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Association between Selected microRNA SNPs and Breast Cancer Risk in a Vietnamese Population

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ABSTRACT MicroRNAs play a central role in the regulation and management of gene expression. miR-196a2, miR-423, and miR-146a were shown to regulate the protein expression in breast cancer signaling pathways. Known SNPs in these miRNAs (rs11614913, rs6505162, and rs2910164) have demonstrated their relationship with breast cancer risk in some populations. This study aimed to identify the association between three selected SNPs and breast cancer risk among Vietnamese women. The HRM and TAPMA genotyping methods were performed in 300 cases and 300 healthy controls, supporting the case/control analysis method. Statistical analysis showed two of three selected SNPs were associated with breast cancer risk in Vietnamese. CC genotype of rs11614913 (OR (95% CI) = 1.64 (1.15 – 2.35), P = 0.006) and CG genotype of rs2910164 (OR (95% CI) = 1.44(1.04 – 1.98), P = 0.027) significantly increase breast cancer risk. Haplotype analysis indicated that two haplotypes ((rs11614913T-rs2910164G-rs6505162A) and (rs11614913T-rs2910164C- rs6505162C)) strongly reduce the risk of the disease.

INTRODUCTION

Breast cancer is the leading cause of cancer deaths among women around the world (Torre et al. 2015). In Vietnam, breast cancer incidence has increased steadily from a crude rate of 13.8 per 100,000 women in 2000 to 20.3 per 100,000 women in 2012, with approximate 11,060 new cases and around 4,670 death cases in the country (GLOBOCAN 2012; Lan et al. 2013). A large number of breast cancer cases in Vietnam are diagnosed at a later stage of development, making them more difficult to treat. The percentage of breast cancer cases in Vietnam with stage (0-I) is 14.7 percent, whereas those with stage II and (III-IV) are 61.2 percent and 27.6 percent, respectively (Trieu et al. 2015). These facts point out the alarm status in the healthy awareness of Vietnamese people, particularly in woman.

Although breast cancer is a complex disease, its development comprises of many biological differences at the molecular level. Several studies have suggested that microRNAs (miRNAs) play important roles in the pathogenesis of breast cancer (Esquela-Kerscher and Slack 2006; Jansson and Lund 2012). MiRNAs are small, single-stranded, non-coding RNAs with between 17 and 25 nucleotides that can bind to mRNAs to regulate expression of over a half of all the protein-coding genes in humans. Hence, miRNAs can affect many processes in the development of cells such as proliferation, proliferation, apoptosis, and tumorigenesis (Esquela-Kerscher and Slack 2006; Jansson and Lund 2012).

Many researchers have shown that SNPs in miRNAs (miRSNPs) could potentially affect the structure, maturation, binding at the target site, as well as the expression level of mature miRNA, and hence influence cancer risk (Mishra and Bertino 2009; Salzman and Weidhaas 2013; Guo et al. 2016). Among miRSNPs in breast cancer susceptibility, rs2910164 in miR-146a, rs6505162 in miR-423, and rs11614913 in miR-196a2 have been found to be associated with an elevated risk of breast cancer in different populations.

Pastrello et al. (2010) found that the breast cancer incidence rate at a young age was higher in Italian women carrying the C allele of rs2910164 (GC + CC vs. GG, $P = 0.024$; GC vs. GG, $P = 0.026$). This SNP has also been shown to be significantly associated with breast cancer risk in Chinese (CC vs GG: OR (95%CI) = 1.85 (1.03-3.32), $P = 0.04$) (Qi and Wang et al. 2015), Australians (G vs C: OR (95% CI) = 1.77 (1.40 – 2.23), $P < 0.0001$) (Upadhyaya and Smith et al. 2016), and Europe-

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ans (CC vs. GG: OR (95% CI) = 1.29 (1.02-1.63), *P* = 0.032) (Lian et al. 2012).

The CC genotype of rs6505162 has a high incidence rate in breast cancer cases among Caucasian populations (CC vs AA: OR (95% CI) = 0.50 (0.27 – 0.92), *P* = 0.03) (Smith and Jedlinski et al. 2012). In South American women, AA genotypes have been shown to increase the disease risk (AA vs CC: OR (95% CI) = 1.4 (1.0 – 1.9); *P* = 0.05) (Morales and Gulppi et al. 2016). However, the association between rs6505162 and breast cancer risk has not been found in Zhang's study (Zhang et al. 2017).

SNP rs11614913 (C/T) SNP has been demonstrated to relate to breast cancer in Chinese (CC vs TT: OR (95%CI) = 1.37 (1.08-1.74), *P* = 0.011) (Hu et al. 2009). Several meta-analyses also showed that rs11614913 was associated with the risk of breast cancer (Hu et al. 2009; Qi et al. 2015; Zhang et al. 2017).

The association between three SNPs and breast cancer just has individually evaluated in some countries, but not in Vietnam. Hence, the crucial purpose for this research is served as the fundamental basement for further breast cancer studies associated to these SNPs, and promote the disease's administration in future not only Vietnam, but also in the world.

Objective

To identify the relationship between rs2910164, rs6505162, and rs11614913 and breast cancer risk in a Vietnamese population.

METHODOLOGY

Study Population

Breast cancer cases were identified by the presence of malignant tumor in the breast of the patients, who underwent surgery in the Oncology Hospital in Ho Chi Minh City, in the South of Vietnam. Average age of the patients was 47.8 ± 4.7 years. Healthy controls were healthy female volunteers who were confirmed to be cancer free by an annual health check. Average age of the healthy control group was 46.3 ± 5.0 years. Blood samples were collected from all participants (controls were matched to the cases according to age and ethnic grouping, that is, Kinh – Vietnamese). This study was approved by the Ethical Committee of Oncology Hospital – HCMC Vietnam under the decision number 177/HĐĐĐ-CĐT, 18th November 2014.

Sample Preparation

Blood samples were collected from 300 breast cancer cases and 300 healthy controls. Genomic DNA was extracted from whole blood using a salting-out method following Hue et al.'s protocol (Hue et al. 2012) with some modifications. DNA samples were evaluated by spectrophotometry using the NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific, USA) to determine DNA concentration and purity. High purity (A260/A280 = 1.7 - 2.0) and high concentration (> 10ng/ul) DNA samples have been used.

SNP Genotyping

In process of designing a genotyping method for a SNP, the nucleotide sequence around the SNP need to be investigated. The sequence of SNPs were identified using Genbank (Hossain and Kuo et al. 2006). Primers were designed using Web-based Primer3plus software (Andreas and Harm 2007). The predicted high-resolution melting curves were established using uMelt (Wittwer 2006). Primer Blast tool and NCBI Blast tool (NCBI 1988) were used to test primers for specificity. Primer secondary structures were checked by OligoAnalyzer 3.1 software (Joseph 1987). The SNP genotyping assays were performed in a LightCycler 96 System (Roche Diagnostics Penzberg Germany).

SNP rs11614913 (C/T) and rs6505162 (A/C)

SNP rs11614913 and rs6505162 were genotyped using real-time PCR high-resolution melting (HRM) analysis (Wittwer and Reed et al. 2003). Optimization was performed for each set of primers to determine suitable conditions for HRM analysis where three different genotypes of each SNP could be recognized easily. The SNP genotyping assays were executed by LightCycler 480 High-Resolution Melting Master (Roche Diagnostics, Germany) and a LightCycler 96 Instrument with a 96-well thermal block (Roche Diagnostics). Sequences of primers are described in Table 1.

PCR amplification and HRM analyses were carried out in a reaction containing 1X HotStar-

Table 1: Primer sequences for two SNPs rs11614913 and rs6505162 genotyped using Real-time PCR high-resolution melt analysis

SNP	*Forward primer sequence (5' – 3')*	*Reverse primer sequence (5' – 3')*
rs11614913	CGCTCAGCTGAT CTGTGGCTTA	GTTGGGGC CCTCGACGAA
rs6505162	TTTTCCAAAAG CTCGGTCTGAGG	GCCCCTGAGAG CATCCAAGC

Taq buffer (Qiagen), 200 µM each dNTP (Promega), 0.2 µM forward primer (Sigma), 0.2 µM reverse primer (Sigma), 2.5 mM $MgCl_2$ (for rs11614913) or 3.5 mM $MgCl_2$ (for rs6505162) (Qiagen), 2.5 units HotStarTaq (Qiagen), LightCycler®480 ResoLight Dye (Roche), 20 ng DNA and molecular H_2O (Qiagen).

Thermal cycles for PCR amplification consisted of an initial pre-incubation at 95°C for 15 minutes followed by 40 cycles of a denaturation step at 95°C for 30 seconds, an annealing step at the optimal annealing temperature for each SNP for 30 seconds (64°C and 66°C for rs11614913 and rs6505162, respectively), and an elongation step at 72°C for 30 seconds. High-resolution melting analysis were followed by heating to 95°C for 1.5 minutes and cooling to 40°C for 60 seconds. Finally, those samples were heated to 65°C for 30 seconds and to 95°C for 1 seconds in continuous acquisition mode with temperature ramp at 0.04°C/s. Finally, the reactions were cooled at 4°C. One negative control and three positive controls of each SNP (genotypes confirmed by Sanger sequencing) were included in each run to ensure the identified genotype accuracy.

The typical HRM curves for rs11614913 and rs6505162 were demonstrated in Figure 1. Based on the melting peak characteristics of DNA amplifications in the Real-time PCR HRM, a criteria was set as standard for genotype identification. The melting temperature difference (ΔTm) of two homozygous melting peaks had to be larger than 0.05 and the heterozygotes was distinguished by their two-peak shape (Figs. 1A and 1B). Besides, three other criteria basing on the melting curve characteristics and threshold value (Ct value) were used for genotype identification. (1) The Ct value must be under 30 cycles. (2) There must be a crossing (at middle) in the normalized melting curves between heterozygote and lower Tm homozygote but does not split the higher Tm homozygote. (3) The difference plot where lower Tm homozygote is served as baseline, there must be an opposite division for heterozygote and higher Tm homozygote is lied on the upper side.

SNP rs2910164 (C/G)

SNP rs2910164 was genotyped using Tetra-Arms-PCR Melting Analysis (TAPMA) (Baris and Etlik et al. 2013). Optimization was performed to determine suitable conditions for Tetra-ARMS PCR where three different genotypes of each SNP could be recognized easily. The SNP genotyping assays were executed by HotStartTaq DNA Polymerase (Qiagen) and a LightCycler®480 ResoLight Dye (Roche). Primers sequences for genotyping are described in Table 2.

Table 2: Primer sequences for SNP rs2910164 genotyped using Tetra-Arms PCR high-resolution melt analysis

Primer	*Sequence (5' -3')*
Forward inner primer (G allele)	TGGGTTGTGTCAGTGTCA GACCTC
Reverse inner primer (C allele)	CCCAGCTGAAGAACTGA ATTTCAC
Forward outer primer	GGGTCTTTGCACCATCTC TGAAAA
Reverse outer primer	TCAAGCCCACGATGACA GAGATA

TAPMA reaction were carried out in a reaction containing 1X HotStarTaq buffer (Qiagen), 200µM each dNTP (Promega), 0.2 µM forward outer primer (Sigma), 0.2 µM reverse outer primer (Sigma), 0.4 µM forward inner primer (G allele) (Sigma), 0.5 µM Reverse inner primer (C allele) (Sigma), 3.5 mM $MgCl_2$ (Qiagen), 2.5 units HotStarTaq (Qiagen), LightCycler®480 ResoLight Dye (Roche), 20 ng DNA and molecular H_2O (Qiagen).

Thermal cycles for PCR amplification consisted of an initial pre-incubation at 95°C for 15 minutes followed by 40 cycles of a denaturation step at 95°C for 30 seconds, an annealing step at 64 °C for 30 seconds, and an elongation step at 72°C for 30 seconds. High-resolution melting analysis were followed by heating to 95°C for 1.5 minutes and cooling to 40°C for 60 seconds. Finally, those samples were heated to 65°C for 30 seconds and to 95°C for 1 seconds in continuous acquisition mode with temperature ramp at

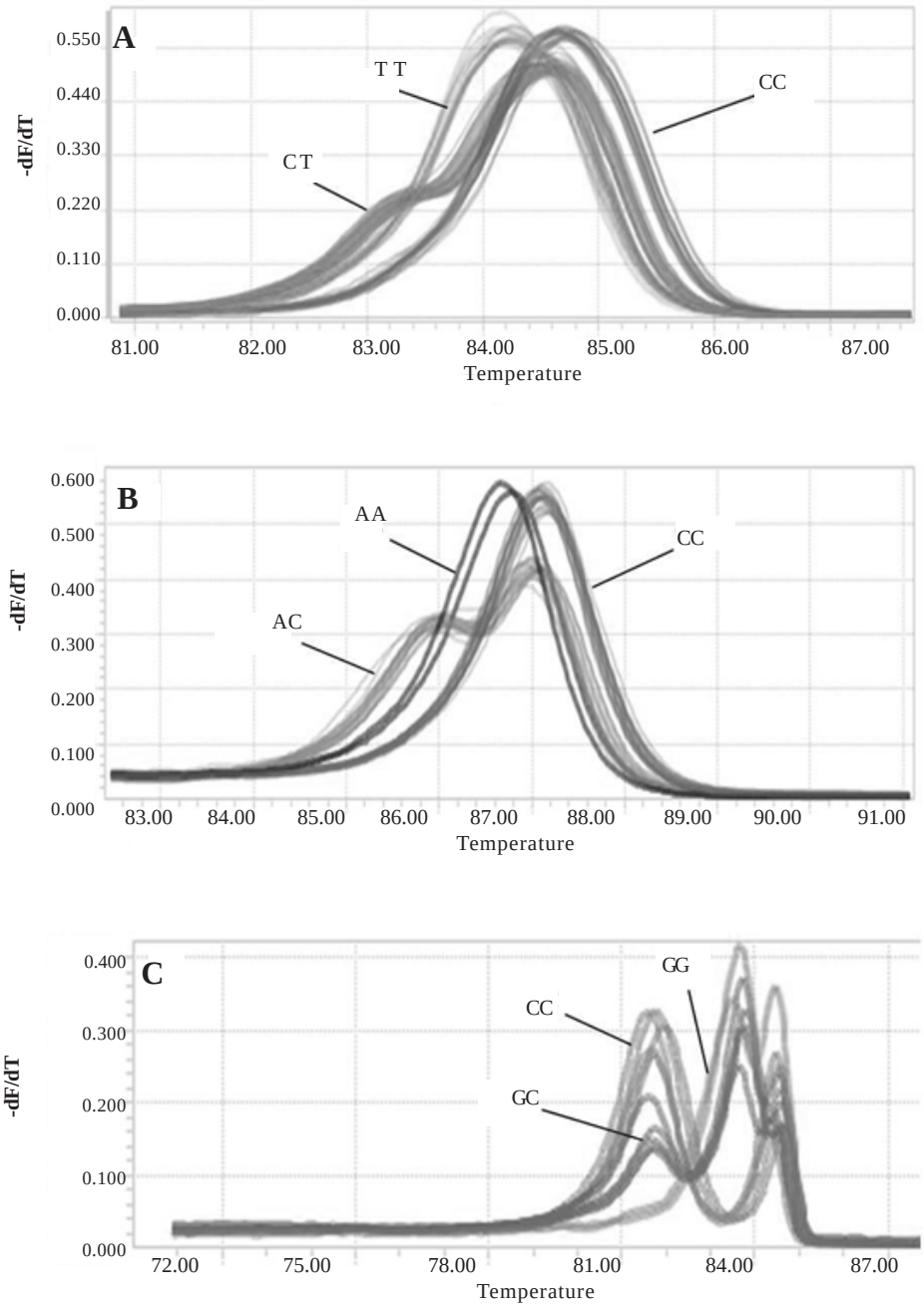

Fig. 1. High resolution melt curves depicting three distinct genotypes for all three SNPs (A) rs11614913, (B) rs6505162, (C) rs2910164

0.04°C/s. Finally, the reactions were cooled at 4°C. One negative control and three positive controls of each SNP (genotypes confirmed by Sanger sequencing) were included in each run to ensure the identified genotype accuracy.

The ideal Tetra-ARMS PCR results for rs2910164 were illustrates in Figure 1C. The heterozygote CG was identified based on three distinct peaks comprising an internal control (Tm = 84.64 ± 0.08°C), allele G product (Tm = 83.80 ± 0.07°C), and allele C amplicon (Tm = 81.80 ± 0.10°C). For homozygote CC, there were only two peaks consists of an internal control (Tm = 84.64 ± 0.08°C), and a peak at 81.80 ± 0.10°C. The last genotype (homozygote GG) was recognized thanks to two separated peaks including an internal control (Tm = 84.64 ± 0.08°C) and a peak at 83.80 ± 0.07°C.

All SNPs in the Primary Set, comprising 106 breast cancer cases and 116 healthy controls that were randomly selected from the Full Set, were initially genotyped to identify genotype and allele frequencies for examination of the potential polymorphism and association. Further validation for potential associated SNPs was performed in the remaining 194 breast cancer cases and 184 healthy controls to make up the Full Set of 300 breast cancer cases and 300 healthy controls.

Statistical Analysis

R i386 3.2.2. was used for statistical analysis of the association between the individual SNPs or the haplotypes and breast cancer risk. Threshold for the significant association was set as P value = 0.05. The frequencies of genotype and allele inside single SNP were presented in percentage. Initially, Hardy-Weinberg equilibrium (HWE) was used to evaluate the distribution for identification of unexpected population or genotyping biases (Hardy 1908; Guo and Thompson 1992). Thereafter, Chi-square test (Fisher correction) was used to determine the differences in allele and genotype frequencies between cases and controls in the populations (Fisher and Yates 1963). The disease risk was evaluate using odds ratio (OR) and 95% confidence interval (95% CI) to figure out whether the allele or genotype can decrease or increase the risk of incidence. Association and haplotype analyses were carried out using 'SNPassoc' and 'haplo.stats' package.

RESULTS

Genotyping and Association Analysis in the Primary Set

All SNPs had a minor allele frequency (MAF) higher than 20 percent (Table 3) indicating their high polymorphism in the Vietnamese population. Cases and controls for the three selected SNPs were in agreement with HWE ($P_{HWE} > 0.05$) (Table 3), especially in the control group, implying that there was a normal distribution between genotypes inside each SNP in the tested population. As also shown in Table 3, the Chi-squared test indicated that there was a significant difference between breast cancer cases and healthy controls for both allelic and genotypic frequency of rs11614913 ($P_{genotype} = 0.04$, $P_{allele} = 0.03$), but not rs6505162 ($P > 0.05$). The genotypic analysis of rs2910164 demonstrated significant differences between cases and controls with a $P_{genotype}$ of 0.02. However, the allelic analysis did not support the difference ($P_{allele} = 0.14$) (Table 3). The small sample size may be a limitation of this analysis. Nevertheless, together with the information about the strong association between the risk allele and the disease, the SNPs rs11614913 and rs2910164 were considered more likely to be potential SNPs for further analysis in the Full Set.

Association Analysis in the Full Set

Genotyping was continued in the remaining samples to evaluate the Full Set of 300 cases and 300 controls, and the frequencies of alleles and genotypes were assessed (Table 4). For rs11614913, there was a significant difference between cases and controls at the allelic ($P_{allele} = 0.05$) and genotypic ($P_{genotype} = 0.02$) level. For rs2910164, there was only a significant difference in genotype distribution between cases and controls ($P_{genotype} = 0.05$).

Further association analysis of genetic models of rs11614913 and rs2920164 was carried out (Table 5). The C allele of rs11614913 was associated with the increased risk of breast cancer (C vs T: OR (95%CI) = 1.28 (1.02 – 1.61), $P = 0.033$). The CC genotype had a 1.55 times higher risk than the reference group (CC vs CT + TT: OR (95%CI) = 1.64 (1.15 – 2.35), $P = 0.006$). As the CT genotype does not appear to be related to disease risk ($P = 0.672$), the C allele might have a

Table 3: Allele and genotype frequencies for three SNPs in the Primary Sset

SNP		*Genotype*		*Allele*			P_{HWE}
		TT	*CC*	*CT*	*C*	*T*	
rs11614913	Case	14	37	49	123	77	1
	(n = 100)	-14%	-37%	-49%	-62.50%	-37.50%	
	Control	28	29	55	113	111	
	(n = 112)	-25%	-25.90%	-49.10%	-50.40%	-49.60%	
	P Chi-square	**0.04**			**0.03**		
		AA	*CC*	*CA*	*A*	*C*	
rs6505162	Case	5	67	34	44	168	0.33
	(n = 106)	-4.70%	-63.20%	-32.10%	-20.80%	-79.20%	
	Control	3	64	49	55	177	
	(n = 116)	-2.60%	-55.20%	-42.20%	-23.70%	-76.30%	
	P Chi-square	0.24			0.53		
		GG	*CC*	*CG*	*G*	*C*	
rs2910164	Case	7	39	54	68	132	0.77
	(n = 100)	-7%	-39%	-54%	-34%	-66%	
	Control	22	41	49	93	131	
	(n = 112)	-19.60%	-36.60%	-43.80%	-41.50%	-58.50%	
	P Chi-square	**0.02**			0.14		

Table 4: Allele and genotype frequencies for rs11614913 and 2910164 in the Full Set

SNP		*Genotype*		*Allele*			P_{HWE}
		TT	*CC*	*CT*	*T*	*C*	
rs11614913	Case	68	104	128	264	336	0.14
	(n = 300)	-22.70%	-34.70%	-42.60%	-44%	-56%	
	Control	74	73	152	300	298	
	(n = 300)	-24.80%	-24.40%	-50.80%	-50.20%	-49.80%	
	P Chi-square	**0.02**			**0.05**		
		GG	*CC*	*CG*	*G*	*C*	
rs2910164	Case	34	115	152	220	382	0.73
	(n = 300)	-11.30%	-38.20%	-50.50%	-36.50%	-63.50%	
	Control	49	127	125	223	379	
	(n = 300)	-16.30%	-42.20%	-41.50%	-37%	-63%	
	P Chi-square	**0.05**			0.96		

recessive effect. For rs2910164, the CG genotype clearly 1.44-fold increased breast cancer risk against the reference group (CG vs CC + GG: OR (95%CI) = 1.44 (1.04 – 1.98), P = 0.027).

Haplotype Analysis

Following this, the researchers combined genotyping results to conduct haplotype association analysis for rs11614913 (C/T), rs6505162 (A/C), and rs2910164 (C/G). Table 6 shows the haplotype frequencies and association analysis for three SNPs. According to the researchers' findings, both cases and controls had the highest frequency of haplotype CCC (35 % in cases and 19.6 % in controls) which could be used as the reference. The statistical analysis have proved that haplotype TCC and TAG decreased

Table 5: Association between genetic models of three SNPs and breast cancer risk in the Full Set

SNP	*Model*	*OR*	*95% CI*	*P*
rs11614913	CC vs. TT	1.55	0.99 – 2.42	0.053
	CT vs. TT	0.92	0.61 - 1.37	0.672
	CC + CT vs. TT	1.12	0.77 – 1.64	0.549
	CC vs. CT + TT	**1.64**	**1.15 – 2.35**	**0.006**
	C vs. T	**1.28**	**1.02 – 1.61**	**0.033**
rs2910164	GG vs. CC	0.77	0.46 – 1.27	0.301
	CG vs. CC	1.34	0.95 – 1.90	0.095
	GG +CG vs. CC	1.18	0.85 – 1.64	0.318
	GG vs. CG + CC	0.65	0.41 – 1.05	0.075
	CG vs. CC + GG	**1.44**	**1.04 – 1.98**	**0.027**
	C vs. G	0.98	0.78 – 1.24	0.858

Table 6: Haplotype frequencies and association analysis for three SNPs

Haplotype	*rs11614913*	*rs2910164*	*rs6505162*	*Frequency (%)*		*OR*	*95% CI*	*P*
				Cases	*Controls*			
1 (intercept)	C	C	C	35	19.6	1	-	-
2	C	C	A	8.4	9.5	0.47	0.16 - 1.38	0.17
3	C	G	C	15.1	20.3	0.43	0.17 – 1.08	0.07
4	C	G	A	3.6	1.9	1.3	0.20 – 8.32	0.78
5	**T**	**C**	**C**	**15.3**	**26**	**0.32**	**0.13 – 0.81**	**0.02**
6	T	C	A	7.5	3.4	1.9	0.32 – 11.25	0.48
7	T	G	C	13.4	11.8	0.62	0.26 – 1.44	0.27
8	**T**	**G**	**A**	**1.7**	**7.5**	**0.12**	**0.02 – 0.60**	**0.01**

the breast cancer risk (TCC vs. CCC: $P = 0.02$ and OR (95% CI) = 0.32 (0.13 – 0.81); TGA vs. CCC: $P = 0.01$ and OR (95% CI) = 0.12 (0.02 – 0.60)). These results suggest that the individuals who carry these two haplotypes seems to be protected against breast cancer risk.

DISCUSSION

The number of breast cancer incidence and mortality are increasing despite the current knowledge on the disease. MicroRNAs are involved in molecular pathways leading to cell growth, differentiation and survival. There is enough evidence to show they play a significant role in the mechanisms that lead to development and progression of different types of cancer, including breast cancer.

In this study, three SNPs in three miRNA genes encoding miRNAs involved in the regulation of cell development have been genotyped in a Vietnamese case-control cohort. Among the genotyped SNPs, rs6505162 were found to not show an association with breast cancer risk, but the remaining two, rs11614913 and rs2910164, showed a significant association with the risk of breast cancer in the Vietnamese population cohort.

In the literature, SNP rs6505162 was shown to be strong associated with breast cancer risk in Australian (CC vs AA: OR (95% CI) = 0.50 (0.27 – 0.92), P = 0.03) and South American (AA vs CC: OR (95% CI) = 1.4 (1.0 – 1.9); P = 0.05) (Smith et al. 2012; Morales et al. 2016). Nevertheless, the meta-analysis in Chinese and Caucasians also failed to support the association between rs6505162 and breast cancer risk (Hu et al. 2014; Zhang et al. 2017). In this study, the researchers found that this SNP was not significantly associated with breast cancer risk in this Vietnamese cohort, with *P* values of genotype and allele frequencies being higher than 0.05 (Table 3). These contradictory results indicate that the SNP is associated with breast cancer risk in a specific ethnicity and population (Mizoo et al. 2013). It is possible that this SNP is related to breast cancer progression, due to their being located in low-penetrance genes coding pre-miR-423. MiR-423 was reported to promote cell cycle progress at the G1/S checkpoint through down-regulating the expression of the tumour suppressor p21 in the p53 pathway (Kasashima et al. 2004). Thus, the presence of rs6505162 may cause disorders in the breast cell cycle via the effect on the expression or pro-

cessing of mature miR-423. These evidences supported a further association study of rs34678647 to evaluate to relation with breast cancer risk in Vietnamese women.

The two remaining SNPs, rs11614913 and rs2910164, were found to be significantly associated with breast cancer risk in the Vietnamese cohort (Table 5). In details, the C allele of rs11614913 was indicated to increase the risk of breast cancer 1.28 times (C vs. T: OR (95% CI) = 1.28 (1.02 – 1.61) and P = 0.033) in Vietnamese women. Particularly, the CC genotype of rs11614913 increased the risk of breast cancer 1.64-fold compared to CT and TT genotypes (CC vs. CT + TT: OR (95% CI) = 1.64 (1.15 – 2.35), *P* = 0.006) with the power analysis at 67.6%. Interestingly, the similar significant association result of this SNP was found in other studies in Chinese population, a with the *P* values being much lower than 0.01 (Hu et al. 2009; Qi et al. 2015; Dai et al. 2016). This similarity can be explained by the close geography and the same ancestry leading to exchange genetic variants.

For rs2910164, carriers of the CG genotype have a 1.44 fold higher risk in the Vietnamese population (CG vs. CC + GG: OR (95% CI) = 1.44 (1.04 – 1.98), *P* = 0.027) with the power analysis at 60.2 percent. Interestingly, the CC genotype of rs2910164 was demonstrated to increase the breast cancer risk in Chinese population (CC vs GG: OR (95% CI) = 1.85 (1.03 - 3.32), *P* = 0.04) (Qi et al. 2015), while the G allele of rs2910164 was observed to be associated with increased risk Australian populations (G vs C: OR (95% CI) = 1.77 (1.40 – 2.23), *P* < 0.0001) (Upadhyaya et al. 2016). This contradictory result indicates that rs2910164 may be evolved in a reverse function under a specific population.

An explanation for the association of rs11614913 and rs2910164 and breast cancer has recently been proposed. SNP rs11614913 (C/T) located in the mature sequence of miR-196-a2, was shown to regulate cell proliferation through down-regulating two tumour suppressors, GADD45G and INHBB, in breast cancer cells (Hoffman et al. 2009). The mutant C allele of rs11614913 may cause changes in the function of miR-196-a2, thereby not allowing the inhibition of expression of two target genes, resulting in altering breast cell development. SNP rs2910164 (C/G) is located in the 3p mature miRNA-146a region. The optimal free energy declined when changed from a G to a C allele, due to a less stable secondary structure for the C allele compared with the G allele. This emphasised that rs2910164 alters the expression of mature miR-146a. The high levels of miR-146a enables enhanced silencing of the *BRCA1* gene. Down regulation of *BRCA1* will reduce the DNA repair function and allow damaged cells to progress into S phase leading to breast cancer cell development (Shen et al. 2008).

Analysis of the eight haplotypes present in the group rs11614913/rs2910164/rs6505162 was conducted. Among them, haplotype 1 is the most common haplotype in both cases and controls, which could be used as the reference. The haplotype TCA and haplotype TGA were strongly associated with the decreased risk (TCA vs. CCC: OR (95%CI) = 0.32 (0.12 – 0.81), P = 0.02; and TGA vs. CCC: OR (95%CI) = 0.11 (0.02 – 0.60), P = 0.01). This strong association indicated these two haplotypes could be potential biomarkers for breast cancer risk in the Vietnamese population.

CONCLUSION

In conclusion via analysis of three SNPs from three miRNAs, rs11614913 and rs2910164 were identified significant association with susceptibility to breast cancer. The interaction between three SNPs, rs11614913(C/T)/rs2910164(C/G)/rs6505162(A/C), at the haplotype level was also strongly associated with breast cancer risk. The presence of the TCA or TGA haplotype significantly decreased the risk of breast cancer in Vietnamese women. A further validation in the larger populations, as well as functional studies are necessary to determine whether or not these individual SNPs and the haplotypes may be used as biomarkers for early prediction of breast cancer risk in Vietnamese population.

RECOMMENDATIONS

Studies with larger sample sizes are necessary to confirm these findings. The outcome will provide information to develop new biomarkers for risk assessment of breast cancer in Vietnam. Further analysis within subgroups of breast cancer should be done to understand in more detail the association between SNPs and each sub-type, such as Her2-positive, ER-positive, PR-positive, or triple negative breast cancers.

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