The miR-146a SNP Rs2910164 and miR-155 SNP rs767649 are risk factors for non- small cell lung cancer in Iranian population

Neda KakaDezfuli
NRITLD
Ian M. Adcock
Imperial College London, The University of Newcastle
Shamila D. Alipour
National Institute of Genetic Engineering and Biotechnology
Sharareh Seyfi
Shahid Beheshti University of Medical Sciences
Babak Salimi
Shahid Beheshti University of Medical Sciences
Maryam Mafi Golchin
Ferdowsi University
Neda Dalil Roofchayee
NRITLD
Mohammad Varhram
NRITLD
Esmaeil Mortaz (emortaz@gmail.com)
NRITLD

Research Article

Keywords: SNPs, Rs2910164, miR-146a, Rs767649, miR-155, lung cancer, NSCLC

DOI: https://doi.org/10.21203/rs.3.rs-68568/v1

Abstract

Background: Lung cancer is a leading cause of death worldwide. MicroRNAs (miRNAs) are small noncoding RNAs that regulate gene expression and may act as both tumor suppressors and as oncogenes. The presence of single nucleotide polymorphisms (SNPs) inside the miRNA genomic region could affect target miRNA maturation, expression and binding to its target mRNA and contribute to cancer development. Previous studies on the SNPs Rs2910164 in miR-146a and Rs767649 in miR-155 showed association with non-small cell lung cancer (NSCLC) development. Thus, the aim of study was to detect any correlation between those SNPs in Iranian NSCLC patients.

Methods: In a small cohort study, 165 NSCLC patients and 147 non-cancer controls were enrolled between Apr 2015 – Sep 2019 at the Masih Daneshvari Hospital, Tehran-Iran. Allele frequencies from genomic DNA of blood PBMC was studied using PCR-RFLP and their association with the risk of lung cancer was evaluated.

Results: The frequency of the C allele of the miR-146a rs2910164 polymorphism was increased in NSCLC patients (OR=1.56, 95%CI=1.10 -2.21, P= 0.012). In contrast, the frequency of the A allele of the miR-155 rs767649 polymorphism was significantly increased in the control group (TT vs. AA+AT: OR=0.58, 95%CI=0.35-0.98, P= 0.043).

Conclusion: The findings of this study suggest that miR-146a rs2910164 and miR-155 rs767649 polymorphisms could be related with the risk of NSCLC in an Iranian population. However, a larger multi center study across Iran is needed to confirm these findings.

Introduction

Lung cancer is the most common cancer worldwide and is associated with high mortality rates [1]. The two main subtypes of lung cancer are small-cell (SCLC) and non-small-cell lung carcinoma (NSCLC). NSCLC encompasses over 80-85% lung cancers although SCLC which totals 12% of all cases is more aggressive than NSCLC [1]. Despite considerable improvement in diagnosis and treatment; lung cancer remains the leading cause of global cancer-related deaths [2]. Improving our knowledge of the molecular pathology of NSCLC is important in achieving earlier diagnosis and successful treatment. Although smoking is one of the major risk factors for lung cancer, nonsmokers are suffer from the disease [3]. Indeed, lung cancer is now considered as a multifactorial disease resulting from a combination of genetic background and lifestyle habits such as diet, smoking and environmental pollution [4].

Previous studies have demonstrated the importance of multiple genes in lung cancer pathology including P53, Myc and BRCA1 [1]. In addition to these genes; microRNAs (miRNAs) have recently been identified as important factors in the development of numerous cancers [5]. miRNAs are a family of endogenous small noncoding RNAs that regulate gene expression post transcriptionally. miRNAs are implicated in the regulation of almost all biological process and may has function as either oncogenes or tumor

suppressor elements. Dysregulation of miRNA expression is reported a wide array of cancers where they play key roles in oncogenesis including the promotion of invasion, metastasis and angiogenesis [5].

Recent attention has focused on the effects of single nucleotide polymorphisms (SNPs) within non-coding regions of miRNAs. SNPs can significantly affect the production or processing of miRNAs by modulating their expression or by altering their association with the 3' untranslated regions (UTRs) of their target mRNAs. Thus, SNP-induced alterations in miRNA maturation, structure and expression may impact on cancer susceptibility [6]. Rs2910164 and rs767649 polymorphisms have been frequently observed in association with lung [7, 8], breast [9], cervical [10] and hepatocellular [11] cancers. In the current study we aimed to assess the possible association between miR-146a rs2910164 and miR-155 rs767649 polymorphisms in an Iranian cohort with newly diagnosed NSCLC.

Materials And Methods

Patients

One hundred and sixty-five patients with newly diagnosed NSCLC (aged 58.5 ± 8.6 years) were recruited at the Masih Daneshvari Hospital, Tehran, Iran between Apr 2015 and Sep 2019. One hundred forty seven age- and gender-matched healthy controls with a negative history of cancer were also enrolled. Demographic information of study participants is shown in **Table1**. The Ethics Committee of the Masih Daneshvari Hospital approved the study and all subjects gave written informed consent (Ethics committee approval number: IR.SBMU.MSP.REC.1397.525).

Genotyping

Genomic DNA was isolated from peripheral blood PBMC using a DNA extraction kit (High Pure PCR Template Preparation Kit, Roche, Germany, Cat.No.11796828001) according to manufacturer instructors. The DNA concentration was measured by Nanodrop 2000 (Thermo Fisher, USA). Specific SNPs were genotyped using polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) with the PCR reaction performed using super PCR master mix (YEKTA TAJHIZ AZMA, Tehran-Iran, Cat NO: YT1553-YT1554) using a Thermal Cycler instrument (Bio-Rad, CA, USA).

The primer sequences for each PCR reaction is shown in **Table 2**. The cycle parameters for the PCR analysis were as follows: initial denaturation at 95°C for 5 min, 35 cycles of denaturation at 94°C for 30 sec, annealing at 58°C for 1min and extension at 72°C for 1 min and a final extension at 72°C for 10 min. To identify the miR-146 C/G polymorphism, the PCR product was digested with the restriction enzyme mnlI (Thermo Fisher, USA, REF: ER1071) by incubating the samples at 37°C for 4h. The miR-155 T/A polymorphism PCR product was incubated at 37°C overnight with the restriction enzyme TSP45I (Thermo Fisher, USA, REF: ER1511) and the digestion products detected by 3% agarose gel electrophoresis.

Statistical analysis

The differences in genotype distribution for the two analyzed SNPs between NSCLC and healthy subjects were analyzed using the Chi-square test. Deviations of the genotype frequencies in the controls from those expected under the Hardy–Weinberg equilibrium (HWE) were assessed by a goodness-of-fit χ^2 test. All statistical tests were carried out using SPSS-25 software (SPSS, Inc.). P values ≤ 0.05 were considered statistically significant.

Results

The study included 165 NSCLC cases and 147 healthy controls (**Table 1**) with a mean age of 58.5 and 52.6 years in cases and controls respectively. The age and gender distributions were similar between the two groups (P>0.05).

Distribution of the rs2910164 and rs767649 genotypes in control group by HWE was (X^2=1.57, P value=0.209; X^2=1.85, P value=0.173 respectively) which indicates for the randomness of control samples.

For rs2910164, the uncut PCR product size was 248bp and digested products in patients with the G allele show bands at 77 and 171bp and for patients with the C allele gave 171, 45 and 32bp products (**Fig. 1A**). The PCR product size for rs767649 was 294bp and digested products in subjects with the T allele were 158, 94 and 42 bp and for those with the A allele were 252 and 42bp (**Fig. 1B**).

Genotypes and allele frequency for rs2910164 and rs767649 are shown in **Table 3**. Statistical analysis using the Chi-square test demonstrated that the C allele and the CC genotype of miR-146a rs2910164 was correlated with NSCLC (OR=1.56, 95%CI=1.10 -2.21, P= 0.012; OR =2.93, 95% CI =1.07-7.9, P=0.034, respectively) compared to the GG genotype.

We also show that TT is a common genotype and that AA is rare genotype of miR-155 rs767649 in Iranian NSCLC patients and control subjects. The AT genotype and the A allele frequency in miR-155 rs767649 variants are lower in NSCLC patients in comparison with the control group (OR=0.57, 95%CI=0.33-0.99, P=0.048; OR=0.58, 95%CI=0.35-0.98, P=0.043). Moreover, the A allele has an inverse association with lung cancer (**Table 3**).

rs2910164 variants were not associated with smoke status (**Table 4**) whereas the AT genotype frequency in smoker controls was higher than in smoking NSCLC patients with the rs767649 variant (OR=0.44, 95%CI=0.21-0.90, P=0.024) (**Table 4**). However, no evidence of association was found between rs2910164 and rs767649 polymorphisms and stage or type of NSCLC (P>0.05, **Table 5**).

The distribution of rs2910164 alleles and rs767649 alleles is summarized in **Table 6** no evidence of association was found.

Discussion

In the current study we found that the miR-146a rs2910164 and miR-155 rs767649 variants were significantly associated with the risk of NSCLC in an Iranian population. In particular, we demonstrate a significant increased risk of NSCLC in subjects with the CC genotype of miR-146a rs2910164 compared to subjects with the GG genotype. In contrast, the AT genotype of miR-155 rs767649 is protective against NSCLC. There was no link between miR-146a polymorphisms and smoking status although the AT genotype frequency of miR-155 was lower in smokers with NSCLC than in non-cancerous smoking controls. No polymorphisms were associated with NSCLC stage or type.

SNPs occur approximately once every three hundred base pairs in both coding and non-coding regions of the genome [12]. Approximately 93% of functional SNPs are located within non-coding regions which are the most common sites for miRNA genes [13]. SNPs located in mature miRNA regions may directly affect binding to target miRNAs and those SNPs occur in pre-miRNA, may interfere with miRNA maturation [13]. Importantly, miRNAs generally do not act individually but as part of a network in order to regulate the expression and/or function of numerous genes whilst variations in miRNA expression induces diverse functional outcomes [6].

miR-146a is known to negatively regulate severe inflammation [14] in an NF-kB-dependent manner and can bind to sequences within the 3'-UTRs of the TNF receptor-associated factor 6 (TRAF 6) and IL-1 receptor-associated kinase 1(IRAC1) genes [14]. NSCLC has been associated with enhanced inflammation [15] and changes in miR-146a expression may enhance lung cancer risk. In previous studies, Jia and colleagues showed that the CC genotype and C allele distribution in NSCLC patients was significantly higher (P = 0.03 and 0.03, respectively) and concluded that the rs2910164 polymorphism of miR-146a was associated with an increased risk of NSCLC in a Chinese population [8]. A meta-analysis study has also reported that the rs2910164 polymorphism is associated with lung cancer (NSCLC and SCLC) [16].

However, there is disparate data concerning miR-146a polymorphisms and the susceptibility to other cancers. Hashemi et al. showed no statistically significant association between miR-146a rs2910164 polymorphisms and prostate cancer in an Iranian population [17] despite a meta-analysis demonstrating that the rs2910164 CC genotype was associated with decreased prostate cancer risk in an Asian population [18]. In addition, whilst the miR-146a rs2910164 GC allele was significantly associated with breast cancer in a Pakistani population [9] this was not the case in Azeri subjects from Iran [19]. Furthermore, Gao and colleagues demonstrated a significant association of the rs2910164 CC genotype or C allele with susceptibility to colorectal cancer in Chinese males [20].

A recent meta-analysis showed that the CC genotype of miR-146a rs2910164 was associated with susceptibility to NSCLC but not to hepatocellular carcinoma and gastric cancer [12]. In stratified analysis by cancer type and ethnicity, is was reported that these polymorphisms were associated with a significant risk of lung cancer, breast cancer and colorectal cancer in Asian, but not Caucasian populations [13].

miR-155 expression is significantly up-regulated in lung cancer tissues, plasma and sputum and is associated with the risk of NSCLC [7]. possibly through a modulatory effect on NF-kB activity [7, 10]. The rs767649A>T polymorphism has been associated with an increased risk and poor prognosis of NSCLC. The T allele increased the transcriptional activity of miR-155 and patients with the rs767649-TT genotype had the highest risk ratio for NSCLC and had a reduced response to radiotherapy or chemotherapy [7].

In contrast, the rs767649 TT genotype is associated with a significantly reduced risk of cervical cancer and was linked with miR-155 overexpression in cancerous tissues compared with normal tissues suggesting an oncogenic role of miR-155 in cervical cancer. However, in vitro miR-155 promoter-luciferase assays indicated that the transition from an A to T allele might lead to miR-155 downregulation at the transcriptional level. This opposing effect on miR-155 expression indicates the importance of other factors in cervical cancer pathogenesis such as the presence or absence of human papillomavirus (HPV) infection. HPV is the main risk factor for cervical cancer and may impact on the direction and magnitude of the association between miR-155 rs767649 polymorphisms and the risk of cervical cancer [10].

miR-155 is overexpressed in hepatocellular carcinoma tissues compared with adjacent healthy tissues [11] . The rs767649 T allele is associated with higher miR-155 expression suggesting that variants of miR-155 contribute to the increased risk and poor prognosis of hepatocellular carcinoma [11]. A study has reported a population-dependent difference in the impact of the rs767649 TT genotype as a risk factor for multiple sclerosis. 93% of patients and 87% of controls had the T allele and none of the patients and controls had the AA genotype in one population but this was the opposite in subjects of Chinese ethnicity [21].

In conclusion, the current study indicates that the miR-146a rs2910164 and miR-155 rs767649 polymorphisms are associated with the risk of NSCLC with the AT genotype of miR-155 rs767649 being protective against NSCLC. Larger sample sizes with diverse ethnicities are needed to extend the findings.

Declarations

Ethics approval and consent to participate:

The study was approved by the Ethics Committee of the Dr. Masih Daneshvari Hospital, and all patients gave signed informed consent.

Consent for publication:

All authors have read the manuscript and consent to publication in the Journal

Availability of data and material:

Not applicable

Competing interests:

The authors confirm that there are no competing interests.

Funding:

This study was supported by internal funding.

Acknowledgement:

This article has been extracted from the thesis written by Neda KakaDezfuli in School of Medicine Shahid Beheshti University of Medical Sciences. (Registration No: 14960). Ethics committee approval ID: IR.SBMU.MSP.REC.1397.525.

EM is funded by the Iran National Science Foundation (INSF) grant number 94003504

IMA was supported by the Wellcome Trust (093080/Z/10/Z), EPSRC (EP/T003189/1) and by the UK MRC (MR/T010371/1).

Abbreviations

BRCA1: Breast cancer 1

miRNA: microRNA

Myc: Transcription factor C-MYC

NSCLC: Non-small cell lung cancer

RFLP: Restriction fragment length polymorphism

PCR: Polymerase chain reaction

SNPs: Single nucleotide polymorphisms

References

1. Travis, W.D., *Pathology of lung cancer.* Clin Chest Med, 2002. **23**(1): p. 65-81.
2. Molina, J.R., et al. , *Non-small cell lung cancer: epidemiology, risk factors, treatment, and survivorship.* in Mayo Clinic Proceedings, 2008. **83**(5): p. 584-94.
3. Mortaz E, A.S., Movassaghi M, Varahram M, Ghorbani J, Folkerts G, *Water-pipe smoke condensate increases the internalization of Mycobacterium Bovis of type II alveolar epithelial cells (A549). .* BMC pulmonary medicine, 2017. **17**(68): p. 1-9.
4. Samet, J.M., *Lung Cancer, Smoking, and Obesity: It's Complicated. .* JNCI: Journal of the National Cancer Institute., 2018. **110**(8): p. 795-796.

5. Alipoor SD, A.I., Garssen J, Mortaz E, Varahram M, Mirsaeidi M.., , *The roles of miRNAs as potential biomarkers in lung diseases. .* Eur J Pharmacol, 2016. **791**: p. 395-404.
6. Gary Wilkand, R.B., *Single nucleotide polymorphisms that modulate microRNA regulation of gene expression in tumors.* arXiv 2018: p. 1-26.
7. Kaipeng Xie , H.M., Cheng Liang , Cheng Wang , Na Qin , Wei Shen , Yayun Gu , Caiwang Yan , Kai Zhang , Ningbin Dai , Meng Zhu , Shuangshuang Wu , Hui Wang , Juncheng Dai , Guangfu Jin , Hongbing Shen , Zhibin Hu, *A functional variant in miR-155 regulation region contributes to lung cancer risk and survival.* Oncotarget, 2015. **6**(40): p. 42781-42792.
8. Youchao Jia , A.Z., Yanhong Shang , Hua Yang , Zizheng Song , Zhiyu Wang , Lili Ren , Yaning Wei , Ling Hu , Hongyun Shi , Hefei Li, *MicroRNA-146a rs2910164 polymorphism is associated with susceptibility to non-small cell lung cancer in the Chinese population.* Med Oncol 2014. **31**(194): p. 1-5.
9. Ahmad, M., et al., *Association of MIR146A rs2910164 variation with a predisposition to sporadic breast cancer in a Pakistani cohort.* Ann Hum Genet, 2019. **83**(5): p. 325-330.
10. ShizhiWang , X.C., Bo Ding , Jinfeng Chen , Mengjing Cui , Yuling Xu , Xiaoyun Lu , Zhengdong Zhang , Aiqin He , Hua Jin, *The rs767649 polymorphism in the promoter of miR-155 contributes to the decreased risk for cervical cancer in a Chinese population.* GENE, 2016. **595**(1): p. 109-114.
11. Jiansong Ji , M.X., Jianfei Tu , Zhongwei Zhao , Jun Gao , Minjiang Chen , Jingjing Song , Haidong Zhu , Xingyao Cheng, Junguo Hui, Xilin Lan, Xiaoming Yang, *MiR-155 and its functional variant rs767649 contribute to the susceptibility and survival of hepatocellular carcinoma.* Oncotarget, 2016. **7**(37): p. 60303-60309.
12. Hao X , X.L., Qu R , Yang X , Jiang M , Zhou B, *Association between miR-146a rs2910164 polymorphism and specific cancer susceptibility: an updated meta-analysis.* Familial cancer, 2018. **17**(3): p. 459-468.
13. Jianbo Wang , Qingwei W., Hong Liu , Na Shao , Bingxu Tan , Guangyu Zhang , Kai Wang , Yibin Jia , Wei Ma , Nana Wang and Yufeng Chen, *The association of miR-146a rs2910164 and miR-196a2 rs11614913 polymorphisms with cancer risk: a meta-analysis of 32 studies.* Mutagenesis, 2012. **27**(6): p. 779-88.
14. Li Zhang , J.W., Di Che , Yanfei Wang , Xing Rong , Lei Pi , Yufen Xu , Wei Li , Ping Huang ,Maoping Chu and Xiaoqiong Gu, *The association between the miR-146a rs2910164 C>G polymorphism and Kawasaki disease in a southern Chinese population.* Bioscience reports, 2018. **38**(4): p. 1-5.
15. Hanne Astrid Eide, A.R.H., Vandana Sandhu, Anne Fåne, Janna Berg ,Vilde Drageset Haakensen, Elin H Kure, Odd Terje Brustugun, Cecilie Essholt Kiserud,Jon Amund Kyte, Åslaug Helland, *Non-small cell lung cancer is characterisedby a distinct inflammatory signature in serumcompared with chronic obstructive pulmonary disease.* Clinical & Translational Immunolog, 2016. **5**(11): p. 1-7.
16. Ren, Y.G., et al., *Effects of common polymorphisms in miR-146a and miR-196a2 on lung cancer susceptibility: a meta-analysis.* J Thorac Dis, 2016. **8**(6): p. 1297-305.

17. Mohammad Hashemi , N.M., Seyed Amir Mohsen Ziaee , Behzad Narouie , Mohammad Hosein Soltani , Maryam Rezaei , Ghazaleh Shahkar , Mohsen Taheri, *Association between single nucleotide polymorphism in miR-499, miR-196a2, miR-146a and miR-149 and prostate cancer risk in a sample of Iranian population.* Journal of Advanced Research, 2016. **7**(3): p. 491-498.
18. Yuanyuan Mi , K.R., Jiangang Zou , Yu Bai, Lifeng Zhang , Li Zuo , Atsushi Okada , Takahiro Yasui *The Association Between Three Genetic Variants in MicroRNAs (Rs11614913, Rs2910164, Rs3746444) and Prostate Cancer Risk.* Cellular Physiology and Biochemistry, 2018. **48**: p. 149-157
19. Nejati-Azar, A. and M.R. Alivand, *miRNA 196a2(rs11614913) & 146a(rs2910164) polymorphisms & breast cancer risk for women in an Iranian population.* Per Med, 2018. **15**(4): p. 279-289.
20. Xueren Gao, Z.Z., Shulong Zhang, *miR-146a rs2910164 polymorphism and the risk of colorectal cancer in Chinese population.* Journal of Cancer Research and Therapeutics, 2018. **14**(8): p. 97-99.
21. Golshani, Z., et al., *Genetic Variation in Intergenic and Exonic miRNA Sequence and Risk of Multiple Sclerosis in the Isfahan Patients.* Iran J Allergy Asthma Immunol, 2018. **17**(5): p. 477-484.

Tables

Table1: Distribution of selected demographic variables of lung cancer and control subjects.

Factors	Lung cancer n=165 (%)	Control n=147 (%)	P-value
Age (Years, Mean±SD)	58.5 ± 8.6	52.6 ± 8.3	
Gender (n, %)			
Male	128 (77.58)	115 (78.23)	0.32
Female	37 (22.42)	32 (21.77)	
Smoking status (n, %)			
Ever	102 (61.82)	93 (63.27)	0.44
Never	63 (38.18)	54 (36.73)	
Histological subtype (n, %)			
ADC	135 (81.81)		
LCC	6 (3.65)		
SCC	24 (14.54)		
Stage (n, %)			
I	4 (2.42)		
II	18 (10.91)		
III	33 (20.00)		
IV	110 (66.67)		

Table 2: PCR primer sequences used and expected fragment sizes.

Polymorphism	Primer sequence	Restriction Enzyme	Product size (bp)
Rs2910164	F: 5’-AGAACTGAATTCCATGGGTTG-3’ R: 5’-TGCTTAGCATAGAATTCAAGTC-3’	mnlI	Uncut product: 248 G Allele: 171+77 C Allele: 171+45+32
Rs767649	F: 5'-CCT GTA TGA CAA GGT TGT GTT TG-3' R: 5'-GCT GGC ATA CTA TTC TAC CCA TAA-3'	TSP451	Uncut product: 294 A Allele: 252+42 T Allele: 158+94+42

Table 3: Genotypic and allelic frequencies of miR-146a rs2910164 and miR-155 rs767649 polymorphisms in non- small cell lung cancer (NSCLC) and control subjects.

Polymorphism	Lung cancer n = 165 subjects (%)	Control n = 147subjects (%)	OR (95% CI)	P-value
miR-146a Rs2910164				
Allele				
G	219 (66.36%)	222(75.55%)	1 (reference)	
C	111 (33.64%)	72(24.45%)	1.56 (1.10-2.21)	0.012
Co-dominant				
GG	69 (41.8%)	81 (55.1%)	1 (reference)	
GC	81 (49.1%)	60 (40.8%)	1.58 (0.99 - 2.51)	0.051
CC	15(9.1%)	6 (4.1%)	2.93 (1.07 - 7.9)	0.034
Dominant				
GG	69 (41.8%)	81 (55.1%)	1 (reference)	
GC+CC	96 (58.2%)	66 (44.9%)	1.7 (1.09 - 2.67)	0.019
Recessive				
GG+GC	150 (90.9%)	141 (95.9%)	1 (reference)	
CC	15 (9.1%)	6 (4.1%)	2.35 (0.88 - 6.22)	0.085
miR-155 Rs767649				
Allele				
T	290 (87.87%)	242 (82.3%)	1 (reference)	
A	40 (12.13%)	52 (17.6%)	0.64 (0.41-1.00)	0.051
Co-dominant				
TT	131 (79.3%)	102 (69.3%)	1 (reference)	
AT	28 (16.9%)	38 (25.8%)	0.57 (0.33-0.99)	0.048
AA	6 (3.6%)	7 (4.7%)	0.66 (0.21-2.04)	0.47
Dominant				
TT	131 79.3%)	102 (69.3%)	1 (reference)	
AA+AT	34 (20.6%)	45 (30.6%)	0.58 (0.35-0.98)	0.043
Recessive				
AT+TT	159 (96.3%)	140 (95.2%)	1 (reference)	

AA	6 (3.6%)	7 (4.7%)	0.75 (0.24-2.29)	0.62

OR = Odds Ratio

Table 4: The association between SNPs and the risk of NSCLC stratified by smoking

SNP	Genotype	Never smokers (n=117)				Ever smokers (n=195)			
		Control (%)	Case (%)	OR (95%CI)	P Value	Control (%)	Case (%)	OR (95%CI)	P Value
miR-146a rs2910164	GG	29 (53.7%)	25 (39.7%)	1		52 (56%)	44 (43%)	1	
	GC	25 (46.3%)	33 (52.3%)	1.53 (0.72-3.22)	0.26	35 (38%)	48 (47%)	1.62 (0.89-2.93)	0.11
	CC	0 (0%)	5 (8.0%)	12.7 (0.67-241)	0.09	6 (6%)	10 (10%)	1.96 (0.66-5.85)	0.22
miR-155 rs767649	TT	42 (77.7%)	48 (76.1%)	1		60 (64.5%)	83 (81.3%)	1	
	TA	12 (22.3%)	12 (19.0%)	0.87 (0.35-2.15)	0.77	26 (27.9%)	16 (15.6%)	0.44 (0.21-0.90)	0.024
	AA	0 (0%)	3 (4.8%)	6.13 (0.30-122)	0.23	7 (7.5%)	3 (2.9%)	0.30 (0.07-1.24)	0.099

Table 5: Association of SNPs with NSCLC according to stage and subtypes

Variable	rs2910164						
	GG	CG	CC	Allele G frequency (%)	Allele C frequency (%)	Adjusted OR (95%CI)	P Value
Stage I (n=4)	2	2	0	6 (0.75)	2 (0.25)		
Stage II (n=18)	7	10	1	24 (0.67)	12 (0.33)	0.66 (0.11-3.81)	0.64
Stage III (n=33)	15	15	3	45 (0.68)	21 (0.32)	0.71 (0.13-3.84)	0.69
Stage IV (n=110)	45	54	11	144 (0.65)	76 (0.35)	0.63 (0.12-3.20)	0.57
ADC Type (n=135)	57	65	13	179 (0.66)	91 (0.34)		
SCC Type (n=24)	11	11	2	33 (0.69)	15 (0.31)	1.11 (0.57-2.16)	0.73
LCC Type (n=6)	1	5	0	7 (0.58)	5 (0.42)	0.71 (0.21-2.30)	0.75
	rs767649						
	TT	TA	AA	Allele T frequency (%)	Allele A frequency (%)	Adjusted OR (95%CI)	P Value
Stage I (n=4)	4	0	0	4 (100)	0 (0%)		
Stage II (n=18)	15	3	0	33 (0.92)	3 (0.08)	1.06 (0.04-24.1)	0.96
Stage III (n=33)	27	6	0	60 (0.91)	6 (0.09)	1.03 (0.04-21.4)	0.98
Stage IV (n=110)	85	19	6	189 (0.86)	31 (0.14)	0.66 (0.03-12.7)	0.78
ADC Type (n=135)	106	25	4	237 (0.88)	33 (0.12)		
SCC Type (n=24)	20	3	1	43 (0.89)	5 (0.11)	1.19 (0.44-3.23)	0.72
LCC Type (n=6)	5	0	1	10 (0.83)	2 (0.17)	0.69 (0.14-3.31)	0.64

Table 6: Association of NSCLC with alleles distribution [rs2910164 G/C; rs767649 T/A]

rs2910164	rs767649	Alleles	NSCLC (n=165)	Control (n=147)	OR (95%CI)	P value
G	T	GT	50% (146)	52% (135)	1 (reference)	
G	A	GA	11% (31)	16% (43)	0.71(0.30- 1.69)	0.44
C	A	CA	7% (21)	7% (18)	1.04 (0.34-3.17)	0.94
C	T	CT	32% (92)	25% (64)	1.33 (0.69- 2.55)	0.38

Figures

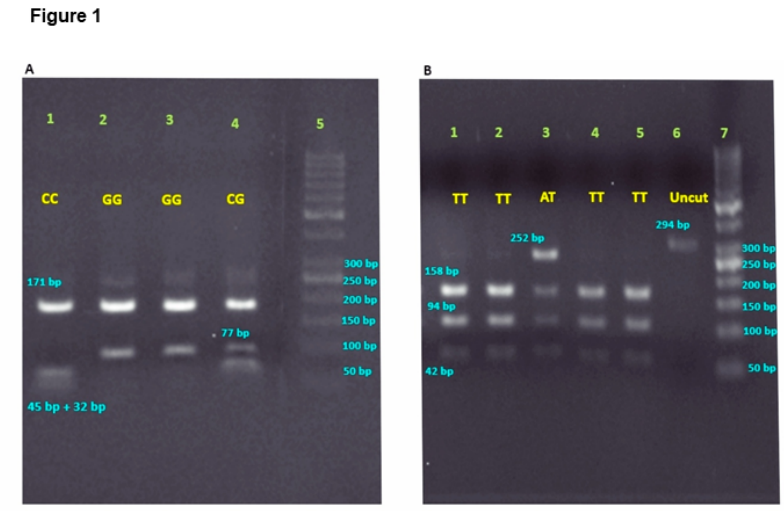

Figure 1

(A) The miR-146a PCR product and the genotypes. Lane 1 shows the CC genotype (bands: 171bp and 45+32bp); Lane 2 ,3 shows the GG genotype (band at 171 and 77bp); lane 4 shows the CG genotype (bands: 171, 77 and 45+32bp) and Lane 5 is the DNA ladder with specific size markers labelled. (B) The miR-155 PCR product and its genotypes. Lane 1,2,4,5 shows the TT genotype (bands: 158,94 and 42bp); lanes 3 shows the AT genotype (bands at 252, 158, 94, 42bp); lane 6 shows uncut PCR product at 294 bp and lane 7 is the DNA ladder.