MMP8 and MMP9 gene polymorphisms were associated with breast cancer risk in a Chinese Han population

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Matrix metalloproteinases (MMPs) are a group of zinc-dependent endopeptidases that can breakdown almost all extracellular matrix components. MMP8 and MMP9 have been shown to be associated with breast cancer (BC) risk in European and American populations. However, few studies have focused on the polymorphisms of MMP8 and MMP9 in Chinese Han BC patients. We investigated nine single nucleotide polymorphisms (SNPs) in 571 BC cases and 578 controls to evaluating their association with risk of BC. The frequency of the "A" allele of rs3787268 was significantly lower in BC cases than in controls ($P = 0.025$). In the genetic model analysis, the minor allele “T” of rs11225394 in MMP8 was associated with increased risk of BC under the recessive model ($P = 0.019$), and the minor allele “A” of rs3787268 was associated with decreased risk of BC under the dominant model ($P = 0.014$). Additionally, the haplotype “AGTCA” constructed by rs3740938, rs2012390, rs1940475, rs11225394, and rs11225395 and the haplotype “CCG” constructed by rs3918249, rs3918254, and rs3787268 were associated with increased risk of BC ($P < 0.05$). Our data showed that polymorphisms of MMP8 and MMP9 may be associated with BC risk in the Chinese Han population.
MMP8 and MMP9 in Chinese Han BC patients. We intended to identify more SNPs in MMP8 and MMP9 gene that may be associated with BC risk.

The SNPs rs3740938, rs2012390 and rs11225394 were reported to be associated with the risk of osteonecrosis of the femoral head in the Chinese Han population. Rs1940475 may take part in modifying the host response to inflammatory stimuli. Rs11225395 and rs2274755 were considered as candidate SNPs in previous association studies on bladder cancer and glaucoma. In addition, the haplotype constructed by rs3918249, rs3918254 and rs3787268 in MMP-9 were associated with primary angle-closure glaucoma in a Chinese Han population. In this case-control study, we selected and genotyped the above nine SNPs in MMP8 and MMP9 to evaluate their association with risk of BC.

Table 1. Basic characteristic of patients with breast cancer and the control individuals. SD: Standard deviation; P value was calculated by Welch’s t test.

| Characteristics | Cases (N = 571) | Controls (N = 578) | P |
|-----------------|-----------------|-------------------|---|
| Age             |                 |                   | 0.517 |
| Mean ± SD       | 50.91 ± 11.23   | 50.21 ± 10.11     |   |

Table 2. Basic information of candidate SNPs in this study. SNP: single-nucleotide polymorphism, Alleles A/B: Minor/major alleles; MAF: minor allele frequency; OR: odds ratio, CI: confidence interval, HWE: Hardy–Weinberg equilibrium; 4HWE P-value < 0.05 was excluded; *p values were calculated using two-sided Chi-squared test; *p < 0.05 indicates statistical significance.

| SNP ID | Gene | Band | Position | Role | Alleles A/B | MAH | P-HWE | MAF | Case | Control | p | OR (95% CI) |
|--------|------|------|----------|------|-------------|-----|-------|-----|------|---------|---|-------------|
| rs3740938 | MMP8 | 11q22.2 | 102587062 | Coding exon | A/G | 0.794 | 0.232 | 0.199 | 0.057 | 1.214 (0.994–1.481) |
| rs2012390 | MMP8 | 11q22.2 | 102590777 | Intron | G/A | 0.349 | 0.264 | 0.231 | 0.063 | 1.197 (0.990–1.447) |
| rs1940475 | MMP8 | 11q22.2 | 102593248 | Coding exon | T/C | 0.713 | 0.380 | 0.343 | 0.068 | 1.172 (0.988–1.390) |
| rs11225394 | MMP8 | 11q22.2 | 102595413 | Intron (boundary) | T/C | 0.065 | 0.118 | 0.115 | 0.813 | 1.031 (0.799–1.332) |
| rs11225395 | MMP8 | 11q22.2 | 102596480 | Promoter | A/G | 0.514 | 0.366 | 0.335 | 0.116 | 1.147 (0.966–1.362) |
| rs3918249 | MMP9 | 20q13.12 | 44638136 | Intron | T/C | 0.325 | 0.307 | 0.345 | 1.089 (0.913–1.298) |
| rs2274755 | MMP9 | 20q13.12 | 44639692 | Intron (boundary) | T/G | 0.001# | 0.147 | 0.121 | 0.067 | 1.252 (0.984–1.593) |
| rs3918254 | MMP9 | 20q13.12 | 44640391 | Intron (boundary) | T/C | 0.579 | 0.184 | 0.183 | 0.960 | 1.006 (0.814–1.242) |
| rs3787268 | MMP9 | 20q13.12 | 44641731 | Intron variant | A/G | 0.184 | 0.332 | 0.377 | 0.025* | 0.822 (0.692–0.975) |

Table 3. Genotype frequencies of the SNPs and their associations with risk of breast cancer. ORs: odds ratios; CI: confidence interval; AIC: Akaike’s Information criterion; BIC: Bayesian Information criterion. *p value < 0.05 indicates statistical significance.

| SNP | Model | Genotype | Case | Control | OR (95% CI) | P | AIC | BIC | OR (95% CI) | P | AIC | BIC |
|-----|-------|----------|------|---------|-------------|---|-----|-----|-------------|---|-----|-----|
| rs11225394 | Codominant | C/C | 445 (78.5%) | 445 (77.5%) | 1 | 0.033* | 1580.9 | 1596 | 1 | 0.042* | 1576.2 | 1596.4 |
| rs11225394 | Dominant | C/C | 445 (78.5%) | 445 (77.5%) | 0.87 (0.65–1.16) | 0.07 | 1585.6 | 1595.6 | 1 | 0.71 | 1580.4 | 1595.5 |
| rs11225394 | Recessive | C/C | 445 (78.5%) | 445 (77.5%) | 0.95 (0.71–1.25) | 0.7 | 1579.8 | 1589.8 | 1 | 0.019* | 1575 | 1590.1 |
| rs3787268 | Codominant | G/G | 253 (44.3%) | 216 (37.5%) | 1 | 0.038* | 1585.7 | 1595.7 | 1.03 (0.80–1.33) | 0.81 | 1580.5 | 1595.6 |
| rs3787268 | Dominant | G/G | 253 (44.3%) | 216 (37.5%) | 0.77 (0.60–0.98) | 0.058 | 1590.4 | 1605.5 | 1 | 0.043* | 1584.5 | 1604.7 |
| rs3787268 | Recessive | G/G | 253 (44.3%) | 216 (37.5%) | 0.75 (0.60–0.95) | 0.019* | 1588.6 | 1598.6 | 0.74 (0.59–0.94) | 0.014* | 1582.8 | 1597.9 |

MMP8 and MMP9 in Chinese Han BC patients. We intended to identify more SNPs in MMP8 and MMP9 gene that may be associated with BC risk. The SNPs rs3740938, rs2012390 and rs11225394 were reported to be associated with the risk of osteonecrosis of the femoral head in the Chinese Han population. Rs1940475 may take part in modifying the host response to inflammatory stimuli. Rs11225395 and rs2274755 were considered as candidate SNPs in previous association studies on bladder cancer and glaucoma. In addition, the haplotype constructed by rs3918249, rs3918254 and rs3787268 in MMP-9 were associated with primary angle-closure glaucoma in a Chinese Han population. In this case-control study, we selected and genotyped the above nine SNPs in MMP8 and MMP9 to evaluate their association with risk of BC.
Results
A total of 571 BC patients and 578 healthy controls were recruited in the study. The ages of the patient and control groups are described in Table 1. The mean age of the participants was 50.91 years in the case group and 50.21 years in the control group. There was no significant difference in the distribution of age between BC patients and healthy controls (P > 0.05).

The basic information of the MMP8 and MMP9 polymorphisms (rs3740938, rs2012390, rs1940475, rs11225394, rs11225395, rs3918249, rs2274755, rs3918254 and rs3787268) is shown in Table 2, including gene, band, position, role, alleles and minor allele frequency (MAF). One SNP (rs2274755) was excluded due to significant deviation from Hardy-Weinberg equilibrium (P < 0.05). By comparing the difference of allele frequencies between two groups, we found that the frequency of the “A” allele of rs3787268 was significantly lower in BC cases than in controls (32.2% versus 37.7%), which suggested that the “A” allele of rs3787268 may be associated with a decreased risk of BC [odds ratios (ORs) = 0.822, and 95% confidence intervals (CIs): 0.692–0.975, P = 0.025].

Next, we investigated the association between each SNP and risk of BC based on different genetic models (Table 3). When the sum of AIC and BIC were at the minimum, the model was considered as the best model. Under the best model-recessive model, the minor allele “T” of rs11225394 in MMP8 was associated with an increased risk of BC (OR = 3.94, 95% CI: 1.10–14.07, P = 0.019). Under the best model-dominant model, the minor allele “A” of rs3787268 was associated with decreased risk of BC (OR = 0.74, 95% CI: 0.59–0.94, P = 0.014).

The association of MMP8 and MMP9 haplotypes with the risk of BC was analysed. Figures 1 and 2 show the linkage disequilibrium (LD) block in MMP8 and MMP9. The association between different haplotypes and BC risk is shown in Table 4. The haplotype “AGTCA” constructed by rs3740938, rs2012390, rs1940475, rs11225394, and rs11225395 was associated with an increased risk of BC after the adjustment (OR = 1.23; 95% CI = 1.00–1.51; P = 0.048). The haplotype “CCG” constructed by rs3918249, rs3918254 and rs3787268 was also associated with an increased risk of BC after the adjustment (OR = 1.37; 95% CI = 1.05–1.77, P = 0.019).

Finally, we summarized the OR and p-values of candidate SNP from previous studies. The results are listed in Table 5.

Discussion
In this study, we investigated the associations between nine candidate SNPs in MMP8 and MMP9 genes and BC risk in a Chinese Han population. We found that rs11225394 in MMP8 and rs3787268 in MMP9 are significantly associated with BC risk. We also found two haplotypes were associated with increased risk of BC: haplotype “AGTCA” constructed by rs3740938, rs2012390, rs1940475, rs11225394, and rs11225395 in MMP8, and haplotype “CCG” constructed by rs3918249, rs3918254 and rs3787268 in MMP9. Our results suggest that the polymorphisms of MMP8 and MMP9 may play an important role in the risk of BC in the Chinese Han population.

MMPs are a family of endopeptidases involved in the degradation of extracellular matrix and basement membrane barriers. As such, they contribute when tumour cells separate from adjacent normal tissues.26,27 Several
studies have revealed that MMPs are associated with the invasion and metastasis of tumour cells. MMP8 and MMP9 are members of the MMPs family. Plasma MMP8 levels were lower in BC patients than in healthy control individuals, and MMP8 was found to protect against lymph node metastasis in BC patients. Differential expression of MMP9 was found in BC cells with different degrees of cellular differentiation. We identified two SNPs in MMP8 and MMP9 that were associated with risk of BC, which further corroborates the association between MMPs and BC risk.

In the present study, we investigated nine SNPs in the MMP8 and MMP9 genes. Among these SNPs, rs3740938, rs2012390, rs11225394, rs2274755 and rs3918254 were identified that have been associated with risk of osteonecrosis of the femoral head in the Chinese Han population. We report for the first time that rs11225394 was associated with BC risk. This association needs to be confirmed in a further study with a larger sample size. Rs1940475 was found to have association with community-acquired pneumonia-associated sepsis and inflammatory response in Caucasians. Rs11225395 was associated with bladder cancer risk in a Polish population. Rs3918249 was demonstrated to be associated with risk for primary angle-closure glaucoma in Chinese Han population and risk for asthma in Mexican paediatric patients. Rs3787268 was also correlated with BC prognosis in previous studies; the “GA” and “AA” genotypes of rs3787268 were significantly associated with 1.52-fold risk of BC in Native American women. However, in our study, we found that the minor allele “A” of rs3787268 was associated with decreased risk of BC in Chinese women, which is inconsistent with previous result. This inconsistency may be due to the limited sample size and different populations.

Our study has some intrinsic limitations. First, the sample size was relatively small, so the results identified here just provide some pilot data for continued in-depth studies on BC. Second, the occurrence of BC was also influenced by BMI, menarche, marital status, parity, and so on. We failed to collect these specific data, which is a weakness of the study. Additionally, although we identified significant SNPs and haplotypes with risk of BC, this is still not enough to explain the molecular mechanism between MMP and the onset and development of BC.

In conclusion, the present study showed that polymorphisms of MMP8 and MMP9 are associated with risk of BC in a Chinese Han population. Further studies will focus on two directions. One is to demonstrate the association between MMP and BC risk in larger sample sizes and different populations; the other is to investigate the mechanisms or pathways by which MMP influence BC risk.

Figure 2. D’ linkage map for the three SNPs in MMP9.
Materials and Methods

Study participants. A total of 571 BC patients and 578 healthy controls were consecutively recruited between June 2012 and July 2016 at the First Affiliated Hospital of Xi’an Jiaotong University, People’s Republic of China. Patients diagnosed with other types of cancers or who underwent radiotherapy or chemotherapy were excluded. The controls with no history of cancer were recruited from the physical examination centre of our hospital.

SNP selection and genotyping. Nine SNPs (rs3740938, rs2012390, rs11225394, rs11225395, rs3918249, rs2274755, rs3918254 and rs3787268) in MMP8 and MMP9 were selected from previous association studies. DNA extraction and concentrations were done as previously described34. SNP genotyping was performed by the Sequenom MassARRAY RS1000, and the data analyses were completed by Sequenom Type 4.0. The polymerase chain reaction (PCR) primers used for the study are listed in Table 6.

Statistical Analyses. All of the statistical analyses were performed with Microsoft Excel (Microsoft Corporation, Redmond, WA, USA) and the SPSS 21.0 statistical package (SPSS, Chicago, IL, USA). SNP allele frequencies in the control subjects were tested for departure from Hardy–Weinberg Equilibrium (HWE) before analysis. Differences in the distributions of allele frequencies between the cases and controls were evaluated using
chi-square tests. Four models (co-dominant, dominant, recessive, and log-additive) were used to assess the association between each genotype and the BC risk. Akaike’s Information criterion (AIC) and Bayesian Information criterion (BIC) were used to select the best model for each SNP. ORs and 95% CIs were calculated with and without adjustment for age. All p values presented in this study were two sided, and p = 0.05 was considered the cutoff for statistical significance.

Haploview software version 4.2 was used to analyse the association between haplotypes and BC. Linkage disequilibrium (LD) analysis was performed using genotype data from all the subjects. Statistical significance was established when p < 0.05.

**Approval.** All of the participants provided written informed consent. The Human Research Committee for Approval of Research Involving Human Subjects, the First Affiliated Hospital of Xi’an Jiaotong University, approved the use of human blood samples in this study. All methods were performed in accordance with the relevant guidelines and regulations.

**Data Availability**
The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

**References**

1. Ferlay, J. *et al.* Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *International journal of cancer* 136(3), E339-E386, E339 (2015).
2. Hirotsu, Y. *et al.* Multigene panel analysis identified germline mutations of DNA repair genes in breast and ovarian cancer. *Molecular Genetics & Genomic Medicine* 3, 459 (2015).
3. Colditz, G. A., Sellers, T. A. & Trapido, E. Epidemiology (indasii) identifying the causes and preventability of cancer? *Nature Reviews Cancer* 6, 75 (2006).
4. Michailidou, K. *et al.* Large-scale genotyping identifies 41 new loci associated with breast cancer risk. *Nature Genetics* 45, 353 (2013).
5. Garcia-Closas, M. *et al.* Genome-wide association studies identify four ER negative-specific breast cancer risk loci. *Nature Genetics* 45, 1–2 (2013).
6. Ren, H. T. *et al.* PD-1rs2227982 Polymorphism Is Associated With the Decreased Risk of Breast Cancer in Northwest Chinese Women: A Hospital-Based Observational Study. *Medicine* 95, e3760 (2016).
7. Xia, P. *et al.* FGFR2 gene polymorphisms are associated with breast cancer risk in the Han Chinese population. *American Journal of Cancer Research* 5, 1854 (2015).
8. Zhou, L. *et al.* Association of five single nucleotide polymorphisms at 6q25.1 with breast cancer risk in northwestern China. *American Journal of Cancer Research* 5, 2467 (2015).
9. Xia, P. *et al.* Polymorphisms in ESR1 and FLJ43663 are associated with breast cancer risk in the Han population. *Tumour Biology the Journal of the International Society for OncoDevelopmental Biology & Medicine* 35, 2187–2190 (2014).
10. Dai, Z. J. *et al.* Association Between Single Nucleotide Polymorphisms in DNA Polymerase Kappa Gene and Breast Cancer Risk in Chinese Han Population: A STROBE-Compliant Observational Study. *Medicine* 95, e2466 (2016).
11. Xia, P. *et al.* Mitochondrial DNA levels in blood and tissue samples from breast cancer patients of different stages. *Asian Pacific Journal of Cancer Prevention* 15, 1339–1344 (2014).
12. Egeland, M. & Werb, Z. New functions for the matrix metalloproteinases in cancer progression. *Nature Reviews Cancer* 2, 161–174 (2002).
13. Detrygina, E. I. & Quigley, J. P. Matrix metalloproteinases and tumor metastasis. *Cancer & Metastasis Reviews* 25, 9–34 (2006).
14. Fan, S. H. *et al.* CERS2 suppresses tumor cell invasion and is associated with decreased V-ATPase and MMP-2/MMP-9 activities in breast cancer. *Journal of Cellular Biochemistry* 116, 502–513 (2015).
15. Tabouret, E. *et al.* MMP2 and MMP9 serum levels are associated with favorable outcome in patients with inflammatory breast cancer treated with bevacizumab-based neoadjuvant chemotherapy in the BEVERLY-2 study. *Oncotarget* 7, 18531–18540 (2016).
16. Darlix, A. *et al.* Serum NSE, MMP-9 and HER2 extracellular domain are associated with brain metastases in metastatic breast cancer patients: predictive biomarkers for brain metastases? *International journal of cancer* 139, 2299–2311 (2016).
17. Decock, J. *et al.* Matrix Metalloproteinase Expression Patterns in Luminal A Type Breast Carcinomas. *Disease Markers* 23, 189–196 (2014).
18. Coskun, U. *et al.* Locally advanced breast carcinoma treated with neoadjuvant chemotherapy: are they in the change levels of YKL-40, MMP-2 and MMP-9 correlated with tumor response? *Neoplasma* 54, 348–352 (2007).
19. Dębniak, T. *et al.* Association of MMP9 gene variation with an increased risk of malignant melanoma. *Melanoma Research* 21, 464–468 (2011).
20. Du, J. *et al.* Association between genetic polymorphisms of MMP8 and the risk of steroid-induced osteonecrosis of the femoral head in the population of northern China. *Medicine* 95, e4794 (2016).
21. An, E. *et al.* MMP8 polymorphism is associated with susceptibility to osteonecrosis of the femoral head in a Chinese Han population. *Oncotarget* 8, 21561–21566 (2017).
22. Rehula, J. M., Jilma, B., Fabry, A., Kaynar, A. M. & Mayr, F. B. MMP-8 Genotypes Influence the Inflammatory Response in Human Endotoxemia. *Inflammation* 37, 451–456 (2014).
23. Wieczorek, E. *et al.* MMP7 and MMP8 genetic polymorphisms in bladder cancer patients. *Central European Journal of Urology* 66, 405–410 (2014).
24. Suh, W., Won, H. H. & Kee, C. The Association of Single-Nucleotide Polymorphisms in the MMP-9 Gene with Normal Tension Glaucoma and Primary Open-Angle Glaucoma. *Current Eye Research*, 1–5 (2017).
25. Xiao-Jin, G., Sheng-Ping, H. & Ping-Hua, L. The association between matrix metalloprotease-9 gene polymorphisms and primary angle-closure glaucoma in a Chinese Han population. *International Journal of Ophthalmology* 7, 397–402 (2014).
26. Yousuf, E. M., Tahir, M. R., Stpierre, Y. & Gouboury, L. A. MMP-9 expression varies according to molecular subtypes of breast cancer. *Bmc Cancer* 14, 609 (2014).
27. Chen, J. *et al.* MMP-3 and MMP-8 single-nucleotide polymorphisms are related to alcohol-induced osteonecrosis of the femoral head in Chinese males. *Oncotarget* 8, 25177 (2017).
31. Kaynar, A. et al. Matrix Metalloproteinase (MMP)-8 is Critical in a Murine Model of Sepsis as well as in Patients with Community-Acquired Pneumonia (CAP)-Associated Sepsis. American Journal of Respiratory & Critical Care Medicine 181, A1132–A1132 (2010).

32. Jiménezmorales, S. et al. Polymorphisms in metalloproteinase-9 are associated with the risk for asthma in Mexican pediatric patients. Human Immunology 74, 998–1002 (2013).

33. Slattery, M. L. et al. Matrix Metalloproteinase Genes Are Associated with Breast Cancer Risk and Survival: The Breast Cancer Health Disparities Study. Plos One 8, e63165 (2013).

34. Jin, T. et al. IL-1RN gene polymorphisms are associated with breast cancer risk in a Chinese Han population. Journal of Gene Medicine 19 (2017).

35. Thakkinstian, A., Mcelduff, P., D’Este, C., Duffy, D. & Attia, J. A method for meta-analysis of molecular association studies. Statistics in Medicine 24, 1291 (2005).

Author Contributions
K.W. and X.Z. conceived and supervised the project. SNP genotyping experiment was conducted by Y.Z., G.L., X.W. and Y.K. Statistical Analyses was conducted by J.Y., Q.Z. and F.X. All the authors contributed to the discussion. K.W. wrote the manuscript with help of J.W.

Additional Information
Competing Interests: The authors declare no competing interests.

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