# Association between the CASC16 rs4784227 polymorphism and breast cancer risk and prognosis in a northeast Chinese Han population

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**Background:** Breast cancer (BC) poses a serious threat to women worldwide. This research was designed to explore the association between the rs4784227 polymorphism of cancer susceptibility candidate gene 16 (*CASC16*) and BC susceptibility and prognosis, aiming to provide further information for the early detection of BC and to accelerate comprehensive cancer management. **Methods:** A total of 1733 subjects were recruited for this case-control study, of which 828 are BC patients and 905 are healthy individuals. The relevance between SNP rs4784227 and BC risk in diverse genetic models was analyzed by using the SNPStats analysis program and was assessed by odds ratios (ORs) and 95% confidence intervals (CIs) using the binary logistic regression model. Pearson's $\chi^2$ test was used to determine the correlation between the polymorphism and clinical characteristics of BC patients. Additionally, univariate survival analysis was performed by the Kaplan-Meier method and log-rank test, and multivariate survival analysis was performed by Cox regression. **Results:** SNP rs4784227 was significantly associated with susceptibility to BC in the dominant model (CT/TT versus CC, OR = 1.237, 95% CI = 1.012–1.513, *P*=0.038). The minor allele of SNP rs4784227 was significantly linked to an increased risk of BC (OR=1.197, 95% CI=1.022–1.401, *P*=0.026). In addition, the rs4784227 polymorphism of *CASC16* was associated with perineural invasion (*P*=0.030), menstrual status (*P*=0.016) and histological grade (*P*=0.001, *P*=0.003, *P*=0.025; respectively) of BC patients. There was no significant association between the genotypes of rs4784227 and disease-free survival (DFS) or overall survival (OS) of breast cancer patients (*P*>0.05) . **Conclusions:** The rs4784227 polymorphism of *CASC16* may affect susceptibility to breast cancer and is associated with perineural invasion, menstrual status and histological grade in BC patients. Additionally, our results could not confirm that this polymorphism was related to breast cancer prognosis.

1 Association between the CASC16 rs4784227 polymorphism and breast cancer risk

2 and prognosis in a northeast Chinese Han population

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17    **Abstract**

18    **Background:** Breast cancer (BC) poses a serious threat to women worldwide. This research was

19    designed to explore the association between the rs4784227 polymorphism of cancer

20    susceptibility candidate gene 16 (*CASC16*) and BC susceptibility and prognosis, aiming to

21    provide further information for the early detection of BC and to accelerate comprehensive cancer

22    management.

23    **Methods:** A total of 1733 subjects were recruited for this case-control study, of which 828 are

24    BC patients and 905 are healthy individuals. The relevance between SNP rs4784227 and BC risk

25    in diverse genetic models was analyzed by using the SNPStats analysis program and was

26    assessed by odds ratios (ORs) and 95% confidence intervals (CIs) using the binary logistic

27    regression model. Pearson's $\chi^2$ test was used to determine the correlation between the

28    polymorphism and clinical characteristics of BC patients. Additionally, univariate survival

29    analysis was performed by the Kaplan-Meier method and log-rank test, and multivariate survival

30    analysis was performed by Cox regression.

31    **Results:** SNP rs4784227 was significantly associated with susceptibility to BC in the dominant

32    model (CT/TT versus CC, OR = 1.237, 95% CI = 1.012–1.513, *P*=0.038). The minor allele of

33    SNP rs4784227 was significantly linked to an increased risk of BC (OR=1.197, 95% CI=1.022–

34    1.401, *P*=0.026). In addition, the rs4784227 polymorphism of *CASC16* was associated with

35    perineural invasion (*P*=0.030), menstrual status (*P*=0.016) and histological grade (*P*=0.001,

36    *P*=0.003, *P*=0.025; respectively) of BC patients. There was no significant association between

37    the genotypes of rs4784227 and disease-free survival (DFS) or overall survival (OS) of breast

38    cancer patients (*P*>0.05).

39    **Conclusions:** The rs4784227 polymorphism of *CASC16* may affect susceptibility to breast

40    cancer and is associated with perineural invasion, menstrual status and histological grade in BC

41    patients. Additionally, our results could not confirm that this polymorphism was related to breast

42    cancer prognosis.

43    **Keywords:** Breast cancer, *CASC16*, Single nucleotide polymorphism, Susceptibility, Prognosis

## 1. Introduction

Breast cancer (BC) is the most common cause of cancer death in women and has become the most common form of cancer worldwide. Similarly, the estimated age-standardized incidence rate of BC is the highest among all cancers in China[1]. The recurrence rate of patients with early breast cancer after surgery is relatively low[2]. According to information from the 2019 Chinese Society of Clinical Oncology (CSCO) Breast Cancer Annual Meeting, the 5-year survival rate of breast cancer in China was more than 80%. The increase in the cure rate of breast cancer in China depends on early detection, early diagnosis and early treatment. To improve the BC survival rate, early screening and early detection are critical and are designed primarily to identify those at high risk.

High-risk groups can be identified by risk factors such as female sex, early menarche, late menopause, increased estrogen exposure and excessive alcohol intake[3-6]. Additionally, gene detection is also feasible. Previous studies have shown that people who carry the breast cancer susceptibility gene 1 (*BRCA1*) or breast cancer susceptibility gene 2 (*BRCA2*) variants are predisposed to breast cancer[7]. This is an indication that single nucleotide polymorphism (SNP) studies could help identify people with high susceptibility to breast cancer.

Cancer susceptibility candidate gene 16 (*CASC16*), is one of these genes that have been studied. *CASC16* is an RNA gene that is located at chromosome 16q12.1 and is affiliated with the lncRNA class. One study showed that the *CASC16* gene had higher expression in breast cancer cells than in normal cells[8], and some loci of *CASC16* have been demonstrated to be significantly associated with BC susceptibility [9-12]. However, the association between this gene

65   and other cancers has not yet been found. Rs4784227 is a locus of the *CASC16* gene and

66   previous studies showed that its MAF in Asian groups was in a range from 0.20 to 0.28[13]. It is

67   well known that *FOXA1* is involved in *ESR1*-mediated transcription, the regulation of cell

68   apoptosis and cell cycle regulation. The place for rs4784227 on *FOXA1* genomic for interaction

69   is on the eighth position of the *FKH* motif recognized via *FOXA1*, thus, affinity DNA site for

70   *FOXA* protein was enhanced for the T allele compared with the C allele in rs4784227, which

71   suggested that the locus may affect a DNA binding sequence change on *FOXA1* and modulate

72   the chromatin affinity for *FOXA1*[11, 14]. However, whether the interaction between *FOXA1* and

73   *CASC16* participates in the above functions and what will be caused by it are not clear. Current

74   studies are limited to the relationship between this locus and BC susceptibility, and the functions

75   of *CASC16* are still unknown[10].

76        Moreover, rs4784227 has been known to be linked to BC risk in previous studies conducted

77   in Chinese populations, such as Shanghai, Tianjin, Nanjing and Taiwan (OR>1)[9]. However,

78   these subject groups are mainly located in southern and coastal cities, and relatively little

79   research has been done on inland areas. Investigations of this locus using a northeast Chinese

80   population may verify whether the findings previously identified are generalizable to other

81   populations. Data from a study published previously indicated that the rs4784227 SNP in

82   *CASC16* promotes lymph node metastasis in BC patients[15]. In addition, stratified analysis using

83   a genetic model indicated that rs4784227 was specific to progesterone receptor positivity[16]. In

84   addition, there have been no studies of the correlation between this locus and BC survival. Thus,

85    in this article, we further explored the clinical features of SNP rs4784227 and its relationship

86    with BC prognosis.

87    **2. Subjects and methods**

88    **2.1 Study population**

89    In total, 828 BC patients confirmed by pathological cytology and histology and 905 healthy

90    individuals from the First Affiliated Hospital of Jilin University (Changchun, Jilin Province,

91    China), who were all genetically unrelated Han Chinese and originated from the Northeast, were

92    recruited in this case-control study. The sample size was calculated by Quanto and the power of

93    our study was above 90%. In addition, the clinical characteristics of patients with BC, including

94    age, menopausal status, family history, pathological type, histological grade, tumor size, lymph

95    node metastasis status, lymph vascular space invasion and perineural invasion, were collected

96    through medical records, which were registered between April 2013 and September 2016.

97    Among these clinical characteristics, breast cancer staging relies on the TNM system based on

98    the sixth edition of AJCC and histological grade was based on Nottingham grading system.

99    **2.2 Selection of SNP and genotyping**

100    Rs4784227 was chosen based on a published paper that reported that this SNP might be

101    related to BC susceptibility.  Genomic DNA was extracted from peripheral blood samples from

102    all the study participants. Moreover, we used the MassAray system (Agena, San Diego,

103    California, United States) as well as the matrix-assisted laser desorption ionization-time of flight

104    (MALDI-TOF) mass spectrometry method to detect genotypes of the rs4784227 locus of

105    *CASC16*. SNP genotyping was conducted without knowing cases and controls' status.

106 **2.3 Ethical approval**

107 Written informed consent was obtained from all participants of this study, and this study

108 was approved by the Institutional Ethics Committee of The First Affiliated Hospital of Jilin

109 University with the ethical approval number 2014-031. All the methods were carried out in

110 accordance with the guidelines of Helsinki's declaration.

111 **2.4 Statistical Analysis**

112 Data analyses were conducted using SPSS 24.0 software (IBM Corp, Armonk, NY, USA)

113 and the online platform SNPStats (www.snpstats.net/start.htm). Hardy-Weinberg equilibrium

114 (HWE) was examined by the chi-squared test in both cases and controls. The optimum model of

115 the locus was determined according to the AIC (Akaike's information criterion) value. The

116 genotype and allele frequency distribution of the rs4784227 genetic polymorphism in the case

117 group and control group in different genetic models was analyzed by the SNPStats analysis

118 program, and corresponding odds ratios (ORs) and 95% confidence intervals (CIs) adjusted by

119 age were computed using the binary logistic regression model. In addition, Pearson's $\chi^2$ test was

120 used to compare the frequency distributions of various clinical characteristics of the different

121 genotypes of rs4784227.

122 In addition, survival analysis was used to further determine the impacts of SNP rs4784227

123 on BC prognosis by selecting disease-free survival (DFS) and overall survival (OS) as prognostic

124 indicators. The time of the surgery was the start of DFS, and the data from follow-ups were

125 retrospectively collected. Univariate survival analysis was performed by the Kaplan–Meier

126 method and log-rank test, and survival curves were drawn by R software (R Foundation for

127  Statistical Computing, Vienna, Austria). Additionally, multivariate survival analysis was

128  performed by Cox's proportional hazards regression model. All statistical tests were two-tailed,

129  and P < 0.05 was considered statistically significant.

130  **3. Results**

131  **3.1 Clinical characteristics of participants**

132      The median age of the case group was 51 (44,58) and that of the control group was 38

133  (32,53). The clinical characteristics of BC cases are presented in Table 1.

134

135  **3.2 Analysis of the association between the rs4784227 polymorphism of *CASC16* and BC**

136  **risk**

137      The genotype distribution of cases and controls suggested that SNP rs4784227 conformed

138  to the HWE (*P* >0.05). The association between SNP rs4784227 and BC susceptibility in

139  different genetic models is shown in Table 2. The results suggested that the rs4784227

140  polymorphism of *CASC16* may be associated with BC risk. After adjusting for age, the results of

141  binary logistic regression analysis showed that the CT/TT genotype of rs4784227 significantly

142  increased susceptibility to BC compared with the CC genotype ($OR_{CT/TT\ vs\ CC}$=1.237, 95%

143  CI=1.012–1.513, *P*=0.038). The T allele was the minor allele of rs4784227, which was

144  significantly associated with an increased risk of BC compared with the C allele (OR=1.197,

145  95% CI=1.022–1.401, P=0.026). Furthermore, the dominant model was the best-fitting model of

146  rs4784227 (AIC=2190.4).

147

**3.3 The association between the rs4784227 polymorphism of *CASC16* and clinical characteristics of breast cancer**

The results of some of these characteristics that were statistically significant or studied previously are shown in Table 3. The findings showed that BC patients who carried the CC genotype were more at risk for perineural invasion than those who carried the CT/TT genotype (P=0.030). Moreover, the proportion of premenopausal people who carried the CT genotype was smaller than that in the CC and TT genotypes and was larger than that in the CC and TT genotypes in postmenopausal people (P=0.016). In addition, the proportion of people who carried the C allele was smaller than that of those carrying the T allele with a histological grade Ⅰ tumor and was larger than that of those carrying the T allele with a histological grade Ⅲ tumor (P=0.001). The proportion of people who carried the CC genotype was smaller than that in those carrying the TT genotype with a histological grade Ⅰ tumor and was larger than that in those carrying the TT genotype with a histological grade Ⅲ tumor (P=0.003). Furthermore, the number of people who carried the CC genotype was less than those carrying the CT/TT genotype with a histological grade Ⅰ tumor and was more than those carrying the CT/TT genotype with a histological grade Ⅲ tumor (P=0.025).

**3.4 The relationship between the rs4784227 polymorphism of *CASC16* and the prognosis of breast cancer**

Among the 828 BC patients, 401 individuals were carrying the CC genotype, and the others were carriers of the CT or TT genotype in the dominant model. By using the log-rank test, we did not find that DFS and OS in BC cases were significantly associated with the rs4784227

5000

169    polymorphism of *CASC16* (DFS: *P*=0.972, OS: *P*=0.727) (Figure 1).

170

171    All the above clinical characteristics of patients were included in the Cox model for

172    multivariate analysis to examine the association between the genotypes of rs4784227 and DFS

173    and OS of BC. The results suggested that the differences in DFS and OS among the different

174    genotypes of the BC patients were not statistically significant (DFS: *HR*=1.037 (0.705–1.526)

175    *P*=0.855, OS: *HR*=1.224 (0.653–2.295) *P*=0.529).

176    **4. Discussion**

177    To date, genome-wide association studies (GWAS) have identified numerous common

178    variants associated with BC risk at multiple genetic loci[16]. Therefore, conducting research on

179    SNPs could help identify people with a high susceptibility to BC. As mentioned before,

180    rs4784227 may affect the DNA binding sequence on *FOXA1* and subsequently increase the

181    *FOXA1*- binding affinity to the *CASC16* gene promoter; *FOXA1* plays an important role in the

182    function of ER and growth of ER$^+$ BC cells[17, 18]. BC patients with ER and/or PR positivity

183    accounted for approximately 75% of BC patients[19], and ER is an important indicator of

184    treatment efficacy prediction and prognosis. Therefore, we wanted to know whether there was a

185    connection between the rs4784227 polymorphism of *CASC16* and susceptibility to BC,

186    especially ER$^+$ BC. Thus, we selected the GWAS-identified SNP rs4784227 to investigate and

187    verify its association with BC susceptibility and prognosis in the northeast Chinese Han

188    population.

189    In our study, we found that the T allele was the minor allele of rs4784227, and the

190    distribution of alleles and genotypes of SNP rs4784227 (C=69.93%, T=30.07%, CC=48.43%,

191    CT=43.00%, TT=8.57%) was consistent with current research. The results indicated that those

192    who carried the T allele had high BC susceptibility, which was in line with Zuo[10], Tajbakhsh[11]

193    and He[20]. In our study, the best-fitting inheritance model of rs4784227 was the dominant model,

194    and in this model, we found that the CC genotype of SNP rs4784227 provided a protective effect

195    against BC, which was consistent with Zuo[10] and Tajbakhsh[11]. However, Sun[15] did not find

196    this association. The difference may be because the sample size in Sun's research was relatively

197    small ($n_{Zuo}$=681, $n_{Tajbakhsh}$ =505, $n_{Sun}$=503), so the results of the data analysis may be biased. The

198    results of the northwest population in our study were the same as the populations in the southern

199    and coastal cities in China, Japan and Europe. Therefore, racial and regional differences may not

200    affect the association between this locus and BC susceptibility.

201    Regarding clinical features, He's study (n=623) found that the T allele of rs4784227

202    exhibited significant associations with the status of ER, PR and HER2 in an additive model [20].

203    In addition, Deng [16] indicated that SNP rs4784227 had a significant association with PR-

204    positive tumor risk. Again, rs4784227 may affect the interactions between *FOXA1* and *CASC16,*

205    and *FOXA1* may affect the status of ER. Thus, we investigated whether SNP rs4784227 could

206    affect the expression of these three receptors, especially ER. Regrettably, in our study, this

207    association could not be found in any of the three receptors. He's research[20] suggested that

208    rs4784227 increased BC risk in a dose-dependent manner, so homozygous carriers have higher

209    susceptibility. Therefore, the difference mentioned above may be because the number of people

210    who carried the TT genotype in our sample was small (8.57%), which was not enough to prove

211    the relationship between this locus and the status of ER. Therefore, studies on larger sample sets

212    and further functional identification of molecular mechanisms are needed to confirm the

213    association mentioned above. Furthermore, the results indicated that the locus was associated

214    with perineural invasion, menstrual status and histological grade of BC patients. Among them,

215    the menstrual status results were consistent with Lin[12]. However, the same findings of

216    perineural invasion and histological grade were not found in other studies. In addition, Zuo[10]

217    and Sun[15] found that there was a significant association between rs4784227 and lymph node

218    metastasis status, but this conclusion was not reached in our study. We propose that this may

219    be because the number of people who carried the TT genotype in our sample was small.

220    Therefore, larger sample sizes and a meta-analysis are required to further verify this relationship.

221        Additionally, our study could not confirm that the rs4784227 polymorphism of *CASC16* had

222    an effect on the DFS and OS of BC patients. This may be because the sample time span was just

223    60 months and the disease-free survival of BC was long, so a longer follow-up period may be

224    necessary.

225    **5. Conclusions**

226        Our findings suggest that the rs4784227 polymorphism of *CASC16* may affect susceptibility

227    to BC and was associated with perineural invasion, menstrual status and histological grade of BC

228    patients. In addition, our results could not confirm that the rs4784227 polymorphism of *CASC16*

229    had an effect on the prognosis of breast cancer.

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Clinical characteristics of breast cancer patients.

1 **Table 1. Clinical characteristics of breast cancer patients (n=828).**

| Characteristics | Case, n (%) |
|---|---|
| Age(years) | |
| ≤35 | 52(6.28) |
| >35 | 776(93.72) |
| Menopausal status | |
| Premenopause | 398(48.07) |
| Postmenopause | 430(51.93) |
| Family history | |
| No | 796(96.14) |
| Yes | 32(3.86) |
| Molecular type | |
| LuminalA | 104(12.56) |
| LuminalB | 520(62.80) |
| HER2 | 103(12.44) |
| Triple negative | 101(12.20) |
| Pathological type | |
| Infiltrating ductal carcinoma | 793(95.77) |
| Other types | 35(4.23) |
| Histological grade | |
| Ⅰ | 31(3.74) |
| Ⅱ | 511(61.72) |
| Ⅲ | 286(34.54) |
| Tumor size | |
| T1 | 422(50.97) |
| T2 | 365(44.08) |
| T3 | 27(3.26) |
| T4 | 14(1.69) |
| Lymph node metastasis | |
| No | 396(47.83) |
| Yes | 432(52.17) |
| Lymph-vascular space invasion | |
| No | 471(56.88) |
| Yes | 357(43.12) |
| Perineural invasion | |
| No | 689 (83.21) |
| Yes | 139(16.79) |
| Treatment protocol | |
| Only anthracycline | 30(3.63) |
| Only taxane | 131(15.84) |

|  |  |
|---|---|
| Anthracycline and taxane | 462(55.86) |
| Others | 204(24.67) |
| Endocrinetherapy | |
| Aromatase inhibitor | 218(26.36) |
| Tamoxifen | 199(24.06) |
| Aromatase inhibitor and tamoxifen | 14(1.69) |
| No | 396 (47.89) |

The relationship between the rs4784227 polymorphism of *CASC16* and the risk of breast cancer.

1    **Table 2. The relationship between the rs4784227 polymorphism of *CASC16* and the risk of breast**

2                                                              **cancer.**

| Allele/Genotype | | Case, n (%) | Control, n (%) | *OR (95%CI)* | *P* | AIC |
|---|---|---|---|---|---|---|
| Allele | C | 1158(69.93) | 1336(73.81) | 1.000 | 0.026 | - |
| | T | 498(30.07) | 474(26.19) | 1.197(1.022-1.401) | | |
| Genotype | | | | | | |
| Codominant | CC | 401(48.43) | 493(54.48) | 1.000 | 0.105 | 2191.7 |
| | CT | 356(43.00) | 350(38.67) | 1.221(0.989-1.507) | 0.063 | |
| | TT | 71(8.57) | 62(6.85) | 1.328(0.904-1.952) | 0.148 | |
| Dominant | CC | 401(48.43) | 493(54.48) | 1.000 | 0.038 | 2190.4 |
| | CT/TT | 427(51.57) | 412(45.52) | 1.237(1.012-1.513) | | |
| Recessive | CC/CT | 757(91.43) | 843(93.15) | 1.000 | 0.306 | 2192.6 |
| | TT | 71(8.57) | 62(6.85) | 1.215(0.837-1.765) | | |
| Overdominant | CC/TT | 472(57.00) | 555(61.33) | 1.000 | 0.121 | 2193 |
| | CT | 356(43.00) | 350(38.67) | 1.175(0.958-1.441) | | |

3    Note: *ORs* and *P* values of the genotypes were adjusted by age.

Correlations between SNP rs4784227 and the clinical characteristics of patients with breast cancer.

1 **Table 3. Correlations between SNP rs4784227 and the clinical characteristics of patients with breast**

2 **cancer.**

| Characteristics | | | Allele | | Genotype | | | Genotype | |
|---|---|---|---|---|---|---|---|---|---|
| | | | C | T | CC | CT | TT | CC | CT/TT |
| ER | Status | Negative | 312(26.94) | 116(23.29) | 113(28.18) | 86(24.16) | 15(21.13) | 113(28.18) | 101(23.65) |
| | | Positive | 846(73.06) | 382(76.71) | 288(71.82) | 270(75.84) | 56(78.87) | 288(71.82) | 326(76.35) |
| | $\chi^2$ | | 2.420 | | 2.494 | | | 2.210 | |
| | $P$ | | 0.120 | | 0.287 | | | 0.137 | |
| PR | Status | Negative | 474(40.93) | 184(36.95) | 168(41.90) | 138(38.76) | 23(32.39) | 168(41.90) | 161(37.70) |
| | | Positive | 684(59.07) | 314(63.05) | 233(58.10) | 218(61.24) | 48(67.61) | 233(58.10) | 266(62.30) |
| | $\chi^2$ | | 2.309 | | 2.519 | | | 1.516 | |
| | $P$ | | 0.129 | | 0.284 | | | 0.218 | |
| HER2 | Status | Negative | 787(67.96) | 339(68.07) | 272(67.83) | 243(68.26) | 48(67.61) | 272(67.83) | 291(68.15) |
| | | Positive | 371(32.04) | 159(31.93) | 129(32.17) | 113(31.74) | 23(32.39) | 129(32.17) | 136(31.85) |
| | $\chi^2$ | | 0.002 | | 0.021 | | | 0.010 | |
| | $P$ | | 0.965 | | 0.989 | | | 0.922 | |
| Lymph node metastasis | Status | No | 553(47.75) | 239(47.99) | 191(47.63) | 171(48.03) | 34(47.89) | 191(47.63) | 205(48.01) |
| | | Yes | 605(52.25) | 259(52.01) | 210(52.37) | 185(51.97) | 37(52.11) | 210(52.37) | 222(51.99) |
| | $\chi^2$ | | 0.008 | | 0.012 | | | 0.012 | |
| | $P$ | | 0.929 | | 0.994 | | | 0.913 | |
| Family history | Status | No | 1120(96.72) | 472(94.78) | 389(97.01) | 342(96.07) | 65(91.55) | 389(97.01) | 407(95.32) |
| | | Yes | 38(3.28) | 26(5.22) | 12(2.99) | 14(3.93) | 6(8.45) | 12(2.99) | 20(4.68) |
| | $\chi^2$ | | 3.525 | | 4.845 | | | 1.592 | |
| | $P$ | | 0.060 | | 0.089 | | | 0.207 | |
| Perineural invasion | Status | No | 951(82.12) | 427(85.74) | 322(80.30) | 307(86.24) | 60(84.51) | 322(80.30) | 367(85.95) |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Yes | 207(17.88) | 71(14.26) | 79(19.70) | 49(13.76) | 11(15.49) | 79(19.70) | 60(14.05) |
| | $\chi^2$ | | 3.264 | | 4.851 | | | 4.724 | |
| | $P$ | | 0.071 | | 0.088 | | | 0.030 | |
| Tumor size | Status | T1 | 590(50.95) | 254(51.00) | 202(50.37) | 186(52.25) | 34(47.89) | 202(50.37) | 220(51.52) |
| | | ≥T2 | 568(49.05) | 244(49.00) | 199(49.63) | 170(47.75) | 37(52.11) | 199(49.63) | 207(48.48) |
| | $\chi^2$ | | <0.001 | | 0.559 | | | 0.109 | |
| | $P$ | | 0.984 | | 0.756 | | | 0.741 | |
| Menstrual status | Status | Premeno-pause | 559(48.27) | 237(47.59) | 203(50.62) | 153(42.98) | 42(59.15) | 203(50.62) | 195(45.67) |
| | | Postmeno-pause | 599(51.73) | 261(52.41) | 198(49.38) | 203(57.02) | 29(40.85) | 198(49.38) | 232(54.33) |
| | $\chi^2$ | | 0.065 | | 8.241 | | | 2.035 | |
| | $p$ | | 0.799 | | 0.016 | | | 0.154 | |
| Histological grade | Status | I | 31(2.68) | 31(6.22) | 8(2.00) | 15(4.21) | 8(11.27) | 8(2.00) | 23(5.39) |
| | | II | 714(61.66) | 308(61.85) | 246(61.35) | 222(62.36) | 43(60.56) | 246(61.35) | 265(62.06) |
| | | III | 413(35.66) | 159(31.93) | 147(36.65) | 119(33.43) | 20(28.17) | 147(36.65) | 139(32.55) |
| | $\chi^2$ | | 13.118 | | 15.751 | | | 7.379 | |
| | $P$ | | 0.001 | | 0.003 | | | 0.025 | |
| Molecular type | Status | LuminalA | 138(11.92) | 70(14.06) | 44(10.97) | 50(14.04) | 10(14.08) | 44(10.97) | 60(14.05) |
| | | LuminalB | 723(62.44) | 317(63.65) | 250(62.35) | 223(62.64) | 47(66.20) | 250(62.34) | 270(63.23) |
| | | HER2 | 141(12.18) | 65(13.05) | 47(11.72) | 47(13.21) | 9(12.68) | 47(11.73) | 56(13.12) |
| | | Triple negative | 156(13.46) | 46(9.24) | 60(14.96) | 36(10.11) | 5(7.04) | 60(14.96) | 41(9.60) |
| | $\chi^2$ | | 6.685 | | 7.372 | | | 6.782 | |
| | $P$ | | 0.083 | | 0.288 | | | 0.079 | |

3    Note: ER: estrogenic receptor, PR: progesterone receptor, HER2: human epidermal growth factor receptor 2.

# Figure 1

DFS and OS for patients with CC, CT and TT genotypes of the rs4784227 polymorphism of *CASC16*.
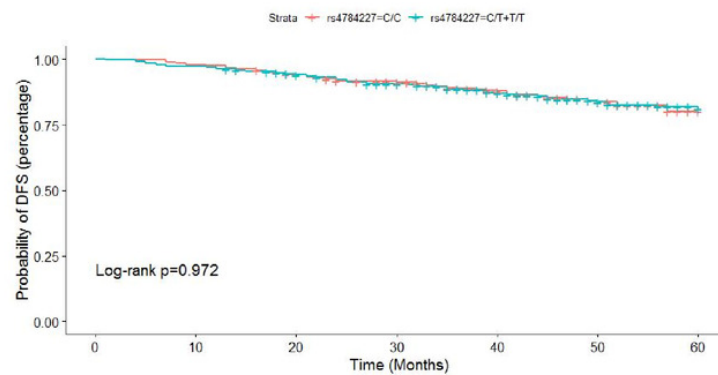
**Figure 1A. DFS for patients with CC, CT and TT genotypes of the rs4784227 polymorphism of**

*CASC16.*
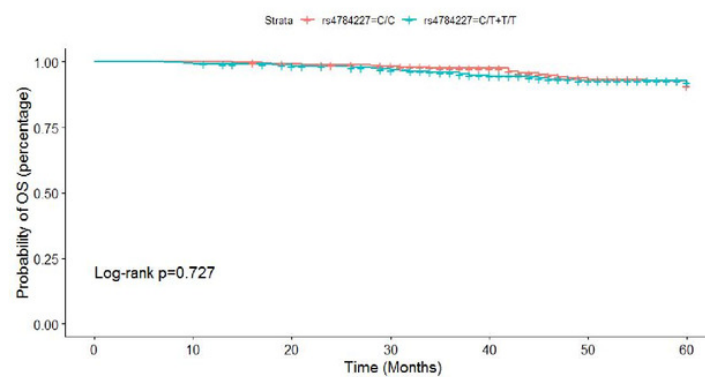
**Figure 1B. OS for patients with CC, CT and TT genotypes of the rs4784227 polymorphism of**

*CASC16.*