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Genetic Predictors and Molecular Insights into Thyroid Cancer Susceptibility

Sarah Salih Hasan[1]; Hasanain Ali Shubbar[2]; Mohammed Jaafar Al-Anssari[3]

[1,2]Jabir Ibn Hayyan University for Medical and Pharmaceutical Sciences, Faculty of Medicine, Iraq
[3]College of Medicine, University of Alkafeel, Al-Najaf, Iraq
[1] https://orcid.org/0009-0000-9275-7067; [2] https://orcid.org/0000-0003-2242-902X
sarah.s.bidany@jmu.edu.iq; hasanain.a.shubbar@jmu.edu.iq; mohammed.alanssari@alkafeel.edu.iq

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Abstract: Thyroid cancer has several histological kinds and subtypes, each defined by unique cellular origins, molecular characteristics, and clinical outcomes. This study sought to examine the relationship between the miR-146a Rs2910164 (G>C) polymorphism and the risk of thyroid cancer in an Iraqi cohort. Approaches: A case-control strategy was employed to assess the association between microRNA (miRNA) and the risk of thyroid cancer, using 148 individuals divided into two groups: 78 cases and 70 controls. Genotyping was conducted with the Tetra-ARMS PCR methodology. Outcomes: The examination of genotypic and allelic frequencies indicated that the GC genotype (odds ratio = 1.68, 95% confidence interval: 1.01-2.78, p = 0.04) and the CC genotype (odds ratio = 1.04-7.97, p = 0.02) exhibited significant associations; conversely, the GG genotype did not reveal any notable connection. The C allele was associated with an elevated risk, but the G allele had no significant correlation with risk (p=0.82). Conclusions: Our research validates and expands upon prior findings, illustrating the significance of rs 2910164, a miRNA, as a genetic risk factor for thyroid cancer. Future study should seek to clarify the functional implications of this polymorphism across many populations and its interplay with environmental variables, therefore advancing precision medicine strategies in the therapy of thyroid cancer.

Keywords: miR-146a, rs2910164 polymorphism, Thyroid cancer, Genetic biomarker, Iraqi population, MicroRNAs (miRNAs), Tetra-ARMS PCR

Introduction

Over the course of the last few decades, there has been a notable increase in the incidence of thyroid cancer, which is classified as an endocrine malignancy. The United States of America has been identified as the country with the largest yearly increase in incidence of thyroid cancer, with an estimated 6.6% increase in incidence between the years 2000 and 2009 (Xing, 2013). There is a very low death rate associated with the condition; nonetheless, it often recurs or continues to

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exist, which results in increased patient morbidity and presents difficulties in obtaining total curability(Cabanillas et al., 2016). Thyroid cancer encompasses a variety of histological types and subtypes, each characterized by distinct cellular origins, molecular profiles, and clinical prognoses (Baloch & LiVolsi, 2018), including differentiated thyroid cancers such as papillary thyroid cancer and follicular thyroid cancer, as well as more aggressive forms like poorly differentiated thyroid cancer and anaplastic thyroid cancer, with medullary thyroid cancer, derived from parafollicular C cells, representing a small fraction of cases and being associated with mutations in the RET gene (Accardo et al., 2017).

In recent years, microRNAs (miRNAs) have emerged as central regulators in the field of cancer biology. These short, non-coding RNA fragments—typically spanning 19 to 25 nucleotides—function at the post-transcriptional level by binding to complementary sequences located in the 3′ untranslated regions (UTRs) of target messenger RNAs.

The interaction its either accelerates the degradation of the mRNA or represses translation, thereby silencing the expression of the gene as well as a single miRNA regulates hundreds of transcripts at the same time the interaction establishes a vast regulatory framework that governs many cellular activities (Braun et al., 2010; Holley & Topkara, 2011). Owing to this vast interaction control and capability to regulate multiple cascades at the same time, miRNAs are also recognized as important players during the initiation as well as the growth of tumours (Matoulkova et al., 2012; Fuziwara & Kimura, 2014; Pu et al., 2019).

In every cellular process, the microRNA function is shown as slender threads that regulate balance during cell proliferation, differentiation, programmed

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death, and immune surveillance (Zhang et al., 2009; Matoulkova et al., 2012; Pu et al., 2019). While their expression patterns deviate from the natural ordering sequences, the disruption precipitates pathological outcomes, leading to cancer, cardiovascular malfunctions, or autoimmune diseases (Bennett et al., 2018). In tumours, an miRNA molecule shows dual functionality—that of constraining malignant growth as a tumour suppressor molecule or that of inducing oncogenesis as a promoter and then acting on the essence of the core networks that signal, the small RNAs direct the processes that range from tumorigenesis initiation to metastasis to even resistance to therapy (Barbu et al., 2020).

This present study is organized to identify the association between the miR-146a rs2910164 polymorphism and thyroid carcinoma dander and its risk among the Iraqi human population. Findings outlined here would be valuable to determine the role of this gene variation as a biomarker to support early diagnosis, identification of the risk, as well as the design of personalized therapeutic regimens.

Materials and Methods

Study Design and Population

In the present study, a case control study design was employed to explore the association between the MIR-146a rs2910164 polymorphism and the predisposition of the population in Iraq to thyroid cancer, for which a total of 148 participants were enrolled, comprising 78 patients with thyroid cancer and 70 healthy controls. The case group included individuals with histopathologically confirmed thyroid malignancy, who were recruited from specialised oncology

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clinics in Najaf, Iraq, namely Al-Hakim General Hospital, Al-Sadr Medical City, and Furat Al-Awsat Hospital. In contrast, the control group consisted of healthy subjects with no prior history of thyroid disorders or cancer.

These patients was selected from the general outpatient population who visited the same medical facilities for regular checks or minor diseases. Participants in both groups were properly matched by age and gender in order to reduce the number of potential factors that could be confusing. The participants, patients who were able to give their informed consent and were between 20 to 70 years old, were eligible to participate. People who were the criteria for exclusion were those suffering from autoimmune thyroid disorders, other cancers, or chronic systemic diseases, as well as those using immunosuppressive or anti-inflammatory drugs in the expansion period who used immunosuppressive or anti-inflammatory medicine in the expulsion period.

DNA Extraction and Genotyping

Sample Collection and Processing

The blood samples (3 mL each) were collected from all participants using EDTA tubes to prevent coagulation(Al-garawi et al., 2022).

DNA Extraction

Genomic DNA was extracted from the collected blood samples using the **SimEXTM Blood DNA Extraction Kit Iran**, 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

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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(Bustani et al., 2024).

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	-

PCR Conditions

The PCR reaction was performed in a total volume of **25 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

promega (50)

Reagent	Volume (µL)
Green Master Mix 2X	25
Upstream outer Primers	2
Downstream outer Primers	2

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Primer (G) allele	2
Primer (G) allele	2
Deoxyribonucleic acid	5
ddH□O	12

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

Table 3: Thermal Cycling Parameters

Step	Temperature (°C)	Duration	No. of Cycle
Pre-denaturation	94	4 min	1
Denaturation	94	20 sec	36
Annealing	59	35 sec	
Elongation	72	40 sec	
Final Elongation	72	6 min	1

Detection of PCR Products

Amplicons were analyzed on a 2% agarose gel stained with ethidium bromide. This method enabled precise identification of the allelic variants of the Rs2910164 polymorphism, providing robust data for subsequent statistical analysis. The entire process was performed under controlled laboratory conditions to ensure reproducibility and accuracy. Genotypic patterns were identified based on band sizes:

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1. GG (Wild Type): Bands at 203 bp and 445 bp.
2. GC (Heterozygote): Bands at 203 bp, 290 bp, and 445 bp.
3. CC (Mutant Type): Bands at 290 bp and 445 bp.

Statistical Analysis

Genotypic and allelic frequencies were compared using Fisher's exact test. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated to determine the strength of associations. A p-value < 0.05 was considered statistically significant.

Results

Genotyping of rs2910164 Polymorphism in miR-146a Gene

The genotyping of the rs2910164 (G>C) polymorphism in the miR-146a gene was successfully performed using the Tetra-ARMS PCR technique. The results were detected on a 2% agarose gel stained with ethidium bromide. The widths of the PCR products indicate that the gel electrophoresis results, shown in Figure 1, reveal distinct band patterns corresponding to the three genotypes (GG, GC, and CC).

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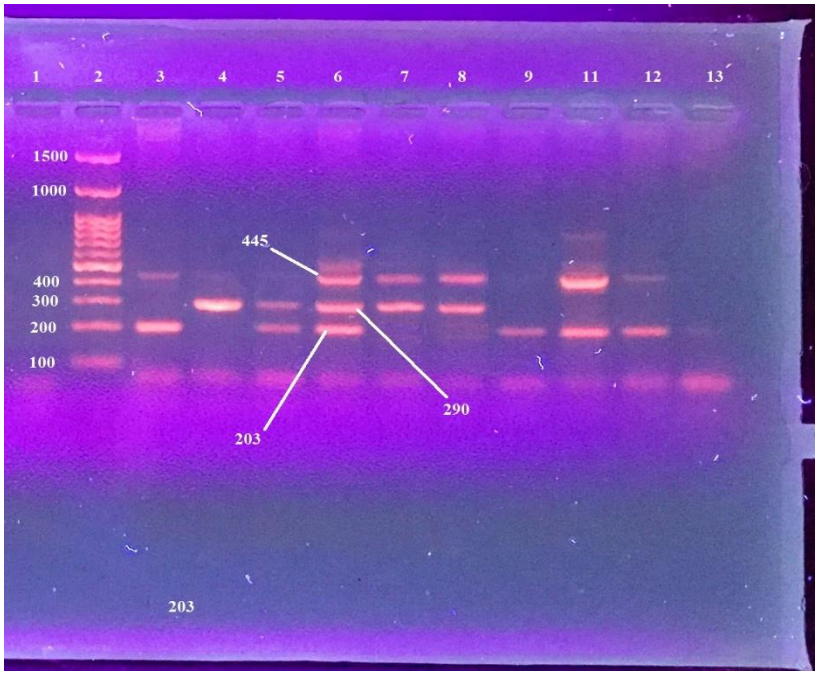

Figure 1: The Tetra-ARMS PCR for the rs2910164 polymorphism in miR-146a are shown. A 100 bp molecular marker (lane 2) was used to verify the sizes of the amplified products. Specific lanes depict the genotypes

In figure-1 the genotyping result showed three unique genotypes according to the banding patterns. The GG homozygous is identified by two bands at 203 bp and 445 bp, with the 445 bp band functioning as an internal reference and the 203 bp band representing the allele (G). On the other hand, GC genotype showed three bands at 203 bp, 290 bp, and 445 bp, where the 445 bp band indicates amplification and the existence of both the (203 bp) and (290 bp) allele. The CC homozygous mutant genotype displays two bands at 290 bp and 445 bp, where the 290 bp band corresponds to the allele (C) and the 445 bp band as the internal control.

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The Figure 2 illustrates the genotypic distribution of the rs2910164 polymorphism in the miR-146a gene, demonstrating distinct band patterns for the GG, GC, and CC genotypes. A 100 bp DNA ladder (Lane 2) was used to confirm product sizes. Lanes with 203 bp and 445 bp bands represent the GG homozygous wild type, where 203 bp indicates the wild-type allele and 445 bp serves as the internal control. Lanes with 203 bp, 290 bp, and 445 bp bands correspond to the GC heterozygous genotype, with 203 bp representing the wild-type allele, 290 bp the mutant allele, and 445 bp as the internal control. Lanes displaying 290 bp and 445 bp bands indicate the CC homozygous mutant type, where 290 bp represents the mutant allele and 445 bp ensures successful amplification. These patterns validate the accuracy and specificity of the Tetra-ARMS PCR method.

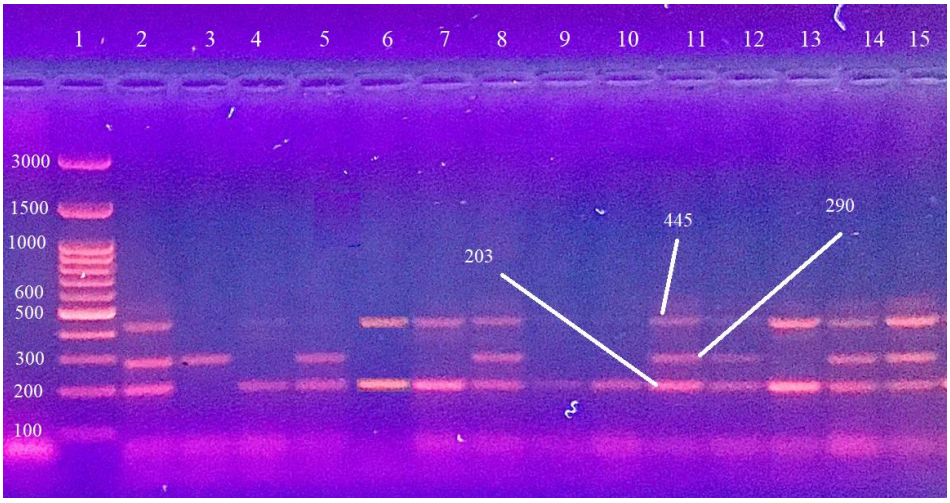

Figure 2: control Tetra ARMS PCR Products Result of rs2910164 (G>C) in miR-146a Gene on 2% Electrophoresis Gel. Molecular marker (100 bp), GC heterozygote (203 bp, 290 bp, 445 bp), CC homozygote (mutant type)(290 bp, 445 bp) and numbers GG homozygote (wild type)(203 bp, 445 bp).

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Genotypic and Allelic Frequencies

The rs2910164 polymorphism in the miR-146a gene demonstrates significant associations with the risk of thyroid cancer, as indicated by the genotypic and allelic frequency analysis shown in Table 3. The result illustrate that is correlation between increased risk and the GC genotype (odds ratio = 1.68, 95% confidence interval: 1.01–2.78, p = 0.04) as well as the CC genotype (odds ratio = 2.88, 95% confidence interval: 1.04–7.97, p = 0.02); however, the GG genotype did not demonstrate any significant association (p = 0.62). The C allele was linked with an increased risk (odds ratio = 1.57, 95% confidence interval: 1.02–2.42, p = 0.03), but the G allele showed no significant association with risk (p = 0.82).

Table 3: Genotypic and Allelic Frequencies and Statistical Analysis

Genetic variant	Affected individuals (n=78)	Unaffected individuals (n=70)	OR (95% CI)	p-value
CC	11	8	2.88 (1.04–7.97)	0.02
GC	38	27	1.68 (1.01–2.78)	0.04
GG	29	35	0.85 (0.45–1.61)	0.62
G Variant	96	97	0.95 (0.62–1.45)	0.82
C Variant	60	43	1.57 (1.02–2.42)	0.03

Discussion

The observed association of the C allele with an increased risk of thyroid cancer is consistent with studies from other populations. For example, research conducted in China reported that the rs2910164 C allele contributes to thyroid cancer susceptibility, suggesting a universal biological mechanism mediated by this polymorphism (Wei et al., 2013; Omer et al., 2024). Similarly, the study in the

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European population identified a significant correlation between CC genotypes and an increased risk of cancer which these findings outline the important role of MIR-146A in tumorigenesis in different ethnic groups. (Lian et al., 2012; Scelo et al., 2014). Several groups have examined the functional effects of the rs2910164 polymorphism in MIR-146A and have shown that the G→C substitution has the ability to re-model MIR-146A processing and reduce its expression (Wei et al., 2013; Xing, 2013; Baloch & LiVolsi, 2018; Omar & Ali, 2019). Reduced MIR-146A expression compromises its tumor-suppressive functions—most importantly its inhibition of the NF-κB pathway and modulation of inflammatory signals—and thus eliminates crucial brakes on cell growth and survival. From a practical standpoint, defective MIR-146A function helps to allow tumors to gain traction, making a plausible mechanistic link to the increased cancer risk that we identified in our population (Xu et al., 2008; Lian et al., 2012).

Notably, individuals carrying the GC genotype also exhibited an elevated risk of thyroid cancer, although the odds ratio was lower than that observed for CC homozygotes. This finding aligns with previous genetic research suggesting a dose-dependent influence of the C allele, whereby each additional copy further heightens susceptibility (Sun et al., 2014). Such patterns highlight the complex interplay between genetic predisposition and environmental factors in shaping disease risk (Cho et al., 2016; Xu et al., 2008). In contrast, our results indicated no significant association between thyroid cancer and either the GG genotype or the G allele, which is consistent with reports describing the G variant as neutral or only mildly protective (Wang et al., 2015; Omer et al., 2024).

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The findings of this study carry significant clinical implications. Identifying individuals who carry the C allele or the CC genotype may support early detection of thyroid cancer and contribute to more personalized treatment approaches. Moreover, the results highlight the importance of conducting additional investigations to develop targeted therapeutic strategies that modulate miR-146a signaling pathways (Rogucki et al., 2022; Bhattacharya et al., 2023).

Conclusion

The present study indicates the significance of miR-146a rs2910164 polymorphism as a genetic predisposing factor to thyroid cancer. It would be the aim of future work to determine the functional outcomes of the polymorphism among various populations and their interaction as regards the environmental factors, which once again open the door to the precise medical strategy in the cancer therapy of the thyroid.

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