

RESEARCH ARTICLE

No Association of the rs17822931 Polymorphism in ABCC11 with Breast Cancer Risk in Koreans

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Abstract

ABCC11 is reported to be associated with breast cancer. However, whether ABCC11 polymorphisms relate to breast cancer risk remains unclear. This study aimed to evaluate any association of a single nucleotide polymorphism (SNP), rs17822931, in ABCC11 with breast cancer in Koreans. Genomic DNA samples of 170 women with breast cancer and 100 controls were assessed for SNP rs17822931 of ABCC11 by single-strand conformation polymorphism (SSCP) and DNA sequencing. A 27-bp deletion ($\Delta 27$) of ABCC11 was analyzed by PCR amplification. The genotype of SNP rs17822931 was confirmed to be AA in all samples from breast cancer patients and $\Delta 27$ was found in none of the samples. Our finding indicated that the SNP rs17822931 in ABCC11 is not associated with breast cancer. However, this study does provide information on fundamental genetic aspects of ABCC11 with regard to breast cancer risk in Koreans.

Keywords: *ABCC11* - breast cancer susceptibility - single nucleotide polymorphism (SNP) - Koreans

Asian Pac J Cancer Prev, 17 (5), 2625-2628

Introduction

Breast cancer is the most common type of epithelial cancer among women, the incidence of breast cancer has been increasing in the world where it accounts for 23% of all cancers and 400,000 deaths each year (Gaudet et al., 2009). Although numerous genetic association studies on breast cancer have been published, there are few candidate genes including HER2, CyclinD1, EBCA1/2. In addition genes, the ABCC11 gene was also reported association with breast cancer risk.

The ABCC11 gene is an ATP-binding cassette (ABC) transporter located on human chromosome 16q12.1 (Yabuuchi et al., 2001). A non-synonymous coding SNP at nucleotide 538 (c.538G/A; rs174822931) of ABCC11 has been shown to underlie the formation of either wet or dry earwax which GG and GA genotypes correspond to the wet type and AA corresponds to the dry type (Yabuuchi et al., 2001). This gene has also strong associations with axillary osmidrosis (Zhu et al., 2015) and apocrine colostrum secretion from the mammary gland (Toyoda et al., 2009).

The SNP rs17822931 of the ABCC11 was first suggested to be associated with breast cancer risk due to an observed relationship between the wet earwax phenotype and breast cancer in Japanese women. In the rs17822931 allele, Breast cancer group showed a higher proportion of the G allele than in normal group (Petrakis,

1971). A potential link with breast cancer susceptibility in the ABCC11 genotype was found among Asian women (Petrakis, 1971; Petrakis et al., 1990). And breast cancer risk was reported to be high in Japanese, the G allele appears to be positively related to breast cancer frequency in this groups (Ota et al., 2010). However, this has controversial results in Europeans. SNP rs17822931 of the ABCC11 has not association for Caucasian breast cancer patients (Beesley et al., 2011) and shown no evidence for relationship of this SNP with breast cancer risk (Lang et al., 2011). Therefore, additional genotyping studies are essential to shed light on an existing disagreement.

In addition, one exceptional mutation, a 27-bp deletion (3939-3965del 27; $\Delta 27$) in exon 29 of ABCC11, showed discordance between phenotype (dry earwax) and genotype (GA heterozygous for each of SNP) (Kitano et al., 2008). The $\Delta 27$ found in the G allele in ABCC11. This finding suggested a loss of function conferred by the G allele, leading to the dry phenotype (Sato et al., 2009).

At the present time, it is uncertain whether G allele of ABCC11 actually influences breast cancer risk. The purpose of this study therefore genotyped the SNP rs17822931 to examine the association between breast cancer and the G allele frequency, and evaluate the frequency of $\Delta 27$ in Korean breast cancer patients.

We have analyzed the genotype at rs17822931 and $\Delta 27$ of ABCC11 in Korean breast cancer patients. The

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results were shown that the SNP rs17822931 was limited to the AA genotype, and $\Delta 27$ was not presented in Korean breast cancer patients. Nevertheless, the present results have addressed fundamental genetic aspects of ABCC11 and useful information as one of genetic markers with breast cancer.

Materials and Methods

Materials

To determine the genotypes of the ABCC11 gene, genomic DNA samples from 170 breast cancer patients and 100 healthy controls were obtained from the Biobank of Dong-san Hospital (Deagu, Korea). These samples were used for experiments after approval of the study by the Institutional Review Board (IRB) of Keimyung University, Daegu, Korea (40525-201511-BR-82-02).

Single-strand conformation polymorphism (SSCP)

For SSCP, genomic DNA samples were used to genotype a SNP rs17822931 of ABCC11 located in exon 4 of chromosome 16. PCR was performed in a final reaction volume of 30 μ l containing ~50 ng of genomic DNA, 0.5 μ M of each primer, 0.2 μ M of each dNTP, 1.5 mM of MgCl₂, 1 \times PCR buffer, and 0.01 U/ μ l Taq DNA polymerase (Toyobo Co., Japan). Amplification was performed with an initial denaturation at 95°C for 5 min followed by 35 cycles of 95°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 1 min, with a final extension at 72°C for 5 min. Amplified PCR products were subjected to SSCP analysis. Electrophoresis was performed in 10% polyacrylamide gel with 1 \times TBE buffer (90 mM Tris-borate, 2 mM EDTA, pH 8.3). Gels were silver stained, dried, and scored manually for SSCP variants (Ha et al., 2005). The primer sequences for the SNP rs17822931 of ABCC11 are listed in Table 1.

DNA sequencing

Purified PCR products were sequenced using a BigDye Terminator Cycle Sequencing kit (Applied Biosystems, USA) on an ABI 3730 Genetic Analyzer, and the raw sequence data was edited manually using Chromas Ver. 2.4. Each of the identified SSCP variants was sequenced from both ends using both forward and reverse primers (Dubey et al., 2015).

Detection of $\Delta 27$ in ABCC11

PCR amplification was performed under standard conditions. A 10 μ l aliquot of each PCR product was subjected to 3% agarose gel electrophoresis. The primer sequences for $\Delta 27$ are listed in Table 1 (Kitano et al., 2008).

Statistical analysis

The distribution of polymorphisms was compared between control and breast cancer using chi-square test. Statistical analysis using the chi-square test was also performed a comparison between total experimental patients and controls.

Results

The genotypes of ABCC11 were AA in Korean breast cancer patients

To determine the genotype of ABCC11, genomic DNA samples were used for the measurement of SNP (538G>A) frequency in breast cancer patients by SSCP and DNA sequencing after PCR amplification (Figure 1).

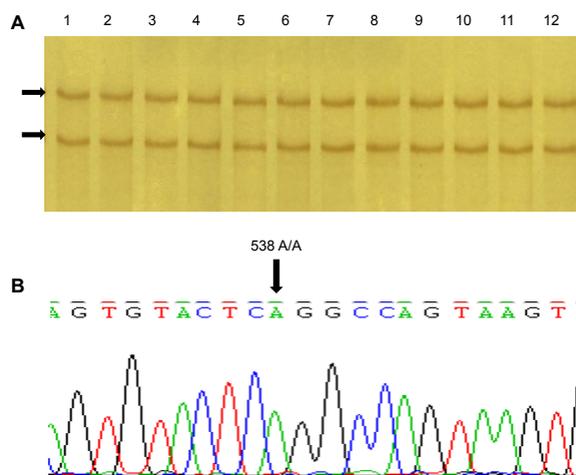


Figure 1. SSCP and DNA Sequences of Chromatograms for the Detection of SNP rs17822931 of ABCC11 in Korean Breast Cancer Patients. (a) SSCP was performed for the detection of a single-strand DNA fragment of the SNP rs17822931 of ABCC11 by electrophoresis on 10% polyacrylamide gel. (b) DNA sequencing of the SNP (538G>A) region was performed for exon 4 of ABCC11 on chromosome 16q12.1

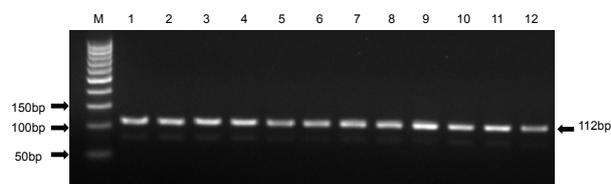


Figure 2. PCR Detection of $\Delta 27$ of ABCC11 in Korean Breast Cancer Patients. PCR products of $\Delta 27$ at exon 29 of ABCC11 were detected in 3% agarose gel (112 bp).

M: molecular size marker (50 bp DNA ladder)

Table 1. Primer Sequences for PCR-SSCP and Detection Assay

| Primer | Location | Sequence | Size |
|----------------|--------------------|--|--------------------------------|
| SNP rs17822931 | Chr. 16 exon 4 | F: 5'-GCT TCT GGT GAT GCT GAG GT-3' R: 5'-ACC ACC ATG CTC TAC TGT CC-3' | 254 bp |
| $\Delta 27$ | Chr. 16 exon 29 | F: 5'-CTT ATC GAT GAA GCC ACA G-3' R: 5'-TGA CAC GGT GGG CAA TGA C -3' | WT: 112 bp $\Delta 27$: 85 bp |

$\Delta 27$ indicates a 27 bp deletion mutation, WT indicates the wild type allele of ABCC11.

We carried the SNP homozygous (AA) alleles from all Korean samples by DNA sequencing ($p = 1.00$). All single-strand DNA fragments showed the same pattern by SSCP electrophoresis (Figure 1A). The sequence of rs17822931 of ABCC11 in exon 4 showed the AA genotype in all breast cancer samples (Figure 1B).

$\Delta 27$ deletion in the ABCC11 was not detected in breast cancer patients

Fragments including the $\Delta 27$ of ABCC11 in exon 29 were PCR amplified from Korean samples. The electrophoretic fragment size of $\Delta 27$ of ABCC11 for wild-type allele was 112 bp and for $\Delta 27$ was 85 bp. All specimen detected as 112bp, and $\Delta 27$ was not found in Korean breast cancer patients (Figure 2).

Discussion

The present study estimated the uncertain association between earwax type associated SNP of ABCC11 and breast cancer risk in Korean. The SNP rs17822931 of ABCC11 in Korean patients with breast cancer was shown to carry the AA genotype in all samples. The studies by Matsunaga (Matsunaga, 1962) and Petrakis et al. (Petrakis et al., 1971) reported that the AA genotype in ABCC11 showed 30%–50% prevalence in South Pacific islands, Central Asia, and North America. In worldwide research, the AA genotype has been frequently observed (80%–95%) among East Asians, but it is uncommon (0%–3%) in European and African populations. Northern Han Chinese and Koreans showed the highest relative frequency (100%) for the A allele. In terms of G allele, frequency of the SNP rs17822931 showed geographical gradient distributions from East Asia to Africa, the GG and GA genotypes predominate in 90% of the Caucasian population, whereas in Asians the G allele was observed at a frequency of <20% (Yoshiura et al., 2006). In our results, all breast cancer samples had the AA genotype at SNP rs17822931 of ABCC11 which consistent with the proportion in Chinese subjects. This result described there is no evidence of association in SNP rs17822931 of ABCC11 with breast cancer.

In previous study, Kim (Kim, 2008) reported frequency of ABCC11 in Korean population related to phenotype of earwax which AA genotype included 98.4% and only 1.6% of AG for genotype. So that, we hypothesized that the distribution of polymorphism for AG genotype was possibly associated with frequency of breast cancer in Koreans. However, our results showed that the AA genotype was completely prevalent in Korean breast cancer patients, and the present data were contrast with a recent study in which GA and GG genotypes were associated with breast cancer susceptibility in a Japanese population. In this respect, our findings are useful as one of genetic information markers with breast cancer, because the frequency of genotype for ABCC11 marked difference among East Asia populations. In addition, $\Delta 27$ of ABCC11 was amplified by PCR from Korean sample with breast cancer. The fragment is known as one of the factors that determine the formation of dry earwax. The prevalence of $\Delta 27$ has been reported as 0.29% in Japanese, 10% in

North Americans and 2% in Bolivians, but 0% in Koreans to date (Yoshiura et al., 2006; Kitano et al., 2008). In the present study, $\Delta 27$ of ABCC11 was not detected in Korean breast cancer samples. However, it remains unknown, where and when $\Delta 27$ of ABCC11 originated.

Since a possible factors with breast cancer risk is histopathology data in the clinical study, clinicopathological features including tumour size, HER2 status, triple negative tumour phenotype, nuclear grade, tumour stage were investigated in the previous studies (Ota et al., 2010; Lang et al., 2011). Neither chi-square tests nor logistic regression analysis revealed any statistically significant difference between the genotype of ABCC11 and clinicopathological features. According to Lang et al. (Lang et al., 2011) study, breast cancer risk was not statistically associated with respect to in general information (menopausal status, family history of breast cancer, use of oral contraceptives, body mass index and smoking). Although ABCC11 mRNA is reportedly overexpressed in breast cancer tissues and cell lines (Bera et al., 2001; Yabuuchi et al., 2001; Bieche et al., 2004), few reports have described the expression of the ABCC11 protein in human cancer (Toyoda et al., 2009; Ishikawa et al., 2012). In this paper, we did not observe differences in breast cancer by the genotype of SNP rs17822931 in Korean samples. In conclusion, our study showed negative results in relationship between ABCC11 and breast cancer, however, the present results will help to provide fundamental genetic aspects including new insight of pharmacogenetics into breast cancer.

Acknowledgements

This work was supported by the National Research Foundation of Korea (NRF) Grant funded by the Korean Government (MSIP) (No. 2017R1A5A2010008).

The research was supported by the Ministry of Science, ICT and Future Planning Through the Development for IT·SW industrial convergence original technology (ID: R0101-15-0147).

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