RESEARCH ARTICLE

# No Association of the TGF-β1 29T/C Polymorphism with Breast Cancer Risk in Caucasian and Asian Populations: Evidence from a Meta-Analysis Involving 55, 841 Subjects

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## Abstract

**The transforming growth factor-β1 (TGF-β1) gene 29 T/C polymorphism is thought to be associated with breast cancer risk. However, reports are largely conflicting and underpowered. We therefore conducted a meta-analysis of all available case-control studies relating the TGF-β1 29T/C polymorphism to the risk of developing breast cancer by including a total of 31 articles involving 24,021 cases and 31,820 controls. Pooled ORs were generated for the allele contrasts, with additive genetic, dominant genetic and recessive genetic models. Subgroup analysis was also performed by ethnicity for the TGF-β1 29T/C polymorphism. No association was found in the overall analysis (C *vs* T: OR=1.028, 95% CI=0.949-1.114, p-value 0.500; CC *vs* TC: OR= 1.022, 95% CI=0.963-1.085, p-value 0.478; CC *vs* TT: OR= 1.054, 95% CI=0.898-1.236, p-value 0.522; CC *vs* TT+ TC: OR= 1.031, 95% CI=0.946-1.124, p-value 0.482; TT *vs* CC+TC: OR= 0.945, 95% CI=0.827-1.080, p-value 0.403). Similarly, in the subgroup analysis by ethnicity, no association was found in Caucasian (C *vs* T: OR= 1.041, 95% CI=0.932-1.162, p-value 0.475; CC *vs* TC: OR= 1.031, 95% CI=0.951-1.118, p-value 0.464; CC *vs* TT: OR= 1.081, 95% CI=0.865-1.351, p-value 0.493; CC *vs* TT+TC: OR= 1.047, 95% CI=0.929-1.180, p-value 0.453; TT *vs* CC+TC: OR= 0.929, 95% CI=0.775-1.114, p-value 0.429;) and Asian populations (C *vs* T: OR= 1.004, 95% CI=0.908-1.111, p-value 0.931; CC *vs* TC: OR= 0.991, 95% CI=0.896-1.097, p-value 0.865; CC *vs* TT: OR= 1.015, 95% CI=0.848-1.214, p-value 0.871; CC *vs* TT+TC: OR= 1.000, 95% CI=0.909-1.101, p-value 0.994; TT *vs* CC+TC: OR= 0.967, 95% CI=0.808-1.159, p-value 0.720;). No evidence of publication bias was detected during the analysis. No significant association with breast cancer risk was demonstrated overall or on subgroup (Caucasian and Asian) analysis. It can be concluded that TGF-β1 29T/C polymorphism does not play a role in breast cancer susceptibility in overall or ethnicity-specific manner.**

**Keywords:** Meta-analysis -TGF-β1 29T/C -cancer - polymorphism - susceptibility

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## Introduction

Breast cancer is the most frequent carcinoma and globally the second leading cause of cancer-related death in women (Ferlay et al., 2010). A host of environmental and genetic factors act synergistically and predispose an individual to cancer (Lichtenstein et al., 2000). Transforming growth factor-b (TGF-β) critically influences the development and progression of breast cancer. It is a member of the transforming growth factor beta family and participates in a number of cell functions involved in cell growth, differentiation and survival (Derynck et al., 2001; Benson, 2004; Bierie and Moses, 2006; Massague, 2008).

TGF-β1 gene located on chromosome 19q13 (Fujii et al., 1986), harbors many SNPs (single nucleotide polymorphisms) that influence TGF β1 protein expression (Watanabe et al., 2002). A functional SNP at the 29th nucleotide in the coding region (exon 1, codon 10, rs1800470; rs1982073; T29C; Leu10Pro) induces a C to T change resulting in amino acid change from proline to leucine (P10L) (Watanabe et al., 2002).

An earlier study has shown that C allele of 29T/C is associated with increased TGF-β1 serum levels (Dunning et al., 2003). Further, elevated TGF-β in tissues and peripheral blood of breast cancer patient is associated with

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an advanced cancer, and a reduced disease-free survival (Shu et al., 2004; Desruisseau et al., 2006; Gonzalez-Zuloeta Ladd et al., 2007; Grau et al., 2008; Zheng, 2009). Thus it is speculated that 29T/C polymorphism may contribute to the development and severity of breast cancer.

A number of case-control studies have investigated the association between TGF-β1 29T/C polymorphism and breast cancer risk, but the results remain inconclusive, controversial and underpowered, attributable to the relatively small sample size previous studies. In order to produce a more precise estimation of the relationship between TGF-β1 polymorphism and breast cancer risk , we performed a meta-analysis of all available case-control studies relating the 29T/C polymorphisms of the TGF-β1 gene to the risk of developing breast cancer in Caucasian and Asian ethnic population.

## Materials and Methods

*Literature search and data extraction strategy*

We carried out a computerized literature search of the PubMed, Web of Science, EBSCO, and CGEMS database (prior to February 2014) covering all research articles published with a combination of the following key words: ''TGF-β1,'' ''polymorphism,'' and ''breast cancer.'' Reference lists of the retrieved articles were also screened for other relevant studies. When, more than one of the same population was included in several publications, only the most recent or complete study was included in this meta-analysis. Since, this is a meta-analysis of published articles based on the association of TGF β1 polymorphism and breast cancer risk, so ethical approval was not required for this study.

The methodological quality assessment and data extraction from each study was done by two reviewers independently adhering stringently to the inclusion-exclusion criteria mentioned below. Decisions of the both reviewers were compared and the cases associated with disagreement on any item of the data from the collected research studies were fully debated by involving a third reviewer to achieve a final consensus.

The main characteristics abstracted from the retrieved studies included: name of the first author, publication year, ethnicity of subjects, the number of cases and controls, study type, and distribution of alleles and genotypes in case and control groups.

*Inclusion and exclusion criteria*

Titles and abstracts of all citations and retrieved literatures were reviewed and in order to minimize heterogeneity and ease the appropriate interpretation of this study, following inclusion criteria were used for the literature selection in our meta-analysis: a) Cross-sectional, case-control or cohort design referring to the association between TGF-β1 29T/C polymorphism and breast cancer in females, b) recruited pathologically or histologically confirmed breast cancer patients and healthy controls, d) and published in English language. The major reasons for study exclusion were, overlapping of the data, case-only studies and review articles.

*Statistical analysis*

To examine the strength of association between TGF-β1 29T/C polymorphism and breast cancer risk, pooled ORs and their corresponding 95% CIs were estimated for each study. In the present meta-analysis, the association between 29T/C polymorphism and the risk of breast cancer was examined using allele compared, additive, recessive, and dominant genetic models. Subgroup analysis was also performed by ethnicity for 29T/C polymorphism defined as Caucasian and Asian. A chi-square-based Q-statistic test was performed to evaluate the between-study heterogeneity of the studies (Wu and Li, 1999).

If p-value < 0.05, the between-study heterogeneity was considered to be significant and the random-effects model was used to calculate the OR (DerSimonian and Laird, 1986). Otherwise, when the between study heterogeneity was not significant, then the data from single comparison was pooled using fixed effects model (Mantel and Haenszel, 1959). Additionally, $I^2$ statistics was employed to quantify inter-study variability where larger values suggested an increasing degree of heterogeneity (Higgins et al., 2003).

The departure of frequencies of TGF-β1 29T/C polymorphism from expectation under Hardy-Weinberg equilibrium (HWE) was calculated by chi-square test in the controls. Possible publication bias was tested by Funnel plot asymmetry using Egger's linear regression test.

The significance of the intercept was determined by the t-test considering p-value < 0.05 as representation of statistically significant publication bias (Egger et al., 1997).

The Comprehensive Meta-Analysis (CMA) Version 2 software program (Biostat, USA) was chosen and utilized to perform all statistical analysis involved in this study after a comparative examination of 'meta-analysis' softwares using url address http://www.meta-analysis.com/pages/comparisons.html.

## Results

*Characteristics of eligible studies*

A total of thirty-one research articles involving 24021 cases and 31820 controls were finally included in the present meta-analysis based on our selection (inclusion-exclusion) criteria (Ziv et al., 2001; Dunning et al., 2003; Hishida et al., 2003; Krippl et al., 2003; Quarmby et al., 2003; Jin et al., 2004; Le Marchand et al., 2004; Saha et al., 2004; Shu et al., 2004; Kaklamani et al., 2005; Lee et al., 2005; Shin et al., 2005; Feigelson et al., 2006; Scola et al., 2006; Cox et al., 2007a; 2007b; Gonullu et al., 2007; Rajkumar et al., 2008; Chen et al., 2011; Joshi et al., 2011; Pooja et al., 2013) (Figure 1). The major characteristics of selected studies are summarized in Table 1. Distribution of genotypes, minor allele frequency (MAF) and HWE in the controls and cases have been presented in Table 2.

*Association of TGF-β1 29T/C polymorphism and breast cancer susceptibility*

All pooled studies together resulted into 24021

controls and 31820 breast cancer cases. Overall pooled analysis did not suggest any correlation between 29T/C polymorphism and breast cancer risk. No association was found in overall analysis (C *vs* T: OR=1.028, 95% CI=0.949-1.114, p-value 0.500; CC *vs* TC: OR=1.022, 95% CI=0.963-1.085, p-value 0.478; CC *vs* TT: OR=1.054, 95% CI=0.898-1.236, p-value 0.522; CC *vs* TT+TC: OR= 1.031, 95% CI=0.946-1.124, p-value 0.482; TT *vs* CC+TC: OR= 0.945, 95% CI=0.827-1.080, p-value 0.403) (Figure 2A-E).

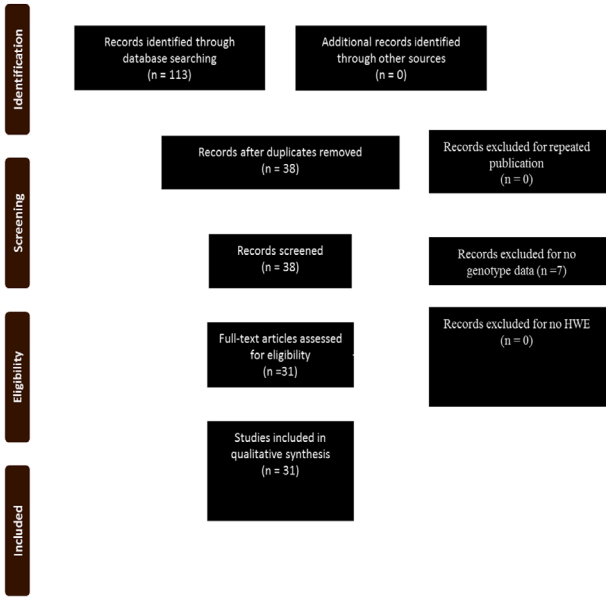


**Figure 1. Prisma Flow Diagram**

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

| Author and Year | Reference number | Ethnicity | Study design | Cases | Controls |
|---|---|---|---|---|---|
| Ziv et al., 2001 | [20] | Caucasian | - | 146 | 2929 |
| Hishida et al., 2003 | [21] | Asian | HB | 232 | 177 |
| Krippl et al., 2003 | [22] | Caucasian | PB | 495 | 499 |
| Quarmby et al., 2003 | [23] | Caucasian | - | 101 | 102 |
| Dunning et al., 2003 | [9] | Caucasian | PB | 2648 | 2902 |
| Jin et al., 2003 | [24] | Caucasian | - | 638 | 439 |
| Shu et al., 2004 | [13] | Asian | - | 180 | 931 |
| Le Marchand et al., 2004 | [25] | Mixed | PB | 1123 | 2314 |
| Saha et al., 2004 | [26] | Asian | - | 26 | 97 |
| Kaklamani et al., 2005 | [27] | Mixed | HB | 658 | 841 |
| Lee et al., 2005 | [28] | Asian | HB | 558 | 501 |
| Shin et al., 2005 | [29] | Asian | PB | 1114 | 1189 |
| Feigelson et al., 2006 | [30] | Caucasian | PB | 485 | 481 |
| Scola et al., 2006 | [31] | Caucasian | PB | 84 | 106 |
| GESBC, 2007 | [32] | Caucasian | PB | 556 | 713 |
| HBCS, 2007 | [32] | Caucasian | HB | 1073 | 1013 |
| IARC-Thai, 2007 | [32] | Asian | HB | 453 | 356 |
| Kuopio et al., 2007 | [32] | Caucasian | PB | 435 | 442 |
| Mayo clinic, 2007 | [32] | Caucasian | HB | 793 | 837 |
| PBCS, 2007 | [32] | Caucasian | PB | 1841 | 2254 |
| SEARCH, 2007 | [32] | Caucasian | PB | 4504 | 5689 |
| Seoul, 2007 | [32] | Asian | HB | 643 | 529 |
| SASBAC, 2007 | [32] | Caucasian | PB | 1303 | 1494 |
| CNIO, 2007 | [32] | Caucasian | HB | 640 | 739 |
| USRT, 2007 | [32] | Caucasian | HB | 705 | 1043 |
| Cox et al., 2007 | [33] | Caucasian | HB | 1185 | 1651 |
| Gonullu et al., 2007 | [34] | Caucasian | - | 38 | 24 |
| Rajkumar et al., 2008 | [35] | Asian | - | 250 | 500 |
| Chen et al., 2011 | [36] | Asian | HB | 447 | 406 |
| Joshi et al., 2011 | [37] | Asian | HB | 203 | 384 |
| Pooja et al., 2013 | [38] | Asian | HB | 464 | 238 |

**Table 2. Distribution of the TGFb1 Polymorphism of Thirty-One Studies Included in the Meta-Analysis**

| Author and Year | Cases | | | | Control | | | | HWEF | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Genotype | | | Minor allele | Genotype | | | Minor allele | | |
| | TT | TC | CC | MAF | TT | TC | CC | MAF | $x^2$ | p-value |
| Ziv et al., 2001 | 56 | 80 | 10 | 0.66 | 1068 | 1413 | 448 | 0.61 | 0.30 | 0.58 |
| Hishida et al., 2003 | 67 | 107 | 58 | 0.52 | 42 | 87 | 48 | 0.48 | 0.04 | 0.83 |
| Krippl et al., 2003 | 196 | 219 | 80 | 0.62 | 182 | 229 | 88 | 0.59 | 1.17 | 0.28 |
| Quarmby et al., 2003 | 44 | 48 | 9 | 0.67 | 54 | 41 | 7 | 0.73 | 0.04 | 0.84 |
| Dunning et al., 2003 | 470 | 1639 | 539 | 0.49 | 1169 | 1354 | 379 | 0.64 | 0.18 | 0.67 |
| Jin et al., 2003 | 270 | 282 | 86 | 0.64 | 189 | 196 | 54 | 0.65 | 0.08 | 0.77 |
| Shu et al., 2004 | 35 | 97 | 48 | 0.46 | 223 | 456 | 252 | 0.48 | 0.35 | 0.55 |
| Le Marchand et al., 2004 | 338 | 550 | 235 | 0.55 | 690 | 1103 | 521 | 0.54 | 4.00 | 0.05 |
| Saha et al., 2004 | 10 | 15 | 1 | 0.67 | 47 | 43 | 7 | 0.71 | 0.45 | 0.50 |
| Kaklamani et al., 2005 | 200 | 339 | 119 | 0.56 | 240 | 419 | 182 | 0.53 | 0.00 | 0.97 |
| Lee et al., 2005 | 135 | 288 | 135 | 0.50 | 148 | 235 | 118 | 0.53 | 1.71 | 0.19 |
| Shin et al., 2005 | 258 | 554 | 302 | 0.48 | 255 | 615 | 319 | 0.47 | 1.67 | 0.20 |
| Feigelson et al., 2006 | 182 | 233 | 70 | 0.62 | 181 | 221 | 79 | 0.61 | 0.69 | 0.41 |
| Scola et al., 2006 | 41 | 27 | 16 | 0.65 | 35 | 52 | 19 | 0.58 | 0.00 | 0.97 |
| GESBC, 2007 | 169 | 284 | 103 | 0.56 | 255 | 338 | 120 | 0.59 | 0.20 | 0.66 |
| HBCS, 2007 | 386 | 506 | 181 | 0.60 | 388 | 471 | 154 | 0.62 | 0.32 | 0.57 |
| IARC-Thai, 2007 | 189 | 213 | 51 | 0.65 | 161 | 162 | 33 | 0.68 | 0.73 | 0.39 |
| Kuopio et al., 2007 | 229 | 175 | 31 | 0.73 | 225 | 189 | 28 | 0.72 | 2.00 | 0.16 |
| Mayo clinic, 2007 | 296 | 404 | 93 | 0.63 | 307 | 409 | 121 | 0.61 | 0.66 | 0.42 |
| PBCS, 2007 | 617 | 890 | 334 | 0.58 | 797 | 1104 | 353 | 0.60 | 0.83 | 0.36 |
| SEARCH, 2007 | 1670 | 2138 | 696 | 0.61 | 2200 | 2716 | 773 | 0.63 | 2.04 | 0.15 |
| Seoul, 2007 | 162 | 327 | 154 | 0.51 | 155 | 253 | 121 | 0.53 | 0.83 | 0.36 |
| SASBAC, 2007 | 539 | 596 | 168 | 0.64 | 657 | 637 | 200 | 0.65 | 5.24 | 0.02* |
| CNIO, 2007 | 160 | 313 | 167 | 0.49 | 217 | 355 | 167 | 0.53 | 0.90 | 0.34 |
| USRT, 2007 | 243 | 339 | 123 | 0.59 | 382 | 494 | 167 | 0.60 | 0.12 | 0.73 |
| Cox et al., 2007 | 469 | 548 | 168 | 0.63 | 613 | 797 | 241 | 0.61 | 0.48 | 0.49 |
| Gonullu et al., 2007 | 20 | 10 | 8 | 0.66 | 11 | 9 | 4 | 0.65 | 0.78 | 0.38 |
| Rajkumar et al., 2008 | 80 | 126 | 44 | 0.57 | 190 | 234 | 76 | 0.61 | 0.08 | 0.78 |
| Chen et al., 2011 | 144 | 186 | 117 | 0.53 | 146 | 170 | 90 | 0.57 | 8.69 | 0.00* |
| Joshi et al., 2011 | 67 | 104 | 32 | 0.59 | 114 | 181 | 89 | 0.53 | 1.09 | 0.30 |
| Pooja et al., 2013 | 214 | 165 | 85 | 0.64 | 64 | 123 | 51 | 0.53 | 0.32 | 0.57 |

*Not consistent with HWE

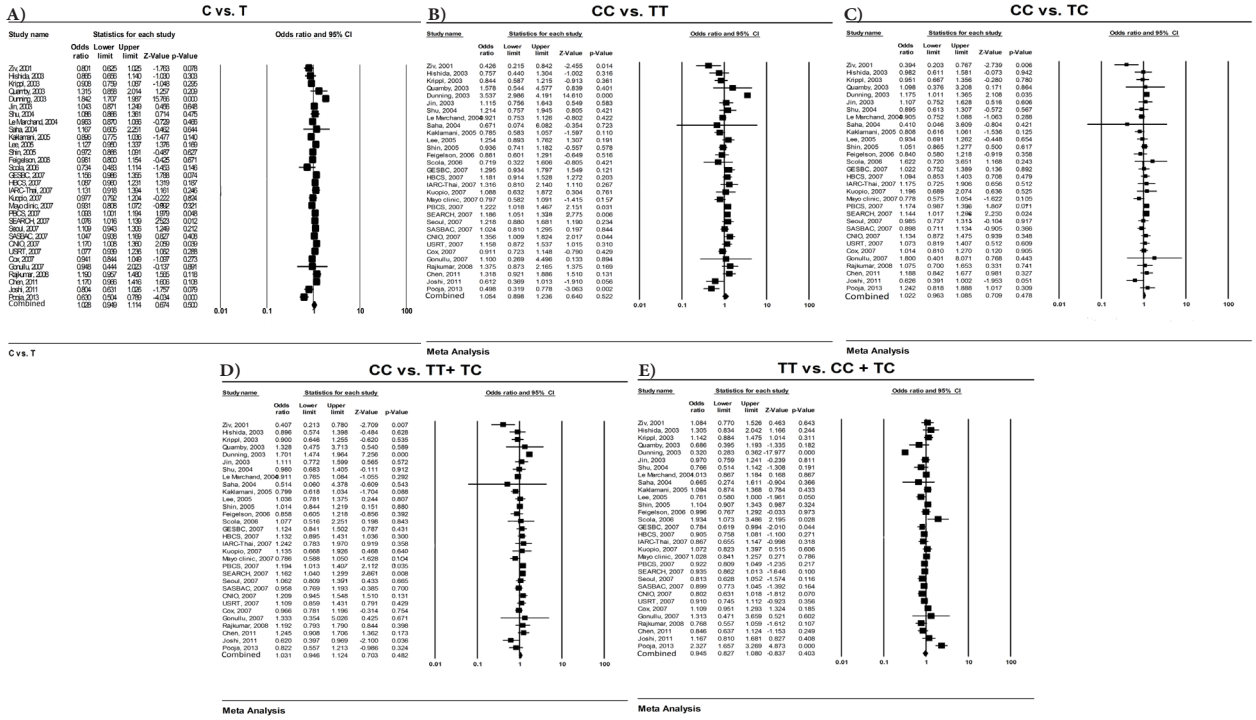


**Figure 2. Forest Plot of OR with 95% CI of Breast Cancer Associated with the TGFb1 C29T Gene Polymorphism in the Mixed Population by Random Effect Model.** Black square represent the value of OR and the size of the square indicates the inverse proportion relative to its variance. Horizontal line is the 95% CI of OR. The studies are listed by year of publication. Forest plot with ORs on breast cancer risk associated with TGFb1 C29T gene polymorphism **A)** C *vs* T; allelic model; **B)** CC *vs* TT; homozygous model; **C)** CC *vs* TC; heterozygous model; **D)** CC *vs* TT+TC; dominant model; **E)** TT *vs* CC+TC; recessive model

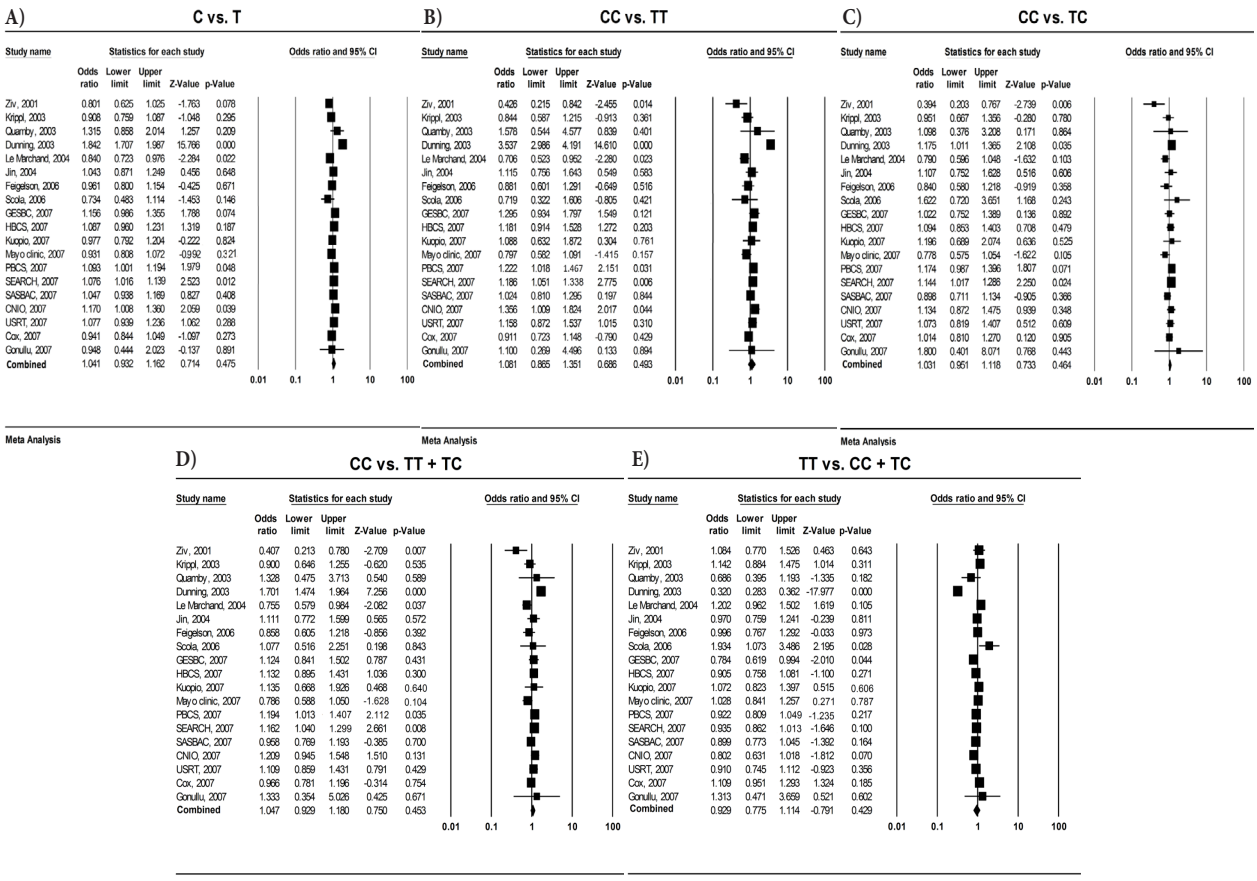


**Figure 3. Forest Plot of OR with 95% CI of Breast Cancer Associated with the TGFb1 C29T Gene Polymorphism in the Caucasian Population By Random Effect Model.** Black square represent the value of OR and the size of the square indicates the inverse proportion relative to its variance. Horizontal line is the 95% CI of OR. The studies are listed by year of publication. Forest plot with ORs on breast cancer risk associated with TGFb1 C29T gene polymorphism **A)** C *vs* T; allelic model; **B)** CC *vs* TT; homozygous model; **C)** CC *vs* TC; heterozygous model; **D)** CC *vs* TT+TC; dominant model; **E)** TT *vs* CC+TC; recessive model

*Subgroup analysis of racial descent*

Caucasian and Asian population was analysed by study design and participants. This meta-analysis included 19 studies pertaining to Caucasian population (18194 cases and 24406 controls), and 12 studies pertaining to Asian population (4873 cases and 5693 controls)

Heterogeneity was observed in all genetic models; thus, random effect model was applied to analyze the data in all groups except in Asian subgroup where fixed effects model was applied to synthesize the data in CC *vs* TC and CC *vs* TT+TC owing to the lack of heterogeneity in both the models. No evidence of publication bias was detected during the analysis.

In the subgroup analysis by ethnicity, no association was found between TGF-β1 29T/C polymorphism and risk of breast cancer in Caucasian population (C *vs* T: OR= 1.041, 95% CI=0.932-1.162, p-value 0.475; CC *vs* TC: OR= 1.031, 95% CI=0.951-1.118, p-value 0.464; CC *vs* TT: OR= 1.081, 95% CI=0.865-1.351, p-value 0.493; CC *vs* TT+TC: OR= 1.047, 95% CI=0.929-1.180, p-value 0.453; TT *vs* CC+TC: OR= 0.929, 95% CI=0.775-1.114, p-value 0.429) (Figure 3A -E).

Similarly, no association was found between TGF-β1 29T/C polymorphism and risk of breast cancer in Asian population (C *vs* T: OR= 1.004, 95% CI=0.908-1.111, p-value 0.931; CC *vs* TC: OR= 0.991, 95% CI=0.896-1.097, p-value 0.865; CC *vs* TT: OR= 1.015, 95% CI=0.848-1.214, p-value 0.871; CC *vs* TT+TC: OR= 1.000, 95% CI=0.909-1.101, p-value 0.994; TT *vs* CC+TC: OR= 0.967, 95% CI=0.808-1.159, p-value 0.720;) (Figure 4A-E).

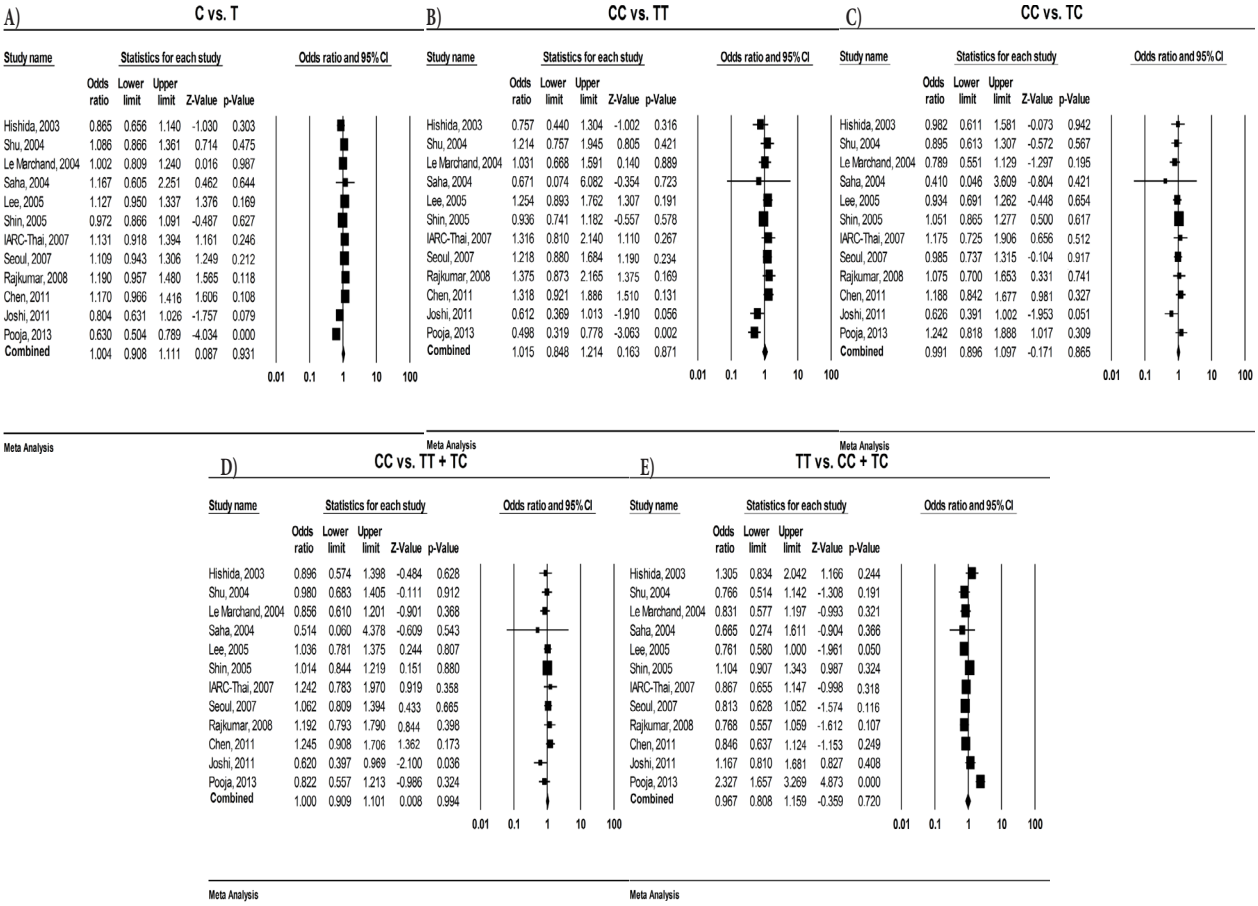


**Figure 4. Forest Plot of OR with 95% CI of Breast Cancer Associated with the TGFb1 C29T Gene Polymorphism in the Asian Population by Random Effect Model.** Black square represent the value of OR and the size of the square indicates the inverse proportion relative to its variance. Horizontal line is the 95% CI of OR. The studies are listed by year of publication. Forest plot with ORs on breast cancer risk associated with TGFb1 C29T gene polymorphism **A)** (C *vs* T; allelic model); **B)** CC *vs* TT; homozygous model; **C)** CC *vs* TC; heterozygous model; **D)** CC *vs* TT+TC; dominant model; **E)** TT *vs* CC+TC; recessive model

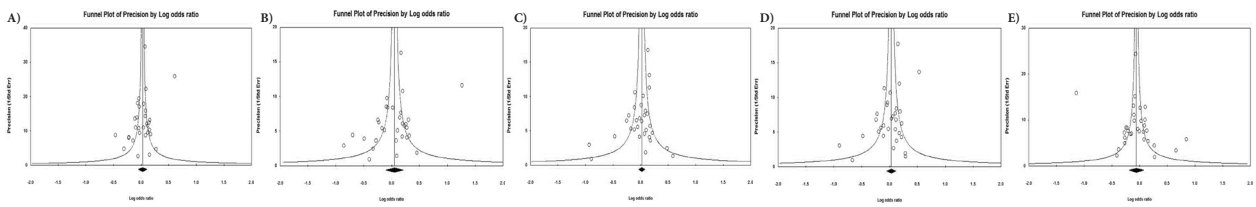


**Figure 5. Assessment of Publication Bias Shown with Funnel Plot in Studies Assaying Odds of Breast Cancer Associated with the TGFb1 C29T gene Polymorphism in the Mixed Population.** Effect size against precision, the inverse of standard error **A)** C *vs* T; allelic model; **B)** CC *vs* TT; homozygous model; **C)** CC *vs* TC; heterozygous model; **D)** CC *vs* TT+TC; dominant model; **E)** TT *vs* CC+TC; recessive model

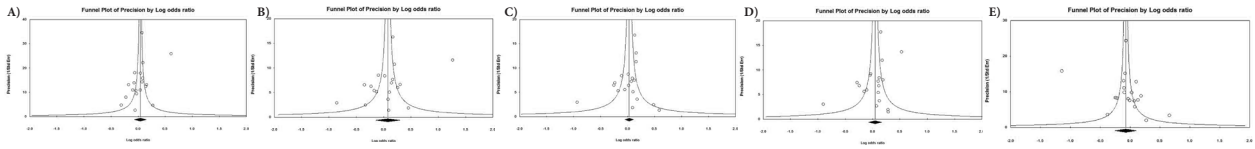

**Figure 6. Assessment of publication bias shown with Funnel plot in studies Assaying Odds of Breast Cancer Associated with the TGFb1 C29T Gene Polymorphism in the Caucasian Population. A)** C *vs* T; allelic model; **B)** CC *vs* TT; homozygous model; **C)** CC *vs* TC; heterozygous model; **D)** CC *vs* TT+TC; dominant model; **E)** TT *vs* CC+TC; recessive model

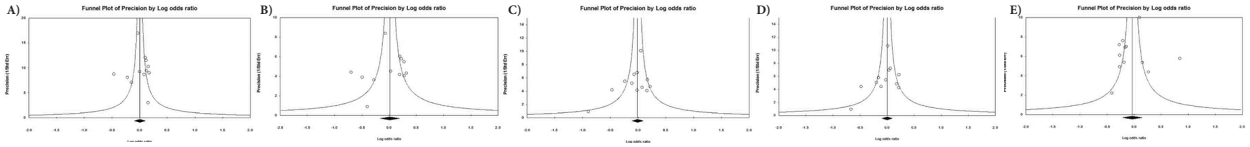

**Figure7. Assessment of Publication Bias Shown with Funnel Plot in Studies Assaying Odds of Breast Cancer Associated with the TGFb1 C29T Gene Polymorphism in the Asian Population.** (A) Effect size against precision, the inverse of standard error (C *vs* T; allelic model). (B) Effect size against precision, the inverse of standard error (CC *vs* TT; homozygous model). (C) Effect size against precision, the inverse of standard error (CC *vs* TC; heterozygous model). (D) Effect size against precision, the inverse of standard error (CC *vs* TT+TC; dominant model). (E) Effect size against precision, the inverse of standard error (TT *vs* CC+TC; recessive model)

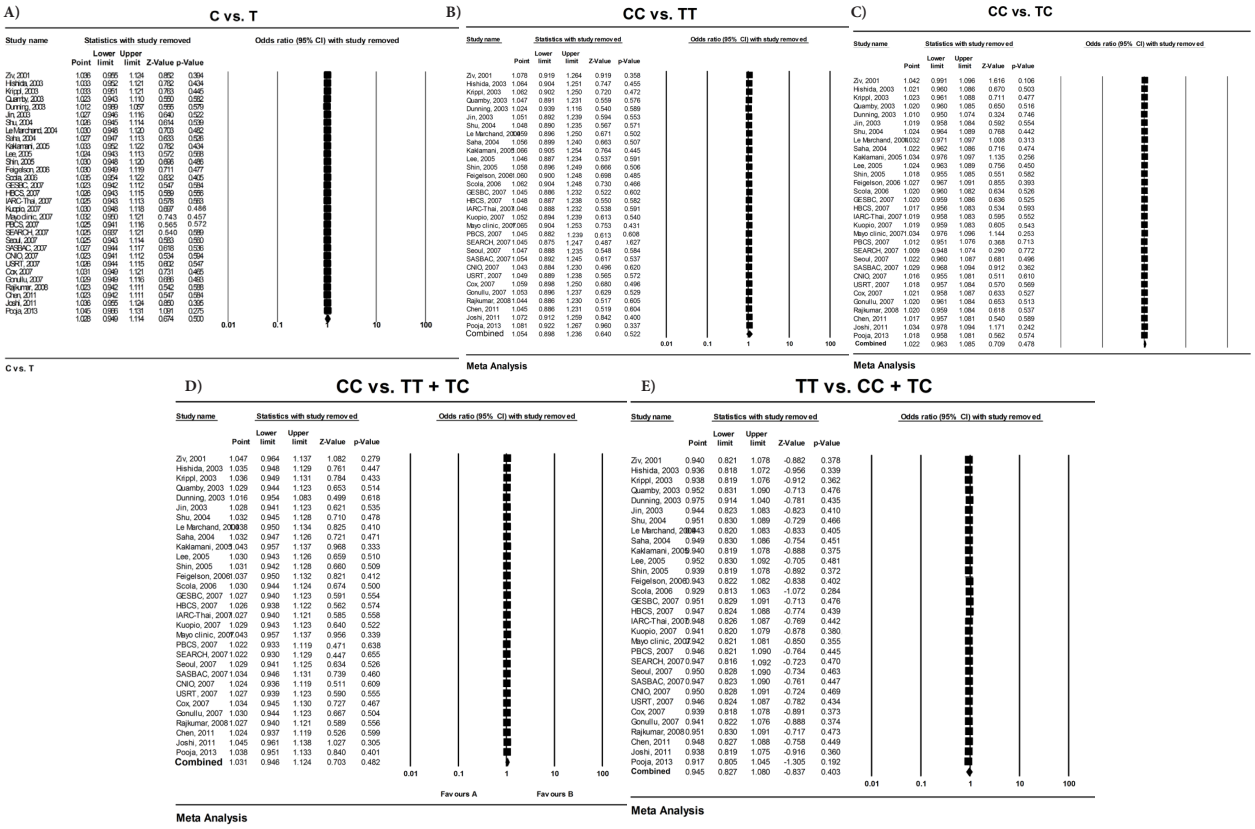


**Figure 8. Sensitivity Analysis by Showing Forest Plot of OR with 95% CI of breast cancer associated with the TGFb1 C29T Gene Polymorphism in the Mixed Population.** Black square represent the value of OR and the size of the square indicates the inverse proportion relative to its variance. Horizontal line is the 95% CI of OR. The studies are listed by year of publication. Forest plot with ORs on breast cancer risk associated with TGFb1 C29T gene polymorphism **A)** C *vs* T; allelic model; **B)** CC *vs* TT; homozygous model; **C)** CC *vs* TC; heterozygous model; **D)** CC *vs* TT+TC; dominant model; **E)** TT *vs* CC+TC; recessive model

**Table 3. Statistics to Test Publication Bias and Heterogeneity in the Cumulative Meta-Analysis**

| Comparison models | Egger's regression analysis | | | Heterogeneity analysis | | | | Model used |
|---|---|---|---|---|---|---|---|---|
| | Intercept | 95% confidence interval | p-value | (2-tailed) | Q-value | df (Q) | $P_{heterogeneity}$ | $I^2$ |
| C *vs* T | -2.10 | -4.33 to 0.12 | 0.06 | 264.20 | 30 | 0.000 | 88.64 | Random |
| CC *vs* TC | -0.75 | -1.58 to 0.08 | 0.07 | 37.87 | 30 | 0.153 | 20.79 | Random |
| CC *vs* TT | -1.89 | -3.97 to 0.19 | 0.07 | 232.40 | 30 | 0.000 | 87.09 | Random |
| CC *vs* TT+TC | -1.35 | -2.55 to -0.16 | 0.02 | 81.15 | 30 | 0.000 | 63.03 | Random |
| TT *vs* CC+TC | 1.63 | -0.97 to 4.24 | 0.21 | 339.03 | 30 | 0.000 | 91.15 | Random |

**Table 4. Statistics to Test Publication Bias and Heterogeneity in the Meta-Analysis Based on Caucasian Ethnicity**

| Comparison models | Egger's regression analysis | | | Heterogeneity analysis | | | | Model used |
|---|---|---|---|---|---|---|---|---|
| | Intercept | 95% confidence interval | p-value | (2-tailed) | Q-value | df (Q) | $P_{heterogeneity}$ | $I^2$ |
| C *vs* T | -2.56 | -5.99 to 0.88 | 0.13 | 221.95 | 18 | 0.00 | 91.89 | Random |
| CC *vs* TC | -0.88 | -2.06 to 0.29 | 0.13 | 27.47 | 18 | 0.07 | 37.48 | Random |
| CC *vs* TT | -2.27 | -5.50 to 0.97 | 0.16 | 198.04 | 18 | 0.00 | 90.91 | Random |
| CC *vs* TT+TC | -1.65 | -3.40 to 0.11 | 0.06 | 65.19 | 18 | 0.00 | 72.38 | Random |
| TT *vs* CC+TC | 1.95 | -2.24 to 6.15 | 0.34 | 292.08 | 18 | 0.00 | 93.83 | Random |

**Table 5. Statistics to Test Publication Bias and Heterogeneity in the Meta-Analysis Based on Asian Ethnicity**

| Comparison models | Egger's regression analysis | | | Heterogeneity analysis | | | | Model used |
|---|---|---|---|---|---|---|---|---|
| | Intercept | 95% confidence interval | p-value | (2-tailed) | Q-value | df (Q) | $P_{heterogeneity}$ | $I^2$ |
| C *vs* T | -0.49 | -4.08 to 3.09 | 0.76 | 31.02 | 11 | 0.001 | 64.54 | Random |
| CC *vs* TC | -0.74 | -2.35 to 0.86 | 0.32 | 9.44 | 11 | 0.581 | 0.00 | Fixed |
| CC *vs* TT | -0.67 | -3.50 to 2.15 | 0.60 | 23.45 | 11 | 0.015 | 53.09 | Random |
| CC *vs* TT+TC | -0.76 | -2.46 to 0.93 | 0.34 | 10.49 | 11 | 0.487 | 0.00 | Fixed |
| TT *vs* CC+TC | -0.07 | -4.55 to 4.41 | 0.97 | 40.92 | 11 | 0.00 | 73.11 | Random |

*Publication bias diagnosis*

Begg's funnel plot and Egger's test were performed to assess the publication bias among the eleigible studies. The shape of funnel plots did not reveal asymmetry in all the genetic models in mixed (Figure 5A-E), Caucasian (Figure 6A-E) and Asian (Figure 7A-E) population. Further, the Egger's test was performed to provide the statistical evidence of funnel plot symmetry. The results did not show any publication bias among all comparison models in mixed (Table 3), Caucasian (Table 4) and Asian (Table 5) population.

*Evaluation of heterogeneity*

Q-test and $I^2$ statistics, used to assess heterogeneity among the eligible studies, showed heterogeneity in all the five genetic models. Therefore, random effects model was applied to synthesize the data in mixed (Table 3) and Caucasian population (Table 4). In Asian population random effects model was applied to synthesize the data in C *vs* T, CC *vs* TT and TT *vs* CC+TC model, while fixed effects model was applied to synthesize the data in CC *vs* TC ($P_{heterogeneity}$ 0.581) and CC *vs* TT+TC ($P_{heterogeneity}$ 0.487) model and $I^2$ value 0.00 for both the models (Table 5).

*Sensitivity analysis*

A single study involved in the meta-analysis was systematically deleted each time to reflect the influence of the individual dataset to the pooled ORs. The results showed that the corresponding pooled ORs were not materially altered (Figure 8A-E; data shown for mixed population), suggesting that our results were statistically robust.

## Discussion

Several early studies suggest that TGFβ acts as both a tumor suppressor and a stimulator of tumor progression, invasion and metastasis (Roberts and Wakefield, 2003; Pardali and Moustakas, 2007). It inhibits tumorigenesis via control of cell-cycle progression and cell proliferation, and by inducing apoptosis in normal tissue and early stage tumors, while improving the invasive and metastatic capabilities of advanced carcinomas by preventing immune surveillance, promoting immune evasion, enhancing angiogenesis, regulating stroma-tumor interactions, and inducing epithelial-mesenchymal transition (Pardali and Moustakas., 2007). TGFβ1 gene polymorphisms are significantly associated with susceptibility to multiple diseases (Yamada., 2001; Shin et al., 2005; Wei et al., 2007; Vishnoi et al., 2008; Zhang et al., 2009).

The 29T/C transition (Leu10Pro substitution) in the signal peptide of the TGF-β1 precursor, is speculated to have an association with protein TGFβ1 secretion and with an increased risk of breast cancer (Dunning et al., 2003; 2006; Gonzalez-Zuloeta Ladd et al., 2007). On the contrary many studies have reported no significant association between this SNP and breast cancer risk (Krippl et al., 2003; Jin et al., 2004; Saha et al., 2004; Cox et al., 2007b; Rajkumar et al., 2008).

Similarly, recent case-control study as well as meta-analysis have shown no association of TGF β1 -509T*C with the risk of hepatocellular carcinoma or overall cancer risk (Liu et al., 2012; Shi et al., 2012). However, some studies have reported that TGF-β1 *29C was protective against breast cancer and suggested that *29C is a protective allele and *29T a risk allele by showing that the individuals with C/C genotype had a significantly lower risk of developing breast cancer compared to those with the T/T or T/C genotype (Ziv et al., 2001; Hishida et al., 2003; Joshi et al., 2011).

Published clinical studies as well as meta-analyses on TGF-β1 29T/C are conflicting in nature so far. As many as five meta-analyses (all in 2010) were conducted on this polymorphism (Gu et al., 2010; Huang et al., 2010; Ma et al., 2010; Qi et al., 2010; Qiu et al., 2010) without reaching a consensus.

Two of these meta-analysis found no association between TGF-β1 29T/C polymorphism and breast cancer risk (Gu et al., 2010; Huang et al., 2010), while the two others showed no overall association between this SNP and breast cancer risk, but an increased risk of breast cancer with 10P allele in Caucasians (Ma et al., 2010; Qi et al., 2010), and another meta-analysis showed significant association of 10P in overall analysis as well as in the Caucasian group (Qiu et al., 2010).

To provide a reliable conclusion, we have undertaken

the present meta-analysis from thirty-one eligible published case-control studies to analyse the association of TGF-β1 29T/C polymorphism and risk of breast cancer. This study shall provide a more robust estimate about the role of this polymorphism with breast cancer risk in overall population and in subgroups defined as Caucasian and Asian population, as combining data from many studies has the advantage of reduced random errors (Bouillon et al., 2008).

The overall pooled results of this meta-analysis revealed that TGF-β1 29T/C polymorphism is not associated with an increased or decreased risk of breast cancer in all the five genetic models as per the eligible studies when compared with wild type allele. Even in the stratified analysis by ethnicity, no statistically significant relationship between TGF-β1 29T/C genotype and breast cancer risk was detected in Caucasian as well as Asian population.

Our findings are in agreement with two earlier meta-analysis (Gu et al., 2010; Huang et al., 2010). which showed no significant breast cancer risk associated with TGF-β1 29T/C. Moreover, many environmental and genetic factors interact closely towards susceptibility to breast cancer and a single genetic variant is usually insufficient to predict the risk of this enigmatic disease.

Heterogeneity between studies is a common aspect of the genetic association studies in meta-analysis. We also found inter-study heterogeneity in overall analysis owing to the genetic backgrounds for cases and controls, diverse genotype distribution of TGF-β1 29T/C in subgroups and uneven selection criteria for the cases and controls in different studies.

Some limitations of the current meta-analysis must be acknowledged. First, only studies published in English language, abstracted and indexed by the selected electronic databases were retrieved and included in this meta-analysis; it is possible that some pertinent reports published in other languages and indexed in other electronic databases may have missed. Second, the role of gene-environment interactions, age at menarche, number of full-term pregnancies, menopausal status was not considered due to absence of available information which may significantly influence the risk of breast cancer. Third, the studies included in the Asian subgroup analysis are limited and the results are sensitive to study selection. More comparative studies are needed to evaluate interactions of TGF-β1 29T/C polymorphism and breast cancer risk in specific populations.

Nevertheless, present meta-analysis also had several strengths. First, although the number of studies involved in the meta-analysis pertaining to Asian population was relatively small, the number of total cases and controls was substantial, which significantly increased the statistical power of the analysis.

Second, no publication biases were detected, indicating that the results may be unbiased. Second, significantly more number of cases and controls were included in the current study when compared to previous meta-analysis study by using effective and efficient searching strategy to increase the statistical power of the analysis.

In conclusion, present meta-analysis pooled both statistically significant and non-significant findings from individual studies to generate a precise conclusion. The findings of current meta-analysis demonstrate that the TGF-β1 29T/C polymorphism may not have an association with breast cancer risk and development. No publication bias among the total studies was detected in overall or subgroups (Caucasian and Asian) analysis. However, larger and well-designed multicentric studies based on the same ethnic group particularly stratified by gene-gene and gene-environment interactions are warranted to validate our findings.

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