544 | 51.66  |
| CC                        | 220 | 18.58  | 32 | 17.68  | 188 | 18.74  | 216 | 20.51  |
| C allele                  | 1,012 | 42.74 | 154 | 42.54  | 858 | 42.77  | 976 | 46.34  |
NSCLC: non-small-cell lung cancer

SCC: squamous cell carcinoma
Table 4  Overall and stratified analyses of miR-146a rs2910164 C>G, miR-196a-2 rs11614913 T>C and miR-499a rs3746444 A>G polymorphisms with NSCLC

| Genotype     | Overall NSCLC cases (n=1,193) vs. Controls (1,056) | Non-SCC cases (n=1,011) vs. Controls (1,056) | SCC cases (n=182) vs. Controls (1,056) |
|--------------|---------------------------------------------------|---------------------------------------------|--------------------------------------|
|              | Crude OR (95%CI) | Adjusted OR (95%CI) | p  | Adjusted OR (95%CI) | OR† | p  | Adjusted OR (95%CI) | OR† | p  |
| miR-146a rs2910164 C>G |                                    |                                              |    |                                              |     |    |                                              |     |    |
| CG vs. CC    | 1.14(0.95-1.36) | 1.11(0.92-1.34) | 0.268 | 1.12(0.93-1.35) | 0.254 | 1.07(0.88-1.30) | 0.498 | 1.26(0.90-1.77) | 0.182 | 1.22(0.82-1.81) | 0.323 |
| GG vs. CC    | 1.11(0.86-1.43) | 1.17(0.90-1.54) | 0.243 | 1.13(0.87-1.48) | 0.368 | 1.15(0.87-1.51) | 0.329 | 0.98(0.58-1.63) | 0.924 | 1.24(0.68-2.27) | 0.477 |
| GG/GC vs. CC | 1.13(0.95-1.34) | 1.13(0.94-1.34) | 0.188 | 1.12(0.94-1.33) | 0.212 | 1.09(0.91-1.31) | 0.367 | 1.19(0.86-1.65) | 0.287 | 1.23(0.84-1.79) | 0.291 |
| GG vs. CC/GG | 1.03(0.82-1.31) | 1.11(0.87-1.42) | 0.415 | 1.07(0.83-1.37) | 0.608 | 1.11(0.86-1.43) | 0.436 | 0.86(0.53-1.39) | 0.536 | 1.12(0.64-1.96) | 0.700 |
| miR-499a rs3746444 A>G |                                    |                                              |    |                                              |     |    |                                              |     |    |
| AG vs. AA    | 1.13(0.94-1.37) | 1.14(0.93-1.39) | 0.201 | 1.15(0.95-1.40) | 0.156 | 1.16(0.94-1.42) | 0.164 | 1.03(0.71-1.47) | 0.891 | 0.92(0.61-1.41) | 0.707 |
| GG vs. AA    | 1.34(0.80-2.25) | 1.63(0.94-2.81) | 0.081 | 1.37(0.80-2.34) | 0.253 | 1.64(0.94-2.88) | 0.083 | 1.18(0.45-3.15) | 0.737 | 1.18(0.37-3.70) | 0.780 |
| GG/AG vs. AA | 1.15(0.96-1.38) | 1.18(0.97-1.42) | 0.098 | 1.17(0.97-1.42) | 0.103 | 1.19(0.98-1.45) | 0.080 | 1.04(0.73-1.47) | 0.829 | 0.94(0.63-1.42) | 0.778 |
| GG vs. AA/AG | 1.29(0.77-2.17) | 1.57(0.91-2.71) | 0.104 | 1.32(0.77-2.24) | 0.315 | 1.58(0.90-2.76) | 0.109 | 1.18(0.44-3.11) | 0.746 | 1.20(0.38-3.76) | 0.752 |
| miR-196a-2 rs11614913 T>C |                                    |                                              |    |                                              |     |    |                                              |     |    |
| TC vs. TT    | 0.79(0.65-0.95) | 0.79(0.65-0.97) | 0.024 | 0.78(0.64-0.95) | 0.014 | 0.79(0.64-0.97) | 0.026 | 0.82(0.58-1.18) | 0.282 | 0.82(0.54-1.24) | 0.336 |
| CC/TC vs. TT | 0.76(0.60-0.97) | 0.77(0.60-0.99) | 0.042 | 0.77(0.60-0.98) | 0.037 | 0.77(0.60-1.00) | 0.052 | 0.74(0.46-1.17) | 0.196 | 0.83(0.48-1.42) | 0.490 |
| CC vs. TT/TC | 0.78(0.65-0.93) | 0.79(0.65-0.95) | 0.014 | 0.78(0.64-0.94) | 0.008 | 0.79(0.65-0.96) | 0.015 | 0.80(0.57-1.12) | 0.190 | 0.82(0.55-1.21) | 0.319 |
| CC/TT vs. TC | 0.88(0.72-1.09) | 0.89(0.71-1.11) | 0.286 | 0.89(0.72-1.11) | 0.314 | 0.90(0.71-1.12) | 0.333 | 0.83(0.55-1.25) | 0.380 | 0.94(0.59-1.51) | 0.795 |

* Adjusted for age, sex, smoking, drinking and body mass index;  
NSCLC: non-small-cell lung cancer;
SCC: squamous cell carcinoma;
Bold values are statistically significant ($P < 0.05$).
Table 5. Stratified analyses between miR-146a rs2910164 C>Г polymorphism and NSCLC risk by age, sex, smoking, drinking and body mass index

| Variable          | miRNA-146a rs2910164 C>G (case/control) a | Adjusted OR b (95% CI); P  |
|-------------------|------------------------------------------|---------------------------|
|                   | CC | CG | GG | CG vs. CC | GG vs. CC | GG/C|GG vs. CC | GG vs. CC/GG |
| Sex               |    |    |    |           |           |     |           |               |
| Male              | 260/249 | 289/255 | 89/80 | 1.06(0.81-1.37); P: 0.685 | 1.19(0.82-1.73); P: 0.361 | 1.09(0.85-1.39); P: 0.508 | 1.16(0.82-1.64); P: 0.411 |
| Female            | 200/191 | 266/212 | 80/66 | 1.15(0.88-1.52); P: 0.309 | 1.21(0.82-1.78); P: 0.347 | 1.17(0.90-1.51); P: 0.247 | 1.12(0.78-1.60); P: 0.550 |
| Age               |    |    |    |           |           |     |           |               |
| <59               | 203/192 | 258/198 | 69/60 | 1.16(0.87-1.54); P: 0.313 | 1.17(0.76-1.78); P: 0.478 | 1.16(0.89-1.52); P: 0.282 | 1.08(0.73-1.60); P: 0.709 |
| ≥59               | 257/248 | 297/269 | 100/86 | 1.06(0.83-1.37); P: 0.627 | 1.22(0.86-1.73); P: 0.272 | 1.10(0.87-1.39); P: 0.426 | 1.18(0.85-1.63); P: 0.323 |
| Smoking status    |    |    |    |           |           |     |           |               |
| Never             | 280/358 | 360/371 | 111/125 | 1.22(0.98-1.52); P: 0.080 | 1.15(0.85-1.57); P: 0.274 | 1.20(0.98-1.48); P: 0.084 | 1.04(0.78-1.38); P: 0.809 |
| Ever              | 180/82 | 195/96 | 58/21 | 0.88(0.61-1.27); P: 0.507 | 1.32(0.74-2.33); P: 0.352 | 0.96(0.68-1.36); P: 0.814 | 1.40(0.82-2.40); P: 0.221 |
| Alcohol consumption |    |    |    |           |           |     |           |               |
| Never             | 354/410 | 447/420 | 139/135 | 1.23(1.01-1.21); P: 0.043 | 1.26(0.94-1.67); P: 0.120 | 1.24(1.02-1.50); P: 0.028 | 1.12(0.86-1.47); P: 0.390 |
| Ever              | 106/30 | 108/47 | 30/11 | 0.59(0.34-1.02); P: 0.061 | 0.77(0.34-1.73); P: 0.527 | 0.63(0.37-1.06); P: 0.079 | 1.02(0.48-2.16); P: 0.956 |
| BMI (kg/m2)       |    |    |    |           |           |     |           |               |
| <24               | 303/236 | 381/260 | 110/73 | 1.12(0.88-1.42); P: 0.373 | 1.27(0.89-1.80); P: 0.191 | 1.15(0.91-1.44); P: 0.236 | 1.19(0.86-1.66); P: 0.292 |
| ≥24               | 157/204 | 174/207 | 59/73 | 1.11(0.82-1.50); P: 0.493 | 1.06(0.70-1.61); P: 0.790 | 1.10(0.83-1.46); P: 0.518 | 1.00(0.68-1.48); P: 0.988 |

* For miRNA-146a rs2910164 C>G, the genotyping was successful in 1,184 (99.25%) NSCLC cases, and 1,053 (99.72%) controls;
Adjusted for age, sex, smoking, drinking and body mass index (besides stratified factors accordingly) in a multiple logistic regression model;
Table 6. Stratified analyses between miR-499a rs3746444 A>G polymorphism and NSCLC risk by age, sex, smoking, drinking and body mass index

| Variable               | miRNA-499a rs3746444 A>G (case/control) a | Adjusted OR b (95% CI); P |
|------------------------|------------------------------------------|---------------------------|
|                        | AA | AG | GG | AG vs. AA | GG vs. AA | GG/AG vs. AA | GG vs. AA/AG |
| Sex                    |    |    |    |           |           |              |              |
| Male                   | 444/415 | 172/152 | 20/17 | 1.05(0.79-1.38); P: 0.744 | 1.59(0.79-3.21); P: 0.199 | 1.09(0.84-1.43); P: 0.509 | 1.57(0.78-3.16); P: 0.209 |
| Female                 | 370/342 | 158/119 | 16/8  | 1.21(0.91-1.61); P: 0.194 | 1.84(0.77-4.41); P: 0.172 | 1.25(0.95-1.65); P: 0.118 | 1.74(0.73-4.17); P: 0.211 |
| Age                    |    |    |    |           |           |              |              |
| <59                    | 367/338 | 144/101 | 15/11 | 1.30(0.95-1.78); P: 0.096 | 1.68(0.72-3.92); P: 0.233 | 1.33(0.99-1.80); P: 0.060 | 1.57(0.67-3.65); P: 0.297 |
| ≥59                    | 447/419 | 186/170 | 21/14 | 1.03(0.79-1.33); P: 0.854 | 1.71(0.84-3.51); P: 0.141 | 1.07(0.84-1.38); P: 0.583 | 1.70(0.83-3.47); P: 0.144 |
| Smoking status         |    |    |    |           |           |              |              |
| Never                  | 511/618 | 209/215 | 28/21 | 1.17(0.93-1.48); P: 0.176 | 1.91(1.08-3.48); P: 0.035 | 1.23(0.99-1.54); P: 0.066 | 1.82(1.00-3.32); P: 0.049 |
| Ever                   | 303/139 | 121/56  | 8/4   | 1.04(0.71-1.52); P: 0.856 | 0.90(0.26-3.13); P: 0.873 | 1.03(0.71-1.49); P: 0.889 | 0.90(0.26-3.09); P: 0.861 |
| Alcohol consumption    |    |    |    |           |           |              |              |
| Never                  | 629/695 | 274/247 | 33/23 | 1.19(0.97-1.47); P: 0.101 | 1.86(1.06-3.29); P: 0.032 | 1.25(1.02-1.53); P: 0.035 | 1.77(1.01-3.12); P: 0.047 |
| Ever                   | 185/62  | 56/24  | 3/2   | 0.75(0.42-1.32); P: 0.314 | 0.43(0.07-2.65); P: 0.360 | 0.72(0.41-1.25); P: 0.245 | 0.46(0.07-2.82); P: 0.398 |
| BMI (kg/m2)            |    |    |    |           |           |              |              |
| <24                    | 535/413 | 230/139 | 25/17 | 1.30(1.01-1.68); P: 0.042 | 1.31(0.68-2.52); P: 0.419 | 1.30(1.02-1.67); P: 0.034 | 1.22(0.64-2.33); P: 0.555 |
| ≥24                    | 279/344 | 100/132 | 11/8  | 0.90(0.65-1.23); P: 0.495 | 2.54(0.98-6.55); P: 0.054 | 0.97(0.71-1.32); P: 0.854 | 2.61(1.02-6.73); P: 0.046 |

a For miR-499a rs3746444 A>G, the genotyping was successful in 1,180 (98.91%) NSCLC cases, and 1,053 (99.72%) controls;

b Adjusted OR for age, sex, smoking, and drinking.
b Adjusted for age, sex, smoking, drinking and body mass index (besides stratified factors accordingly) in a multiple logistic regression model;
### Table 7 Stratified analyses between miR-196a-2 rs11614913 T>C polymorphism and NSCLC risk by age, sex, smoking, drinking and body mass index

| Variable               | mIR-196a-2 rs11614913 T>C (case/control) | Adjusted OR (95% CI); P       |
|------------------------|-----------------------------------------|-------------------------------|
|                        | TT           | TC           | CC           | TT vs. TC | CC vs. TT | CC/TC vs. TT | CC vs. TT/TC |
| **Sex**                |              |              |              |           |          |              |              |
| Male                   | 204/176      | 315/287      | 119/121      | 0.96(0.73-1.26); P: 0.761 | 0.87(0.61-1.23); P: 0.428 | 0.93(0.72-1.21); P: 0.594 | 0.89(0.66-1.21); P: 0.461 |
| Female                 | 188/117      | 257/257      | 101/95       | **0.66(0.49-0.88); P: 0.005** | **0.68(0.47-0.98); P: 0.038** | **0.66(0.50-0.87); P: 0.004** | 0.88(0.64-1.22); P: 0.445 |
| **Age**                |              |              |              |           |          |              |              |
| <59                    | 184/141      | 246/218      | 100/91       | 0.81(0.60-1.09); P: 0.165 | 0.81(0.56-1.19); P: 0.279 | 0.81(0.61-1.07); P: 0.142 | 0.92(0.66-1.29); P: 0.625 |
| ≥59                    | 208/152      | 326/326      | 120/125      | 0.79(0.60-1.03); P: 0.083 | 0.74(0.53-1.04); P: 0.081 | 0.77(0.60-1.00); P: 0.050 | 0.86(0.64-1.15); P: 0.317 |
| **Smoking status**     |              |              |              |           |          |              |              |
| Never                  | 246/237      | 365/436      | 140/181      | 0.83(0.66-1.05); P: 0.121 | **0.73(0.55-0.98); P: 0.038** | **0.80(0.64-1.00); P: 0.049** | 0.82(0.64-1.06); P: 0.131 |
| Ever                   | 146/56       | 207/108      | 80/35        | 0.73(0.49-1.08); P: 0.116 | 0.88(0.53-1.47); P: 0.624 | 0.77(0.53-1.11); P: 0.163 | 1.07(0.69-1.67); P: 0.765 |
| **Alcohol consumption**|              |              |              |           |          |              |              |
| Never                  | 312/264      | 456/501      | 172/200      | **0.78(0.63-0.97); P: 0.024** | **0.72(0.55-0.95); P: 0.018** | **0.76(0.62-0.94); P: 0.009** | 0.84(0.67-1.07); P: 0.151 |
| Ever                   | 80/29        | 116/43       | 48/16        | 0.97(0.55-1.70); P: 0.908 | 1.19(0.58-2.45); P: 0.640 | 1.03(0.61-1.74); P: 0.923 | 1.21(0.64-2.30); P: 0.558 |
| **BMI (kg/m2)**        |              |              |              |           |          |              |              |
| <24                    | 258/165      | 382/282      | 154/122      | 0.83(0.64-1.08); P: 0.167 | 0.82(0.59-1.12); P: 0.207 | 0.83(0.65-1.06); P: 0.128 | 0.91(0.69-1.20); P: 0.505 |
| ≥24                    | 134/128      | 190/262      | 66/94        | 0.75(0.55-1.03); P: 0.079 | 0.70(0.47-1.07); P: 0.097 | 0.74(0.55-1.00); P: 0.051 | 0.84(0.59-1.21); P: 0.358 |

*For mIR-196a-2 rs11614913 T>C, the genotyping was successful in 1,184 (99.25%) NSCLC cases, and 1,053 (99.72%) controls*;
Adjusted for age, sex, smoking, drinking and body mass index (besides stratified factors accordingly) in a multiple logistic regression model;
| Genotype | case | Control | OR (95% CI) | P-value |
|----------|------|---------|-------------|---------|
|          | n    | %       | n           | %       |         |
| rs11614913/rs2910164 |      |         |             |         |
| TT/CC    | 159  | 13.43   | 122         | 11.59   | 1.00    |
| TT/CG    | 177  | 14.95   | 133         | 12.63   | 1.02(0.74-1.41) | 0.900 |
| TT/GG    | 56   | 4.73    | 38          | 3.61    | 1.13(0.70-1.82) | 0.612 |
| TC/CC    | 227  | 19.17   | 224         | 21.27   | 0.78(0.58-1.02) | 0.110 |
| TC/CG    | 268  | 22.64   | 239         | 22.70   | 0.86(0.64-1.15) | 0.315 |
| TC/GG    | 77   | 6.50    | 81          | 7.69    | 0.73(0.49-1.08) | 0.113 |
| CC/CC    | 74   | 6.25    | 94          | 8.93    | **0.60(0.41-0.89)** | **0.010** |
| CC/CG    | 110  | 9.29    | 95          | 9.02    | 0.89(0.62-1.28) | 0.522 |
| CC/GG    | 36   | 3.04    | 27          | 2.56    | 1.02(0.59-1.78) | 0.936 |
| rs11614913/rs3746444 |      |         |             |         |
| TT/AA    | 283  | 23.98   | 200         | 18.99   | 1.00    |
| TT/AG    | 97   | 8.22    | 86          | 8.17    | 0.80(0.57-1.12) | 0.194 |
| TT/GG    | 11   | 0.93    | 7           | 0.66    | 1.11(0.42-2.91) | 0.831 |
| TC/AA    | 384  | 32.54   | 397         | 37.70   | **0.68(0.54-0.86)** | **0.001** |
| TC/AG    | 166  | 14.07   | 137         | 13.01   | 0.86(0.64-1.14) | 0.294 |
| TC/GG    | 19   | 1.61    | 10          | 0.95    | 1.34(0.61-2.95) | 0.462 |
| CC/AA    | 147  | 12.46   | 160         | 15.19   | **0.65(0.49-0.87)** | **0.003** |
| CC/AG    | 67   | 5.68    | 48          | 4.56    | 0.99(0.65-1.49) | 0.948 |
| CC/GG    | 6    | 0.51    | 8           | 0.76    | 0.53(0.18-1.55) | 0.239 |
| rs2910164/rs3746444 |      |         |             |         |
| CC/AA    | 322  | 27.29   | 324         | 30.77   | 1.00    |
|       |       |       |       |       |       |
|-------|-------|-------|-------|-------|-------|
| CC/AG |      124 | 10.51 | 108  | 10.26 | 1.16(0.86-1.56) | 0.346 |
| CC/GG  |       13  | 1.10  | 8    | 0.76  | 1.64(0.67-4.00) | 0.277 |
| CG/AA  |      374 | 31.69 | 320  | 30.89 | 1.18(0.95-1.46) | 0.139 |
| CG/AG  |      161 | 13.64 | 135  | 12.82 | 1.20(0.91-1.58) | 0.195 |
| CG/GG  |      18  | 1.53  | 12   | 1.14  | 1.51(0.72-3.18) | 0.277 |
| GG/AA  |      118 | 10.00 | 113  | 10.73 | 1.05(0.78-1.42) | 0.747 |
| GG/AG  |       45  | 3.81  | 28   | 2.66  | 1.62(0.98-2.66) | 0.056 |
| GG/GG  |       5   | 0.42  | 5    | 0.47  | 1.01(0.29-3.51) | 0.992 |
| rs11614913/rs2910164/rs3746444 |         |       |       |       |       |
| TT/CC/AA  |   114 | 9.66  | 86   | 8.17  | 1.00  |
| TT/CC/AG  |      41 | 3.47  | 35   | 3.32  | 0.88(0.52-1.50) | 0.648 |
| TT/CC/GG  |       4  | 0.34  | 1    | 0.09  | 3.02(0.33-27.55)| 0.304 |
| TT/CG/AA  |     128 | 10.85 | 89   | 8.45  | 1.08(0.74-1.60) | 0.681 |
| TT/CG/AG  |      44 | 3.73  | 40   | 3.80  | 0.83(0.50-1.38) | 0.475 |
| TT/CG/GG  |       5  | 0.42  | 4    | 0.38  | 0.94(0.25-3.62) | 0.932 |
| TT/GG/AA  |      41 | 3.47  | 25   | 2.37  | 1.24(0.70-2.19) | 0.464 |
| TT/GG/GG  |       12 | 1.02  | 11   | 1.04  | 0.82(0.35-1.95) | 0.658 |
| TT/GG/AG  |       2  | 0.17  | 2    | 0.19  | 0.75(0.10-5.47) | 0.780 |
| TC/CC/AA  |     155 | 13.14 | 167  | 15.86 | 0.70(0.49-1.00) | 0.049 |
| TC/CC/AG  |      64 | 5.42  | 54   | 5.13  | 0.89(0.57-1.41) | 0.632 |
|       |       |       |       |       |       |
|-------|-------|-------|-------|-------|-------|
|       |       |       |       |       |       |
| TC/CC/GG | 7 | 0.59 | 3 | 0.28 | 1.76(0.44-7.01) | 0.417 |
| TC/CG/AA | 174 | 14.75 | 163 | 15.48 | 0.81(0.57-1.15) | 0.228 |
| TC/CG/AG | 83 | 7.03 | 71 | 6.74 | 0.88(0.58-1.35) | 0.560 |
| TC/CG/GG | 9 | 0.76 | 5 | 0.47 | 1.36(0.44-4.20) | 0.594 |
| TC/GG/AA | 55 | 4.66 | 67 | 6.36 | **0.62(0.39-0.97)** | **0.038** |
| TC/GG/AG | 19 | 1.61 | 12 | 1.14 | 1.19(0.55-2.59) | 0.653 |
| TC/GG/GG | 3 | 0.25 | 2 | 0.19 | 1.13(0.18-6.92) | 0.894 |
| CC/CC/AA | 53 | 4.49 | 71 | 6.74 | **0.56(0.36-0.89)** | **0.013** |
| CC/CC/AG | 19 | 1.61 | 19 | 1.80 | 0.75(0.38-1.51) | 0.426 |
| CC/CC/GG | 2 | 0.17 | 4 | 0.38 | 0.38(0.07-2.11) | 0.250 |
| CC/CG/AA | 72 | 6.10 | 68 | 6.46 | 0.80(0.52-1.23) | 0.310 |
| CC/CG/AG | 34 | 2.88 | 24 | 2.28 | 1.07(0.59-1.93) | 0.826 |
| CC/CG/GG | 4 | 0.34 | 3 | 0.28 | 1.01(0.22-4.61) | 0.994 |
| CC/GG/AA | 22 | 1.86 | 21 | 1.99 | 0.79(0.41-1.53) | 0.484 |
| CC/GG/AG | 14 | 1.19 | 5 | 0.47 | 2.26(0.79-6.47) | 0.119 |
| CC/GG/GG | 0 | 0.0 | 1 | 0.09 | 0.25(0.01-6.26) | 0.251 |
