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Potential Functions of MicroRNA Biomarkers as a Prognostic Factor in Urothelial Bladder Carcinoma in a Saudi Community

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Abbreviations: BCG: Bacillus Calmette-Guérin; miRNA: microRNA; PCR: Polymerase Chain Reaction; SNP: Single Nucleotide Polymorphism; TaqMan Real-Time PCR; TNM: Tumor, Node, And Metastasis; TURBT: Transurethral Resection of Bladder Tumors; UBa; Urinary Bladder; UBCa: Urothelial Bladder Carcinoma

Abstract

Purpose: Growing knowledge supports the importance of microRNAs in cell growth regulation, differentiation, apoptosis, and tumorigenesis. We addressed the associations between miRNA variants and risk of urothelial bladder carcinoma (UBCa).

Subjects and Methods: Sixty-six cases with UBCa (33 pTa, 13 pT1, 20 pT2) of low and high tumor grade (21 and 45 cases, respectively), and 156 healthy controls were genotyped for miR-196a2 rs11614913(C>T), miR-146a rs2910164(C>G), and miR-499 rs3746444(A>G) using TaqMan genotyping assays. Patients were stratified according to tumor stage, tumor grade, and risk of recurrence for UBCa. We used the SNPStats (https://www.snpstats.net) to choose best interactive model(s) of inheritance in selected miRNAs.

Results: The rs3746444 SNP showed a potential association with risk of UBCa (odds ratio [OR]= 1.9; P= 0.012). Significant associations were found for rs3746444 (P= 0.013) in codominant models, and for rs11614913 (P= 0.048) in an additive model. The rs11614913T allele had a protective effect, while homozygotes for the C allele were associated with a significantly increased risk of UBCa compared with controls ($\chi 2$= 3.7; P= 0.05). Homozygosity of rs3746444A/A was more frequent in cases than controls (43.9% versus 30.8%). Despite its significant impact in diverse ethnic populations, the rs29101164 SNP did not show clear effects on UBCa. Besides, the presence of rs3746444A/A was associated with tumors of high grade (OR= 2.7; P= 0.046).

Conclusion: This study was the first among the Saudi community to present a substantial function of miRNA biomarkers to predict UBCa, identify at-risk patients, and suggest a potential therapeutic target for UBCa.

Keywords: MicroRNAs; Urothelial Carcinoma of Bladder; Biomarkers; Genotyping

Introduction

Bladder cancer (BCa) is one of the most frequent malignancies worldwide. It has been characterized by a high recurrence rate, thus reflecting a substantial public health burden. In Saudi Arabia, almost 83% of all diagnosed BCa is urothelial bladder carcinoma (UBCa), and one-third of UBCa is described as invasive UBCa with a high risk for distant metastases [1]. Approximately 70% to 80% of patients are diagnosed with non-invasive UBCa (pTa-pT1), and the remaining patients have invasive UBCa that often leads to recurrence [2]. The recurrence rate within five years following the transurethral resection of bladder tumors (TURBT) can be estimated as 75% [3]. Thus, the disease may invade the muscle layer very rapidly. UBCa is not easy to cure because of its high recurrence and metastasis rates, with a five-year survival rate of about 57%. [4]. The great impact of microRNA (miRNA) has revolutionized current cell biology and medical science. miRNAs are a group of highly conserved small noncoding RNA molecules with 18-24 nucleotides [5]. They can post-transcriptionally regulate gene expression by directly targeting messenger RNAs (mRNAs). miRNAs can bind to the 3` untranslated regions of mRNAs, resulting in the degradation or translational repression of mRNAs. Previous reports revealed that miRNAs are widely involved in various processes of cell proliferation, differentiation, and apoptosis [6-8]. However, miRNAs can function as either oncogenes or tumor suppressors in different carcinomas, including BCa [6,9-17]. Reports on candidate genes and their impact on the risk of UBCa are still inadequate in Saudi population. Recently, we investigated associations between combinations of genetic variants of glutathione transferases, cytochrome *P450, TP53* and *MTHFR*, and *MTRR* genes with the risk of UBCa among Saudi patients [18]. Here, we have extended our work to examine the effect of single nucleotide polymorphisms (SNPs) in miRNAs— the rs2910164 SNP in miR-146a, the rs11614913 SNP in miR-196a2, and the rs3746444 SNP in miR-499 genes—on the risk of UBCa in Saudi patients.

Subjects and Methods

Ethics Statement and Consent

The Institutional Biomedical Ethics Committee of Medicine College at Umm Al-Qura University approved the study protocols (reference #HAPO-02-K-012), licensed from the National Committee of Medical & Bioethics, KACST, Riyadh (http://bioethics.kacst.edu.sa/About.aspx?lang=en-US). All study participants signed an informed consent form.

Study Population

The study included 66 patients (ages 39-94 years) diagnosed with UBCa who were referred to the Urology Department at King Abdullah City Hospital (Mecca city) between June 2014 and January 2017. Epidemiologic and clinical characteristics regarding sex, age at examination, family history of cancer, cigarette smoking habits, alcohol consumption, pathologic tumor stage, tumor grade, and metastasis were recorded for each patient for statistical analyses (Figure 1). Patients were stratified according to: 1) pathological tumor staging using the tumor, node, and metastasis (TNM) classification system (pTa, pT1, pT2), 2) tumor grades according to the 2014 World Health Organization grading system,[19] and 3) recurrence risk according to the European Organization for Research and Treatment of Cancer risk criteria [20]. In the TNM system, 'pTa' is defined as a noninvasive papillary urothelial neoplasm of low malignancy, and the small section of tissue can be easily removed with TURBT. In contrast, 'pT1' describes a tumor that invades the subepithelial connective muscle but does not involve the bladder, and 'pT2' describes a tumor that has spread to the muscle of the bladder wall.

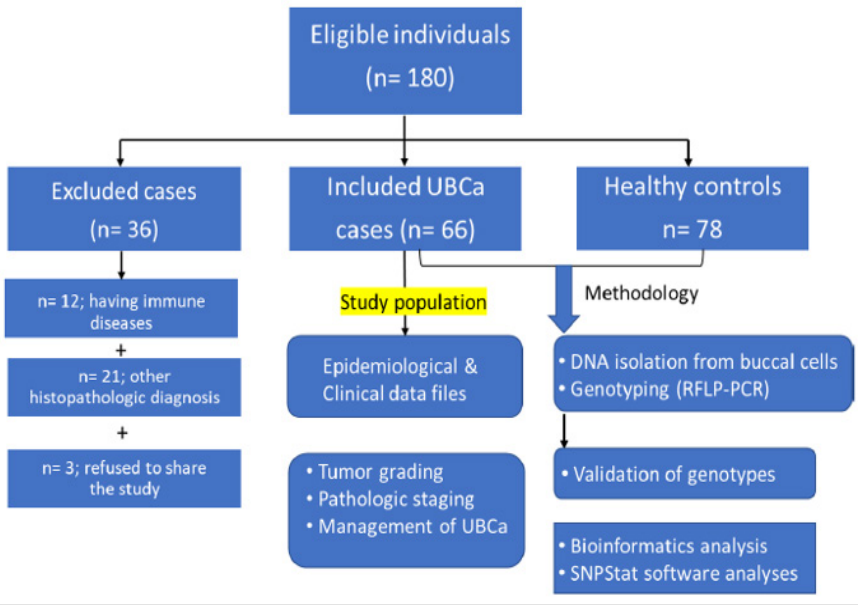

Figure 1: Flow chart of the eligible sample individuals, and applied methodology in the study population.

The management of the disease—through intravesical bacillus Calmette-Guérin (BCG), immunotherapy, a conservative TURBT–BCG therapy (injection), chemotherapy, chemoradiotherapy, or radical cystectomy—was also recorded. After TURBT was performed, the high-grade tumor cases were treated with BCG. Patients who previously had cancer, radiotherapy, and chemotherapy, or metastasized cancer from non-Saudi or unknown origins were excluded. Patients (n = 36) with immune disease or any histopathologic diagnosis other than UBCa were also excluded. Healthy individuals (n = 78) having no evidence of any clinical phenotypes of malignancies were selected as controls (ages 44-89 years) in routine follow-up at governmental hospitals in Makkah (Figure 1).

TaqMan Genotyping Analysis

Genomic DNA was extracted from peripheral blood (200 µL) using the QIAamp DNA blood kit as recommended by the manufacturer (Qiagen, Hilden, GmbH, Germany). In some cases, DNA samples were taken from buccal mucosa using the Oragene. DNA-OGR-575 kit (DNA Genotek Inc., Ottawa, ON, Canada) with some modifications [21]. We adopted TaqMan real-Time PCR analysis (Thermo, Applied Biosystems, USA) to genotype individuals for the selected SNPs of the miRNA 164a (rs2910164; C__15946974_10), miRNA 196a2 (rs11614913; C__31185852_10), and miRNA 499a (rs3746444; C___2142612_40) using a 7500 Fast-Dx Real-Time PCR System (Thermo, Applied Biosystems, Life Technologies Inc., USA). To ensure the accuracy of genotyping, all DNA samples, as well as negative controls, were included in the assays. We repeatedly genotyped 10% of the samples, and the results were 100% concordant. Some genotypes were validated by genotyping using a Genetic Analyzer 3500 (ABI, Life Technologies, Jeddah, Saudi Arabia).

Statistical Analysis

SNPs from all participants were tested for Hardy-Weinberg equilibrium (HWE) using the χ^2 test, and a *P* value > 0.05 was considered consistent with HWE. We conducted the statistical analysis considering the interactive models of inheritance—codominant, dominant, recessive, over dominant, and additive—using the SNPStats software (https://www.snpstats.net). Logistic regressions for genotypic distributions and allelic frequencies for UBCa cases and controls were measured in terms of odds ratios (ORs) and 95% confidence intervals (CIs). The less *Akaike* information criterion (*AIC*) value that corresponded to the minimally expected entropy was adopted to assess the best model of inheritance. We used the MedCalc statistical software version 16.4.3 (MedCalc Software bvba, Ostend, Belgium; https://www.medcalc.org) to perform *t*-tests and *chi*-square tests for epidemiologic and clinical characteristics. A two-tailed P < 0.05 was considered statistically significant.

Results

Characteristics of the Study Population

Sixty-six eligible Saudi individuals with UBCa (60 men, 6 women; 10:1 ratio) and 78 healthy controls (70 men, 8 women; 8.75:1 ratio) were enrolled. The mean age of patients was 62.6 ± 10.84 years, with no significant difference when compared with controls (t = 0.4, P = 0.7). There was no significant difference between cases and controls in terms of the percentage of current cigarette smokers (72.7% versus 71.8%, respectively; χ^2 = 0.014, P = 0.9). A significant difference between the proportion of cases with a high tumor grade (45 cases, 68.2%) and the proportion with a low tumor grade (21 cases, 31.8%) was found (χ^2 = 17.4, 95% CI 19.5-50.5; P < 0.0001). The percentages of UBCa cases with specific tumor stages were 50.0% for pTa, 19.7% for pT1, and 30.3% for pT2 (χ^2 = 13.9; P = 0.0002) (Table 1). Among all cases, 18% (12 cases) with pTa, 20% (13 cases) with pT1, and 30% (20 cases) with pT2 tumors had high-grade tumors. No cases with pT1 or pT2 tumors had low-grade tumors, and 21 (32%) of cases with milder pTa tumors had low-grade tumors (Figure 2). However, there was a highly significant difference between pathologic tumor stages with response to tumor grades among our cases with UBCa (χ^2 = 30.8, P ≤ 0.001). The predominant management course for our cases with UBCa was BCG immunotherapy (76.9%; z = 23.8, P < 0.0001). The proportion of cases managed with TURBT-BCG injection (conservative therapy) was higher than the proportions managed with other strategies (74.2% versus 13.6% for radical cystectomy, 7.6% for chemo-radiotherapy, and 4.5% for chemotherapy) (Table 1).

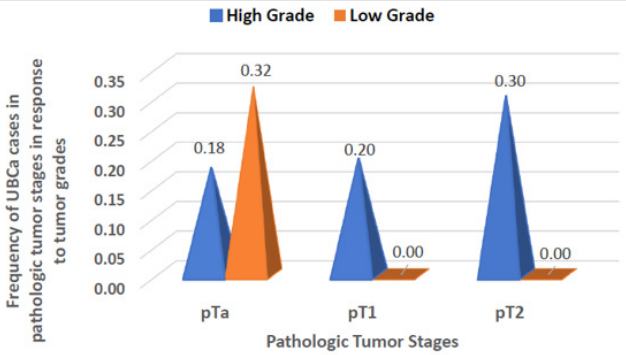

Figure 2: Pathological stages and tumor grades in cases with urothelial bladder carcinoma (UBCa). The number of cases with tumors of "high grade" increased from pTa to pT2. There were no cases with tumors of "low grade" linked to pTa or pT2.

Table 1: Epidemiologic and clinical characteristics in UBCa cases.

Parameter	UBCa cases, no. (%) n = 66	z (95% CI)	P-value
Age at examination (range, years) Mean age ± SD (years)	39–94 62.6 ± 10.84 [a]	43.2 (59.9-65.3)	<0.0001
Gender, male	60 (90.9)	32.0 (81.2-96.6)	<0.0001
Cigarette smoking (current)	48 (72.7)	25.2 (60.3-82.9)	<0.0001
Alcohol consumption (yes)	0 (0.0)	–	–
Tumor grade: Low-grade High-grade	21 (31.8) 45 (68.2)	17.4 [b]	<0.0001
Pathological stage: pTa pT1 pT2	33 (50.0) 13 (19.7) 20 (30.3)	13.9 [b]	0.0002
Metastasis (yes):	0 (0.0)		
Management: BCG (yes) BCG (no) Conservative therapy Radical cystectomy Chemo-radiotherapy Chemotherapy	38 (57.6) 28 (42.4) 49 (74.2) 9 (13.6) 5 (7.6) 3 (4.5)	19.6 (44.8-69.7) 25.8 (62.0-84.2) 3.2 (6.4-24.3) 1.0 (2.5-16.8) 0.2 (0.9-12.7)	<0.0001 <0.0001 0.0013 0.33 0.85
Number of Recurrences: Rec (1) Rec (2) Rec (3) Non-recurrence	3 (4.5) 11 (16.7) 6 (9.1) 46 (69.7)	0.2 (0.9-12.7) 4.4 (8.7-27.9) 1.5 (3.4-18.8) 24.1 (57.2-80.4)	**0.85** **<0.0001** **0.126** **<0.0001**

Note: BCG: bacillus Calmette-Guérin; a weakened bacterium intravesically introduced via a catheter, Conservative therapy: TURBT plus BCG, CI: confidence interval, SD: standard deviation, UBCa: urothelial bladder carcinoma, pTa, no invasive papillary carcinoma and the small section of tissue can be easily removed with TURBT, pT1: tumor invades the subepithelial connective muscle but does not involve the bladder, pT2: tumor has spread to the muscle of the bladder wall. Bold numbers indicate statistically significant associations ($P < 0.05$). Rec (1, 2, 3) denotes the number of recurrences of UBCa.

[a]Student's t-test. Values are mean ± SD

[b]Chi-square value.

HWE of SNPs in miRNA Regions

Three SNPs in the miRNAs' regions—rs2910164 G>C, rs11614913 C>T, and rs3746444 A>G—were successfully genotyped in 66 patients with UBCa and 78 control subjects. All cases and controls were in HWE at the examined SNPs in the miRNAs ($P > 0.05$) (Table 2).

Table 2: Genotype distribution and allele frequencies of selected SNPs in microRNA regions and their associations with UBCa risk among cases and control.

SNP	Geno-type	UBCa, no. (%) *n* = 66	Control, no. (%) *n* = 78	χ^2 (*P* value)	Model	Comparison	Logistic regression		
							OR (95% CI)	*P*-value	*AIC*
***miR-146a* G>C (+) (rs2910164):**									
Genotype:						G/G vs. C/C			
					Codominant-1	G/G vs. C/G	0.9 (0.2-5.2)	0.94	115.4
					Codominant-2	G/G vs. C/G-C/C	1.2 (0.4-3.0)	0.87	
	G/G	27 (40.9)	30 (38.5)	0.1 (0.77)	Dominant	G/G-C/G vs. C/C	1.1 (0.4-2.8)	0.83	113.5
	C/G	33 (50.0)	42 (53.9)	0.2 (0.64)	Recessive	G/G-C/C vs. C/G	0.8 (0.2-4.4)	0.83	113.5
	C/C	6 (9.1)	6 (7.7)	0.1 (0.76)	Overdominant		1.2 (0.6-2.9)	0.74	113.4
					log-additive		1.0 (0.5-2.12)	0.94	113.5

HWE, χ^2 (*P* value):		0.43	0.66						
Allele:	G	87 (0.66)	102 (0.65)				1		
	C	45 (0.34)	54 (0.35)				1.0 (0.6-1.7)	0.93	
***miR-196a2* C>T (+) (rs11614913):**									
Genotype:									
						C/C vs. T/T			
					Codominant-1	C/C vs. C/T	3.4 (0.3-34.3)	0.30	113.4
					Codominant-2	C/C vs. C/T-T/T	1.9 (0.9-3.6)	0.07	
	C/C	41 (62.1)	36 (46.1)	3.7 (0.05)	Dominant	C/C-C/T vs. T/T	1.9 (0.8-4.8)	0.057	111.6
	C/T	24 (36.4)	39 (50.0)	2.7 (0.10)	Recessive	C/C-T/T vs. C/T	2.6 (0.2-43.2)	0.41	113.1
	T/T	1 (1.5)	3 (3.8)	0.7 (0.34)	Overdominant		1.8 (0.7-4.38)	0.10	112.1
					log-additive		1.9 (0.8-4.3)	0.048	111.4

HWE, χ^2 (*P* value):		0.43	0.63						
Allele:	C	106 (0.80)	111 (0.71)				1		
	T	26 (0.20)	45 (0.29)				1.7 (1.0-2.9)	0.074	
***miR-499* A>G (+) (rs3746444):**									
Genotype:									
						A/A vs. G/G			
					Codominant-1	A/A vs. A/G	3.6 (1.3-9.97)	0.013	109.0
					Codominant-2	A/A vs. A/G-G/G	1.3 (0.6-2.8)	0.044	109.6
	A/A	29 (43.9)	24 (30.8)	2.6 (0.106)	Dominant	A/A-A/G vs. G/G	1.8 (0.9-3.5)	0.10	112.1
	A/G	30 (45.5)	33 (42.3)	0.1 (0.701)	Recessive	A/A-G/G vs. A/G	3.1 (1.2-7.9)	0.017	109.8
	G/G	7 (10.6)	21 (26.9)	6.0 (0.010)	Overdominant		0.9 (0.5-1.7)	0.70	113.4
					log-additive		2.5 (0.99-4.8)	0.015	109.2

HWE, χ^2 (*P* value):		1	0.45						
Allele:	A	88 (0.67)	81 (0.52)				1		
	G	44 (0.33)	75 (0.48)				1.9 (1.1-3.0)	0.012	

Note: UBCa: urothelial bladder carcinoma, SNP: single nucleotide polymorphism, HWE: Hardy-Weinberg Equilibrium, OR: odds ratio, CI: confidence interval. *AIC* values refer to the model with the less AIC value that corresponds to the minimal expected entropy. Bold numbers indicate statistically significant associations (P < 0.05). Underlined numbers represent the best model of inheritance with the less *AIC* value.

Allele Frequencies and Interactive Genotypic Models

Regarding allele frequencies, the ORs of the allelic variants were 1.0 (95% CI, 0.6-1.7) for rs2910164, 1.7 (95% CI, 1.0-2.9) for rs11614913, and 1.9 (95% CI, 1.1-3.0) for rs3746444 (Table 2). However, the rs11614913C and rs3746444A alleles were more frequent in cases than in controls (0.80 versus 0.71; P = 0.074, and 0.67 versus 0.52; P = 0.012, respectively). In contrast, neither G allele of the rs2910164 SNP in cases or controls showed a clear effect on UBCa (0.66 versus 0.65, P = 0.93). In Table 2, the best interactive model of inheritance, described with the less AIC value, corresponds to the minimal expected entropy. Thus, evidence suggested that the best model was over dominant for rs2910164 (OR = 1.2; 95% CI 0.6-2.9; P = 0.74) and codominant-1 for rs11614913 and rs3746444 (OR = 1.9; 95% CI 0.8-4.3; P = 0.048, and OR = 3.6; 95% CI 1.3-9.97; P = 0.013, respectively). With slightly larger AIC values, the examined rs3746444 SNP showed that the codominant-2 (OR = 1.3; 95% CI 0.6-2.8; P = 0.044), recessive (OR = 3.1; 95% CI 3.1-7.9; P = 0.017), and log-additive (OR = 2.5; 95% CI 0.99-4.8; P = 0.015) models of inheritance were significantly associated with risk of UBCa. Consequently, examination of the rs3746444 SNP suggested an apparent role for homozygosity (codominant-1 model) in increasing risk for cases with UBCa compared to controls (43.9% versus 30.8%). As for rs11614913 C>T, the additive model reflected that each copy of T modified the protective effect in cases (additive model, 2T/T+C/T to C/C).

Genotyping associations of miRNAs and tumor grades

The interactive genotypic models of the selected SNPs in miRNAs with the response of tumor grades after adjusting for age and gender are shown in Table 3. There was a significant difference between the tumor grades of cases with UBCa in rs3746444 A>G in an overdominant model (OR = 2.7; 95% CI 0.9-7.8; P = 0.046), but not in rs2910164 (OR = 2.3; 95% CI 0.7-7.1; P = 0.11) or rs11614913 (OR = 1.4; 95% CI 0.0-3.8; P = 0.13). The proportions of the homozygotes in C/C in rs11614913 and A/A in rs3746444 were much higher in cases with high-grade tumors than those with low-grade tumors (62.1% versus 46.1%, and 43.9% versus 30.8%, respectively).

Table 3: Genotypic associations between selected SNPs in miRNAs and tumor grades among cases with UBCa.

SNP	Genotype	High grade, no. (%)	Low grade, no. (%)	Model	Comparison	Logistic regression		
						OR (95% CI)	*P*-value	*AIC*
		45 (68.2)	21 (31.8)			17.4 (19.5-50.5)	0.0001	
***miR-146a* G>C (rs2910164):**								
Genotype:	G/G	21 (46.7)	6 (28.6)	Codominant-1	G/G vs. C/C	2.3 (0.7-7.2)	0.36	86.5
	C/G	20 (44.4)	13 (61.9)	Codominant-2	G/G vs. C/G	2.3 (0.7-7.1)	0.11	84.5
	C/C	4 (8.9)	2 (9.5)	Dominant	G/G vs. C/G-C/C	2.2 (0.7-6.7)	0.16	84.6
				Recessive	G/G-C/G vs. C/C	1.1 (0.2-6.4)	0.93	86.6
				Overdominant	G/G-C/C vs. C/G	2.0 (0.7-5.9)	0.18	84.8
				log-additive	---	1.6 (0.7-3.6)	0.26	85.3
Allele	G	62 (0.69)	25 (0.6)			1		
	C	28 (0.31)	17 (0.4)			1.5 (0.7-3.2)	0.29	
***miR-196a2* C>T (rs11614913):**								
Genotype:	C/C	29 (64.4)	12 (57.1)	Codominant-1	C/C vs. T/T	1.21 (0.41-3.57)	0.3	86.1
	C/T	16 (35.6)	8 (38.1)	Codominant-2	C/C vs. C/T	1.2 (0.4-3.6)	0.73	86
	T/T	0 (0.0)	1 (4.8)	Dominant	C/C vs. C/T-T/T	1.4 (0.5-3.9)	0.57	86.2
				Recessive	C/C-C/T vs. T/T	1.36 (0.0-3.8)	0.13	84.2
				Overdominant	C/C-T/T vs. C/T	1.1 (0.4-3.3)	0.84	86.5
				log-additive	---	1.55 (0.6-4.2)	0.38	85.8

Allele:	C	74 (0.82)	32 (0.76)			1		
	T	16 (0.18)	10 (0.24)			1.4 (0.6-3.5)	0.42	
***miR-499* A>G (rs3746444):**								
Genotype:	A/A	22 (48.9)	7 (33.3)	Codominant-1	A/A vs. G/G	2.40 (0.8-7.33)	0.16	84.9
	A/G	17 (37.8)	13 (61.9)	Codominant-2	A/A vs. A/G	0.52 (0.1-5.1)		
	G/G	6 (13.3)	1 (4.8)	Dominant	A/A vs. A/G-G/G	1.9 (0.7-5.6)	0.23	85.1
				Recessive	A/A-A/G vs. G/G	0.3 (0.04-2.9)	0.26	85.3
				Overdominant	A/A-G/G vs. A/G	2.7 (0.9-7.8)	0.046	83.2
				log-additive	---	1.2 (0.5-2.6)	0.69	86.4
Allele:	A	61 (0.68)	27 (0.64)			1		
	G	29 (0.32)	15 (0.36)			1.2 (0.5-2.5)	0.7	

Note: UBCa: urothelial bladder carcinoma, SNP: single nucleotide polymorphism, OR: odds ratio, CI: confidence interval. AIC values refer to the model with the less *AIC* value that corresponds to the minimal expected entropy. Bold numbers indicate statistically significant associations (P < 0.05). Underlined numbers represent the best model of inheritance with the less *AIC* value.

Discussion

This hospital-based case-control study presents the first evidence that common SNPs in miRNAs regions may be used as candidate genetic markers for UBCa vulnerability in a Saudi population. Our results show that the miR-499 rs3746444 SNP, but not the miR-164a rs2910164 or miR-196a2 rs11614913 SNP, is associated with risk for UBCa. Meta-analyses have been inconclusive regarding the association between the rs2910164, rs11614913, and rs3746444 SNPs in the miRNA genes and cancer risk [22-24]. Several reports have provided no evidence of an association between rs2910164 and overall cancer risk in diverse populations [22,23,25]. In our Saudi cases, the lack of association between the rs2910164 SNP and UBCa is consistent with previous results in Caucasian and Asian populations [26-28]. In addition, for the rs2910164C allele, reduced risks have been found for various types of cancer and in variable ethnic populations: cervical cancer in Chinese populations;[29] prostate in Iranian;[30] liver in Chinese, [31-34] Egyptian, [35] and Turkish [36]; colorectal cancer in Czech [37] and South Korean [38]; and gastric in Japanese [39] and Caucasian [40].

In contrast, the rs2910164C variant was found to be associated with increased risks of developing lung cancer, [41] and of developing head/neck cancer among Caucasian populations [42]. These discrepancies cannot be explained by variable ethnicity, but instead are likely due to organ-specific effects as well as different living environments, diets, climates, and lifestyles. Some previous results have suggested that rs11614913 and rs3746444 SNPs confer susceptibility to UBCa risk among different ethnic populations. However, consistent with our study, Mittal et al. [43] reported no association between the rs11614913 SNP and BCa. For rs11614913, some previous studies reported a significantly increased risk of certain cancers when T/T for C/C or combined C/C-C/T was present: breast cancer, [44] gliomas, [45] prostate cancer, [46] lung cancer, [47] colorectal cancer, [48] and liver cancer in patients infected with hepatitis B virus [49]. In agreement with our study, a meta-analysis found significant associations between allele frequency or different genotypic models within miR-499 rs3746444 SNP and risk of BCa in studies among Chinese populations [23].

In contrast, several reports could not find any associations between the rs3746444 SNP and BCa, [50] but did find associations with other types of cancer, including colorectal, liver, gallbladder, and breast cancer [38,51-53]. Based on the Human Gene Mutation Database, [54] human related-disease genes can be categorized into cancer disease genes and Mendelian disease genes. Several miRNAs have been found to regulate cancer migration, invasion, metastasis, and growth, and could be the targets for cancer therapy in about 30% of cancer genes [15,24,55-58]. Unfortunately, few articles have dealt with the regulation of BCa cells by these oncogene-targeted miRNAs, including miR-940/INPP4A, miR-940/GSK3b, miR-146a-3p/PTTG1, and miR-145/PAK1 [59-61]. However, Xiang et al.60 found that overexpression of miR-146a-3p could inhibit BCa progression by targeting PTTG1 (OMIM 604147)—an oncogene that is expressed in many tumors and is correlated with tumor size, TNM stage, lymphatic invasion, and distant metastasis of BCa. Besides, small interfering RNA and miRNA can serve to increase the sensitivity of BCa cells to chemotherapeutic drugs [62-64]. For instance, miR-164a, miR-196a2, and miR-499 have been shown to hinder chemotherapeutic resistance in BCa [65,66].

Limitations

Meta-analysis has revealed that ethnicity could affect the association of miRNA polymorphisms with cancer risk Qiu et al. [67] reported that the significance of the sample size (P = 0.02), but not cancer type (P = 0.89) or source of controls (P = 0.97), contributed to the source of heterogeneity. Pinning down the miRNA polymorphisms for UBCa has been difficult because of poor replication of studies. Firstly, some studies have had different admixed populations, different sources of controls (hospital-based or population-based), or very small sample sizes, which would lower the strength of the overall results. Secondly, various studies included different SNPs of miRNAs that were not consistent with HWE in either controls or cases, which may have given rise to biased results of positive or negative associations. Thirdly, in our study, inclusion of more subjects in a realistic time frame from a specific hospital or clinical center would have been challenging, but replication of our results through larger, multicentric investigations will be of interest. Also, the literature includes other pathological types of cancer (e.g., squamous carcinoma or adenocarcinoma) in addition to UBCa, which might have influenced the outcomes of associations of the genetic markers with BCa. However, the present study focused only on UBCa, which consequently strengthens the reliability of our outcomes of associations [68,69].

Conclusion

Our results present the first evidence that miRNAs have great potential for clinical use as diagnostic biomarkers for UBCa susceptibility in a Saudi population. Our data show that the miR-499 rs3746444 A>G SNP contributes to an increased susceptibility to UBCa, as compared with the miR-164a rs2910164 G>C or the miR-196a2 rs11614913 C>T SNPs. Furthermore, increased expression of miR-499 rs3746444 is significantly associated with a stage of UBCa with high-grade tumors. Our results regarding the rs3746444 SNP support roles for homozygosity and heterozygosity in affecting risk for UBCa, as one copy of the allelic variant rs3746444G could be necessary to produce a considerable damaging effect. In contrast, each copy of the T allele in rs11614913 can double the protective effect on individuals. Even though we found no association between the variant genotypes of miR-146a and UBCa risk in our Saudi population, miR-146a could exert a prominent role in carcinogenesis via marginal significance of heterozygosity in the debate of Asian and Caucasian ethnic studies. The inconclusive studies of cancer risk and the susceptibility conferred by the examined miRNAs may be due not only to admixture ethnicities and different living environments, diets, climates or lifestyles but also to organ-specific effects. Based on the results of previous clinical trials, some miRNAs under investigation can be used as biomarkers for risk prevention and therapeutic intervention. Ongoing analyses of UBCa patient DNA using whole-exome sequencing are being performed to discover more cancer genes that are targeted predominantly by several miRNAs. Thus, the miRNA targets on cancer genes analyzed will help enhance the knowledge UBa cancer genes and therapeutic improvements in treating cancer.

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Ethics Approval and Consent to Participate

Published work complies with the guidelines for human studies and the research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. Subjects have given their written informed consents and the study protocol on human research was approved by the Institutional Biomedical Ethics Committee of Umm Al-Qura University based on the National Committee of Biomedical Ethics at King Abdulaziz City for Sciences and Technology (KACST) (http://bioethics.kacst.edu.sa/About.aspx?lang=en-US).

Conflict of Interest

The authors have no economic or any conflicts of interest exists.

Consent for Publication

Written informed consent was obtained from all study participants to publish the results.

Data Availability

The data sets analyzed during the current study are available from the corresponding author.

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Authors' Contributions

NAE, and AMN designed the research; AMN, ASB, HAS, SNE, and HS made the clinical investigations and managements; NAE, AHM, MTT, and IAS, SNE, and ENE performed the practical work; NAE, IAS, AHM, and ENE work for in-silico predictions and statistical analyses; All authors shared in writing the draft, and reviewing and approving the final manuscript.

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