RESEARCH ARTICLE

# Association Between VDR Polymorphisms and Breast Cancer: An Updated and Comparative Meta-analysis of Crude and Adjusted Odd Ratios

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## Abstract

**There is a lot of debate on the relationship between vitamin D receptor polymorphisms and risk of breast cancer. Herein, we quantitatively analyzed the published case-control studies on this relationship by meta-analysis, performing a bibliographic search from Pubmed and CNKI up to July 31, 2013. The included case-control studies for Fok1, Bsm1, Taq1, Apa1, Cdx2 and Poly-A were 16, 19, 20, 10, 4, 6, respectively. Crude and adjusted odd ratios and 95% confidence intervals were calculated to present and compare the strength of any associations. The results of combined analyses indicated that Fok1, Bsm1, Apa1, Cdx2 and Poly-A were not significantly associated with the risk of breast cancer. In contrast, the tt genotype of Taq1 was a modest risk factor for breast cancer development (tt vs. TT: OR = 1.21, 95% CI: 1.01-1.44). To further confirm the above results, adjusted effects for the six polymorphisms were pooled based on adjusted ORs reported in the original studies. Adjusted ORs of Fok1, Apa1, Cdx2 and Poly-A were similar to the crude ORs. However, Bsm1 and Taq1 showed inconsistent results. For Bsm1, OR for BB vs. bb was 0.85, 95% CI: 0.74-0.98; for Taq1, OR for tt vs. TT was 1.03, 95% CI: 0.92-1.15, and not associated with risk. Subgroup analyses for crude ORs showed some association between Bsm1, Taq1 and breast cancer in Caucasians only, but for adjusted ORs, no associations were found. This meta-analysis suggests that the roles that Fok1, Apa1, Cdx2 and Poly-A polymorphisms play in breast cancer risk are negligible, with Bsm1 and Taq1 as possible exceptions. To be conservative, we still assumed that they may play a modest role in determining breast cancer risk. Further studies are needed to validate our findings.**

**Keywords:** Vitamin D receptor - polymorphism - breast cancer - risk - meta-analysis

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## Introduction

Vitamin D receptor (VDR) is a nuclear transcriptional factor which is expressed in most normal and cancer cells. It participates in a wide variety of biological process including bone metabolism, immune response modulation, and regulation of cell proliferation and differentiation in its active form of vitamin D (1,25(OH)D3), and all these collectively play an important role in the carcinogenesis of cancer (McCullough et al., 2009). Previous studies have demonstrated that VDR expression was decreased in breast cancer cell (Lopes et al., 2010), and the expression and/or function of the VDR protein is influenced by the polymorphism in the VDR gene (Tang et al., 2009).

The human VDR gene, located on chromosome 12q13, includes more than 470 single-nucleotide polymorphisms (SNPs) (McCullough et al., 2009). Among them, the following six were intensively studied: Fok1 (rs2228570), Bsm1 (rs1544410), Taq1 (rs731236), Apa1 (rs7975232), Cdx2 (rs11568820) and Poly A (rs17878969). Currently, there are still a lot of debates on the relationship between VDR polymorphism and the risk of breast cancer development. Case-control studies on Fok1 in breast cancer showed some evidence of increased risk among ff carriers (Sinotte et al., 2008; Gapska et al., 2009; McKay et al., 2009), which was confirmed by some later meta-analysis (McCullough et al., 2009; Tang et al., 2009; Wang et al., 2013). However, these studies also reported some decreased risk among ff carriers(Anderson et al., 2011), or no association with breast cancer (Curran et al., 1999; Guy et al., 2004; John et al., 2007; Abbas et al., 2008; Engel et al., 2012; Rollison et al., 2012; Fuhrman et al., 2013; Mishra et al., 2013; Shahbazi et al., 2013). Similarly, mixed results have been observed concerning the relationship between other polymorphisms and the risk of breast cancer development. For example, Bsm1 was reported to be associated with breast cancer in some studies (Guy et al., 2004; Lowe et al., 2005; Fuhrman et al.,

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2013; Shahbazi et al., 2013) but not in others (Buyru et al., 2003; Hefler et al., 2004; VandeVord et al., 2006; Trabert et al., 2007; Sinotte et al., 2008; Gapska et al., 2009; McKay et al., 2009; Anderson et al., 2011; Rollison et al., 2012; Mishra et al., 2013); Apa1 was found with positive relationship in some breast cancer studies (Curran et al., 1999; Sillanpaa et al., 2004; Dalessandri et al., 2012), but negative in others (Cui et al., 2001; Hou et al., 2002; Chakraborty et al., 2009; Anderson et al., 2011; Engel et al., 2012; Mishra et al., 2013); the same as Taq1, with associations in some studies (Cui et al., 2001; Wang et al., 2013), but not in others (Curran et al., 1999; Dunning et al., 1999; Lundin et al., 1999; Hou et al., 2002; Newcomb et al., 2002; Buyru et al., 2003; Sillanpaa et al., 2004; John et al., 2007; Abbas et al., 2008; Chakraborty et al., 2009; Gapska et al., 2009; Anderson et al., 2011; Engel et al., 2012; Mishra et al., 2013); Cdx2, positive in some studies (Anderson et al., 2011; Yao et al., 2012; Huang et al., 2013) but not in others (Abbas et al., 2008; Zhou et al., 2013); and Poly-A, some found positive relationship (Ingles et al., 2000; Guy et al., 2004; Chakraborty et al., 2009) and others found negative (Trabert et al., 2007; Wedren et al., 2007; Rollison et al., 2012; Huang et al., 2013).

Given the small number of related case-control studies and their inconsistency, we aimed to perform a comparative meta-analysis to obtain a more prudential estimate to strengthen the postulated genetic association between VDR polymorphisms and breast cancer development. We pooled and calculated the crude and adjusted odd ratios to compare their different effects. We also quantify and explain the heterogeneity between studies and investigate the existence of potential bias.

## Materials and Methods

*Study Selection*

We focused on six well-characterized polymorphisms of VDR: Bsm1, Fok1, Taq1, Apa1, Cdx2, and Poly-A. Studies were included if they met the following criteria: 1) evaluation of the above variants of VDR and the risk of breast cancer, 2) the use of the methodology of a case–control study, 3) studies that provided the frequencies of the variants in the cases and controls or provided sufficient data to calculate the estimate risk for the variants, 4) the confirmed histopathological diagnosis of breast cancer patients. 5) If overlapping populations were identified between studies, only the latest one was included. 6) A study including two case-control groups (this was considered as two studies in the research).

*Literature Search Strategy*

In literature search, we retrieved the articles using the keywords "vitamin D receptor or VDR", "polymorphisms" and "breast cancer" from PubMed and Chinese National Knowledge Infrastructure (CNKI) databases (Q. Huang and Y. Liao, last search update: July 31, 2013). The languages were limited to English and Chinese. Reference lists were manually examined to further identify potentially relevant studies. We contacted the corresponding authors by e-mail when there was uncertainty about the genotyping or when we could not get the full text. If there was no reply or the author refused to provide the data required, the study was excluded. All studies matching the inclusion criteria were retrieved for further examination and data extraction. All of the investigators have received training in literature search, statistics and evidence-based medicine.

*Quality assessment*

The quality of all studies was assessed using the Newcastle-Ottawa Quality Assessment Scales for case-control studies (Wells et al., 2011). In brief, the scores of the scale were based on areas related to the selection of subjects, comparability of groups and reliability of outcomes (exposures). Those areas were accessed by a total of 9 categories with a star awarded for the qualified study in each category. We regarded rating > 5 stars as high-quality studies, 3-4 stars as medium quality, and < 3 stars as low quality. The study was removed if it was rated less than 3 stars.

*Data extraction*

Two investigators (QH and YL) independently extracted the data and reached consensus on all items. From each report, the following data were extracted: the last name of the first author, publication year, country in which the study was performed, ethnicity, the source of controls, genotyping method, sample size, SNPs, genotypes distribution, adjusted odd ratio (OR) and 95% confidence level (95% CI) if presented and level of adjustment. Detailed information is shown in Table S1-S6. To stay consistent with previous literature, five VDR SNPs are reported here using restriction fragment length polymorphism (RFLP) nomenclature (See Table S7) (Shab-Bidar et al., 2011). The other Poly-A polymorphism is named L/S, which is based on 17A's (Long (L) ≥ 17A's; short (S) < 17A's) (Huang et al., 2013).

*Statistical analysis*

For each study, Hardy-Weinberg equilibrium (HWE) was evaluated by the Chi-square test in control. Crude ORs and 95% CIs were calculated to assess the strength of the association between VDR polymorphism and susceptibility to breast cancer. Pooled ORs were calculated for allele frequency comparison (e.g., Bsm1: B vs. b), homozygote comparison (e.g., Bsm1: BB vs. bb), dominant model (e.g., Bsm1: BB vs. Bb + bb), and recessive model (e.g., Bsm1: bb vs. Bb + BB), respectively. In addition, to better understand the relationship between the variants and breast cancer, we stratified data which had reported adjusted OR (95% CI) of a genetic comparison (e.g., Bsm1: BB vs. bb) with confounders adjustment, and pooled out adjusted OR (95% CI) to compare with the crude one. Moreover, subgroup analyses were conducted if more than three primary studies reported certain ethnicity (Caucasian, African-American, Hispanic, Asian and others).

All ORs were pooled by either fixed-effects model or random-effects model, depending on the overall heterogeneity among studies (fixed if $P > 0.1$, random if $P \leq 0.1$). Sensitivity analysis was carried out by deleting one single study each time to examine the influence of individual data set on the pooled ORs. Publication bias of literatures was assessed using funnel plots and Egger's

**Table 1. Characteristics of Studies Included in the Meta-analysis**

| NO. | Author | Year | Country | Ethnicity | Source of controls | Genotyping method | Case | Control | SNP | Adjusted or not |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Shahbazi | 2013 | Iran | Iranian | HB | PCR-RFLP | 140 | 156 | Bsm1, Fok1 | Yes |
| 2 | Mishra | 2013 | America | AA and HP | HB | PCR-RFLP | 232 | 349 | Fok1, Bsm1, Taq1, Apa1 | Yes |
| 3 | Fuhrman | 2013 | America | NR | PB | TaqMan | 484 | 845 | Bsm1, Fok1 | Yes |
| 4 | Dalessandri | 2012 | America | Caucasian | PB | microbead-based ASPE | 164 | 174 | Apa1 | No |
| 5 | Engel | 2012 | America | 98%white | PB | Mass Array and Pyrosequencing | 270 | 554 | Fok1, Apa1, Taq1 | Yes |
| 6 | Rollison | 2012 | America | HP and NHPW | PB | PCR-RFLP | 2318 | 2521 | Bsm1, Fok1, Poly-A | Yes |
| 7 | Yao | 2012 | America | AA<br>EA | PB | Illumina Golden Gate assay | 553<br>383 | 466<br>382 | Cdx2 | Yes |
| 8 | Huang | 2012 | China | Han Chinese | HB | PCR-RFLP | 146 | 320 | Bsm1, Apa1, Taq1 | Yes |
| 9 | Ye | 2012 | China | Chinese | HB | PCR-RFLP | 200 | 200 | Taq1 | No |
| 10 | Anderson | 2011 | America | Caucasian | PB | MassArray | 1560 | 1633 | Taq1, Bsm1, Fok1, Apa1, Cdx2 | Yes |
| 11 | Li | 2010 | China | Han Chinese | HB | PCR-RFLP | 81 | 78 | Fok1 | No |
| 12 | Chakraborty | 2009 | India | Hindus and Muslims | HB | PCR-RFLP | 160 | 140 | Apa1, Taq1, Poly-A | No |
| 13 | McKay | 2009 | USA | HP,AA,JA, Caucasian | PB | TaqMan | 6473<br>6355 | 8397<br>8149 | Fok1<br>Bsm1 | Yes |
| 14 | Gapska | 2008 | Poland | Polish | PB | PCR-TaqMan | 960<br>800 | 960<br>550 | Bsm1, Taq1, Fok1 | No |
| 15 | Sinotte | 2008 | Canada | NR | HB & PB<br>PB | allele-specific PCR | 255<br>622 | 463<br>974 | Fok1, Bsm1 | Yes |
| 16 | Abbas | 2008 | Germany | NR | PB | Pyrosequencing and PCR-RFLP | 1408 | 2612 | Taq1, Fok1, Cdx2 | Yes |
| 17 | Barroso | 2008 | Spain | Caucasian | HB | TaqMan | 549 | 556 | Fok1, Taq1 | Yes |
| 18 | John | 2007 | America | HP, AA, NHPW | PB | TaqMan | 814 | 910 | Fok1, Taq1 | No |
| 19 | Wedren | 2007 | Swedish | NR(resident) | PB | PCR-RFLP | 1502 | 1510 | Poly-A | Yes |
| 20 | Trabert | 2007 | America | Caucasian and AA | PB | PCR-RFLP | 1631 | 1435 | Bsm1, Poly-A | Yes |
| 21 | VandeVord | 2006 | America | white and AA | HP & PB | PCR-RFLP | 220 | 192 | Bsm1 | No |
| 22 | Lowe | 2005 | UK | Caucasian | HB | PCR-RFLP | 179 | 179 | Bsm I | Yes |
| 23 | Guy | 2004 | UK | Caucasian | HB | PCR-RFLP | 398 | 427 | Bsm1, Fok1, Poly-A | Yes |
| 24 | Hefler | 2004 | Germany | Caucasian | HB | MicroArray | 396 | 2090, | Bsm1 | No |
| 25 | Sillanpaa | 2004 | Finnish | Caucasian | HB | PCR-RFLP | 483 | 482 | ApaI, TaqI | Yes |
| 26 | Buyru | 2003 | Turkey | NR | NR | PCR-RFLP | 78 | 27 | BsmI, TaqI | No |
| 27 | Newcomb | 2002 | America | NR | PB | TaqMan | 420 | 405 | Taq1 | Yes |
| 28 | Hou | 2002 | Taiwan | NR | HB | PCR-RFLP | 34 | 215 | Apa1, Bsm1, Taq1 | No |
| 29 | Cui | 2001 | China | NR | HB | PCR-RFLP | 86 | 134 | Apa1, Taq1 | No |
| 30 | Ingles | 2000 | America | HP | PB | PCR-RFLP | 143 | 300 | Poly-A | Yes |
| 31 | Curran | 1999 | Australian | NR | HB | PCR-RFLP | 135 | 110 | Fok1, Apa1, Taq1 | No |
| 32 | Dunning | 1999 | UK | Caucasian | PB | PCR-RFLP | 288<br>672 | 288<br>384 | Taq1 | No |
| 33 | Lundin | 1999 | Swedish | NR | HB | PCR-RFLP | 111 | 130 | Taq1 | No |
| 34 | Ruggiero | 1998 | Italy | NR | HB | PCR-RFLP | 88 | 167 | Bsm1 | No |

SNP, Single nucleotide polymorphism; HB, Hospital based; PB, Population based; NR, Not reported; AA, African-American; EA, European-American; JA, Japanese-American; HP, Hispanic; NHPW, Non-Hispanic White

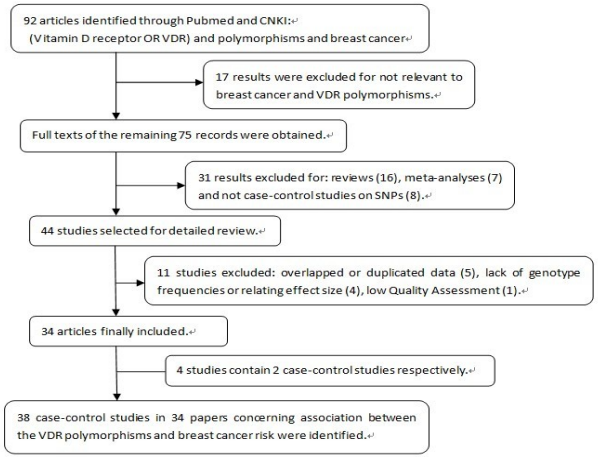


**Figure 1. Flow Diagram of Included/Excluded Studies**

test (significant at $P \leq 0.1$). Additionally, the trim-and-fill method was used to adjust the risk estimates when the tests for publication bias were statistically significant (Duval et al., 2000). All of the statistical tests were performed with STATA software version 10.0 (STATA Corporation, College Station, TX, USA).

## Results

*Characteristics of studies*

A total of 38 eligible case-control studies met the pre-specified inclusion criteria (See Figure 1), in which 16, 19, 20, 10, 4 and 6 studies were pooled for the analyses of the Fok1, Bsm1, Taq1, Apa1, Cdx2 and Poly-A, respectively (Table 1). Eighteen studies did not provide the adjusted OR; therefore, all of them were excluded. Finally, 11 studies on Fok1, 12 studies on Bsm1, 7 studies on Taq1, 5 studies on Apa1, 4 studies on Cdx2, and 5 on Poly-A were enrolled to take a secondary meta-analysis for adjusted ORs.

For the subgroup analyses, 8 studies did not provide the race-based data, and 4 studies were mixed population that cannot be divided into different races. Finally, 13 studies with Caucasian background, 4 studies with African-American, 3 with Hispanic and 5 with Asian background

**Table 2. Summary Crude and Adjusted OR (95%CI) for Various Contrasts in VDR Polymorphisms**

| SNP | Contrast | Crude OR (95%CI) | Model | *P* for egger's test | N (case/control) | Adjusted OR (95%CI) | Model | *P* for egger's test | N (case/control) |
|---|---|---|---|---|---|---|---|---|---|
| Fok1 | F vs. f | 0.98 (0.91-1.05) | random | 0.92 | | | | | |
| | ff vs. FF | 1.05 (0.91-1.22) | random | 0.97 | 16237/20909 | 1.01 (0.86-1.15) | random | 0.8 | 13521/18378 |
| | FF+Ff vs. ff | 0.98 (0.87-1.10) | random | 0.68 | | | | | |
| | Ff+ff vs. FF | 1.05 (0.96-1.14) | random | 0.59 | | | | | |
| Bsm1 | B vs. b | 0.97 (0.93-1.02) | fixed | 0.32 | | | | | |
| | BB vs. bb | 0.91 (0.82-1.01) | random | 0.35 | 16122/20645 | 0.85 (0.74-0.98)* | random | 0.02 | 13684/16899 |
| | BB+Bb vs. bb | 0.94 (0.86-1.02) | random | 0.22 | | | | | |
| | Bb+bb vs. BB | 1.04 (0.98-1.11) | fixed | 0.81 | | | | | |
| Taq1 | T vs. t | 0.96 (0.90-1.02) | fixed | 0.67 | | | | | |
| | tt vs. TT | 1.21 (1.01-1.44)* | random | 0.93 | 8681/10190 | 1.03 (0.92-1.15) | fixed | 0.77 | 4488/6310 |
| | TT+Tt vs. tt | 0.87 (0.80-0.95)* | fixed | 0.72 | | | | | |
| | Tt+tt vs. TT | 1.10 (0.97-1.26) | random | 0.96 | | | | | |
| Apa1 | A vs. a | 0.97 (0.89-1.07) | fixed | 0.21 | | | | | |
| | aa vs. AA | 1.03 (0.90-1.18) | fixed | 0.37 | 3246/4089 | 1.10 (0.80-1.40) | random | 0.44 | 2667/3321 |
| | AA+Aa vs. aa | 0.92 (0.82-1.03) | fixed | 0.2 | | | | | |
| | Aa+aa vs. AA | 1.02 (0.86-1.21) | random | 0.73 | | | | | |
| Cdx2 | G vs. A | 0.95 (0.86-1.06) | fixed | 0.53 | | | | | |
| | AA vs. GG | 1.22 (0.98-1.50) | fixed | 0.75 | 3841/5039 | 1.09 (0.83-1.35) | fixed | 0.93 | 3841/5039 |
| | GG+GA vs. AA | 0.83 (0.63-1.08) | random | 0.7 | | | | | |
| | GA+AA vs. GG | 1.03 (0.87-1.21) | random | 0.53 | | | | | |
| Poly-A | S vs. L | 0.99 (0.87-1.13) | random | 0.8 | | | | | |
| | SS vs. LL | 0.95 (0.73-1.25) | random | 0.65 | 5456/5653 | 0.93 (0.67-1.18) | random | 0.79 | 5296/5513 |
| | SS+SL vs. LL | 0.95 (0.78-1.17) | random | 0.46 | | | | | |
| | SL+LL vs. SS | 0.98 (0.88-1.09) | fixed | 0.71 | | | | | |

*Significance values; VDR, Vitamin D receptor; OR, odd ratios; 95%CI, 95% confidential interval

**Table 3. Comparison of Crude and Adjusted OR (95%CI) in Subgroup Analysis**

| Ethnicity | SNP | Contrast | Crude OR (95%CI) | Model | P for egger's test | N (case/control) | Adjusted OR (95%CI) | Model | P for egger's test | N (case/control) |
|---|---|---|---|---|---|---|---|---|---|---|
| Caucasian | Bsm1 | B vs. b | 0.90 (0.82-1.00) | random | 0.14 | | | | | |
| | | BB vs. bb | 0.83 (0.69-0.99)* | random | 0.21 | 11494/13939 | 0.85 (0.68-1.02) | random | 0.06 | 9388/10627 |
| | | BB+Bb vs. bb | 0.80 (0.67-0.95)* | random | 0.06 | | | | | |
| | | Bb+bb vs. BB | 1.06 (0.99-1.14) | fixed | 0.75 | | | | | |
| | Taq1 | T vs. t | 0.95 (0.89-1.02) | fixed | 0.43 | | | | | |
| | | tt vs. TT | 1.12 (1.01-1.25)* | fixed | 0.89 | 6176/6905 | 1.02 (0.90-1.14) | fixed | 0.89 | 3987/5268 |
| | | TT+Tt vs. tt | 0.92 (0.83-1.01) | fixed | 0.9 | | | | | |
| | | Tt+tt vs. TT | 1.06 (0.98-1.13) | fixed | 0.19 | | | | | |

*Significance values; VDR, Vitamin D receptor; OR, odd ratios; 95%CI, 95% confidential interval

were included to take the race subgroup analysis.

*Analyses for Fok1 polymorphisms and breast cancer risk*

We analyzed 16 case-control studies on the relationship of Fok1 polymorphism and breast cancer risk. Eleven of them reported adjusted ORs, which contain 83% and 88% (case/control) population size of the total (Table 2).

Results from neither the pooled crude OR nor the adjusted OR showed significant association between the genotypes ff vs. FF with breast cancer (Crude OR = 1.05, 95% CI: 0.91-1.22; Adjusted OR = 1.01, 95% CI: 0.86, 1.15). There was also no significant association in the allele contrast (OR = 0.98, 95% CI: 0.91-1.05), recessive (FF + Ff vs. ff, OR = 0.98, 95% CI: 0.87-1.10) and dominant models (Ff + ff vs. FF, OR = 1.05, 95% CI: 0.96-1.14).

All ethnic groups did not demonstrate a link between Fok1 and the risk of breast cancer (data not shown).

*Analyses for Bsm1 polymorphisms and breast cancer risk*

Twelve case-control studies reported adjusted ORs. Sample size was 15% and 18% less than the total cases and controls. According to the pooled adjusted OR, individuals carrying BB genotype had a decreased risk of breast cancer risk compared to those with the bb genotype (OR = 0.85, 95% CI: 0.74-0.98). However, results from crude ORs showed no significant association in all kind of contrasts (Table 2).

In subgroup analysis by race, we found a decreased risk of BB carriers in the Caucasian in pooled crude OR (OR = 0.83, 95% CI: 0.69-0.99), but not in the adjusted one (OR = 0.85, 95% CI: 0.68-1.02) (Table 3). B allele and recessive model (BB + Bb vs. bb) showed similar protective effect of B allele compared to b allele (OR = 0.90, 95% CI: 0.82-1.0) and bb genotype (OR = 0.80, 95% CI: 0.67-0.95). No association was found in African-American and Hispanic groups between Bsm1 and breast cancer.

Between-study heterogeneity for Bsm1 existed in both overall and subgroup analyses, random effect model was selected (Table 2-3).

*Analyses for Taq1 polymorphisms and breast cancer risk*

Seven studies out of 20 reported adjusted ORs, the proportion of adjusted population size were only 52% and 62% of the total (8681/10190). Significant genetic association was identified in comparisons of tt vs. TT and TT + Tt vs. tt when pooling the crude ORs ($OR_{tt\ vs.\ TT}$

= 1.21, 95% CI: 1.01-1.44; $OR_{TT + Tt vs. tt}$ = 0.87, 95% CI: 0.80-0.95). Nevertheless, the result from the adjusted ORs was inconsistent ($OR_{tt vs. TT}$ = 1.03, 95% CI: 0.92-1.15) (Table 2).

We performed subgroup analyses in Caucasians and Asians. In crude ORs, among 6176 Caucasian case and 6905 Caucasian control, tt genotype showed a moderate relationship with breast cancer compared to TT genotype (OR = 1.12, 95% CI: 1.01-1.25) (Table 3). Heterogeneity was eliminated, which indicated that studies in Asians were the main source of between-study heterogeneity. However, adjusted OR showed that the relationship between tt and breast cancer risk was null (Table 3).

*Analyses for Cdx2 polymorphisms and breast cancer risk*

The risk of breast cancer was the only variant in our study that all 4 previous relevant studies have presented adjusted OR. In the 3841 case and 5039 control, results from crude OR and adjusted OR was the same. No significant association with breast cancer risk was found (crude $OR_{AA vs. GG}$ = 1.22, 95% CI: 0.98-1.50; adjusted $OR_{AA vs. GG}$ = 1.09, 95% CI: 0.83-1.35) (Table 2).

*Analyses for Apa1 and Poly-A polymorphisms and breast cancer risk*

Five out of ten case-control studies (82% and 81% sample size to the whole population) reported adjusted OR. Results from crude ORs and adjusted ORs were consistent in aa vs. AA group (crude OR = 1.03, 95% CI: 0.90-1.18; adjusted OR = 1.10, 95% CI: 0.80-1.40) (Table 2). We did not find any significant association between the Apa1 variants and the risk of breast cancer in overall and subgroup analyses.

Five out of six studies (97% and 98% to total case and control size) presented adjusted OR. Compared to LL, SS play no association with the risk of breast cancer (crude OR = 0.95, 95% CI: 0.73-1.25; adjusted OR = 0.93, 95% CI: 0.67-1.18) (Table 2). No association was found in subgroup analyses as well (data not shown).

*Test for the sensitivity of analysis and publication bias*

When every study was omitted one at a time, the results of re-analyses for Bsm1, Fok1, Taq1, Apa1, Cdx2 and Poly-A polymorphisms were persistent, which indicated that the results of our meta-analysis were reliable (data not shown).

Funnel plot and Egger's test were performed to estimate the publication bias of literature. In the process of pooling crude OR, no publication bias was presented in all six polymorphisms ($P > 0.1$; Table 2). When pooling adjusted OR, only ORs adjusted from the original paper could be analyzed, and so we found published bias in Bsm1 ($P < 0.05$; Table 2). In this case, we use the trim-and-fill method to adjust its effect size.

## Discussion

In this meta-analysis, an association between the six common SNPs in VDR (Fok1, Bsm1, Taq1, Apa1, Cdx2, Poly-A) and the risk of breast cancer development was evaluated by the pooled results from 34 published studies. Results were consistent in the conclusion that Fok1, Apa1, Cdx2 and Poly-A polymorphisms had no relationship with the development of breast cancer. However, the evidence is not sufficiently robust to draw conclusions regarding whether the Bsm1 and Taq1 polymorphisms were associated with the risk of breast cancer, because the results from the pooled crude OR and the pooled adjusted OR at the variants was inconsistent.

The Fok1 polymorphism does not show any association with the risk of breast cancer. The pooled crude OR is consistent with the adjusted OR, and this makes our conclusion more robust. The subgroup analyses of Caucasian and Hispanic showed similar results. However, our result was not supported by previous meta-analyses, which considered ff genotype of Fok1 as a risk factor (Tang et al., 2009; Wang et al., 2013). The reasons of the difference described could be as followed: 1) we have a much bigger sample size. We updated 7 more studies compared to Tang et al. (Tang et al., 2009), and the total sample size was 16237 / 20909 (case/control) compare to Tang's (854 / 1096); 2) we have operated a more careful work. Wang et al. (Wang et al., 2013) have brought overlapping data from Guy et al. (Guy et al., 2004) and Bretherton-Watt et al. (Bretherton-Watt et al., 2001); Chan et al. (Chen et al., 2005), McCullough et al. (McCullough et al., 2007) and McKay et al. (McKay et al., 2009). In a word, these efforts make our results more convincing.

Bsm1 alleles, genotypes, recessive and dominant models did not show significant differences with the risk of breast cancer with pooling the crude OR. Previous meta-analyses pooled crude ORs and their results were similar to ours (Tang et al., 2009; Wang et al., 2013). But we found some differences using pooled adjusted ORs, which showed that BB may decrease the risk of breast cancer compared to bb genotype.

In the subgroup analyses, BB genotype exerted a moderate protective affect on breast cancer development in Caucasians, while heterogeneity existed. After adjusted for confounders, result showed no statistical relationship between BB and the risk of breast cancer. However, the heterogeneity cannot be eliminated. In these cases, we could not confirm whether or not Bsm1 polymorphism confers risk effect on the breast cancer development, other factors affected heterogeneity should be considered.

Taq1 showed a significant difference in tt vs. TT and TT + Tt vs. tt groups when pooling crude ORs. It seemed that tt genotype was a risk factor to breast cancer development, which was supported by Wang et al. (Wang et al., 2013). Interestingly, after 48% and 38% reduction of total case and control size, our analyses found heterogeneity disappeared and the tt genotype was no longer related to the risk of breast cancer.

Our results suggest that by grouping and pooling adjusted ORs, heterogeneity and publication bias might be eliminated. Nevertheless, due to the reduced sample size, we could not make a conclusion about the association of Taq1 polymorphism to the risk of breast cancer.

Cdx2 is the only one that all included studies reported adjusted OR, and results from crude OR and adjusted OR about AA vs. GG to breast cancer were consistent. Compared to previous studies, with the same included

data, Huang et al. (Huang et al., 2013)found the same results with ours but the results from Zhou et al (Zhou et al., 2013) didn't agree. The reason for the discrepancy could due to different *P* value to heterogeneity. When regarded $P < 0.5$ as heterogeneous, fixed effect model was selected, effect size went to be 0.81 (95% CI: 0.69-0.96). In this case, setting higher standard seems to be more rigorous (Lau et al., 1997).

Apa1 and Poly-A were the rest of SNPs for which we could not find any association with the risk of breast cancer. The results were consistent between groups with crude and adjusted ORs and consistent with previous meta-analyses (Tang et al., 2009; Huang et al., 2013; Wang et al., 2013).

There are some critical advantages of this meta-analysis. The comparatively low statistical power of a single study probably causes potential bias because of the limited number of participants. Like all meta-analysis, ours can get a more precise result by greatly increasing the statistical power based on all primary studies. In addition, the sample size of the primary studies was comparatively large, ranging from 159 to 14870, which might encounter less chance of bias compared with small-size studies. Therefore, our meta-analysis also encountered less chance of bias introduced from primary studies. Furthermore, genetic meta-analysis was always performed without adjustment due to limited data in primary studies. In this meta-analysis, besides quantitative analyses for all SNPs without adjustment, adjusted analyses were also performed for Fok1, Bsm1, Taq1, Apa1, Cdx2 and Poly-A polymorphisms. Compared to the crude analyses, the results from adjusted ones for Fok1, Apa1, Cdx2 and Poly-A were persistent. While the adjusted analyses found some differences in Bsm1 and Taq1, which made us hard to confirm their relationship with breast cancer. Finally, up to our knowledge, six VDR polymorphisms had been studied, the highest number compared to other published meta-analyses.

Similar to other studies, possible limitations of this meta-analysis should be considered when interpreting the results. Firstly, selection bias is a possible major source of heterogeneity resulting from nonsystemic and arbitrary acquisition of different background of controls. Secondly, in order to reduce heterogeneity, we made additional analyses on data which had reported adjusted odd ratio and pooled out an overall effect. To some extent, this is a gene-environment consideration, however, adjusted factors such as age, age at menarche, menopausal status, body mass index, hormone replacement treatment usage, family history, race, smoking etc. were different from original studies, which could bring bias in our study; Meanwhile, the relatively small sample size of studies may lead to reduced statistical power after this stratification. Lastly, the comparison between pool crude OR and adjusted OR was only limited in homozygote group, but not available in allele contrast or dominant and recessive models.

In summary, our study provides the evidence that Fok1, Apa1, Poly-A, Cdx2 were not associated to the risk of breast cancer in general and more specifically in the Caucasian population; Bsm1 and Taq1 could be potential modest factor affecting the risk of breast cancer, conservatively. Further studies are underway to clarify the results given in the current meta-analysis.

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