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Association study of hsa-mir-603 rs11014002 polymorphism and risk of breast cancer in a sample of Iranian population

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Abstract

Accumulated evidence have proposed that single nucleotide polymorphisms (SNPs) in microRNAs (miRNAs) are connected to breast cancer (BC) risk. We have done a case-control study with 258 BC patients and 209 control women to examine the potential association of Hsa-mir-603 rs11014002 C>T polymorphisms with BC susceptibility. The polymorphisms were genotyped by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) method. Our findings showed that the rs11014002 C>T variant was not associated with an increased risk of BC in codominant (OR=0.67, 95%CI=0.42-1.08, P=0.121, CT vs CC; and OR=0.18, 95%CI=0.02-1.67, P=0.170, TT vs CC), dominant (OR=0.64, 95%CI=0.41-1.01, P=0.062, CT+TT vs CC), and recessive (OR=0.20, 95%CI=0.02-1.81, P=0.178, TT vs CC+CT) inheritance models tested. While, the T allele significantly decreased the risk of BC (OR= 0.63; 95% CI =0.41-0.95; P=0.032) compared to C allele. In conclusion, the findings indicated that Mir603 rs11014002 T allele might contribute to decrease the risk of BC in a sample of Iranian population. Further studies with larger sample sizes and different ethnicities are warranted to confirm our findings.

Key words: Hsa-mir-603, Cancer, PCR-RFLP, Breast, polymorphism.

Introduction

Breast cancer (BC) is one of the most prevalent cancers in women worldwide which affecting more than 1 million women annually (1-4). It comprises 21.4% of all malignancies among Iranian females. Though the etiology of BC is relatively unknown, it has been proposed that genetic factors play important roles in the pathogenesis and progression of BC (5-8).

miRNAs genes are transcribed by RNA polymerase II. The primary miRNA transcripts (pri-miRNAs) contain cap structures as well as poly(A) tails. It is cleaved by the Drosha ribonuclease III enzyme to produce an approximately 70-nt stem-loop precursor miRNA (pre-miRNA), which is further cleaved by the cytoplasmic Dicer ribonuclease to create the mature miRNA (9). Single nucleotide polymorphisms (SNPs) are abundant form of genetic variations. SNPs in miRNAs may effect miRNAs biogenesis, processing, target sequence binding, or the expression level of mature miRNAs (10). miRNAs are a class of short (18–24 nucleotides) noncoding RNA molecules that modulate gene expression at post-transcriptional level by targeting mRNAs for direct cleavage or translation repression (11, 12). Such regulatory mechanisms enable miRNAs to play key roles in a variety of physiological and pathological processes, such as development, differentiation, cell proliferation, apoptosis, as well as stress responses (13, 14). It has been proposed that genetic polymorphisms in miRNAs implicated in the initiation and progression of various cancers (10, 15-21).

The rs11014002 C>T variant in precursor sequence of hsa-miR-603 (pre-miR-603) may affect the biogenesis of mature miR-603 and potentially could be involved in cancer development. It has been shown that miR-603 binds to the O^6-methylguanine methyl transferase (MGMT) 3`UTR and results in a loss of MGMT protein expression both *in vitro* and *in vivo* (22). It as been proposed that insulin-like growth factor 1 (IGF1) is a target for miR-603 it has shown that insulin-like growth factor 1 (IGF1) is a target for miR-603 (23).

Currently, only one study available concerning the association between hsa-mir-603 polymorphism and colorectal cancer (CRC) risk (18). To the best of our knowledge, there is no report regarding the impact of hsa-mir-603 polymorphism on BC susceptibility. Accordingly, in this study, we aimed to evaluate the possible association between hsa-mir-603 rs11014002 polymorphism and BC risk in a sample of Iranian women.

Materials and Methods

Patients

This population based case-control study was done on 258 BC patients referred to university-affiliated hospital (Ali Ebneh Abitaleb hospital, Zahedan, Iran) from February 2009 until November 21014 and 209 ages matched healthy women with no history of cancer of any type. The enrollment process and study design have been described in our previous investigations (5,

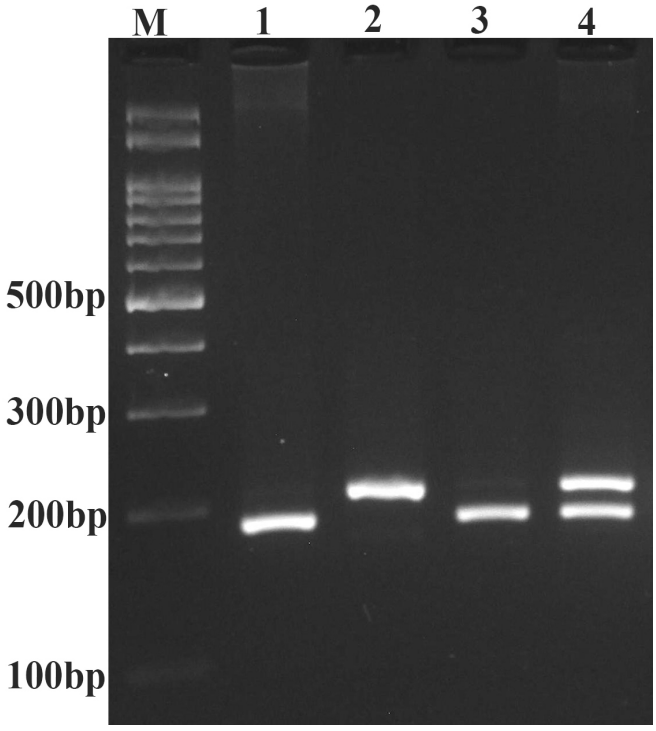

Figure 1. Photograph of the hsa-mir-603 rs11014002 C>T polymorphism using PCR-RFLP. The C allele was digested by HhaI restriction enzyme and produced 25-bp and 187-bp fragments, while the T allele was undigested (212-bp). M: DNA marker; Lanes 1 and 3: CC; Lane 2: TT; Lane 4: CT.

8, 24). Ethical approvals for recruitment were taken from local Ethics Committee of Zahedan University of Medical Sciences, and informed consent was obtained from all patients and healthy individuals. Blood samples were collected in EDTA-containing tubes from case and control subjects, and DNA were extracted using salting out method (25).

Genotyping

We designed PCR-RFLP method for genotyping of hsa-mir-603 rs11014002 polymorphism. The forward and reverse primers were 5′- GGTTTGGTGCAAAAG-TAATTGCAGcG -3′ and 5′- AATATCAGGACCA-GAAGGGAGAACTG-3′, respectively. In each 0.20 ml PCR reaction tube, 1 µl of genomic DNA (~100 ng/ml), 1 µl of each primer (10 µM) and 10 µl of 2X Prime Taq Premix (Genet Bio, Korea) and 7 µl ddH2O were added. The PCR conditions were justified as follows: 5 min preheating at 95°C, 30 cycles of 95°C for 30s, 63°C for 30s, and 72 °C for 30 s followed by a final extension step for 10 min at 72 °C (T100 ™ Thermal Cycler, Bio-Rad laboratories, Inc.). The PCR product (10 µl) was digested using HhaI restriction enzyme (Fermentas, Lithuania). The C allele was digested and produced 25-bp and 187-bp fragments, while the T allele was undigested (212-bp) (Figure 1). To verify genotyping quality, we randomly re-genotyped 20% of the samples and found no genotyping mistakes.

Statistical analysis

Statistical analysis was calculated using statistical package SPSS 20 software. Independent sample t-test and the X2 test were used for continuous and categorical data, respectively. The association between genotypes and BC were assessed by computing the odds ratio (OR) and 95% confidence intervals (95% CI) from logistic regression analyses. The statistical level of significance was defined as $p<0.05$. We estimated the Hardy Weinberg equilibrium (HWE) separately for cases and controls.

Results

The study group consisted of 258 BC patients with an average age of 49.1 ±11.0 years and 209 healthy women with a mean age of 50.1±13.1 years. No significant difference was found between the groups regarding age (p=0.390).

The genotype and allelic frequency of hsa-mir-603 rs11014002 C>T polymorphism are shown in table 1. Our finding showed that the rs11014002 C>T variant was not associated with an increased risk of BC in codominant (OR=0.67, 95%CI=0.42-1.08, P=0.121, CT vs CC; and OR=0.18, 95%CI=0.02-1.67, P=0.170, TT vs CC), dominant (OR=0.64, 95%CI=0.41-1.01, P=0.062, CT+TT vs CC), and recessive (OR=0.20, 95%CI=0.02-1.81, P=0.178, TT vs CC+CT) inheritance models tested. While, the T allele significantly decreased the risk of BC (OR= 0.63; 95% CI =0.41-0.95; P=0.032) compared with C allele.

The genotype of hsa-mir-603 rs11014002 polymorphism in controls and cases were in HWE ($\chi2=0.099$, $P=0.752$ and $\chi2=0.489$, $P=0.484$, respectively).

As shown in table 2, no significant association

Table 1. Association of hsa-mir-603 rs11014002 polymorphism and the risk of breast cancer.

hsa-mir-603 rs11014002 C>T	Case n (%)	Control n (%)	OR (95%CI)	p-value
Codominant				
CC	215 (83.3)	159 (76.1)	1.00	-
CT	42 (16.3)	46 (22.0)	0.67 (0.42-1.08)	0.121
TT	1 (0.4)	4 (1.9)	0.18 (0.02-1.67)	0.170
Dominant				
CC	215 (83.3)	159 (76.1)	1.00	-
CT+TT	43 (16.7)	50 (23.9)	0.64 (0.41-1.01)	0.062
Recessive				
CC+CT	257 (99.6)	205 (98.1)	1.00	-
TT	1 (0.4)	4 (1.9)	0.20 (0.02-1.80)	0.178
Allele				
C	472 (91.5)	364 (87.1)	1.00	-
T	44 (8.5)	54 (12.9)	0.63 (0.41-0.95)	0.032

Table 2. Correlation between hsa-mir-603 rs11014002 C>T genotypes and clinical characteristics of breast cancer (BC) patients.

Variables	hsa-mir-603 rs11014002 genotypes			p-value
	CC	CT	TT	
Age				0.175
≤50	123	19	0	
>50	80	22	1	
Tumor Size (cm)				0.392
≤ 2	70	9	0	
>2	130	27	1	
Node metastasis status				0.128
Yes	125	19	0	
No	55	4	1	
Grade				0.432
I	36	4	0	
II	111	13	0	
III+IV	38	5	1	
Stage				0.805
I	35	4	0	
II	74	16	1	
III	59	13	0	
IV	35	7	0	
Histology				0.890
Ductal carcinoma	141	27	0	
Other	59	11	0	
Estrogen Receptor				0.357
Positive	125	26	0	
Negative	68	12	1	
Progesterone Receptor				0.202
Positive	122	20	0	
Negative	70	18	1	
HER2				0.488
Positive	105	18	0	
Negative	98	21	1	

between hsa-mir-603 rs11014002 genotypes and clinical characteristics of BC patients were found (P>0.05).

Discussion

In the present study, for the first time, we investigated the impact of hsa-mir-603 rs11014002 polymorphism on BC risk in a sample of Iranian population. Our data revealed that rs11014002 genotypes were not associated with BC in any inheritance model tested. While, the carriers of rs11014002 T allele were associated with decreased risk of developing BC compared with carriers of C allele. Our findings suggest no significant association between hsa-mir-603 rs11014002 variant and clinical characteristics of BC. Rs11014002 is located within the precursor sequence of hsa-mir-603. It has been proposed that this variant may be a functional SNP and may have the potential to influence an individual's susceptibility to colorectal cancer (CRC) (18).

To the best of our knowledge, there is only one report concerning the impact of hsa-mir-603 polymorphism on cancer risk. In a case control study, Wang et al (18) investigated the possible association between hsa-mir-603 rs11014002 polymorphism and risk of colorectal cancer (CRC). They found that subjects carrying rs11014002 CT/TT genotype had an increased susceptibility for CRC (CT vs. CC: OR=2.352, 95% CI: 1.142–4.840, P=0.020; CT+TT vs. CC: OR=2.031, 95% CI: 1.063-3.883, P=0.032). Their findings propose that genetic polymorphism in hsa-mir-603 is associated with CRC susceptibility.

Several studies reported that miRNAs expression patterns such as miR-21, miR-200, miR-21, miR-31, miR-127, and miR-27a were altered in breast tumors and might have great potential to serve as novel, non-invasive biomarkers for early detection of breast cancer (26-35).

Many epidemiological studies have evaluated the associations between SNPs in miRNAs and BC risk. It has been reported that miR-146a rs2910164 G>C, miR-196a2 rs11614913 T>C, miR-499 rs3746444 T>C, mir-27a rs895819 A>G, as well as rs462480 and rs1053872 polymorphisms in the flank regions of miR-101-2 increased the BC risk (16, 36-40). While miR-27a rs895819 A>G, miR-196a2 rs11614913 C>T reduced the risk of BC (37, 41).

KIAA1217 gene mapped on 10p12.31 and hsa-miR-603 lies in an intron of this gene. It has been shown that KIAA1217 gene is involved in prostate cancer (42), hepatocellular carcinomas (43) as well as non-small cell lung cancer (44).

Altered expression of miR-603 in human neoplasias implying its important role in the process of carcinogenesis (45-47). D'Angelo et al (45) have found an inverse correlation between the expression of miR-603 and high-mobility group A1 (HMGA1), and HMGA2 protein levels in GH adenomas, whose overexpression and/or activation plays a critical role in pituitary tumorigenesis.

Guo et al (47) found that miR-603 is upregulated in

glioma. Abnormal overexpression of miR-603 in glioma causes increased Wnt/β-catenin signaling in glioma cells and clinical tissues. This aberrant signaling further permits cells to escape checkpoints in the G/S phase transition resulting in glioma cell proliferation, differentiation and tumorigenesis.

Hsa-miR-603 (MI0003616) lies in an intron of *KIAA1217* gene. The rs11014002 variant in pre-miR-603 may affect the biogenesis of mature miR-603 and may be involved in cancer development. Furthermore, it has shown that insulin-like growth factor 1 (IGF1) is a target for miR-603. The rs6218 C to T polymorphism in *IGF1* 3'-UTR disrupting the regulatory role of miR-603 in IGF1 expression (48). Up-regulation of *IGF1* have been shown to be related to carcinogenesis and metastasis (48-51).

In conclusion, our finding indicated that has-mir-603 rs11014002 T allele decreased the risk of BC risk in a sample of Iranian population. Further studies on larger sample sizes with different ethnicities as well as laboratory-based functional studies are needed to certify our findings.

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