Tumor Biol. (2014) 35:10503–10513
DOI 10.1007/s13277-014-2323-0

RESEARCH ARTICLE

Promoter methylation and polymorphism of *E-cadherin* gene may confer a risk to prostate cancer: a meta-analysis based on 22 studies

Zheng Chang · Hongbing Zhou · Yi Liu

Received: 13 May 2014 / Accepted: 7 July 2014 / Published online: 24 July 2014

Abstract Emerging evidence has suggested that −160C/A polymorphism and promoter methylation of *E-cadherin* gene may contribute to the risk of prostate cancer. However, the results are still conflicting. We aim to systematically evaluate the potential of promoter methylation and polymorphism in *E-cadherin* gene to confer a risk to prostate cancer through meta-analysis. PubMed, Embase, Web of Science, Cochrane Library, and Chinese National Knowledge Infrastructure (CNKI) databases were searched to identify eligible studies published before April 1, 2014. Pooled odds ratios (ORs) with their 95 % confidence intervals (95 % CIs) were calculated by using the random-effect model or the fixed-effect model, according to heterogeneity test. Subgroup analyses were also performed to explore the potential sources of heterogeneity. Sensitivity and publication bias analyses were used to test the robustness of our results. We performed a meta-analysis of 22 included studies, with 11 on −160C/A polymorphism and another 11 on promoter methylation of *E-cadherin* gene. Our meta-analysis results suggested that *E-cadherin* −160C/A polymorphism may be a potential risk factor for prostate cancer. Furthermore, we observed that the frequencies of promoter methylation of *E-cadherin* gene in the prostate cancer tissues were significantly higher than those of normal tissues, indicating that promoter methylation of *E-cadherin* gene may play an important role in prostate carcinogenesis. In conclusion, the present meta-analysis provides further evidence that promoter methylation and −160C/A polymorphism of *E-cadherin* gene may confer a risk to prostate cancer. Identifying these risk factors for prostate cancer will improve early detection, allow for selective chemoprevention, and provide further insights into its disease mechanisms.

Keywords *E-cadherin* · Polymorphism · Promoter methylation · Prostate cancer · Meta-analysis

Introduction

Prostate cancer is one of the most common cancers among men in developed countries, which has become a major public health challenge. Traditionally considered as a disease of elderly men, an increasing proportion of prostate cancer cases now occur in men of pre-retirement ages. Established risk factors include age, ethnicity, and family history. In addition, a few low-penetrance susceptibility genes, including *E-cadherin* with a higher population frequency, may be relevant to prostate cancer risk in combination with environmental factors. Prostate cancer is the second most prevalent cancer and the sixth leading cause of death in males [1, 2]. More prostate cancer cases now occur in younger men [3, 4], not only in elderly men. New markers for identifying high-risk populations as well as novel strategies for early detection and preventive care are urgently needed. Due to the high incidence and low survival rate, novel biomarkers for early diagnosis of prostate cancer are urgently needed. The mechanism of prostatic tumorigenesis is still not fully understood. Although the casual mechanisms of prostate cancer are still not fully understood, several risk factors have been suggested to be associated with prostate cancer, including ethnic variance, environmental factors, family history, and lifestyles [5–7]. It has been suggested that low-penetrance susceptibility genes combining

Zheng Chang and Hongbing Zhouhave the same contributions to this study and should be considered as co-first authors

Z. Chang · Y. Liu (✉)
Department of Urology, General Hospital of Jinan Military Command, 25 Shifan Road, Jinan 250031, People's Republic of China
e-mail: yiliu_jn@yeah.net

H. Zhou
Department of General Surgery, PLA 456th Hospital, 25 Wuyingshan Road, Jinan 250031, People's Republic of China

with environmental factors may be important in the development of cancer. Emerging evidence has suggested potential genetic risks for prostate cancer [8, 9]. It has also been reported that promoter methylation of related genes may also play an important role in prostate carcinogenesis [10, 11].

In recent years, several common low-penetrate genes have been identified as potential prostate cancer susceptibility genes. An important one is *E-cadherin* gene, which locates on chromosome 16q22.1 and consists of 16 coding exons [12, 13]. E-cadherin, which is encoded by *E-cadherin* gene in epithelial cells [14, 15], plays an important role in the establishment and maintenance of intercellular adhesion, cell polarity, and tissue architecture [16, 17]. But its expression is largely reduced in undifferentiated cancers. The E-cadherin/catenin complex is important for cellular polarity and maintenance of normal tissue morphology and cellular differentiation. Decreased E-cadherin expression is supposed to be associated with various malignancies including prostate cancer [14, 18–20]. In addition, disruption of E-cadherin with rare mutations may also be involved in carcinogenesis through a modified Wnt signaling pathway. Several polymorphisms and somatic mutations have been identified in *E-cadherin.* The −160C/A polymorphism in the promoter region of the *E-cadherin* gene has been reported to have a direct effect on its transcriptional regulation and therefore may influence susceptibility to cancers.

E-cadherin −160C/A polymorphism has been identified in the promoter region related to the transcriptional start site [21]. It has been shown that the A allele decreased transcriptional efficiency by about 10–68 % compared with the wild-type C allele. It was also observed that the C allele showed much higher binding affinity to transcriptional factor than the mutant allele, indicating that the −160C/A variant may alter transcriptional activity of the *E-cadherin* gene and be responsible for decreased E-cadherin expression and increased susceptibility to epithelial cancers. Furthermore, aberrant E-cadherin functions have been reported to be associated with malignant transformation of prostatic epithelium as well as metastasis and poor prognosis of prostate cancer.

A number of studies have reported the function of *E-cadherin* −160C/A polymorphism in prostate cancer risk, but the results are inconclusive, partially because of the possible small effect of the polymorphism on prostate cancer risk and the relatively small sample size in each of published studies. Li et al. first reported that the −160C/A polymorphism directly affects the *E-cadherin* gene transcriptional regulation [22]. Kallakury et al. [23] found that promoter methylation of *E-cadherin* gene is more frequently in prostate carcinoma and may play a role in the decreased expression of the E-cadherin protein. Thereafter, accumulating epidemiological and molecular studies have reported results about the association of *E-cadherin* −160C/A polymorphism and promoter methylation with prostate cancer risk, although their results have been conflicting. Thus, these observations raised quite a controversial question regarding the significance of −160C/A in prostate cancer pathogenesis. Obviously, statistical power of an individual study could be very limited for efficient assessment of the −160A allele. Integration of these data sets may provide improved statistical power to detect the significance. Therefore, we performed the current meta-analysis to derive a relatively comprehensive assessment of the relationship between −160C/A polymorphism and promoter methylation of *E-cadherin* gene and the risk of prostate cancer.

Materials and methods

Literature search

To identify relevant studies, we conducted a literature search in PubMed, Embase, Web of Science, Cochrane Library, and Chinese National Knowledge Infrastructure (CNKI) databases without language limitation. The last retrieval was conducted on April 1, 2014. The search strategy was formulated using the following keywords: ("E-cadherin" or "CDH1" or "−160C/A" or "rs16260") and ("single nucleotide polymorphism" or "SNP") and ("methylation" and "hypermethylation") and ("prostate cancer" or "prostatic neoplasm"). References of relevant studies were also manually searched to explore additional eligible studies.

Selection criteria

Included candidate studies had to be original and had to report the genotype frequencies in cases and controls or their estimated odds ratios (ORs) with 95 % confidence intervals (95 % CIs). Moreover, all patients had to meet the diagnostic criteria for prostate cancer and control subjects should be cancer- or disease-free. When duplicate publications from the same study were found, only the one with the most complete data was included in this meta-analysis.

Data extraction

Two investigators independently extracted the following data using a standardized table: surname of first author, year of publication, country of origin, ethnicity, basic characteristics of subjects (number, age, and sex), genotype frequency, genotyping method, methylation frequency, detection method, clinical characteristics, etc. For data not available in the publications, required information was obtained by contacting corresponding authors.

Quality assessment

The strengthening the reporting of genetic association studies (STREGA) quality score system was used to assess the

qualities of all included studies [24]. The STREGA system includes 22 assessment aspects, with STREGA scores higher than 14 indicating a moderate high quality. Two investigators independently evaluated the quality of included studies. Discrepancies about the quality score were resolved by consensus among all the authors.

Statistical analysis

All analyses were conducted using STATA 12.0 software. Hardy–Weinberg equilibrium (HWE) in the control group was tested for each data set to ensure that genotype distribution was not significantly deviated from HWE [25]. The association between *E-cadherin* −160C/A polymorphism and promoter methylation and the risk of prostate cancer was evaluated using the pooled ORs with their corresponding 95 % CIs. The *Z* test was used to assess the significance of the pooled estimates, with a *P* value less than 0.05 as a cut-off value. Between-study heterogeneity was tested using the Cochran's *Q* statistic and the I^2 metric [26, 27]. When $P>0.05$ for *Q* statistic or $I^2<50$ % indicates no significant heterogeneity, the fixed-effect model was applied to calculate the pooled ORs and 95 %CIs. Otherwise, a random-effect model was used. In addition to an overall analysis, subgroups analyses were performed based on ethnicity, source of control, detection method, Gleason score (GS), and tumor grade (TG), where applicable. Pathologic grade was determined according to the Gleason pattern and classified as low GS (6 or lower) and high GS (7 or higher). Localized cancer was defined as low TG, advanced or metastasis cancer as high TG. One-way sensitivity analyses were also conducted by omitting individual studies in turn to reflect the influence of individual data sets on the pooled results [28]. Potential publication bias was tested by Begg's funnel plot and Egger's publication plot [29, 30].

Results

Baseline characteristics of included studies

The review process of the literature research is illustrated in Fig. 1. The initial screening identified 84 potentially relevant articles and six were excluded as duplicate publications. After the title and abstract review, 48 were excluded, among which 22 were letters or reviews, 13 were not human studies, and 11 were not related to our research topic, leaving 30 articles available for further full text review. In accordance with the inclusion criteria, 22 articles were selected for this meta-analysis, 11 for *E-cadherin* −160C/A polymorphism [21, 31–40], and another 11 for promoter methylation of *E-cadherin* gene [23, 41–50]. The publication year of included studies ranged from 2000 to 2013. There were 14 studies from 11 articles that investigated the association between *E-cadherin* −160C/A polymorphism and prostate cancer risk, with eight in Caucasians, three in Asians, and the other three in Africans. Eight studies used normal controls, whereas four employed the benign prostatic hyperplasia (BPH) as controls. Eight studies differentiated prostate cancer patients based on low Gleason score (GS) or high GS, while ten classified the patients as low tumor grade (TG) and high TG. Among the 11 studies considering the association between promoter methylation of *E-cadherin* gene and the risk of prostate cancer, seven compared the methylation frequency of prostate cancer tissues with that of normal tissues, and six investigated the methylation frequency difference between low GS and high GS patients, while seven focused on the variance between low TG and high TG. The qualities of all included studies were moderately high, with STREGA scores higher than 14. Further details on the main characteristics of each study can be found in Table 1 and Table 2.

Association between *E-cadherin* −160C/A polymorphism and prostate cancer risk

Since between-study heterogeneity was obvious ($I^2>50$ % and $P<0.01$), random-effect model was applied for the overall analysis. As shown in Table 3, the overall analysis suggested that *E-cadherin* −160C/A was significantly associated with an increased risk of prostate cancer (AA vs. CC: OR=1.21, 95 % CI=1.05–1.70, $P=0.011$; CA vs. CC: OR=1.28, 95 % CI=1.05–1.55, $P=0.013$; AA+CA vs. CC: OR=1.29, 95 % CI=1.07–1.56, $P=0.009$). Stratified analysis by ethnicity found a significant association for Caucasians and Asians (Table 3; Fig. 2a). In addition, subgroup analysis based on source of control suggested that the comparison between prostate cancer patients and BPH controls was more significant than normal controls (Fig. 2b). It is worth noting that the risk allele of *E-cadherin* −160C/A was only associated with high pathologic grade of prostate cancer, including high GS (Fig. 2c) and high TG (Fig. 2d).

Association between promoter methylation of *E-cadherin* gene and the risk of prostate cancer

Random-effect model was applied for the analysis of the association between promoter methylation of *E-cadherin* gene and the risk of prostate cancer, since significant heterogeneity was also observed. The comparison between prostate cancer tissues and normal tissues suggested that the frequencies of promoter methylation in prostate cancer tissues were significantly higher than normal tissues (OR=4.85, 95 % CI=1.58–14.95, $P=0.006$), either for Caucasians (OR=4.25, 95 % CI=1.15–15.71, $P=0.030$) or Asians (OR=8.87, 95 % CI=1.48–53.06, $P=0.017$) (Fig. 3a). Furthermore, we observed a significant association in subgroups based on methylation-specific PCR (MSP), but not in non-MSP subgroups,

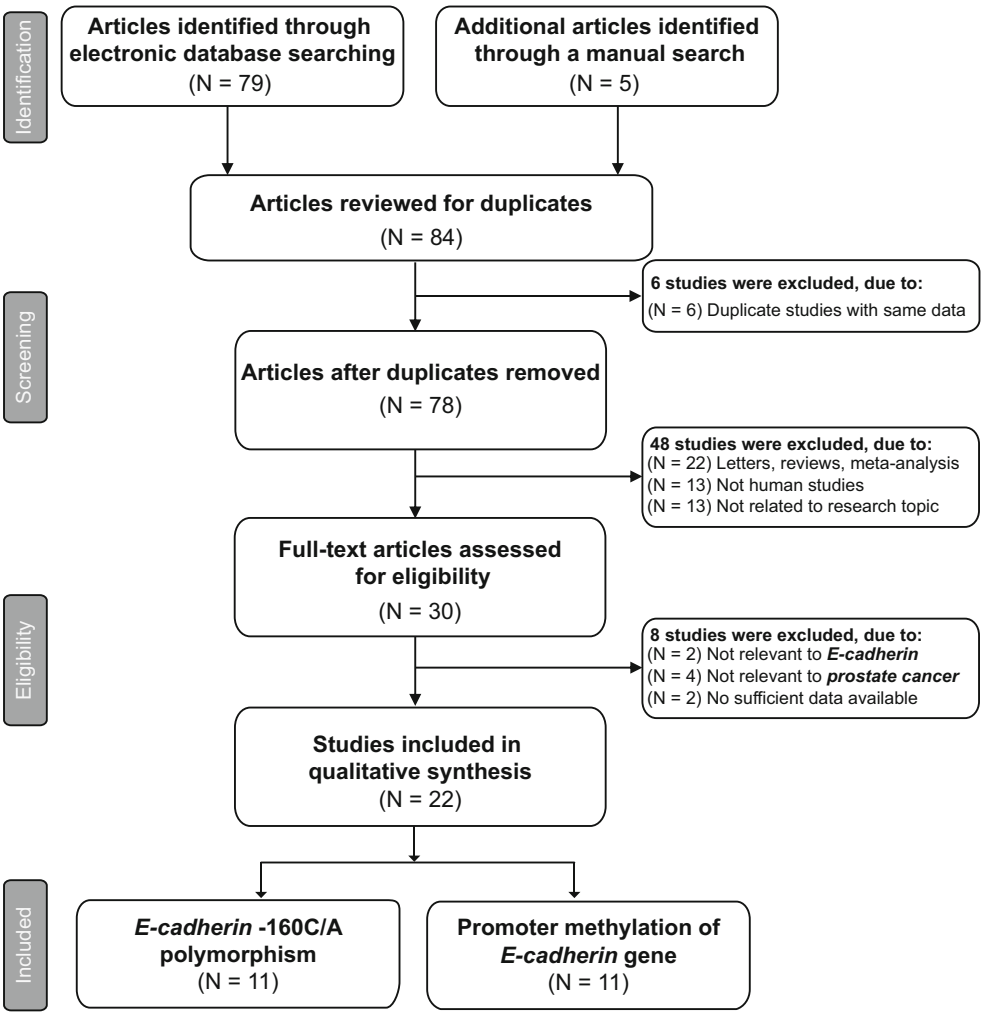

Fig. 1 Flow diagram of the selection of studies and specific reasons for exclusion from the present meta-analysis

Table 1 Main characteristics of all eligible studies for the *E-cadherin* −160C/A polymorphism

Reference	Year	Country	Ethnicity	Cases [*n*, age (year)]	Control [*n*, age (year)]	Source of control	Genotyping method	Quality score
Verhage et al. [40]	2002	Dutch	Caucasian	82, 63.8±5.8	188, 50.0±13.8	BPH	PCR-RFLP	17/22
Hajdinjak and Toplak [35]	2004	Slovenia	Caucasian	183, NA	198, NA	BPH	TaqMan	15/22
Jonsson et al. [21]	2004	Sweden	Caucasian	1,038, [43–85]	669, [48–80]	Normal	TaqMan	19/22
Tsukino et al. [39]	2004	Japan	Asian	219, mean 72.4	219, mean 72.2	Normal	PCR-RFLP	18/22
Kamoto et al. [36]	2005	Japan	Asian	236, 72.2±7.9	348, NA	BPH	PCR-RFLP	16/22
Lindstrom et al. [37]	2005	Sweden	Caucasian	1,636, NA	801, mean 67.8	Normal	DASH-PCR	16/22
Bonilla et al. [31], C-01	2006	USA	African	119, 65.1±0.9	112, 67.2±1.1	Normal	PCR-RFLP	20/22
Bonilla et al. [31], C-02	2006	Jamaica	African	89, 67.1±1.4	123, 65.4±1.0	Normal	PCR-RFLP	20/22
Bonilla et al. [31], C-03	2006	USA	Caucasian	219, 61.0±0.6	102, 63.9±1.0	Normal	PCR-RFLP	20/22
Pookot et al. [38], D-01	2006	USA	African	49, NA	117, NA	Normal	PCR-RFLP	18/22
Pookot et al. [38], D-02	2006	USA	Caucasian	86, NA	120, NA	Normal	PCR-RFLP	18/22
Cybulski et al. [32]	2007	Poland	Caucasian	506, [43–92]	511, NA	Normal	PCR-RFLP	16/22
Goto et al. [34]	2007	Japan	Asian	200, 72.7±7.5	159, 74.5±8.6	BPH	PCR-RFLP	17/22
Forszt et al. [33]	2009	Poland	Caucasian	100, 75.0±7.6	100, NA	Normal	Multiplex-PCR	16/22

NA not available, *BPH* benign prostatic hyperplasia, *PCR* polymerase chain reaction, *RFLP* restriction fragment length polymorphism, *DASH* dynamic allele-specific hybridization

Table 2 Main characteristics and methylation data of all eligible studies for the promoter methylation of *E-cadherin* gene

Reference	Year	Country	Ethnicity	Sample size	Method	Prostate cancer		Normal control		High GS		Low GS		High TG		Low TG		Quality score
						Meth	Unmeth	Meth	Unmeth	Meth	Unmeth	Score	Unmeth	Meth	Unmeth	Meth	Unmeth	
Li et al. [42]	2000	China	Asian	40	BGS	NA	NA	NA	NA	NA	NA	NA	NA	14	6	5	15	14/22
Kallakury BV [23]	2001	USA	Caucasian	12	MSP	NA	NA	NA	NA	5	1	4	2	6	1	3	1	16/22
Li et al. [43]	2001	USA	Caucasian	35	BGS	NA	NA	NA	NA	NA	NA	NA	NA	5	10	14	6	15/22
Maruyama et al. [44]	2002	USA	Caucasian	133	MSP	27	74	8	24	17	48	9	27	11	23	6	20	20/22
Singal et al. [45]	2004	USA	Caucasian	123	MSP	49	32	6	36	28	15	17	13	16	8	30	21	19/22
Woodson et al. [49]	2004	USA	Caucasian	83	MSP	NA	NA	NA	NA	11	16	9	47	5	18	14	49	16/22
Hoque et al. [41]	2005	USA	Caucasian	143	QMSP	40	12	5	86	4	5	36	9	24	4	16	8	20/22
Yao et al. [50]	2006	China	Asian	43	MSP	6	14	1	22	4	7	2	9	5	4	1	10	18/22
Tilandyova et al. [46]	2010	Slovakia	Caucasian	188	MSP	35	50	21	82	NA	NA	NA	NA	NA	NA	NA	NA	14/22
Vasiljevic et al. [48]	2011	Multi	Caucasian	56	Pyrosequencing	9	18	10	19	NA	NA	NA	NA	NA	NA	NA	NA	15/22
Tong et al. [47]	2013	China	Asian	50	Pyrosequencing	25	0	22	3	NA	NA	NA	NA	NA	NA	NA	NA	14/22

GS Gleason score, *TG* tumor stage, *Multi* multination, *Meth* methylation number, *Unmeth* unmethylation number, *NA* not applicable, *BGS* bisulfite genome sequencing, *QMSP* quantitative methylation specific PCR, *MSP* methylation specific PCR

indicating MSP may be a more promising method to detect methylation status (Fig. 3b). However, no significant results were found in the comparison between low GS and high GS tissues (Fig. 3c), as well as the comparison between low TG and high TG tissues (Fig. 3d).

Sensitivity analyses and publication bias

One-way sensitivity analyses were performed to determine the influence of individual data sets on the pooled ORs by sequentially removing each eligible study. With the omission of each study, pooled estimates remained virtually the same, indicating that no single study heavily influenced summary ORs, either for polymorphism analysis (Fig. 4a) or methylation analysis (Fig. 4b). Begg's funnel plots and Egger's publication plots were used to assess the potential for publication bias in included studies. The shapes of the plots did not reveal any evidence of obvious asymmetry, with the *P* value larger than 0.10 for each plot (Fig. 5). The above tests indicated a promising level of robustness and accuracy for the results of this meta-analysis.

Discussion

Recently, genetic variants of the *E-cadherin* gene in the etiology of several cancers have drawn increasing attention. Growing number of studies have suggested that −160A in the promoter region of the *E-cadherin* gene was emerging as a low penetrance tumor susceptibility allele in the development of several kinds of cancer, such as prostate cancer, urothelial cancer, and gastric cancer. Prostate cancer is still one of the most common male malignancies [1, 2]. Heredity factors are supposed to play important roles in the tumorigenesis of prostate cancer [51, 52]. It has been generally accepted that gene mutation and promoter methylation can suppress transcriptional expression of tumor suppressor gene and result in the development of malignant tumors including prostate cancer. A number of studies have reported a function of the *E-cadherin* −160 C/A polymorphism in prostate cancer risk with inconclusive results. Emerging evidence has reported the role of *E-cadherin* −160C/A polymorphism and promoter methylation in the risk of prostate cancer. However, their results have been inconclusive, possibly partially due to the small effects of these risk factors on prostate carcinoma and limited statistical power resulting from the relatively small sample sizes of individual studies. To our knowledge, the present meta-analysis is the most comprehensive overview of the association between *E-cadherin* −160C/A polymorphism and promoter methylation and the risk of prostate cancer.

For the polymorphism of *E-cadherin* gene, the overall analysis of our meta-analysis showed that *E-cadherin* −160C/A was significantly associated with an increased risk of prostate

Table 3 Meta-analysis of the association between the *E-cadherin* −160C/A polymorphism and the risk of prostate cancer

SNP	No. of study	AA vs. CC				CA vs. CC				AA+CA vs. CC			
		OR (95 % CI)	P_{OR}	P_h	I^2	OR (95 % CI)	P_{OR}	P_h	I^2	OR (95 % CI)	P_{OR}	P_h	I^2
Overall	14	1.21 (1.05–1.70)	0.011	<0.001	70.3 %	1.28 (1.05–1.55)	0.013	<0.001	70.7 %	1.29 (1.07–1.56)	0.009	<0.001	72.8 %
Ethnicity													
Caucasian	8	1.25 (1.06–1.47)	0.007	0.002	69.1 %	1.40 (1.13–1.74)	0.002	0.002	68.9 %	1.40 (1.13–1.74)	0.002	0.001	71.5 %
Asian	3	1.47 (1.15–1.89)	0.002	0.210	36.0 %	1.51 (1.14–2.00)	0.004	0.247	28.5 %	1.56 (1.16–2.09)	0.003	0.198	38.2 %
African	3	0.70 (0.44–1.11)	0.131	0.126	51.7 %	0.59 (0.31–1.11)	0.102	0.073	61.7 %	0.62 (0.34–1.12)	0.110	0.079	60.7 %
Source of control													
Healthy	10	1.10 (0.94–1.28)	0.247	0.004	63.2 %	1.14 (0.94–1.39)	0.175	0.007	60.6 %	1.14 (0.94–1.38)	0.188	0.003	64.0 %
BPH	4	1.56 (1.23–1.96)	<0.001	0.153	43.1 %	1.78 (1.08–2.94)	0.024	0.002	79.5 %	1.83 (1.17–2.85)	0.008	0.006	75.6 %
Genotype method													
PCR-RFLP	10	1.23 (0.97–1.56)	0.089	<0.001	75.6 %	1.25 (0.92–1.70)	0.157	<0.001	76.3 %	1.28 (0.94–1.74)	0.113	<0.001	78.3 %
Non-PCR-RFLP	4	1.10 (0.97–1.26)	0.149	0.192	36.7 %	1.25 (1.01–1.54)	0.037	0.100	52.0 %	1.21 (1.00–1.46)	0.045	0.131	46.8 %
Gleason score (GS)													
Low GS	4	0.88 (0.48–1.64)	0.692	0.004	77.2 %	1.41 (0.73–2.71)	0.304	0.261	25.1 %	1.01 (0.74–1.38)	0.966	0.002	80.2 %
High GS	4	1.61 (1.07–2.42)	0.023	0.053	61.1 %	2.55 (1.44–4.52)	0.001	0.718	0.00 %	1.59 (1.18–2.15)	0.003	0.014	71.8 %
Tumor grade (TG)													
Low TG	5	1.20 (0.80–1.80)	0.383	0.014	68.0 %	1.03 (0.58–1.84)	0.908	0.004	73.6 %	1.12 (0.65–1.92)	0.678	0.004	74.1 %
High TG	5	1.56 (1.17–2.08)	0.003	0.165	38.4 %	1.56 (1.12–2.18)	0.009	0.237	27.7 %	1.62 (1.14–2.30)	0.007	0.162	38.9 %

OR odd ratio, *CI* confidence interval, P_{OR} *P* value of odd ratio, P_h *P* value of heterogeneity test, *PCR* polymerase chain reaction, *RFLP* restriction fragment length polymorphism, *BPH* benign prostatic hyperplasia, *GS* Gleason score, *TS* tumor stage

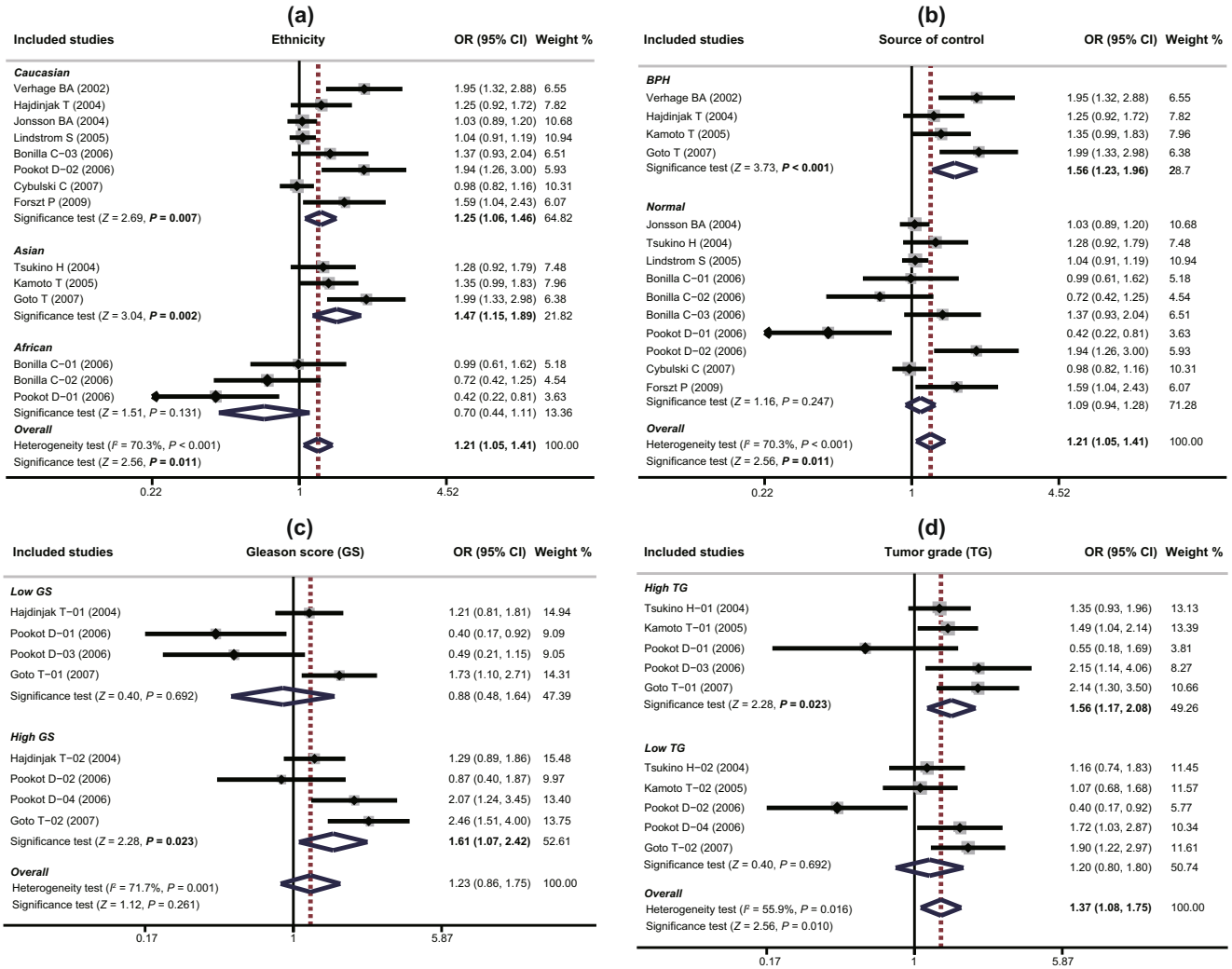

Fig. 2 Forest plots of ORs for the association between *E-cadherin* −160C/A polymorphism and prostate cancer risk (**a** ethnicity, **b** source of control, **c** Gleason score, **d** tumor grade)

cancer. Since the same polymorphism may have different roles in cancer susceptibility for different ethnicities, subgroup analyses based on ethnicity were performed. Our results indicated that risk appeared to be significant in Europeans and Asians but not in Africans, suggesting a possible role of ethnic differences in their heredity background and living environment. The influence of the risk allele in Africans might be masked by the presence of other unidentified causal genes involved in prostate tumorigenesis. In addition, this variance may be just due to chance since small sample size studies lack sufficient statistical power to detect a slight effect. Considering the limited number and sample sizes of studies on Africans included in this meta-analysis, more large-scale studies are warranted to confirm our results. Interestingly, we also found that the risk allele of *E-cadherin* −160C/A was only associated with high pathologic grade of prostate cancer. A potential explanation may be that this genetic mutation may begin to play a role only at the advanced stage of prostate cancer and thus further aggravate the progression of this disease.

Our analyses of the association between promoter methylation of *E-cadherin* gene and the risk of prostate cancer suggested that the frequencies of promoter methylation in prostate cancer tissues were significantly higher than normal tissues. It is reasonable to consider that transcriptional inactivation attributed to promoter methylation may lead to the malignant proliferation of prostate tissues. No ethnicity variance was found for the methylation analysis, suggesting that promoter methylation of *E-cadherin* gene may threaten the health of men all over the world. Our results suggested that MSP appears to be superior in comparison to the other methods compared in this meta-analysis, which also provide evidence for the extensive use of this method in clinical context. Unfortunately, we did not find any significant difference when comparing different pathologic grade tissues of prostate cancer, although we are expecting to find a correlation between the clinical stage or pathologic grade of prostate cancer and promoter methylation of *E-cadherin* gene. A possible explanation to this result may be that promoter methylation status of *E-cadherin* gene is irrelevant to the

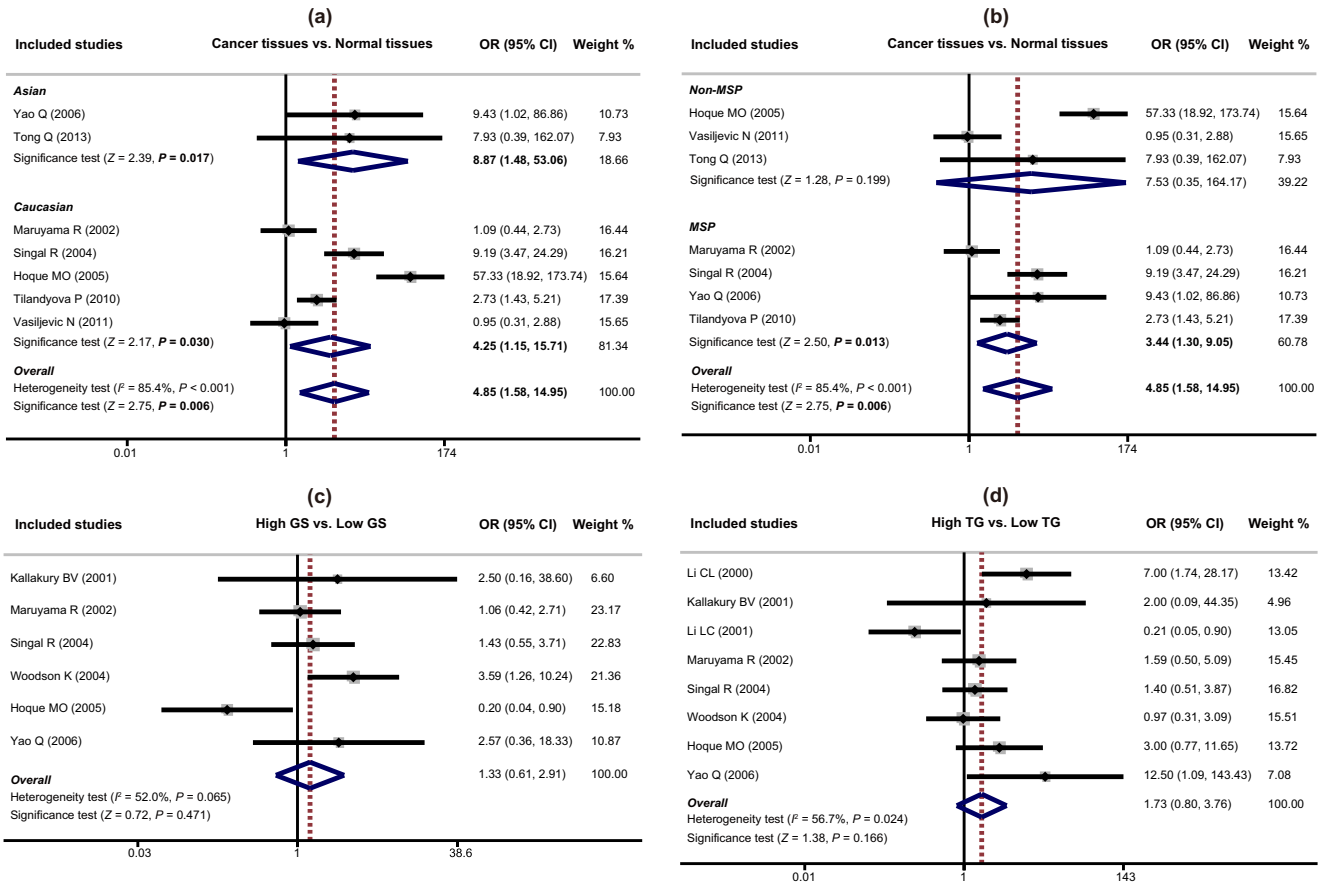

Fig. 3 Forest plots of ORs for the association between promoter methylation of *E-cadherin* gene and the risk of prostate cancer (**a** ethnicity, **b** detection method, **c** Gleason score, **d** tumor grade)

progression of prostate cancer, but just related to its tumorigenesis. However, it should be mentioned that classification for prostate cancer based on Gleason score or tumor stage as low and high may be not accurate to detect a correlation between clinical stage and promoter methylation of E-cadherin. Hence, further investigations should be conducted to differentiate prostate cancer more specifically.

Although several meta-analyses about this association between the *E-cadherin* −160C/A polymorphism and prostate cancer have been conducted previously, it is the most comprehensive systematic review of this association. For instance, Wang et al. [53] conducted the first general overview to assess susceptibility of *E-cadherin* −160C>A to seven types of cancers. For prostate cancer, eight studies were included in

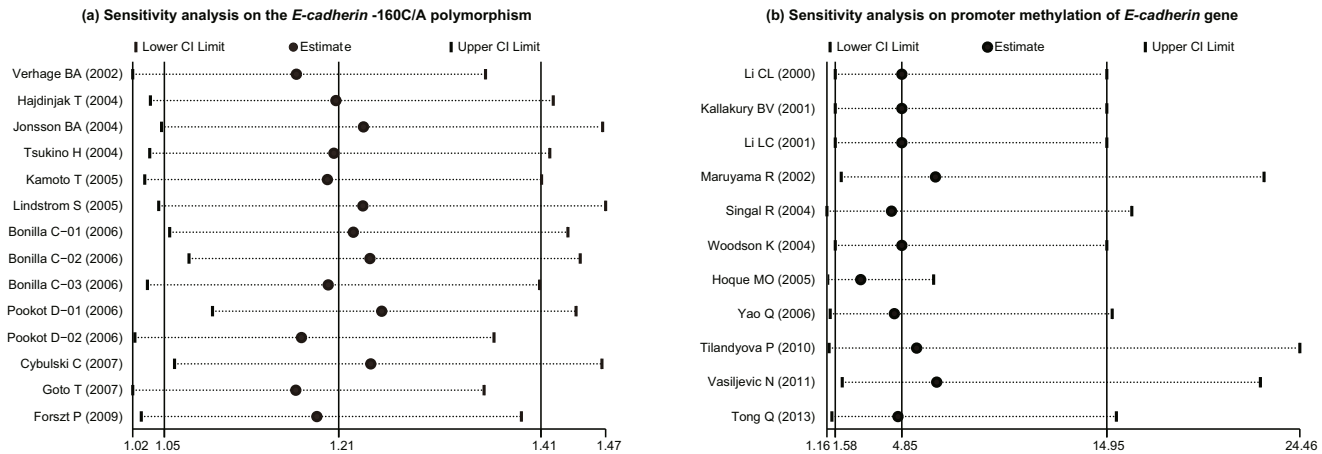

Fig. 4 One-way sensitivity analysis for the robustness of the results in the meta-analysis (**a** *E-cadherin* −160C/A polymorphism, **b** promoter methylation of *E-cadherin* gene)

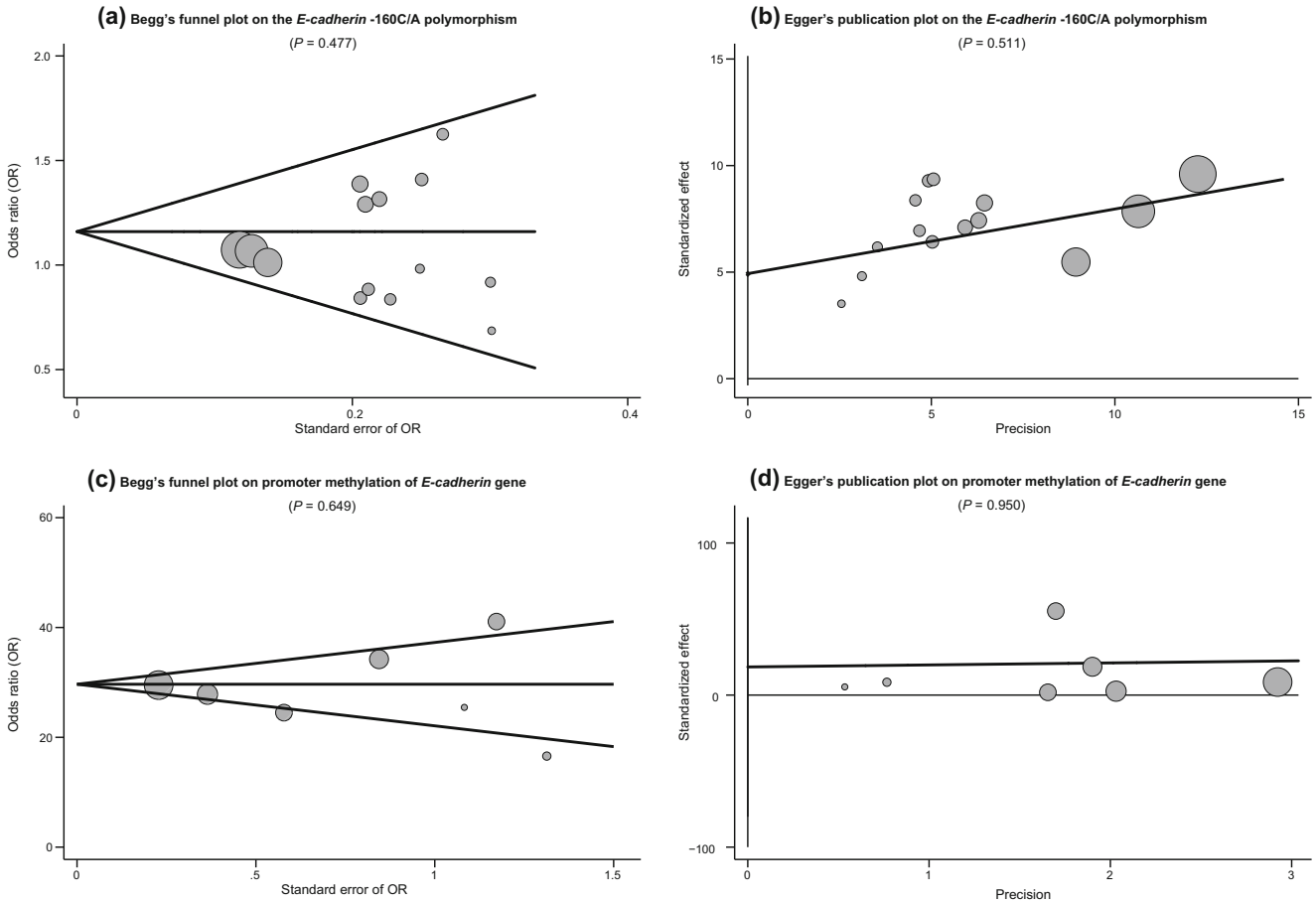

Fig. 5 Begg's funnel plots and Egger's publication plots for the association between *E-cadherin* gene and susceptibility to prostate cancer (**a** Begg's funnel plot on the *E-cadherin* −160C/A polymorphism, **b** Egger's publication plot on the *E-cadherin* −160C/A polymorphism, **c** Begg's funnel plot on the promoter methylation of *E-cadherin* gene, **d** Egger's publication plot on the promoter methylation of *E-cadherin* gene)

their meta-analysis. Since then, four new studies have been conducted and should be included in the updated meta-analysis. Wang et al. [54] further performed a meta-analysis of 47 case–control studies on the association between *E-cadherin* −160C>A and 16 types of cancers. Still, a study conducted by Forszt et al. [33] was left out, which should be included according to inclusion criteria. Although two more meta-analyses focusing on the association between *E-cadherin* 160C/A polymorphism and prostate cancer risk were conducted, their included studies are far from comprehensive. Qiu et al. [55] missed three eligible studies, while the most recent meta-analysis conducted by Deng et al. [56] even left out four eligible ones. Our meta-analysis comprehensively searched all related studies, and 14 studies from 11 publications were involved.

Our study has several methodological advantages. This study is the first meta-analysis attempt to explore the interaction between promoter methylation of *E-cadherin* gene and the risk of prostate cancer, although there are some systematic reviews considering the role of *E-cadherin* −160C/A polymorphism in prostate cancer susceptibility. We also are the first to investigate variances in source of control, genotyping method, and pathologic grade on this association in our meta-analysis. Furthermore, sensitivity analyses and publication bias were used to confirm the robustness of our results. Several potential limitations of our study also warrant mention. First, although we tried to incorporate all relevant studies, we may still have left out useful publications during screening process. Second, in the subgroup analysis by ethnicity, the number of Africans was relatively small and did not have enough statistical power to analyze the association. Last, our results were based on unadjusted estimates, while a more precise analysis should be conducted if individual data were available, which would allow for adjustment by other covariates including age, environmental factors, and lifestyle. Hence, further investigations are warranted to combine these covariates into analysis.

Despite these limitations, the present meta-analysis supports growing evidence of *E-cadherin* −160C/A polymorphism and promoter methylation in the risk of prostate cancer, especially in Europeans and Asians. We believe that identifying these risk factors would be informative in prostate cancer progression. Moreover, gene–gene and gene–environment interactions should also be considered in further investigations.

Such studies taking these factors into account may eventually lead to a more comprehensive understanding of this association.

Conflicts of interest None

References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011;61:69–90.
2. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. CA Cancer J Clin. 2012;62:10–29.
3. Filella X, Alcover J, Molina R. Active surveillance in prostate cancer: the need to standardize. Tumour Biol. 2011;32:839–43.
4. Wong YN, Mitra N, Hudes G, Localio R, Schwartz JS, Wan F, et al. Survival associated with treatment vs observation of localized prostate cancer in elderly men. JAMA. 2006;296:2683–93.
5. Gotay CC, Holup JL, Pagano I. Ethnic differences in quality of life among early breast and prostate cancer survivors. Psychooncology. 2002;11:103–13.
6. Gronberg H. Prostate cancer epidemiology. Lancet. 2003;361:859–64.
7. Sankpal UT, Pius H, Khan M, Shukoor MI, Maliakal P, Lee CM, et al. Environmental factors in causing human cancers: emphasis on tumorigenesis. Tumour Biol. 2012;33:1265–74.
8. Thomas G, Jacobs KB, Yeager M, Kraft P, Wacholder S, Orr N, et al. Multiple loci identified in a genome-wide association study of prostate cancer. Nat Genet. 2008;40:310–5.
9. Zheng SL, Sun J, Wiklund F, Smith S, Stattin P, Li G, et al. Cumulative association of five genetic variants with prostate cancer. N Engl J Med. 2008;358:910–9.
10. Hunter DJ, Riboli E, Haiman CA, Albanes D, Altshuler D, Chanock SJ, et al. A candidate gene approach to searching for low-penetrance breast and prostate cancer genes. Nat Rev Cancer. 2005;5:977–85.
11. Shields PG, Harris CC. Cancer risk and low-penetrance susceptibility genes in gene–environment interactions. J Clin Oncol. 2000;18:2309–15.
12. Berx G, Becker KF, Hofler H, van Roy F. Mutations of the human E-cadherin (CDH1) gene. Hum Mutat. 1998;12:226–37.
13. More H, Humar B, Weber W, Ward R, Christian A, Lintott C, et al. Identification of seven novel germline mutations in the human E-cadherin (CDH1) gene. Hum Mutat. 2007;28:203.
14. Kuefer R, Hofer MD, Gschwend JE, Pienta KJ, Sanda MG, Chinnaiyan AM, et al. The role of an 80 kDa fragment of E-cadherin in the metastatic progression of prostate cancer. Clin Cancer Res. 2003;9:6447–52.
15. Onder TT, Gupta PB, Mani SA, Yang J, Lander ES, Weinberg RA. Loss of E-cadherin promotes metastasis via multiple downstream transcriptional pathways. Cancer Res. 2008;68:3645–54.
16. Wijnhoven BP, Dinjens WN, Pignatelli M. E-cadherin–catenin cell–cell adhesion complex and human cancer. Br J Surg. 2000;87:992–1005.
17. Boggon TJ, Murray J, Chappuis-Flament S, Wong E, Gumbiner BM, Shapiro L. C-cadherin ectodomain structure and implications for cell adhesion mechanisms. Science. 2002;296:1308–13.
18. Baranwal S, Alahari SK. Molecular mechanisms controlling E-cadherin expression in breast cancer. Biochem Biophys Res Commun. 2009;384:6–11.
19. Chen B, Zhou Y, Yang P, Liu L, Qin XP, Wu XT. CDH1–160C>A gene polymorphism is an ethnicity-dependent risk factor for gastric cancer. Cytokine. 2011;55:266–73.
20. von Burstin J, Eser S, Paul MC, Seidler B, Brandl M, Messer M, et al. E-cadherin regulates metastasis of pancreatic cancer in vivo and is suppressed by a SNAIL/HDAC1/HDAC2 repressor complex. Gastroenterology. 2009;137:361–71, 71 e1–5.
21. Jonsson BA, Adami HO, Hagglund M, Bergh A, Goransson I, Stattin P, et al. −160C/A polymorphism in the E-cadherin gene promoter and risk of hereditary, familial and sporadic prostate cancer. Int J Cancer. 2004;109:348–52.
22. Li LC, Chui RM, Sasaki M, Nakajima K, Perinchery G, Au HC, et al. A single nucleotide polymorphism in the E-cadherin gene promoter alters transcriptional activities. Cancer Res. 2000;60:873–6.
23. Kallakury BV, Sheehan CE, Winn-Deen E, Oliver J, Fisher HA, Kaufman Jr RP, et al. Decreased expression of catenins (alpha and beta), p120 CTN, and E-cadherin cell adhesion proteins and E-cadherin gene promoter methylation in prostatic adenocarcinomas. Cancer. 2001;92:2786–95.
24. Little J, Higgins JP, Ioannidis JP, Moher D, Gagnon F, von Elm E, et al. Strengthening the reporting of genetic association studies (STREGA): an extension of the STROBE statement. Hum Genet. 2009;125:131–51.
25. Teo YY, Fry AE, Clark TG, Tai ES, Seielstad M. On the usage of HWE for identifying genotyping errors. Ann Hum Genet. 2007;71:701–3. author reply 4.
26. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med. 2002;21:1539–58.
27. Jackson D, White IR, Riley RD. Quantifying the impact of between-study heterogeneity in multivariate meta-analyses. Stat Med. 2012;31:3805–20.
28. Sacks HS, Berrier J, Reitman D, Ancona-Berk VA, Chalmers TC. Meta-analyses of randomized controlled trials. N Engl J Med. 1987;316:450–5.
29. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ. 1997;315:629–34.
30. Peters JL, Sutton AJ, Jones DR, Abrams KR, Rushton L. Comparison of two methods to detect publication bias in meta-analysis. JAMA. 2006;295:676–80.
31. Bonilla C, Mason T, Long L, Ahaghotu C, Chen W, Zhao A, et al. E-cadherin polymorphisms and haplotypes influence risk for prostate cancer. Prostate. 2006;66:546–56.
32. Cybulski C, Wokolorczyk D, Jakubowska A, Gliniewicz B, Sikorski A, Huzarski T, et al. DNA variation in MSR1, RNASEL and E-cadherin genes and prostate cancer in Poland. Urol Int. 2007;79:44–9.
33. Forszt P, Pilecka A, Malodobra M, Markowska J, Maksymowicz K, Dobosz T. Single-nucleotide polymorphism association study of VDR and CDH1 genes and the risk of prostate cancer. Adv Clin Exp Med. 2009;18:215–20.
34. Goto T, Nakano M, Ito S, Ehara H, Yamamoto N, Deguchi T. Significance of an E-cadherin gene promoter polymorphism for risk and disease severity of prostate cancer in a Japanese population. Urology. 2007;70:127–30.
35. Hajdinjak T, Toplak N. E-cadherin polymorphism—−160 C/A and prostate cancer. Int J Cancer. 2004;109:480–1.
36. Kamoto T, Isogawa Y, Shimizu Y, Minamiguchi S, Kinoshita H, Kakehi Y, et al. Association of a genetic polymorphism of the E-cadherin gene with prostate cancer in a Japanese population. Jpn J Clin Oncol. 2005;35:158–61.
37. Lindstrom S, Wiklund F, Jonsson BA, Adami HO, Balter K, Brookes AJ, et al. Comprehensive genetic evaluation of common E-cadherin sequence variants and prostate cancer risk: strong confirmation of functional promoter SNP. Hum Genet. 2005;118:339–47.
38. Pookot D, Li LC, Tabatabai ZL, Tanaka Y, Greene KL, Dahiya R. The E-cadherin −160 C/A polymorphism and prostate cancer risk in white and black American men. J Urol. 2006;176:793–6.

39. Tsukino H, Kuroda Y, Imai H, Nakao H, Qiu D, Komiya Y, et al. Lack of evidence for the association of E-cadherin gene polymorphism with increased risk or progression of prostate cancer. Urol Int. 2004;72:203–7.
40. Verhage BA, van Houwelingen K, Ruijter TE, Kiemeney LA, Schalken JA. Single-nucleotide polymorphism in the E-cadherin gene promoter modifies the risk of prostate cancer. Int J Cancer. 2002;100:683–5.
41. Hoque MO, Topaloglu O, Begum S, Henrique R, Rosenbaum E, Van Criekinge W, et al. Quantitative methylation-specific polymerase chain reaction gene patterns in urine sediment distinguish prostate cancer patients from control subjects. J Clin Oncol. 2005;23:6569–75.
42. Li C, Zhang X, Chen Z, Ouyang J, Du G, Yang W, et al. A specific methylation pattern of the E-cadherin gene promoter is associated with its expression and the pathological grades in prostate cancer. Chin J Exp Surg. 2000;17:47–8.
43. Li LC, Zhao H, Nakajima K, Oh BR, Ribeiro Filho LA, Carroll P, et al. Methylation of the E-cadherin gene promoter correlates with progression of prostate cancer. J Urol. 2001;166:705–9.
44. Maruyama R, Toyooka S, Toyooka KO, Virmani AK, Zochbauer-Muller S, Farinas AJ, et al. Aberrant promoter methylation profile of prostate cancers and its relationship to clinicopathological features. Clin Cancer Res. 2002;8:514–9.
45. Singal R, Ferdinand L, Reis IM, Schlesselman JJ. Methylation of multiple genes in prostate cancer and the relationship with clinicopathological features of disease. Oncol Rep. 2004;12:631–7.
46. Tilandyova P, Grobarcikova S, Kajo K, Kliment J, Lasabova Z, Zubor P, et al. Detection of methylation of GSTP1, E-cad and PTGS2 in prostate adenocarcinoma and benign prostate hyperplasia. European Urology. 2010;Supplement 9:626–7.
47. Tong Q, Qiu J, Yao L, Huang J, Liu J, Sun R, et al. Pyrosequencing detection of aberrant methylation of GSTP1, CDH1 and p16 genes in prostate cancer. Chin J Med Off. 2013;41:577–9.
48. Vasiljevic N, Wu K, Brentnall AR, Kim DC, Thorat MA, Kudahetti SC, et al. Absolute quantitation of DNA methylation of 28 candidate genes in prostate cancer using pyrosequencing. Dis Markers. 2011;30:151–61.
49. Woodson K, Hanson J, Tangrea J. A survey of gene-specific methylation in human prostate cancer among black and white men. Cancer Lett. 2004;205:181–8.
50. Yao Q, He XS, Zhang JM, He J. Promotor hypermethylation of E-cadherin, p16 and estrogen receptor in prostate carcinoma. Zhonghua Nan Ke Xue. 2006;12:28–31.
51. Abate-Shen C, Shen MM. Molecular genetics of prostate cancer. Genes Dev. 2000;14:2410–34.
52. Shen MM, Abate-Shen C. Molecular genetics of prostate cancer: new prospects for old challenges. Genes Dev. 2010;24:1967–2000.
53. Wang GY, Lu CQ, Zhang RM, Hu XH, Luo ZW. The E-cadherin gene polymorphism 160C→A and cancer risk: a HuGE review and meta-analysis of 26 case–control studies. Am J Epidemiol. 2008;167:7–14. doi:10.1093/aje/kwm264.
54. Wang L, Wang G, Lu C, Feng B, Kang J. Contribution of the −160C/A polymorphism in the E-cadherin promoter to cancer risk: a meta-analysis of 47 case–control studies. PLoS One. 2012;7:e40219. doi:10.1371/journal.pone.0040219.
55. Qiu LX, Li RT, Zhang JB, Zhong WZ, Bai JL, Liu BR, et al. The E-cadherin (CDH1)—−160 C/A polymorphism and prostate cancer risk: a meta-analysis. Eur J Hum Genet. 2009;17:244–9. doi:10.1038/ejhg.2008.157.
56. Deng QW, He BS, Pan YQ, Sun HL, Xu YQ, Gao TY, et al. Roles of E-cadherin (CDH1) genetic variations in cancer risk: a meta-analysis. Asian Pac J Cancer Prev. 2014;15:3705–13.