The rs9340799 polymorphism of the estrogen receptor alpha (ESR1) gene and its association with breast cancer susceptibility

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The ESR1 rs9340799 polymorphism has been frequently investigated with regard to its association with breast cancer (BC) susceptibility, but the findings have been inconclusive. In this work, we aimed to address the inconsistencies in study findings by performing a systematic review and meta-analysis. Eligible studies were identified from the Web of Science, PubMed, Scopus, China National Knowledge Infrastructure, VIP and Wanfang databases based on the predefined inclusion and exclusion criteria. The pooled odds ratio (OR) was then calculated under five genetic models: homozygous (GG vs. AA), heterozygous (AG vs. AA), dominant (AG + GG vs. AA), recessive (GG vs. AA + AG) and allele (G vs. A). Combined results from 23 studies involving 34,721 subjects indicated a lack of significant association between the polymorphism and BC susceptibility (homozygous model, OR = 1.045, 95% CI 0.887–1.231, P = 0.601; heterozygous model, OR = 0.941, 95% CI 0.861–1.030, P = 0.186; dominant model, OR = 0.957, 95% CI 0.875–1.045, P = 0.327; recessive model, OR = 1.053, 95% CI 0.908–1.222, P = 0.495; allele model, OR = 0.987, 95% CI 0.919–1.059, P = 0.709). Subgroup analyses by ethnicity, menopausal status and study quality also revealed no statistically significant association (P > 0.05). In conclusion, our results showed that the ESR1 rs9340799 polymorphism was not associated with BC susceptibility, suggesting its limited potential as a genetic marker for BC.

According to the World Health Organization statistics, breast cancer (BC) is the most common malignant tumor type, as well as a leading cause of mortality in the female population.1 Like other malignancies, risk factors of BC are primarily genetic predisposition and environmental influences.1 It has been reported that genetic background or familial history accounts for ~20–25% of overall BC incidence.1 Among the ~80 genetic loci known to be associated with susceptibility to BC, the BRCA1 and BRCA2 loci carry the highest risk.5,6 Together with other high- and medium-penetration germline mutations located at the loci of TP53, PTEN, ATM, BRIP1, CHEK2 and PALB2, they made up ~15–20% of the genetic risk of BC.7–9 Common low-penetration genetic polymorphisms account for the remaining risk for BC susceptibility.10,11 On the other hand, environmental and lifestyle risk factors for BC include the consumption of oral contraceptive, cigarette smoking, alcohol consumption, breastfeeding and delayed age at first childbirth.12–14

Among these risk factors, it has been specifically pointed out that estrogen can act as a carcinogen, not only by causing chromosome segregation errors as well as structural chromosomal alterations, but also by stimulating the uncontrolled proliferation of mutated breast cells.4,5,15,16 Mounting evidence from population-based studies has corroborated the association of endogenous and exogenous circulating estrogen in BC etiology and the increased risk of BC in premenopausal women.17 The physiological receptors for estrogen are estrogen receptors (ER), which function to mediate the effect of estrogen on breast cells. Binding of estrogen to ER promotes the growth and differentiation of the normal breast cells and can lead to breast carcinogenesis.18 There are two ER isoforms, i.e., ERα and ERβ. These two ER isoforms are respectively encoded by two distinct genes, ESR1 and ESR2.19 ERα has a higher level of expression in the breast tissue between these two isoforms, hence it is frequently implicated in BC development.20

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The focus of this meta-analysis is the ERα-encoding gene, ESR1, which is highly polymorphic. Among the many polymorphisms in ESR1, the two best-studied ones are rs2234693 (also known as PvuII or 397T>C) and rs9340799 (also known as XbaI or 351A>G) polymorphisms. Both polymorphisms are located in intron 1, respectively at 1,397 bp and 351 bp upstream of exon 2 of the gene, and have been associated with several female cancers, including BC and endometrial cancer21–23. However, the association of the two polymorphisms with BC susceptibility has been described with conflicting results in many studies24–27. To sort out this inconsistency, a meta-analysis on rs2234693 was performed in 2018, and showed that the polymorphism was significantly associated with a decreased BC susceptibility28. As for rs9340799, a meta-analysis based on seven previous studies was reported by Zhang et al. in 2015, and found no significant association between the polymorphism and BC susceptibility under all three genetic models examined, even when the data were stratified into subgroups according to the ethnicity and source of controls29. In this current work, we attempted to perform an updated meta-analysis on the relationship between ESR1 rs9340799 polymorphism and BC susceptibility, by including a large number of additional studies that have been left out by Zhang et al. or have only been published after 2015.

Results

Study selection and characteristics. The study selection process is depicted in Fig. 1. The initial database and bibliographic searches identified 236 records (PubMed, N = 51; Scopus, N = 44; WoS, N = 114; Wanfang, N = 20; CNKI, N = 7; VIP, N = 0). After duplicated records were removed, 153 articles were screened by full-text review. Of these, 13 articles that did not meet the eligibility criteria and one article that had an inappropriate study design (as male controls were included in the analysis)30 were excluded. In addition, two articles were found to contain overlapping data18,31, and the one with the smaller sample size was excluded31. Ultimately, 22 articles comprising 23 studies were included for the quantitative synthesis of data3,18,21,24,25,27,32–47.

The 23 included studies involved a total of 34,721 subjects (12,766 cases and 21,955 controls). Among the included studies, eight (from seven articles) reported data for pre- and postmenopausal women separately3,18,21,25,34,38,41, and four other studies included only postmenopausal women24,27,39,40. The remaining studies either did not mention the menopausal status or did not perform separate analyses for pre- and postmenopausal women. In terms of ethnicity, nine studies were conducted on Asians3,18,25,32,34,37,38,41,44, nine on Caucasian21,24,27,33,35,36,39,40,47, three on other ethnicities43,45,46, and two on mixed ethnicities21,42. All studies were case–control in design. Fifteen (15) of the studies were considered as having high quality, whereas eight had low quality (Supplementary Table S1 online). The characteristics of the included studies are summarized in Table 1.

Meta-analysis results. The meta-analysis results are shown in Table 2. Overall, no statistically significant association was observed between ESR1 rs9340799 polymorphism and BC susceptibility (homozygous model, OR = 1.045, 95% CI 0.887–1.231, P = 0.601; heterozygous model, OR = 0.941, 95% CI 0.861–1.030, P = 0.186; dominant model, OR = 0.957, 95% CI 0.875–1.045, P = 0.327; recessive model, OR = 1.053, 95% CI 0.908–1.222, P = 0.495; allele model, OR = 0.987, 95% CI 0.919–1.059, P = 0.709). The random-effects model was used in the
above analyses as significant heterogeneity was present in all genetic models. The forest plots of the associations are presented in Fig. 2. Sensitivity analysis revealed that none of the individual studies had significant impact on the pooled OR (Supplementary Fig. S1 online).

Subgroup analyses. Subgroup analyses were performed based on the ethnicity (Asian vs. Caucasian) and menopausal status (premenopause vs. postmenopause) of the study subjects, as well as the quality of the studies (high quality vs. low quality). No statistical significant association was observed for all subgroups under all genetic models (P > 0.05; Table 2).

Although significant heterogeneity was observed in the overall analysis, several subgroups were found to have low heterogeneity based on the $I^2$ value. In the homozygous model, low heterogeneity was found for Asians ($I^2 = 0.0\%$), premenopause ($I^2 = 0.0\%$), postmenopause ($I^2 = 0.0\%$) and low quality ($I^2 = 19.8\%$) subgroups. A similar observation was observed for the recessive model (Asians, $I^2 = 16.9\%$; premenopause, $I^2 = 0.0\%$; postmenopause, $I^2 = 0.0\%$; low quality, $I^2 = 45.4\%$). In heterozygous model, the Caucasian ($I^2 = 18.8\%$) and high quality ($I^2 = 34.9\%$) subgroups showed low heterogeneity, whereas in allele model, low heterogeneity was noted in premenopause ($I^2 = 24.5\%$), postmenopause ($I^2 = 47.5\%$) and low quality subgroups ($I^2 = 46.7\%$). All subgroups in the dominant model showed high heterogeneity ($I^2 > 50\%$).

Publication bias. No evidence of asymmetry was detected in the funnel plots of all genetic models (Fig. 3), indicating the absence of publication bias. This observation was corroborated by the results of Beggs’s and Egger’s tests (homozygous model, Beggs’s test P = 0.529, Egger’s test P = 0.625; heterozygous model, Beggs’s test P = 0.978, Egger’s test P = 0.152; dominant model, Beggs’s test P = 0.366; recessive model, Beggs’s test P = 0.488, Egger’s test P = 0.303; allele model, Beggs’s test P = 0.636, Egger’s test P = 0.937).

Discussion

ERα, a member of the nuclear receptor superfamily, is encoded by a ~300 kb gene, ESR1, which is mapped to chromosomal locus 6q25.1 and contains eight exons. It has been documented that the human ESR1 gene contains at least nine promoters, whereby each promoter harbors multiple transcription factors-binding sites. The ERα protein possesses DNA- and ligand-binding domains which are highly conserved. It is depicted that ERα can mediate the effect of estrogen via several molecular pathways. Among these, the classical pathway is the best-known. In this direct pathway, unliganded ERα forms a cytosolic complex with Hsp90. Upon estrogen binding to the ligand-binding domains of ERα, the ERα-Hsp90 complex dissociates. Subsequently, ERα dimerizes and translocates to the nucleus. Following that, the DNA-binding domains of ERα, consisting of two functionally

Table 1. Characteristics of the included studies. HWE Hardy–Weinberg equilibrium, PCR–RFLP polymerase chain reaction-restriction fragment length polymorphism.
distinct zinc finger motifs, bind to a characteristic stretch of DNA sequence named the estrogen response elements in the promoters of the target genes to influence the process of transcription50. Meanwhile, the tethered pathway entails protein–protein interaction or heterodimerization of ERα with other transcription factors such as AP1 or NF-kB after ligand activation. This results in the indirect binding of DNA by ERα, contributing to the regulation of target genes including insulin-like growth factor 1, cathepsin D, progesterone receptor, transforming growth factor α, pS2, retinoic acid receptor α1, c-myc, etc., which are essential for cell proliferation and survival51. The nongenomic pathway typically involves a small plasma membrane population- and cytoplasm-based ERα52, which interacts with signaling proteins such as Src, mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase. These signaling molecules can activate the phosphorylation of ERα and its coregulators53,54. This subsequently triggers signaling cascades via second messengers (SM), and eventually, it enhances nuclear ERα signaling without involving gene regulation. The last ER pathway is the ligand-independent pathway. In this case, ERs can become activated via crosstalk with other signaling pathways, e.g. the insulin-like growth factor-1 receptor and the epidermal growth factor receptor pathways55. In these instances, ERs are activated by phosphorylation to form dimers, to bind DNA, and regulate the expression of genes.

### Table 2. Summary of the association between ESR1 rs9340799 polymorphism and breast cancer susceptibility. *OR odds ratio, CI confidence interval.

| Comparison model | No. of studies | No. of cases | No. of controls | Effect model | OR (95% CI)* | P-value |
|------------------|----------------|--------------|-----------------|--------------|---------------|---------|
| **Homozygous model** | | | | | | |
| Overall          | 23             | 7296         | 12,323          | Random       | 1.045 (0.887–1.231) | 0.601   |
| Asian            | 9              | 2211         | 2800            | Fixed        | 0.845 (0.677–1.054) | 0.135   |
| Caucasian        | 9              | 4270         | 8716            | Random       | 1.042 (0.845–1.285) | 0.701   |
| Premenopause     | 6              | 788          | 980             | Fixed        | 0.825 (0.561–1.213) | 0.327   |
| Postmenopause    | 10             | 1697         | 5663            | Fixed        | 0.970 (0.819–1.149) | 0.725   |
| High quality     | 15             | 5593         | 10,581          | Random       | 1.109 (0.903–1.362) | 0.322   |
| Low quality      | 8              | 1703         | 1742            | Fixed        | 0.921 (0.741–1.145) | 0.461   |
| **Heterozygous model** | | | | | | |
| Overall          | 23             | 11,385       | 18,482          | Random       | 0.941 (0.861–1.030) | 0.186   |
| Asian            | 9              | 3329         | 4206            | Random       | 0.866 (0.707–1.061) | 0.164   |
| Caucasian        | 9              | 6861         | 12,952          | Fixed        | 1.020 (0.956–1.089) | 0.546   |
| Premenopause     | 6              | 1294         | 1558            | Random       | 0.891 (0.682–1.163) | 0.396   |
| Postmenopause    | 10             | 2547         | 7921            | Random       | 0.908 (0.734–1.124) | 0.375   |
| High quality     | 15             | 8713         | 15,674          | Fixed        | 0.994 (0.942–1.057) | 0.939   |
| Low quality      | 8              | 2672         | 2808            | Random       | 0.851 (0.673–1.075) | 0.176   |
| **Dominant model** | | | | | | |
| Overall          | 23             | 12,766       | 21,955          | Random       | 0.957 (0.875–1.045) | 0.327   |
| Asian            | 9              | 3480         | 4428            | Random       | 0.857 (0.713–1.031) | 0.102   |
| Caucasian        | 9              | 7931         | 16,077          | Random       | 1.011 (0.905–1.130) | 0.842   |
| Premenopause     | 6              | 1345         | 1637            | Random       | 0.812 (0.557–1.183) | 0.278   |
| Postmenopause    | 12             | 4000         | 11,631          | Random       | 0.915 (0.785–1.066) | 0.253   |
| High quality     | 15             | 9907         | 18,945          | Random       | 0.991 (0.897–1.095) | 0.861   |
| Low quality      | 8              | 2859         | 3010            | Random       | 0.869 (0.714–1.058) | 0.162   |
| **Recessive model** | | | | | | |
| Overall          | 23             | 12,766       | 21,955          | Random       | 1.053 (0.908–1.222) | 0.495   |
| Asian            | 9              | 3480         | 4428            | Fixed        | 0.858 (0.690–1.066) | 0.166   |
| Caucasian        | 9              | 7931         | 16,077          | Random       | 1.023 (0.872–1.200) | 0.784   |
| Premenopause     | 6              | 1345         | 1637            | Fixed        | 0.812 (0.557–1.183) | 0.278   |
| Postmenopause    | 10             | 2917         | 10,265          | Fixed        | 1.003 (0.868–1.158) | 0.967   |
| High quality     | 15             | 9907         | 18,945          | Random       | 1.091 (0.916–1.299) | 0.330   |
| Low quality      | 8              | 2859         | 3010            | Fixed        | 0.954 (0.773–1.179) | 0.664   |
| **Allele model** | | | | | | |
| Overall          | 23             | 12,766       | 21,955          | Random       | 0.987 (0.919–1.059) | 0.709   |
| Asian            | 9              | 3480         | 4428            | Random       | 0.888 (0.782–1.009) | 0.068   |
| Caucasian        | 9              | 7931         | 16,077          | Random       | 1.015 (0.924–1.114) | 0.760   |
| Premenopause     | 6              | 1345         | 1637            | Fixed        | 0.952 (0.845–1.074) | 0.426   |
| Postmenopause    | 10             | 2917         | 10,265          | Fixed        | 0.972 (0.904–1.046) | 0.451   |
| High quality     | 15             | 9907         | 18,945          | Random       | 1.018 (0.933–1.111) | 0.687   |
| Low quality      | 8              | 2859         | 3010            | Fixed        | 0.949 (0.875–1.030) | 0.212   |
### (A) Homozygous model (GG vs. AA)

| Study ID | ES (95% CI) | % Weight |
|----------|-------------|----------|
| Other    |             |          |
| Carrillo-Moreno 2019 | 0.87 (0.53, 1.45) | 5.10 |
| Sierra-Martinez 2018 | 3.04 (1.12, 8.27) | 2.11 |
| Atoum 2017 | 4.80 (2.10, 10.96) | 2.81 |
| Stัทเทอรี (mixed) 2007 | 1.04 (0.70, 1.56) | 6.21 |
| Conings 2003 | 1.48 (0.90, 2.37) | 2.45 |
| Subtotal (I²-squared = 75.0%, p = 0.003) | 1.66 (0.92, 3.02) | 18.67 |
| Asian    |             |          |
| Dai 2019 | 1.56 (0.82, 2.71) | 4.30 |
| Lu 2014  | 0.59 (0.35, 0.99) | 5.03 |
| Javed 2011 | 0.63 (0.27, 1.47) | 2.74 |
| Sàtalik 2011 | 1.06 (0.60, 1.86) | 4.55 |
| Hu 2007  | 0.36 (0.10, 1.54) | 1.22 |
| Shen 2006 | 0.75 (0.37, 1.53) | 3.45 |
| Lu 2005  | 0.77 (0.24, 2.51) | 1.62 |
| Cac 2003 | 0.84 (0.54, 1.31) | 5.71 |
| Shin 2003 | 1.04 (0.39, 2.79) | 2.16 |
| Subtotal (I²-squared = 98.0%, p = 0.434) | 0.84 (0.06, 2.53) | 3.75 |
| Caucasian |             |          |
| Ramalhandsi 2013 | 5.61 (2.19, 14.36) | 2.33 |
| Dunning 2009 | 1.10 (0.96, 1.27) | 6.24 |
| González-Zúñiga Ladd 2008 | 1.18 (0.73, 1.86) | 5.42 |
| Stàłłtey (non-Hispanic) 2007 | 1.00 (0.77, 1.29) | 7.96 |
| Wang 2007  | 0.62 (0.41, 0.94) | 6.08 |
| Omland-Moët 2005 | 0.91 (0.56, 1.41) | 5.19 |
| Modugno 2005 | 1.24 (0.80, 1.92) | 5.76 |
| Wadieek 2004 | 0.87 (0.58, 1.22) | 8.01 |
| Subtotal (I²-squared = 68.5%, p = 0.002) | 1.04 (0.64, 1.29) | 50.58 |
| Overall (I²-squared = 59.7%, p = 0.000) | 1.04 (0.89, 1.23) | 100.00 |

NOTE: Weights are from random effects analysis.

### (B) Heterozygous model (AG vs. AA)

| Study ID | ES (95% CI) | % Weight |
|----------|-------------|----------|
| Other    |             |          |
| Carrillo-Moreno 2019 | 0.78 (0.58, 1.05) | 4.67 |
| Sierra-Martinez 2018 | 0.66 (0.36, 1.24) | 1.71 |
| Atoum 2017 | 0.96 (0.58, 1.60) | 2.39 |
| Stàłłtey (mixed) 2007 | 0.90 (0.73, 1.10) | 6.21 |
| Conings 2003 | 1.54 (0.81, 2.92) | 1.06 |
| Subtotal (I²-squared = 14.1%, p = 0.324) | 0.89 (0.74, 1.07) | 16.64 |
| Asian    |             |          |
| Dai 2019 | 0.97 (0.74, 1.27) | 5.40 |
| Lu 2014  | 0.80 (0.65, 1.03) | 6.23 |
| Javed 2011 | 1.27 (0.67, 2.34) | 1.77 |
| Sàtalik 2011 | 1.02 (0.82, 1.28) | 3.36 |
| Hu 2007  | 0.67 (0.48, 1.54) | 1.98 |
| Shen 2006 | 1.09 (0.75, 1.58) | 3.74 |
| Lu 2005  | 0.94 (0.78, 1.17) | 7.64 |
| Cac 2003 | 1.11 (0.94, 1.32) | 7.64 |
| Shin 2003 | 0.93 (0.76, 1.13) | 3.18 |
| Subtotal (I²-squared = 72.8%, p = 0.000) | 0.87 (0.71, 1.06) | 36.63 |
| Caucasian |             |          |
| Ramalhandsi 2013 | 1.25 (0.71, 2.22) | 1.99 |
| Dunning 2009 | 1.07 (0.96, 1.17) | 9.50 |
| González-Zúñiga Ladd 2008 | 1.27 (0.93, 1.74) | 4.56 |
| Stàłłtey (non-Hispanic) 2007 | 1.01 (0.86, 1.19) | 7.67 |
| Wang 2007  | 0.85 (0.66, 1.10) | 5.64 |
| Omland-Moët 2005 | 1.14 (0.74, 1.77) | 2.59 |
| Modugno 2005 | 0.90 (0.77, 1.06) | 7.82 |
| Wadieek 2004 | 1.01 (0.93, 1.10) | 44.33 |
| Subtotal (I²-squared = 18.8%, p = 0.281) | 0.94 (0.86, 1.03) | 100.00 |

NOTE: Weights are from random effects analysis.

Figure 2. Forest plots of the association between ESRI rs9340799 polymorphism and breast cancer susceptibility.
### C Dominant model (AG + GG vs. AA)

| Study                     | ID               | Effect Size (95% CI) | % Weight |
|---------------------------|------------------|----------------------|----------|
| Other                     |                  | 0.80 (0.60, 1.05)    | 4.94     |
| Carrillo-Moreno 2019      |                  | 0.98 (0.56, 1.71)    | 2.00     |
| Sierra-Martinez 2018      |                  | 1.34 (0.82, 2.16)    | 2.40     |
| Aboul 2017                |                  | 0.94 (0.75, 1.17)    | 6.15     |
| Stalley (mixed) 2007      |                  | 1.53 (0.83, 2.80)    | 1.76     |
| Comings 2003              |                  | 0.99 (0.81, 1.21)    | 17.34    |
| Subtotal (I^2-squared = 31.3%, p = 0.213) | |  | |
| Asian                     |                  | 1.03 (0.79, 1.33)    | 5.39     |
| Dai 2019                  |                  | 0.78 (0.63, 0.97)    | 6.15     |
| Lu 2014                   |                  | 1.05 (0.59, 1.87)    | 1.93     |
| Javed 2011                |                  | 1.03 (0.83, 1.27)    | 6.23     |
| Salceta 2011              |                  | 0.79 (0.45, 1.37)    | 2.06     |
| Hu 2007                   |                  | 1.02 (0.72, 1.40)    | 3.87     |
| Shani 2006                |                  | 1.01 (0.63, 1.63)    | 5.73     |
| Lu 2005                   |                  | 1.09 (0.62, 1.26)    | 7.32     |
| Ca 2003                   |                  | 0.93 (0.26, 0.60)    | 3.23     |
| Shani 2003                |                  | 0.89 (0.71, 1.03)    | 38.75    |
| Subtotal (I^2-squared = 66.7%, p = 0.001) | |  | |
| Caucasian                 |                  | 1.71 (1.00, 2.94)    | 2.12     |
| Ramalhinho 2013           |                  | 1.08 (0.99, 1.17)    | 9.02     |
| Dunning 2009              |                  | 1.25 (0.83, 1.93)    | 6.93     |
| González-Zufiola Laid 2008|                  | 1.01 (0.86, 1.16)    | 7.46     |
| Stalley (non-Hispanic) 2007|                  | 0.80 (0.63, 1.02)    | 5.63     |
| Wang 2007                 |                  | 0.88 (0.64, 1.21)    | 4.34     |
| Crand-Morit 2005           |                  | 1.19 (0.78, 1.80)    | 3.10     |
| Modugno 2005              |                  | 0.89 (0.77, 1.04)    | 7.90     |
| Wedeni 2004               |                  | 1.01 (0.51, 1.33)    | 43.91    |
| Subtotal (I^2-squared = 52.5%, p = 0.040) | |  | |
| Overall (I^2-squared = 58.2%, p = 0.000) | | 0.96 (0.88, 1.05) | 100.00 |

NOTE: Weights are from random effects analysis.

### D Recessive model (GG vs. AA + AG)

| Study                     | ID               | Effect Size (95% CI) | % Weight |
|---------------------------|------------------|----------------------|----------|
| Other                     |                  | 0.98 (0.60, 1.59)    | 4.81     |
| Carrillo-Moreno 2019      |                  | 3.51 (1.32, 9.33)    | 1.88     |
| Sierra-Martinez 2018      |                  | 4.93 (2.29, 10.90)   | 2.73     |
| Aboul 2017                |                  | 1.08 (0.74, 1.60)    | 5.93     |
| Stalley (mixed) 2007      |                  | 1.16 (0.51, 2.66)    | 2.44     |
| Comings 2003              |                  | 1.71 (0.95, 3.11)    | 17.90    |
| Subtotal (I^2-squared = 77.4%, p = 0.001) | |  | |
| Asian                     |                  | 1.51 (0.84, 2.72)    | 3.88     |
| Dai 2019                  |                  | 0.63 (0.38, 1.05)    | 4.81     |
| Lu 2014                   |                  | 0.56 (0.26, 1.21)    | 2.67     |
| Javed 2011                |                  | 1.05 (0.80, 1.38)    | 4.12     |
| Salceta 2011              |                  | 0.40 (0.10, 1.59)    | 1.04     |
| Hu 2007                   |                  | 0.73 (0.36, 1.47)    | 3.11     |
| Shani 2006                |                  | 1.02 (0.52, 2.02)    | 1.42     |
| Lu 2005                   |                  | 0.80 (0.51, 1.23)    | 5.33     |
| Ca 2003                   |                  | 1.55 (0.59, 4.10)    | 1.91     |
| Shani 2003                |                  | 0.86 (0.67, 1.10)    | 28.09    |
| Subtotal (I^2-squared = 16.9%, p = 0.292) | |  | |
| Caucasian                 |                  | 0.96 (0.41, 2.25)    | 2.31     |
| Madares 2014              |                  | 4.97 (2.05, 12.03)   | 2.20     |
| Dunning 2009              |                  | 0.94 (0.41, 1.31)    | 2.33     |
| González-Zufiola Laid 2008|                  | 1.04 (0.67, 1.61)    | 5.32     |
| Stalley (non-Hispanic) 2007|                  | 1.00 (0.78, 1.26)    | 7.89     |
| Wang 2007                 |                  | 0.68 (0.46, 1.03)    | 5.38     |
| Crand-Morit 2005           |                  | 0.98 (0.66, 1.47)    | 5.75     |
| Modugno 2005              |                  | 1.12 (0.86, 1.45)    | 7.63     |
| Wedeni 2004               |                  | 0.92 (0.57, 1.47)    | 7.89     |
| Subtotal (I^2-squared = 56.1%, p = 0.019) | |  | |
| Overall (I^2-squared = 58.4%, p = 0.006) | | 1.02 (0.87, 1.20) | 54.11 |

NOTE: Weights are from random effects analysis.

Figure 2. (continued)
Notwithstanding, all models of ERα signaling pathways point to the vital role of ERα in the proliferation and survival of breast epithelial cells, as well as mammary tumorogenesis. ER has been used as a molecular classifier for breast tumors, whereby BC can be graded as ER-positive and ER-negative. A large proportion (~75%) of BC are known to be ER-positive. ERα-positive cases are often associated with more optimistic prognosis as they generally respond more positively to endocrine therapies, and are also sensitive to CDK4/6 inhibitors. In contrast, ERα-negative BC is generally regarded as aggressive and metastatic malignancies.

Given the important role of ERα in BC, its level and structure need to be tightly regulated to ensure an optimal functionality. The level and structure of a protein are known to be influenced by, among others, genetic polymorphisms. For this reason, many genetic association studies have investigated the relationship between ESR1 polymorphisms and BC susceptibility. These polymorphisms include, but not limited to, rs9340799, rs3020364, rs9322335, rs2234693, rs1801132, rs2046210, rs3020314, rs1514348, rs3020314, rs1514348, and rs3020314.

Among these many polymorphisms, we have chosen to focus on rs9340799, an intronic polymorphism located just upstream of exon 2 of ESR1. This is because the rs9340799 polymorphism has been widely studied and conflicting results have been frequently obtained, and no recent meta-analysis has been carried out to address the inconsistencies in the study findings. For instance, while Wang et al. reported that the GG genotype of the polymorphism was associated with a reduced susceptibility to BC, Sierra-Martínez et al. reported that the same genotype was associated with an increased susceptibility to BC. Besides, Sakoda et al. did not find any significant association between the polymorphism and BC susceptibility. The difference in the study findings could be attributed to the variations in allele frequency across different studies. These variations are particularly relevant in populations consisting of different ethnicities, as interethnic differences in allele frequencies have long been known. Taking the examples above, while Wang et al. noted in a Caucasian population that the minor allele frequency (MAF) of the polymorphism was 0.369, Sakoda et al. found that the MAF was merely 0.192 in an Asian population. These variations can account for differences in gene expression and therefore, disease susceptibility. It is thus important to take into account the population variations in the allele frequency when attempting to identify a genetic biomarker for early identification of a disease. For this reason, heterogeneity tests and subgroup analysis by ethnicity need to be performed when pooling the results from different studies together, as were done in our meta-analysis.

It is noteworthy that most of these studies have centered on genetic association rather than deciphering the exact biological mechanisms. Nonetheless, it has been postulated that intronic polymorphisms such as the rs9340799 polymorphism of ESR1 may influence the cancer susceptibility by (i) being in linkage disequilibrium with another functional polymorphism in the same locus; (ii) influencing the expression of other genes through alterations to their transcriptional activity or mRNA stability; (iii) containing regulatory sequences which can impact gene expression via transcriptional regulation. For these reasons, in this meta-analysis, we attempted to precisely re-examine the relationship between the ESR1 rs9340799 polymorphism and the susceptibility to BC.
In doing so, we included 23 case–control studies from 22 systematically selected published articles. We performed the meta-analysis under five different genetic models, namely the homozygous, heterozygous, dominant, recessive, and allele models. Importantly, our analyses with all five genetic models failed to detect any significant association between the rs9340799 polymorphism and BC susceptibility. Under each genetic model, we further stratified our analysis based on the following subgroups: (i) ethnicity (Asian vs. Caucasian), (ii) menopausal status (premenopause vs. postmenopause), and (iii) study quality (high quality vs. low quality). Again, none of these subgroups showed any significant association. Notably, our finding was in agreement with that of the Zhang et al. even though we have included more studies (N = 23 vs. N = 7)29.

The major strength of our study is that we have analyzed data from a large population of meticulously selected studies; therefore, this study has strong statistical power. Besides, the chosen exposure, i.e., the rs9340799

Figure 3. Funnel plots for assessing publication bias.
polymorphism, is a discrete and well-defined parameter that can be genotyped with high precision using the available technologies. This allows a fair comparison to be made among independent studies, contributing to more consistent inter-laboratory or inter-study comparison. On the other hand, the major limitation of this study is that gene–gene or gene-environment interactions were not measured as most of the included studies did not report this information. Furthermore, our meta-analysis has so far focused on one polymorphism from ESR1. The analyses of more polymorphisms of ESR1 in future, either individually or in tandem, may further reveal the synergistic effects of such polymorphisms in influencing BC susceptibility.

In conclusion, our overall results revealed no significant association between the rs9340799 polymorphism of ESR1 and the susceptibility to BC, despite the different genetic models considered. Each genetic model was further divided into subgroups based on ethnicity, study quality and menopausal status, but similarly, no statistically significant association was observed. Nevertheless, our conclusion warrants further studies, given that the ESR1 harbors many polymorphisms that await detailed investigation.

Methods

Literature search. A comprehensive literature search was performed in the Web of Science (WoS), PubMed, Scopus, China National Knowledge Infrastructure (CNKI), VIP and Wanfang databases up to January 21st, 2021, without language restriction. The following search terms were used: (ESR1 OR estrogen receptor) AND (Xbal OR rs9340799) AND (polymorphism or variant) AND (breast cancer OR breast neoplasm). Studies were selected if they fulfilled the following inclusion criteria: (i) were case–control and/or cohort studies which have investigated the association between ESR1 rs9340799 polymorphism and BC susceptibility, and (ii) reported the genotype and allele frequencies or contained necessary data to obtain the information. Studies were excluded if (i) they were not original research papers (e.g. review articles or commentaries), and (ii) the investigations were not performed on human subjects. The reference lists of the eligible studies were also manually screened to identify additional relevant studies. When overlapping data were found, we included only the study with the largest sample size. The study protocol was pre-registered with PROSPERO (registration number: CRD42021231912).

Data extraction and quality assessment. Three investigators independently extracted the following data from the included studies: name of the first author, publication year, location, ethnic group, sample size, genotype and allele frequencies, menopausal status, genotyping method, blinding status, genotyping success rate, and sources of controls. Discrepancies were resolved through discussion until a mutual agreement was reached. The P-values of the Hardy–Weinberg equilibrium (HWE) among the control group was calculated using a goodness-of-fit test. The Modified Newcastle–Ottawa Scale for Case–Control Studies of Genetic Association was used to assess the quality of the included studies. Studies rated ≥ 6 stars were considered high quality.

Statistical analysis. STATA version 16.0 (StataCorp, College Station, Texas, USA) was used for the quantitative synthesis of the data. The association between ESR1 rs9340799 polymorphism and BC susceptibility was evaluated using the odds ratio (OR) for various genetic models, i.e. homozygous (GG vs. AA), heterozygous (AG vs. AA), dominant (AG + GG vs. AA), recessive (GG vs. AA + AG) and allele (G vs. A). A forest plot was also generated to graphically represent the findings. The associations were performed according to ethnicity (Asian vs. Caucasian), study quality (high quality vs. low quality), and menopausal status (premenopausal or postmenopausal). In most included studies, the ethnicity was explicitly stated, although the standards of classification (i.e. self-reported or via genetic analyses) was not known. However, when such information was not available, the populations were classified into different ethnicities based on the major ethnic group of the countries in which the subjects were recruited. Publication bias was evaluated using the Begg's and the Egger's tests, and through visual inspection of the funnel plot for asymmetry. For all analyses, the result was considered to be statistically significant when P < 0.05, unless otherwise stated.

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Author contributions
S.C.T. conceived and designed the study, screened and selected the eligible studies for meta-analysis, collected and extracted the data, appraised the study quality, performed statistical analysis. E.A.M.H. and M.A.K.S. independently screened and selected the studies, and extracted the data for analysis. H.K.-V. independently performed the quantitative data synthesis. T.Y.L. analyzed and interpreted the data and wrote the manuscript. M.A.I. critically revised the manuscript and provided significant input and feedback on the draft manuscript. All authors have read and approved the final manuscript.

Competing interests
The authors declare no competing interests.

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