Functional Variants in *Linc-ROR* are Associated with mRNA Expression of *Linc-ROR* and Breast Cancer Susceptibility

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Functional polymorphisms in *Linc-ROR* may change its ability of regulation by regulating *Linc-ROR* expression. However, these functional polymorphisms in *Linc-ROR* and their associations with breast cancer (BC) susceptibility were scarcely reported. In this molecular epidemiological study, four SNPs (rs6420545, rs4801078, rs1942348 and rs9636089) were selected in *Linc-ROR* by bioinformatics method. Unconditional logistic regression model was performed to analyze the associations between four SNPs and BC susceptibility adjusted for reproductive factors. Quantitative real-time (qRT) PCR was used to evaluate relative expression of *Linc-ROR* in plasma. The interactions of gene reproductive factors were assessed by Multifactor Dimensionality Reduction (MDR) method. A novel finding showed TT (OR: 1.79; 95%CI: 1.20–2.68) genotype of rs4801078 in *Linc-ROR* had a significant association with the higher risk of BC and the expression of *Linc-ROR* mRNA was closely related with the alleles of rs4801078. In addition, we found the interaction of rs4801078, number of pregnancy and menopausal status might increase BC risk (OR: 2.78; 95%CI: 2.74–3.61). Our results suggest that interactions of SNPs in *Linc-ROR* and reproductive factors might contribute to BC risk, and alleles of rs4801078 might affect *Linc-ROR* expression level.

Breast cancer is the most common diagnosed cancer and the leading cause of cancer death in Chinese women. It alone is expected to account for fifteen percent of all new cancers in women with about 4292,000 newly diagnosed invasive cancer cases in 2015 in China.

Noncoding RNAs (ncRNAs) which function by means other than directing the production of proteins were a distinguishing feature of metazoan genomes. Numerous studies have underlined the regulatory role of microRNAs (miRNAs) in the development of cancers, and their variants are reported to be related to various cancer. Long noncoding RNAs ranging from 200 nucleotides (nt) to over 10 kb are spliced, polyadenylated, and are roughly as diverse in a given cell type as protein-coding mRNAs. In addition to the small regulatory RNAs, emerging studies indicate that IncRNAs play critical roles in various biological processes ranging from embryonic development to human diseases, including controlling cell cycle progression, apoptosis, invasion, and migration. The aberrant expressions of several IncRNAs in various cancers indicate that IncRNAs may be play roles in tumor carcinogenesis. Moreover, recent studies have shown the important roles of IncRNAs and their genetic variants played in tumor carcinogenesis. Yan et al. found that TC genotype of rs10463297 in IncRNA SRA could increase BC risk compared with CC genotype. Reactivation of the H19 gene has been observed in bladder tumors, and TC genotype of rs2839698 in H19 was found to decrease bladder cancer risk. Yao et al. suggested that individuals with rs7958904 CC genotype in HOTAIR had significantly decreased risk of colorectal cancer. Linc-ROR was first identified as a regulator for reprogramming of differentiated cells to induced pluripotent stem cells (iPSCs). Linc-ROR also functions as a microRNA sponge to prevent the core transcription factors (TFs) Oct4, Sox2, and Nanog from binding to their target sites, thereby silencing their expression.

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Nanog, and Sox2 from miRNA-mediated suppression in self-renewing human embryonic stem cells (ESCs) and studies indicated that Linc-ROR could act as a p53 repressor in response to DNA damage. All the evidence intrigued us to speculate that Linc-ROR might also have a role in cancer progression and we found several studies have focused the role of Linc-ROR in the development of cancers, including breast cancer.

Reproductive factors are closely related to the development of breast cancer and the research on interaction between reproductive factors and susceptibility gene has been done. However, up to now, research combining the SNPs in Linc-ROR and reproductive factors on BC risk has not been reported. In our current case-control study, we selected four tagger SNPs (rs6420545, rs4801078, rs1942348 and rs9636089) in Linc-ROR by bioinformatics method and explored a potential correlation between those potentially functional polymorphisms and risk of BC in Chinese women. Furthermore, analysis of qRT-PCR was applied to detect the relative expression level of Linc-ROR in plasma and the interactions of gene reproductive factors were analyzed using MDR method.

### Results

#### Characteristics of patients and controls.

The demographic and clinical characteristics of 968 subjects were displayed in Table 1. The mean age of patients and matched controls were 48.41 ± 10.21 and 49.23 ± 10.15 respectively ($P = 0.21$). The mean age at menarche of BC patients (14.48 ± 1.82) was significantly higher than mean age at menarche of controls (14.08 ± 1.78). More than 4 pregnancies for women increased the risk of BC ($OR: 2.01, 95\% CI: 1.32–3.06$). However, there is no difference in the distributions of the other characteristics.

#### The SNPs genotypes were associated with BC.

Table 2 showed the distributions of genotypes in BC patients and control group for four selected SNPs. All of SNPs genotypes in control group meet Hardy-Weinberg equilibrium ($P = 0.99$ for rs1942348, 0.77 for rs4801078, 0.94 for rs6420545 and 0.99 for rs9636089). TT (OR: 1.79; 95\% CI: 1.20–2.68) genotype of rs4801078 in Linc-ROR increased BC risk in codominant model. Also, TT (OR: 1.84, 95\% CI: 1.28–2.66) genotype of rs4801078 in Linc-ROR showed increased risk of BC in recessive model.

![Table 1](image)

**Table 1.** The baseline characteristics of total 484 BC patients and 484 cancer-free controls. *Student’s t test. bTwo-sided χ² test, $P < 0.05$ was considered to be statistically significant.**

![Table 2](image)

**Table 2.** The distributions of genotypes in BC patients and control group for four selected SNPs. All of SNPs genotypes in control group meet Hardy-Weinberg equilibrium ($P = 0.99$ for rs1942348, 0.77 for rs4801078, 0.94 for rs6420545 and 0.99 for rs9636089). TT (OR: 1.79; 95\% CI: 1.20–2.68) genotype of rs4801078 in Linc-ROR increased BC risk in codominant model. Also, TT (OR: 1.84, 95\% CI: 1.28–2.66) genotype of rs4801078 in Linc-ROR showed increased risk of BC in recessive model.
Stratified analysis. We evaluated the relationship of SNPs genotypes and BC risk stratified by the reproductive factors (Table 3). The positive effect of rs6420545 CT + TT genotype was more significant in the subjects ($P = 0.005$) with age at menarche $\leq 13$ ($OR: 0.95$, 95% CI: 0.92–0.99) and rs19 CT + TT genotype were more

| SNPs      | Genetic model | Genotype | Case (%) | Control (%) | $p^a$ | Adjusted OR(95%CI)$^b$ | $p^b$ |
|-----------|---------------|----------|----------|-------------|-------|------------------------|-------|
| rs1942348 | Codominant    | TT       | 184(0.38) | 183(0.38)   | 0.99  | 1                      |       |
|           |               | CT       | 227(0.47) | 227(0.47)   | 0.91  | 0.67–1.23              | 0.529 |
|           |               | CC       | 73(0.15)  | 74(0.15)    | 0.99  | 0.65–1.51              | 0.959 |
|           | Dominant      | TT       | 1        |             |       | 1                      |       |
|           |               | CT + CC  | 300(0.62) | 301(0.62)   | 0.93  | 0.70–1.23              | 0.602 |
|           |               | CC       | 104(0.21) | 104(0.21)   | 1     | 0.71–1.54              | 0.827 |
|           | Recessive     | TT + TC  | 411(0.85) | 410(0.85)   | 1     | 1                      |       |
|           |               | CC       |           |             |       | 1.04(0.71–1.54)        | 0.507 |
|           | Over-dominant | TT + CC  | 257(0.53) | 257(0.53)   | 1     | 0.91(0.69–1.20)        | 0.981 |
|           |               | TC       |           |             |       | 1                      |       |
|           | Allele        | T        | 595(0.61) | 593(0.61)   | 1     | 1.01(0.83–1.20)        | 0.005 |
|           |               | C        | 373(0.39) | 375(0.39)   | 1     | 0.98(0.78–1.26)        | 0.022 |
| rs4801078 | Codominant    | CC       | 162(0.33) | 176(0.36)   | 0.77  | 1                      |       |
|           |               | TT       | 211(0.44) | 238(0.49)   | 0.95  | 0.70–1.30              | 0.740 |
|           |               | TT       | 111(0.23) | 70(0.15)    | 1.79  | 1.20–2.68              | 0.005 |
|           | Dominant      | CC       | 1        |             |       | 1                      |       |
|           |               | CT + TT  | 322(0.67) | 308(0.64)   | 1.14  | 0.85–1.52              | 0.386 |
|           | Recessive     | CC + CT  | 373(0.77) | 414(0.85)   | 1     | 1.14(0.83–1.57)        | 0.408 |
|           |               | TT       |           |             |       | 1.08(0.78–1.51)        | 0.408 |
|           | Over-dominant | CC + TT  | 273(0.56) | 246(0.51)   | 1     | 0.78(0.59–1.02)        | 0.078 |
|           |               | CT       |           |             |       | 1                      |       |
|           | Allele        | C        | 535(0.55) | 590(0.61)   | 1     | 1.09(0.74–1.62)        | 0.661 |
|           |               | T        | 433(0.45) | 378(0.39)   | 1.24  | 1.03–1.48              | 0.005 |
| rs6420545 | Codominant    | CC       | 126(0.26) | 120(0.25)   | 0.94  | 1                      |       |
|           |               | TT       | 228(0.47) | 236(0.49)   | 0.95  | 0.63–1.24              | 0.462 |
|           |               | TT       | 130(0.27) | 128(0.26)   | 1.05  | 0.72–1.54              | 0.794 |
|           | Dominant      | CC       | 1        |             |       | 1                      |       |
|           |               | CT + TT  | 358(0.74) | 364(0.75)   | 0.94  | 0.68–1.29              | 0.695 |
|           | Recessive     | CC + CT  | 354(0.73) | 356(0.74)   | 1     | 1.14(0.83–1.57)        | 0.408 |
|           |               | TT       |           |             |       | 1.14(0.83–1.57)        | 0.408 |
|           | Over-dominant | CC + TT  | 256(0.53) | 248(0.51)   | 1     | 1.28(0.84–2.05)        | 0.386 |
|           |               | CT       |           |             |       | 0.86(0.65–1.13)        | 0.282 |
|           | Allele        | C        | 480(0.50) | 476(0.49)   | 1     | 1.09(0.74–1.62)        | 0.518 |
|           |               | T        | 488(0.50) | 492(0.51)   | 1     | 1.04(0.79–1.39)        | 0.518 |
| rs9636089 | Codominant    | TT       | 187(0.39) | 191(0.39)   | 0.99  | 1                      |       |
|           |               | CT       | 223(0.46) | 226(0.47)   | 0.90  | 0.66–1.21              | 0.475 |
|           |               | CC       | 74(0.15)  | 67(0.14)    | 1.03  | 0.67–1.58              | 0.896 |
|           | Dominant      | TT       | 1        |             |       | 1                      |       |
|           |               | CT + CC  | 297(0.61) | 293(0.61)   | 0.93  | 0.70–1.23              | 0.596 |
|           | Recessive     | TT + TC  | 410(0.85) | 417(0.86)   | 1     | 1.09(0.74–1.62)        | 0.661 |
|           |               | CC       |           |             |       | 1.09(0.74–1.62)        | 0.661 |
|           | Over-dominant | TT + CC  | 261(0.54) | 258(0.53)   | 1     | 0.89(0.67–1.18)        | 0.408 |
|           |               | TC       |           |             |       | 1                      |       |
|           | Allele        | T        | 597(0.62) | 608(0.63)   | 1     | 1.04(0.86–1.25)        | 0.680 |
|           |               | C        | 371(0.38) | 360(0.37)   | 1     | 1.04(0.86–1.25)        | 0.680 |

Table 2. The association between four SNPs genotypes and risk of breast cancer. $^a$Value of Hardy-Weinberg equilibrium in controls; $^b$Value of logistic regression analysis with adjusted for age, age at menarche, menopausal status, number of pregnancy and abortion, breast-feeding status, and family history of BC in first-degree relatives.
| variables | case | control | rs6420545 | rs6420545 | rs4801078 | rs4801078 | rs1942348 | rs1942348 | rs9636089 | rs9636089 |
|-----------|------|---------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| OR (95%CI) | OR (95%CI) | OR (95%CI) | OR (95%CI) | OR (95%CI) | OR (95%CI) | OR (95%CI) | OR (95%CI) | OR (95%CI) | OR (95%CI) | OR (95%CI) |
| TT vs CT + CC | P | OR (95%CI) | TT vs CT + CC | P | OR (95%CI) | TT vs CT + CC | P | OR (95%CI) | TT vs CT + CC | P |
| age | | | | | | | | | | |
| ≤50 | 302 | 285 | 0.70 (0.46–1.05) | 0.083 | 0.79(0.54–1.14) | 0.206 | 1.16(0.81–1.67) | 0.41 | 1.24 (0.87–1.78) | 0.238 |
| >50 | 182 | 199 | 1.50(0.90–2.52) | 0.125 | 2.14(1.33–3.45) | 0.002 | 0.60(0.37–0.99) | 0.045 | 0.57(0.35–0.92) | 0.021 |
| Age at menarche | | | | | | | | | | |
| ≤13 | 152 | 201 | 0.95 (0.92–0.99) | 0.005 | 0.69(0.42–1.13) | 0.14 | 1.38(0.85–2.26) | 0.196 | 1.19(0.74–1.93) | 0.476 |
| >13 | 332 | 283 | 1.46 (0.98–2.16) | 0.061 | 1.52(1.06–2.19) | 0.025 | 0.77(0.54–1.11) | 0.162 | 0.83(0.58–1.19) | 0.31 |
| Age at menopause | | | | | | | | | | |
| ≤50 | 352 | 337 | 0.91(0.64–1.32) | 0.630 | 1.16 (0.83–1.62) | 0.4 | 1.01(0.72–1.42) | 0.946 | 1.03(0.74–1.44) | 0.85 |
| >50 | 132 | 147 | 1.13 (0.58–2.19) | 0.726 | 1.13(0.62–2.06) | 0.68 | 0.67(0.38–1.20) | 0.18 | 0.66 (0.37–1.16) | 0.147 |
| No. of pregnancy | | | | | | | | | | |
| ≤2 | 176 | 243 | 0.98 (0.58–1.66) | 0.95 | 1.32 (0.82–2.12) | 0.155 | 0.96(0.61–1.52) | 0.866 | 0.86 (0.55–1.35) | 0.512 |
| >2 | 308 | 241 | 0.95(0.63–1.43) | 0.807 | 1.08 (0.74–1.59) | 0.679 | 0.89(0.61–1.31) | 0.577 | 0.96(0.66–1.41) | 0.848 |
| No. of abortion | | | | | | | | | | |
| ≤2 | 417 | 438 | 0.95(0.68–1.33) | 0.776 | 1.16 (0.86–1.57) | 0.339 | 0.91(0.68–1.24) | 0.559 | 0.93(0.69–1.25) | 0.622 |
| >2 | 67 | 46 | 1.10(0.44–2.73) | 0.784 | 1.13(0.46–2.77) | 0.784 | 0.86(0.37–2.02) | 0.729 | 0.84(0.36–1.98) | 0.691 |
| Breast-feeding | | | | | | | | | | |
| no | 27 | 42 | 46.67 (2.46–884.21) | 0.010 | 18.72(1.17–300.7) | 0.039 | 0.56(0.16–1.96) | 0.366 | 0.60(0.17–2.11) | 0.422 |
| yes | 457 | 442 | 0.89 (0.64–1.23) | 0.473 | 1.11 (0.82–1.49) | 0.513 | 0.95(0.71–1.28) | 0.735 | 0.94(0.70–1.27) | 0.696 |
| Family history | | | | | | | | | | |
| no | 462 | 463 | 1.01(0.73–1.40) | 0.947 | 1.21(0.90–1.64) | 0.212 | 0.92(0.69–1.24) | 0.599 | 0.90(0.67–1.21) | 0.473 |
| yes | 22 | 21 | 0.29(0.05–1.55) | 0.147 | 0.52(0.10–2.80) | 0.448 | 0.65(0.14–2.92) | 0.573 | 1.33 (0.31–5.77) | 0.701 |

Table 3. Stratification analysis of the five SNPs and BC susceptibility. *Pvalue of logistic regression analysis with adjusted for age, age at menarche, menopausal status, number of pregnancy and abortion, breast-feeding status, family history of BC in first-degree relatives (the stratified factor in each stratum excluded).

evident in the subjects (P = 0.045) with age >50 (OR: 0.60, 95% CI: 0.37–0.99). The protective role of CT + CC genotypes for rs9636089 were more obvious in the women (P = 0.021) with age >50 (OR: 0.57, 95% CI: 0.35–0.92). In addition, the CT + TT genotypes of rs4801078 revealed a significant higher risk of BC in the participant with age >50 (OR: 2.14, 95% CI: 1.33–3.45) and age of menarche >13 (OR: 1.52, 95% CI: 1.06–2.19).

The association of receptor status and the four SNPs genotypes. The association between SNPs in Linc-ROR and the receptors status in cases were displayed in Table 4. After adjusted for reproductive factors, only a boundary significant association between rs6420545 CT (OR: 0.66, 95% CI: 0.99–2.76) genotype with PR status of patients was detected.

Haplotype analysis. The Haplotype analysis for polymorphisms in Linc-ROR was showed in Table 5. Four haplotypes were showed in the table (a total of 16 haplotypes) and all those frequencies <0.03 in cases or controls has been dropped in this analysis. Trs6420545 Trs4801078 Trs1942348 Trs9636089 was the most frequent haplotype in the cases (38.5%) and controls (35.3%). Compared with the controls, the frequency of haplotype Trs6420545 C rs4801078 Trs1942348 Trs9636089 was lower in cases (OR: 0.72, 95% CI: 0.54–0.97).

The analysis of Gene reproductive factors interactions. The interactions of gene and reproductive factors among four SNPs (rs6420545, rs4801078, rs1942348 and rs9636089) and reproductive factors were revealed in Table 6. Four models in MDR were showed and we found the interaction of rs4801078, number of pregnancy and menopausal status (Trs4801078, number of pregnancy ≥2 and post-menopausal) might increase risk for breast cancer by 2.78 times (OR: 2.78; 95% CI: 2.74–3.61).

The results of Benjamini-Hochberg (BH) correction for false discovery rate (FDR). We applied the BH correction to control FDR (Table 7). The q-value indicated TT genotype and T allele of rs4801078 in Linc-ROR could still increase BC risk. Besides, the relationship between CT + TT genotypes of rs4801078 and BC was still noteworthy in the participant with age >50.

The relative expression of Linc-ROR in plasma. We investigated the association between rs4801078 genotypes and Linc-ROR mRNA expression level by the real-time PCR amplification reactions in 150 subjects. There were 38 subjects with CC genotype, 64 with CT genotype and 48 with TT genotype (Fig 1). The relative expression of Linc-ROR mRNA in CT + TT group (1.94 ± 0.27) was significantly higher than the CC group (1.21 ± 0.19) and that indicated the SNPs in Linc-ROR might play a role in the expression level of Linc-ROR mRNA.
Discussion

This case-control study was the first to demonstrate the association between potential regulatory variants in Linc-ROR and BC risk, and we found TT (OR: 1.79; 95%CI: 1.195–2.68) genotype of rs4801078 in Linc-ROR had a significant association with the higher risk of BC in Chinese population. Furthermore, analysis of qRT-PCR showed the relative expression of Linc-ROR mRNA in rs4801078CT + TT group (1.94 ± 0.27) was higher than the CC group (1.21 ± 0.19). In addition, we found the frequency of haplotype T rs6420545C rs4801078Trs1942348Trs9636089 was lower in cases than in controls (OR: 0.72, 95%CI: 0.54–0.97) and the interactions of rs4801078, number of pregnancy and menopausal status might increase BC risk (OR: 2.78; 95%CI: 2.74–3.61).

LncRNAs have received widespread attention and are observed to play pivotal roles in tumorigenesis and progression of human cancers. It has already been revealed that some lncRNAs, such as HOTAIR, H19 and MALAT1, are potential biomarkers in cancer diagnosis and prognosis. Among them, Linc-ROR, first discovered in 2010, are also found to have strong association with tumorigenesis, metastasis and poor therapeutic response of malignant tumors. Recent studies found that Linc-ROR was upregulated in pancreatic cancer tissues and decreased Linc-ROR expression could inhibit pancreatic cancer cell proliferation, invasion, and tumourigenic growth. In another study, the researchers found Linc-ROR confers gemcitabine resistance to pancreatic cancer cells at least partly via inducing autophagy. Zhou et al. found that Linc-ROR had an important role during endometrial carcinogenesis by acting as a miR-145 "sponge" to inhibit mediation of the differentiation of endometrial...
tumorspheres. A recent study suggested the function of Linc-ROR exerted in LAD cells depended on the sponging of miR-145 and it led to the chemotherapy resistance and EMT phenotypes of docetaxel-resistant LAD cells. The qRT-PCR showed a significant up-regulation of Linc-ROR and its variants 2 (P = 0.025) and 4 (P = 0.0002) in esophageal squamous cell carcinoma. Li et al. found that Linc-ROR was significantly upregulated in nasopharyngeal carcinoma tissues and the enrichment of Linc-ROR played a critical functional role in chemoresistance by suppressing P53 signal pathway. A recent study also provided several new mechanistic insights into acquired chemoresistance in HCC and they found Linc-ROR acting as mediators are involved in modulation of cellular responses to chemotherapy. However, the role of Linc-ROR in glioma is the opposite of other tumors. Feng et al. suggested that Linc-ROR might act as a novel tumor suppressor gene in glioma by inhibiting the proliferation of cancer cell, self-renewal of GSCs and the KLF4 expression.

In addition to the mentioned malignant tumor, the role of Linc-ROR in breast cancer has also been reported. In 2014, Hou et al. found that Linc-ROR could function as an important regulator of epithelial-to-mesenchymal transition and promote breast cancer progression and metastasis through regulation of miRNAs. In 2016, the study of Chen et al. investigated the role of Linc-ROR in the chemoresistance of human BC cells and its mechanism. The effect of the Linc-ROR on epithelial-to-mesenchymal transition was verified to contribute to the chemoresistance and invasion of breast cancer cells. In the same year, one study concluded that Linc-ROR suppressed gemcitabine-induced autophagy and apoptosis in breast cancer cells by silencing miR-34a expression. Recently, Zhang et al. found down-regulated Linc-ROR could enhance the sensibility of breast cancer cells to tamoxifen by increasing miR-205 expression and suppressing the expressions of ZEB1 and ZEB2.

Zhao et al. suggested the expression levels of Linc-ROR were significantly higher in breast cancer and combination of the Linc-ROR with the conventional biomarkers might produce better diagnostic ability. Together, these results indicate that Linc-ROR might have a crucial impact on the development of BC and it is necessary to investigate the association between regulatory variants in Linc-ROR and BC.

In recent years, a large amount of SNPs in lncRNAs have been found to be related to carcinogenesis. For example, genetic variants in MALAT1 were suggested to be associated with breast cancer and colorectal cancer.

| Genotype | Stratiﬁed factors | Adjusted P | q-value |
|----------|------------------|------------|---------|
| rs4801078 | CC/TT            | 0.005      | 0.015   |
|          | CC + CT/TT       | 0.001      | 0.006   |
|          | C/T              | 0.022      | 0.044   |
| rs6420545 | TT/CT + CC       | 0.005      | 0.070   |
|          | TT/CT + CC       | 0.010      | 0.070   |
| rs4801078 | CC/CT + TT       | 0.002      | 0.028   |
|          | CC/CT + TT       | 0.025      | 0.175   |
| rs1942348 | CC/CT + TT       | 0.039      | 0.182   |
| rs9636089 | TT/CT + CC       | 0.045      | 0.630   |

Table 7. Results of Benjamini-Hochberg (BH) correction.
A combined analysis of genome-wide association study (GWAS) and meta-analysis identified a novel and significant association between rs16941835 in lncRNA RP11-58A18.1 and CRC susceptibility. Not only that, but those SNPs in lncRNAs were implied to have function in regulating the expression level of lncRNAs in the process of cancer. A genome-wide association study indicated that variant SNPs in a long noncoding RNA MIR2052HG showed a dose-dependent increase in MIR2052HG expression as well as increased binding of ERs to the EREs by performing estradiol treatment. Another research showed the rs2147578 in lnc-LAMC2–1:1 were significantly associated with increased CRC risk by influencing the binding of lnc-LAMC2–1:1 and miR-128–3p. In addition, the latest findings were reported that the SNP rs2027701 of Linc-ROR in the lncRNA-p53 regulatory network had significant associations with the risk of neutropenia. These results inspire us to study the role of Linc-ROR tagger SNPs in the expression of Linc-ROR mRNA and the the process of breast cancer.

However, to date, no study about the tagger SNPs of Linc-ROR has ever been reported in BC. In our case-control study, we found TT (OR: 1.79; 95% CI: 1.20–2.68) genotype of rs4801078 in Linc-ROR had a significant association with the higher BC risk in codominant model (OR: 1.79; 95% CI: 1.20–2.68) and recessive model (OR: 1.80, 95% CI: 1.28–2.66). Moreover, the expression of Linc-ROR mRNA was closely related with the alleles of rs4801078. The results of qRT-PCR revealed the relative expression of Linc-ROR mRNA in rs4801078 CT + TT group (1.94 ± 0.27) was significantly higher than the CC group (1.21 ± 0.19). No meaningful association between the other three SNPs (rs1942348, rs6420545 and rs9636089) and the risk of BC was observed in the major models, however, women with the mutant alleles of rs1942348, rs6420545 and rs9636089 had a lower BC risk in the subgroup with age at menarche ≤ 13 (OR: 0.95, 95% CI: 0.92–0.99 for rs6420545) and age > 50 (OR: 0.60, 95% CI: 0.37–0.99 for rs1944238; OR: 0.57, 95% CI: 0.35–0.92 for rs9636089). In addition, the Haplotype analysis showed haplotype rs4801078TT+rs9636089TT+rs1942348TT could decrease BC risk (OR: 0.72, 95% CI: 0.54–0.97). Our results suggest that the regulatory polymorphisms in Linc-ROR should influence breast cancer risk and large sample studies carrying in other races are needed to verify our discovery.

A total of 968 participants were enrolled in the genetic epidemiology study between 2013 and 2015 from a community-based study in Henan province attended by 20000 individuals. Due to the low prevalence of breast cancer in the program, most of BC patients came from the First Affiliated and the Third Affiliated Hospital of Zhengzhou University. The inclusion criteria included the newly pathological diagnosed BC patients in Henan province without any other malignant tumor. 484 frequency matched cancer-free controls were selected from a community-based study in Henan province. The demographic data and some potential BC risk factors were collected from a structured questionnaire by uniformly trained investigators and information was double entered in the database; moreover, randomly selected ten percent DNA samples were directly sequenced and each sample was repeated three times independently in qRT-PCR tests for the repeatability; nevertheless, in some subgroups, the statistical power is reduced due to the relatively small sample size; in addition, since early menarche was an established risk factor for breast cancer, the higher mean age at menarche in cases showed that there was still selection bias in our study.

Methods

Methods: Subjects. A total of 968 participants were enrolled in the genetic epidemiology study between 2013 and 2015 from a community-based study in Henan province attended by 20000 individuals. Due to the low prevalence of breast cancer in the program, most of BC patients came from the First Affiliated and the Third Affiliated Hospital of Zhengzhou University. The inclusion criteria included the newly pathological diagnosed BC patients in Henan province without any other malignant tumor. 484 frequency matched cancer-free controls were selected from the program randomly. The inclusion criteria of the control group was healthy people without chronic diseases history and age-appropriate frequency matched (±2 years). All the participants were unrelated.

The demographic data and some potential BC risk factors were collected from a structured questionnaire by face to face interviews. The information of receptor status (estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor-2 (HER-2) status) came from pathological report. Some potential BC risk factors in the current study included age, age of menarche, menopause age, menopausal state and hormone levels for women. Recent research suggested that estrogen-independent growth of ER+ breast cancer by the MAPK/ERK signaling pathway. Moreover, another study suggested that the estrogen-independent growth of ER+ breast cancer by the MAPK/ERK signaling pathway. Moreover, another study suggested that the estrogen-independent growth of ER+ breast cancer by the MAPK/ERK signaling pathway.
−ΔΔ provided GAPDH as a reference gene and the 2 selected 10% of the DNA samples to be sequenced (BGI Sequencing, Beijing). In order to control the quality, we randomly enzymes of NsiI, MwoI, BlpI and BsmAI (Thermo Scientific) respectively which were selected by WATCUT with minor allele frequency (MAF) on Chinese Han population data of HapMap Project (HapMapRel 28, NCBI B36) as well as 1000 Genome Project 0.05 was regarded as significant.

DNA extraction. We collected 5 ml venous blood from each subject in ethylene diamine tetra acetic acid (EDTA), part of the blood cells were used to extract genomic DNA with the DNA Extraction Kit (TIANGEN BIOTECH, Beijing). Separated plasma and DNA samples were stored at −80°C.

SNP selection and genotyping. All tagger SNPs of Linc-ROR were selected by Haplovieview software basing on Chinese Han population data of HapMap Project (HapMapRel 28, NCBI B36) as well as 1000 Genome Project with minor allele frequency (MAF) > 0.1 in Chinese Han population (Table 8).

| SNP ID     | Allele | MAF | Genotyping assay | Tm(°C) | Primers(5'-3')                                      |
|------------|--------|-----|------------------|--------|---------------------------------------------------|
| rs1942348  | T/C    | 0.44| CRS-RFLP         | 59.6   | Sense: TTTCCCTCTTGCTAATGCTGCTGA                    |
|            |        |     |                  |        | Antisense: TTACATAACCTGGCGAGAAGGA                  |
| rs6420545  | C/T    | 0.45| PCR-RFLP         | 56.1   | Sense: TTCCAGCCTGATGACAGA                         |
|            |        |     |                  |        | Antisense: CACAGGACACTATTTCTAT                   |
| rs4801078  | C/T    | 0.41| CRS-RFLP         | 56.1   | Sense: ATTTCAGTCAGATCCTATAGAG                     |
|            |        |     |                  |        | Antisense: TCTAAGGCGCAAATAAATAATCTG              |
| rs9636089  | T/C    | 0.41| PCR-RFLP         | 59.6   | Sense: GCACAGTTCACAGATGGA                         |
|            |        |     |                  |        | Antisense: CAGGAGATTGGCTTGGT                      |

Table 8. PCR information of the four SNPs.

The relative expression of the Linc-ROR in plasma. One hundred and fifty individuals from the controls were selected and qRT-PCR test was used to detect the relative expression of Linc-ROR in plasma by the Eco Real-Time PCR System (Illumina, USA). The conditions for the reaction of real-time PCR are as follows: 1) initial denaturation: 95°C for 30 s; 2) 40 cycles of denaturation: 95°C for 10 s; 3) anneal: 60°C for 30 s. Our assay reactions was performed to standardize the DNA amplification conditions and optimize the annealing temperature for the primers set (Table 1). Rs6420545, rs9636089 rs1942348 and rs4801078 were digested by restriction enzymes of NsiI, MwoI, BlpI and BsmAI (Thermo Scientific) respectively which were selected by WATCUT website (http://watcut.uwaterloo.ca/watcut/watcut/template.php). In order to control the quality, we randomly selected 10% of the DNA samples to be sequenced (BGI Sequencing, Beijing).

Analysis. The sample size (n = 459) of the study was calculated by the software power analysis and sample size (PASS) with the minimum allele frequency (0.25) and the study power (0.9). We assess the representativeness of the control population using a goodness-of-fit χ2-test (Hardy–Weinberg equilibrium). Categorical variables and continuous variables were calculated by Chi-squared and Student’s t test respectively to assess distribution departure in two groups. Unconditional logistic regression analysis was applied to estimate the relationship between SNPs and BC (or receptor status) with adjusted for the potential BC risk factors. Stratified analysis for the potential risk factors mentioned was made in different subgroups to estimate the relationship between SNPs and BC risk. MDR method was conducted to calculate the gene-reproductive factors interactions and online SHEsis (http://analysis.bio-x.cn/myAnalysis.php) was used to analyze the difference of haplotype frequencies in both patients and controls. Relative expressions of the gene Linc-ROR were presented as mean ± standard deviation (X ± SD) and the one-way ANOVA was applied to assess the difference. The Benjamini-Hochberg (BH) correction was used to control false discovery rate (FDR). The SPSS 21.0 statistical software package (Analysis software, Shanghai, co., LTD, 6761805c6989326cbf14) was used for all statistics analyses, and two-side P value less than 0.05 was regarded as significant.

Data availability. The datasets generated during and analysed during the current study are available from the corresponding author on reasonable request.

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Acknowledgements

This work was supported by the National Natural Science Foundation of China under Grant (U1604168); Major Science and Technology programs in Henan Province under Grant (161100311400); and Medical Science and Technology Key Projects of Henan Province and Zhengzhou under Grant (201602295 and 20150374). We also acknowledge the Key Laboratory of cancer epidemiology in Henan for providing research platform.

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Additional Information

Competing Interests: The authors declare no competing interests.

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