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Genetic Predictors of Lung Cancer Risk: A Molecular Approach Involving microRNA Signatures

*[1]Hasanain Ali Shubbar

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About Author

[1] Jabir Ibn Hayyan University for Medical and Pharmaceutical Sciences, Faculty of Medicine, Iraq

Contact @ Hasanain Ali Shubbar
hasanain.a.shubbar@jmu.edu.iq

ABSTRACT

Lung cancer is a leading cause of cancer-related deaths worldwide, with both environmental and genetic factors contributing to its development. Among genetic determinants, microRNA (miRNA) dysregulation has emerged as a critical mechanism in tumorigenesis. MiR-146a plays a pivotal role in modulating immune and inflammatory responses, and its rs2910164 polymorphism (G>C) has been implicated in cancer susceptibility. This study aimed to investigate the association between the miR-146a rs2910164 polymorphism and lung cancer risk in an Iraqi population. A case-control design was employed, involving 72 patients with histopathologically confirmed lung cancer and 70 age- and gender-matched healthy controls. Genomic DNA was extracted from peripheral blood samples, and the rs2910164 polymorphism was genotyped using Tetra-ARMS PCR. Genotypes were determined by analyzing distinct band patterns on 2% agarose gels. Statistical analyses were performed to compare genotypic and allelic frequencies between cases and controls, calculating odds ratios (OR) with 95% confidence intervals (CI) and p-values. The GC genotype showed a significantly higher frequency among lung cancer cases compared to controls (OR = 2.22, 95% CI: 1.19–4.14; p = 0.004), suggesting a potential risk factor. The C allele also demonstrated a notable association with increased lung cancer susceptibility (OR = 1.79, 95% CI: 1.11–2.89; p = 0.017). In contrast, neither the GG genotype nor the G allele exhibited a significant relationship with disease risk ($p > 0.05$). Collectively, our findings confirm that carriers of the miR-146a rs2910164 C allele, especially the GC carriers, are at a heightened risk of developing lung cancer in the Iraqi population. To confirm such a finding and elucidate the molecular circuitry of Iraqi carcinogenesis, however, future investigations must be extended: larger, multiethnic cohorts with control for other genetic markers, lifestyle factors, and environmental factors will be required.

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1. INTRODUCTION

Globally, lung cancer is the leading cause of cancer morbidity and mortality. It accounts for nearly 1.8 million new cases every year, making it the most diagnosed cancer as well as a major health challenge (Ren *et al.*, 2016). Notwithstanding new developments in screenings and treatments, the outcomes in regard to survival are poor because the patients are in an advanced stage when presenting and the tumour's biology is aggressively malignant (Zhang *et al.*, 2020). Its aetiology is not straightforward. Environmental insults tobacco smoke and air pollution are the worst offenders meet inherited susceptibility to cause the cancer (Jia *et al.*, 2014). Though still the leading perpetrator, many instances occur in long-term nonsmokers, illustrating the way gene and molecular programs control lung-cancer susceptibility (Yin *et al.*, 2017). To be a leading player in them, microRNAs have emerged as regulators of initiation and tumour progression, and their action in lung cancer is now well established (Wang *et al.*, 2012).

MicroRNAs (miRNAs) are small, non-coding strands of RNA that modulate gene expression by binding to messenger RNAs and speeding up their degradation or inhibiting their translation. If the regulation equilibrium is disrupted, the ripple is transmitted to all the primary cellular programs growth, differentiation, apoptosis and can set the stage for tumour formation (Dezfuli *et al.*, 2020). One of them, miR-146a, is a critical intersection between inflammation, immune homeostasis, and cancer vulnerability. A single-nucleotide polymorphism in its gene, rs2910164, exchanges a G for a C, slightly altering the precursor transcript's stem-loop and diminishing its maturation efficacy (Jia *et al.*, 2014). The polymorphism relaxes miR-146a's grip over the targeted genes making the cell susceptible to malignant transformation (Wistuba & Gazdar, 2006). Studies of the rs2910164 variant have drawn a mixed picture of its role in lung cancer. Some studies associate the CC genotype with a sizable increase in cancer hazard, especially in groups with a high prevalence of smokers (Ren *et al.*, 2016; Dezfuli *et al.*, 2020), yet other studies show the very same change is weakly protective in certain groups non-smoking women, say (Yin *et al.*, 2017; Omar & Ali, 2019).

Confronted with such opposing results, our case-control analysis compares the genotype of lung-cancer patients with that of volunteer controls to make an estimate of the variant's true effect. Elucidation of the impact of rs2910164 on disease risk will help towards an understanding of the aetiology of tumour formation and can lead to the identification of early-detection targets or targets for tailored therapies.

2. LITERATURE REVIEW

Lung cancer remains a leading cause of cancer-related mortality worldwide, and recent advances have shifted focus toward the regulatory roles of non-coding RNAs, particularly microRNAs, in tumor biology. Among these, miR-146a has attracted considerable attention due to its involvement in modulating inflammatory pathways and immune responses two hallmarks of tumorigenesis (Jia *et al.*, 2014; Wistuba & Gazdar, 2006). The rs2910164 polymorphism in the miR-146a gene, characterized by a G>C nucleotide substitution, has been proposed to alter the maturation and functional expression of this microRNA, thereby weakening its tumor-suppressive potential (Dezfuli *et al.*, 2020; Yin *et al.*, 2017). While several studies report a statistically significant association between the C allele and increased lung cancer susceptibility, particularly among smokers or genetically predisposed populations (Ren *et al.*, 2016; Zhang *et al.*, 2020), others note conflicting or population-specific outcomes, highlighting the complexity of gene-environment interactions (Wang *et al.*, 2012). Despite the volume of data, most existing literature tends to describe findings in isolation, lacking a unified theoretical model to interpret how miR-146a polymorphisms mediate carcinogenic risk. A useful lens may be provided by systems biology, which emphasizes the interplay between genetic regulation and systemic inflammatory cues, thus offering a more integrative perspective for future research on microRNA-driven oncogenesis.

3. METHODOLOGY

3.1. Study design and population

The study carried out a hospital-based case–control investigation of the involvement of the miR-146a rs2910164 polymorphism in the Iraqi population. We recruited 142 subjects, with 72 newly diagnosed, histologically confirmed lung carcinoma cases and 70 controls who did not have cancer. We recruited the cases from the oncology wards of Al-Sadr Medical City, Al-Furat Al-Awsat Hospital, and Al-Hakim General Hospital in Najaf, and the controls from the outpatient wards of the aforementioned hospitals.

The control group consisted of age- and gender-matched healthy individuals with no personal or family history of lung cancer or other malignancies. Controls were recruited from the general outpatient clinics of the same hospitals during routine health checkups or treatment for non-cancer-related minor illnesses. To ensure the validity of the study, participants were carefully matched to minimize confounding variables, such as smoking history and occupational exposure to carcinogens.

3.2. DNA Extraction and genotyping

3.2.1. Sample collection and processing

The blood samples (3 mL each) were collected from all participants using EDTA tubes to prevent coagulation 1

3.3. DNA Extraction

Genomic DNA was extracted from the collected blood samples using the Wizard Genomic DNA Purification Kit (Promega/USA) , following the manufacturer's protocol, which involved a series of critical steps. First, cell lysis was performed to break open red blood cells and other cellular components, releasing nuclear material. Following this step, protein precipitation was performed, by removal of proteins and other impurities. In the end, the DNA was isolated by precipitating it with alcohol and then purifying it. After that, the DNA was washed and resuspended in an appropriate buffer so that it could be used for further investigations.

Table 1. The primer sequences and expected product sizes.

Primer	Sequence (5' ⟶ 3')	Product Size (bp)
Primer-F (Allele-C)	tccatgggttgtgtcagtgtcagagctc	290 bp
Primer-R (Allele-G)	atatcccagctgaagaactgaattacac	203 bp
Common Primer-F	tagacctggtactaggaagcagctgcat	445 bp
Common Primer-R	gagtagcagcagcagcaagagagactt	-

3.4. PCR Conditions

The PCR reaction was performed in a total volume of 20 µL containing (Table 2). The thermal cycling conditions were optimized to ensure the specific amplification of the target polymorphism are detailed in Table 3.

Table 2. PCR Reaction Mixture

Intron Maxime PCR PreMix Kit for 20µl rxn

Reagent	Volume (µL)
Template DNA	2
Primer outer (F) 10 pmol/ µL	1.5
Primer outer (R) 10 pmol/ µL	1.5
F inner	1.5
R inner	1.5
Distilled Water	12

The thermal cycling conditions were optimized to ensure the specific amplification of the target polymorphism:

Table 3. Thermal cycling parameters

Step	Temp. (°C)	Duration	Cycles
Pre-denaturation	92	2 min	1
Denaturation	92	20 sec	34
Annealing	59	45 sec	
Extension	72	35 sec	
Final Extension	72	7 min	1

3.5. Detection of PCR products

PCR amplification products were separated in 2% agarose gel with ethidium bromide, which segregated the rs2910164 alleles well and offered accurate genotype calls for further studies. All the runs are held under strict standardised laboratory conditions to be accurate, and to ensure relevance. The banding was sharp: homozygous wild type, (GG), samples showed 445 bp and 203 bp fragments, heterozygous (GC), samples showed 4 bands in total 445 bp, 290 bp and 203 bp, homozygotes (CC) showed 4 bands in total 445 bp and 290 bp.

3.6. Statistical analysis

Fisher's exact test was used to compare genotype and allele frequencies and the measurement of each of the associations was provided as an odds ratio with 95 % confidence interval. Significance was determined if the p-value was < 0.05.

4. RESULTS AND DISCUSSION

The Tetra-ARMS PCR of the rs2910164 (G>C) miR-146a gene polymorphism resulted in readily resolvable banding patterns in a 2% agarose gel, using which the genotypes could be determined for certain. The GG type (wild type) had 203 bp and 445 bp bands, GC heterozygote had 203 bp, 290 bp, and 445 bp bands, and the CC genotype (mutant) by 290 bp and 445 bp bands. In the gel under analysis, lanes 3, 5, 7, and 18 contained the GC genotypes; lanes 4, 8, 10, 14, 16, 19, and 21 contained GG patterns, and lanes 6, 9, 12, 13, 15, and 20 contained the CC profile. Lane 1 consisted of a 100 bp DNA ladder, which was used for verification of the fragment sizes; amplification of the internal control band, at 445 bp, in each sample confirmed a successful amplification. The data give a clear visual impression of genotype distribution within the lung cancer cohort population and represent a baseline data set from which to define the contribution of this SNP towards genetic susceptibility.

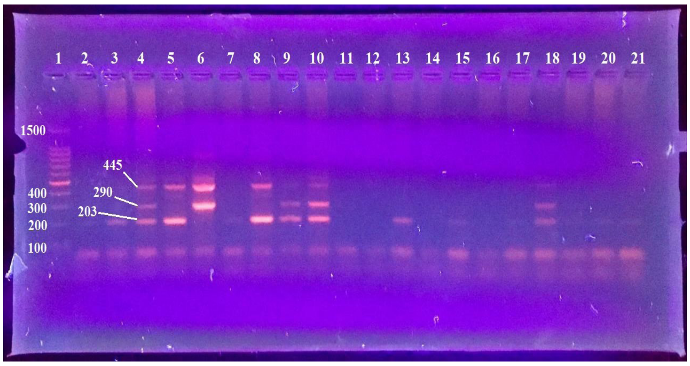

Figure 1. Tetra-ARMS PCR Results of rs2910164 | (G>C) Polymorphism in miR-146a Gene

Visualized on a 2% Agarose Gel. The molecular marker (100 bp ladder) serves to acquire size reference. Band patterns as evidence of genotypes: GC heterozygote - 203 bp, 290 bp, 445 bp; CC homozygote - mutant type, 290 bp, 445 bp; GG homozygote - wild type, 203 bp, 445 bp, lung cancer samples. Screening of Gel electrophoresis of Tetra-ARMS PCR products for miR-146a variant rs2910164 in 2 % agarose gel electrophoresis to create clean and readable gel electrophoresis gel. The wild-type GG genotype had 2 fragments --445 bp and 203 bp-- and GC heterozygotes extra 290bp band, CC homozygotes had only one cataract 490 bp fragment and control 445 bp fragment. Lane 1 contained a 100 bp ladder to hold the size calls in place and there was the universal 445 bp band in every lane to assure that there was successful amplification. The calls for this gel were straightforward: lanes 2, 5, 8, 12, and 16 were GC; lanes 3, 6, 10, and 14 were GG; lanes 4, 7, 9, 11, and 13 were CC. The clean and unambiguous profiles are a testament to Tetra-ARMS PCR has been designed to provide robust genotype calls for directly typing the rs2910164 SNP and give ample power to our planned lung-cancer association analysis.

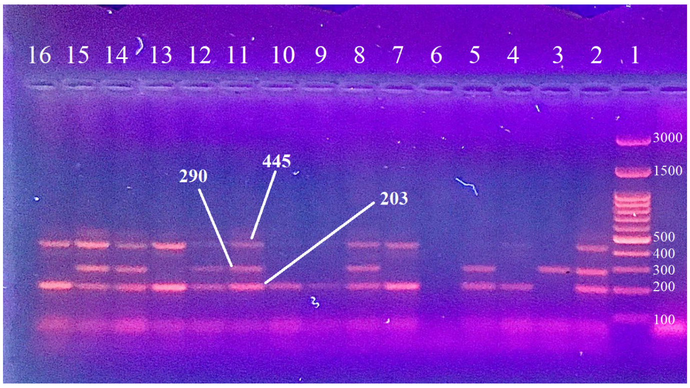

Figure 2. Comparisons of Control Tetra-ARMS PCR Results with rs2910164 (G>C) Polymorphism in miR-146a

Gene Visualized on a 2% Agarose Gel. The molecular marker (100 bp ladder) is used for size reference. Band patterns correspond to genotypes: GC heterozygote (203 bp, 290 bp, 445 bp), CC homozygote (mutant type, 290 bp, 445 bp), and GG homozygote (wild type, 203 bp, 445 bp) in lung cancer samples. The distribution of genotypes and alleles among cases and controls is summarized in Table 3, along with the corresponding odds ratios (OR), 95% confidence intervals (CI), and p-values. The GG genotype was observed in 27 cases and 40 controls, with an OR of 0.70 (95% CI: 0.36–1.34; p = 0.285), suggesting no significant association. The GC genotype was present in 30 cases and 20 controls, showing a significant association with lung cancer susceptibility (OR = 2.22, 95% CI: 1.19–4.14; p = 0.004). The CC genotype was observed in 15 cases and 10 controls, with no significant association (OR = 1.97, 95% CI: 0.80–4.84; p = 0.134). Allelic analysis revealed that the G allele was more frequent in controls (100) than in cases (84), with an OR of 0.74 (95% CI: 0.50–1.11; p = 0.138), indicating no significant difference. In contrast, the C allele was significantly more frequent in cases (60) compared to controls (40), with an OR of 1.79 (95% CI: 1.11–2.89; p = 0.017). The gel electrophoresis results confirmed the specificity of the PCR, with distinct band patterns for each genotype visualized clearly (Figure 1). These findings suggest that the GC genotype and the C allele are significantly associated with increased lung cancer susceptibility.

Table 3. Allelic along with genotypic frequencies and analysis of statistical significance

Allelic variant	Disease cohort (n=72)	Control group (n=70)	Odds R. (95% CI)	p-value
GC	30	20	2.22 (1.19–4.14)	0.004
CC	15	10	1.97 (0.80–4.84)	0.134
GG	27	40	0.70 (0.36–1.34)	0.285
G Nucleotide Variant	84	100	0.74 (0.50–1.11)	0.138
C Nucleotide Variant	60	40	1.79 (1.11–2.89)	0.017

4.1. Discussion

Our Iraqi cohort provides powerful evidence for a role resonance variable in miR-146a (rs2910164) to be a susceptibility factor for lung cancer. Women and men who had the GC genotype were more than twice as likely as the GG homozygotes to have the condition (OR = 2.22, p = 0.004), and the presence of the C allele by itself increased susceptibility to the condition by a factor of approximately 80 percent (OR = 1.79, p = 0.017). The trend holds across other groups, too Jia *et al.* (2014) found an excess of the C allele in Chinese non-small-cell lung cancer patients – and implies that this single nucleotide substitution blunts the tumour-suppressive effect of miR-146a via a similarly shared mechanism. With the evidence, the case for rs2910164 as an actual lung cancer susceptibility factor is strengthened and deserving of further elucidation of its mechanistic action in a wide array of ethnic groups.

One large meta-analysis by Ren *et al.* (2016) reaffirmed evidence that the C allele of the rs2910164 variant, especially in CC homozygosity, is associated with increased lung-cancer susceptibility in various populations, emphasizing the usefulness of the variant as a susceptibility factor. The GG genotype and the G allele in the same review also did not have a strong association with the condition (p > 0.05), consistent with the finding of Yin *et al.* (2017) that GG might even be mildly protective, yet not always to a statistically significant degree. Differences from one study to the other most likely are caused by differences in the ancestral background, local stresses, and differences in the sizes of the samples.

For example, Wang *et al.*'s (2012) meta-analysis revealed a discordant view and did not find any overall significant relationship between the rs2910164 polymorphism and lung cancer susceptibility. The heterogeneity indicates that the impact of the SNP is mediated by other gene polymorphisms or the external environment, which supports the hypothesis of multifactor susceptibility to cancer. Mechanistically, the stemloop of the pre-miRNA precursor 146a seems to change conformation, leading to an altered maturation and reduced productive rate as consequence of the G-to-C transition rs2910164 (Dezfuli *et al.*, 2020; Omar *et al.*, 2024). In addition, it can upset the regulation of the target genes of the inflammatory and oncogenic pathways. To prove the importance of functional, Wistuba and Gazdar (2006) and Zhang *et al.*, (2020) established that miR-146a performs an important function in immunological regulation and signals brought on by neoplasms. These results provide further biological plausibility that rs2910164 is involved in risk for the pathogenesis of lung cancer.

There is a necessity to be mindful of the limitations of this study. The limited number of samples, the absence of long-term follow up and of adequate exposure to all environmental pollutants affected a power of weak significance of the findings and their generalisation. The results can be replicated and expanded in larger samples and in diverse cases as well as analyses of the combined data from molecular and environmental approaches is necessary to validate the work. In contrast, analysis of a single SNP reflects only a small portion of the regulatory network of lung cancer. Completion of such studies must await future widespread genome-wide genome scanning combined with the proper characterization of interactions of heritable variation with the environment, before a full picture of an individual's susceptibility to the disease is in place.

5. CONCLUSION

The study validates the high association of miR-146a GC genotype and C allele of polymorphic marker rs2910164 with increased risk of lung cancer in Iraqi population. These findings are consistent with previous studies in other populations, suggesting that the rs2910164 polymorphism may serve as a genetic risk marker for lung cancer. Further research is required to elucidate the underlying mechanisms and validate these associations in larger and more diverse cohorts.

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