Association of Single-Nucleotide Polymorphisms of CD44 Gene with Susceptibility to Breast Cancer in Chinese Women

Background: This study aimed to evaluate the association of CD44 gene single-nucleotide polymorphisms with susceptibility to breast cancer.

Material/Methods: This case-control study included 242 breast cancer patients and 252 normal people without disease. The single-nucleotide polymorphisms of the CD44 gene in the 2 groups were genotyped by PCR-LDR method. The OR and its 95% CI was calculated by chi-square test and logistic regression analysis. The construction of haplotypes and their interaction analysis with relevant factors were carried out by SHEsis and SNPStats online.

Results: The genotype distribution of CC and CT, CC and CC+CT, and CC+CT and TT in rs13347 showed a significant difference between cases and controls, and the difference in distribution of alleles C and T was statistically significant. The genotype and alleles distribution of rs4756195 and rs8193 showed no statistically significant difference (P>0.05). The haplotypes distribution of CAC, CGT, TAC, and TGT showed a significant difference between the 2 groups (P<0.05). The results of analysis of haplotypes and their interactions with relevant factors showed that breast cancer risk in the PR-negative group was significantly higher than that in the PR-positive group (P=0.016). We found an interaction between haplotypes and PR status.

Conclusions: The genotypes CT, CT+TT, TT, and allele T in rs13347 may be risk factors for breast cancer. The haplotype CAC may be a protective factor against breast cancer, and CGT, TAC, and TGT may be risk factors for breast cancer. The PR status interacts with CD44 gene SNP.

MeSH Keywords: Antigens, CD44 • Polymorphism, Single Nucleotide • Triple Negative Breast Neoplasms

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**Background**

Breast cancer is one of the most common tumors in women [1]. According to data released by the World Health Organization (WHO), the new cases of breast cancer reported in 2012 accounted for 25.2% of all female primary cancers. In China, the number of new cases of breast cancer was recently reported as 187,000, ranking first in the incidence rate of female cancers and posing a serious threat to the health and quality of life of Chinese women [2].

Cluster of Differentiation 44 (CD44) is a multi-structural and multi-functional transmembrane glycoprotein, which acts as the receptor of hyaluronic acids and many other extracellular matrix components, and is also the receptor of multiple growth factors and cytokines [3]. Most studies have shown that CD44 is involved in many key cellular processes, including cell growth, reproduction, differentiation, adhesion, and migration [4–8]. CD44 is also a common tumor marker closely related to the proliferation, migration, and invasion of tumor cells, as well as being involved in tumor-related angiogenesis. Studies have found that CD44 is widely expressed in a variety of cancer cells, affecting the prognosis of the disease, and that specific knockdown of CD44 inhibits the genesis and development of cancer [9].

There have been many studies on the single-nucleotide polymorphisms (SNPs) of breast cancer susceptibility genes. However, these SNP genes, including BRCA1 and BRCA2, can only explain about 25% of breast cancer susceptibility and morbidity. Previous studies have shown that the CD44 gene SNPs are detected in 84% of breast cancer patients and 68% of healthy people. Therefore, the CD44 gene may be one of the high-penetration genes affecting breast cancer susceptibility [10]. There have been a few studies on the correlation of CD44 single-nucleotide polymorphism and the risk of breast cancer, which showed that CD44 gene SNPs can affect the susceptibility to breast cancer [11,12].

Therefore, in this study we selected 3 sites of CD44 single-nucleotide polymorphism: rs13347, rs4756195, and rs8193, and performed genotyping among the population in Beijing. Case-control studies were carried out to investigate the relationship between SNPs of CD44 gene and the susceptibility of breast cancer in the population of Beijing, China.

**Material and Methods**

**Research objectives**

For the case group, we randomly selected 225 breast cancer patients who were without family history and who were pathologically diagnosed in the Ruian People’s Hospital of Zhejiang Province from January 2016 to December 2016. For the control group, we included 237 healthy women who came to the hospital for physical examination during the same period of time. The patients in the case group were 35–75 (53±7.93) years old, and the people in the control group were 34–78 (52.03±8.11) years old. The healthy people in the control group showed normal clinical and laboratory examination results and were without tumor history or family history. All the people in the case group and the control group signed informed consent and the study was approved by the Ethics Committee of Ruian People’s Hospital of Zhejiang Province.

**Sample collection**

Subjects in the case group and the control group were instructed to fast for the morning before 2 ml of peripheral venous blood was collected. After ethylene diamine tetraacetic acid (EDTA) anticoagulation, the blood sample was sub-packed and stored in a freezer at −80°C.

**Sample size estimation**

According to the preliminary experiments and literature reports, the mutant allele frequency of CD44 in the control group (Pc) was set as 0.1, the odds ratio of the case group and the control group (OR) was set as 2, with α=0.05 and the power 1-β=0.85. The sample size was calculated by use of PASS 11.0, and the resulting sample size of each group was 196. There were more than 196 subjects in both groups, thus meeting the test requirements.

**Polymorphic loci selection**

We selected 3 SNPs on the CD44 gene based on 1 of the following screen conditions: I. It has been reported to be associated with breast cancer; II. It has heterozygosity and the minimum allele frequency (MAF) >0.05; III. It locates on the gene fragment that can cause functional changes. We selected the CD44 gene label tag SNPs in the Chinese population through the online site http://gvs.gs.washington.edu/GVS144/ and searched for those with the minor allele frequency (MAF) >0.05 while r² >0.8. Finally, we found 3 representative SNP loci on the CD44 gene: rs13347, rs8193, and rs4756195. Detailed information on the selected SNP loci is shown in Table 1.

**Genotyping**

Polymerase chain reaction and ligase detection reaction (PCR-LDR) method was used to detect the polymorphism of the 3 SNP loci on CD44. PCR amplification products containing the target DNA were cut at the specific-recognition sites by restriction endonucleases, and the SNP loci were determined by gel
electrophoresis according to the size and number of restriction fragments. Primers were designed using data from the National Center for Biotechnology Information (NCBI) and the primer design software Primer Premier 5, and primers were synthesized by Invitrogen, Shanghai. The primer sequences are listed in Table 2.

**Statistical analysis**

IBM SPSS 21.0 software was used to analyze the results. The genotype and outcomes were tested by Hardy-Weinberg (H-W) equilibrium. The chi-square test was used to compare the phenotype and allele frequencies as well as the degree of coincidence between the 2 groups. The odds ratio (OR) and the 95% confidence interval (CI) were calculated according to the characteristics of the data using the chi-square test and single-factor unconditional logistic regression analysis, and the results were used to compare the relative risks of breast cancer of the genotype with polymorphic loci mutations and that with wild-type homozygosity. Linkage disequilibrium analysis was conducted using SHEsis online software (http://analysis.bio-x.cn/myAnalysis.php). SNPStats online software (http://bioinfo.iconcologia.net/snpstats/start.htm) was used to construct haplotypes and analyze the interactions with related factors. The 2-sided hypothesis test was used and the significance level was set as α=0.05.

**Results**

**Comparison of general data between groups**

A total of 494 research subjects were enrolled in this study, including 242 patients with breast cancer and 252 healthy people as controls. The general data of the case group and the control group is shown in Table 3, and the group comparison results showed that there was no significant difference in age, body mass index (BMI), estrogen receptor (ER) status, progesterone receptor (PR) status, or human epidermal growth factor receptor-2 (HER-2) status between the 2 groups (P>0.05) (Table 3).

**Hardy-Weinberg equilibrium analysis of the polymorphism genotypes in the 2 groups**

The 3 SNPs of the CD44 gene were tested by Hardy-Weinberg equilibrium and the results were all in line with the equilibrium (P>0.05), suggesting that the subjects in this study had no significant natural selection or migration and that they were representative of the population (Table 4).

**Distribution of the genotype and allele frequencies of the 3 SNP loci on CD44 gene in the case group and the control group**

Single-factor logistic regression analysis showed that the distribution of the genotypes CC and CT, CC and DT+TT, and CC+CT and TT, as well as the frequency of C allele and T allele of locus rs13347, exhibited significant differences between the case group and the control group (P<0.05), suggesting that T allele might be a risk factor for breast cancer. There was no statistically significant difference between the distribution of genotypes and allele frequencies of loci rs4756195 and rs8193 between the 2 groups (P>0.05) (Table 5).

**Correlation of CD44 haplotype with breast cancer risks**

We analyzed the differences in haplotype distribution between the case group and the control group, and the results suggested that the haplotype CAC (OR=0.656, 95%CI: 0.44–0.95) was significantly associated with breast cancer risk.

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**Table 1.** Detailed information on the 3 SNP loci on CD44.

| SNP     | W>M* | Mutation position | MAF  | Location         |
|---------|------|-------------------|------|------------------|
| rs13347 | C>T  | 3’UTR             | 0.29 | 11: 35231725     |
| rs4756195 | A>G  | Intron            | 0.14 | 11: 35176485     |
| Rs8193  | C>T  | 3’UTR             | 0.44 | 11: 35229771     |

* W means wild-type gene, M means mutant gene.

**Table 2.** The SNP primer sequences and product sizes.

| SNP     | Primer L                  | Primer R                  | Length |
|---------|---------------------------|---------------------------|--------|
| rs13347 | 5’-AGGCTGAGAGAGGTAT-3’     | 3’-CCAGAGTTAGCCCTTGAGA-5’ | 250 bp |
| rs4756195 | 5’TGCCCTCGTCCGAAGTATG-3’  | 3’-CGAGACCTCTGGCTAACA-5’  | 314 bp |
| Rs8193  | 5’TGGAAACATAACCATTACGGGAG-3’ | 3’-CCAAGGTGGAACGTAACCA-5’ | 220 bp |
0.507–0.848, P=0.001) was a protective factor against breast cancer while the haplotypes CGT (OR=2.235, 95%CI: 1.161–4.304, P=0.014), TAC (OR=1.974, 95%CI: 1.347–2.893, P<0.001) and TGT (OR=2.313, 95%CI: 1.080–4.957, P=0.027) showed significantly higher distributions in the case group than in the control group, and therefore are risk factors for breast cancer (Table 6).

Interactive analysis of genotype and the related factors

We performed interactive analysis of CD44 haplotype and the related factors by analyzing the interactions of the 8 haplotypes of 3 SNPs loci with age, BMI, ER status, PR status, and HER-2 status, while the haplotype CAC served as the control. The results showed that there were interactions between haplotypes and PR (P =0.016), but there was no interaction between haplotypes and age, BMI, ER, or HER-2 (P>0.05). Compared with the PR-positive haplotype CAC, the risk of breast cancer was increased by the haplotype CAC in the PR-negative group. The analysis results are shown in Table 7.

### Table 3. The distribution of general physiological and biochemical indexes between the 2 groups.

| Index                  | Grouping | Case group | Control group | t/χ² | P     |
|------------------------|----------|------------|---------------|------|-------|
| Ages                   |          | 53.00±7.93 | 52.03±8.11    | 1.341 | 0.181 |
| Age groups             | ≤50      | 93         | 111           | 1.607 | 0.205 |
|                        | >50      | 149        | 141           |      |       |
| BMI                    | ≤25      | 143        | 165           | 2.144 | 0.143 |
|                        | >25      | 99         | 87            |      |       |
| Tumor grading          | I        | 106        |               |      |       |
|                        | II       |             | 96            |      |       |
|                        | III      |             | 40            |      |       |
| Lymph node status      | Positive | 114        |               |      |       |
|                        | Negative | 128        |               |      |       |
| Tumor size (cm)        | T1: ≤2   | 96         |               |      |       |
|                        | T2: >2, ≤5 | 78       |               |      |       |
|                        | T3: >5   | 68         |               |      |       |
| ER* status             | Positive | 123        | 114           | 1.545 | 0.214 |
|                        | Negative | 119        | 138           |      |       |
| PR** status            | Positive | 139        | 135           | 0.747 | 0.387 |
|                        | Negative | 103        | 117           |      |       |
| Her-2* status          | Positive | 114        | 130           | 0.991 | 0.319 |
|                        | Negative | 128        | 122           |      |       |

*ER – estrogen receptor; **PR – progesterone receptor; * Her-2 – human epidermal growth factor receptor 2.

### Table 4. Hardy-Weinberg equilibrium analysis of the 3 SNP loci in the case group and the control group.

| Grouping | SNP       | WW | WM | MM | W  | M  | HWE χ² | P     |
|----------|-----------|----|----|----|----|----|--------|-------|
| Case group | Rs13347   | 102| 104| 36 | 308| 176| 1.235  | 0.266 |
|           | Rs4756195 | 123| 107| 12 | 353| 131| 3.480  | 0.062 |
|           | Rs8193    | 116| 97 | 29 | 329| 155| 1.524  | 0.387 |
| Control group | Rs13347 | 138| 94 | 20 | 370| 134| 0.498  | 0.480 |
|           | Rs4756195 | 140| 102| 10 | 382| 122| 2.670  | 0.102 |
|           | Rs8193    | 135| 99 | 18 | 369| 135| 0.001  | 0.979 |
The human CD44 gene is located on the short arm of chromosome 11, and consists of 20 highly conserved exons. Hyaluronic acid (HA) is an important component of the extracellular matrix in mammalian tissues, and all CD44 isoforms contain HA structures in the extracellular domain. The combination of HA and CD44 not only affects cell adhesion, but also stimulates specific functions of various tumor cells to promote breast cancer progression [13].

The results of this study showed that there were no significant differences in the distribution of alleles at the rs13347 locus on the CD44 gene between the case group and the control group. Compared with the CC genotype, the TT and CT+TT genotypes appeared to increase the risk of breast cancer. Previous studies on single-nucleotide polymorphisms in the CD44 gene have found that SNPs at the rs13347 locus can significantly increase the risk of breast cancer in the Chinese Han population [14–16], which is consistent with our results in the present study. However, other studies showed that rs13347...
single-nucleotide polymorphisms were not significantly correlated with risk of breast cancer in the northern population of India [12], which may be explained by the different susceptibility to breast cancer of people from different countries with different genetic backgrounds.

In addition, our results showed that the distribution of alleles at locus rs4756195 and locus rs8193 exhibited no statistically significant difference between the case group and the control group. Analysis of SNPs of locus rs4756195 has not been reported abroad, but it has been reported in China [17] that the polymorphisms of locus rs4756195 on CD44 are associated with breast cancer risks among women of Han ethnicity in Chongqing, China, and that the AG and GG genotypes are susceptible genotypes of breast cancer in these women, while the GG genotype may be associated with poor prognosis of breast cancer. In the present study, we did not find an impact of rs4756195 on breast cancer susceptibility, which may be due to polygenic influences and multiple environmental factors. The expression of traits is influenced not only by genotypes, but also by lifestyle, nutritional status, geographical environment, economic level, and difference in sample quantities, as well as the introduction of possible confounding factors and sampling errors, all of which potentially affect the results.

There have been few studies on this locus, so further research is needed to verify our results. We also found that locus rs8193 was not associated with breast cancer susceptibility. There have been relatively few studies on locus rs8193, and to date there is no report on the relationship between rs8193 single-nucleotide polymorphisms and breast cancer risks. However, a study found that the rs8193 allele T can increase the incidence of skin adverse reactions after radiotherapy in breast cancer patients [18], while another group found that the SNP at locus rs8193 is a risk factor for colon cancer prognosis [19].

### Table 6. Relationship between haplotype of CD44 gene and breast cancer risks.

| Haplotype* | Case group (n=484) | Control group (n=504) | $\chi^2$ | OR (95% CI) | P |
|------------|-------------------|----------------------|--------|-------------|---|
| CAC*       | 166.15 (0.343)    | 223.52 (0.443)       | 10.379 | 0.656 [0.507–0.848] | 0.001 |
| CAT        | 66.90 (0.138)     | 75.06 (0.149)        | 0.230  | 0.917 [0.642–1.309] | 0.632 |
| CGC        | 46.34 (0.096)     | 57.64 (0.114)        | 0.910  | 0.820 [0.545–1.234] | 0.340 |
| CGT*       | 28.61 (0.059)     | 13.78 (0.027)        | 6.070  | 2.235 [1.161–4.304] | 0.014 |
| TAC*       | 82.00 (0.169)     | 47.21 (0.094)        | 12.463 | 1.974 [1.347–2.893] | <0.001 |
| TAT        | 37.95 (0.078)     | 36.21 (0.072)        | 0.153  | 1.099 [0.685–1.765] | 0.696 |
| TGC        | 34.52 (0.071)     | 40.63 (0.081)        | 0.305  | 0.876 [0.546–1.234] | 0.340 |
| TGT*       | 21.54 (0.045)     | 9.95 (0.020)         | 4.909  | 2.313 [1.080–4.957] | 0.027 |

OR = odds ratio; CI = confidence interval. * Haplotypes were sorted according to the order of rs13347, rs4756195, and rs8193. * The haplotype was significantly correlated with breast cancer risks (p<0.05).

### Table 7. Interactive analysis of CD44 SNP haplotype and PR status.

| Haplotype | frequency | PR+ group | PR– group |
|-----------|-----------|-----------|-----------|
| CAC       | 0.3932    | 1.00      | 2.63 [1.08–6.44] |
| CAT       | 0.1447    | 1.09 [0.58–2.05] | 1.93 [0.80–4.68] |
| TAC       | 0.1292    | 0.77 [0.36–1.66] | 0.84 [0.18–1.07] |
| CGC       | 0.1073    | 0.86 [0.37–2.00] | 1.96 [0.82–4.70] |
| TGC       | 0.0767    | 1.49 [0.57–3.94] | 1.67 [0.55–5.04] |
| TAT       | 0.0767    | 0.46 [0.19–1.09] | 2.77 [0.73–10.47] |
| CGT       | 0.0410    | 0.45 [0.11–1.93] | 0.41 [0.04–4.47] |
| TGT       | 0.0311    | 0.88 [0.16–4.69] | —         |

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SNP of CD44 with breast cancer
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The genesis and development of diseases is usually not the result of a single allele, but instead is the consequence of interactions of multiple SNP loci, which could be passed on to offspring in the form of haplotypes. The phenotype is also influenced by other non-genetic factors. Therefore, constructing haplotypes and analyzing their interactions with non-genetic factors might contribute to the study of disease pathogenesis [20]. By analyzing distribution differences of haplotypes in the case group and the control group, we found that the haplotype CAC is a protective factor against breast cancer (OR=0.656, 95%CI: 0.507–0.848, P=0.001), while the haplotypes CTG, TAC, and TGT are risk factors for breast cancer (the distributions of these 3 haplotypes were significantly higher in the case group than in the control group). In addition, through interactive analysis of haplotypes and other factors, we found that there were interactions between haplotype CAC of the 3 loci on CD44 and PR (P = 0.016), and that the breast cancer risk of the PR-negative group with haplotype CAC was 2.63 times higher than in the PR-positive group (OR=2.63, 95%CI: 1.08–6.44). There were no interactions between haplotypes and other factors such as age, BMI, ER, or HER-2 (P>0.05). The mammary gland is one of the main target organs of sex hormones. Both endogenous and exogenous estrogen and progesterone can affect mammary gland and may participate in the pathogenesis and development of breast cancer [21]. Breast cancer is a hormone-dependent tumor. ER/PR-negative breast cancers lack the target for endocrine therapy and therefore are associated with worse prognoses than are ER/PR-positive breast cancers. In this study, we found that CD44 SNPs have potential interactions with PR status, which influences breast cancer susceptibility. Further research is needed to validate these results.

Conclusions

By comparing the distribution patterns of each haplotype in the case group and the control group, we found a haplotype that might reduce the risk of breast cancer. The interactive analysis of haplotypes and age, BMI, or other related factors contributed to the further discovery of the combined effects of genetic factors and non-genetic factors on breast cancer susceptibility. In addition, since there are few studies on the relationship between the 3 SNP loci on the CD44 gene and breast cancer risks, this study may provide a theoretical basis for clinical and scientific research, but further studies are needed to validate our findings.

Conflict of interest

None.

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