Correlation between IL-7R gene variants and breast cancer susceptibility in Chinese Han women

CURRENT STATUS: POSTED

Miao Li
The First Affiliated Hospital of Xi'an Jiaotong University

Chenli Yue
Shaanxi Provincial Crops Hospital of Chinese Peoples Armed Police Force

Xiaoxiao Zuo
The First Affiliated Hospital of Zhegzhou University

Guoquan Jin
The Fifth People's Hospital of Qinghai Province

Guanying Wang
The Fist Hospital of Xi'an Jiaotong University

Hulin Guo
The Fifth People's Hospital of Qinghai Province

Fang Wu
The First Affiliated Hospital of Xi'an Jiaotong University

Shangke Huang
The Affiliated Hospital of Southwest Medical University

Xinhan Zhao
The First Affiliated Hospital of Xi'an Jiaotong University

zhaohanxinC21@163.com Corresponding Author
ORCiD: https://orcid.org/0000-0002-6970-0692

DOI:
10.21203/rs.2.20360/v1

SUBJECT AREAS
Immunology Allergy & Immune Disorders

KEYWORDS
IL-7R, Polymorphisms, Breast cancer, Cancer susceptibility
Abstract

Introduction

IL-7R is involved in the occurrence and development of breast cancer by binding to its ligand IL-7. This study aimed to explore the potential relationships of IL-7R polymorphisms with breast cancer susceptibility in Chinese Han women.

Methods

Five polymorphisms (rs969129, rs10213865, rs10053847, rs118137916, and rs6451231) of IL-7R genewere genotyped in 553 patients and 550 healthy healthy individuals from the in Chinese Han women using the Agena MassARRAY platform. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated used to evaluate the relationship.

Results

IL-7R rs10213865, rs969129 and rs6451231 was correlated with an increased the risk of breast cancer in multiple genetic models. Age stratified analysis revealed that rs6451231 was correlated with an increased breast cancer risk at age > 52 years. Additionally, rs10213865 was correlated with tumor site and ER expression, rs969129 was related to tumor size and Ki67 expresses status, and rs6451231 was related to tumor size. Haplotype CGAG (rs969129, rs10213865, rs10053847 and rs118137916) were observed to a decrease breast cancer risk.

Conclusions

IL-7R polymorphism were significantly correlated with an increased breast cancer susceptibility in Chinese Han women.

Introduction

Breast cancer, with about 2.1 million new cases and 0.6 million deaths in GLOBOCAN 2018 data, has been recognized as the most common type of cancer and a main cause of cancer death among women worldwide [1]. The data from China’s urban cancer registration institutions showed that the incidence of breast cancer has increased by 20–30%, and the annual growth rate was 35% in the past 30 years. It is estimated that by 2021, the number of breast cancer cases in Chinese women aged 35 and 49 will reach 2.2 million [2]. Although the current development of medical technology has
contributed to reduce the death rate of breast cancer, the specific cause of breast cancer has not been clarified. In addition to environmental factors, including radiation exposure and lifestyle, genetic factors are also crucial to the development of breast cancer. These genetic factors include single nucleotide polymorphism (SNPs) in the genome, and genetic variations in susceptible genes resulting in about 5-10% breast cancer [3, 4]. Interleukin 7 receptor (IL-7R) is one of the I type cytokines receptors family members secreted by stromal cells, and its coding protein is interleukin-7 (IL-7) receptor [5]. Previous studies have shown that IL-7 binds to IL-7R can was essential for inflammatory or the immune response [6, 7]. Recently, increasing studies have shown that IL-7R influences the occurrence of various tumors, such as breast cancer, by forming signal complexes with its ligand IL-7 [8]. Binding IL-7R to IL-7 triggers a cascade of phosphorylation induced by signaling molecules that also participated in cell behavior such as cell division, cell adhesion, and cell differentiation [9, 10]. Rawi et al. pointed out the abnormal expression of IL-7R and IL-7 in breast cancer, and also found that IL-7 could accelerated the growth of breast cancer cells through wortmannin-sensitive pathway [11, 12]. These studies suggested that IL-7R is closely related to the occurrence of breast cancer. Moreover, a large amount of evidence has been explored that single nucleotide polymorphisms (SNPs) affect an individual’s risk of breast cancer [13, 14]. However, the relationship between IL-7R polymorphisms and breast cancer risk remains unclear. Therefore, we conducted an association study based on Chinese Han population to clarify the correlation between IL-7R SNPs and the risk of breast cancer, which is of great significance for the early molecular diagnosis of breast cancer.

Materials And Methods
Ethics statement
Our research was approved by the First Affiliated Hospital of Xi’an Jiaotong University and conducted in accordance with the ethical standards of the Helsinki declaration and national and international norms. All subjects signed informed consent forms after full understanding of the purpose of this research, agreeing to provide personal and relevant clinical information.

Participates statement
The study finally included 681 cases and 756 controls recruited from the the First Affiliated Hospital of Xi’an Jiaotong University.
Xi’an Jiaotong University. All subjects of cases were confirmed as breast cancer by pathology, excluding those who had received radiotherapy, chemotherapy, and other types of cancers. Data on the patient’s clinicopathological characteristics (such as tumor location, tumor size, progesterone receptor (PR) status, estrogen receptor (ER) status, and Ki-67 status etc) were obtained from the patient’s medical records. The control groups were healthy women with no disease and no history of any disease who were recruited from the same hospital for physical examination at the same time. All the participants were Chinese Han population and were not related to each other.

**SNP selection and genotyping**

In this study, five IL-7R SNPs (rs10213865, rs969129, rs118137916, rs10053847 and rs6451231) with minor allele frequency (MAF) value > 0.05 were selected in present study based on 1000 Genomes Project (http://www.1000genomes.org/) and dbSNP database (https://www.ncbi.nlm.nih.gov/projects/SNP/). Participants’ blood samples were stored at -80°C in a test tube containing EDTA reagent until analyzed. Whole-blood genomic DNA extraction was performed using the GoldMag whole-blood genomic DNA purification kit (GoldMag Co. Ltd., Xi’an, China), and its quality was detected by NanoDrop 2000C spectrophotometer (Thermo Scientific, Waltham, MA, USA) according to the manufacturer’s guidelines. Primer design was conducted with Agena MassARRAY Assay Design 3.0 Software (San Diego, California, USA) [15]. The corresponding primers of the selected SNPs in this study were listed in Supplementary Table S1. The SNPs genotyping was performed by two professionals using the Agena MassARRAY system (Agena, San Diego, CA, U.S.A.) software in a double-blind manner [16].

**Statistical analysis**

This study used SPSS 20.0 version and Microsoft Excel to analyze all relevant data, and $P < 0.05$ was considered statistically significant. The variation frequency of the controls was evaluated by the hardy weinberg-equilibrium (HWE) calculated by Fisher’s exact test. The frequency of alleles and genotypes in cases and controls was assessed by the Pearson’s $\chi^2$ test, and the relationship between IL-7Rs SNPs and breast cancer risk was analyzed using Odds ratios (OR) and 95% confidence intervals (CI) in multiple genetic model (allele, co-dominant, dominant, recessive and log-additive). Linkage
disequilibrium and haplotype analysis were performed using the 4.2 version of the Haplovew
software package.

Results
The Basic information about the subject
Basic clinical information of the cases and the control groups were summarized in Table 1. Our study
containing 553 patients with an average age of 52.00 ± 9.83 years and 550 controls with an average
age of 52.11 ± 9.51 years. There was no statistically significant difference in age distribution between
the case group and the control group, and they were matched by age (P = 0.884).

Table 1
The general characteristics of study subjects

| Variable               | Cases (553) | Control (550) | P     |
|------------------------|-------------|---------------|-------|
| Age                    |             |               | 0.844 |
| Age (Mean ± SD, years) | 52.00 ± 9.83| 52.11 ± 9.51  |       |
| Age > 52               | 263 (48%)   | 271 (49%)     |       |
| Age ≤ 52               | 290 (52%)   | 279 (51%)     |       |
| LN metastasis          |             |               |       |
| Positive               | 268 (48.5%) |               |       |
| Negative               | 258 (46.7%) |               |       |
| Unavailable            | 27 (4.9%)   |               |       |
| T stage                |             |               |       |
| T1 + T2                | 361 (65.3%) |               |       |
| T3 + T4                | 156 (28.2%) |               |       |
| Unavailable            | 36 (6.5%)   |               |       |
| PR status              |             |               |       |
| Positive               | 334 (60.4%) |               |       |
| Negative               | 209 (37.8%) |               |       |
| Unavailable            | 10 (1.8%)   |               |       |
| ER status              |             |               |       |
| Positive               | 371 (67.1%) |               |       |
| Negative               | 159 (28.8%) |               |       |
| Unavailable            | 10 (1.8%)   |               |       |
| Tumor size             |             |               |       |
| > 2 cm                 | 311 (56.2%) |               |       |
| ≤ 2 cm                 | 105 (19.0%) |               |       |
| Unavailable            | 137 (24.8%) |               |       |
| Tumor site             |             |               |       |
| Left                   | 224 (40.5%) |               |       |
| Right                  | 215 (38.9%) |               |       |
| Unavailable            | 114 (20.6%) |               |       |
| Ki-67 expresses state  |             |               |       |
| Overexpression (Ki67 > 25%) | 360 (65.1%) |               |       |
| Low expression (Ki67 < 25%) | 134 (24.2%) |               |       |
| Unavailable            | 59 (10.7%)  |               |       |

Abbreviations: LN, lymph node; ER estrogen receptor, RP progesterone receptor;
Notes: P value was calculated by independent samples t test. P < 0.05 in bold indicates a statistically significant
difference.

Information about the IL7R SNPs locus
The specific information of the IL-7R polymorphisms were shown in Table 2. All SNPs in the control
group were complied with Hardy-Weinberg equilibrium (HWE). In addition, we used the HaploRegv4.1
database to predict the function of IL-7R polymorphisms, and found that the SNPs sites were involved
in the Proteins bound, DNAse, and motifs changed etc., suggesting that these SNPs sites had potential biological functions.

| SNPs   | Chr | Position | Gene(s) | Role       | Alleles (A/B) | Frequency (MAF) | P - HWE | HaploReg |
|--------|-----|----------|---------|------------|---------------|----------------|---------|----------|
|        |     |          |         |            |               | Cases        | Controls |          |
| rs10213865 | 5   | 35857748 | IL7R    | intronic   | A/C           | 237          | 198      | 0.772    | DNAse, Proteins bound, Motifs changed, GRASP QTL hits, Selected eQTL hits |
| rs969129   | 5   | 35861166 | IL7R    | intronic   | G/T           | 541          | 482      | 1.000    | Promoter histone marks, Enhancer histone marks, DNAse, Motifs changed, Selected eQTL hits |
| rs118137916 | 5   | 35863436 | IL7R    | intronic   | A/G           | 82           | 88       | 0.770    | Promoter histone marks, Enhancer histone marks, DNAse, Proteins bound, Motifs changed |
| rs10053847  | 5   | 35878038 | IL7R    | UTR3       | A/G           | 168          | 166      | 0.136    | Motifs changed, GRASP QTL hits, Selected eQTL hits |
| rs6451231   | 5   | 35878825 | IL7R    | UTR3       | C/T           | 457          | 404      | 0.854    | Enhancer histone marks, DNAse, Motifs changed, Selected eQTL hits |

Abbreviations: SNP: single nucleotide polymorphism, Alleles A/B: minor/major alleles, MAF: minor allele frequency, HWE: Hardy-Weinberg equilibrium, OR: odds ratio, 95% CI: 95% confidence interval;

**Table 3**
Genotypes frequencies of the SNPs and their associations with risk of breast cancer under multiple models of inheritance

| SNP     | Model     | Genotype | Control (N, %) | Case (N, %) | With adjustment |
|---------|-----------|----------|----------------|-------------|-----------------|
|         |           |          |                |             | OR (95% CI)     | P-value        |
| rs10213865 | Allele    | A        | 900 (82.0%)    | 869 (78.6%)  | 1               |               |
|         |           | C        | 198 (18.0%)    | 237 (21.4%)  | 1.24 (1.00-1.53)| 0.045          |
|         | Co-dominant | A/A      | 370 (67.4%)    | 340 (61.5%)  | 1               |               |
|         |           | A/C      | 160 (29.1%)    | 189 (34.2%)  | 1.37 (0.74–2.55)| 0.317          |
|         |           | C/C      | 40 (7.5%)      | 34 (6.2%)    | 1.26 (0.54–2.93)| 0.654          |
| Allele      | C/C | C/T | T/T | Log-additive |
|------------|-----|-----|-----|--------------|
| rs969129   |     |     |     | 1.24 (1.00-1.53) | 0.045 |
| rs118137916|     |     |     | 1.22 (1.03-1.44) | 0.019 |
| rs10053847 |     |     |     | 1.27 (1.00-1.53) | 0.054 |
| rs6451231  |     |     |     | 1.25 (1.02-1.53) | 0.016 |
Association of IL7R SNPs with breast cancer susceptibility

Pearson’s \( \chi^2 \) test was used to assess the effect of IL-7R SNPs on breast cancer risk in a variety of genetic models. In the overall analysis, IL-7R - rs10213865 was related to an increased breast cancer risk in allele (C vs A: OR = 1.24, 95% CI = 1.00–1.53, \( P = 0.045 \)), dominant (A/C-C/C vs A/A: OR = 1.30, 95% CI = 1.01–1.66, \( P = 0.040 \)), and log-additive models (OR = 1.24, 95% CI = 1.00–1.53, \( P = 0.029 \)).

As for polymorphism rs969129, an increased risk of breast cancer was found in the allele (G vs T: OR = 1.22, 95% CI = 1.04–1.45, \( P = 0.018 \)), co-dominant (T/G vs T/T: OR = 1.50, 95% CI = 1.07–2.1, \( P = 0.017 \)), recessive (G/G vs T/T-G/G: OR = 1.37, 95% CI = 1.02–1.82, \( P = 0.034 \)), and additive models (OR = 1.22, 95% CI = 1.03–1.44, \( P = 0.019 \)). Rs6451231 was related to an increased breast cancer risk under allele (C vs T: OR = 1.23, 95% CI = 1.04–1.46, \( P = 0.018 \)), co-dominant (T/C vs T/T: OR = 1.52, 95% CI = 1.07–2.18, \( P = 0.021 \)), and log-additive models (OR = 1.23, 95% CI = 1.03–1.46, \( P = 0.019 \)).

The other two SNPs rs118137916 and rs10053847 were not related to breast cancer susceptibility (\( P > 0.05 \)).

Stratification analysis of IL7R polymorphisms and breast cancer risk risk by age

To further investigate the impact of age on the potential breast cancer risk of selected IL7R polymorphisms, we stratified analysis by age under multiple genetic, we performed a stratified analysis according to age under multiple genetic models (Table 4). The results showed that rs6451231 was a breast cancer risk SNP locus at age > 52 years under allele (C vs T: OR = 1.30, 95% CI = 1.02–1.66, \( P = 0.034 \)), co-dominant (T/C vs T/T: OR = 1.69, 95% CI = 1.03–2.79, \( P = 0.039 \)) and log-additive models (OR = 1.30, 95% CI = 1.02–1.65, \( P = 0.036 \)).
### Table 4
Association analysis of the IL7R polymorphisms and risk of breast cancer stratified by age

| SNP      | Model       | Genotype | Age (years) ≤ 52 | Age (years) > 52 |
|----------|-------------|----------|------------------|------------------|
|          |             | Control  | Case  | OR (95% CI) | P-value | Control  | Case  | OR (95% CI) | P-value |
| rs6451231 | Allele      | T        | 354 (64.1%) | 346 (60.5%) | 1.00     | 335 (61.8%) | 287 (55.4%) | 1.00 |
|          |             | C        | 198 (35.9%) | 226 (39.5%) | 1.17 (0.92–1.49) | 0.208 | 207 (38.2%) | 231 (44.6%) | 1.30 (1.02–1.66) | 0.034 |
|          | Co-dominant | T/T      | 113 (40.9%) | 105 (36.7%) | 1.00     | 105 (38.7%) | 82 (31.7%) | 1.00 |
|          |             | T/C      | 128 (46.4%) | 136 (47.6%) | 1.38 (0.83–2.32) | 0.217 | 125 (46.1%) | 123 (47.5%) | 1.69 (1.03–2.79) | 0.039 |
|          |             | C/C      | 35 (12.7%) | 45 (15.7%) | 1.14 (0.8–1.64) | 0.465 | 41 (15.1%) | 54 (20.8%) | 1.27 (0.87–1.86) | 0.220 |
|          | Dominant    | T/T      | 113 (40.9%) | 105 (36.7%) | 1.00     | 105 (38.7%) | 82 (31.7%) | 1.00 |
|          |             | T/C-C/C  | 163 (57.1%) | 181 (63.3%) | 1.2 (0.85–1.68) | 0.305 | 166 (71.3%) | 313 (76.3%) | 1.38 (0.96–1.97) | 0.082 |
|          | Reccessive  | T/T-T/C  | 241 (87.3%) | 241 (84.3%) | 1.00     | 230 (84.9%) | 205 (79.2%) | 1.00 |
|          |             | C/C      | 35 (12.7%) | 45 (15.7%) | 1.29 (0.8–2.07) | 0.301 | 41 (15.1%) | 54 (20.8%) | 1.48 (0.94–2.31) | 0.089 |
| Log-additive | ---      | ---      | ---    | 1.17 (0.92–1.49) | 0.209 | ---      | ---    | 1.30 (1.02–1.65) | 0.036 |

Abbreviations: SNP: single nucleotide polymorphism, OR: odds ratio, 95% CI: 95% confidence interval.
Notes: P values were calculated using two-sided Chi-squared test; P < 0.05 in bold indicates a statistically significant difference.

### Table 5
Association analysis of the IL7R polymorphisms and risk of breast cancer stratified by Clinical features
(tumor site)

| SNP      | Model       | Genotype | Tumor site-left | Tumor site-right |
|----------|-------------|----------|------------------|------------------|
|          |             | Control  | Case  | OR (95% CI) | P-value | Control  | Case  | OR (95% CI) | P-value |
| rs10213865 | Allele      | A        | 900 (82.0%) | 339 (75.7%) | 1.00     | 900 (82.0%) | 354 (82.3%) | 1.00 |
|          |             | C        | 198 (18.0%) | 109 (24.3%) | 1.46 (1.12–1.91) | 0.005 | 198 (18.0%) | 76 (17.7%) | 0.98 (0.7–3.13) | 0.870 |
|          | Co-dominant | A/A      | 370 (74.7%) | 128 (57.1%) | 1.00     | 370 (74.7%) | 145 (67.4%) | 1.00 |
|          |             | A/C      | 160 (29.1%) | 83 (37.1%) | 1.95 (0.94–4.07) | 0.075 | 160 (29.1%) | 64 (29.8%) | 0.81 (0.32–2.06) | 0.651 |
|          |             | C/C      | 19 (3.5%) | 13 (5.8%) | 1.51 (1.01–2.11) | 0.015 | 19 (3.5%) | 6 (2.8%) | 1.02 (0.72–1.45) | 0.907 |
|          | Dominant    | A/A      | 370 (74.7%) | 128 (57.1%) | 1.00     | 370 (74.7%) | 145 (67.4%) | 1.00 |
|          |             | A/C-C/C  | 179 (32.6%) | 96 (42.9%) | 1.56 (1.13–2.15) | 0.006 | 179 (32.6%) | 70 (32.6%) | 1.07 (0.71–1.4) | 0.991 |
|          | Recessive   | A/A-A/C  | 530 (96.5%) | 201 (94.2%) | 1.00     | 530 (96.5%) | 209 (97.2%) | 1.00 |
|          |             | C/C      | 19 (3.5%) | 13 (5.8%) | 1.69 (0.82–3.49) | 0.154 | 19 (3.5%) | 6 (2.8%) | 0.8 (0.32–2.03) | 0.639 |
| Log-additive | ---      | ---      | ---    | 1.46 (1.12–1.9) | 0.005 | ---      | ---    | 0.98 (0.73–1.31) | 0.870 |
| rs96912   | Allele      | T        | 616 (56.1%) | 121 (48.9%) | 1.00     | 616 (56.1%) | 234 (54.4%) | 1.00 |
Relationship of IL7R SNPs with clinicopathological features of breast cancer

The effect of IL7R SNPs on the clinical characteristics of breast cancer was also investigated. In subgroups stratified by tumor site, we found that rs10213865 significantly increases the risk of tumors on the left side in allele (C vs A: OR = 1.46, 95% CI = 1.12–1.91, P = 0.005), co-dominant (C/C vs AA: OR = 1.51, 95% CI = 1.08–2.11, P = 0.015), dominant (A/C-C/C vs A/A: OR = 1.56, 95% CI = 1.13–2.15, P = 0.006), and log-additive models (OR = 1.46, 95% CI = 1.12–1.90, P = 0.005). Rs969129 significantly increases the risk of tumors on the left side in allele (C vs A: OR = 1.34, 95% CI = 1.07–1.67, P = 0.010), dominant (A/C-C/C vs A/A: OR = 1.78, 95% CI = 1.15–2.74, P = 0.009), recessive (G/G vs T/T-T/G: OR = 1.90, 95% CI = 1.28–2.83, P = 0.001) and log-additive genetic models (OR = 1.25, 95% CI = 1.01–1.56, P = 0.044). Rs6451231 was correlated with an increased risk of tumor on the

|   | Allele |   |   |   |   |   |
|---|--------|---|---|---|---|---|
|   | T      | C      | T/C | C/C | T/T | C/C |
|   | 688(63.0%) | 404(37.0%) | 252(46.2%) | 76(13.9%) | 218(39.9%) | 328(60.1%) |
|   | 256(57.4%) | 190(42.6%) | 86(38.6%) | 52(23.3%) | 85(38.1%) | 138(61.9%) |
|   | 1.00 | 1.26(1.01-1.58) | 1.78(1.17-2.71) | 0.92(0.63-1.33) | 1.00 | 1.16(0.82-1.63) |
|   |   |   |   |   |   |   |
|   | T/T   | T/C   | C/C |   |   |   |
|   | 173(31.5%) | 64(28.6%) | 470(76.1%) | 76(13.9%) | 328(60.1%) | 328(60.1%) |
|   | 62(31.5%) | 229(51.1%) | 138(61.9%) | 52(23.3%) | 138(61.9%) | 138(61.9%) |
|   | 1.00 | 1.34(1.07-1.67) | 1.16(0.82-1.63) | 1.87(1.31-2.67) | 1.00 | 1.15(0.82-1.63) |
|   |   |   |   |   |   |   |
|   | Co-dominant | Dominant | Recessive | Log-additive |   |   |
|   |   | T/T   | T/T-T/C |   |   |   |
|   | 173(31.5%) | 173(31.5%) | 470(76.1%) | 328(60.1%) | 328(60.1%) | 328(60.1%) |
|   | 62(31.5%) | 62(31.5%) | 138(61.9%) | 138(61.9%) | 138(61.9%) | 138(61.9%) |
|   | 1.00 | 1.16(0.82-1.63) | 1.15(0.82-1.63) | 1.87(1.31-2.67) | 1.00 | 1.15(0.82-1.63) |
|   |   |   |   |   |   |   |
|   |   | C/C   |   |   |   |   |
|   | 76(13.9%) | 76(13.9%) | 76(13.9%) | 76(13.9%) | 76(13.9%) | 76(13.9%) |
|   | 52(23.3%) | 52(23.3%) | 52(23.3%) | 52(23.3%) | 52(23.3%) | 52(23.3%) |
|   | 1.00 | 1.16(0.82-1.63) | 1.15(0.82-1.63) | 1.87(1.31-2.67) | 1.00 | 1.15(0.82-1.63) |
|   |   |   |   |   |   |   |
|   |   |   |   |   |   |   |

Abbreviations: SNP: single nucleotide polymorphism, OR: odds ratio, 95% CI: 95% confidence interval; Notes: P values were calculated using two-sided Chi-squared test; P < 0.05 in bold indicates a statistically significant difference.
left in allele (C vs T: OR = 1.24, 95% CI = 1.01–1.58, P = 0.041), co-dominant (T/C vs T/T: OR = 1.78, 95% CI = 1.17–2.71, P = 0.007), recessive (C/C vs T/T-T/C: OR = 1.87, 95% CI = 1.31–2.67, P = 0.001), and log-additive genetic models (OR = 1.32, 95% CI = 1.07–1.64, P = 0.011).

As shown in Table 6, we observed that individuals with rs10213865 mutational genotype had a higher risk of breast cancer in ER positive patients than in ER negative patients in allele model (C vs A: OR = 0.69, 95% CI = 0.51–0.92, P = 0.012), co-dominant model (C/C vs A/A: OR = 0.62, 95% CI = 0.43–0.90, P = 0.011), dominant model (A/C-C/C vs A/A: OR = 0.62, 95% CI = 0.43–0.88, P = 0.008) and log-additive model (OR = 0.68, 95% CI = 0.50–0.92, P = 0.011). The mutation genotype frequency of rs969129 in patients with high expression of Ki-67 was significantly increased (co-dominant model, G/G vs T/T: OR = 0.56, 95% CI = 0.34–0.92, P = 0.023).

Table 6
Association analysis of the IL7R polymorphisms and risk of breast cancer stratified by Clinical features (ER status and Ki67 expresses status)

| Clinical features                          | SNP         | Model   | Genotype | Control (N, %) | Case (N, %) | With adjustment | OR (95% CI) | P-value |
|--------------------------------------------|-------------|---------|----------|----------------|-------------|-----------------|------------|---------|
| ER status (positive vs. negative)          | rs10213865  | Allele  | A        | 313(74.9%)     | 543(81.3%)  | 1.00            | 0.69(0.51–0.92) | 0.012    |
|                                            |             |         | C        | 105(25.1%)     | 125(18.7%)  |                 | 1.00       |         |
|                                            |             | Co-dominant | A/A     | 115(55%)      | 222(66.5%)  |                 | 0.59(0.26–1.37) | 0.219    |
|                                            |             |         | A/C     | 83(39.7%)     | 99(29.6%)   |                 | 0.62(0.43–0.90) | 0.011    |
|                                            |             |         | C/C     | 11(5.3%)      | 13(3.9%)    |                 | 0.62(0.43–0.90) | 0.011    |
|                                            |             | Dominant | A/A     | 115(55.0%)    | 222(66.5%)  |                 | 0.62(0.43–0.88) | 0.008    |
|                                            |             |         | A/C-C/C | 94(45.0%)     | 112(33.5%)  |                 | 0.62(0.43–0.88) | 0.008    |
|                                            |             | Recessive | A/A-A/C | 198(94.7%)    | 312(33.5%)  |                 | 0.7(0.31–1.61)  | 0.406    |
|                                            |             |         | C/C     | 11(5.3%)      | 13(3.9%)    |                 | 0.7(0.31–1.61)  | 0.406    |
|                                            |             | Log-additive | ---    | ---           | ---         |                 | 0.68(0.50–0.92) | 0.011    |
| Ki67 expresses status (Ki67 > 25% vs. Ki67 < 25%) | rs969129   | Allele  | T        | 136(50.7%)    | 373(51.8%)  | 1.00            | 0.96(0.72–1.27) | 0.767    |
|                                            |             |         | G        | 132(49.3%)    | 347(48.2%)  |                 | 0.96(0.72–1.27) | 0.767    |
|                                            |             | Co-dominant | T/T     | 28(20.9%)     | 104(28.9%)  |                 | 0.95(0.52–1.74) | 0.868    |
|                                            |             |         | T/G     | 80(59.7%)     | 165(45.8%)  |                 | 0.56(0.34–0.92) | 0.023    |
|                                            |             |         | G/G     | 26(19.4%)     | 91(25.3%)   |                 | 0.56(0.34–0.92) | 0.023    |
|                                            |             | Dominant | T/T     | 28(20.9%)     | 104(28.9%)  |                 | 0.66(0.41–1.06) | 0.084    |
|                                            |             |         | T/G-G/G | 106(79.1%)    | 256(70.1%)  |                 | 0.66(0.41–1.06) | 0.084    |
|                                            |             | Recessive | T/T-T/G | 108(90.6%)    | 269(74.7%)  | 1.00            | 1.41(0.86–2.3)  | 0.175    |
|                                            |             |         | G/G     | 26(19.4%)     | 91(25.3%)   | 1.41(0.86–2.3)  | 0.175    |
|                                            |             | Log-additive | ---    | ---           | ---         |                 | 0.96(0.73–1.28) | 0.793    |

Abbreviations: SNP: single nucleotide polymorphism, OR: odds ratio, 95% CI: 95% confidence interval;
Notes: P values were calculated using two-sided Chi-squared test; P < 0.05 in bold indicates a statistically significant difference.
Associations between haplotype analyses and the risk of breast cancer
Finally, haplotype analysis was performed to explore the relationship between interactions of the IL-7R SNPs and breast cancer risk. As shown in Fig. 1, rs10213865, rs969129, rs118137916 and rs10053847 consisted of one block in IL-7R gene. As shown in Table 7, haplotype CGAG was observed to prominently reduce the risk of breast cancer through $\chi^2$ test analysis (OR = 0.80, 95% CI, 0.65–0.99; $P$ = 0.043).

| SNPs                      | Haplotype | Freq (Case) | Freq (Control) | Without adjusted OR (95% CI) | $P$ | With adjusted OR (95% CI) | $P$ |
|---------------------------|-----------|-------------|----------------|------------------------------|-----|--------------------------|-----|
| rs10213865/rs969129/rs118137916/rs10053847 | AGAA      | 0.848       | 0.849          | 0.99 (0.79–1.24)             | 0.95| 0.99 (0.79–1.24)         | 0.945|
|                           | ATGG      | 0.926       | 0.92           | 1.09 (0.79–1.49)             | 0.602| 1.09 (0.79–1.49)         | 0.604|
|                           | CGAG      | 0.785       | 0.82           | 0.80 (0.65–0.99)             | 0.043| 0.80 (0.65–0.99)         | 0.043|
|                           | AGAG      | 0.877       | 0.893          | 0.85 (0.65–1.11)             | 0.243| 0.85 (0.65–1.11)         | 0.243|
|                           | ATAG      | 0.436       | 0.481          | 0.83 (0.7–0.99)              | 0.034| 0.83 (0.7–0.99)          | 0.033|

Abbreviations: SNP: single nucleotide polymorphism, OR: odds ratio, 95% CI: 95% confidence interval; Notes: $P$ values were calculated using two-sided Chi-squared test; $P < 0.05$ in bold indicates a statistically significant difference.

Discussion
Genetic variations, including single nucleotide polymorphism, were closely related to the occurrence of breast cancer. Genome-wide association studies (GWAS) have detected many SNPs were related to breast cancer susceptibility in tumor-related genes such as PD-1, FGFR2, ESR1, PTEN, and MAP3K1 [17–19]. The effect of IL-7R SNPs on breast cancer susceptibility in Chinese Han population is not clear at present. In this present work, we revealed that the allele and genotype frequency distribution of IL-7R SNPs (rs969129, rs10213865 and rs6451231) was significantly different between the case and control groups, and they increased the risk of breast cancer. In the age stratified analysis subgroup, IL-7R-rs6451231 significantly increased breast cancer risk among those older than 50 years. Rs10213865, rs969129 and rs6451231 were also significantly correlated with some clinical parameters, such as tumor size, ER status and Ki67 stress status. We have also observed that
haplotype CGAG increases breast cancer susceptibility. These results provided some support for the impact of genetic variation of IL-7R on breast cancer risk among Chinese Han population.

IL-7R, located on chromosome 5p13, is a heterodimer consisting of two strands (IL-7Rα and common γ chain). The binding of IL-7R and IL-7 leads to the activation of tumor-related signaling pathways, including PEDF Induced Signaling and Interleukin-7 signaling pathway, etc [20, 21]. At present, there are many studies on the relevant mechanism of IL-7R in lung cancer. Researchs have reported that IL-7/IL-7R can affect the production level of vascular endothelial growth factor in lung cancer and the formation of lymphatic vessels through the c-fos/c-jun pathway [22]. It can also affect the production of cyclin D1 by activating the AP1 pathway, thus accelerating the proliferation of breast cancer cells [23]. However, there are few researchs has been done on the mechanism of IL-7R in breast cancer. Only studies reported the expression levels of IL-7 and IL-7R have been observed to increase significantly in breast cancer [8, 11], and IL-7 can affect the proliferation rate of breast cancer cells [12]. Therefore, further studies on the mechanism of IL-7R and breast cancer are still needed.

Genetic variation polymorphisms of IL-7R has been reported to affect the occurrence of many diseases. Sinha et al. [24] found that polymorphisms of IL-7R (rs1494555 and rs6897932) were associated with asthma in the northern Indian population. IL-7R -rs6897932 (C/T) was related to the susceptibility of systemic lupus erythematosu [25]. In our study, we explored the influence of IL-7R SNPs (rs6451231, rs10213865, rs118137916, rs969129, and rs10053847) on breast cancer susceptibility in Chinese Han population. Bai et al. [26] that IL-7R SNPs (rs6451231 and rs969129) were significantly correlated with an increased rheumatoid arthritis (RA) susceptibility under multiple genetic model, and the haplotype GAGC constructed by IL-7R SNPs (rs118137916, rs969129, rs6451231 and rs10053847) also increased the RA risk.

Zhang et al. [27] explored the effect of IL-7R polymorphism on lung cancer risk. The rs10053847 and rs10213865 variant was observed to be correlated with a decreased breast cancer risk under multiple genetic model, and AGAA haplotypes (rs10213865, rs969129, rs118137916, and rs10053847) also increased breast cancer risk. However, our study observed that IL-7R SNPs (rs969129, rs10213865 and rs6451231) were significantly increased breast cancer risk, and haplotype CGAG (rs10213865,
rs118137916, rs969129 and rs10053847) significantly increased breast cancer susceptibility. As far as we know, our study is the first to explore the relationship between IL-7R polymorphism and susceptibility to breast cancer.

There are some limitations to this study that cannot be ignored. First, this was a hospital-based case-control study involving only Han women, and the applicability of our conclusions should be explored in other ethnic populations. Second, our sample size was relatively small, and false negative results cannot be excluded. Despite the above limitations, the results of this study provide a scientific basis for future research on IL-7R gene and breast cancer.

In conclusion, our study was the first to found that IL-7R SNPs (rs6451231, rs10213865 and rs969129) were significantly increased breast cancer risk, which provides a basis for further mechanism research of IL-7R and breast cancer.

Abbreviations

IL-7: Interleukin 7; SNPs: single nucleotide polymorphisms; OR: Odds ratio; CIs: 95% confidence intervals.

Declarations

ACKNOWLEDGMENTS

We thank all the participants in this study, including clinicians and hospital staff who provided samples for the study and those who contributed to the writing.

Consent for publication

Written informed consent was obtained from the patient for publication of this report.

Availability of data and materials

The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Authors’ contributions

Miao Li and Chenli Yue: analyzed the data. Xiaoxiao Zuo and Guoquan Jin: performed the
experiments. Guanying Wang, Hulin Guo and Fang Wu: contributed reagents/materials/analysis tools.
Xinhan Zhao and Shangke Huang: conceived and designed the experiments. All authors contributed
significantly to the final draft of the paper and agreed to submit the manuscript for publication.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A: Global cancer
   statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide
   for 36 cancers in 185 countries. CA: a cancer journal for clinicians 2018,
   68(6):394-424.

2. Li T, Mello-Thoms C, Brennan PC: Descriptive epidemiology of breast cancer in
   China: incidence, mortality, survival and prevalence. Breast cancer research
   and treatment 2016, 159(3):395-406.

3. Tan DS, Marchio C, Reis-Filho JS: Hereditary breast cancer: from molecular
   pathology to tailored therapies. Journal of clinical pathology 2008, 61(10):1073-
   1082.

4. Mavaddat N, Antoniou AC, Easton DF, Garcia-Closas M: Genetic susceptibility to
   breast cancer. Molecular oncology 2010, 4(3):174-191.

5. Wu L, Li J, Xu HL, Xu B, Tong XH, Kwak-Kim J, Liu YS: IL-7/IL-7R signaling pathway
   might play a role in recurrent pregnancy losses by increasing inflammatory
   Th17 cells and decreasing Treg cells. American journal of reproductive
   immunology (New York, NY : 1989) 2016, 76(6):454-464.

6. Galarza-Munoz G, Briggs FBS, Evsyukova I, Schott-Lerner G, Kennedy EM, Nyanhete T,
   Wang L, Bergamaschi L, Widen SG, Tomaras GD et al: Human Epistatic Interaction
   Controls IL7R Splicing and Increases Multiple Sclerosis Risk. Cell 2017,
   169(1):72-84.e13.

7. Lundtoft C, Afum-Adjei Awuah A, Rimpler J, Harling K, Nausch N: Aberrant plasma
IL-7 and soluble IL-7 receptor levels indicate impaired T-cell response to IL-7 in human tuberculosis. 2017, 13(6):e1006425.

8. Al-Rawi MA, Mansel RE, Jiang WG: Interleukin-7 (IL-7) and IL-7 receptor (IL-7R) signalling complex in human solid tumours. Histology and histopathology 2003, 18(3):911-923.

9. Toker A, Cantley LC: Signalling through the lipid products of phosphoinositide-3-OH kinase. Nature 1997, 387(6634):673-676.

10. Vanhaesebroeck B, Leevers SJ, Panayotou G, Waterfield MD: Phosphoinositide 3-kinases: a conserved family of signal transducers. Trends in biochemical sciences 1997, 22(7):267-272.

11. Al-Rawi MA, Rmali K, Watkins G, Mansel RE, Jiang WG: Aberrant expression of interleukin-7 (IL-7) and its signalling complex in human breast cancer. European journal of cancer (Oxford, England : 1990) 2004, 40(4):494-502.

12. Al-Rawi MA, Rmali K, Mansel RE, Jiang WG: Interleukin 7 induces the growth of breast cancer cells through a wortmannin-sensitive pathway. The British journal of surgery 2004, 91(1):61-68.

13. Shiovitz S, Korde LA: Genetics of breast cancer: a topic in evolution. Annals of oncology : official journal of the European Society for Medical Oncology 2015, 26(7):1291-1299.

14. Michailidou K, Lindstrom S, Dennis J, Beesley J, Hui S, Kar S, Lemacon A, Soucy P, Glubb D, Rostamianfar A et al: Association analysis identifies 65 new breast cancer risk loci. Nature 2017, 551(7678):92-94.

15. Gabriel S, Ziaugra L, Tabbaa D: SNP genotyping using the Sequenom MassARRAY iPLEX platform. Current protocols in human genetics 2009, Chapter 2:Unit 2.12.
16. Dai ZJ, Liu XH, Kang HF, Wang XJ, Jin TB, Zhang SQ, Feng T, Ma XB, Wang M, Feng YJ et al: Genetic Variation in Metastasis-Associated in Colon Cancer-1 and the Risk of Breast Cancer Among the Chinese Han Population: A STROBE-Compliant Observational Study. Medicine 2016, 95(6):e2801.

17. Xia P, Li B, Geng T, Deng Z, Dang C, Chang D, Kang L, Jin T, Chen C: FGFR2 gene polymorphisms are associated with breast cancer risk in the Han Chinese population. American journal of cancer research 2015, 5(5):1854-1861.

18. Ren HT, Li YM, Wang XJ, Kang HF, Jin TB, Ma XB, Liu XH, Wang M, Liu K, Xu P et al: PD-1 rs2227982 Polymorphism Is Associated With the Decreased Risk of Breast Cancer in Northwest Chinese Women: A Hospital-Based Observational Study. Medicine 2016, 95(21):e3760.

19. Xia P, Jin T, Geng T, Sun T, Li X, Dang C, Kang L, Chen C, Sun J: 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 and Medicine 2014, 35(3):2187-2190.

20. Wu TH, Bolt AM, Chou H, Plourde D, De Jay N, Guilbert C, Young YK, Kleinman CL, Mann KK: Tungsten Blocks Murine B Lymphocyte Differentiation and Proliferation Through Downregulation of IL-7 Receptor/Pax5 Signaling. Toxicological sciences : an official journal of the Society of Toxicology 2019, 170(1):45-56.

21. Jian M, Yunjia Z, Zhiying D, Yanduo J, Guocheng J: Interleukin 7 receptor activates PI3K/Akt/mTOR signaling pathway via downregulation of Beclin-1 in lung cancer. 2019, 58(3):358-365.

22. Ming J, Zhang Q, Qiu X, Wang E: Interleukin 7/interleukin 7 receptor induce c-Fos/c-Jun-dependent vascular endothelial growth factor-D up-regulation: a
mechanism of lymphangiogenesis in lung cancer. *European journal of cancer* (Oxford, England : 1990) 2009, **45**(5):866-873.

23. Ming J, Jiang G, Zhang Q, Qiu X, Wang E: Interleukin-7 up-regulates cyclin D1 via activator protein-1 to promote proliferation of cell in lung cancer. *Cancer immunology, immunotherapy : CII* 2012, **61**(1):79-88.

24. Sinha S, Singh J, Jindal SK: Association of interleukin 7 receptor (rs1494555 and rs6897932) gene polymorphisms with asthma in a north Indian population. *Allergy & rhinology (Providence, RI)* 2015, **6**(3):168-176.

25. Wang XS, Wen PF, Zhang M, Hu LF, Ni J, Qiu LJ, Liang Y, Zhao W, Huang Q, Tao SS et al: Interleukin-7 receptor single nucleotide polymorphism rs6897932 (C/T) and the susceptibility to systemic lupus erythematosus. *Inflammation* 2014, **37**(2):615-620.

26. Bai M, He X, He Y, Yuan D, Jin T, Wang L: IL-7R gene polymorphisms among patients with rheumatoid arthritis: A case-control study. 2019, **7**(7):e00738.

27. Zhang C, Su P, Chen W, Li Q, Dai R, Cheng Y, Yang J: Genetic polymorphisms in IL-7 and IL-7R are correlated with lung cancer risk in the Chinese Han population. *Cancer management and research* 2019, **11**:5393-5401.

Figures
rs10213865, rs969129, rs118137916 and rs10053847 consisted of one block in IL-7R gene.

Supplementary Files
This is a list of supplementary files associated with this preprint. Click to download.
Supplementary Tables.docx