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

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About Article

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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.

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

The products of PCR amplification were resolved in a 2 % agarose gel with ethidium bromide, which separated the rs2910164 alleles efficiently and provided reproducible genotype calls for further analyses. All the runs are carried out under rigid standardised laboratory conditions for accuracy and for the sake of relevance. The banding was sharp: wild-type samples (GG) contained 445 bp and 203 bp fragments, heterozygotes (GC) contained three fragments—445 bp, 290 bp, and 203 bp, and homozygous mutants (CC) contained 445 bp and 290 bp fragments.

3.6. Statistical analysis

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

4. RESULTS AND DISCUSSION

The Tetra-ARMS PCR for the rs2910164 (G>C) miR-146a gene polymorphism produced easily discriminable banding patterns in a 2% agarose gel, from which the genotypes could be identified with certainty. The GG genotype (wild type) was characterized by 203 bp and 445 bp bands, the GC heterozygote by 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 contained a 100 bp DNA ladder for the validation of fragment sizes, and the 445 bp internal control band in each of the samples testified to successful amplification. The data provide an obvious visually-oriented overview of genotype distribution across the lung cancer cohort, which represents a foundation dataset for establishing the gene contribution of this SNP towards susceptibility.

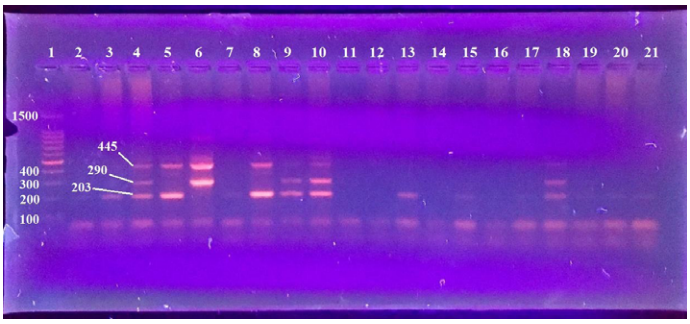

Figure 1. Tetra-ARMS PCR Results for rs2910164 (G>C) Polymorphism in miR-146a Gene

Visualized on a 2% Agarose Gel. The molecular marker (100 bp ladder) is used for size reference. Band patterns indicate 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.

Gel electrophoresis of Tetra-ARMS PCR products for the miR-146a variant rs2910164 in 2 % agarose produced clean, readable gels. The wild-type GG genotype had two fragments—445 bp and 203 bp—and GC heterozygotes an extra 290 bp band, the CC homozygotes having only the 290 bp fragment and control 445 bp fragment. Lane 1 contained a 100-bp ladder to hold the size calls in place, and the universal 445 bp band in every lane assured us 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, unambiguous profiles are a testament to

the ruggedness of Tetra-ARMS PCR for typing rs2910164 and provide us with the strong genotype counts we desire for our lung-cancer association analysis.

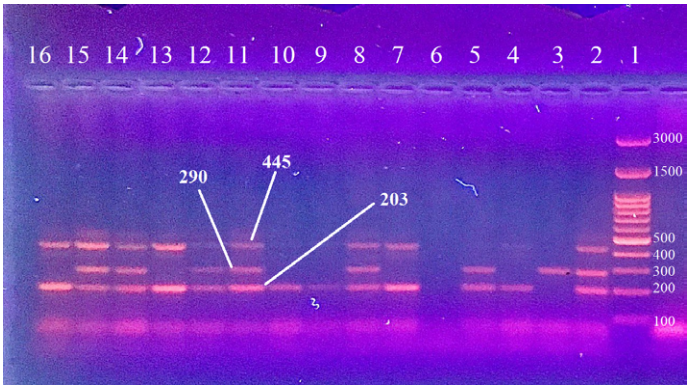

Figure 2. Control Tetra-ARMS PCR Results for rs2910164 (G>C) Polymorphism in miR-146a

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. Genotypic and allelic frequencies and statistical analysis

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 demonstrates robust evidence that the rs2910164 variant in miR-146a is a susceptibility factor for lung cancer. Women and men with the GC genotype had over twice the probability as GG homozygotes of having the condition (OR = 2.22, p = 0.004), and the presence of the C allele alone raised the susceptibility by a factor of about 80 percent (OR = 1.79, p = 0.017). The trend is confirmed in other groups as well—Jia *et al.* (2014) identified a preponderance of the C allele among Chinese non-small-cell lung cancer patients—and suggests that this single-nucleotide substitution blunts the tumour-suppressive activity of miR-146a by 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 variation proves that the effect of the SNP is controlled by other gene variants or the external environment, corroborating the concept of the multifactoriality of susceptibility to cancer. Mechanistically, the G-to-C transition caused by rs2910164 alters the conformation of the precursor miR-146a's stem-loop, which interferes with its maturation and lowers its functional yield (Dezfuli *et al.*, 2020; Omar *et al.*, 2024). The disturbance can interfere with the regulation of the inflammatory and oncogenic pathways' target genes.

To validate the functional relevance, studies by Wistuba and Gazdar (2006) and by Zhang *et al.* (2020) established that miR-146a plays an essential role in immune regulation as well as regulation of the signals induced by tumors. These findings increase the biological plausibility that rs2910164 plays a role in the pathogenesis of lung cancer.

Yet there is a need to acknowledge the limitations of this research. The relatively small sample size, lack of long-term follow-up, and lack of complete environmental exposure measures are factors that can impact the power and generalizability of the results. More research is required in larger and more diverse groups as well as synthesis analyses of both the genetic and the environmental data to replicate and extend the findings.
Investigation of a single SNP, in addition, reveals only a small part of the complex gene map of lung cancer. Completion must be achieved by future studies with genome-wide scans and with appropriate investigation of the interaction of heritable variation with the environment to set the stage for a complete portrait of an individual's susceptibility to the disease.

5. CONCLUSION

In conclusion, this study confirms the significant association of the GC genotype and C allele of the rs2910164 polymorphism in miR-146a with an increased risk of lung cancer in an 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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