**Research Article**

# The Development of Rs2853826 Genotyping and the Initial Association with Breast Cancer Risk in Vietnamese Women

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## ABSTRACT

Breast cancer (BC) is known as the leading cause of dead in women through worldwide including Vietnam. Recently, mitochondrial genome mutations concerned to breast cancer has been screened in order to propose new potential biomarkers. The SNP rs2853826 located at 10398-nucleotide position within the NADH-dehydrogenase3 has been demonstrated to be associated with BC in populations such as Malaysian, Northern Indian, Poland, Banglasdesh, African-American, Eropean-American, but Vietnam. Thus, rs2853826 was selected as the candidate SNP for investigation in Vietnamese population. In this study, the High Resolution Melting method is optimized for genotyping 100/100 cases/controls samples, then determined the association between this SNP and the disease. The genotyping results revealed SNP rs2853826 has high polymorphism with the minor allele frequency, G allele, was 17.2%. risk allele G was 17.2 %. The association result between rs2853826 and BC revealed that G allele tend to increases risk of BC among Vietnamese when comparing A allele (OR[95%CI]=1.224[0.818-1.832]); and homozygote and heterozygote model also showed this trend when compared to dominant model. Since the p-values were larger 0.05 (p=0.326), our results only show a trend correlation rather than a significant association between SNP rs2853826 and BC risk. However, the reliability of this association analysis result is low (7.57%), so this research needs to be conducted with a larger sample size to reach the greater power (>90%) for therelation.

**Keywords:** Breast cancer, ND3, rs2853826, HRM

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# INTRODUCTION

Breast cancer is globally considered as the leading cause of dead in woman. According to WHO, there was an alarming estimation about 2.1 million (11.6%) diagnosed female breast cancer cases throughout the world in 2018, accounting for almost 1 in 4 cancer cases among women [1]. This disease is now diagnosed more frequently among Asians, especially in younger generations. It is predicted to continue to increase in less developed and developing countries over the next decade and become the leading cause of cancer-related deaths among women. In Vietnam, the breast cancer incidence has significantly increased over last years from rate of 23.0 per 100,000 women in 2012 (GLOBOCAN 2012) to 26.4 per 100,000 in 2018 (GLOBOCAN 2018). The latest statistics in 2018 of the WHO has reported that Vietnam has approximately 15,229 new breast cancer cases are detected, with the mortality number of 6,103 cases [1]. Unlike in Western countries where women typically have early stages of the disease, a large number of breast cancers in Vietnam occur at a later stage of development, making the treatment more difficult. In Hanoi and Ho Chi Minh City, as much as 70 percent breast cancer patients are diagnosed in late stages of the disease [2]. In which, Hanoi has a higher incidence rate (29.7 per 100,000), which is more than 1.5 higher than the rate in HCMC (19.4 per 100,000) [3]. This obviously demonstrates that the late diagnostic, which makes the treatment less effective, is the main cause of the increasing mortality [3, 4]. In the other words, breast cancer patients are able to improve their life expectancy in case of early detection [3]. Thus, it is critical to quickly identify new potential biomarkers to enhance early diagnosis and to predict patient prognosis, drug resistance development and suitable treatment choice.

Breast cancer has marked its name as one of the most globally common malignancies. Susceptibility genes, including high-penetrance genes whose mutations rarely appear in the population (<0.1%) such as BRCA1 and BRCA2 [5, 6] and medium and low-penetrant genes which possess high-frequency variations (>0.1%), plays a vital role in the etiology [7, 8]. The interactions among SNPs may poses a more significant effect when each of them mildly affect the risk of breast cancer [9]. Therefore, a higher frequency and association with the other risk SNPs in low-penetrant genes make them potential genetic markers in the prediction of the breast cancer risk [6].

In this study, a SNP in mitochondrial genome were investigated. Mitochondria are small organelles which are in main charge of producing most of cellular energy in form of ATP molecules through the oxidative phosphorylation (OXPHOS) system and importantly involved in energy metabolism, reactive oxygen species (ROS) generation and initiation of apoptosis. If there is an imbalance between the production of ROS and their antioxidant defenses, it will consequently lead to the oxidative stress on cells, promotion of cancer growth, cancerous transformation and metastasis through induction of DNA damage, cellular structure, somatic mutations [10, 11]. Thus, any SNPs that occur in mitochondrial genes may affect to generate energy and the imbalance in ROS production in the cell, thereby contributing to the initiation and progression of the cancer, including breast cancer [12, 13].

Several association studies have identified numerous genetic susceptibility SNPs on mitochondrial genes that are associated with breast cancer risk. Among them, SNP rs2853826 in NADH-dehydrogenase3 (*MTND3*) gene encoding for a protein evolving in OXPHOS system has been reported to be associated with breast cancer risk in different populations, not only Asian populations but also American and European, with the majority of the *P* values being much lower than 0.01 [14-18].

The mtDNA SNP rs2853826 has been reported in many association studies on Breast cancer

patients in many populations around the world including African-American, Polish and some Asian countries, which are closely to Vietnamese population such as Northern Indian or Malaysian. Among them, A allele was indicated to increase the risk of invasive breast cancer African-American and Northern Indian women (P = 0.013, P = 0.01 respectively) with odds ratios (ORs) (95% confidence interval (95% CI) ranging from 1.6 (1.1-2.31) to 1.73 (1.13 – 2.66) [14, 15]. Whereas, the opposite was observed that allele G is highly significantly associated with increased risk of breast cancer in Bangladesh, European-American, Malaysian and Polish population (P= 0.0182, P = 0.01, P = 0.007 and P=0.0008, respectively) with ORs (95% CI) ranging from 1.79 (1.14 – 2.81) to 9.51 (2.64 – 33.88) [16-18]. Nevertheless, there has not yet been an adequate evaluation of the SNP in other populations, including Vietnamese. Thus, in this study, the genotyping method was developed and applied for the investigation of rs2853826.

High Resolution Melting (HRM) analysis is a powerful technique for the detection of polymorphisms in double-stranded DNA samples. The HRM process is achieved by using a fluorescent dye, which is then measured and plotted as a graph known as a melt curve, showing the level of fluorescence versus the temperature. Each SNP has three genotypes including two homozygotes and one heterozygote. Each genotype gives a melt curve that is slightly different. These tiny differences are accurately documented and therefore identified based on the high resolution process in the HRM machine. Thus, with a high quality HRM assay it is possible to distinguish between all three genotypes [19-21]. HRM has been showed to be suitable in principle for the detection of SNPs in the breast cancer susceptibility genes [22-27].

In this study, HRM was used to investigate the presence of rs2853826 in *MTND3* gene. The association between rs2853826 and breast cancer risk was then determined in a sample of the Vietnamese population with the aim to add to the literature and to improve the management of breast cancer in the future, not only in Vietnam, but all over the world.

## MATERIALS AND METHODS

### Subjects

Blood samples of case group were collected from patients before their surgeries who clinically have tumors. Meanwhile, healthy samples were from people who were free of any types of cancer at the time their blood was collected. All the samples are from Kinh people, have their ages matched (47.8 ± 4.7 and 46.3 ± 5.0 years old), and were taken under the approval of Ethical Committee of Oncology Hospital – HCMC Vietnam under the decision number 177/HĐĐĐ-CĐT, 18th November 2014.

A set of 100 breast cancer cases and 100 healthy controls have been selected for this study. Genomic DNA was extracted from whole blood using salting–out method following Hue et al.'s protocol [28] with some modifications. DNA samples were evaluated by spectrophotometry using the NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific, USA). High purity (A 260/A 280 = 1.7-2.0) and high concentration (>= 30ng/ul) DNA samples have been used.

### SNP genotyping development

*Primer design*

The reference sequence for SNP rs2853826 could be obtained through the SNP database on NCBI (http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=2853826#fasta).

Primers were designed using Primer3plus (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi). The predicted high-resolution melting curves were established using uMelt (https://www.dna.utah.edu/hets/umh.php). Primer-Blast (http://blast.ncbi.nlm.nih.gov/Blast.cgi) were used to test primers' specificity. OligoAnalyzer 3.1 (http://sg.idtdna.com/calc/analyzer) was

applied to check the secondary structure of primers.

*Optimization*

The optimization of annealing temperature (Ta), was carried out by the thermal gradient to determine the optimal Ta. A reaction consisted of 1X Toptaq Mastermix, 0.2µM forward primer, 0.2µM reverse primer, 10ng/µl DNA and molecular $H_2O$. The result was analyzed by electrophoresis. The optimal gel electrophoresis procedure was performed on 2% agarose gel, at 90V in 30 minutes, and 100bp DNA Ladder (ThermoFisher) was used as reference.

With the optimal Ta, the amplified PCR products were then analyzed to see the melting curve. Several randomized samples were performed HRM. The different melting curves are considered as different genotypes. HRM components in a reaction consisted 1 X Brilliant HRM Ultra-Fast Loci Master Mix, 0.2 µM forward primer, 0.2 µM reverse primer, 5 ng DNA and molecular $H_2O$. The PCR amplification of specific regions of mtDNA was performed on the basis of following cycling conditions: initial denaturing at 95 °C for 3 min followed by 95 °C for 5 s, 63 °C for 30 s for 40 cycles and HRM thermal condition at 95 °C for 30 s, the optimal annealing temperature for 30s and 95 °C for 30s for 1 cycles.

To provide positive genotype controls for further optimization and genotyping after that the possible genotypes samples selected from primary analysis melting curve were then sequencing using 3130 ABI system. Three genotype of SNP rs2853826 (AA, AG, GG) is expected to obtained.

*Evaluation of HRM method*

Stability (Tm range) and sensitivity were two criteria to determine whether the method was successful by running HRM unrepeatedly for 200 samples. Unlike the heterozygote which has distinct curve shape, two types of homozygotes yield similar shapes of curve but have different Tm. For melting peaks and different plot, the peak of each genotype of the SNP rs2853826 must be different from the others.

**Statistical analysis**

The optimal HRM condition obtained from the previous optimization stage above was applied to genotype 100 case and 100 control DNA samples with 3 positive controls AA, AG and GG. Finally, the frequencies of allele would be calculated by the following formula: $f(A) = f(AA) + ½ f(AG)$ and $f(G) = f(GG) + ½ f(AG)$ in which f(CC, CT or TT) is equal a number of individuals having the specific genotype divided by the sample population.

The association was calculated using the STATA version 12. The significant association was concluded based on the P-value (< 0.05). The genotypic and allelic frequencies were presented in percentage and their significant difference between case and control group was determined using the chi-squared test (Fisher correction) [29]. Odd ratios (OR) and 95% confident interval (95%) were used to evaluate the disease risk, which is either to decrease or increase the risk of breast cancer.

**RESULTS**

**SNP genotyping development**

*Design primer*

The most specific pair of primers for the detection of SNP rs2853826 were identified (Table 1).

**Table 1. Primers for HRM analysis and sequencing**

| Primer | Sequence (5'-3') | Tm ($^0$C) | Length | Amplicon length |
|--------|------------------|-----------|--------|-----------------|
| HRM-F | GCCCTAAGTCTGGCCTATGAGTGAC | 65.5 | 25bp | 90bp |
| HRM-R | TGAGTCGAAATCATTCGTTTTGTTT | 63.0 | 25bp | |

| SEQ-F | TTACGAGTGCGGCTTCGACCCTAT | 66.8 | 24bp | 392bp |
|-------|--------------------------|------|------|-------|
| SEQ-R | GGGAGGATATGAGGTGTGAGCGATA | 66.5 | 25bp | |

*Optimization*

For Ta optimization, PCR reaction with gradient temperature ranged from 57 $^0$C to 67 $^0$C was run and the result was analyzed using gel electrophoresis (figure 1). Gel electrophoresis result showed a bright band from 57 $^0$C to 65 $^0$C. However, 63 $^0$C was considered to be the most ideal Ta for the HRM analysis because this temperature might be the potential condition for primers to anneal and specificity of primers is increased.
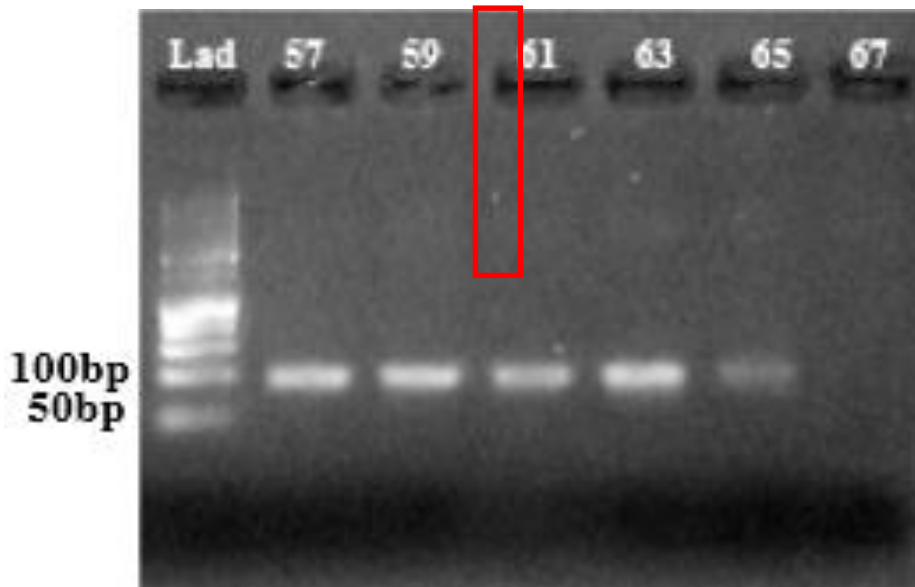


**Figure 1. Agarose gel electrophoresis of PCR products with different Ta.**

The optimal Ta at 63 $^0$C was applied for HRM to identify the positive control samples. Several DNA samples were chosen for HRM to analyze the melting curve as primary analysis. Three controls were identified and confirmed by sequencing (figure 2)
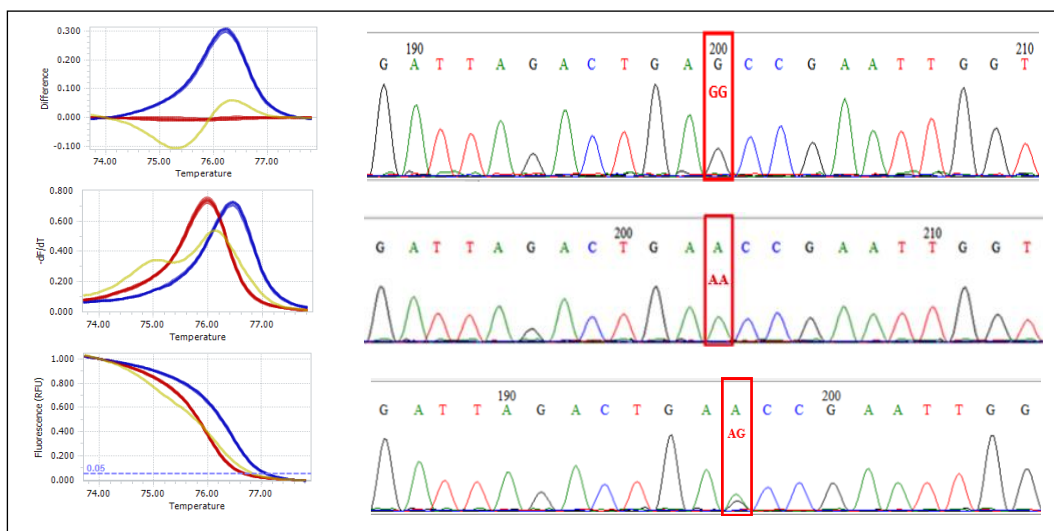


**Figure 2. Three Control screening at Ta = 63$^0$C**

Despite obtaining optimal condition for the HRM-PCR reaction, only 197 successful samples were able to produce data, including 98 case and 99 control. Most of sample having

clear curve and they are distributed into three distinctive groups which are presented for three different genotypes (Figure 3**)**.
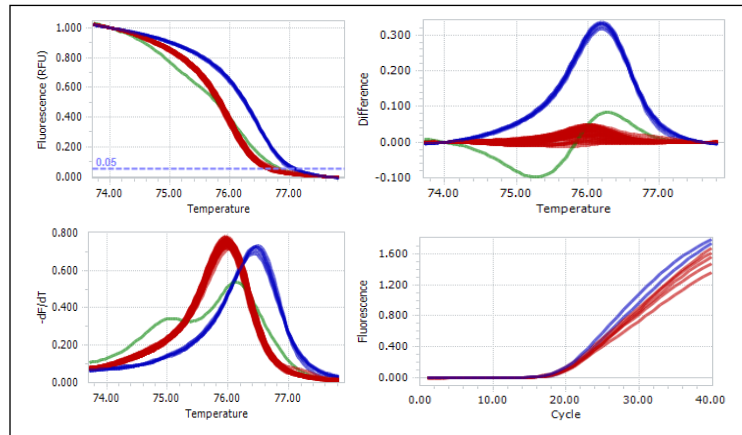


**Figure 3. The melting curves analysis of rs2853826 by HRM showed 3 different genotypes. The red, blue and green curve presented AA, GG and AG genotype, respectively.**

*Genotyping method evaluation*

*Sensitivity*: The optimal protocol was applied to genotype 200 DNA samples. Most of sample having clear curve and they are distributed into three distinctive groups which are presented for three different genotypes (Figure 3). With 197

successful samples, and 2 samples did not express any amplification signal and 1 sample having curve did not cluster into the three genotypes (Figure 4). With 3/200 samples are not counted we calculate the sensitivity of the HRM assay as 98.5%.
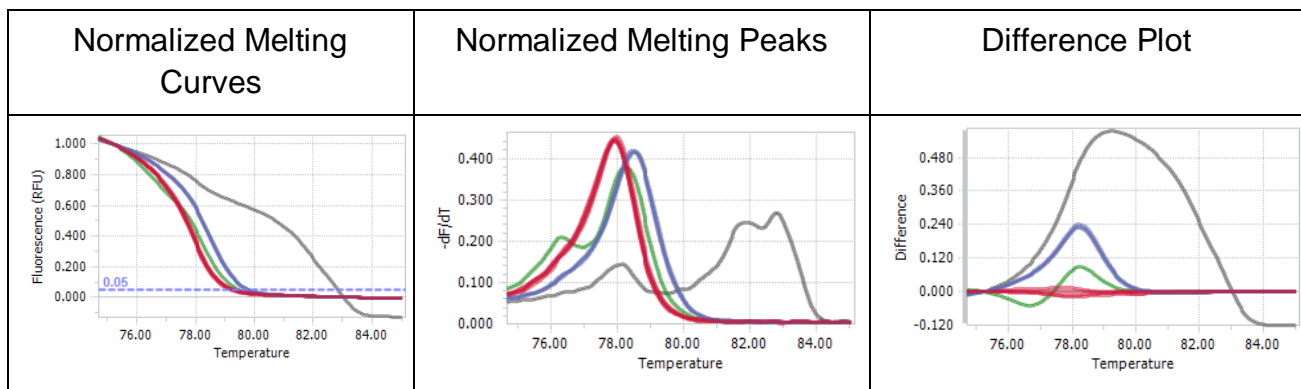


**Figure 4. The failed samples in black curve analyzed by HRM.**

*Stability:*

The stability of the method was determined by the Tm difference among control samples and distinct runs. As being shown in table 2, the difference in the average Tm values is hardly seen (0.01 – 0.06). These differences are not significant (0.01 – 0.04) between two

genotypes, according to Table 3. Therefore, the stability must be high to ensure a better distinction of all three genotypes of this SNP. The average Tms of the two homozygous genotypes (AA vs AG) are 0.44 away from each other. Meanwhile, heterozygous (AG) melting peak's curve was presented with two peaks,

which could be easily distinguished among the others. As a result, the genotypes of a large mount of samples can be easily determined by HRM method.

**Table 2. Average melting curve peak Tm value after HRM runs for SNP rs2853826**

|  | $\overline{T}m(^0C)$ | $\Delta\overline{T}m\ (^0C)$ |
|---|---|---|
| Tm (AA) | 75.74 | 0.06 |
| Tm (GG) | 76.18 | 0.05 |
| Tm (AG) | 75.89 | 0.01 |

**Table 3. Average melting curve peak ΔTm value after HRM runs for SNP rs2853826**

|  | $\Delta Tm(^0C)$ | $\Delta(\Delta\overline{T}m)(^0C)$ |
|---|---|---|
| AA-GG | 0.44 | 0.04 |
| AA-AG | 0.16 | 0.03 |
| AG-GG | 0.33 | 0.01 |

## Statistical analysis

### Allelic and Genotypic analysis

The main results of this analysis are listed in Table 4. The results show that the frequencies genotype of AA, AG, and GG were 82.8 %, 0.0 % and 17.2 % in cases group and 86.7 %, 2.0%, and 11.2 % in controls group, respectively. Additionally, the higher percentage (17.2 %) of allele G in 98 cases, compared with 99 controls (12.2 %), was strong evidence to conclude that allele G is a risk allele in the Vietnamese population. Therefore, Vietnamese people with a higher percentage of G allele in SNP rs2853826 may be expected to develop breast cancer.

**Table 4:  Genotypes and alleles frequency for rs2853826**

|  | Genotypes | | | Alleles | |
|---|---|---|---|---|---|
|  | **AA** | **AG** | **GG** | **A** | **G** |
| **Cases** **98** | 82 (82.8 %) | 0 (0 %) | 17 (17.2 %) | 164 (82.8 %) | 34 (17.2 %) |
| **Controls** **99** | 85 (86.7 %) | 2 (2 %) | 11 (11.2 %) | 172 (87.8 %) | 24 (12.2 %) |
| $P_{chi-square}$ | 0.19 | | | 0.17 | |

Results of $P_{chi-square}$ test for both genotypes and alleles were greater than 0.05 indicating that the frequency difference between the control and control groups was not statistically significant. This suggests that the polymorphism of rs2853826 does not affect the risk of breast cancer in Vietnam. Therefore, genetic models will be analyzed to determine the association of this SNP with breast cancer more clearly.

## *Association analysis*

The association analysis between this SNP and breast cancer was conducted by STATA version 12. The results are shown in Table 5. In allelic model, revealing that the G allele may enhance breast cancer risk 1.22 fold higher compared to A allele (OR [95%CI] =1.22[0.82-1.83]). However, the Chi-squared test of p-value showed higher than 0.05 (p=0.33) for allele difference between case and control group. It suggested that SNP rs2853826 was not significantly associated with breast cancer risk in the Vietnamese population. In addition, genotypic association analysis revealed that GG and GA carriers in genotype also tends to enhance the breast cancer risk compared to AA carriers (OR$_{additive}$[95%CI]=1.60[0.71-3.63]; OR$_{dominant}$ [95%CI]= 1.36 [0.62-2.97]). It seems that A allele has a recessive effect on the disease, but logistic analysis showed that SNP rs2853826 has no relationship with breast cancer risk (p=0.26 and p=0.23, respectively). In other hands, results analysis for additive model and recessive model compared with reference group were 0.17 and 0.44, respectively. Therefore, there was a non-significant association between this SNP and breast cancer in the Vietnamese population.

### Table 5: Association analysis of SNP rs2853826

| Association analysis | Analysis model | | OR | 95%CI | P-value | Power |
|---|---|---|---|---|---|---|
| Allelic | G vs A | | 1.22 | 0.82-1.83 | 0.33 | |
| Genotypic | Additive | GG vs AA | 1.60 | 0.71-3.63 | 0.26 | 7.57 % |
| | | GA vs AA | 0.21 | 0.01-4.38 | 0.17 | |
| | Recessive | GG vs [GA+AA] | 0.61 | 0.27-1.38 | 0.44 | |
| | Dominant | (GA+GG) vs AA | 1.36 | 0.62-2.97 | 0.23 | |

## IV.    DISCUSSION

This is case-control study, which is the first study investigated the genotype frequency and the association between SNP rs2853826 with breast cancer in Vietnamese population, using the optimal High Resolution Melting method. In terms designing primers, many powerful and available tools were applied in order to test the specificity. An appropriate pair of primers for amplification of the SNP was obtained and satisfied all the requirement such as the SNP located in the center position, primers sizes were from 20bp to 25bp or the Tm were from 57ºC to 67ºC. A new pair of primers with the high specificity was also designed with the larger product size to perform the sequencing analysis. Sequencing results of two positive controls also confirmed that there was no other polymorphism beside our interest SNP. Highly specificity primers are presented in Table 1. In terms of the genotyping method, with optimal HRM conditions were just 3 failed samples (3%) detected in 200 BC samples. One of them was illustrated by an abnormal melting curve (Figure 4). The possible reason was that the DNA sample was contaminated with the other primers during the preparation the HRM mix to form the nonspecific product. The two other failed ones were shown to be non-amplification signal, which meant no melt curve was exhibited. That mistake could be due to the problem of DNA sample and results to non-amplification of DNA. Meanwhile, the melting curves of 97 successful genotyped samples (98.5%) were always well separated into 3 groups. There was not any curves shifted away the control more than 0.06 $^{0}$C (Table 2). This strongly demonstrated the accuracy as well as the sensitivity of HRM analysis. The discrimination between two genotypes was significantly distinguishable (Tables 2, 3).

Furthermore, three genotypes could be discerned by the shape of their melting curves, melting peaks, and/or different plots. In this study, we successfully genotyped all samples by HRM with high stability and accuracy.

In this study, the frequency of a minor allele (G allele) was 12.2 % in control group and increase to 17.2 % in case group. Compared with previous studies in Asia, the frequency of G allele ranged from 53.3 % to 54.0 % [16, 30], these results suggest that the Vietnamese population are distinct from other populations concerning this SNP frequency.

According to previous studies, there are different conclusions about the association between SNP rs2853826 and breast cancer in different populations. It suggests that association of SNP rs2853826 with breast cancer depends on the ethnic group. According to a study in Malaysian population has sample size similar to our study (101 case/90 control) showed that allele G significantly increased the risk of invasive breast cancer (P = 0.007, OR = 2.29 , 95 % CI = [1.25-4.20]), but allele risk accounted for 73 % in the cases group compared to controls group (54 %) [16], significantly difference with Vietnamese population (17.2 % and 12.2 %, respectively). In 2005, Canter et al., reported A allele increase the risk of invasive breast cancer African-American OR = 1.6, 95% CI = [1.1-2.31], P = 0.013, but no correlation with white women OR = 1.03, 95% CI = 0.81- 1.31] [31]. Until 2008, a further studies of Setiawan et al., on a larger sample including 1456 breast cancer patients and 978 healthy African American women (with data pooled across the studies) had not found the correlation between this SNP and breast cancer risk (OR=1.14, 95%CI=0.80-1.62, P=0.47) as previously concluded of Canter [32]. The other evidences also showed that the mitochondrial DNA (mtDNA) G10398A polymorphism significantly increased breast cancer risk including Northern Indian women (P = 0.01, OR = 1.73 (95% CI [1.13 - 2.66]) [15], European-American (P=0.01,

OR=1.79, 95%CI=1.14–2.81]) [18], Poland P=0.0008, OR [95% CI]=9.51 [2.64–33.88]) [17] and Bangladesh P = 0.0182, OR [95% CI] = 5.5 [1.53-20.5] [33]. Moreover, the evidences also suggests that rs2853826 causes functional defects in I complex of the respiratory chain, therefore affect the apoptosis through increased levels expression of Bax protein (pro-apoptotic) compared with Bcl-2 (anti-apoptotic protein) in cells breast cancer of patient carrying G allele (P = 0.016) [16]. In contrast, many studies observed that SNP rs2853826 has not association with disease. As according a study in the Chinese Han population (506 cases and 520 controls) failed to support the association of rs2853826 and breast cancer risk (P = 0.545), allele G was no difference in both the control and case groups (51.4 % and 53.3 %, respectively) [30]. The genotype of rs2853826 varied with histological types and Her2 status of breast cancer patients, but was not associated with other clinical parameters such as age, menopausal status, age at menarche, number of pregnancies, number of live births, tumor size, lymph node metastasis, or hormone receptor status. It seems that both A and G alleles are not deleterious alleles for breast cancer risk in Han Chinese women. A recent meta-analysis showed a similar negative overall correlation between this polymorphism and breast cancer risk [34]. Besides, the study of Ismaeel et al. has not found variants of rs2853826 in malignant tumor group and control groups but it determined in benign tumors group (9 %), provides further evidence of this SNP unrelated breast cancer [35]. In addition, some other studies also show rs2853826 was no relationship to ER-positive and PR-positive samples [15, 30]. Moreover, ND3 is one of seven subunits encoded by mitochondrial genome of complex I, which is the largest and the most complicated enzyme of the mitochondrial respiratory chain. The A to G polymorphism at 10398-nucleotide position in ND3 causes the amino acid substitution from threonine—a polar, neutral amino acid for

alanine-a nonpolar [36]. This alteration could leads to structural change in mtDNA-encoded protein ND3 subunit and improper protein folding. It then consequently alters Complex I structure and function; therefore it promotes the impaired electron transport function and the increased ROS production. Thus polymorphism in ND3 subunit has been proposed to contributing in developing cancer. During the process of ROS-associated OXPHOS, certain mtDNA mutations may generate increased superoxide and nitric oxide and lead to aberrant mitochondrial biogenesis. It was reported that mtDNA rs2853826 polymorphism in the ND3 gene influenced breast cancer susceptibility in African-American women [14]. The studies proposed that the A allele may be deleterious in African-American populations because African mitochondrial haplotypes are prone to generate more ROS than mitochondria in other ethnic populations, or because coexistent mutations in other mitochondrial or nuclear genes decrease cellular capacity to manage oxidative stress. However, results of later research regarding this polymorphism were contradictory, as mentioned above [15, 17, 18, 37].

In this study, although both the additive and dominant models proved that G allele tended to enhance the disease risk as odd ratio range from 1.36 to 1.6, but the GG genotype had no effect on breast cancer susceptibility (p>0.05). Compared to AA, GG may increased 1.6-folds the risk of breast cancer. However, P>0.05 illustrated no significantly statistical effect. Besides, in terms of additive models, allele A may confer an adverse effect when GA vs AA with the OR=0.21, which implied that allele G may act as recessive role. Result from recessive model has supported this, when A appear, the OR is less than 1. Meanwhile, GG increased 1.6 times the risk compared to AA, but when allele A also presented along with G in dominant model, the effect decrease to 1.36 (P=0.23). Therefore, allele G confers a relatively weak susceptibility in the breast cancer risk when G vs A resulted in an OR=1.22 with no significant effect 95%CI=0.82-1.83, P=0.33, no statistical significance. Therefore, no significant association between SNP rs2853826 and the risk of breast cancer in Vietnam has been found in this study. Nevertheless, the humble sample size (98/99 cases/controls) indicates an unreliable result, for the power of this study is only 7.57% and the lack of available information leading to the inaccurate evaluation of the affect of aging, smoking status, alcohol consumption, etc. with the risk of breast cancer. Thus, it is necessary to conduct the study in the future on a larger scale so as to achieve a more precise association status between the studied SNP and the breast cancer risk in Vietnam.

## V. CONCLUSION

In this study, HRM method was successfully optimized and proved to be a powerful technique for genotyping SNP rs2853826 with the very high sensitivity of 98.5 % (197/200 samples identified genotypes). Our study is the first to examine the SNPs rs2853826 and their effect on breast cancer risk. This SNP have been annotated in the NCBI SNP database, but no frequency data is currently available. With sample size including 99 controls and 98 cases, our study suggested that SNP rs2853826 was not associated with breast cancer in Vietnamese women, but the power of this study is very low. Hence, the future study needs to be investigated with a larger sample size in order to confirm the result of our study.

## VI. ACKNOWLEDGMENT

## VII. AUTHOR CONTRIBUTION

Hanh T.H. Duong contributed to acquisition, analysis, interpretation of data, drafting of manuscript. Thanh T.N. Nguyen reviewed and

edited the manuscript for intellectual content. Hue T. Nguyen oriented, gave important idea and revised the manuscript of this review. All authors gave final approval of the version to be published.

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