eNOS Genetic Polymorphisms and Cancer Risk
A Meta-Analysis and a Case–Control Study of Breast Cancer
Xueren Gao, PhD, Jie Wang, PhD, Wenjun Wang, MS, Mingxi Wang, MD, and Jianqiong Zhang, PhD

Abstract: The association between endothelial nitric oxide synthase (eNOS) polymorphisms (intron 4a/b, -786T>C and 894G>T) and cancer risk remains elusive. In addition, no studies focused on their associations with the risk of breast cancer in Chinese Han population. Thus, a meta-analysis was conducted to determine the relationship between eNOS polymorphisms and cancer risk, and then a case–control study in Chinese Han population was performed to assess their associations with breast cancer susceptibility.

Odds ratios (ORs) with 95% confidence intervals (CIs) were used to assess the strength of association. The pooled analysis indicated that eNOS intron 4a/b and -786T>C polymorphisms were significantly associated with an increased risk of overall cancer. In subgroup analyses based on cancer type, the significant association was found between eNOS intron 4a/b polymorphism and prostate cancer risk, eNOS -786T>C polymorphism and risk of prostate, bladder and breast cancers, and eNOS 894G>T polymorphism and breast cancer risk. In subgroup analyses based on ethnicity, eNOS intron 4a/b and -786T>C polymorphisms were associated with an increased risk of cancer in Caucasians. In consistent with our meta-analysis results, a case–control study in Chinese Han population showed significant associations of eNOS -786T>C and 894G>T polymorphisms with the increased risk of breast cancer. In addition, stratified analyses based on pathological type showed that eNOS 894G>T polymorphism was only associated with the risk of infiltrative ductal carcinoma. Stratified analyses by tumor stage showed that eNOS -786T>C polymorphism was only associated with the risk of tumor stage III and IV.

In conclusion, our meta-analysis and case–control study suggest that eNOS -786T>C and 894G>T polymorphisms are associated with the increased risk of breast cancer.

(Medicine 94(26):e972)

INTRODUCTION
	nitric oxide (NO) is a short-lived and small molecule, which is closely related to inflammatory status and regarded as a key inflammation mediator. Overproduction of NO can cause DNA damage and inhibit DNA repair.1 In addition, NO also promotes tumor angiogenesis and metastasis.7 Therefore, NO plays a significant role in the development of cancer. NO is produced from L-arginine by nitric oxide synthases (NOSs), which have 3 different isoforms and are divided into 2 functional classes. Constitutive class includes endothelial nitric oxide synthase (eNOS) and neuronal-NOS (nNOS) while the other class contains inducible form of NOS (iNOS).2 eNOS is a Ca2+ dependent enzyme and firstly defined in the vascular endothelial cells. Increased expression of eNOS has been noted in the vasculature of various tumor tissues, including bladder, colon, and pancreatic cancers.4–6 Previous studies have shown that eNOS can modulate cancer-related events, such as angiogenesis, invasion, and metastasis.7–9 eNOS is a central mediator of several endothelium growth stimulators, such as vascular endothelial growth factor (VEGF) and prostaglandin E2. The former (VEGF) can increase angiogenesis in both iNOS+/− and iNOS−/− mice but not in eNOS−/− mice, suggesting a predominant role of eNOS in VEGF-induced angiogenesis.7 In addition, an in vivo study has indicated that high eNOS expression is correlated to trophoblast cell cancer vascular invasion.8 Tumor cells in lung metastatic sites are always strongly eNOS-positive, suggesting that eNOS expression facilitates metastasis.9

The gene-encoding eNOS is located on chromosome 7q35 and has more than 168 polymorphisms.10 Among these polymorphisms, intron 4a/b, -786T>C (rs2070744), and 894G>T (rs1799983) polymorphisms seem to be functional and have been widely investigated for their associations with cancer risk.11–24 However, results were inconsistent. Therefore, we performed a comprehensive meta-analysis to derive a more precise estimation of the relationship between eNOS intron 4a/b, -786T>C, and 894G>T polymorphisms and cancer risk. Additionally, considering that no studies focused on the association of eNOS intron 4a/b, -786T>C, and 894G>T polymorphisms with the risk of breast cancer in Chinese Han population, we performed a case–control study to assess the association.

MATERIALS AND METHODS

Meta-Analysis

A comprehensive literature search was performed by PubMed and EMBASE databases with the following key words “endothelial nitric oxide synthase or eNOS or NOS3,” “polymorphism or variation,” and “cancer or tumor” (up to October
In addition, references of retrieved articles were also screened. The inclusion criteria were as follows: evaluation of the association between eNOS polymorphisms and cancer risk; case–control studies; detailed genotype data for estimating of odds ratios (ORs) and 95% confidence intervals (CIs); and no deviation from Hardy-Weinberg equilibrium (HWE) among the controls. As described previously, data were independently extracted from all eligible studies by 2 investigators, and any disagreement was resolved by discussion.43 The following information was collected from each study: first author, publication year, ethnicity, cancer type, total number of cases and controls, and number of different genotypes in cases and controls.

Case–Control Study

All recruited subjects were ethnically homogenous Han Chinese. A total of 873 patients (age 50.62 ± 10.20) with histopathologically diagnosed breast cancer and 1034 age-matched healthy women (age 51.02 ± 10.79) were consecutively recruited between October 2013 and May 2014 at the Affiliated Hospital of Bengbu Medical College. Clinicopathologic information were collected from medical records and pathology reports (Table 1). Written informed consent was obtained from all participants. The research protocol was approved by the ethics committee of the Affiliated Hospital of Bengbu Medical College. Genotyping method and material of eNOS polymorphisms, including primer sequences, PCR program, selected restriction enzymes, and fragment sizes, were presented in Table 2. PCR-RFLP assay was performed for genotyping of -786T>C and 894G>T loci. PCR products were digested by restriction enzyme MspI for -786T>C and BanII for 894G>T overnight according to the manufacture’s protocols. The digestion products of -786T>C and 894G>T as well as the amplifying product of intron 4a/b locus were analyzed by electrophoresis on a 3% agarose gel.

Statistical Analysis

Crude ORs with 95% CIs were calculated by the Stata version 12.0 software. Heterogeneity among studies was evaluated using the χ²-based Cochran Q statistic test. The random effect model was used to estimate a pooled OR when there was heterogeneity between studies (P<0.05); otherwise, the fixed effect model was adopted. HWE among the controls was verified using a goodness-of-fit χ² test. The association between eNOS polymorphisms and cancer risk was examined under

### Table 1. Distribution of Clinicopathologic Features Among Breast Cancer Cases

| Variables                  | Cases (n = 873) |
|----------------------------|----------------|
| N%                         |               |
| Pathological type           |               |
| Infiltrative ductal carcinoma | 713 (81.7)   |
| Other carcinoma             | 160 (18.3)    |
| Stage                      |               |
| I                          | 226 (25.9)    |
| II                         | 476 (54.5)    |
| III                        | 120 (13.7)    |
| IV                         | 51 (5.8)      |

### Table 2. Genotyping Method and Material of eNOS Polymorphisms

| Polymorphism | Primer Sequences | PCR Program | Restriction Enzyme | Fragment Size, bp |
|--------------|------------------|-------------|--------------------|------------------|
| intron 4a/b  |                  |             |                    |                  |
| -786T>C      | F-5'-AGGCCCCTAATGCTGTGTCAC-3' | 94°C 5 min, 35 cycles | MspI              | T allele: 138 +42 |
|              | R-5'-GCCTGCCACCCCCACCCTGTC-3' | 72°C 30 seconds, 72°C 5 minutes |                 |                  |
| 894G>T       | F-5'-AGGCTCTAGGAACTGGTTGG-3' | 94°C 5 min, 35 cycles | BanII             | G allele: 248 +85 |
|              | R-5'-ATGCTGCTGCACCGGACA-3' | 72°C 30 seconds, 72°C 5 minutes |                 |                  |

---

Gao et al Medicine Volume 94, Number 26, July 2015

Copyright © 2015 Wolters Kluwer Health, Inc. All rights reserved.
allele contrast (4a vs 4b, C vs T, and T vs G), recessive model (4a/4a vs 4a/4b + 4b/4b, CC vs CT + TT, and TT vs TG + GG), dominant model (4a/4a + 4a/4b vs 4b/4b, CC + CT vs TT, and TT + TG vs GG), homozygote contrast (4a/4a vs 4b/4b, CC vs TT, and TT vs GG), and heterozygote contrast (4a/4b vs 4b/4b, CT vs TT, and TG vs GG). Additionally, sensitivity analysis was performed by removing each individual study in turn from the total and reanalyzing the remainder. Finally, the Begg funnel plot and Egger test were employed to investigate the potential publication bias. In case–control study, logistic regression was used to analyze the association between 

**RESULTS**

**Meta-Analysis of eNOS Polymorphisms and Cancer Risk**

A total of 33 articles met the inclusion criteria and were included in the meta-analysis (Figure 1 and Table 3). For eNOS intron 4a/b polymorphism, 16 studies with 3850 cases and 4180 controls met the inclusion criteria. Among these studies, there were 4 studies of Asians and 12 studies of Caucasians. For eNOS -786T>C polymorphism, 10 studies with 4593 cases and 4355 controls met the inclusion criteria. Among these studies, there were 2 studies of Asians, 21 studies of Caucasians, and 1 study of mixed population.

As shown in Table 4, meta-analysis for eNOS intron 4a/b polymorphism showed significant associations in overall cancer. In the subgroup analysis based on cancer type, significant associations were found in prostate cancer. In the subgroup analysis based on ethnicity, significant associations were found in prostate cancer. In the subgroup analysis based on cancer type, significant associations were found in overall cancer, but stratified analysis by cancer type revealed that eNOS 894G>T polymorphism was associated with the risk of breast cancer.

The sensitivity analysis was performed to assess the influence of an individual study on the overall OR. For eNOS -786T>C polymorphism, the omission of Lu J study slightly affected the overall OR under recessive model (CC vs CT + TT: OR = 1.22, 95%CI: 0.99–1.50), and so did Jang MJ study under heterozygote contrast (CT vs TT: OR = 1.10, 95%CI: 0.99–1.23).

Both Begg funnel plot and Egger test were conducted to assess the publication bias. The shape of the funnel plot for the overall results seemed symmetrical. Similarly, Egger test showed no evidence of publication bias in the eNOS intron 4a/b, -786T>C, and 894G>T polymorphisms (Table 4).

Subsequently, subgroup analysis by cancer type showed statistically significant associations in breast, prostate, and bladder cancers. Subgroup analysis by ethnicity showed significant associations among Caucasians. For eNOS 894G>T polymorphism, no significant associations were found in overall cancer, but stratified analysis by cancer type revealed that eNOS 894G>T polymorphism was associated with the risk of breast cancer.

**Stratification of eNOS Polymorphisms With Breast Cancer Risk**

As summarized in Tables 6 and 7, stratified analysis based on pathological type indicated that eNOS -786T>C polymorphism was associated with the risk of infiltrative ductal carcinoma (C vs T: OR = 1.34, 95%CI: 1.02–1.76, P = 0.03) and other carcinoma (CC vs TT: OR = 3.40, 95%CI: 1.36–8.46, P = 0.01; CC vs CT + TT: OR = 3.37, 95%CI: 1.36–8.37, P = 0.01). However, eNOS 894G>T polymorphism was only associated with the risk of infiltrative ductal carcinoma (T vs G: OR = 1.25, 95%CI: 1.02–1.52, P = 0.03; TT vs GG: OR = 1.73, 95%CI: 1.20–2.50, P = 0.00; TT vs TG + GG: OR = 1.71, 95%CI: 1.19–2.47, P = 0.00). Furthermore, stratified analysis by tumor stage suggested that eNOS -786T>C polymorphism was only associated with the risk of tumor stage III and IV (C vs T: OR = 1.99, 95%CI: 1.35–2.93, P = 0.00; CC vs TT: OR = 4.42, 95%CI: 1.97–9.89, P = 0.00; CC + CT vs TT: OR = 1.74, 95%CI: 1.12–2.68, P = 0.01; CC vs CT + TT: OR = 4.34, 95%CI: 1.94–9.71, P = 0.00). However, eNOS 894G>T polymorphism was associated not only with the risk of tumor stage I and II (TT vs GG, OR = 1.57, 95%CI: 1.07–2.29, P = 0.02; TT vs TG + GG, OR = 2.45, 95%CI: 1.15–5.23, P = 0.02), but also with tumor stage III and IV (TT vs GG, OR = 2.14, 95%CI: 1.34–3.40, P = 0.00; TT vs TG + GG, OR = 4.56, 95%CI: 1.81–11.52, P = 0.00).
| Study         | Year | Ethnicity       | Cancer Type               | Number of Cases/Controls | Case | Control | PHWE |
|--------------|------|-----------------|---------------------------|--------------------------|------|---------|------|
| Yuan F       | 2013 | Asian           | Hepatocellular carcinoma | 293/384                  | 3    | 59      | 231  | 2    | 94   | 288  | 0.05 |
| Safarinejad MR | 2013 | Caucasian       | Prostate cancer           | 170/340                  | 15   | 54      | 101  | 3    | 88   | 249  | 0.11 |
| Ramirez-Patino R | 2013 | Caucasian       | Breast cancer             | 428/280                  | 3    | 94      | 331  | 2    | 34   | 244  | 0.50 |
| Jang MJ      | 2013 | Asian           | Colorectal cancer         | 528/509                  | 7    | 87      | 434  | 2    | 98   | 409  | 0.13 |
| Amsysli AS   | 2012 | Caucasian       | Bladder cancer            | 123/201                  | 8    | 63      | 52   | 5    | 59   | 137  | 0.65 |
| Oztürk E     | 2011 | Caucasian       | Endometrial cancer        | 89/60                    | 10   | 31      | 48   | 1    | 16   | 43   | 0.72 |
| Sanli O      | 2011 | Caucasian       | Prostate cancer           | 132/158                  | 5    | 40      | 87   | 6    | 48   | 104  | 0.88 |
| Tecder Ünal M | 2010 | Caucasian       | Gastric cancer            | 46/98                    | 1    | 10      | 35   | 4    | 28   | 66   | 0.64 |
| Zintzaras E  | 2010 | Caucasian       | Breast cancer             | 100/100                  | 3    | 27      | 70   | 4    | 37   | 59   | 0.54 |
| Yeh CC       | 2009 | Asian           | Colorectal cancer         | 713/723                  | 7    | 115     | 591  | 6    | 98   | 409  | 0.75 |
| Lu J         | 2006 | Caucasian       | Breast cancer             | 421/423                  | 15   | 113     | 293  | 13   | 110  | 300  | 0.46 |
| Heffler LA   | 2006 | Caucasian       | Breast cancer             | 269/270                  | 5    | 68      | 196  | 2    | 75   | 193  | 0.07 |
| Rierne EK    | 2004 | Caucasian       | Vulvar cancer             | 65/227                   | 0    | 17      | 48   | 3    | 53   | 171  | 0.62 |
| Mediros R    | 2002 | Caucasian       | Prostate cancer           | 125/153                  | 6    | 32      | 87   | 3    | 29   | 121  | 0.43 |
| Heffler LA   | 2002 | Caucasian       | Ovarian cancer            | 130/133                  | 0    | 40      | 90   | 2    | 34   | 97   | 0.61 |
| Cheon KT     | 2000 | Asian           | Lung cancer               | 218/121                  | 2    | 19      | 197  | 1    | 29   | 91   | 0.42 |
| Safarinejad MR | 2013 | Caucasian       | Prostate cancer           | 170/340                  | 52   | 93      | 25   | 150  | 159  | 31   | 0.22 |
| Jang MJ      | 2013 | Asian           | Colorectal cancer         | 528/509                  | 392  | 128     | 5    | 418  | 87   | 4    | 0.82 |
| Brankovic A  | 2013 | Caucasian       | Prostate cancer           | 150/100                  | 54   | 68      | 28   | 34   | 51   | 15   | 0.56 |
| Lee SA       | 2012 | Asian           | Breast cancer             | 504/508                  | 409  | 88      | 7    | 415  | 85   | 8    | 0.14 |
| Ryk C        | 2011 | Caucasian       | Bladder cancer            | 334/155                  | 152  | 142     | 40   | 84   | 63   | 8    | 0.38 |
| Yeh CC       | 2009 | Asian           | Colorectal cancer         | 683/726                  | 566  | 110     | 7    | 604  | 116  | 6    | 0.87 |
| Lee KM       | 2007 | Asian           | Breast cancer             | 136/956                  | 1025 | 22      | 22   | 766  | 177  | 13   | 0.45 |
| Lu J         | 2006 | Caucasian       | Breast cancer             | 421/423                  | 167  | 200     | 54   | 203  | 185  | 35   | 0.43 |
| Conde M      | 2006 | Caucasian       | Colorectal cancer         | 368/547                  | 107  | 184     | 77   | 152  | 273  | 122  | 0.98 |
| Ghiardini G  | 2003 | Caucasian       | Breast cancer             | 71/91                    | 22   | 35      | 14   | 37   | 42   | 12   | 0.99 |
| Ziaei SA     | 2013 | Caucasian       | Prostate cancer           | 78/87                    | 11   | 23      | 44   | 7    | 33   | 48   | 0.92 |
| Safarinejad MR | 2013 | Caucasian       | Prostate cancer           | 170/340                  | 2    | 48      | 120  | 3    | 89   | 248  | 0.10 |
| Verim L      | 2013 | Caucasian       | Bladder cancer            | 66/88                    | 10   | 49      | 7    | 13   | 44   | 31   | 0.68 |
| Jang MJ      | 2013 | Asian           | Colorectal cancer         | 528/509                  | 9    | 102     | 417  | 2    | 76   | 431  | 0.48 |
| Brankovic A  | 2013 | Caucasian       | Prostate cancer           | 150/100                  | 9    | 65      | 76   | 6    | 40   | 54   | 0.69 |
| Arikan S     | 2012 | Caucasian       | Colorectal cancer         | 84/99                    | 7    | 42      | 35   | 22   | 50   | 27   | 0.90 |
| Oztürk E     | 2011 | Caucasian       | Endometrial cancer        | 89/60                    | 11   | 31      | 47   | 0    | 18   | 42   | 0.17 |
| Ryk C        | 2011 | Caucasian       | Bladder cancer            | 262/150                  | 28   | 106     | 128  | 13   | 62   | 75   | 0.97 |
| Zintzaras E  | 2010 | Caucasian       | Breast cancer             | 100/100                  | 39   | 46      | 15   | 38   | 50   | 12   | 0.47 |
| Funke S      | 2009 | Caucasian       | Colorectal cancer         | 632/604                  | 58   | 285     | 289  | 61   | 272  | 271  | 0.55 |
| Yeh CC       | 2009 | Asian           | Colorectal cancer         | 702/728                  | 10   | 124     | 568  | 10   | 143  | 575  | 0.74 |
| Li Y         | 2009 | Mixed           | Breast cancer             | 489/485                  | 47   | 200     | 242  | 40   | 209  | 236  | 0.51 |
| Lee KM1      | 2009 | Caucasian       | Prostate cancer           | 1088/1293                | 103  | 468     | 517  | 129  | 557  | 607  | 0.94 |
| Lee KM2      | 2009 | African-American| Prostate cancer           | 97/373                   | 0    | 20      | 77   | 5    | 88   | 280  | 0.51 |
| Rajaraman P  | 2008 | Caucasian       | Acoustic neuroma, Glioma, Meningioma | 526/467  | 54  | 230     | 242  | 61  | 202  | 204  | 0.33 |

$P_{HWE} > 0.05$ was considered consistent with HWE. Mixed = White, African-American, Hispanic, Asian, and other/unknown.
| Polymorphism | Comparison      | Subgroup       | N  | $P_{HT}$ | $P_{Z}$ | $P_{L}$ | Random   | Fixed   |
|--------------|----------------|----------------|----|----------|---------|---------|----------|---------|
| Intron 4a/b  | 4a vs 4b       | Overall        | 16 | 0.00     | 0.24    | 0.74    | 1.13     | 1.15    |
|              |                | Breast cancer  | 4  | 0.02     | 0.62    |         | 1.09     | 1.11    |
|              |                | Colorectal cancer | 2  | 0.58     | 0.92    |         | 1.01     | 1.01    |
|              |                | Prostate cancer | 3  | 0.03     | 0.07    |         | 1.51     | 1.56    |
|              |                | Other cancer   | 7  | 0.00     | 0.85    |         | 1.04     | 1.11    |
|              |                | Caucasian      | 12 | 0.00     | 0.03    |         | 1.29     | 1.31    |
|              |                | Asian          | 4  | 0.01     | 0.21    |         | 0.82     | 0.90    |
|              | 4a/4b vs 4b/4b | Overall        | 16 | 0.00     | 0.50    | 0.97    | 1.13     | 1.15    |
|              |                | Breast cancer  | 4  | 0.01     | 0.76    |         | 1.07     | 1.11    |
|              |                | Colorectal cancer | 2  | 0.30     | 0.64    |         | 0.95     | 0.95    |
|              |                | Prostate cancer | 3  | 0.39     | 0.04    |         | 1.34     | 1.34    |
|              |                | Other cancer   | 7  | 0.00     | 0.92    |         | 1.03     | 1.06    |
|              |                | Caucasian      | 12 | 0.00     | 0.06    |         | 1.26     | 1.27    |
|              |                | Asian          | 4  | 0.01     | 0.11    |         | 0.73     | 0.83    |
|              | 4a/4a vs 4a/4b| Overall        | 16 | 0.18     | 0.00    | 0.50    | 1.73     | 1.81    |
|              |                | Breast cancer  | 4  | 0.75     | 0.55    |         | 1.18     | 1.19    |
|              |                | Colorectal cancer | 2  | 0.28     | 0.21    |         | 1.72     | 1.75    |
|              |                | Prostate cancer | 3  | 0.03     | 0.13    |         | 3.00     | 3.27    |
|              |                | Other cancer   | 7  | 0.38     | 0.08    |         | 1.71     | 1.77    |
|              |                | Caucasian      | 12 | 0.07     | 0.00    |         | 1.72     | 1.84    |
|              |                | Asian          | 4  | 0.73     | 0.16    |         | 1.65     | 1.71    |
|              | 4a/4a + 4a/4b | Overall        | 16 | 0.00     | 0.33    | 0.93    | 1.12     | 1.12    |
|              |                | Breast cancer  | 4  | 0.01     | 0.72    |         | 1.08     | 1.12    |
|              |                | Colorectal cancer | 2  | 0.40     | 0.84    |         | 0.98     | 0.98    |
|              |                | Prostate cancer | 3  | 0.13     | 0.00    |         | 1.47     | 1.50    |
|              |                | Other cancer   | 7  | 0.00     | 0.84    |         | 1.06     | 1.09    |
|              |                | Caucasian      | 12 | 0.00     | 0.04    |         | 1.31     | 1.32    |
|              |                | Asian          | 4  | 0.01     | 0.15    |         | 0.76     | 0.86    |
|              | 4a/4a vs 4b/4b | Overall        | 16 | 0.08     | 0.11    | 0.46    | 1.81     | 1.90    |
|              |                | Breast cancer  | 4  | 0.71     | 0.57    |         | 1.17     | 1.19    |
|              |                | Colorectal cancer | 2  | 0.30     | 0.22    |         | 1.69     | 1.74    |
|              |                | Prostate cancer | 3  | 0.02     | 0.12    |         | 3.23     | 3.49    |
|              |                | Other cancer   | 7  | 0.21     | 0.03    |         | 1.72     | 2.00    |
|              |                | Caucasian      | 12 | 0.03     | 0.06    |         | 1.83     | 1.97    |
|              |                | Asian          | 4  | 0.73     | 0.19    |         | 1.60     | 1.66    |
|              | -786T>C        | C vs T         | 10 | 0.04     | 0.11    | 0.16    | 1.11     | 1.15    |
|              |                | Breast cancer  | 4  | 0.16     | 0.03    |         | 1.15     | 1.15    |
|              |                | Colorectal cancer | 3  | 0.04     | 0.41    |         | 1.11     | 1.07    |
|              |                | Prostate cancer | 2  | 0.10     | 0.01    |         | 1.28     | 1.32    |
|              |                | Bladder cancer | 1  | –        | 0.02    |         | 1.46     | 1.46    |
|              |                | Caucasian      | 6  | 0.03     | 0.02    |         | 1.25     | 1.21    |
|              |                | Asian          | 4  | 0.16     | 0.14    |         | 1.11     | 1.10    |
|              | CT vs TT       | Overall        | 10 | 0.02     | 0.01    | 0.36    | 1.18     | 1.16    |
|              |                | Breast cancer  | 4  | 0.40     | 0.21    |         | 1.10     | 1.10    |
|              |                | Colorectal cancer | 3  | 0.05     | 0.13    |         | 1.15     | 1.14    |
|              |                | Prostate cancer | 2  | 0.05     | 0.56    |         | 1.22     | 1.33    |
|              |                | Bladder cancer | 1  | –        | 0.28    |         | 1.25     | 1.25    |
|              |                | Caucasian      | 6  | 0.23     | 0.02    |         | 1.21     | 1.21    |
|              |                | Asian          | 4  | 0.10     | 0.17    |         | 1.12     | 1.10    |
|              | CC vs TT       | Overall        | 10 | 0.12     | 0.01    | 0.32    | 1.16     | 1.15    |
|              |                | Breast cancer  | 4  | 0.40     | 0.21    |         | 1.10     | 1.10    |
|              |                | Colorectal cancer | 3  | 0.05     | 0.13    |         | 1.15     | 1.14    |
|              |                | Prostate cancer | 2  | 0.05     | 0.56    |         | 1.22     | 1.33    |
|              |                | Bladder cancer | 1  | –        | 0.28    |         | 1.25     | 1.25    |
|              |                | Caucasian      | 6  | 0.23     | 0.02    |         | 1.21     | 1.21    |
|              |                | Asian          | 4  | 0.10     | 0.17    |         | 1.12     | 1.10    |
|              | CC vs CT + TT  | Overall        | 10 | 0.36     | 0.01    | 0.36    | 1.30     | 1.28    |
| Polymorphism/Comparison | Subgroup | N  | $P_H$  | $P_Z$  | $P_E$ | Random  | Fixed  |
|--------------------------|----------|----|--------|--------|-------|---------|-------|
| **Breast cancer**        |          |    |        |        |       |         |       |
| CC + CT vs TT            |          | 4  | 0.67   | 0.03   | 1.43  | (1.03–1.98) | 1.43 (1.03–1.97) |
| T vs G                   |          | 8  | 0.84   | 0.11   | 1.07  | (0.98–1.16) | 1.07 (0.98–1.16) |
| TG vs GG                 |          | 25 | 0.37   | 0.37   | 1.03  | (0.96–1.10) | 1.03 (0.97–1.10) |
| TT vs GG                 |          | 25 | 0.03   | 0.28   | 1.09  | (0.93–1.27) | 1.04 (0.93–1.15) |
| TT + TG vs GG            |          | 25 | 0.12   | 0.33   | 1.04  | (0.96–1.12) | 1.03 (0.97–1.10) |
| TT vs TG + GG            |          | 25 | 0.09   | 0.73   | 1.05  | (0.92–1.21) | 1.02 (0.92–1.13) |
| **Colorectal cancer**    |          |    |        |        |       |         |       |
| CC + CT vs TT            |          | 3  | 0.82   | 0.77   | 0.96  | (0.71–1.29) | 0.96 (0.71–1.29) |
| T vs G                   |          | 7  | 0.80   | 0.72   | 0.99  | (0.92–1.06) | 0.99 (0.91–1.06) |
| TG vs GG                 |          | 25 | 0.75   | 0.40   | 1.04  | (0.94–1.16) | 1.04 (0.94–1.16) |
| TT vs GG                 |          | 25 | 0.63   | 0.03   | 1.23  | (1.02–1.49) | 1.24 (1.02–1.50) |
| TT + TG vs GG            |          | 25 | 0.85   | 0.71   | 1.02  | (0.92–1.12) | 1.02 (0.92–1.12) |
| TT vs TG + GG            |          | 25 | 0.91   | 0.51   | 1.04  | (0.93–1.16) | 1.04 (0.93–1.16) |
| **Prostate cancer**      |          |    |        |        |       |         |       |
| CC + CT vs TT            |          | 2  | 0.54   | 0.06   | 1.54  | (0.99–2.37) | 1.53 (0.99–2.37) |
| T vs G                   |          | 3  | 0.14   | 0.11   | 1.29  | (0.85–1.96) | 1.23 (0.96–1.60) |
| TG vs GG                 |          | 25 | 0.02   | 0.74   | 1.02  | (0.54–1.55) | 0.95 (0.74–1.22) |
| TT vs GG                 |          | 25 | 0.67   | 0.28   | 0.91  | (0.76–1.08) | 0.91 (0.76–1.08) |
| TT + TG vs GG            |          | 25 | 0.03   | 0.99   | 1.00  | (0.80–1.25) | 1.01 (0.89–1.15) |
| TT vs TG + GG            |          | 25 | 0.02   | 0.99   | 1.00  | (0.80–1.25) | 1.01 (0.89–1.15) |
| **Bladder cancer**       |          |    |        |        |       |         |       |
| CC + CT vs TT            |          | 1  | –      | –      | 2.50  | (1.14–5.48) | 2.50 (1.14–5.48) |
| T vs G                   |          | 1  | –      | –      | 1.14  | (0.85–1.53) | 1.13 (0.96–1.34) |
| TG vs GG                 |          | 25 | 0.13   | 0.11   | 1.29  | (0.85–1.96) | 1.23 (0.96–1.60) |
| TT vs GG                 |          | 25 | 0.13   | 0.11   | 1.29  | (0.85–1.96) | 1.23 (0.96–1.60) |
| TT + TG vs GG            |          | 25 | 0.09   | 0.32   | 2.07  | (0.49–8.81) | 1.65 (0.89–3.03) |
| TT vs TG + GG            |          | 25 | 0.09   | 0.32   | 2.07  | (0.49–8.81) | 1.65 (0.89–3.03) |
| **Caucasian**            |          |    |        |        |       |         |       |
| CC + CT vs TT            |          | 6  | 0.10   | 0.01   | 1.43  | (1.05–1.95) | 1.31 (1.07–1.61) |
| T vs G                   |          | 6  | 0.10   | 0.01   | 1.43  | (1.05–1.95) | 1.31 (1.07–1.61) |
| TG vs GG                 |          | 25 | 0.04   | 0.39   | 1.14  | (0.85–1.53) | 1.13 (0.96–1.34) |
| TT vs GG                 |          | 25 | 0.04   | 0.39   | 1.14  | (0.85–1.53) | 1.13 (0.96–1.34) |
| TT + TG vs GG            |          | 25 | 0.04   | 0.39   | 1.14  | (0.85–1.53) | 1.13 (0.96–1.34) |
| TT vs TG + GG            |          | 25 | 0.04   | 0.39   | 1.14  | (0.85–1.53) | 1.13 (0.96–1.34) |
| **Asian**                |          |    |        |        |       |         |       |
| CC + CT vs TT            |          | 4  | 0.06   | 0.62   | 1.13  | (0.70–1.81) | 1.13 (0.70–1.81) |
| T vs G                   |          | 4  | 0.06   | 0.62   | 1.13  | (0.70–1.81) | 1.13 (0.70–1.81) |
| TG vs GG                 |          | 25 | 0.07   | 0.07   | 1.42  | (0.97–2.08) | 1.42 (0.97–2.08) |
| TT vs GG                 |          | 25 | 0.07   | 0.07   | 1.42  | (0.97–2.08) | 1.42 (0.97–2.08) |
| TT + TG vs GG            |          | 25 | 0.07   | 0.07   | 1.42  | (0.97–2.08) | 1.42 (0.97–2.08) |
| TT vs TG + GG            |          | 25 | 0.07   | 0.07   | 1.42  | (0.97–2.08) | 1.42 (0.97–2.08) |
| **Other cancer**         |          |    |        |        |       |         |       |
| CC + CT vs TT            |          | 1  | –      | –      | 1.29  | (0.85–1.93) | 1.22 (0.85–1.17) |
| T vs G                   |          | 1  | –      | –      | 1.29  | (0.85–1.93) | 1.22 (0.85–1.17) |
| TG vs GG                 |          | 25 | 0.11   | 0.40   | 1.54  | (0.99–2.37) | 1.53 (0.99–2.37) |
| TT vs GG                 |          | 25 | 0.11   | 0.40   | 1.54  | (0.99–2.37) | 1.53 (0.99–2.37) |
| TT + TG vs GG            |          | 25 | 0.11   | 0.40   | 1.54  | (0.99–2.37) | 1.53 (0.99–2.37) |
| TT vs TG + GG            |          | 25 | 0.11   | 0.40   | 1.54  | (0.99–2.37) | 1.53 (0.99–2.37) |

*Note: P-values are calculated based on the given data.*
TABLE 5. Association of eNOS Polymorphisms With Breast Cancer Risk Among Chinese Han Population

| Genotype Description | Cases (n=873) | Controls (n=1034) | Comparison | OR (95% CI) | P* |
|----------------------|---------------|-------------------|------------|-------------|----|
| Intron 4a/b          |               |                   |            |             |    |
| 4b/4b                | 722           | 858               | 4a vs 4b   | 1.02 (0.82–1.28) | 0.84 |
| 4a/4b                | 140           | 164               | 4a/4a vs 4b/4b | 1.08 (0.47–2.46) | 0.86 |
| 4a/4a                | 11            | 12                | 4a/4a vs 4a/4b | 1.02 (0.79–1.30) | 0.91 |
| 4b/4b                |               |                   | 4a/4a vs 4b/4b | 1.02 (0.80–1.29) | 0.88 |
| P_HWE                | 0.19          |                   | 4a/4a vs 4a/4b | 1.08 (0.47–2.45) | 0.86 |
| -786T>C              |               |                   |            |             |    |
| TT                   | 751           | 917               | CC vs TT   | 2.19 (1.01–4.76) | 0.05 |
| CT                   | 114           | 115               | CT vs TT   | 1.21 (0.92–1.60) | 0.17 |
| CC                   | 8             | 2                 | CC + CT vs TT | 1.28 (0.97–1.67) | 0.08 |
| P_HWE                | 0.41          |                   | CC vs CT+TT | 2.17 (1.00–4.72) | 0.50 |
| 894G>T               |               |                   |            |             |    |
| TT                   | 652           | 791               | TT vs GG   | 1.69 (1.19–2.42) | 0.00 |
| TG                   | 195           | 232               | TG vs GG   | 1.02 (0.82–1.27) | 0.87 |
| TT                   | 26            | 11                | TT + TG vs GG | 1.10 (0.89–1.36) | 0.36 |
| P_HWE                | 0.18          |                   | TT vs TG + GG | 1.69 (1.18–2.41) | 0.00 |

* Adjusted for age.

TABLE 6. Genotype Distribution of eNOS Polymorphisms in Stratification Analysis

| Pathological type               | Cases (n=873) | Controls (n=1034) |
|---------------------------------|---------------|-------------------|
| Infiltrative ductal carcinoma   |               |                   |
| Other carcinoma                 | 136           | 164               |
| Stage                           |               |                   |
| I + II                         | 584           | 858               |
| III + IV                       | 138           | 858               |
| 894G>T                          | 140           | 858               |
| Pathological type               |               |                   |
| Infiltrative ductal carcinoma   | 610           | 917               |
| Other carcinoma                 | 141           | 917               |
| Stage                           |               |                   |
| I + II                         | 611           | 917               |
| III + IV                       | 140           | 917               |
| 894G>T                          | 129           | 917               |
| Pathological type               |               |                   |
| Infiltrative ductal carcinoma   |               |                   |
| Other carcinoma                 | 126           | 791               |
| Stage                           |               |                   |
| I + II                         | 526           | 791               |
| III + IV                       | 126           | 791               |

P_E = P-value of Egger test, P_H = P-value of heterogeneity test, P_Z = P-value of Z test.
| Pathological type     | Stage       | Infiltrative ductal carcinoma | Other carcinoma | Infiltrative ductal carcinoma | Other carcinoma | Infiltrative ductal carcinoma | Other carcinoma | Infiltrative ductal carcinoma | Other carcinoma | Infiltrative ductal carcinoma | Other carcinoma |
|-----------------------|-------------|-------------------------------|-----------------|-------------------------------|-----------------|-------------------------------|-----------------|-------------------------------|-----------------|-------------------------------|-----------------|
| 4a/b                  | I + II      | 0.99 (0.78–1.25)              | 0.91            | 0.97 (0.39–2.39)              | 0.95            | 0.99 (0.76–1.28)              | 0.91            | 0.98 (0.76–1.27)              | 0.90            | 0.97 (0.40–2.39)              | 0.95            |
|                       | III + IV    | 1.18 (0.81–1.71)              | 0.40            | 1.55 (0.43–5.57)              | 0.50            | 1.14 (0.74–1.75)              | 0.56            | 1.17 (0.77–1.76)              | 0.47            | 1.52 (0.42–5.44)              | 0.52            |
| -786T>C               | I + II      | 1.16 (0.88–1.54)              | 0.29            | 1.22 (0.46–3.25)              | 0.69            | 1.16 (0.87–1.56)              | 0.32            | 1.17 (0.87–1.57)              | 0.30            | 1.21 (0.45–3.23)              | 0.71            |
|                       | III + IV    | 1.99 (1.35–2.93)              | 0.00            | 4.42 (1.97–9.89)              | 0.00            | 1.42 (0.89–2.27)              | 0.14            | 1.74 (1.12–2.68)              | 0.01            | 4.34 (1.94–9.71)              | 0.00            |
| 894G>T                | I + II      | 1.15 (0.94–1.40)              | 0.19            | 1.57 (1.07–2.29)              | 0.02            | 1.02 (0.81–1.29)              | 0.85            | 1.09 (0.87–1.36)              | 0.46            | 2.45 (1.15–5.23)              | 0.02            |
|                       | III + IV    | 1.31 (0.95–1.81)              | 0.10            | 2.14 (1.34–3.40)              | 0.00            | 1.00 (0.68–1.49)              | 0.99            | 1.16 (0.80–1.68)              | 0.42            | 4.56 (1.81–11.52)             | 0.00            |

*Adjusted for age.
DISCUSSION

Multiple lines of evidence supported an important role for genetics in determining cancer risk, and understanding polymorphisms associated with cancer risk may be valuable for providing personalized diagnosis and therapy of certain cancers. Since the identification of eNOS intron 4a/b, -786T>C, and 894G>T polymorphisms, an increasing number of studies suggested that eNOS intron 4a/b, -786T>C, and 894G>T polymorphisms may play important roles in cancer risk. Epidemiological studies of eNOS intron 4a/b, -786T>C, and 894G>T polymorphisms, if large and unbiased, can provide insight into the association between the gene and cancer risk. However, previous results are inconclusive. To derive a more precise estimation of the association, we performed this meta-analysis. The eNOS intron 4a/b and -786T>C polymorphisms were significantly associated with overall cancer risk. In subgroup analyses based on cancer type, significant associations were found between eNOS intron 4a/b polymorphism and the risk of prostate, bladder and breast cancers, and eNOS -786T>C polymorphisms may play important roles in cancer risk. In subgroup analyses by tumor stage, we found that -786T>C polymorphisms were associated with cancer risk in Caucasians. In current case–control study of 873 patients with breast cancer and 1034 healthy women, we found that eNOS -786T>C and 894G>T polymorphisms were associated with breast cancer risk in Chinese Han population, which were consistent with our meta-analysis results. Furthermore, stratified analyses based on pathological type showed that eNOS 894G>T polymorphism was only associated with risk of infiltrative ductal carcinoma. In stratified analyses by tumor stage, we found that eNOS -786T>C polymorphism was only associated with the risk of tumor stage III and IV.

To a certain extent, our meta-analysis and case–control study still include some limitations, which should be interpreted and taken into consideration. For the present meta-analysis, we did not have original data for all studies to adjust estimates and perform a more precise analysis. For the case–control study, all participants were from hospital, which may result in inherent selection bias.

In conclusion, our meta-analysis and case–control study suggest that eNOS -786T>C and 894G>T polymorphisms are associated with the risk of breast cancer. However, our findings need to be further validated in well-designed studies.

ACKNOWLEDGMENTS

The authors thank “the Fundamental Research Funds for the Central Universities,” “Postgraduate Research and Innovation Project in University of Jiangsu Province (KYZZ_0071),” and “National Nature Science Foundation of China 81371609” for the support.

REFERENCES

1. Chien YH, Bai DT, Jan KY. Nitric oxide inhibits DNA-adduct excision in nucleotide excision repair. Free Radic Biol Med. 2004;36:1011–1017.

2. Jadeski LC, Chakraborty C, Lala PK. Nitric oxide-mediated promotion of mammary tumour cell migration requires sequential activation of nitric oxide synthase, guanylate cyclase and mitogen-activated protein kinase. Int J Cancer. 2003;106:496–504.

3. Albrecht EW, Stegeman CA, Heeringa P, et al. Protective role of endothelial nitric oxide synthase. J Pathol. 2003;199:8–17.

4. Klotz T, Bloch W, Jacobs G, et al. Immunolocalization of inducible and constitutive nitric oxide synthases in human bladder cancer. Urology. 1999;54:416–419.

5. Chhatwal VJ, Ngoi SS, Chan ST, et al. Aberrant expression of nitric oxide synthase in human polyps, neoplastic colonic mucosa and surrounding peritumoral normal mucosa. Carcinogenesis. 1994;15:2081–2085.

6. Nussler AK, Gansauge S, Gansauge F, et al. Overexpression of endothelium-derived nitric oxide synthase isoform 3 in the vasculature of human pancreatic tumor biopsies. Langenbecks Arch Surg. 1998;383:474–480.

7. Duda DG, Fukumura D, Jain RK. Role of eNOS in neovascularization: NO for endothelial progenitor cells. Trends Mol Med. 2004;10:143–145.

8. Ariel I, Hochberg A, Shochina M. Endothelial nitric oxide synthase immunoreactivity in early gestation and in trophoblastic disease. J Clin Pathol. 1998;51:427–431.

9. Jadeski LC, Hum KO, Chakraborty C, et al. Nitric oxide promotes murine mammary tumour growth and metastasis by stimulating tumour cell migration, invasiveness and angiogenesis. Int J Cancer. 2000;86:30–39.

10. Lu J, Wei Q, Bondy ML, et al. Promoter polymorphism (-786T>C) in the endothelial nitric oxide synthase gene is associated with risk of sporadic breast cancer in non-Hispanic white women age younger than 55 years. Cancer. 2006;107:2245–2253.

11. Yuan F, Zhang LS, Li HY, et al. Influence of angiotensin I-converting enzyme gene polymorphism on hepatocellular carcinoma risk in China. DNA Cell Biol. 2013;32:268–273.

12. Sarafinnejad MR, Sarafinnejad S, Shafiei N, et al. Effects of the T-786C, G894T, and Intron 4 VNTR (4a/b) polymorphisms of the endothelial nitric oxide synthase gene on the risk of prostate cancer. Urol Oncol. 2013;31:1132–1140.

13. Ramirez-Patino R, Figuera LE, Puebla-Perez AM, et al. Intron 4 VNTR (4a/b) polymorphism of the endothelial nitric oxide synthase gene is associated with breast cancer in Mexican women. J Korean Med Sci. 2013;28:1587–1594.

14. Jang MJ, Jeon YJ, Kim JW, et al. Association of eNOS polymorphisms (-786T>C, 4a4b, 894G>T) with colorectal cancer susceptibility in the Korean population. Gene. 2013;512:275–281.

15. Amasyali AS, Kucugkergin C, Erdem S, et al. Nitric oxide synthase (eNOS4a/b) gene polymorphism is associated with tumor recurrence and progression in superficial bladder cancer cases. J Urol. 2012;188:2398–2403.

16. Oztürk E, Dikenozy E, Balat O, et al. Association of endothelial nitric oxide synthase gene polymorphisms with endometrial carcinoma: a preliminary study. J Turk Ger Gynecol Assoc. 2011;12:229–233.

17. Sanli O, Kucukgkergin C, Gokpinar M, et al. Despite the lack of association between different genotypes and the presence of prostate cancer, endothelial nitric oxide synthase b/a (eNOS4a/b) polymorphism may be associated with advanced clinical stage and bone metastasis. Urol Oncol. 2011;29:183–188.

18. Tecder Ünal M, Karabulut HG, Gümüş-Akay G, et al. Endothelial nitric oxide synthase gene polymorphism in gastric cancer. Turk J Gastroenterol. 2010;21:338–344.

19. Zintzaras E, Grammatikou M, Kitsios GD, et al. Polymorphisms of the endothelial nitric oxide synthase gene in breast cancer: a genetic association study and meta-analysis. J Hum Genet. 2010;55:743–748.

20. Yeh CC, Santella RM, Hsieh LL, et al. An intron 4 VNTR polymorphism of the endothelial nitric oxide synthase gene is
associated with early-onset colorectal cancer. *Int J Cancer.*
2009;124:1565–1571.

21. Hefler LA, Grimm C, Lantzsch T, et al. Polymorphisms of the endothelial nitric oxide synthase gene in breast cancer. *Breast Cancer Res Treat.* 2006;98:151–155.

22. Hefler LA, Ludwig E, Lampe D, et al. Polymorphisms of the endothelial nitric oxide synthase gene in ovarian cancer. *Gynecol Oncol.* 2002;86:134–137.

23. Rieker EK, Hefler LA, Grimm C, et al. Polymorphisms of the endothelial nitric oxide synthase gene in women with vulvar cancer. *Gynecol Oncol.* 2004;93:686–690.

24. Medeiros R, Morais A, Vasconcelos A, et al. Endothelial nitric oxide synthase gene polymorphisms and genetic susceptibility to prostate cancer. *Eur J Cancer Prev.* 2002;11:343–350.

25. Cheon KT, Choi KH, Lee HB, et al. Gene polymorphisms of endothelial nitric oxide synthase and angiotensin-converting enzyme in patients with lung cancer. *Lang.* 2000;178:351–360.

26. Lee A, Brajuskovic G, Nikolic Z, et al. Endothelial nitric oxide synthase gene polymorphisms and prostate cancer risk in Serbian population. *Int J Exp Pathol.* 2013;94:355–361.

27. Arikan S, Cacina C, Guler E, et al. The effects of NOS3 Glu298Asp variant on colorectal cancer risk and progression in Turkish population. *Mol Biol Rep.* 2012;39:3245–3249.

28. Lee KM, Choi JY, Lee JE, et al. Genetic polymorphisms of NOS3 are associated with the risk of invasive breast cancer with lymph node involvement. *Breast Cancer Res Treat.* 2007;106:433–438.

29. Lee KM, Kang D, Park SK, et al. Nitric oxide synthase gene polymorphisms and prostate cancer risk. *Carcinogenesis.* 2009;30:621–625.

30. Lee SA, Lee KM, Yoo KY, et al. Combined effects of antioxidant vitamin and NOS3 genetic polymorphisms on breast cancer risk in women. *Clin Nutr.* 2012;31:93–98.

31. Li Y, Ambrosone CB, McCullough MJ, et al. Oxidative stress-related genotypes, fruit and vegetable consumption and breast cancer risk. *Carcinogenesis.* 2009;30:777–784.

32. Rajaraman P, Hutchinson A, Rothman N, et al. Oxidative response gene polymorphisms and risk of adult brain tumors. *Neuro Oncol.* 2008;10:709–715.

33. Ryk C, Wiklund NP, Nyberg T, et al. Polymorphisms in nitric-oxide synthase 3 may influence the risk of urinary-bladder cancer. *Nitric Oxide.* 2011;25:338–343.

34. Conde MC, Ramirez-Lorca R, Lopez-Jamar JM, et al. Genetic analysis of caveolin-1 and eNOS genes in colorectal cancer. *Oncol Rep.* 2006;16:353–359.

35. Funke S, Hoffmeister M, Brenner H, et al. Effect modification by smoking on the association between genetic polymorphisms in oxidative stress genes and colorectal cancer risk. *Cancer Epidemiol Biomarkers Prev.* 2009;18:2336–2338.

36. Verim L, Toptas B, Ozkan NE, et al. Possible relation between the NOS3 gene GLU298ASP polymorphism and bladder cancer in Turkey. *Asian Pac J Cancer Prev.* 2013;14:665–668.

37. Yang J, Ambrosone CB, Hong CC, et al. Relationships between polymorphisms in NOS3 and MPO genes, cigarette smoking and risk of post-menopausal breast cancer. *Carcinogenesis.* 2007;28:1247–1253.

38. Ziaei SA, Samzadeh M, Jamaldini SH, et al. Endothelial nitric oxide synthase Glu298Asp polymorphism as a risk factor for prostate cancer. *Int J Biol Markers.* 2013;28:43–48.

39. Ghiardi G, Bianchi ML, Cecchini F, et al. Vascular invasion in human breast cancer is correlated to T-/-786C polymorphism of NOS3 gene. *Nitric Oxide.* 2003;9:118–122.

40. Hong CC, Ambrosone CB, Ahn J, et al. Genetic variability in iron-related oxidative stress pathways (Nrf2, NQ01, NOS3, and HO-1), iron intake, and risk of postmenopausal breast cancer. *Cancer Epidemiol Biomarkers Prev.* 2007;16:1784–1794.

41. Jacobs EJ, Hsing AW, Bain EB, et al. Polymorphisms in angiogenesis-related genes and prostate cancer. *Cancer Epidemiol Biomarkers Prev.* 2008;17:972–977.

42. Royo JL, Moreno-Nogueira JA, Galan JJ, et al. Lack of association between NOS3 Glu298Asp and breast cancer risk: a case-control study. *Breast Cancer Res Treat.* 2006;100:331–333.

43. Liu ZL, Zhu WR, Zhou WC, et al. Traditional Chinese medicinal herbs combined with epidermal growth factor receptor tyrosine kinase inhibitor for advanced non-small cell lung cancer: a systematic review and meta-analysis. *J Integr Med.* 2014;12:346–358.