Association Between Single Nucleotide Polymorphism Rs11614913 (C>T) on Mir-196a2 and Breast Cancer in Vietnamese Population

Tran Thi Hong Minh, Nguyen Thi Ngoc Thanh, Tran Van Thiep, and Nguyen Thi Hue

Abstract

Breast cancer (BC) is a major health problem throughout the world. It is one of the most common cancer among women both in developed and developing countries. To raise the survival of BC patients, genetic factors are used for early diagnosis because they are non-changed factors and give ability of cells to proliferate and metastasize. MiR-196a2 targets many genes which are vital for development, apoptosis, differentiation, motility and angiogenesis. The SNP rs11614913 (C/T) affects the processing of the pre-miRNA to its mature form and the ability to regulate target genes. This SNP has been demonstrated to relate to breast cancer in Asian, especially in Chinese [OR (95% CI) = 0.73 (0.57–0.93), p = 0.011]. This study aimed to investigate the correlation between the SNP rs11614913 and BC in Vietnamese population. 113 cases and 127 controls were genotyped using optimized high resolution melting method (HRM) then statistical analysis was applied to examine the association of the SNP. The results show that the frequencies of TT, CT, and CC were 26.54, 30.97 and 42.49% in cases group and 25.19, 50.39, and 24.42% in controls group, respectively. Statistic result revealed an obvious decreased risk of BC among Vietnamese population when compared of heterozygote model and dominant model (CT vs. CC: OR = 0.35, 95% CI: 0.19–0.65, p = 0.00074; TT + CT vs. CC: OR = 0.44, 95% CI: 0.25–0.76, p = 0.00295). Our results shown that rs11614913 could be a potential biomarker for early detection and diagnosis of breast cancer for Vietnamese patients.

Keywords

rs11614913 • miR-196a2 • Breast cancer • Polymorphism • High resolution melting method

1 Introduction

Breast cancer is a major health problem throughout the world. It is one of the most common cancer among women both in developed and developing countries. It is also the leading cause of cancer death in female, with nearly 1.7 million new cases diagnosed and more than 520,000 breast cancer deaths in 2012 [1]. In Vietnam, the breast cancer incidence has increased steadily with an approximate 11,067 new cases in the country. The mortality rates ranked first place in Vietnamese women (account for 13% deaths) [1, 2].

Reducing the burden of disease mortality and morbidity can be done by early detection of individual having breast cancer risk. Breast tumorigenesis proceeds through the accumulation of genetic and epigenetic alteration. Some of these changes are expressed during early stage of the tumor development and thus provide a chance for diagnosis and treatment strategies. Among them, microRNAs have recently emerged as novel biomarkers that can help detect

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tumor at early stage. MicroRNAs (miRNAs) are small, single-stranded, non-coding RNAs with sizes of 17–25 nucleotides. A single miRNA might bind to as many as 200 gene targets and these targets can be diverse in their function [3]. Tumor formation may arise from reduction of a tumor suppressive miRNA and amplification of an oncogenic miRNA. Single nucleotide polymorphisms (SNPs) in pre-miRNAs or mature miRNAs can modify the biological processes of miRNA and thus are highly potential targets for study of cancer, including breast cancer.

The SNP rs11614913 results in a change from cytosine to thymine. This polymorphism is located at the 3p mature miR-196a region and affects the processing of the pre-miR-196a2 to its mature form. MiR-196a2 targets many genes which are vital for development, apoptosis, differentiation, motility and angiogenesis. Four members of the HOX gene family (HOXB2, HOXB3, HOXC13, and HOXB5) were significantly down-regulated in cells treated with pre-miR-196a2 C allele. Furthermore, two tumor suppressor, GADD45G and INHBB, were significantly down-regulated following pre-miR-196a2 C allele introduction [4, 5].

Although SNP rs11614913 on miR-196a2 gene is reported to be associated with breast cancer in Caucasian and Asian, conclusions of the relevant studies remain inconsistent because of heterogeneity of small sample size and ethnicity of the patients. This study has been conducted to analyze the correlation between SNP rs11614913 and the risk for developing breast cancer in Vietnamese women.

2 Materials and Methods

2.1 Study Population

The population of interest in this study was Vietnamese population. Blood samples were collected from Oncology Hospital, Ho Chi Minh City. Sample population included 113 cases and 127 controls. All of the patients were given consent forms to sign on. The collected blood was stored in EDTA containing tubes at −20 °C for further use.

2.2 DNA Extraction

DNA from blood samples were extracted by salting-out method followed protocol by Hue et al. [6]. First, white blood cells are isolated from the whole blood. After treatment with various reagents. the DNA will be collected by ethanol precipitation before ethanol is also removed. Finally, the DNA pellet is resuspended in molecular or RNAse-free water.

2.3 HRM Design and Optimization

Primer design. Identification of the SNP's location and its nucleotide sequence on NCBI. In order obtain the primer pair, the Web-based Primer3plus software (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi) was used. Next, the specificity of the designed primers was tested with NCBI Blast tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to limit any PCR undesired product. The sequences of the forward and reverse primer are described in Table 1.

In silico prediction. The amplicon of HRM analysis was retrieved using in–silico PCR of UCSC database (https://genome.ucsc.edu/). uMELT HETS (https://www.dna.utah.edu/hets/) was exploited to predict melting curves corresponding to three different genotypes of rs11614913 at various concentration of $MgCl_2$, monocation, DMSO and Betaine.

Initial optimization for annealing temperature (T_a). T_a optimization was performed in range 60–68 °C by thermal cycle PCR Eppendorf instrument and HostarTaq DNA polymerase, Qiagen. Components for 20μL—PCR reaction included: 1X PCR buffer, 200 μM each dNTP, 0.2 μM forward primer, 0.2 μM reverse primer, 2.5 units HotstarTaq, 10 ng DNA and molecular H_2O.

Controls identification. Control identification was performed in several randomized samples. HRM components for a 10 μL reaction consisted of: 1X PCR buffer, 200 μM each dNTP, 2.0 mM $MgCl_2$, 0.2 μM forward primer, 0.2 μM reverse primer, 2.5 units HotstarTaq, 10 ng DNA and molecular H_2O.

Table 1 Primers for HRM analysis and sequencing

Primer	Sequence (5′–3′)	Tm
HRM-F	CGCTCAGCTGATCTGTGGCTTA	68.8
HRM-R	GTTGGGGCCCTCGACGAA	70.8
SEQ-F	CCCTTACCCACCCAGCAACC	57.9
SEQ-R	CTGGACCCTCTTTGTCTGTCTC	56.7

Post optimization for $MgCl_2$ concentration. The optimization was conducted at 2, 2.5, 3.0 and 3.5 mM $MgCl_2$ to achieve the best discrimination between identified controls.

2.4 Genotyping

After having controls, the condition of reaction was continued to optimize to having best curves for discriminating three genotypes. The optimal condition for HRM was applied to genotype 113 cases and 127 controls. The results were displayed using LightCycler® 96 SW 1.1 software. Frequencies of allele C (f(C)) and allele T (f(T)) were calculated by the following formula: $f(C) = f(CC) + ½\ f(CT)$ *and* $f(T) = f(TT) + ½\ f(CT)$ in which f(CC, CT or TT) is equal a number of individuals having the specific genotype divided by the sample population.

2.5 Statistical Analysis

Hardy-Weinberg equilibrium (HWE) in controls was analyzed. $P < 0.05$ was considered representative of departure from HWE. To determine whether the SNP frequency in case and control group is significant difference, allele and genotype frequencies were compared using Chi-square test. Furthermore, OR with 95% CI was used to assess the strength of association between rs11614913 and Vietnamese patients.

3 Results

3.1 Designed Primer and in Silico Prediction

The designed primers had been selected based on the criteria of Primer3Plus and the predicted melting curves. The selected primers could distinguish three genotypes of rs11614913 easily. The HRM primer set suitable for amplifying the amplicon containing the selected SNP and the other set required for sequencing to confirm the presence of each allele were designed as described in Table 1 and (Fig. 1).

3.2 Initial Optimization

For Ta optimization, PCR reaction with gradient temperature ranged from 60 to 68 °C was run and the result was analyzed using gel electrophoresis (Fig. 2). Gel electrophoresis result showed a bright band from 60 to 64 °C. As increasing Ta, specificity of primers is increased, so 64 °C was chosen as Ta for further HRM analysis.

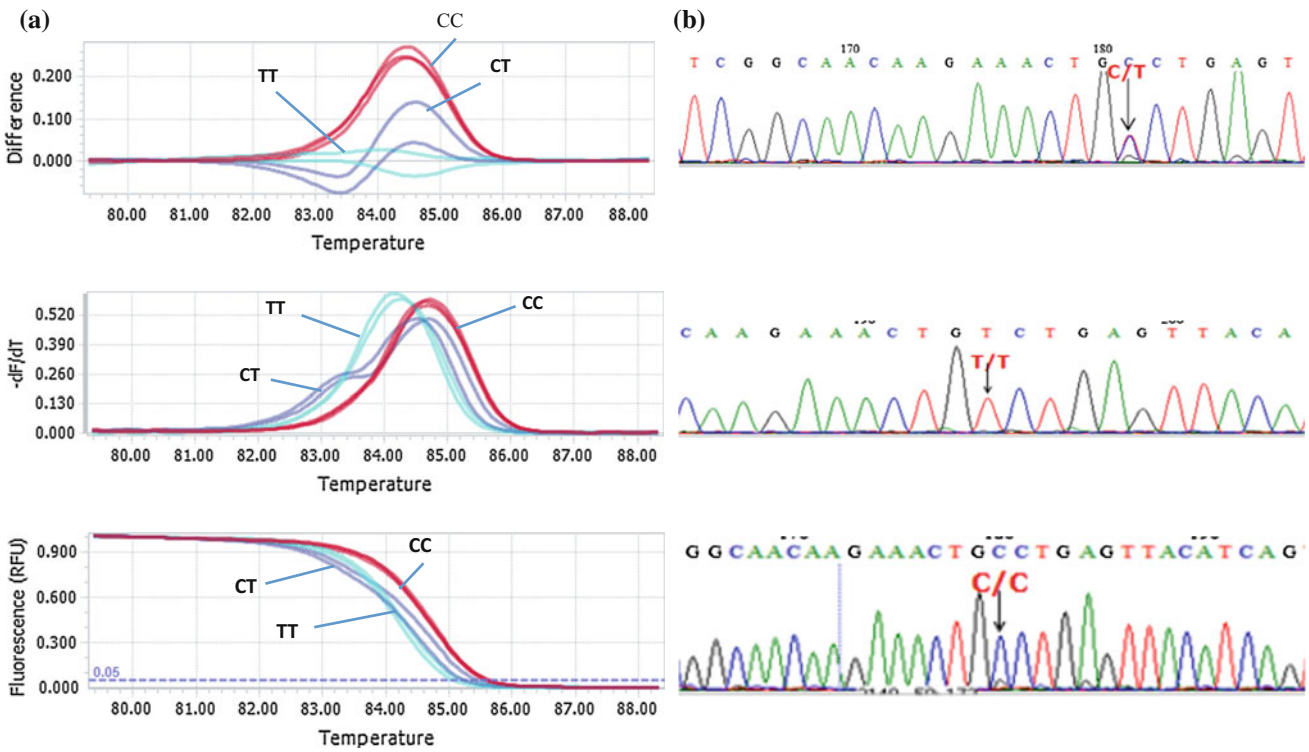

Fig. 1 The predicted melting curves (**a**) and melting peaks (**b**) of rs11614913 on UmeltHets at [Mg2 +] = 2 (mM), %DMSO = 0. The *blue*, *black* and *red curve* presented TT, CC and TC genotype, respectively

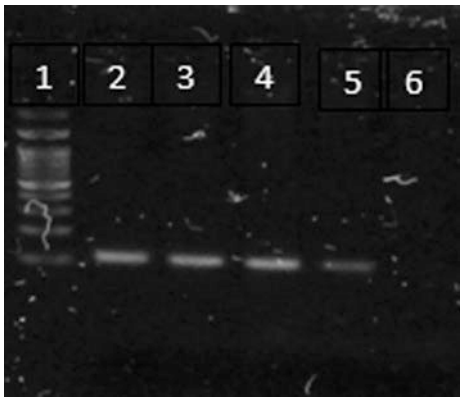

Fig. 2 Agarose gel electrophoresis of PCR products with different Ta (*Lane 1* the DNA ladder, *lanes 2*, *3*, *4*, *5* and *6* 60, 62, 64, 66 and 68 °C)

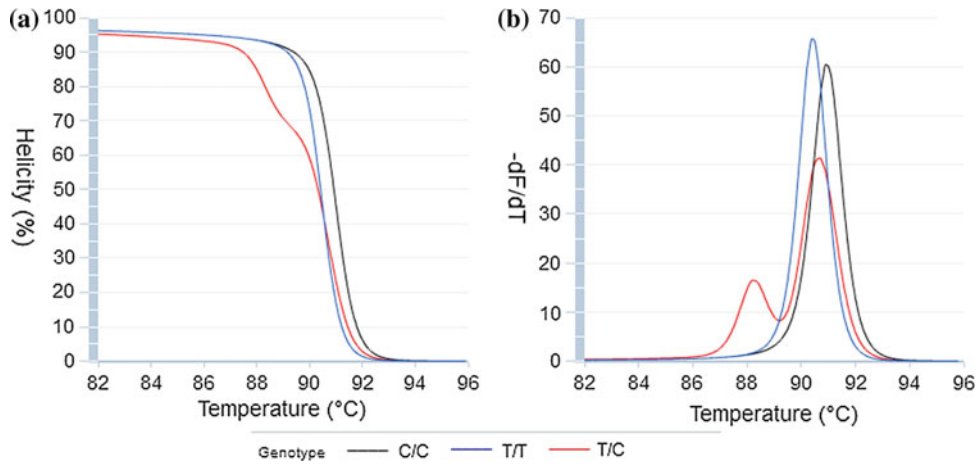

Fig. 3 Control screening at [Mg2 +] = 2 mM, Ta = 64 °C

3.3 Controls Identification

After determining Ta, the predicted HRM condition by Umelt was applied to running random samples for finding controls: 2 mM $MgCl_2$, %DMSO = 0, and Ta of 64 °C. Three controls were identified and confirmed by sequencing (Fig. 3).

3.4 Post Optimization for $MgCl_2$ Concentration

Since $MgCl_2$ was the component that might influence the organization of melting curve between three genotypes, $MgCl_2$ concentration was optimized. HRM was run on three controls with different $MgCl_2$: 2, 2.5, 3 and 3.5 mM. The result revealed that at $MgCl_2$ 2.5 mM, three melting curve exhibited the best shape and discrimination.

3.5 Genotyping

Total 240 samples were genotyped, including 127 controls and 113 cases. Figure 4 is an example of HRM result with three different genotypes.

Tables 2 and 3 lists the main results of this analysis. The controls were in agreement with HWE. The results show that the frequencies of TT, CT, and CC were 26.54, 30.97 and 42.49% in cases group and 25.19, 50.39, and 24.42% in controls group, respectively. The CT genotype and T allele were more common in the control population. However, Chi-square test indicated that there was only significant difference between breast cancer group and control group for genotypes (χ^2 = 11.44, P = 0.00328). Overall, significantly reduced breast cancer risk was associated with miR-196a2 T allele (CT vs. CC: OR = 0.35, 95% CI: 0.19–0.65, p = 0.00074; dominant model TT + CT vs. CC: OR = 0.44, 95% CI: 0.25–0.76, p = 0.00295).

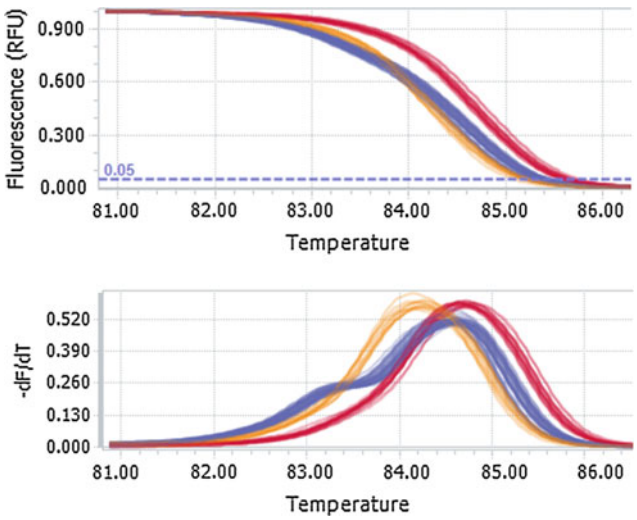

Fig. 4 The melting curves and melting peaks analysis of rs11614913 by HRM showed 3 different genotypes. The *red*, *blue* and *indigo* curve presented CC, TT and TC genotype, respectively

Table 2 Allele and Genotype frequencies for rs11614913

	Genotypes			Alleles		HWE
	TT	CT	CC	T	C	
Cases 113	30 (26.5%)	35 (31%)	48 (42.5%)	95 (42%)	131 (58%)	0.929
Controls 127	32 (25.2%)	64 (50.4%)	31 (24.4%)	128 (50.4%)	126 (49.6%)	
Chi-square *p*-value	11.44, 0.00328			3.0312, 0.08168		

Table 3 Analysis results for SNP rs11614913

Cases/controls	T versus C		CT versus CC		TT versus CC		(CT + TT) versus CC	
	OR(95% CI)	P	OR(95% CI)	P	OR(95% CI)	P	OR(95% CI)	P
113/127	0.714 (0.498–1.024)	0.067	0.353 (0.192–0.651)	**0.0007**	0.605 (0.309–1.186)	0.142	0.437 (0.252–0.758)	**0.003**

4 Discussion

In this case-control study of breast cancer in Vietnamese population, we examine the association of the SNP rs11614913 with the aid of HRM analysis and found that variant genotypes of miR-196a2 was associated with significantly decreased risk of breast cancer. The study provides more evidence that common SNPs in miRNAs may be used as breast cancer biomarkers.

MiR-196a2 is well known for its function in diverse cellular processes and various diseases including breast cancer. Recently, genetic variants of the miR-196a2 gene in the etiology of several cancers have drawn more attention. According to many data, miR-196a2 may function as an oncogenic factor which blocks translation of several tumor suppressor genes. Rs11614913 locates in the mature sequence of miR-196a2 but can affect processing of the pre-miRNA, as well as influence capacity to regulate target genes. Hoffman et al. study showed that the variant allele T decreased miR-196a levels compared with miR-196a-C [7]. Several studies have proved that rs11614913 was associated with many different types of cancers [8–10]. This SNP has been demonstrated to relate to breast cancer in Chinese, American (Connecticut), and Brazilian [7, 8, 11]. In Hu's study, significantly decreased breast cancer risk was found to be associated with TT/CT genotypes [TT vs. CC: OR (95% CI) = 0.731(0.575–0.930), p = 0.011; (TT + CT) vs. CC: OR(95% CI) = 0.803(0.652–0.988), p = 0.037] [8].

When studied in Brazilian, Linhares et al. also showed the difference in genotype distribution between groups (healthy and breast cancer) but the present of TT or CT variants increased the risk of breast cancer [10]. The contradictory between those study and our result might be explained by population-specific factors, such as genetic background, lifestyle, or environmental factors. T is the common allele in Asian, but in Caucasian, it is the minor allele. The controls group of our study and Han Chinese study by Hu et al. also showed the high proportion of T allele. Whereas study of Linhares indicated that C was the dominant allele in Brazilian population. Moreover, the frequency of C allele in cases group of our study is greater than T allele (58 and 42%, respectively). These results contrast with Linhares' study, T allele made up to 53% while C allele was just about 47%.

Besides, the study of Catucci et al. in Italian/German and Jedlinski et al. in Australian failed to support the association of rs11614913 and breast cancer risk [12, 13]. This may be explained that the participants in Linhares and Hoffman' study were not match for ethnicity including Caucasian, African-American and others.

There are still some complications in this study. Although both the heterozygous and dominant models proved that T allele reduced the disease risk, the TT genotype had no effect on breast cancer susceptibility ($p = 0.142$). Furthermore, when comparing T and C allele to determine whether T allele may contribute to breast cancer risk, the p-value was just nearly borderline ($p = 0.067$). This could be due to some limitations of the study. First, because of small sample size, the power was not high (power = 36.234%). Second, lack of available information prevented a more precise evaluation including age, smoking status, alcohol consumption, menopausal status, etc.

5 Conclusions

In conclusion, this case-control study suggests that SNP rs11614913 contribute to breast cancer susceptibility among Vietnamese population. However, conducting studies with larger sample size and using well matched controls is necessary to confirm these findings. The outcome will provide information to develop a new molecular diagnostic test for early detection of breast cancer in Vietnam.

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References

1. Torre LA et al (2015) Global cancer statistics,2012. CA Cancer J Clin
2. Lan NH et al (2012) Survival probability and prognostic factors for breast cancer patients in Vietnam. Glob Health Action 6:18860
3. Kerscher AE et al (2006) Oncomirs—microRNAs with a role in cancer. Nat Rev 6:259–269
4. Raman V et al (2000) Compromised HOXA5 function can limit p53 expression in human breast tumours. Nature 405:974–978
5. Ying J et al (2005) The stress-responsive gene GADD45G is a functional tumor suppressor, with its response to environmental stresses frequently disrupted epigenetically in multiple tumors. Clin Cancer Res 11:6442–6449
6. Hue NT et al (2012) Extraction of human genomic DNA from dried blood spots and hairs roots. Int J Biosci Biochem Bioinform 2:21–26
7. Hoffman AE et al (2009) MicroRNA miR-196a-2 and breast cancer: a genetic and epigenetic association study and functional analysis. Can Res 69:5970–5977
8. Hu Z et al (2009) Common genetic variants in pre-microRNAs were associated with increased risk of breast cancer in chinese women. Hum Mutat 30:79–84
9. Qiu LX et al (2011) miR-196a2 C allele is a low-penetrant risk factor for cancer development. Cytokine 56:589–592
10. Wang F et al (2012) A genetic variant in microRNA-196a2 is associated with increased cancer risk: a meta-analysis. Mol Biol Rep 39:269–275
11. Linhares JJ et al (2012) Evaluation of single nucleotide polymorphisms in microRNAs (hsa-miR-196a2 rs11614913 C/T) from Brazilian women with breast cancer. BMC Med Genet 13:119
12. Catucci I et al (2009) Evaluation of SNPs in miR-146a, miR196a2 and miR-499 as low-penetrance alleles in German and Italian familial breast cancer cases. Hum Mutat 31:1052–1057
13. Jedlinski DJ et al (2011) Single nucleotide polymorphism in hsa-mir-196a-2 and breast cancer risk: a case control study. Twin Res Hum Genet 14:417–421