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# Association between *KRAS* gene polymorphisms and genetic susceptibility to breast cancer in a Chinese population

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## Abstract

**Objective:** The *KRAS* gene has a pathophysiological role in the development of many cancers. This study aims to investigate the relationship between *KRAS* polymorphisms and genetic susceptibility to breast cancer.

**Method:** The rs712, rs12587 and rs9266 gene loci in the *KRAS* gene of 421 subjects (141 breast cancer patients, 141 benign breast tumours and 139 healthy controls) were analysed by the polymerase chain reaction and SNaPshot sequencing. Transcriptomic information on *KRAS* and corresponding clinical information was downloaded from the TCGA and GTEx databases. Differences in *KRAS* expression between breast cancer tissues and control tissues were analysed.

**Results:** We found no significant association between *KRAS* rs712 and rs12587 locus gene polymorphisms and an increased risk of developing benign breast tumours and breast cancer ($p > 0.05$). The *KRAS* rs9266 locus mutation heterozygous model CT and dominant model CT + TT were significantly associated with an increased risk of breast cancer (both $p < 0.05$). In addition, the TAT haplotype was expressed at an increased frequency, and the GAC haplotype was expressed at a reduced frequency in breast cancer compared with controls (both $p < 0.05$). We found that *KRAS* was over expressed in breast cancer tumour tissues compared with the control tissues ($p < 0.0001$).

**Conclusion:** The *KRAS* rs9266 gene polymorphism and the TAT haplotype may be associated with an increased risk of breast cancer in Chinese women. The GAC haplotype may be a protective factor against breast cancer.

KEYWORDS
breast cancer, Chinese population, *KRAS*, single-nucleotide polymorphism

## 1 | INTRODUCTION

Breast cancer (BRCA) is a malignant tumour that begins in the glandular epithelial tissue of the breast, and the majority of patients are women.[1] The global incidence of BRCA has risen since the late 1970s and is the second leading cause of death in Western countries.[2] The incidence of BRCA among Chinese women is growing twice as fast as the global rate, and it is the fifth leading cause of cancer-related

Min Jin and Fengke Lu contributed equally to this work and share first authorship.

deaths in China.[3] At present, medical researchers studying the early diagnosis and treatment of cancer have made some progress, but BRCA still poses a severe threat to the health of women, and its morbidity and mortality rates are expected to continue to increase in the next few years.

*KRAS* is one of the most frequently mutated oncogenes in cancer. This oncogene can be used as an early diagnosis of malignant tumours and as a predictive biomarker for response to treatment.[4] A signal transduction protein located in the downstream area of the intracellular signal transduction pathway is encoded by *KRAS*. This protein has an essential role in regulating cell growth, apoptosis, survival, differentiation and other biological processes.[5] Generally, the expression of the *KRAS* genotype can be regular (wild type) or abnormal (mutant type). The protein activation and inactivation effects of regular *KRAS* gene expression are controlled. The growth of potential tumour cells is inhibited by normal *KRAS* genes belonging to tumour suppressor genes. It has been shown in studies that *KRAS* has gradually evolved from a biomarker with predictive value to a more promising therapeutic target.[6,7]

The 3'untranslated region (3'UTR) is a gene sequence not involved in translation and expression, located downstream of the mature messenger ribonucleic acid (mRNA) coding region. It has been shown in previous studies that single-nucleotide polymorphisms (SNPs) in the 3'UTR are related to susceptibility to a variety of diseases.[8–11] The let-7 microRNA (miRNA) family, as a small, noncoding RNA molecule, has a vital role in the process of carcinogenesis by targeting tumour suppressor genes or as highly expressed oncogenes.[12] According to existing reports, the functional SNP of the miRNA let-7 binding site in the 3'UTR of *KRAS* mRNA may be related to the risk of various cancers, including colorectal cancer, lung nasopharyngeal cancer and ovarian cancer.[13–17] Therefore, studying the 3'UTR-related gene polymorphism of *KRAS* will have an essential role in the early diagnosis, treatment and prognosis of cancer.

At present, the research results on the association between the 3'UTR gene polymorphism of *KRAS* and BRCA susceptibility have not been uniform, and the association between *KRAS* rs12587 and rs9266 and BRCA genetic susceptibility has not been reported. Therefore, we will further study and analyse the partial binding sites of the 3'UTR of *KRAS* and plan to explore the relationship between SNPs at three *KRAS* sites—rs712, rs12587 and rs9266—and genetic BRCA susceptibility.

## 2 | MATERIALS AND METHODS

### 2.1 | Study population

The patients included in this case–control study were all admitted to the First Affiliated Hospital of Guangxi Medical University in China from August to December 2019. All study participants provided written informed consent. This study was approved by the Ethics Committee of the First Affiliated Hospital of Guangxi Medical University.

The included case groups were confirmed by histopathological examination. All patients were unrelated females, and blood specimens were collected prior to antineoplastic treatment, such as chemotherapy or radiotherapy. The inclusion criteria of the BRCA group refer to the Guidelines and Norms for the Diagnosis and Treatment of Breast Cancer of the Chinese Anti-Cancer Association (2019 edition).[18] The inclusion criteria of the benign breast tumour group were breast fibroids, intraductal or intracystic papillomas, lymphangiomas, ductal papillomas, lipomas and other benign tumours. The exclusion criteria of the case group were no prominent organic diseases and no previous serious diseases, such as tumours, endocrine disorders, diseases of the cardiovascular system or severe hepatic or renal insufficiency.

The healthy control group was selected from the health examination centre of the First Affiliated Hospital of Guangxi Medical University during the same period. The exclusion criteria were no prominent organic or severe diseases, such as tumours, endocrine disorders, diseases of the cardiovascular system or severe hepatic or renal insufficiency.

### 2.2 | *KRAS* gene polymorphism detection

#### 2.2.1 | DNA extraction and primer design

An AxyPrep Blood Genomic DNA Mini Kit (Corning Life Sciences Ltd.) was used to extract genomic DNA from 2 ml of peripheral blood. All samples were stored at −80°C.

The oligonucleotide primers of rs712 and rs9266 were forward, 5′-TAGCATTTGTTTTAGCATTACC-3′ and reverse, 5′-CAGTGGAAAGGAGACAAAAC-3′. For rs12587, the primers used were forward, 5′-GTAGGCATTCTAGGCTCTATTT-3′ and reverse, 5′-TATCCCAAACAGGCACTTC-3′. The polymerase chain reaction (PCR) amplification primers were designed and synthesised by Guangzhou Tsingke Biotechnology Co. Ltd.

#### 2.2.2 | PCR

The PCR reaction conditions were pre-denaturation at 95°C for 15 min, followed by 15 cycles of 0.5°C drop per cycle: denaturation at 94°C for 40 s; annealing at 63°C for 1 min; extension at 72°C for 8 min. This was followed by denaturation at 94°C for 40 s; annealing at 56°C for 40 s; extension at 72°C for 90 s for 25 cycles. The final extension was at 72°C for 8 min. PCR amplification products were subjected to constant pressure (120 V) electrophoresis on 1.0% agarose. After 45 min, the electrophoretic bands were observed under a gel imaging system. The lengths of the genomic fragments amplified by rs712, rs12587 and rs9266 were 489 bp, 536 bp and 489 bp, respectively (Figure S1).

#### 2.2.3 | Pre-treatment of PCR amplification products

After PCR amplification, 4.05 ul of a mixture of Exonuclease I (Exo I) and shrimp alkaline phosphatase (SAP) was added to the PCR amplification product and incubated at 37°C for 1 h, followed by a 15-min hold at 75°C to inactivate the SAP and Exo I.

#### 2.2.4 | Genetic sequencing and validation

Two microlitre of each pre-treated PCR product was mixed and used as a template for SNaPshot PCR. SNaPshot PCR reaction conditions were pre-denaturation at 96°C for 2 min, followed by 25 cycles: denaturation at 96°C for 10 s; annealing at 50°C for 10 s; extension at 60°C for 30 s. Final extension was performed at 4°C. The purified SNaPshot PCR product was obtained by adding 0.5 U SAP to 5 μl of the above SNaPshot PCR product, incubating at 37°C for 1 h and 75°C for 15 min. The SNaPshot method was used to detect alleles and genotypes of the three SNP loci of the *KRAS* gene in the samples for analysis (Figures S2–S4) (Guangzhou Tsingke Biotechnology Co. Ltd.).

A total of 10% of the samples were randomly selected from each site to use the first-generation common sequencing method (Sanger) for gene sequencing verification (Nanning Guotuo Biotechnology Co.).

### 2.3 | Bioinformatics analysis

The expression of *KRAS* mRNA in BRCA and control tissues was further explored by bioinformatic analysis. The RNAseq data (level 3) and corresponding clinical information for BRCA tissues and paracancerous tissues were downloaded from the Cancer Genome Atlas (TCGA) database (https://portal.gdc.com). Transcriptomic data for normal tissues were obtained from the current version (V8) of the GTEx dataset (https://www.gtexportal.org/home/datasets). All the data used were standardised TPM data, and the data distribution was close to the normal distribution. The "ggplot2" R package was used to integrate and analyse these data.

### 2.4 | Statistical analysis

The SPSS 25.0 software was used for statistical analysis. The goodness-of-fit $\chi^2$ test was used to analyse whether the gene frequency distribution in each group conforms to the Hardy–Weinberg Equilibrium (HWE) law. The K-S normality test was conducted on each data group. Measurement data subject to normal distribution were represented by $\bar{X} \pm SD$, and measurement data of non-normal distribution were represented by the median M (P25, P75). A comparison between multiple groups was carried out using a one-way analysis of variance. The pairwise comparison between groups was performed using the LSD test, and the counting data were conducted using the $\chi^2$ test. The correlation between *KRAS* gene polymorphism and the risk of BRCA was analysed by binary logistic regression. SHEsis online software was used to build a haploid model for SNPs at three sites and analyse the correlation between each haploid and the risk of BRCA. Wilcox tests were performed on the database information using R software v4.0.3 (R Foundation for Statistical Computing). A $p$-value $< 0.05$ was considered statistically significant.

## 3 | RESULTS

### 3.1 | Comparison of age parameters in the study population

A total of 421 subjects were successfully genotyped and divided into three groups: 141 patients with BRCA, 141 patients with benign breast tumours and 139 healthy controls. The mean age of the BRCA, benign breast tumour and control groups was $48.47 \pm 8.84$, $39.52 \pm 10.98$ and $45.83 \pm 8.66$, respectively. The difference in age between the three groups of subjects was statistically significant ($p < 0.05$).

### 3.2 | HWE test results for *KRAS* genes rs712, rs12587 and rs9266

The genotypes and allele frequencies of *KRAS* rs712, rs12587 and rs9266 between the BRCA group and the benign breast tumour group are shown in Table 1. The predicted and actual values of the genotype frequencies at the three SNPs of the *KRAS* in each group were in accordance with the HWE equilibrium law ($p > 0.05$) after goodness-of-fit $\chi^2$ test analysis. The results are shown in Table 2.

### 3.3 | Risk assessment of various gene models at three loci of *KRAS* gene in breast tumour group and control group

We examined the genotypes of the three *KRAS* loci using the SNaPshot method and validated them with Sanger sequencing. The sequencing results of the samples taken were consistent with the results of this SNaPshot experiment.

*KRAS* rs712 and rs12587 mutation genotypes and alleles had no correlation with the risk of BRCA ($p > 0.05$). The dominant model and recessive model had no correlation with the risk of BRCA ($p > 0.05$). However, the rs9266 mutation genotype CT and the dominant model CT + TT were associated with the risk of BRCA ($p < 0.05$), whereas rs9266 was not associated with BRCA risk under the homozygous comparison, allelic model and recessive model ($p > 0.05$).

Taking the benign breast tumour group as a control, the genotypes, alleles, dominant models and recessive models of the three SNPs of *KRAS* were not correlated with the risk of BRCA ($p > 0.05$). The results are shown in Table 1.

**TABLE 1** Genotype and allele frequencies of *KRAS* polymorphisms and their association with risk of BRCA and Benign breast tumour.

| SNPs/Models | Genotypes | Controls $n = 139$ (%) | Breast cancer group versus controls | | | Benign breast tumour group versus controls | | | Breast cancer group versus benign breast tumour group | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | $n = 141$ (%) | OR (95% CI) | $P_{OR}$ | $n = 141$ (%) | OR (95% CI) | $P_{OR}$ | OR (95% CI) | $P_{OR}$ |
| rs712 | | | | | | | | | | |
| Allele | G | 241 (86.7) | 228 (80.9) | ref | | 234 (83.0) | ref | | ref | |
| | T | 37 (13.3) | 54 (19.1) | 1.52 (0.958–2.411) | 0.075 | 48 (17.0) | 1.491 (0.912–2.438) | 0.111 | 1.142 (0.714–1.828) | 0.579 |
| Codominant | GG | 106 (76.3) | 92 (65.3) | ref | | 97 (68.8) | ref | | ref | |
| | GT | 29 (20.9) | 44 (31.2) | 1.718 (0.990–2.983) | 0.054 | 40 (28.4) | 1.64 (0.913–2.946) | 0.098 | 1.177 (0.668–2.072) | 0.573 |
| | TT | 4 (2.8) | 5 (3.5) | 1.412 (0.361–5.518) | 0.620 | 4 (2.8) | 1.457 (0.339–6.257) | 0.612 | 1.176 (0.282–4.913) | 0.824 |
| Dominant | GG | 106 | 92 | ref | | 97 | ref | | ref | |
| | GT+TT | 33 | 49 | 1.681 (0.991–2.851) | 0.054 | 44 | 1.619 (0.924–2.837) | 0.092 | 1.177 (0.682–2.030) | 0.559 |
| Recessive | GT+GG | 135 | 136 | ref | | 137 | ref | | ref | |
| | TT | 4 | 5 | 1.222 (0.315–4.741) | 0.772 | 4 | 1.284 (0.302–5.459) | 0.735 | 1.119 (0.271–4.625) | 0.877 |
| rs12587 | | | | | | | | | | |
| Allele | C | 235 (84.5) | 229 (81.2) | ref | | 234 (83.0) | ref | | ref | |
| | A | 43 (15.5) | 53 (18.8) | 1.216 (0.778–1.902) | 0.390 | 48 (17.0) | 1.29 (0.802–2.076) | 0.294 | 1.1 (0.686–1.762) | 0.693 |
| Codominant | CC | 98 (70.5) | 92 (65.3) | ref | | 97 (68.8) | ref | | ref | |
| | CA | 39 (28.1) | 45 (31.9) | 1.171 (0.696–1.973) | 0.552 | 40 (28.4) | 1.173 (0.673–2.044) | 0.573 | 1.178 (0.671–2.067) | 0.569 |
| | AA | 2 (1.4) | 4 (2.8) | 2.058 (0.357–11.858) | 0.419 | 4 (2.8) | 2.953 (0.495–17.621) | 0.235 | 0.934 (0.205–4.252) | 0.929 |
| Dominant | CC | 98 | 92 | ref | | 97 | ref | | ref | |
| | CA+AA | 41 | 49 | 1.214 (0.729–2.200) | 0.456 | 44 | 1.252 (0.729–2.150) | 0.416 | 1.153 (0.669–1.988) | 0.608 |
| Recessive | CA+CC | 137 | 137 | ref | | 137 | ref | | ref | |
| | AA | 2 | 4 | 1.961 (0.343–11.229) | 0.449 | 4 | 2.812 (0.476–16.626) | 0.254 | 0.887 (0.197–4.001) | 0.876 |
| rs9266 | | | | | | | | | | |
| Allele | C | 241 (86.7) | 227 (80.5) | ref | | 234 (83.0) | ref | | ref | |
| | T | 37 (13.3) | 55 (19.5) | 1.558 (0.983–2.467) | 0.059 | 48 (17.0) | 1.491 (0.912–2.946) | 0.098 | 1.173 (0.734–1.873) | 0.504 |
| Codominant | CC | 106 (76.3) | 91 (64.5) | ref | | 97 (68.8) | ref | | ref | |
| | CT | 29 (20.8) | 45 (31.9) | 1.781 (1.027–3.087) | **0.040** | 40 (28.4) | 1.64 (0.913–2.946) | 0.098 | 1.223 (0.696–2.150) | 0.484 |
| | TT | 4 (2.9) | 5 (3.5) | 1.428 (0.365–5.583) | 0.608 | 4 (2.8) | 1.457 (0.339–6.257) | 0.612 | 1.191 (0.285–4.977) | 0.810 |
| Dominant | CC | 106 | 91 | ref | | 97 | ref | | ref | |
| | CT+TT | 33 | 50 | 1.738 (1.026–2.944) | **0.040** | 44 | 1.619 (0.924–2.837) | 0.092 | 1.22 (0.708–2.102) | 0.474 |
| Recessive | CT+CC | 135 | 136 | ref | | 137 | ref | | ref | |
| | TT | 4 | 5 | 1.222 (0.315–4.741) | 0.772 | 4 | 1.284 (0.302–5.459) | 0.735 | 1.119 (0.271–4.625) | 0.877 |

*Note*: The predicted and actual values of genotype frequencies in the controls were consistent with the HWE law (Table S1). Bold text indicates a statistically significant analysis.

Abbreviations: ref, reference; SNPs, single-nucleotide polymorphisms.

**TABLE 2** HWE test of three *KRAS* gene loci genotypes.

| | Breast cancer group ($n = 141$) | | Benign breast tumour group ($n = 141$) | |
|---|---|---|---|---|
| Genotypes | Measured value | Predicted value | Measured value | Predicted value |
| rs712 | | | | |
| GG | 92 | 92.2 | 97 | 97.1 |
| GT | 44 | 43.7 | 40 | 39.8 |
| TT | 5 | 5.2 | 4 | 4.1 |
| $\chi^2$ | 0.009 | | 0.003 | |
| $p$ | 0.926 | | 0.960 | |
| rs12587 | | | | |
| CC | 92 | 93.0 | 97 | 97.1 |
| CA | 45 | 43.0 | 40 | 39.8 |
| AA | 4 | 5.0 | 4 | 4.1 |
| $\chi^2$ | 0.293 | | 0.003 | |
| $p$ | 0.588 | | 0.960 | |
| rs9266 | | | | |
| CC | 91 | 91.4 | 97 | 97.1 |
| CT | 45 | 44.3 | 40 | 39.8 |
| TT | 5 | 5.4 | 4 | 4.1 |
| $\chi^2$ | 0.038 | | 0.003 | |
| $p$ | 0.845 | | 0.960 | |

### 3.4 | Haplotype analyses of *KRAS* gene polymorphisms and BRCA, benign breast tumour risk

The haplotypes constructed in the control, benign and BRCA groups included GAC, GCC, TAT, TCT and GAT. The GAC haploid was expressed more frequently in the controls than in the BRCA and benign breast tumour groups ($p < 0.05$). It may be one of the protective factors for BRCA and benign breast tumours. The TAT haplotype may be related to the risk of BRCA compared with controls (OR = 1.629, $p = 0.040$), and the other haplotypes did not correlate with the risk of BRCA, as shown in Table 3.

### 3.5 | The different expressions of *KRAS* in BRCA and control tissues

To evaluate the *KRAS* expression in BRCA and control tissues, mRNA expression data were collected from 1101 BRCA tissues and 572 control tissues (including 113 paraneoplastic tissues and 459 normal tissues). The results show that *KRAS* was overexpressed in BRCA tissues compared with controls ($p < 0.0001$) (Figure 1).

## 4 | DISCUSSION

Breast cancer is a heterogeneous disease associated with genetic and environmental factors affecting women worldwide.[19] Among the RAS gene family, *KRAS* has a significant impact on the developmental process and prognosis of cancer through its involvement in intracellular signalling. In this study, we evaluated three SNPs for *KRAS* 3'UTR in patients with BRCA and benign breast tumours and in healthy controls. At the same time, haploid constructs were performed for the SNPs at the *KRAS* rs712, rs12587 and rs9266 loci. Finally, the expression of *KRAS* in BRCA tissues and normal tissues was compared by database.

We found that *KRAS* was overexpressed in BRCA tumour tissues, and the *KRAS* rs9266 CT genotype (OR = 1.781; 95% CI = 1.027–3.087; $p = 0.040$), dominant gene model CT + TT (OR = 1.738; 95% CI = 1.026–2.944; $p = 0.040$) was significantly associated with BRCA susceptibility. Kim et al. found that *KRAS* rs712 and rs9266 were correlated with the risk and prognosis of lung and ovarian cancers.[20] In the let-7 miRNA complementary site of the 3'UTR of the *KRAS* gene, a specific SNP variation was associated with the risk and prognosis of various cancers. The rs9266 site has a functional role in regulating *KRAS* by destroying the complementary sites of various miRNAs (including let-7 and miR-181), indicating that the biological mechanism of rs9266 in the region is related to the occurrence and development of cancer.[20] This finding is consistent with the conclusions of this study.

However, no genotype or allele association between *KRAS* rs712 and BRCA risk was found in this study. This finding is consistent with the conclusion of Huang et al.[17] In contrast, in the results found by Sanaei et al. in Iran, the *KRAS* rs712 TT vs. GG + GT genotypes were shown to be associated with a reduced BRCA risk (OR = 0.560;

TABLE 3 Haplotype distribution in cases and healthy control subjects.

| Haplotype | Controls (n = 139) | Breast cancer group (n = 141) | | | Benign breast tumour group (n = 141) | | | Breast cancer group versus benign breast tumour group | |
|---|---|---|---|---|---|---|---|---|---|
| | Frequency | Frequency | OR (95% CI) | p | Frequency | OR (95% CI) | p | OR (95% CI) | p |
| GAC | 0.033 | 0.000 | - | **0.002** | 0.000 | - | **0.002** | - | - |
| GCC | 0.834 | 0.805 | 0.809 (0.519–1.261) | 0.349 | 0.830 | 0.904 (0.576–1.417) | 0.660 | 0.895 (0.581–1.380) | 0.617 |
| TAT | 0.122 | 0.184 | 1.629 (1.019–2.604) | **0.040** | 1.170 | 1.458 (0.907–2.345) | 0.118 | 1.117 (0.724–1.721) | 0.617 |
| TCT | 0.011 | 0.007 | - | - | 0.000 | - | - | - | - |
| GAT | 0.000 | 0.004 | - | - | - | - | - | - | - |

*Note*: Bold text indicates a statistically significant analysis.

95% CI = 0.380–0.840; $p = 0.006$). Additionally, the rs712 T allele reduced BRCA risk compared with the G allele (OR = 0.750, 95% CI = 0.580–0.970, $p = 0.031$).[21] The inconsistency with the results of this study may be related to the fact that the study subjects originated from different populations and differences in sample sizes. In a study of the association between *KRAS* rs712 and other tumours, according to the results of a meta-analysis by Gholami et al, the risk of colorectal cancer in the East Asian population was increased by rs712 polymorphism. At the same time, in a European population, rs712 may have a protective effect. It has been shown that this locus may have different effects on different ethnic groups, and the results of research on different groups based on population stratification are of great value.[22]

Similarly, no correlation between *KRAS* rs12587 and BRCA risk was found in this study. There is no report about the association between the two at present, and there are also many controversies in other cancer-related reports. Fu et al. showed that the risk of nephroblastoma was increased by rs12587 gene polymorphism in *KRAS*. In further stratified analyses, it was demonstrated that the rs12587 gene polymorphism was associated with nephroblastoma risk in children over 18 years of age (corrected OR = 1.390, 95% CI = 1.020–1.890, $p = 0.037$).[23] However, no significant correlation between the two was found by Lin et al.[24] Therefore, in future studies, analyses of research objects from multiple centres and large sample sources of different populations are still needed.

Furthermore, compared with the control group, in this study, it was found that the TAT haplotypes constructed at three sites were associated with increased BRCA susceptibility in the BRCA group, and the GAC haplotypes may be a protective factor in BRCA ($p < 0.05$). However, the relationship between these haplotypes and BRCA has not been reported in any other studies.

The findings of this study should be interpreted with caution, considering its limitations. First, the sample size of the genetic association study was relatively small, although the sample size was sufficient to assess risk. Second, this study sampled the Guangxi population using participants from only one hospital. Therefore, our results may not be generalisable to other populations. Third, BRCA pathogenesis is influenced by multiple factors. Various environmental factors and individual exposure to genetic susceptibility may influence the function of SNPs. Finally, the exact mechanisms by which BRCA susceptibility is affected by *KRAS* polymorphisms remain unknown. Further studies are needed to address these questions thoroughly.

In summary, the polymorphism of *KRAS* rs9266 and the TAT haplotype constructed at three sites may be related to increased BRCA risk in Chinese women. The GAC haplotype may be a protective factor against BRCA. BRCA aetiology is complex. Therefore, larger sample sizes and multicentre studies are needed to gain insights into the impact of *KRAS* gene polymorphisms on the BRCA development mechanism, to screen people with genetic risk and to provide a reference for the early diagnosis and prognosis of BRCA treatment.

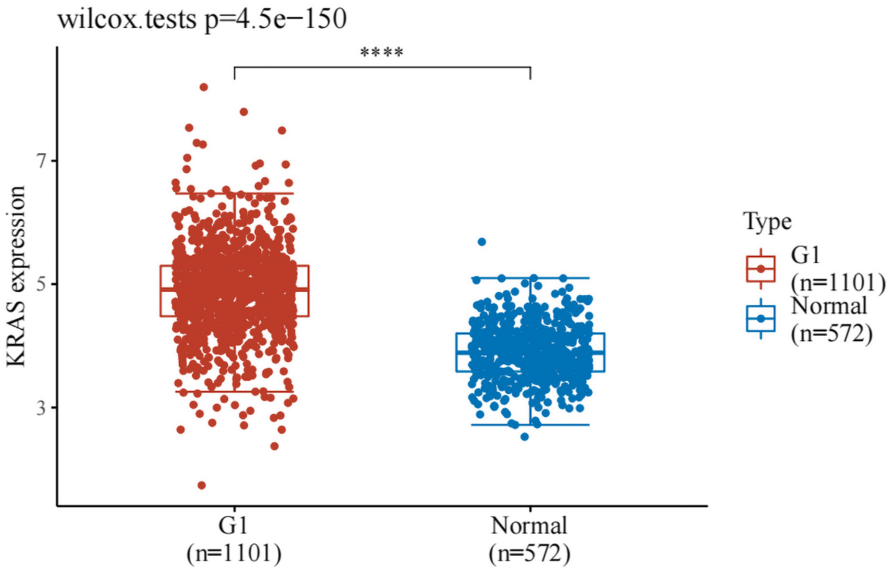


**FIGURE 1** The expression distribution of *KRAS* gene in BRCA tissues and control tissues. G1: BRCA tissues; Normal: paraneoplastic and normal tissues. ****$p < 0.0001$.

## AUTHOR CONTRIBUTIONS

All the authors were involved in the design of this study. MJ and FL wrote the article experimental and were responsible for the procedures, and statistical analyses; XL, YJ, WZ and SL were responsible for the collection of samples, and HW and JW were responsible for the evaluation of clinical data. All the authors contributed to the article and approved the submitted version.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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