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*Original Article*

# Association between MSMB rs10993994 polymorphism and susceptibility to prostate cancer: a meta-analysis and trial sequential analysis

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**Abstract:** Background: Previous studies remained controversial results related to the relationship between microseminoprotein beta gene (MSMB) rs10993994 polymorphism and prostate cancer risk. Therefore, this meta-analysis was performed to summarize such association. Methods: We searched for relevant available literatures on rs10993994 and prostate cancer until March 1st, 2016 on the databases Pubmed, Embase and web of science. The pooled odds ratios (ORs) with 95% confidence intervals (CIs) were used to assess the strength of the association. Subgroup analyses were conducted based on ethnicity and source of controls. Then, trial sequential analysis was performed to reduce the risk of type I error and evaluate whether the results were based on firm evidence. Results: Overall, our results indicated that significant increased risk of prostate cancer was associated with rs10993994 for dominant model OR=1.28 (95% CI: 1.21-1.36), recessive model OR=1.41 (95% CI: 1.25-1.58) and homozygote model OR=1.57 (95% CI: 1.45-1.70) and heterozygote model OR=1.19 (95% CI: 1.12-1.26). In the subgroup analysis by ethnicity, significant results were detected only in Caucasian populations (*dominant model*: OR=1.29, 95% CI: 1.22-1.37; *recessive model*: OR=1.46, 95% CI: 1.33-160; *homozygote model*: OR=1.62, 95% CI: 1.49-1.77; *heterozygote model*: OR=1.19, 95% CI: 1.12-1.27). Moreover, when stratified by source of controls, statistically significant increased risks were found among both population-based control group and hospital-based control group. In the present study, such association was confirmed by trial sequential analyses. Conclusions: This meta-analysis suggests the T allele of the MSMB rs10993994 polymorphism increases prostate cancer susceptibility, which holds potential as biomarkers for prostate cancer risk.

**Keywords:** MSMB polymorphism, rs10993994, prostate cancer, meta-analysis

## Introduction

As one of the most common malignancies among men in the western countries, prostate cancer (PCa) is considered the second leading cause of cancer-related deaths in men [1, 2]. Although previous studies have reported a series of potential risk factors such as smoking, inflammation, diet, environment, age, and genetic factors that might increase PCa susceptibility, the accurate etiology of PCa is still unclear [3-8]. A study revealed that malignant transformation of prostate cells was associated with somatic genomic changes, including deletions, amplifications, or point mutations [9, 10]. Genetic factors, particularly single-nucleotide genetic polymorphisms (SNPs), have been reported to play an important role in the development of PCa [11].

Among these SNPs, genome-wide association studies (GWAS) have recently identified a SNP, rs10993994: C>T, locating on chromosome 10q11, which is located in the proximal promoter region (-59 bp) of the microseminoprotein beta gene (MSMB) that encodes for β-microsemino protein (MSP) [12-14]. Besides, MSP is one of the major secreted proteins from the

prostate gland [15]. More importantly, early studies suggested that the replacement of T allele by C allele might destroy a potential binding site of cAMP response element binding protein (CREB), the T allele therefore had much lower promoter activity than the C allele [16, 17]. In addition, a growing number of studies discovered the association between rs10993994 causal variant and PCa susceptibility [17-24]. Hence, this SNP, rs10993994: C>T may play a vital role in prostate carcinogenesis.

Subsequently, a number of studies were performed to elucidate the possible relationship between rs10993994 and the risk of PCa. However, the results remained unclear or even contradictory. Moreover, due to lack of meta-analysis on comprehensive understanding of the relationship between rs10993994 and the risk of PCa, this meta-analysis was for the first time conducted by including all eligible articles to clarify the real association and identify statistical evidence. Furthermore, trial sequential analyses (TSA) were used to clarify whether the evidence for the results was sufficient.

## Materials and methods

We conducted a comprehensive search based on PubMed, EMBASE and Web of Science to identify relevant studies, with the last search update on March 1st, 2016.

The following search items were utilized: "microseminoprotein beta gene" or "rs10993994", "MSMB", "variants" or "polymorphism", and "prostate cancer". Additional eligible studies were collected by a manual search from the references of original studies identified or recent review articles for the meta-analysis. Only the latest or more comprehensive publication was included, if the same data existed in more than one publication. Furthermore, ethical approval and informed consent were not required because our meta-analysis was based on data from previously published studies.

Eligible studies were selected if they met the following inclusion criteria: (1) An independent case-control design; (2) The association between rs10993994 polymorphism and PCa susceptibility was evaluated; (3) The data on frequency of genotypes of the polymorphisms must be clearly presented. In addition, in case of the violation of the aforementioned requirements, this study was certainly excluded from this meta-analysis.

### *Data extraction*

Based on the above the inclusion criteria, data were extracted from the identified studies by two investigators (Qin ZQ and Tang JY) independently, and any disagreement was resolved by a discussion with a third reviewer and a ultimate decision was based on the main point of view. All the extracted information were recorded in a standardized form: first author's last name, year of publication, ethnicity, source of controls, genotyping assay, number of cases and controls, genotype frequency of rs10993994 gene polymorphism between cases and controls respectively, and the results of the Hardy-Weinberg equilibrium (HWE) test.

### *Statistical analysis*

The strength of association between rs10993994 and PCa susceptibility was evaluated by the pooled odds ratios (ORs) with 95% confidence intervals (CIs) based on four genetic comparison models: dominant model (CT+TT versus CC), recessive model (TT versus CC+CT), homozygous model (TT versus CC) and heterozygous model (CT versus CC). The goodness-of-fit chi-square test was adopted to assess HWE in controls and $P<0.05$ was regarded as significant disequilibrium [25]. The pooled ORs were calculated either with fixed-effects model (a Mantel-Haenszel method) or with the random-effects model (a DerSimonian-Laird method) according to the *P* values of study heterogeneities [26]. If there was no indication of substantial heterogeneity, the fixed-effects model would be conducted. Otherwise, the random effects model was selected to perform meta-analysis. After that, subgroup analysis according to ethnicity and source of controls was further carried out to explore the potential sources of heterogeneity. To examine the stability and reliability of the overall meta-analysis results, sensitivity analysis was performed by excluding one single study one by one and recalculating their ORs. In addition, Begg's funnel plots and Egger's linear regression test were employed to search for publication bias between the studies, and *P* values were deemed as a significantly selective bias when less than 0.05 [27]. STATA software (version 12.0; StataCorp LP,

**Table 1.** Characteristics of individual studies included in the meta-analysis

| MSMB | rs10993994 | | | | | | Case (n) | | | Control (n) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Year | Surname | Ethnicity | SOC | Genotyping | Case | Control | CC | CT | TT | CC | CT | TT | HWE |
| 2016 | Sjoblom | Caucasian | HB | Sequenom | 368 | 901 | 154 | 160 | 54 | 394 | 396 | 111 | Y |
| 2015 | Mhatre | Asian | PB | PCR | 50 | 30 | 9 | 24 | 17 | 5 | 10 | 15 | Y |
| 2013 | Stott-Miller | Caucasian | PB | Taqman | 1239 | 1232 | 377 | 621 | 241 | 465 | 599 | 168 | Y |
| 2013 | FitzGerald | Caucasian | PB | Taqman | 1257 | 1253 | 382 | 633 | 242 | 472 | 608 | 173 | Y |
| 2012 | Haiman | Mixed | PB | AutoDELFIA | 1221 | 1230 | 314 | 588 | 319 | 359 | 585 | 286 | Y |
| 2012 | Ho | Caucasian | PB | PCR | 242 | 264 | 83 | 94 | 65 | 102 | 119 | 43 | Y |
| 2010 | Xu | Asian | HB | TaqMan | 251 | 258 | 57 | 122 | 72 | 71 | 140 | 47 | Y |
| 2009 | Chang-a | Caucasian | PB | PCR | 2863 | 1701 | 963 | 1354 | 546 | 627 | 810 | 264 | Y |
| 2009 | Chang-b | Caucasian | HB | PCR | 4444 | 3350 | 1380 | 2129 | 935 | 1275 | 1584 | 491 | Y |

SOC: Source of controls; PB: Population-based controls; HB: Hospital-based controls.

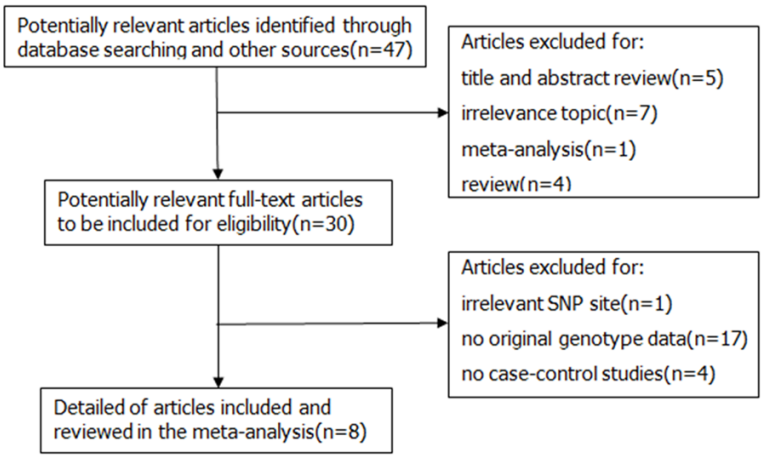


**Figure 1.** Flow diagram of literature search and selection process.

College Station, TX) was utilized to deal with all above statistical analyses.

*Trial sequential analysis*

when a cumulative meta-analyses was updated with addition of new publishing trials, repeated significance testing and sparse data might result in type I and type II errors owing to an increased risk of random error [28-30]. Thus, TSA was introduced to control the risk of type I error by estimation of required information size and with an adjusted threshold for statistical significance [31, 32]. TSA was performed with a desire to maintain a 20% relative risk reduction, an overall 5% risk a type I error of and 15% risk of the type II error (a statistical test power of 85%) [33]. When the blue line (the cumulative Z-curve) crosses the sloping red (the line trial sequential monitoring boundary), a sufficient level of evidence may have been reached and further studies are unnecessary. If the blue line does not cross any of the boundaries and the vertical red line (the required information size) has not been reached, additional clinical trials are needed to reach a sufficient conclusion [34]. The trial sequential analysis software (TSA, version 0.9; Copenhagen Trial Unit, Copenhagen, Denmark, 2011) was applied in this study.

## Results

*Studies characteristics*

Finally, A total of nine case-control studies from eight articles including 11935 cases and 10219 controls were selected based on the inclusion and exclusion criteria and were combined in the current meta-analysis [17-24]. Additionally, due to different source of controls in an article by Chang et al. [17], we divided it into two research studies. The detailed characteristics and genotype distribution of the selected studies are listed in **Table 1**. The process of literature search and exclusion was shown in **Figure 1**. Among these previous studies, there were three different ethnic groups, including 6 studies conducted in Caucasians population, 2 studies based on Asian population and a study from mixed population. Furthermore, in order to distinguish between different sources of control group, we consisted of 7 population-based studies and 2 hospital-based studies.

**Table 2.** Meta-analysis results of association between rs10993994 polymorphism and prostate cancer risk

| | | | Dominant model | | Recessive model | | Homozygote model | | Heterozygote model | |
|---|---|---|---|---|---|---|---|---|---|---|
| | N[a] | Sample Size | OR (95% CI) | P[b] | OR (95% CI)* | P[b] | OR (95% CI) | P[b] | OR (95% CI) | P[b] |
| Total | 9 | 22154 | 1.28 (1.21-1.36) | 0.334 | 1.41 (1.25-1.58) | 0.031 | 1.57 (1.45-1.70) | 0.057 | 1.19 (1.12-1.26) | 0.615 |
| Ethnicity | | | | | | | | | | |
| Caucasian | 6 | 19114 | 1.29 (1.22-1.37) | 0.154 | 1.46 (1.33-1.60) | 0.306 | 1.62 (1.49-1.77) | 0.125 | 1.19 (1.12-1.27) | 0.311 |
| Asian | 2 | 589 | 1.25 (0.85-1.82) | 0.589 | 1.03 (0.31-3.51) | 0.015 | 1.64 (1.03-2.61) | 0.118 | 1.11 (0.74-1.66) | 0.771 |
| Mixed | 1 | 2451 | 1.19 (1.00-1.42) | - | 1.17 (0.97-1.40) | - | 1.28 (1.02-1.59) | - | 1.15 (0.95-1.39) | - |
| SOC | | | | | | | | | | |
| PB | 7 | 13091 | 1.36 (1.16-1.58) | 0.045 | 1.25 (1.16-1.35) | 0.397 | 1.48 (1.34-1.65) | 0.103 | 1.17 (1.08-1.27) | 0.526 |
| HB | 2 | 9063 | 1.52 (1.33-1.74) | 0.331 | 1.32 (1.21-1.44) | 0.218 | 1.71 (1.51-1.93) | 0.211 | 1.21 (1.10-1.32) | 0.387 |

Dominant model: CT/TT vs CC; recessive model: TT vs CT/CC; homozygote model: TT vs CC; heterozygote model: CT vs CC. [a]Number of studies. [b]*P* value of Q test for heterogeneity. *Random-effects model was used when *P* value for heterogeneity test <0.05; otherwise, fixed-effects model was used.

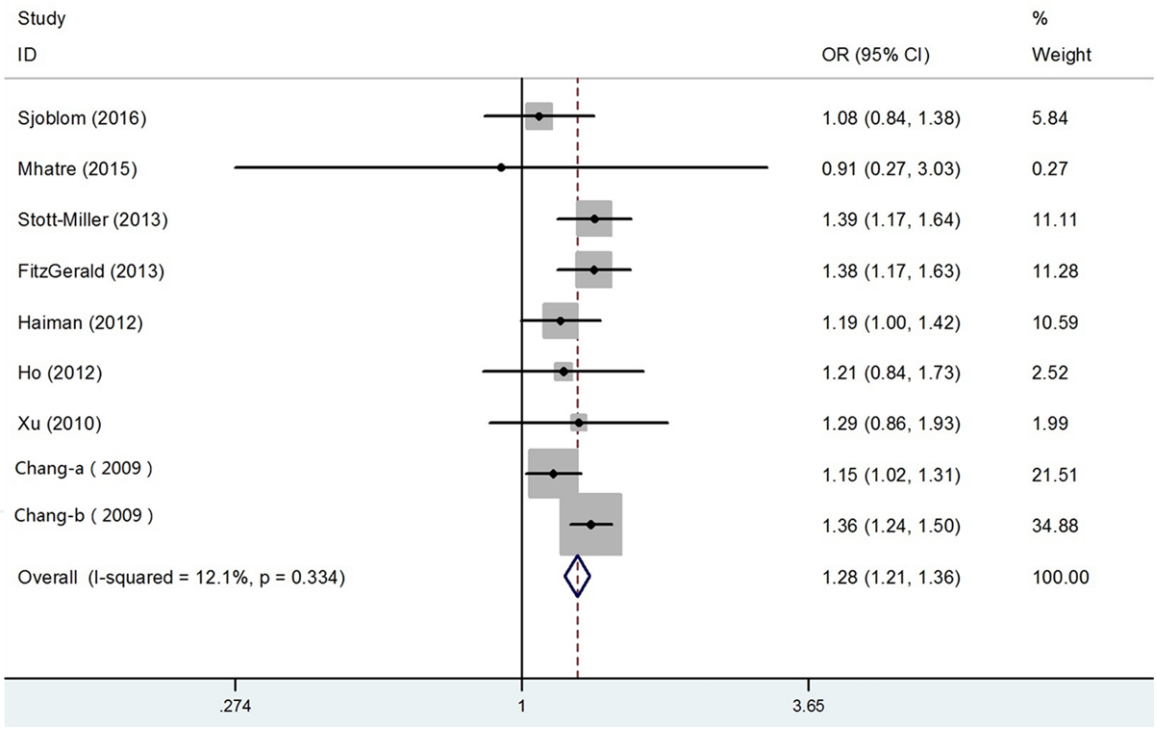


**Figure 2.** Forest plots of the association between rs10993994 polymorphism and prostate cancer susceptibility in dominant model.

*Quantitative synthesis results*

In this meta-analysis, we conducted analyses using fixed-effect models except in recessive model, when *P* value for heterogeneity test >0.05. Besides, the combined results indicated that rs10993994 polymorphism was significantly associated with risk of PCa. Overall, the main results of this meta-analysis about the associations between rs10993994 polymorphism and PCa were shown in **Table 2**. The pooled OR was 1.28 (95% CI: 1.21-1.36) for dominant model, 1.41 (95% CI: 1.25-1.58) for recessive model 1.57 (95% CI: 1.45-1.70) for homozygote model and 1.19 (95% CI: 1.12-1.26) for heterozygote model (**Figure 2**). In the subgroup analysis by ethnicity, the results were significant only in Caucasian populations (*dominant model*: pooled OR=1.29, 95% CI: 1.22-1.37; *recessive model*: pooled OR=1.46, 95% CI: 1.33-1.60; *homozygote model*: OR=1.62, 95% CI: 1.49-1.77; *heterozygote model*: pooled OR=1.19, 95% CI: 1.12-1.27) (**Figure 3A**). Moreover, when the studies were stratified by source of controls, the positive result was detected in both population-based control group (*dominant model*: pooled OR=1.25, 95% CI: 1.16-1.35; *recessive model*: pooled OR= 1.36, 95% CI: 1.16-1.58; *homozygote model*: pooled OR=1.48, 95% CI: 1.34-1.65; *heterozygote model*: pooled OR=1.17, 95% CI: 1.08-1.27) and hospital-based controls (*dominant model*: pooled OR=1.32, 95% CI: 1.21-1.44; *recessive model*: pooled OR=1.52, 95% CI: 1.33-1.74; *homozygote model*: pooled OR=1.71, 95% CI: 1.51-1.93; *heterozygote model*: pooled OR= 1.21, 95% CI: 1.10-1.32) (**Figure 3B**). In general, with the effect of rs10993994 gene polymorphism, the carriers of T allele held higher PCa risk than carriers of C allele, especially in Caucasian ethnicity.

*Test of heterogeneity*

Heterogeneity was observed in overall genetic models, but it was interesting that subgroup analyses could decrease the heterogeneity. Thus, neither ethnicity nor source of controls were performed to contribute to substantial heterogeneity. **Figure 4** showed the analysis of a Galbraith radial plot in dominant model, suggesting that there is no obvious heterogeneity between studies.

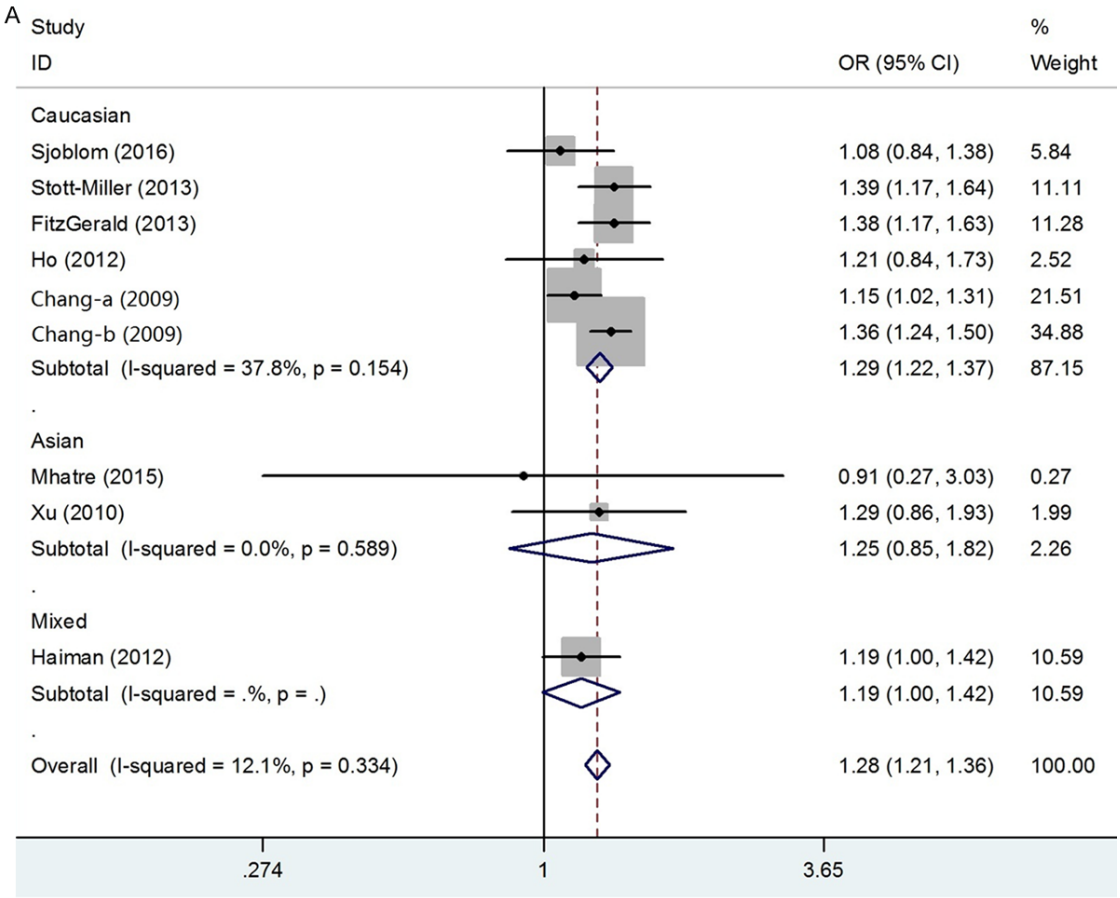
A
Study
ID
OR (95% CI)
%
Weight
Caucasian
Sjoblom (2016) 1.08 (0.84, 1.38) 5.84
Stott-Miller (2013) 1.39 (1.17, 1.64) 11.11
FitzGerald (2013) 1.38 (1.17, 1.63) 11.28
Ho (2012) 1.21 (0.84, 1.73) 2.52
Chang-a (2009) 1.15 (1.02, 1.31) 21.51
Chang-b (2009) 1.36 (1.24, 1.50) 34.88
Subtotal (I-squared = 37.8%, p = 0.154) 1.29 (1.22, 1.37) 87.15
Asian
Mhatre (2015) 0.91 (0.27, 3.03) 0.27
Xu (2010) 1.29 (0.86, 1.93) 1.99
Subtotal (I-squared = 0.0%, p = 0.589) 1.25 (0.85, 1.82) 2.26
Mixed
Haiman (2012) 1.19 (1.00, 1.42) 10.59
Subtotal (I-squared = .%, p = .) 1.19 (1.00, 1.42) 10.59
Overall (I-squared = 12.1%, p = 0.334) 1.28 (1.21, 1.36) 100.00
.274
1
3.65
B
Study
ID
OR (95% CI)
%
Weight
HB
Sjoblom (2016) 1.08 (0.84, 1.38) 5.84
Xu (2010) 1.29 (0.86, 1.93) 1.99
Chang-b ( 2009 ) 1.36 (1.24, 1.50) 34.88
Subtotal (I-squared = 34.4%, p = 0.218) 1.32 (1.21, 1.44) 42.71
PB
Mhatre (2015) 0.91 (0.27, 3.03) 0.27
Stott-Miller (2013) 1.39 (1.17, 1.64) 11.11
FitzGerald (2013) 1.38 (1.17, 1.63) 11.28
Haiman (2012) 1.19 (1.00, 1.42) 10.59
Ho (2012) 1.21 (0.84, 1.73) 2.52
Chang-a ( 2009 ) 1.15 (1.02, 1.31) 21.51
Subtotal (I-squared = 3.0%, p = 0.397) 1.25 (1.16, 1.35) 57.29
Overall (I-squared = 12.1%, p = 0.334) 1.28 (1.21, 1.36) 100.00
.274
1
3.65

**Figure 3.** Forest plots of subgroup analysis of the association between rs10993994 polymorphism and prostate cancer susceptibility in dominant model. A: Stratified by ethnicity; B: Stratified by source of controls.

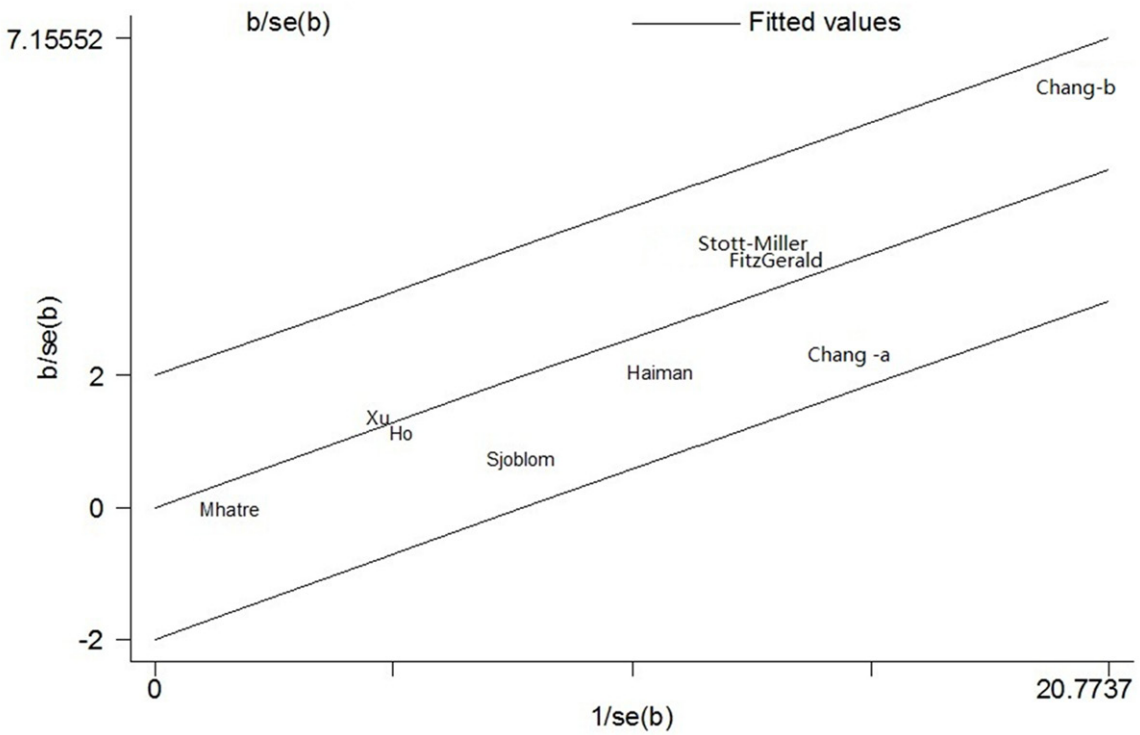


**Figure 4.** Galbraith plot of the association between rs10993994 polymorphism and prostate cancer susceptibility in dominant model.

*Sensitivity analysis*

Sensitivity analysis was carried out by omitting one single study one by one to check their influence of each individual study on the recalculated ORs by repeating the meta-analysis. The sensitivity analysis on association between rs10993994 polymorphism with PCa for dominant model was listed in **Figure 5**, demonstrating that the pooled ORs were not significantly influenced. Therefore, the sensitivity analysis suggested that our meta-analysis results were robust and stability.

*Publication bias*

The Begg's funnel plot and Egger's test were applied to assess the publication bias for all data. The shapes of the funnel plots seemed symmetrically distributed in the funnel plots of rs10993994 polymorphism, indicating little evidence of significant publication bias across studies, which was also confirmed by Egger's test (dominant model: P=0.602) (**Figure 6**).

*Trial sequential analysis results*

In our present study, **Figure 7** showed that not only the cumulative Z-curve crosses the trial sequential monitoring boundary, but also the total number of cases and controls were more than the required information size, showing the results were firm evidence of effect.

## Discussion

The SNP rs10993994:C>T on chromosome 10q11.2, is located in a putative CREB-binding site of the promoter region of MSMB gene, which encodes MSP. Moreover, MSP, as a member in the immunoglobulin binding factor family, is synthesized by epithelial cells of the prostate gland before secretion into the seminal plasma [12, 13]. MSMB might act as a serum marker for early diagnosis of high-risk PCa. In addition, MSMB was considered as a tumor suppressor gene, which expression of MSMB progressively decreases during occurrence and development of PCa from early to late stages. Meanwhile,

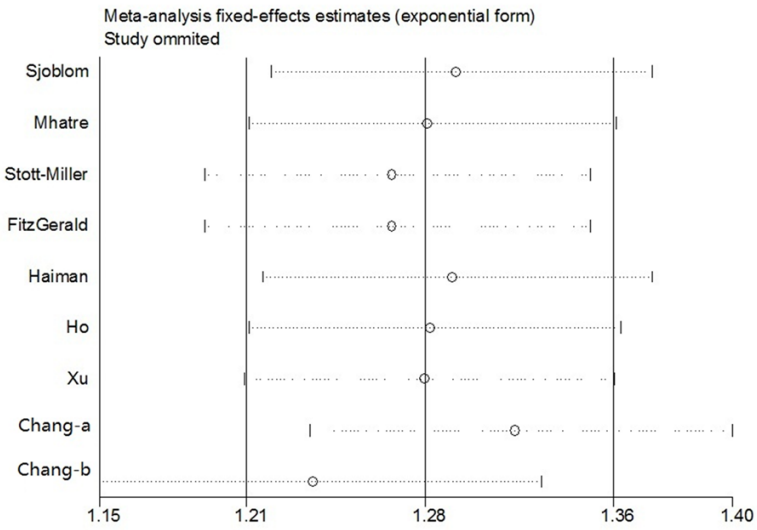


**Figure 5.** Sensitivity analysis under the dominant model.

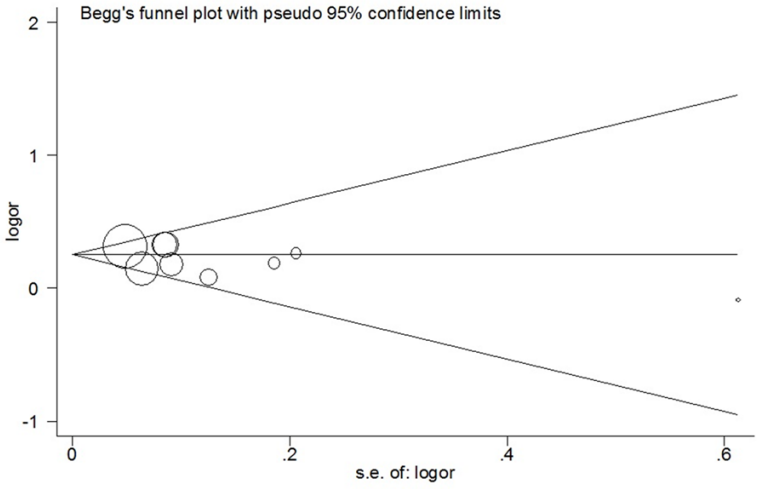


**Figure 6.** Begg's funnel plot of publication bias test in the dominant model.

over expression of MSMB has been described as a protective element, implying MSMB might induce PCa cell apoptosis and suppress PCa growth, invasion and metastasis [35-38]. Thus, these findings support further exploratory studies that the risk T allele of rs10993994 might be predicted to result in the production of PCa with lower amounts of this putative tumor suppressor gene in individuals carrying this variant allele.

In summary, the outcomes of previous case-control studies depicting the association between rs10993994 polymorphism and PCa risk remained inconclusive and controversial. The causes of these conflicting among them might be the relatively small sample size of individual studies, the various ethnicities, the different methodologies, the limited statistical power in the published studies and the possible small effect of the rs10993994 polymorphism on the risk of PCa. However, since then, no meta-analyses explored the relationship between rs10993994 and PCa susceptibility. Recently, as the case-control studies published about such association has continued to increase, our meta-analysis were adopted for the first time to conduct a comprehensive assessment of the relationship between rs10993994 polymorphism and the risk of PCa. Last but not least, TSA was performed to effectively reduce the risk of type I error and assess whether the evidence of our results is reliable. Therefore, it was significant to investigate the association of rs10993994 polymorphisms with PCa susceptibility.

To make the conclusion more credible, we performed meta-analysis as a powerful tool to explain controversial conclusions and provide the most comprehensive understanding of the association between rs10993994 polymorphism and the risk of PCa through different subgroup analysis [39]. All these factors contributed to the development of the current meta-analysis. As a consequence, we took advantage of meta-analysis to illustrate this possible association. In the current meta-analysis, our results revealed the T allele of rs10993994 polymorphism increases PCa susceptibility, especially among Caucasian ethnicity.

These findings of subgroup analyses based on ethnicity and control source can be explained as follows. After stratified analysis was per-

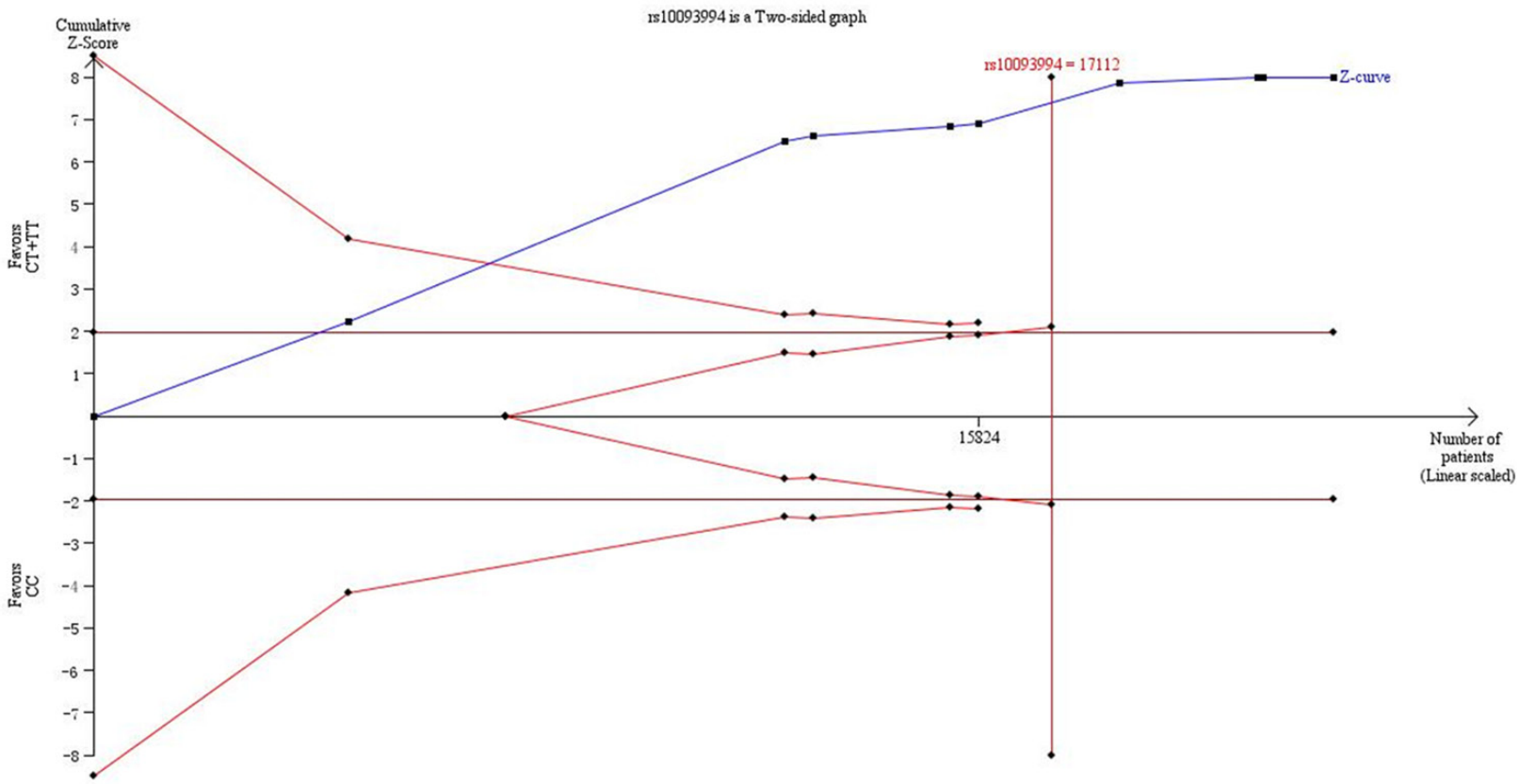


**Figure 7.** Trial sequential analysis of the association between rs10993994 polymorphism and the risk of prostate cancer. The required information size was calculated based on a two side α=5%, β=15% (power 85%), and a relative risk reduction of 20%.

formed by ethnicity, and statistically significantly increased PCa risk was only in Caucasian populations instead of Asian or Mixed populations. Though the exact mechanism was unclear, it was likely that different ethnic groups with various genetic backgrounds might have different gene polymorphisms risk of developing PCa. In addition, we conducted stratified analysis by source of controls and the result was detected significant both in population-based and hospital-based populations. In this meta-analysis, the results were in concordance with these hypotheses of previous studies, which needed to further prove that rs10993994 played an important role in PCa susceptibility.

TSA, as an useful tool, is similar to interim analyses in a single trial, where trial monitoring boundaries are drawn for each outcome whether to continue additional trials to evaluate for evidence when a *P* value is sufficiently small to show the anticipated effect or for futility. In some previous studies, it is believed that the application of TSA is more reliable compared to traditional meta-analysis [40, 41]. when the cumulative Z-curve crosses by the monitoring boundaries, it shows firm evidence for such study. In consequence, we took advantage of TSA to control the risk of type I error and estimate whether further trials are necessary. In the current meta-analyses, the cumulative Z-curve crossed the monitoring boundaries and larger sample size were included than the required information size. Thus, it was strongly of the view that our results were based on firm evidence of effect.

Notably, this is the first meta-analysis to comprehensively illustration the impact of rs10993994 polymorphism in response to PCa risk. Nevertheless, several limitations should be taken into consideration and interpreted. Firstly, certain results, especially those in each stratified analyses, are still indefinitive and remain to be further validated due to relatively insufficient sample size, contributing to potentially limiting the statistical power to explore the real association. Secondly, the pathogenesis of PCa, as a multi-factorial disease, is closely related to environmental backgrounds as well as the interaction with various genetic factors instead of the influence of any single gene. Therefore, additional studies about exploring the risk effects of this polymorphism in susceptibility to PCa needed to be further validated in subsequent studies. What's more, in the present meta-analysis, we did not have enough data for all studies to adjust estimates by other covariates, such as age, gender, life-style and so on. Thereby, a more precise analysis would have been performed if more detailed individual data were available. Additionally, the majority studies used were investigated in Caucasian population, suggesting analysis result might exist some merits. Hence, to guaranty reliability of our meta-analysis, more researches should focus on the influence of different factors in the future.

## Conclusion

The results of the present meta-analysis indicated that the rs10993994 gene polymorphism is significantly associated with susceptibility to PCa. Meanwhile, the variant C allele may be a strong risk factor of PCa, especially in Caucasian populations. More importantly, our findings need to be further validated whether rs10993994 polymorphism might be a potential etiology and detecting marker for the risk of PCa in the future.

## Disclosure of conflict of interest

None.

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