Association between Vitamin D Receptor Gene Polymorphisms and Breast Cancer Risk: A Meta-Analysis of 39 Studies

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Abstract

Background: The associations between vitamin D receptor (VDR) gene polymorphisms and breast cancer risk were comprehensively investigated to clarify issues that remain controversial.

Methodology/Principal Findings: An electronic search was conducted of several databases, including PubMed, the Cochrane library, Web of Science, EMBASE, CBM and CNKI, for papers that describe the association between Fok1, poly-A repeat, Bsm1, Taq1 or Apa1 polymorphisms of the VDR gene and breast cancer risk. Summary odds ratios and 95% confidence intervals (CI) were estimated based on a fixed-effect model (FEM) or random-effect model (REM), depending on the absence or presence of significant heterogeneity. A total of 39 studies met the inclusion criteria. A meta-analysis of high-quality studies showed that the Fok1 polymorphism of the VDR gene was associated with an increased risk of breast cancer (ff vs. Ff+FF, OR: 1.09, 95%CI: 1.02 to 1.16, p = 0.007). No significant associations were observed between the other polymorphisms and breast cancer risk. No positive results were detected by pooling the results of all relevant studies.

Conclusion: A meta-analysis of high-quality studies demonstrated that the Fok1 polymorphism of the VDR gene was closely associated with breast cancer risk.

Introduction

Laboratories investigations and epidemiological studies have suggested that the level of vitamin D, and the expression of the vitamin D receptor (VDR), might be associated with an increased risk of breast cancer [1,2]. However, based on the current available data, these relationships need to be further evaluated. Vitamin D from all sources undergoes hydroxylation in the liver to become 25-hydroxyvitamin D [25(OH) D], which is then further hydroxylated in the kidneys and other tissues to an active form of vitamin D (1,25-dihydroxyvitamin D, 1,25(OH)2D) [3,4]. In several studies, 1,25(OH)2D has been demonstrated to promote cell differentiation and inhibit cell proliferation, potentially modifying cancer risk via binding to the VDR [5,6]. The VDR is an intracellular hormone receptor that specifically binds to 1,25(OH)2D and interacts with specific nucleotide sequences (response elements) of target genes to produce a variety of biological effects. As vitamin D exerts its activity by binding to the VDR, the finding that normal breast epithelial cells [7] and most breast cancer cells [8] express VDR suggests the possibility that VDR gene polymorphism may be associated with breast cancer risk.

The gene that encodes VDR maps to the long arm of chromosome 12 (12q12-14), and harbors approximately 200 single nucleotide polymorphisms (SNPs). Some are linked to differences in 1-25(OH)2D uptake and can therefore be considered as latent disease risk variants. A series of characterized VDR gene polymorphisms, including Fok1 (rs2228570) [9–24], a poly-adenosine (poly-A) repeat variant [10,19,22,25–27], Bsm1 (rs1544110) [10,11,13,15–17,19–22,24,25,28–33], Taq1 (rs731236) [9,12–14,17,18,24,26,29,30,34–38] and Apa1 (rs7975232) [9,13,17,18,24,29,36,38–40], have been extensively studied with regard to their association with breast cancer risk, but with conflicting results. To clarify the association between breast cancer risk and VDR gene polymorphisms, we performed a meta-analysis of 39 existing studies to clarify the relationship between genetic variations in VDR and the risk of breast cancer.

Materials and Methods

Search Strategies
A comprehensive literature search of numerous databases, including PubMed, the Cochrane library, Web of Science, EMBASE, CBM (China Biology Medicine) and CNKI (China...
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National Knowledge Infrastructure), was conducted up until December 21st, 2013. Publications with the following search words in the titles, abstract or key words of the original studies were included: vitamin D receptor’, ‘VDR’, ‘Fok1’, ‘Poly A’, ‘Bsm1’, ‘Tag1’, ‘Apa1’, ‘polymorphism’ or ‘variant’ or ‘mutation’ coupled with the term ‘breast cancer’. Additional studies that were not captured by the database search were identified by reviewing the bibliographies of relevant articles.

Inclusion Criteria
All identified studies were reviewed independently by two investigators. The following criteria were used for a publication to be included in the meta-analysis: (1) any study published as an original study that evaluated the association between VDR gene polymorphisms (Fok1, poly A, Bsm1, Tag1 and Apa1) and breast cancer risk; (2) cases of breast cancer were confirmed by medical records or linkage with population-based tumor registries; (3) the numbers of case and control groups for each genotype were reported or the relevant data was available, and adequate data was provided to calculate the odds ratio (OR); and (4) publications in both English and Chinese were included.

Data Extraction and Quality Assessment
Two investigators conducted the search, extracted and tabulated all the relevant data independently. If a study was referenced more than once, the most complete and newly released study was used. If one article reported two or more different case-control studies, it was considered as two or more studies, respectively. Data extracted from each study were as followings: name of the first author, publication year, ethnic origin of the studied population, numbers of case and controls, and the genotype frequency of the polymorphisms. To maintain consistency with the previously published literature, SNPs of the VDR gene were reported using restriction fragment length polymorphism (RFLP) nomenclature for the major and minor alleles, as follows: Fok1 (rs228570) alleles C = F and T = f; Bsm1 (rs1544410) alleles G = b and A = B; Tag1 (rs731236) alleles T = T, and C = c; and Apa1 (rs7975232) A = A and C = a. The allele counts were calculated from the genotype counts when needed.

The quality of studies was assessed according to the STrengthening the REporting of Genetic Association Studies (STREGA) criteria [41], and studies according to STREGA criteria were defined as high-quality studies. An independent review and decision was made by a senior investigator if there were disagreements between the two initial reviewers.

Statistical Analysis
The strengths of the associations between five polymorphisms of the VDR gene and the risk of breast cancer were assessed for the contrast between two groups of homozygotes (ff vs. FF, SS vs. LL, bb vs. BB, tt vs. TT, aa vs. AA), the recessive (ff vs. Ff+FF, SS vs. SL+LL, bb vs. Bb+BB, tt vs. Tt+TT, aa vs. Aa+AA), dominant (ff+Ff vs. FF, SS+SL vs. LL, bb+Bb vs. BB, tt+Tt vs. TT, aa+Aa vs. AA) and allelic (f vs. F, s vs L, b vs. B, t vs. T, a vs. A) models by calculating the pooled OR and its 95% confidence interval (CI). The pooled ORs were obtained using either the fixed-effects (Mantel-Haenszel method) model [42] or the random-effect (DerSimonian and Laird method) model [43], depending on the absence or presence of significant heterogeneity. The significance of pooled ORs was determined by the Z test. Heterogeneity among studies was assessed by the Chi-square test -based Q statistic and was quantified using the I$^2$ statistic [44]. A significant Q statistic (P-value <0.10) or I$^2$ statistic (I$^2$>50%) indicated significant heterogeneity existed across studies.

A Sensitivity analysis was performed to evaluate the key studies that had substantial impacts on between-study heterogeneity levels by removing the individual studies sequentially. To further explore the cause of heterogeneity, a meta-regression was performed, which included covariates such as ethnicity and sample size of the studies. If the origin of heterogeneity was found, subgroup analyses were conducted according to the origin. All statistical analyses, except the meta-regression, were performed using RevMan version 5.1.6 software (Review Manager, Copenhagen: the Nordic Cochrane Centre, The Cochrane Collaboration, 2011). The meta-regression procedure was conducted using STATA statistical software (version 12.0; Stata Corporation, College Station, USA).

The possibility of publication bias was assessed using Begger’s linear regression and funnel plots. An asymmetrical funnel plot suggested a possible publication bias.

Results
Baseline Characteristics
A flow chart of the literature search is shown in Figure 1. According to the criteria eligibility, 39 studies was identified regarding the associations between the Fok1, poly-A, Bsm1, Tag1 or Apa1 polymorphisms of VDR gene and breast cancer risk. Among these studies, 22 studies [9–24] concerned the association of the Fok1 polymorphism with breast cancer, including 16,353 cases and 21,881 controls, while seven studies [10,19,22,25–27] investigated the association between the poly-A repeat variation and breast cancer risk, with 5,493 cases and 5,566 controls. For the Bsm1 polymorphism, 25 studies [10,11,13,15–17,19–22,24,25,28–33] included 16,160 cases and 21,023 controls, while 16 studies [9,12–14,17,18,24,26,29,30,34–38] on the Tag1 polymorphism included 6,940 cases and 8,267 controls. For the Apa1 polymorphism, 11 studies were included [9,13,17,18,26,29,36,39] with 3,738 cases and 4,489 controls. All of these 39 studies provided sufficient data to calculate the possible relationship between the five polymorphisms of the VDR gene and breast cancer risk. The general characteristics of the selected studies are summarized in Table 1. More detailed information is shown in Tables S1, S2, S3, S4, S5. The pooled results are shown in Table 2.

Meta-analysis
Fok1 polymorphism and breast cancer risk. Sixteen of 22 studies were in accordance with STREGA criteria and were therefore defined as high-quality studies [11–16,18,19,21,22]. The meta-analysis of these studies showed a significant effect of the ff genotype on risk of breast cancer (ff vs. Ff+FF OR: 1.09, 95%CI: 1.02 to 1.16, p = 0.007; I$^2$ = 18%, p$H$ = 0.24) (Figure 2). No significant associations were found for the other comparisons (ff vs. FF OR: 1.10, 95% CI: 1.00 to 1.20, p = 0.06; fF+FF vs. FF OR: 1.03, 95% CI: 0.97 to 1.01, p = 0.33; fF vs. F OR: 1.04, 95% CI: 0.99 to 1.09, p = 0.12 (Figures S1, S2, S3). No positive results were detected by pooling the data from all 22 studies.

Poly-A variant and breast cancer risk. Four of the seven studies complied with the STREGA criteria [22,25,27]. No significant association was detected between the poly-A variant and breast cancer by pooling the results of all studies or only high-quality studies.

Bsm1, Tag1 and Apa1 polymorphism and breast cancer risk. For all studies reviewed, the pooled results did not illustrate any significant correlation between Bsm1, Tag1 or Apa1 polymorphisms and breast cancer risk. Twelve high-quality studies did not show a significant association between the Bsm1 polymorphism and breast cancer risk [11,13,15–17,19,21,24]. As less than three studies complied with the STREGA criteria for the Tag1 and Apa1
polymorphisms, and therefore meta-analyses were not performed by pooling the results of high-quality studies alone.

Meta-regression
To detect the origin of study heterogeneity, the random effects meta-regression method was used [45]. In the regression procedure, an independent variable, the logarithm OR, and two covariates, ethnicity and sample size was included. The results of all the meta-regressions showed that the two covariates were not the origin of the heterogeneity.

Sensitivity Analysis and Publication Bias Evaluation
A sensitivity analysis was performed by removing the individual studies sequentially to assess the effect of individual studies. The results detected did not differ from the initial analysis. Begger’s linear regression showed that no publication bias existed in relationship to any variation (P > 0.05). The funnel plot for the recessive model of Fok1 polymorphism was symmetrical (Figure 3). The other funnel plots were not shown.

Discussion
Vitamin D regulates a variety of independent biological processes including bone metabolism, the innate immune response, cell proliferation and cell differentiation [46,47]. Several studies have suggested that adequate vitamin D levels may provide protection against chronic diseases, such as cancers, and could improve cancer prognosis [48]. The important roles that VDR polymorphisms play in the pathogenesis of breast cancer have been investigated across the world. Many studies have been carried out to investigate the relationship between VDR gene polymorphisms and the risk of breast cancer [9–39]. As a result of the limitations of sample sizes and the low statistical power of individual studies, research results have been conflicting and inconsistent. A previous meta-analysis involved only four SNPs (Fok1, Bsm1, Taq1 and Apa1) and contained relevant studies that were published before October 2008 [49]. Another review published in 2009 summarized the association between VDR polymorphisms and breast cancer risk, but no definitive quantitative results were obtained [50]. Our current meta-analysis included almost all studies that had investigated the associations between Fok1, poly-A, Bsm1, Taq1 and Apa1 polymorphisms and breast cancer risk. This is the most comprehensive meta-analysis to data, to the best of our knowledge.

This meta-analysis included data from 39 relevant studies. The meta-analysis of high-quality studies showed that individuals with homozygous ff genotype were responsive to the increased risk of breast cancer compared to patients with Ff or FF genotypes. The overall data from all genetic models did not demonstrate that there was a significant association between the poly-A repeat, Bsm1, Taq1 and Apa1 polymorphisms and breast cancer risk. A sensitivity analysis was performed by removing the individual studies sequentially, and the overall genetic effects were consistent with those of the corresponding sensitivity analyses for the poly-A,
Table 1. Characteristics of studies included in the meta-analysis of the relation between the Fok1, Poly A, Bsm1, Taq1 and Apa1 polymorphisms in the vitamin D receptor gene and breast cancer.

| Author[Ref]          | Year | Country | Racial descent | Breast cancer | Control | Genotyping method | SNPs               |
|----------------------|------|---------|----------------|--------------|---------|-------------------|--------------------|
| Ruggiero et al. [28] | 1998 | Italy   | European       | 88           | 167     | PCR-RFLP         | Bsm1               |
| Curran et al. [9]    | 1999 | Australia | European       | 135          | 110     | PCR-RFLP         | Fok1, Taq1, Apa1   |
| Dunning et al. [34]  | 1999 | UK      | European       | 211          | 268     | PCR-RFLP         | Taq1               |
| Dunning et al. [34]  | 1999 | UK      | European       | 740          | 359     | PCR-RFLP         | Taq1               |
| Lundin et al. [35]   | 1999 | Sweden  | European       | 111          | 130     | PCR-RFLP         | Taq1               |
| Ingel et al. [10]    | 2000 | America | European       | 143          | 300     | TaqMan            | Fok1, poly A, Bsm1 |
| Cui et al. [8]       | 2001 | China   | Asian          | 86           | 134     | PCR-RFLP         | Taq1, Apa1         |
| Hou et al. [29]      | 2002 | Taiwan  | Asian          | 34           | 169     | PCR-RFLP         | Bsm1, Taq1, Apa1   |
| Buyu et al. [30]     | 2003 | Turkey  | European       | 78           | 27      | PCR-RFLP         | Taq1               |
| Guy et al. [22]      | 2004 | UK      | European       | 398          | 427     | PCR-RFLP         | Fok1, poly A       |
| Heffer et al. [31]   | 2004 | Germany | European       | 290          | 1699    | PCR-RFLP         | Bsm1               |
| Sillanpaa et al. [36]| 2004 | Finland | European       | 472          | 479     | PCR-RFLP         | Taq1, Apa1         |
| Chen et al. [11]     | 2005 | Turkey  | European       | 1234         | 1676    | TaqMan            | Fok1, Bsm1         |
| Lowe et al. [32]     | 2005 | UK      | European       | 179          | 179     | PCR-RFLP         | Bsm1               |
| Vandevorid et al. [33]| 2006 | America | Mixed          | 220          | 192     | PCR-RFLP         | Bsm1               |
| John et al. [12]     | 2007 | America | Mixed          | 764          | 865     | PCR-RFLP         | Fok1, Taq1         |
| McCullough et al. [13]| 2007| America | European       | 475          | 480     | TaqMan            | Fok1, Bsm1, Taq1, Apa1 |
| Trabert et al. [25]  | 2007 | America | European       | 1139         | 905     | PCR-RFLP         | poly A, Bsm1       |
| Trabert et al. [25]  | 2007 | America | European       | 441          | 417     | PCR-RFLP         | poly A, Bsm1       |
| Wedren et al. [27]   | 2007 | Sweden  | European       | 1801         | 1712    | TaqMan            | poly A             |
| Abbas et al. [14]    | 2008 | Germany | European       | 1408         | 2612    | PCR-RFLP         | Fok1, Taq1         |
| Sinotte et al. [15]  | 2008 | Canada  | European       | 255          | 463     | TaqMan            | Fok1, Bsm1         |
| Sinotte et al. [15]  | 2008 | Canada  | European       | 622          | 974     | TaqMan            | Fok1, Bsm1         |
| Chakraborty et al. [26]| 2009| India   | Asian          | 160          | 140     | PCR-RFLP         | poly A, Taq1, Apa1 |
| Mckay et al. [16]    | 2009 | Unknown | European       | 1677         | 2795    | TaqMan            | Fok1, Bsm1         |
| Mckay et al. [16]    | 2009 | Unknown | European       | 1598         | 1952    | TaqMan            | Fok1, Bsm1         |
| Mckay et al. [16]    | 2009 | America | European       | 1073         | 1108    | TaqMan            | Fok1, Bsm1         |
| Mckay et al. [16]    | 2009 | America | European       | 685          | 683     | TaqMan            | Fok1, Bsm1         |
| Mckay et al. [16]    | 2009 | America | European       | 499          | 504     | TaqMan            | Fok1, Bsm1         |
| Mckay et al. [16]    | 2009 | America | European       | 1257         | 1748    | TaqMan            | Fok1, Bsm1         |
| Li et al. [23]       | 2010 | China   | Asian          | 81           | 78      | PCR-RFLP         | Fok1               |
| Anderson et al. [17] | 2011 | Canada  | European       | 1560         | 1633    | PCR-RFLP         | Fok1, Bsm1, Taq1, Apa1 |
| Dalessandri et al. [36]| 2012| Canada  | European       | 164          | 174     | PCR-RFLP         | Apa1               |
| Liu et al. [37]      | 2011 | China   | Asian          | 80           | 80      | PCR-RFLP         | Taq1               |
Table 1. Cont.

| Author/Ref       | Year | Country  | Race          | Breast cancer | Control    | Genotyping method | SNPs   |
|------------------|------|----------|---------------|---------------|------------|-------------------|--------|
| Engel et al. [18]| 2012 | America  | European      | 293           | 586        | PCR-RFLP          | Fok1, Taq1, Apa1 |
| Huang et al. [40]| 2012 | China    | Asian         | 146           | 320        | TaqMan            | Apa1   |
| Rollison et al. [19] | 2012 | America  | European      | 1740          | 2051       | PCR-RFLP          | Fok1, PolyA, Bsm1 |
| Fuhrman et al. [21]| 2013 | America  | European      | 477           | 842        | TaqMan            | Fok1, Bsm1    |
| Mirash et al. [24]| 2013 | America  | Mixed         | 252           | 389        | PCR-RFLP          | Fok1, Bsm1, Taq1, Apa1 |
| Shahabazi et al. [20]| 2013 | Iran     | Asian         | 140           | 156        | PCR-RFLP          | Fok1, Bsm1    |

PCR-RFLP: Polymerase chain restriction fragment length polymorphism.

The poly-A repeat in the 3′-untranslated region of the VDR gene which is strongly linked with Bsm1, Taq1 and Apa1 has an important impact on VDR mRNA stability [52]. Several studies have indicated that LL genotype (long/long) confers susceptibility to breast cancer risk compared with the SS genotype [10,22,26]. Chakraborthy et al. revealed that the LL genotype is significantly associated with high-grade breast cancer in northern Indians [(unadjusted OR [95% CI]: 4.45[1.87, 10.63]; adjusted OR [95% CI]: 4.66 [1.88, 11.53]) [26]. However, this result conflicted with the report from Ingles et al., where breast cancer risk was found to increase with increasing numbers of A alleles [10]. Our finding did not show any significant association between the poly-A variation and breast cancer risk in any genetic model. These inconsistent results might result from differences of ethnicity, sample size, study design, amongst other factors.

The Bsm1 polymorphism is located at the 3′ end of the VDR gene. It does not appear to change the nature of the translated VDR protein [53]. However, this polymorphism is linked in a haplotype with the variable-length poly A sequence within the 3′-untranslated region, which affects the VDR mRNA stability [54]. On the other hand, the Bsm1, Taq1 and Apa1 polymorphisms are all in the same linkage disequilibrium block. These polymorphisms have been widely investigated, but with differing results. Consistent with a previous meta-analysis, our finding showed no significant association of these three genetic variations with breast cancer risk. Several studies have been performed to examine the VDR haplotypes [9,13,14,18,35], but these results were also conflicting. McCullough et al. has investigated haplotypes that involved Bsm1(B/b), Apa1(A/a), Taq1(T/t) and a poly-A repeat(S/L), but they failed to find significant association between any haplotype and breast cancer risk [13]. However, in a Caucasian population the baTL has been reported to increase the risk of breast cancer [9,35]. It is unclear whether chance or underlying differences in populations led to these inconsistencies. Due to the limited information available about these polymorphisms, we could not conduct an analysis for linkage disequilibrium and haplotypes.

A few studies have investigated the association of VDR polymorphisms with breast cancer survival, but their results were also inconsistency. An analysis conducted among 111 Swedish breast cancer patients younger than 37 years of age found a trend

Bsm1, Taq1 and Apa1 variants. These findings further indicated the robustness of the lack of association between these four polymorphisms and breast cancer risk.
Table 2. The pooled measures on the relation of Fok1, Poly A, Bsm1, Taq1 and Apa1 polymorphisms with breast cancer.

| VDR Polymorphism | Studies                  | Comparisons       | Numbers of cases/controls | Pooled OR (95% CI) | P    | I²    | P_h  |
|------------------|--------------------------|-------------------|---------------------------|--------------------|------|-------|------|
|                  |                          |                   |                           |                    |      |       |      |
| Fok1             | ALL relevant studies     | ff vs. FF         | 16353/21881               | 1.06 (0.95–1.17)   | 0.30 | 57%   | 0.0005 |
|                  |                          | ff+Ff vs. FF      | 16353/21881               | 1.03 (0.97–1.09)   | 0.34 | 35%   | 0.06  |
|                  |                          | ff vs. Ff+FF      | 16353/21881               | 1.04 (0.96–1.14)   | 0.34 | 50%   | 0.004 |
|                  |                          | f vs. F           | 16353/21881               | 1.03 (0.98–1.08)   | 0.29 | 29%   | 0.01  |
|                  | Studies with high-quality| ff vs. FF         | 14076/19267               | 1.10 (1.00–1.21)   | 0.06 | 45%   | 0.03  |
|                  |                          | f vs. F           | 14076/19267               | 1.04 (0.99–1.09)   | 0.12 | 52%   | 0.009 |
| Poly-A           | ALL relevant studies     | SS vs. LL         | 5493/5566                 | 0.99 (0.77–1.29)   | 0.96 | 74%   | 0.0009 |
|                  |                          | SS+SL vs. LL      | 5493/5566                 | 0.99 (0.83–1.20)   | 0.96 | 76%   | 0.0003 |
|                  |                          | S vs. L           | 5493/5566                 | 1.00 (0.85–1.18)   | 0.98 | 77%   | 0.0005 |
|                  | Studies with high-quality| SS vs. LL         | 3474/3089                 | 0.94 (0.71–1.25)   | 0.69 | 67%   | 0.03  |
|                  |                          | SS+SL vs. LL      | 3474/3089                 | 0.95 (0.80–1.14)   | 0.60 | 64%   | 0.04  |
|                  |                          | S vs. L           | 3474/3089                 | 0.98 (0.78–1.23)   | 0.84 | 59%   | 0.006 |
|                  | Bsm1                     | bb vs. BB         | 16160/21203               | 1.07 (0.97–1.17)   | 0.18 | 44%   | 0.01  |
|                  |                          | bb+Bb vs. BB      | 16160/21203               | 1.03 (0.94–1.13)   | 0.49 | 54%   | 0.0007 |
|                  |                          | b vs. B           | 16160/21203               | 1.05 (0.97–1.14)   | 0.21 | 66%   | <0.0001 |
|                  | Studies with high-quality| bb vs. BB         | 11594/14404               | 1.03 (0.93–1.14)   | 0.53 | 41%   | 0.06  |
|                  |                          | bb+Bb vs. BB      | 11594/14404               | 1.03 (0.94–1.14)   | 0.50 | 49%   | 0.02  |
|                  | Taq1                     | tt vs. TT         | 6940/8267                 | 1.02 (0.92–1.13)   | 0.66 | 62%   | 0.05  |
|                  |                          | tt+Tt vs. TT      | 6940/8267                 | 1.03 (0.92–1.15)   | 0.61 | 47%   | 0.02  |
|                  |                          | tt vs. Tt+TT      | 6940/8267                 | 0.98 (0.90–1.08)   | 0.94 | 49%   | 0.02  |
|                  |                          | t vs. T           | 6940/8267                 | 1.00 (0.92–1.08)   | 0.94 | 49%   | 0.02  |
|                  | Apa1                     | aa vs. AA         | 3738/4489                 | 0.99 (0.87–1.13)   | 0.89 | 15%   | 0.31  |
|                  |                          | aa+Ax vs. AA      | 3738/4489                 | 0.98 (0.82–1.17)   | 0.82 | 61%   | 0.004 |
|                  |                          | aa vs. Aa+AA      | 3738/4489                 | 1.00 (0.90–1.22)   | 0.99 | 0%    | 0.56  |
|                  |                          | a vs. A           | 3738/4489                 | 0.95 (0.82–1.10)   | 0.52 | 75%   | <0.0001 |

HWE: Hardy Weinberg Equilibrium,

P_h for heterogeneity, heterogeneity was checked by the chi square based Q test.
The symbol *shows the positive result.
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towards a higher survival rate, especially among those estrogen receptor-positive tamoxifen-treated patients that were homozygous for the rare Taq1 allele [35]. However, Perna et al. reported that homozygous carriers of the rare Taq1 homozygous genotype had a 2.8-fold increase in the probability of death from breast cancer compared to homozygous carriers with the common allele (OR: 2.8, 95% CI: 1.1–7.2) [55].

Our meta-analysis illustrates strong evidence for the association between a VDR gene polymorphism in Fok1 and an increased risk of breast cancer. The obvious evidence of between-study heterogeneity in this meta-analysis should be discussed. Although a meta-regression procedure that included two covariates was performed, the origin of the heterogeneity among the studies was not found. The heterogeneity might have been due to other factors, such as diversity in the population characteristics (ethnicity, age, sun exposure and dietary vitamin D intake, etc.), genotyping methods and study design. Previous studies have shown that the ethnic (genetic) background, gene-gene or gene-environment interactions and life-style (sun exposure, dietary vitamin D intake and smoking) might play a major role in the increased risk of breast cancer in association with genetic variations. Our meta-analysis was based on estimates without adjusting the data for these factors, which is another potential limitation of this study.

In conclusion, this comprehensive meta-analysis of high-quality studies provides substantial evidence that the Fok1 polymorphism
in the VDR gene is significantly associated with an increased risk of developing breast cancer. Furthermore, individuals that were homozygous for the minor allele genotype of FokI were more likely to develop breast cancer. No correlations were found between the poly-A variation, BsmI, TaqI and ApaI polymorphisms in the VDR gene and the risk of breast cancer in this study.

Supporting Information

Figure S1 Forest plots of association of FokI polymorphism with breast cancer (ff vs. FF).

Figure S2 Forest plots of association of FokI polymorphism with breast cancer (ff+ff’ vs. FF).

Figure S3 Forest plots of association of FokI polymorphism with breast cancer (f’ vs. F).

Table S1 Characteristics of studies included in this meta-analysis between the FokI polymorphism in the vitamin D receptor gene and breast cancer.

| Study                              | Country | Sample Size | Genotype | Odds Ratio (95% CI) |
|------------------------------------|---------|-------------|----------|--------------------|
| Study 1                            | Country | Sample Size | Genotype | Odds Ratio (95% CI) |
| Study 2                            | Country | Sample Size | Genotype | Odds Ratio (95% CI) |

Table S2 Characteristics of studies included in this meta-analysis between the poly-A polymorphism in the vitamin D receptor gene and breast cancer.

| Study                              | Country | Sample Size | Genotype | Odds Ratio (95% CI) |
|------------------------------------|---------|-------------|----------|--------------------|
| Study 1                            | Country | Sample Size | Genotype | Odds Ratio (95% CI) |
| Study 2                            | Country | Sample Size | Genotype | Odds Ratio (95% CI) |

Table S3 Characteristics of studies included in this meta-analysis between the BsmI polymorphism in the vitamin D receptor gene and breast cancer.

| Study                              | Country | Sample Size | Genotype | Odds Ratio (95% CI) |
|------------------------------------|---------|-------------|----------|--------------------|
| Study 1                            | Country | Sample Size | Genotype | Odds Ratio (95% CI) |
| Study 2                            | Country | Sample Size | Genotype | Odds Ratio (95% CI) |

Table S4 Characteristics of studies included in this meta-analysis between the TaqI polymorphism in the vitamin D receptor gene and breast cancer.

| Study                              | Country | Sample Size | Genotype | Odds Ratio (95% CI) |
|------------------------------------|---------|-------------|----------|--------------------|
| Study 1                            | Country | Sample Size | Genotype | Odds Ratio (95% CI) |
| Study 2                            | Country | Sample Size | Genotype | Odds Ratio (95% CI) |

Table S5 Characteristics of studies included in this meta-analysis between the ApaI polymorphism in the vitamin D receptor gene and breast cancer.

| Study                              | Country | Sample Size | Genotype | Odds Ratio (95% CI) |
|------------------------------------|---------|-------------|----------|--------------------|
| Study 1                            | Country | Sample Size | Genotype | Odds Ratio (95% CI) |
| Study 2                            | Country | Sample Size | Genotype | Odds Ratio (95% CI) |

Appendix S1 PRISMA Checklist.

Author Contributions

Conceived and designed the experiments: KZ LS. Performed the experiments: KZ. Analyzed the data: KZ LS. Contributed reagents/materials/analysis tools: KZ LS. Wrote the paper: KZ LS.

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