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Genetic predictors and molecular insights into thyroid cancer susceptibility

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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) [21]. There is a very low death rate associated with the condition; nonetheless, it often recurs or continues to exist, which results in increased patient morbidity and presents difficulties in obtaining total curability (Cabanillas *et al*., 2016) [9]. Thyroid cancer encompasses a variety of histological types and subtypes, each characterized by distinct cellular origins, molecular profiles, and clinical prognoses (Baloch & LiVolsi, 2018) [3], 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) [1].

Over the past decade, microRNAs (miRNAs) have risen to prominence as key molecular conductors in cancer biology. These slender, non-coding RNA strands—usually just 19-25 nucleotides long—operate after transcription, attaching themselves to complementary sequences in the 3′-untranslated regions of target mRNAs. When tied, they are either unstable messenger RNA or prevent the translation and effectively silence the voice of the gene. Because a single miRNA can change dozens of ribosomes, these molecules form a complex regulatory net that shapes a wide variety of cellular processes. When their widespread impact and ability to coordinate multiple signal routes reflect miRNA in the center of tumor development and progress (Braun *et al*., 2010; Holley & Topkara, 2011;

Matoulkova *et al*., 2012; Fuziwara & Kimura, 2014; Pu *et al*., 2019) [7, 12, 14, 11, 17]. Through almost each route, the micro's thread that holds a cell in equilibrium, complication, offspring, a programmed cell death, and even guides different programs in the form of immune monitoring (Zhang *et al*., 2009; Matoulkova *et al*., 2012; Pu *et al*., 2019) [23, 14, 17]. When their right expressions are outside the slips sequence, the resulting ripple leads to physiological disease, which can tip normal physiology, just like cancer, unheard of heart function, or autoimmune disease (Bennett *et al*., 2018) [5]. In the tumor, the same micro-grain can serve as a break or accelerator: a reference makes it a guardian that suppresses deadly growth, another receives it as an oncogene driver. By starting the central signal circuit, these small RNAs first shape everything from fatal sparks to metastatic lookups and therapy resistance (Barbu *et al*., 2020) [4].

Thus, the current study aims to check the relationship between miR-146a Rs2910164polymorphism and sensitivity to thyroid cancer in the Iraqi population. The findings of this study will provide insight into the potential role that this polymorphism can play as a genetic biomarker for early detection for thyroid cancer, risk stratification and individual therapeutic strategies.

Materials and Methods

Study Design and Population

In the current study, a case control design was used to evaluate the relationship between MIR-146A, RS2910164-Polymorphism and the Iraqi population's tendency to develop cancer in the thyroid gland, with 148 people divided into two groups: 78 cases and 70 controls.

Patients who had histopathologically proven thyroid cancer were included in the case group. These patients were admitted to the expert's oncology clinics located in Najaf, Iraq, at Al-Hakim General Hospital, Al-Sadar Medical City, and Furat Al-Awsat Hospital. On the other hand, the control group consisted of individuals who were in good health and had no personal history of thyroid or cancer-related problems. These people were 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 confused. The participants who were able to give their informed consent and were between 20 and 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) [2].

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 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) [8].

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
Primer (G) allele	2
Primer (G) allele	2
Deoxyribonucleic acid	5
ddH_2O	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:

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).

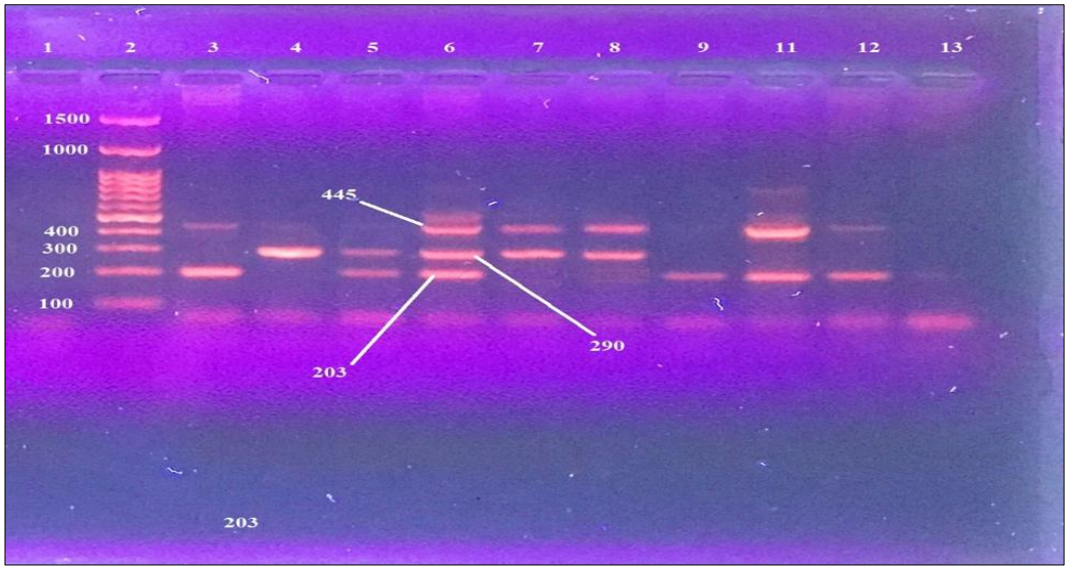

Fig 1: The Tetra-ARMS PCR for the rs2910164 polymorphism in miR-146a are shown. A 100 bp molecular marker (lane 2) was used to verify the sizes of the amplified products. Specific lanes depict the genotypes

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.

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.

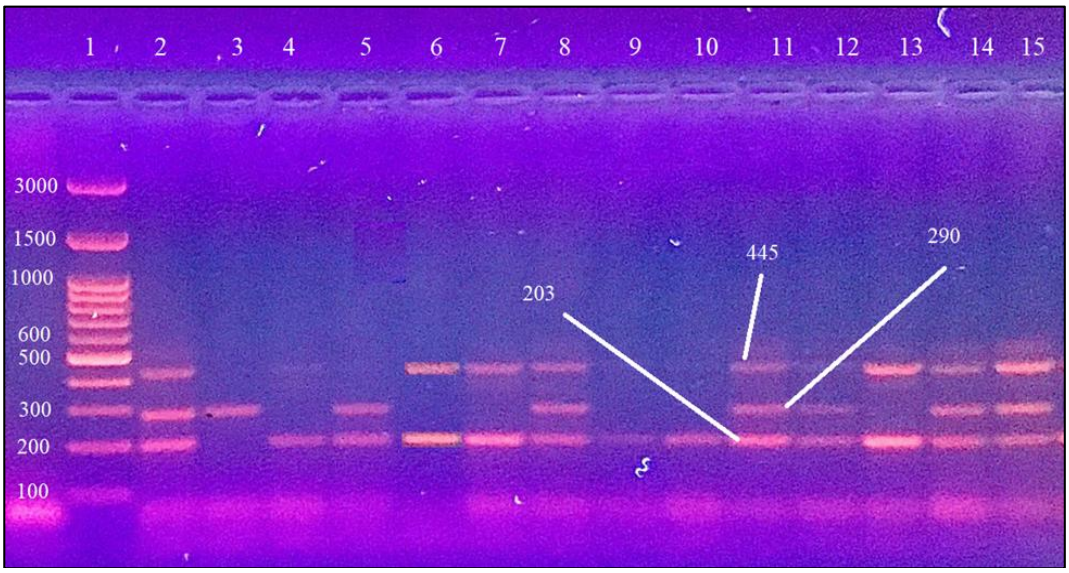

Fig 2: Control Tetra ARMS PCR Products Result of rs2910164 (G>C) in miR-146a Gene on 2% Electrophoresis Gel. Molecular marker (100 bp), GC heterozygote (203 bp, 290 bp, 445 bp), CC homozygote (mutant type) (290 bp, 445 bp) and numbers GG homozygote (wild type) (203 bp, 445 bp).

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) [20, 16]. Similarly, the study in the 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) [13, 19]. 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) [20, 21, 4, 15]. 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) [22, 13].

Interestingly, the GC genotype also demonstrated increased risk of the disease but with a reduced odds ratio compared to that of CC homozygotes. The trend is concordant with earlier genetic studies that demonstrated a dose-dependent effect of the C allele, with each additional copy increasingly enhancing susceptibility (Sun *et al*., 2014). These subtleties reflect the intricate interactions between environmental exposures and genetic susceptibilities to regulate disease susceptibility (Cho *et al*., 2016; Xu *et al*., 2008) [10, 22]. On the other hand, the GG genotype and the G allele in our study did not significantly correlate with thyroid-cancer risk, which is concordant with studies that have found the G variant to be neutral or weakly protective (Wang *et al*., 2015; Omer *et al*., 2024) [16].

The results of the study hold major clinical relevance. The establishment of C allele or CC genotype carriers can assist in the diagnosis of thyroid cancer at an early stage and individualize the treatment. The results also demonstrate the utility of further studies to identify the medical therapeutic interventions aimed at managing miR-146a pathways (Rogucki *et al*., 2022; Bhattacharya *et al*., 2023) [18, 6].

In summary, our study expands on previous findings, which demonstrate the relevance of miR-146a rs2910164 polymorphism as a genetic risk factor for thyroid cancer. The purpose of future research would be to clarify the functional results of this polymorphism in diverse populations and its interaction with environmental factors, which further paves the way for the exact medical approach in the cancer management of the thyroid.

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