Genetic polymorphisms in inflammatory response genes and their associations with breast cancer risk

**Aim** To explore the association of \( \text{NFKB1} \) c.-798_-795delAT-TG (rs28362491), \( \text{NFKBIA} \) c.-949C>T (rs2233406), \( \text{IL-8} \) c.-352-A>T (rs4073), \( \text{IL-10} \) c.-854T>C (rs1800871), \( \text{TNF} \) c.-418G>A (rs361525), and \( \text{TNF} \) c.-488G>A (rs1800629) polymorphisms with breast cancer risk in an East Chinese population.

**Methods** We conducted a case-control study including 975 study participants (474 breast cancer patients and 501 female controls without cancer) and genotyped the polymorphisms employing polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Logistic regression was used to assess the association of the polymorphisms with breast cancer risk.

**Results** We found that the ins/del and del/del genotypes of \( \text{NFKB1} \) polymorphism and TT genotype of \( \text{IL-10} \) polymorphism significantly increased breast cancer risk (\( \text{NFKB1} \) ins/del odds ratio [OR] 1.69, 95% CI 1.23-2.33, \( P = 0.001 \); \( \text{NFKB1} \) del/del OR 2.42, 95% CI 1.72-3.42, \( P < 0.001 \); \( \text{IL-10} \) TT OR 2.36, 95% CI 1.58-3.52, \( P < 0.001 \)). On the other hand, the TT genotype of \( \text{IL-8} \) polymorphism, GA and AA genotypes of \( \text{TNF} \) c.-418G>A polymorphism, and GA genotype of \( \text{TNF} \) c.-488G>A polymorphism significantly reduced breast cancer risk (\( \text{IL-8} \) TT OR 0.48, 95% CI 0.33-0.72, \( P < 0.001 \); \( \text{TNF} \) c.-418GA OR 0.58, 95% CI 0.41-0.80, \( P = 0.001 \); \( \text{TNF} \) c.-418AA OR 0.38, 95% CI 0.14-0.98, \( P = 0.044 \); \( \text{TNF} \) c.-488GA OR 0.68, 95% CI 0.48-0.96, \( P = 0.029 \)). When stratified by menopausal status, the CT genotype of \( \text{NFKBIA} \) polymorphism significantly reduced the risk among pre-menopausal women (OR 0.63, 95% CI 0.40-0.99, \( P = 0.043 \)), but not among post-menopausal women.

**Conclusions** \( \text{NFKB1} \), \( \text{NFKBIA} \), \( \text{IL-8} \), \( \text{IL-10} \), and \( \text{TNF} \) polymorphisms could serve as useful predictive biomarkers for breast cancer risk among women in East China.
Breast cancer is the most frequent form of cancer and leading cause of cancer-related deaths among women around the world (1). The cancer accounts for almost one quarter of new cancer cases annually (2), and the incidence continues to increase rapidly, both in China and worldwide (3). Although it has been well-established that breast carcinogenesis is a result of the complex interactions between multiple environmental and genetic factors, the mechanisms of the oncogenesis at the molecular level remain poorly understood. Genetic factors can serve as a susceptibility variable for breast cancer development, and their identification can help to reduce the incidence of breast cancer (4). However, several breast cancer susceptibility genes identified so far, such as BRCA1 and BRCA2, account for only less than 5% of the total breast cancer incidence (5).

Single nucleotide polymorphisms (SNPs) have been extensively investigated for their associations with the risk of various cancers (6-11). As inflammation is caused by a molecular network underlying breast carcinogenesis (12), we propose that SNPs within inflammatory response genes could modify breast cancer predisposition risk. The associations of various inflammatory response gene polymorphisms with breast cancer risk in the Chinese population, especially the East Chinese population, have been understudied. In the current study, we investigated the associations of NFKB1 c.-798_-795delATTG (rs28362491), NFKBIA c.-949C>T (rs2233406), IL-8 c.-352A>T (rs4073), IL-10 c.-854T>C (rs1800871), TNF c.-418G>A (rs361525), and TNF c.-488G>A (rs1800629) polymorphisms with breast cancer risk in East China. Since all these polymorphisms are located in the promoter region, they could affect the transcriptional activity of the gene, resulting in enhanced or reduced cDNA, and eventually protein levels, among their carriers (6,7,13). In addition, despite the relatively well established associations of the polymorphisms with cancer risks in other populations (6-9), little is known about their association with breast cancer risk in East China population, which further motivated us to undertake this research.

PATIENTS AND METHODS

Study participants and ethical considerations

A total of 1032 female study participants – 514 breast cancer patients and 518 controls without cancer were identified at the Jiujiang First People’s Hospital. 474 breast cancer patients and 501 female controls without cancer agreed to participate in the study. The participants were interviewed by trained professionals and data related to smoking, oral contraceptive use, and menopausal status were collected. The patients’ histopathological types and cancer grading were retrieved from their medical records. All the participants were Han Chinese. The study received approval from the Ethics of Human Research Board of Jiujiang First People’s Hospital. Informed consent was obtained from the participants before inclusion in the study.

Genotyping

Polymorphisms were genotyped on the DNA isolated from the peripheral blood samples using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique and the genotypes were verified by direct sequencing of PCR products. For NFKB1 c.-798_-795delATTG (rs28362491), the PCR primers used were 5’-TGG GCA CAA GTC GTT TAT GA-3’ and 5’-CTG GAG CCG GTA GGG AAG T3’ (6) and the annealing temperature was 63.5°C. The PCR product 281 bp (deletion allele) or 285 bp (insertion allele) was digested with Pfml (Van91I) restriction enzyme. The insertion genotype was identified as 2 bands on agarose gel, at 240 bp and 45 bp.

For NFKBIA c.-949C>T (rs2233406) polymorphism, the forward primer was 5’-GGT CCT TAA GGT CCA ATC G-3’ and the reverse primer was 5’-CTG GAG CCG GTA GGG AAG T3’ (7). The annealing temperature was also 63.5°C; the 200 bp product was digested with BflI restriction enzyme; and the CC genotype was identified as 180 + 20 bp bands.

For IL-8 c.-352A>T (rs4073) polymorphism, the forward primer was 5’-CCA TCA TGA TAG CAT CTG T-3’ and the reverse primer was 5’-GCC CAA TTT GTG GAA TTA TTA A-3’ (8). The annealing temperature was 57°C; the 173 bp PCR product was digested with AseI restriction enzyme; and the AA genotype was identified as 152 + 21 bp bands.

For IL-10 c.-854T>C (rs1800871) polymorphism, the primers used were 5’-GTT GTG GAT ACC TTG CAC TA-3’ and 5’-GGT CCT TAA GGT CCA ATC G-3’ and the annealing temperature was 59°C. The PCR product 281 bp (deletion allele) or 285 bp (insertion allele) was digested with PflMI (Van91I) restriction enzyme. The CC genotype was identified as 180 + 20 bp bands.

For TNF c.-418G>A (rs361525) polymorphism, the primes used were 5’-AAA CAG ACC ACA GAC CTG GTC-3’ and 5’-CTC ACA CTC CCC ATC CTC CGG GAT C-3’ (15). Annealing temperature was 59°C; the 150 bp PCR product was digested with BnaHI restriction enzyme; and the GG genotype was identified as 130 + 20 bp bands.
For TNF c.-488G>A (rs1800629) polymorphism, the primers used were 5’-GAG GCA ATA GGT TTT GAG GGC CAT-3’ and 5’-GGG ACA CAC AAG CAT CAA G-3’ (15). The annealing temperature was 61°C; the 107 bp product was digested with Ncol restriction enzyme; and the GG genotype was identified as 87 + 20 bp bands.

**Statistical analysis**

Statistical analysis was done by using SPSS, version 17.0 (SPSS Inc., Chicago, IL, USA) The differences in age, smoking habit, oral contraceptive use, menopausal status, and genotypic distribution between cases and controls were assessed using a χ2 test. Risk association between the polymorphisms and breast cancer was evaluated using logistic regression analysis. *P* values of <0.05 were considered significant.

**RESULTS**

There were no significant differences in mean age, smoking, oral contraceptives use, and menopausal status between patients and controls (Table 1).

**Genotype distribution**

Significant differences between cases and controls were observed for *NFKB1* ins/del and del/del genotypes, *IL-8* TT genotype, *IL-10* CC and TT genotypes, and *TNF* c.-418 and c.-488 GG and GA genotypes (Table 2). The two *TNF* polymorphisms were in strong linkage disequilibrium with Ncol restriction enzyme; and the GG genotype was identified as 87 + 20 bp bands.

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### Table 1. Demographic characteristics of cases with breast cancer and control participants

| Variable                        | Cases   | Controls | *P*  |
|----------------------------------|---------|----------|------|
| Mean age, mean ± standard deviation | 59.1 ± 7.9 | 59.4 ± 8.0 | 0.567 |
| Smoking, n                       |         |          |      |
| Yes                              | 138     | 137      | 0.540 |
| No                               | 336     | 364      |      |
| Oral contraceptive, n           |         |          |      |
| Use                              | 135     | 159      | 0.268 |
| No                               | 339     | 342      |      |
| Menopausal status, n             |         |          |      |
| Pre                              | 179     | 213      | 0.130 |
| Post                             | 295     | 288      |      |
| Histopathological type, n *      |         |          |      |
| IDC                              | 346     | -        |      |
| DCIS                             | 71      | -        |      |
| ILC                              | 57      | -        |      |
| Grade, n†                        |         |          |      |
| 1                                | 42      | -        |      |
| 2                                | 228     | -        |      |
| 3                                | 204     | -        |      |

*IDC – invasive ductal carcinoma; DCIS – ductal carcinoma in situ; ILC – invasive lobular carcinoma.

*Grade 1 – well differentiated; Grade 2 – moderately differentiated; Grade 3 – poorly differentiated.

### Table 2. Genotype distribution of the polymorphisms in cases with breast cancer and control participants

| Gene   | Genotype | Case, n/% | Controls, n/% | *P*    |
|--------|----------|-----------|---------------|--------|
| NFKB1  | ins/ins  | 93/19.6   | 162/32.2      | <0.001 |
|        | ins/del  | 210/44.3  | 216/43.1      | 0.708  |
|        | del/del  | 171/36.1  | 123/24.6      | <0.001 |
| NFKB1A | CC       | 288/60.8  | 297/59.3      | 0.637  |
|        | CT       | 147/31.0  | 162/32.3      | 0.657  |
|        | TT       | 39/8.2    | 42/8.4        | 0.930  |
| IL-8   | AA       | 192/40.5  | 186/37.1      | 0.281  |
|        | AT       | 231/48.7  | 213/42.5      | 0.052  |
|        | TT       | 51/10.8   | 102/20.4      | <0.001 |
| IL-10  | CC       | 186/39.2  | 234/46.7      | 0.018  |
|        | CT       | 198/41.8  | 219/43.7      | 0.054  |
|        | TT       | 90/19.0   | 48/9.6        | <0.001 |
| TNF c.-418 | GG     | 399/84.2  | 374/74.7      | 0.774  |
|        | GA       | 69/14.6   | 112/22.4      | 0.002  |
|        | AA       | 6/1.3     | 15/3.0        | 0.071  |
| TNF c.-488 | GG     | 404/85.2  | 397/79.2      | 0.015  |
|        | GA       | 66/13.9   | 95/19.0       | 0.034  |
|        | AA       | 4/0.8     | 9/1.8         | 0.206  |

### Table 3. Association between the polymorphisms and breast cancer risk in cases with breast cancer and control participants

| Gene   | Genotype | Cases, n/% | Controls, n/% | Odds ratio (95% confidence interval) | *P*    |
|--------|----------|------------|---------------|-------------------------------------|--------|
| NFKB1  | ins/ins  | 93/19.6    | 162/32.2      | -                                   | -      |
|        | ins/del  | 210/44.3   | 216/43.1      | 1.69 (1.23-2.33)                     | 0.001  |
|        | del/del  | 171/36.1   | 123/24.6      | 2.42 (1.72-3.42)                     | <0.001 |
| NFKB1A | CC       | 288/60.8   | 297/59.3      | -                                   | -      |
|        | CT       | 147/31.0   | 162/32.3      | 0.94 (0.71-1.23)                     | 0.637  |
|        | TT       | 39/8.2     | 42/8.4        | 0.96 (0.60-1.52)                     | 0.855  |
| IL-8   | AA       | 192/40.5   | 186/37.1      | -                                   | -      |
|        | AT       | 231/48.7   | 213/42.5      | 1.14 (0.87-1.50)                     | 0.054  |
|        | TT       | 51/10.8    | 102/20.4      | 0.48 (0.33-0.72)                     | <0.001 |
| IL-10  | CC       | 186/39.2   | 234/46.7      | -                                   | -      |
|        | CT       | 198/41.8   | 219/43.7      | 1.14 (0.87-1.50)                     | 0.354  |
|        | TT       | 90/19.0    | 48/9.6        | 2.36 (1.58-3.52)                     | <0.001 |
| TNF c.-418 | GG     | 399/84.2   | 374/74.7      | -                                   | -      |
|        | GA       | 69/14.6    | 112/22.4      | 0.58 (0.41-0.80)                     | 0.001  |
|        | AA       | 6/1.3      | 15/3.0        | 0.38 (0.14-0.98)                     | 0.044  |
| TNF c.-488 | GG     | 404/85.2   | 397/79.2      | -                                   | -      |
|        | GA       | 66/13.9    | 95/19.0       | 0.68 (0.48-0.96)                     | 0.029  |
|        | AA       | 4/0.8      | 9/1.8         | 0.44 (0.13-1.43)                     | 0.171  |
(\(R^2 = 0.819\)). All the genotypic distributions followed Hardy-Weinberg equilibrium.

**Association between the polymorphisms and breast cancer risk**

Significant associations were observed for at least one genotype of all the polymorphisms, with the exception of \(NFKBIA\) polymorphism. \(NFKB1\) c.-798_-795delATTG ins/del and del/del genotypes, and \(IL-10\) c.-854 TT genotype were associated with increased breast cancer risk, while \(IL-8\) c.-

**TABLE 4.** Combination of polymorphisms and their associations with breast cancer risk in cases with breast cancer and control participants

| Genotype combination                      | Odds ratio (95% confidence interval) | P    |
|------------------------------------------|--------------------------------------|------|
| Cases Controls                          | dence interval                       |      |
| 50                                       | 92                                   |      |
| 127                                      | 127                                  | 1.84 (1.21-2.81) | 0.004 |
| 111                                      | 78                                   | 2.62 (1.67-4.11) | <0.001 |
| 33                                       | 57                                   | 1.07 (0.61-1.85) | 0.822 |
| 66                                       | 68                                   | 1.79 (1.10-2.99) | 0.019 |
| 46                                       | 37                                   | 2.29 (1.32-3.98) | 0.003 |
| 9                                        | 13                                   | 1.27 (0.51-3.19) | 0.604 |
| 16                                       | 21                                   | 1.40 (0.67-2.93) | 0.369 |
| 14                                       | 8                                    | 3.22 (1.26-8.20) | 0.014 |
| 18                                       | 76                                   | -     |      |
| 32                                       | 48                                   | 0.82 (0.48-1.40) | 0.460 |
| 82                                       | 75                                   | 1.34 (0.87-2.07) | 0.191 |
| 101                                      | 101                                  | 1.22 (0.81-1.84) | 0.334 |
| 13                                       | 43                                   | 0.43 (0.19-0.74) | 0.005 |
| 33                                       | 18                                   | 2.24 (1.17-4.29) | 0.014 |
| 48                                       | 19                                   | 3.09 (1.68-5.70) | <0.001 |
| 11                                       | 6                                    | 0.67 (0.24-1.89) | 0.446 |
| 399                                      | 374                                  | -     |      |
| 3                                        | 17                                   | 0.17 (0.05-0.57) | 0.004 |
| 2                                        | 6                                    | 0.31 (0.62-1.56) | 0.156 |
| 0                                        | 0                                    | N/A   | N/A  |
| 66                                       | 95                                   | 0.65 (0.46-0.92) | 0.014 |
| 0                                        | 0                                    | N/A   | N/A  |
| 0                                        | 0                                    | N/A   | N/A  |
| 0                                        | 0                                    | N/A   | N/A  |
| 4                                        | 9                                    | 0.42 (0.13-1.36) | 0.148 |

352 TT genotype, \(TNF\) c.-418 GA and AA genotypes, and c.-488 GA genotype were significantly associated with a reduced risk (Table 3).

**Combinations of polymorphisms and their associations with breast cancer risk**

When \(NFKB1\) and \(NFKBIA\) polymorphic genotypes were combined, positive ORs were observed for all the combinations.

**TABLE 5.** Association between the polymorphisms and breast cancer risk among pre- and post-menopausal women with and without breast cancer

| Menopause | Genotype | Odds ratio (95% confidence interval) | P    |
|-----------|----------|--------------------------------------|------|
| Pre       | \(NFKB1\) ins/ins | 34                                      | 67     | - |      |
| Pre       | \(NFKB1\) ins/del | 84                                      | 90     | 1.84 (1.11-3.06) | 0.019 |
| Pre       | \(NFKB1\) del/del | 61                                      | 56     | 2.15 (1.24-3.72) | 0.006 |
| Post      | \(NFKB1\) ins/ins | 59                                      | 95     | - |      |
| Post      | \(NFKB1\) ins/del | 126                                     | 126    | 1.61 (1.07-2.42) | 0.022 |
| Post      | \(NFKB1\) del/del | 110                                     | 67     | 2.64 (1.69-4.12) | <0.001 |
| Post      | \(NFKBIA\) CC | 119                                      | 124    | - |      |
| Post      | \(NFKBIA\) CT | 44                                      | 73     | 0.63 (0.40-0.99) | 0.043 |
| Post      | \(NFKBIA\) TT | 16                                      | 16     | 1.04 (0.50-2.18) | 0.913 |
| Post      | \(NFKBIA\) CC | 169                                     | 173    | - |      |
| Post      | \(NFKBIA\) CT | 103                                     | 89     | 1.18 (0.83-1.69) | 0.348 |
| Post      | \(NFKBIA\) TT | 23                                      | 26     | 0.91 (0.50-1.65) | 0.746 |
| Pre       | \(IL-8\) AA | 72                                      | 79     | - |      |
| Pre       | \(IL-8\) AT | 85                                      | 86     | 1.08 (0.70-1.68) | 0.717 |
| Pre       | \(IL-8\) TT | 22                                      | 48     | 0.50 (0.28-0.91) | 0.024 |
| Post      | \(IL-8\) AA | 120                                     | 107    | - |      |
| Post      | \(IL-8\) AT | 140                                     | 127    | 0.98 (0.69-1.40) | 0.924 |
| Post      | \(IL-8\) TT | 29                                      | 54     | 0.48 (0.28-0.81) | 0.006 |
| Post      | \(IL-10\) CC | 73                                      | 104    | - |      |
| Post      | \(IL-10\) CT | 72                                      | 92     | 1.11 (0.73-1.71) | 0.620 |
| Post      | \(IL-10\) TT | 28                                      | 17     | 2.35 (1.20-4.60) | 0.013 |
| Post      | \(IL-10\) CC | 113                                     | 130    | - |      |
| Post      | \(IL-10\) CT | 120                                     | 127    | 1.09 (0.76-1.55) | 0.644 |
| Post      | \(IL-10\) TT | 62                                      | 31     | 2.30 (1.40-3.79) | 0.011 |
| Pre       | \(TNF\) c.-418 GG | 150                                     | 162    | - |      |
| Pre       | \(TNF\) c.-418 GA | 26                                      | 45     | 0.62 (0.37-1.06) | 0.082 |
| Pre       | \(TNF\) c.-418 AA | 3                                       | 6      | 0.54 (0.13-2.20) | 0.389 |
| Post      | \(TNF\) c.-418 GG | 249                                     | 212    | - |      |
| Post      | \(TNF\) c.-418 GA | 43                                      | 67     | 0.55 (0.36-0.84) | 0.005 |
| Post      | \(TNF\) c.-418 AA | 3                                       | 9      | 0.28 (0.08-1.06) | 0.061 |
| Pre       | \(TNF\) c.-488 GG | 154                                     | 173    | - |      |
| Pre       | \(TNF\) c.-488 GA | 24                                      | 37     | 0.73 (0.42-1.27) | 0.266 |
| Pre       | \(TNF\) c.-488 AA | 1                                       | 3      | 0.37 (0.04-3.64) | 0.397 |
| Post      | \(TNF\) c.-488 GG | 252                                     | 224    | - |      |
| Post      | \(TNF\) c.-488 GA | 41                                      | 58     | 0.63 (0.41-0.97) | 0.038 |
| Post      | \(TNF\) c.-488 AA | 2                                       | 6      | 0.30 (0.06-1.48) | 0.139 |
nations. However, 5 out of 8 combinations showed significant association with breast cancer risk (Table 4) and only three combinations of IL-8 and IL-10 polymorphisms showed significant association with breast cancer risk (Ta-

**Table 6. Association between the polymorphisms and breast cancer risk according to histopathological type of patients**

| Histo-pathological type* | Genotype     | Cases | Controls | Odds ratio (95% confidence interval) | P    |
|--------------------------|--------------|-------|----------|-------------------------------------|------|
| IDC                      | NFKB1 ins/ins | 64    | 162      |                                     |      |
| IDC                      | NFKB1 del/del | 152   | 216      | 1.78 (1.25-2.54)                    | 0.002|
| IDC                      | NFKB1 del/del | 130   | 123      | 2.68 (1.83-3.91)                    | <0.001|
| DCIS                     | NFKB1 ins/ins | 12    | 162      |                                     |      |
| DCIS                     | NFKB1 ins/del | 33    | 216      | 2.06 (1.03-4.12)                    | 0.040|
| DCIS                     | NFKB1 del/del | 26    | 123      | 2.85 (1.38-5.88)                    | 0.005|
| ILC                      | NFKB1 ins/ins | 17    | 162      |                                     |      |
| ILC                      | NFKB1 ins/del | 25    | 216      | 1.10 (0.58-2.11)                    | 0.767|
| ILC                      | NFKB1 del/del | 15    | 123      | 1.16 (0.56-2.42)                    | 0.688|
| ILC                      | NFKB1A CC     | 212   | 297      |                                     |      |
| ILC                      | NFKB1A CT     | 102   | 162      | 0.88 (0.61-1.21)                    | 0.419|
| ILC                      | NFKB1A TT     | 32    | 42       | 1.07 (0.65-1.75)                    | 0.795|
| DCIS                     | NFKB1A CC     | 46    | 297      |                                     |      |
| DCIS                     | NFKB1A CT     | 25    | 162      | 0.99 (0.59-1.68)                    | 0.989|
| DCIS                     | NFKB1A TT     | 0     | 42       | N/A                                 |      |
| ILC                      | NFKB1A CC     | 30    | 297      |                                     |      |
| ILC                      | NFKB1A CT     | 20    | 162      | 1.22 (0.67-2.22)                    | 0.510|
| ILC                      | NFKB1A TT     | 7     | 42       | 1.65 (0.68-3.99)                    | 0.266|
| IDC                      | IL-8 AA       | 137   | 186      |                                     |      |
| IDC                      | IL-8 AT       | 174   | 213      | 1.10 (0.82-1.49)                    | 0.496|
| IDC                      | IL-8 TT       | 35    | 102      | 0.46 (0.29-0.72)                    | 0.001|
| DCIS                     | IL-8 AA       | 29    | 186      |                                     |      |
| DCIS                     | IL-8 AT       | 33    | 213      | 0.99 (0.58-1.69)                    | 0.981|
| DCIS                     | IL-8 TT       | 9     | 102      | 0.56 (0.25-1.24)                    | 0.156|
| ILC                      | IL-8 AA       | 26    | 186      |                                     |      |
| ILC                      | IL-8 AT       | 24    | 213      | 0.80 (0.44-1.45)                    | 0.473|
| ILC                      | IL-8 TT       | 7     | 102      | 0.49 (0.20-1.17)                    | 0.108|
| IDC                      | IL-10 CC      | 140   | 234      |                                     |      |
| IDC                      | IL-10 CT      | 147   | 219      | 1.12 (0.83-1.50)                    | 0.446|
| IDC                      | IL-10 TT      | 59    | 48       | 2.05 (1.33-3.17)                    | 0.001|
| DCIS                     | IL-10 TT      | 29    | 234      |                                     |      |
| DCIS                     | IL-10 CT      | 29    | 219      | 1.06 (0.61-1.84)                    | 0.812|
| DCIS                     | IL-10 TT      | 13    | 48       | 2.18 (1.05-4.50)                    | 0.034|
| ILC                      | IL-10 CC      | 17    | 234      |                                     |      |
| ILC                      | IL-10 CT      | 22    | 219      | 1.38 (0.71-2.71)                    | 0.335|
| ILC                      | IL-10 TT      | 18    | 48       | 5.16 (2.48-10.73)                   | <0.001|
| IDC                      | TNF c.-418 GG  | 298   | 374      |                                     |      |
| IDC                      | TNF c.-418 GA  | 43    | 112      | 0.48 (0.32-0.71)                    | 0.001|
| IDC                      | TNF c.-418 AA  | 5     | 15       | 0.41 (0.15-1.16)                    | 0.095|
| DCIS                     | TNF c.-418 GA  | 61    | 374      |                                     |      |
| DCIS                     | TNF c.-418 AA  | 10    | 112      | 0.54 (0.27-1.10)                    | 0.092|
| ILC                      | TNF c.-418 AA  | 0     | 15       | N/A                                 |      |
| ILC                      | TNF c.-418 GG  | 40    | 374      |                                     |      |
| ILC                      | TNF c.-418 GA  | 16    | 112      | 1.33 (0.72-2.47)                    | 0.358|
| ILC                      | TNF c.-418 AA  | 1     | 15       | 0.62 (0.06-4.84)                    | 0.651|
| IDC                      | TNF c.-488 GG  | 302   | 397      |                                     |      |
| IDC                      | TNF c.-488 GA  | 41    | 95       | 0.56 (0.38-0.84)                    | 0.005|
| IDC                      | TNF c.-488 AA  | 3     | 9        | 0.43 (0.11-1.63)                    | 0.219|
| DCIS                     | TNF c.-488 GA  | 61    | 397      |                                     |      |
| DCIS                     | TNF c.-488 AA  | 10    | 95       | 0.68 (0.33-1.38)                    | 0.293|
| ILC                      | TNF c.-488 AA  | 0     | 9        | N/A                                 |      |
| ILC                      | TNF c.-488 GG  | 41    | 397      |                                     |      |
| ILC                      | TNF c.-488 GA  | 15    | 95       | 1.52 (0.81-2.87)                    | 0.188|
| ILC                      | TNF c.-488 AA  | 1     | 9        | 1.07 (0.13-8.70)                    | 0.945|

*IDC – invasive ductal carcinoma; DCIS – ductal carcinoma in situ; ILC – invasive lobular carcinoma.
SNPs in inflammatory genes and breast cancer risk

Patients). Decreased risk associations were observed for Grade 1 patients), and heterozygous and variant genotypes, and TT genotype of IL-10 (Table 4). Only four combinations of TNF c.-418 and c.-488 were analyzed due to the absence of other combinations in the study participants and two of them showed a significant association with breast cancer risk (Table 4).

Stratification of breast cancer risk association according to menopausal status

For pre-menopausal women, significant associations with breast cancer risk were observed for NFKB1 ins/del and del/del genotypes, NFKBIA CT genotype, IL-8 TT genotype, IL-10 TT genotype, and TNF c.-418 GA and AA genotypes. For post-menopausal women, significant associations with breast cancer risk were observed for NFKB1 ins/del and del/del genotypes, IL-8 TT genotype, IL-10 TT genotype, TNF c.-418 GA and AA genotypes, and TNF c.-488 GA genotype (Table 5).

Risk association according to patient histopathological types

NFKB1 heterozygous and variant genotypes were associated with breast cancer risk in invasive ductal carcinoma (IDC) and ductal carcinoma in situ (DCIS), but not in invasive lobular carcinoma (ILC). IL10 variant genotype was associated with increased breast cancer risk in all three types of breast cancers. On the other hand, IL8 variant genotype and heterozygous genotypes of both TNF polymorphisms were associated with decreased risk of IDC but not of other types of breast cancer (Table 6).

Risk association according to patient cancer grading

Increased risk associations were observed for NFKB1 heterozygous genotype (in Grade 2 and 3 patients), NFKB1 variant genotype (in all patients), NFKBIA variant genotype (in Grade 1 patients), IL10 heterozygous genotype (in Grade 1 patients), IL10 variant genotype (in all patients), and TNF c.-488 heterozygous genotype (in Grade 1 patients). Decreased risk associations were observed for IL8 heterozygous genotypes and variant genotypes, TNF c.-418 heterozygous genotype, and TNF c.-488 heterozygous genotype (all in Grade 2 and 3 patients) (Table 7).

DISCUSSION

This study established that the ins/del and del/del genotypes of NFKB1 polymorphism and TT genotype of IL-10 polymorphism significantly increased breast cancer risk, while the TT genotype of IL-8 polymorphism, GA and AA genotypes of TNF c.-418G>A polymorphism, and GA genotype of TNF c.-488G>A polymorphism significantly reduced breast cancer risk. Various lines of evidence have found that chronic inflammation was a risk factor for breast cancer development (16-18). Inflammation can cause DNA damage, and hence carcinogenesis, by inducing and activating oxidant-producing enzymes (19). Events that are linked to inflammation, such as postmenopausal status and obesity, have also been associated with an increased breast cancer risk (6). If inflammation represents an important pathway in carcinogenesis, polymorphisms in the inflammatory response genes could potentially modify cancer predisposition risk.

We analyzed not only the association of individual polymorphisms and breast cancer risk, but also the effects of combinations of functionally related polymorphisms (NFKB1 and NFKBIA; IL-8 and IL-10; and TNF c.-418 and c.-488), menopausal status, histopathological type, and cancer grading. To our knowledge, this is the first study investigating the association between NFKB1 polymorphism and breast cancer risk although there are a few reports on its association with several other cancers. Our findings are in agreement with a study from East China that found that del/del genotype increased the risk of bladder cancer (20). However, a study in Southern Chinese population (21) found that the ins/ins genotype increased the risk of colorectal cancer. Our report also presents the first evidence for the association of NFKBIA polymorphism with the risk of breast cancer in any Asian population. Thus far, only one study has examined this association but it was conducted in a Caucasian population (22). Similarly to our study, they found no association between NFKBIA polymorphism and breast cancer risk. For IL-8 polymorphism, one study conducted in East China showed no association with breast cancer risk (23). Our results are in disagreement with this study, whose genotype distribution deviate significantly from the Hardy-Weinberg equilibrium. However, our results are similar to an Iranian study, which also found an association between the variant genotype of the polymorphism and breast cancer risk (24). On the other hand, a study from East China showed no association between IL-10 polymorphism and breast cancer risk (25), which is different from our results. For TNF c.-418 and c.-488 polymorphisms, an Indian study (26), reported that the AA genotype resulted in an increased breast cancer risk, which is also different from our results. It should be noted, however, that this study had a small sample size with only 40 cases. Similar to our study, Park et al (27) reported a reduced risk of breast cancer among carriers of the A allele of the
| Grade* | Genotype   | Cases | Controls | Odds ratio (95% confidence interval) | P     |
|--------|------------|-------|----------|-------------------------------------|-------|
| 1      | NFKB1 ins/ins | 10    | 162      | -                                   | -     |
| 1      | NFKB1 ins/del | 12    | 216      | 0.90 (0.37-2.13)                    | 0.81  |
| 1      | NFKB1 del/del | 20    | 123      | 2.63 (1.19-5.83)                    | 0.017 |
| 2      | NFKB1 ins/ins | 44    | 162      | -                                   | -     |
| 2      | NFKB1 ins/del | 101   | 216      | 1.72 (1.14-2.59)                    | 0.009 |
| 2      | NFKB1 del/del | 83    | 123      | 2.48 (1.60-3.83)                    | <0.001|
| 3      | NFKB1 ins/ins | 39    | 162      | -                                   | -     |
| 3      | NFKB1 ins/del | 97    | 216      | 1.86 (1.22-2.84)                    | 0.004 |
| 3      | NFKB1 del/del | 68    | 123      | 2.29 (1.45-3.63)                    | <0.001|
| 1      | NFKB1A CC    | 14    | 297      | -                                   | -     |
| 1      | NFKB1A CT    | 16    | 162      | 2.09 (0.99-4.40)                    | 0.051 |
| 1      | NFKB1A TT    | 12    | 42       | 6.06 (2.62-13.98)                   | <0.001|
| 2      | NFKB1A CC    | 144   | 297      | -                                   | -     |
| 2      | NFKB1A CT    | 67    | 162      | 0.81 (0.57-1.15)                    | 0.253 |
| 2      | NFKB1A TT    | 17    | 42       | 0.83 (0.45-1.51)                    | 0.554 |
| 3      | NFKB1A CC    | 130   | 297      | -                                   | -     |
| 3      | NFKB1A CT    | 64    | 162      | 0.90 (0.63-1.28)                    | 0.571 |
| 3      | NFKB1A TT    | 10    | 42       | 0.54 (0.26-1.11)                    | 0.097 |
| 1      | IL-8 AA      | 17    | 186      | -                                   | -     |
| 1      | IL-8 AT      | 16    | 213      | 0.82 (0.40-1.67)                    | 0.588 |
| 1      | IL-8 TT      | 9     | 102      | 0.96 (0.41-2.24)                    | 0.935 |
| 2      | IL-8 AA      | 90    | 186      | -                                   | -     |
| 2      | IL-8 AT      | 111   | 213      | 0.54 (0.33-0.89)                    | 0.017 |
| 2      | IL-8 TT      | 27    | 102      | 1.07 (0.76-1.51)                    | 0.669 |
| 3      | IL-8 AA      | 85    | 186      | -                                   | -     |
| 3      | IL-8 AT      | 104   | 213      | 1.06 (0.75-1.51)                    | 0.709 |
| 3      | IL-8 TT      | 15    | 102      | 0.32 (0.17-0.58)                    | <0.001|
| 1      | IL-10 CC     | 5     | 234      | -                                   | -     |
| 1      | IL-10 CT     | 28    | 219      | 5.98 (2.26-15.77)                   | <0.001|
| 1      | IL-10 TT     | 9     | 48       | 8.77 (2.81-27.34)                   | <0.001|
| 2      | IL-10 CC     | 24    | -        | -                                   | -     |
| 2      | IL-10 CT     | 91    | 219      | 1.06 (0.75-1.50)                    | 0.706 |
| 2      | IL-10 TT     | 46    | 48       | 2.46 (1.53-3.94)                    | <0.001|
| 3      | IL-10 CC     | 90    | 234      | -                                   | -     |
| 3      | IL-10 CT     | 79    | 219      | 0.93 (0.65-1.33)                    | 0.722 |
| 3      | IL-10 TT     | 35    | 48       | 1.89 (1.15-3.12)                    | 0.012 |
| 1      | TNF c.-418 GG | 30    | 374      | -                                   | -     |
| 1      | TNF c.-418 GA | 12    | 112      | 1.33 (0.66-2.69)                    | 0.419 |
| 1      | TNF c.-418 AA | 0     | 15       | N/A                                 | N/A   |
| 2      | TNF c.-418 GG | 190   | 374      | -                                   | -     |
| 2      | TNF c.-418 GA | 33    | 112      | 0.58 (0.37-0.88)                    | 0.012 |
| 2      | TNF c.-418 AA | 5     | 15       | 0.65 (0.23-1.83)                    | 0.421 |
| 3      | TNF c.-418 GG | 179   | 374      | -                                   | -     |
| 3      | TNF c.-418 GA | 24    | 112      | 0.44 (0.27-0.72)                    | 0.001 |
| 3      | TNF c.-418 AA | 1     | 15       | 0.13 (0.01-1.06)                    | 0.057 |
| 1      | TNF c.-488 GG | 21    | 397      | -                                   | -     |
| 1      | TNF c.-488 GA | 21    | 95       | 4.17 (2.19-7.96)                    | <0.001|
| 1      | TNF c.-488 AA | 0     | 9        | N/A                                 | N/A   |
| 2      | TNF c.-488 GG | 199   | 397      | -                                   | -     |
| 2      | TNF c.-488 GA | 27    | 95       | 0.56 (0.35-0.89)                    | 0.016 |
| 2      | TNF c.-488 AA | 2     | 9        | 0.44 (0.09-2.07)                    | 0.301 |
| 3      | TNF c.-488 GG | 184   | 397      | -                                   | -     |
| 3      | TNF c.-488 GA | 18    | 95       | 0.40 (0.23-0.69)                    | 0.001 |
| 3      | TNF c.-488 AA | 2     | 9        | 0.48 (0.10-2.24)                    | 0.350 |

*Grade 1 – well differentiated; Grade 2 – moderately differentiated; Grade 3 – poorly differentiated.
polymorphisms. However, this risk reduction was not statistically significant.

In conclusion, our study provided evidence for the association of various inflammatory response gene polymorphisms with the risk of breast cancer in East China. The strengths of the present study are the reasonably large sample size and the detailed combination and stratification analyses performed. The limitations of the study are the small number of polymorphisms studied within each gene and the small sample sizes obtained by stratification according to menopausal status, histopathological type, and cancer grading, which might have led to misleading interpretation. Therefore, further studies by independent research groups are needed to confirm our findings.

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Declaration of authorship ZW and QLL recruited study participants and collected the samples, isolated DNA from the samples, validated genotyping results and drafted the manuscript. WS and CJY genotyped the polymorphisms and performed statistical analysis. LT and XZ were involved in recruitment of participants and sample collection, including briefing of all the participants about the research study and obtaining informed consent from them. XMZ conceived of the study, participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

Competing interests All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: no support from any organization that might have an interest in the submitted work; no financial relationships with any organizations for the submitted work; no other relationships or activities that could appear to have influenced the submitted work.

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