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ORIGINAL RESEARCH

Effects of four single nucleotide polymorphisms of *EZH2* on cancer risk: a systematic review and meta-analysis

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Background: Although the relationship between several single nucleotide polymorphisms (SNPs) of the oncogene *EZH2* and cancer risk has been assessed by some case–control studies, results of subsequent studies are controversial. Sample sizes from single-center studies are also limited, thereby providing unreliable findings. Hence, we conducted a comprehensive search and meta-analysis to evaluate the associations between *EZH2* SNPs and cancer risk.

Materials and methods: A comprehensive literature search for studies focusing on *EZH2* SNPs and cancer risk was conducted on PubMed, Web of Science, Embase, and China National Knowledge Infrastructure online databases. Genotype data were extracted and examined through a meta-analysis, and pooled odds ratios (ORs) with 95% CIs were used to assess the corresponding associations. Sensitivity analysis, publication bias assessment, and heterogeneity test were performed using STATA 12.0.

Results: Twelve eligible studies were included in this meta-analysis. The association of 4 SNPs, namely, rs887569, rs2302427, rs3757441, and rs41277434, in the *EZH2* locus with cancer risk was evaluated. Five studies (1,794 cases and 1,878 controls) indicated that rs887569 was related to a decreased cancer risk (CTTT/CC: OR =0.849, 95% CI: [0.740 to 0.973], *P*=0.019; TT/CCCT: OR =0.793, 95% CI: [0.654 to 0.962], *P*=0.019). Seven studies (2,408 cases and 2,910 controls) showed that rs2302427 was linked to a decreased cancer risk (GG/CC: OR =0.562, 95% CI: [0.400 to 0.792], *P*=0.001; CGGG/CC: OR =0.856, 95% CI: [0.748 to 0.980], *P*=0.024; GG/CCCG: OR =0.733, 95% CI: [0.571 to 0.940], *P*=0.015). No relationships were observed between rs3757441 or rs41277434 and cancer risk.

Conclusion: rs887569 and rs2302427 in *EZH2* may be correlated with a decreased cancer risk. Although rs3757441 and rs41277434 are independent risk factors of cancer, further large-scale and functional studies are warranted to validate our findings.

Keywords: EZH2, single nucleotide polymorphism, cancer risk, meta-analysis

Introduction

Approximately 1,688,780 new cancer cases and 600,920 cancer deaths are projected to occur in the USA in 2017.[1] Cancer is caused by uncontrolled cell division or inappropriate survival of a cell with DNA damage, which is critical for tumor initiation and progression.

Thousands of genes that are either transcriptionally upregulated or downregulated in tumor samples have been identified through microarray analysis, indicating that cancer is a disease with extreme heterogeneity. These deregulations act as the main drivers that enable tumors to invade cellular barriers, proliferate, and metastasize.[2] The dynamic regulation of histone modifications in promoters and enhancers plays a vital role in the

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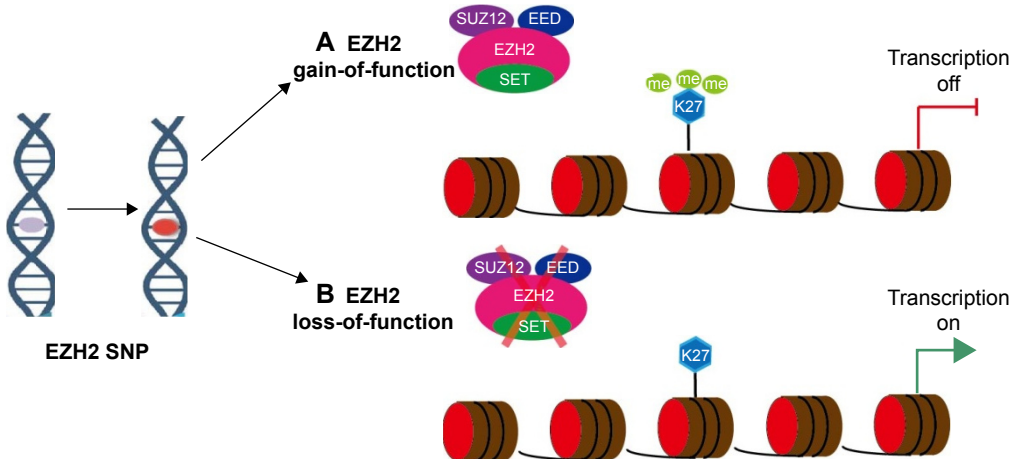

Figure 1 EZH2 polymorphism affects transcription of downstream targets.
Abbreviation: SNP, single nucleotide polymorphism.

control of gene expression and consequently affects disease susceptibility. EZH2 has been widely investigated because it serves as a master regulator of cancer epigenetics.[3] It is also a core component of Polycomb repressive complex 2, which mainly methylates lysine 27 of histone H3 (H3K27) to induce transcriptional gene silencing.[4] EZH2 overexpression causes epigenetic alterations in tumor suppressor genes, and such changes are required for cancer proliferation, migration, invasion, and metastasis.[5–7] Therefore, aberrant EZH2 activities may participate in increasing the risk of tumorigenesis.

The oncogenic role of EZH2 has been observed in numerous cancers, including prostate cancer, bladder cancer, breast cancer, and melanoma, whose high EZH2 expression levels are positively correlated with poor survival rate and aggressiveness.[8–11] The function of EZH2 in cancer progression may also be affected by mutations. For example, the mutation of tyrosine 641 (Y641) within the C-terminal catalytic SET domain of EZH2 increases the levels of trimethylated H3K27 (H3K27me3) and thus represses the expression of Polycomb targets.[12] The loss-of-function mutations of *EZH2* may occur during cancer development. The frequency of missense mutations of *EZH2* in the pediatric subtype of human T-cell acute lymphoblastic leukemia (T-ALL) and early T-cell precursor (ETP) ALL is higher than that in non-ETP pediatric T-ALL.[13,14] Similarly, single nucleotide polymorphisms (SNPs) of *EZH2* may have different effects on disease susceptibility through the transcriptional regulation of genes involved in cancer initiation and progression (Figure 1). Although several studies have investigated the relationship of 4 SNPs (rs887569 C>T, rs2302427 C>G, rs3757441 T>C, and rs41277434 A>C) of *EZH2* and cancer risk, results are inconsistent. This relationship has yet to be systematically investigated, and definitive conclusions have yet to be presented. Hence, comprehensive reviews and meta-analyses should be performed. Here, we conducted a meta-analysis to precisely assess and provide a comprehensive conclusion about the associations between *EZH2* variations and cancer risk from all eligible case–control studies published to date.

Materials and methods

Search strategy and identification of eligible studies

Two reviewers (Ling and You) searched the online databases PubMed, Google Scholar, Web of Science, Embase, China National Knowledge Infrastructure (CNKI), and Wangfang Data to identify relevant articles published until September 2017. The following search terms were used either separately or in combination: "EZH2, enhancer of zeste homolog 2," "rs887569, rs2302427, rs3757441, rs41277434," "cancer, carcinoma, neoplasm," "tumor, tumour," and "SNP, polymorphism, allele, variation." Studies were limited to articles published in Chinese or English, and the references of pertinent articles were manually screened and checked. Articles that satisfied the following criteria were included: 1) studies that assessed the association between a SNP from EZH2 (rs887569, rs2302427, rs3757441, and rs41277434) and cancer risk; 2) case–control or population-based studies; and 3) studies with available genotype frequencies. Studies were excluded according to the following criteria: 1) articles that were presented as a systematic review or focusing on animals; 2) studies that involved DNA extracted from cancer tissues rather than blood samples, or studies that did not provide usable data for meta-analysis; and 3) studies that

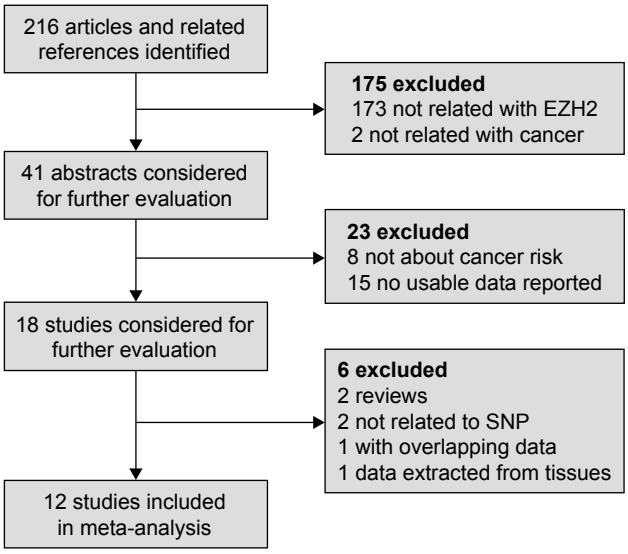

Figure 2 Studies identified with criteria of inclusion and exclusion.
Abbreviation: SNP, single nucleotide polymorphism.

reported data overlapping with those described in the included studies.

Data extraction

Two reviewers (Ling and You) independently extracted the following information from each study: first author, year of publication, cancer types, country or region, ethnicity, genotype detection method, control source of each study, number of cases and controls, polymorphism site included in each study, and results of Hardy–Weinberg equilibrium (HWE). Inconsistencies were resolved by discussion until a consensus was obtained. Newcastle–Ottawa Quality Assessment Scale was used to examine the quality of the articles included in this study.[15]

Statistical analysis

The strength of the association between SNPs and cancer risk was evaluated by determining the odds ratio (OR) with 95% CI, which was calculated by *Z*-test, and the result of the pooled OR was considered significant when $P<0.05$. This association was also examined by using homozygote, heterozygote, dominant genetic, and recessive genetic models. Subgroup analyses were conducted according to cancer types and ethnic groups. Heterogeneity between articles was identified with *Q*-test and I^2 index.[16] When heterogeneity was observed ($P<0.05$ or $I^2>50\%$), a random-effect model (DerSimonian–Laird method) was applied; otherwise, a fixed-effect model (Mantel–Haenszel method) was utilized.[17,18] Publication bias was evaluated by Egger's test and Begg's test, with a *P*-value >0.05 considered evidence

Table 1 Characteristics of studies included in the meta-analysis

First author	Years	Cancer type	Region	Ethnicity	Methods	Controls	Case	Control	Polymorphism site	HWE	NOS
Bachmann et al[19]	2005	Prostate cancer	Germany	Caucasian	SNaPshot	PB	287	96	rs2302427	Yes	8
Breyer et al[20]	2009	Prostate cancer	America	Caucasian	Illumina	PB	523	523	rs2302427	Yes	8
Yoon et al[21]	2010	Lung cancer	Korea	Asian	Illumina	PB	335	335	rs887569, rs2302427, rs3757441, rs41277434	Yes	8
Zhou et al[22]	2014	Gastric cancer	China	Asian	Sequenom	PB	311	425	rs3757441	Yes	8
Yu et al[23]	2013	Hepatocellular carcinoma	Taiwan	Asian	TaqMan	HB	220	552	rs2302427, rs3757441, rs41277434	Yes	7
Wang et al[24]	2014	Colorectal cancer	China	Asian	PCR-RFLP	HB	512	576	rs887569, rs3757441, rs41277434	Yes	7
Yu et al[25]	2014	Urothelial cell carcinoma	Taiwan	Asian	TaqMan	HB	233	552	rs2302427, rs3757441, rs41277434	Yes	7
Ma et al[26]	2014	Esophageal squamous cell carcinoma	China	Asian	PCR-RFLP	HB	476	492	rs887569, rs3757441, rs41277434	Yes	7
Huang et al[27]	2015	Colorectal cancer	China	Asian	PCR-RFLP	PB	96	100	rs887569	Yes	8
Su et al[28]	2015	Oral squamous cell cancer	Taiwan	Asian	TaqMan	HB	576	552	rs2302427, rs3757441, rs41277434	Yes	7
Tao et al[29]	2015	Breast cancer	China	Asian	SNaPshot	PB	234	300	rs2302427, rs3757441	Yes	8
Chang et al[30]	2016	Bladder cancer	Taiwan	Asian	PCR-RFLP	PB	375	375	rs887569, rs3757441, rs41277434	Yes	8

Abbreviations: HB, hospital-based controls; HWE, Hardy–Weinberg equilibrium; Illumina, Illumina GoldenGate platform; NOS, Newcastle–Ottawa Quality Assessment Scale; PB, population-based controls; PCR-RFPL, polymerase chain reaction-restriction fragment length polymorphism; Sequenom, Sequenom MassARRAY iPLEX platform; SNaPshot, multiplex-PCR SNaPshot assay; TaqMan, TaqMan Real-Time PCR Assays.

Table 2 Analysis of associations between SNPs of *EZH2* and cancer risk

Comparisons	Study	N[a]	Cases/ controls	WM vs WW[b]	*P*-value[c]	I^2, %	MM vs WW[b]
				OR (95% CI)			OR (95% CI)
rs887569 C>T	Overall (Asian)	5	1,794/1,878	0.889 (0.771 to 1.026)	0.466	0.0	0.738 (0.520 to 1.047)
Cancer type	DSC	3	1,084/1,168	0.923 (0.764 to 1.115)	0.260	25.7	0.878 (0.533 to 1.445)
rs2302427 C>G	Overall	7	2,408/2,910	0.866 (0.696 to 1.077)	0.051	52.0	**0.562 (0.400 to 0.792)**
Ethnicity	Asian	5	1,598/2,291	0.937 (0.733 to 1.197)	0.093	49.8	**0.550 (0.384 to 0.787)**
	Caucasian	2	810/619	**0.686 (0.511 to 0.921)**	**0.601**	**0.0**	0.723 (0.226 to 2.313)
Cancer type	DSC	2	796/1,104	1.132 (0.925 to 1.385)	0.958	0.0	**0.618 (0.394 to 0.970)**
	USC	3	1,033/1,362	**0.684 (0.546 to 0.857)**	**0.872**	**0.0**	**0.484 (0.248 to 0.943)**
rs3757441 T>C	Overall (Asian)	9	3,272/4,159	0.938 (0.849 to 1.036)	0.202	27.2	0.827 (0.555 to 1.231)
Cancer type	DSC	5	2,905/2,579	0.947 (0.806 to 1.177)	0.068	54.2	0.947 (0.513 to 1.748)
	USC	2	608/927	0.937 (0.751 to 1.169)	0.538	0.0	0.811 (0.563 to 1.170)
rs41277434 A>C	Overall (Asian)	7	2,727/3,403	1.050 (0.908 to 1.213)	0.990	0.0	1.044 (0.812 to 1.240)
Cancer type	DSC	4	1,784/2,172	1.041 (0.872 to 1.242)	0.855	0.0	0.971 (0.755 to 1.247)
	USC	2	608/927	1.045 (0776 to 1.408)	0.996	0.0	1.705 (0.717 to 1.595)

Notes: [a]Number of comparisons; [b]W, major allele; M, minor allele. [c]*P*-value of *Q*-test of heterogeneity test. DSCs, including hepatocellular carcinoma, oral squamous cell cancer, colorectal cancer, esophageal squamous cell carcinoma, or gastric cancer; USCs, including urothelial cell carcinoma, prostate cancer, bladder cancer. Random-effects models were used if heterogeneity between articles was reported ($P<0.10$, $I^2>50\%$), otherwise fixed-effects models were applied. WM, WW, MM represent heterozygote, homozygote for major allele and homozygote for minor allele, respectively. Bold data is statistically significant.
Abbreviations: DSC, digestive system cancer; USC, urogenital system cancer.

for no potential publication bias. Begg's or Egger's test was performed only for SNPs involved in 5 or more studies. Statistical tests were 2-sided, and analyses were carried out with Stata 12.0 at least twice.

Results

Characteristics of the included studies

After PubMed, Google Scholar, Web of Science, Embase, CNKI, and Wangfang Data online databases were extensively screened, 216 relevant articles were identified. As shown in the flowchart in Figure 2, 12 case–control studies involving the 4 EZH2 SNPs were finally included for further meta-analysis after ineligible articles were excluded according to our inclusion and exclusion criteria.[19–30] The characteristics of the included studies are summarized in Table 1. Of the 12 included studies, 6 focused on digestive system cancers (DSCs; gastric cancer, hepatocellular carcinoma, colorectal cancer [CRC], and esophageal squamous cell carcinoma), 4 examined urogenital system cancers (USCs; prostate cancer, urothelial cell carcinoma, and bladder cancer), and 2 investigated other types of cancers. The detailed information of the analyzed articles for each SNP is shown in Table S1.

Quantitative synthesis

The associations between *EZH2* SNPs and human cancer risks were evaluated (Table 2; Figures 3 and 4). Overall, the *EZH2* rs887569 C>T polymorphism was significantly associated with a decreased cancer risk in the dominant and recessive models (CTTT/CC: OR =0.849, 95% CI: [0.740 to 0.973], *P*=0.019; TT/CCCT: OR =0.793, 95% CI: [0.654 to 0.962], *P*=0.019). EZH2 rs2302427 C>G polymorphism was also related to the decreased overall cancer risk in the homozygote dominant genetic and recessive genetic models (GG/CC: OR =0.562, 95% CI: [0.400 to 0.792], *P*=0.001; CGGG/CC: OR =0.856, 95% CI: [0.748 to 0.980], *P*=0.024; GG/CCCG: OR =0.733, 95% CI: [0.571 to 0.940], *P*=0.015). In other genotype models, such a relationship remains controversial.

Subgroup analysis revealed that the variant CG (OR =0.686, 95% CI: [0.511 to 0.921], *P*=0.012) and CG/GG (OR =0.688, 95% CI: [0.515 to 0.917], *P*=0.01) genotypes of rs2302427 C>G polymorphism were associated with a decreased cancer risk compared with the wild-type CC genotype in individuals of Caucasian descent. rs2302427 C>G polymorphism in Asian descent was linked to the decreased overall cancer risk in the homozygote and recessive genetic models (GG/CC: OR =0.550, 95% CI: [0.384 to 0.787], *P*=0.001; GG/CCCG: OR =0.731, 95% CI: [0.566 to 0.944], *P*=0.016).

We also conducted a stratified analysis of the data in terms of cancer types, namely, USCs and DSCs. With regard to subgroup analysis of USCs, our results did not show any association of rs887569 C>T polymorphism with cancer risk in any genotype model. However, rs2302427 C>G polymorphism was correlated with a decreased cancer risk in homozygote and recessive genetic models for DSCs. As for USCs, similar results were observed in homozygote, heterozygote, and dominant genetic models.

For rs3757441 T>C and rs41277434 A>C polymorphisms, 9 and 7 studies were included, respectively.

P-value[c]	I^2, %	WM + MM vs WW[b] OR (95% CI)	*P*-value[c]	I^2, %	MM vs WM + WW[b] OR (95% CI)	*P*-value[c]	I^2, %
0.058	56.2	**0.849 (0.740 to 0.973)**	**0.29**	**19.6**	**0.793 (0.654 to 0.962)**	**0.162**	**38.9**
0.068	62.8	0.894 (0.746 to 1.071)	0.179	41.9	0.877 (0.696 to 1.104)	0.186	40.6
0.967	**0.0**	**0.856 (0.748 to 0.980)**	**0.089**	**45.4**	**0.733 (0.571 to 0.940)**	**0.621**	**0.0**
0.881	**0.0**	0.911 (0.782 to 1.061)	0.098	49.0	**0.731 (0.566 to 0.944)**	**0.356**	**0.0**
0.914	0.0	**0.688 (0.515 to 0.917)**	**0.627**	**0.0**	0.768 (0.240 to 2.456)	0.898	0.0
0.558	**0.0**	1.045 (0.862 to 1.267)	0.927	0.0	**0.593 (0.380 to 0.925)**	**0.547**	**0.0**
0.733	**0.0**	**0.664 (0.534 to 0.826)**	**0.837**	**0.0**	0.533 (0.274 to 1.035)	0.769	0.0
0.000	81.3	0.915 (0.774 to 1.081)	0.002	67.0	0.846 (0.599 to 1.193)	0.000	77.6
0.000	87.7	0.976 (0.743 to 1.282)	0.001	80.0	0.946 (0.562 to 1.592)	0.000	85.0
0.881	0.0	0.912 (0.739 to 1.125)	0.625	0.0	0.817 (0.575 to 1.160)	0.845	0.0
0.986	0.0	1.037 (0.905 to 1.187)	0.988	0.0	0.957 (0.791 to 1.158)	0.948	0.0
0.928	0.0	1.017 (0.860 to 1.203)	0.881	0.0	0.920 (0.738 to 1.148)	0.827	0.0
0.851	0.0	1.049 (0.807 to 1.365)	0.913	0.0	1.056 (0.718 to 1.554)	0.856	0.0

No evidence suggested that these 2 SNPs might be associated with cancer risk either in overall or subgroup analysis ($P>0.05$; Table 2; Figures S1 and S2).

Sensitivity analysis and publication bias assessment

Sensitivity analyses were conducted by omitting each individual article to measure its specific effect on the pooled ORs (Figure S3). The sensitivity analysis forest plot indicated that no single study significantly affected the pooled ORs for any genetic models of the 4 SNPs. A random-effect model was used when obvious heterogeneity was observed ($P<0.05$ or $I^2>50\%$); otherwise, a fixed-effect model was applied. Considering the small number of studies included in the meta-analysis, we conducted Begg's and Egger's tests to assess the publication bias for each genetic model of the 4 SNPs. No evidence of publication bias was detected in any of the homozygote, heterozygote, and dominant and recessive models of each SNP except rs3757441 and rs41277434 (Table 3).

Discussion

EZH2 overexpression is a marker of advanced and metastatic diseases in many solid tumors, including prostate,[8] bladder,[31] gastric,[32] lung,[33] and breast cancer.[34] EZH2 has also been implicated in cancer initiation, promotion, and progression.[35] Therefore, genetic mutations may significantly influence the function of EZH2 in cancer initiation and risk.[36] Cumulative studies have suggested that recurrent heterozygous point mutations affecting tyrosine 641 (Y641) in germinal center B-cell and point mutations at alanine 687 or 677 in non-Hodgkin's lymphomas can increase H3K27me3 levels, thereby repressing the expression of Polycomb targets.[37–39]

SNPs, as the most common genetic sequence variation, can affect the function of *EZH2* and its downstream targets by altering *EZH2* transcription and H3K27 trimethylation. For example, the rs3757441 polymorphism C/C genotype is associated with strong EZH2 and H3K27me3 immunoreactivity in primary CRC, indicating that this genotype can be a promising biomarker for EZH2-targeting agents.[27] The rs887569 TT genotype is correlated with a significantly increased overall survival and a reduced risk of mortality in patients with cholangiocarcinoma.[40] Zhou et al[22] found that the haplotypes of *EZH2* genes with minor alleles of rs12670401 and rs6464926 or major alleles of rs2072407, rs734005, and rs734004 significantly increase the risk of gastric cancer, whereas the haplotypes of *EZH2* genes with major alleles of rs12670401 and rs6464926 or minor alleles of rs2072407, rs734005, and rs734004 can reduce the risk of gastric cancer. These studies have demonstrated that the SNPs of *EZH2* are closely related to cancer risk and prognosis. Although studies have revealed that *EZH2* polymorphisms are associated with cancer risk, results are inconsistent. Therefore, we systematically reviewed the literature through a meta-analysis of the association between *EZH2* gene polymorphisms and cancer risk. To the best of our knowledge, this study is the first meta-analysis to investigate the relationship between *EZH2* SNPs and cancer risk.

While searching for eligible studies, we found 11 *EZH2* SNPs that were reported to be associated with cancer risk:

rs887569, rs2302427, rs375441, rs41277434, rs6950683, rs2072407, rs734005, rs734004, rs6464926, rs12670401, and rs1880357. However, only the first 4 SNPs were examined in at least 5 individual studies. We then performed 4 genotype distributions between cases and controls. Our study included 5 articles, with a pooled total of 1,794 cases and 1,878 controls, which were relevant to the relationship between the rs887569 SNP and cancer risk. The cancer risk was significantly reduced in CT/TT genotype relative to CC genotype (CTTT/CC: OR =0.849, 95% CI: [0.740 to 0.973], *P*=0.019). This association was also detected in the recessive genetic model (TT/CCCT: OR =0.793, 95% CI: [0.654 to 0.962], *P*=0.019). *Z*-scores and *P*-values were calculated to evaluate the reliability of our results, and the *P*-values of the dominant and recessive genetic models of rs887569 were 0.019, which might strengthen our findings. We also found a significant link between rs2302427 polymorphism and cancer susceptibility in the homozygote genotype, dominant genetic, and recessive genetic models (GG/CC: OR =0.562, 95% CI: [0.400 to 0.792], *P*=0.001; CGGG/CC: OR =0.856, 95% CI: [0.748 to 0.980], *P*=0.024; GG/CCCG: OR =0.733, 95% CI: [0.571 to 0.940], *P*=0.015). In the subgroup analysis of ethnicity, rs2302427 CG or CG/GG genotype was significantly related to a decreased prostate cancer risk in the Caucasian population, whereas the GG genotype was closely linked to a decreased overall cancer risk in the Asian

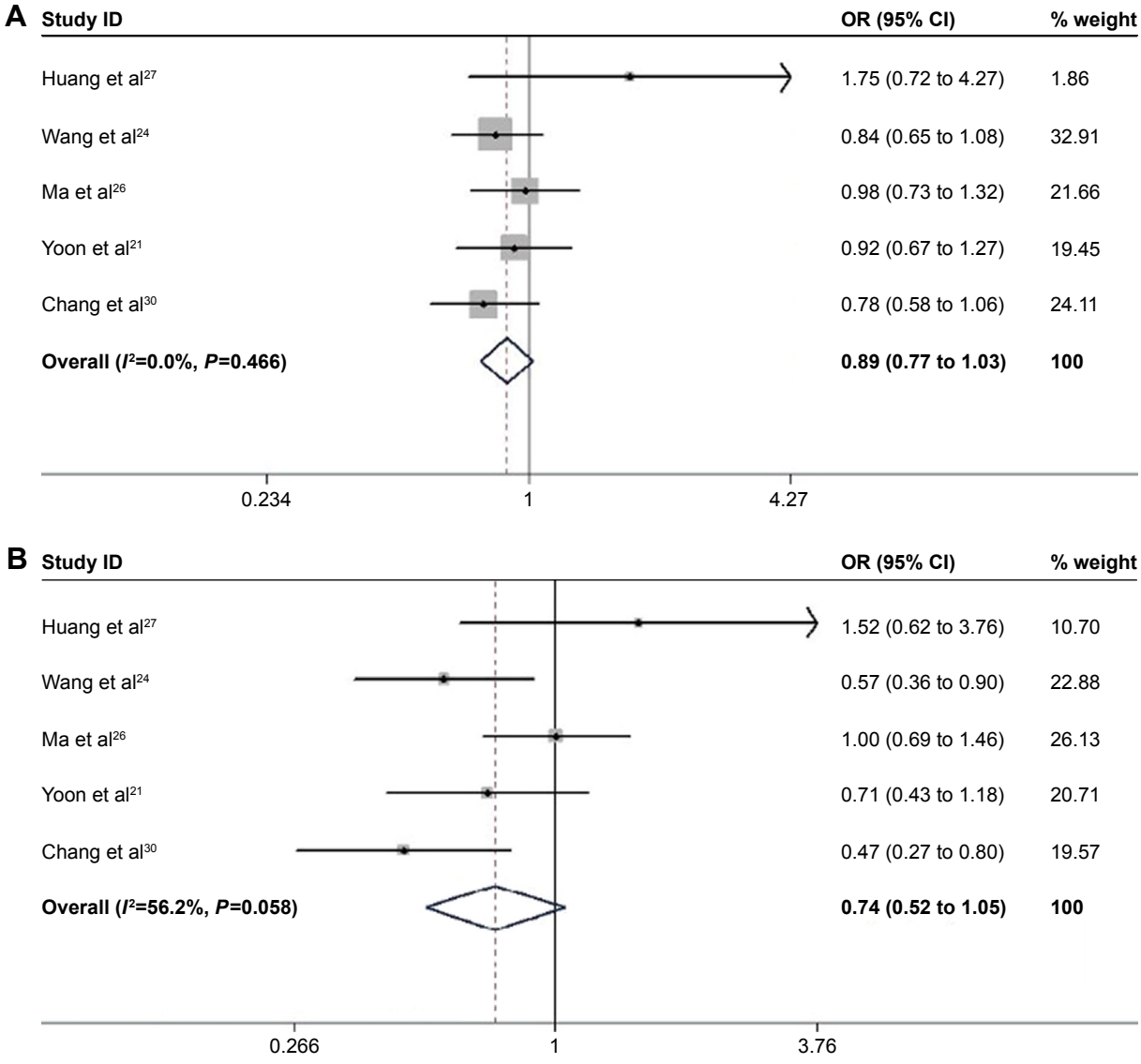

Figure 3 (*Continued*)

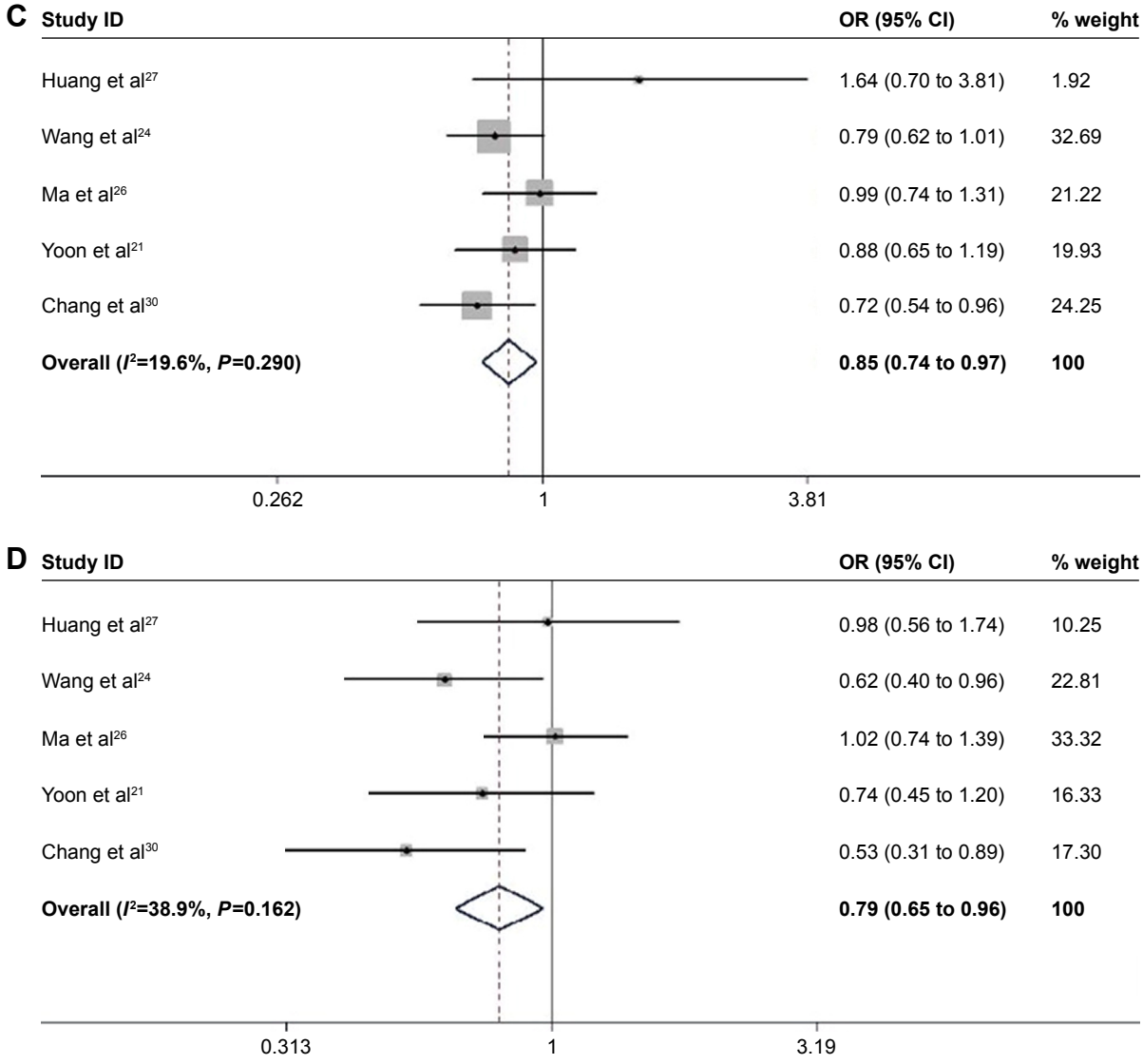

Figure 3 Forest plot for the relationship between rs887569 and cancer risk: (**A**) CT/CC; (**B**) TT/CC; (**C**) CTTT/CC; (**D**) TT/CCCT.
Note: Weights are from random effects analysis.

population. However, the reliability of our data would have improved had we enrolled more eligible studies and a larger sample size than the obtained data.

We subsequently examined the effect of *EZH2* SNP rs3757441, which is a key indicator of poor prognosis in metastatic CRC, on overall cancer risk by analyzing 9 eligible studies.[27] However, in our current meta-analysis, the association between rs3757441 and cancer risk is controversial. We also performed a stratified analysis by cancer types, but no association was observed between rs3757441 and USC or DSC. These inconsistent results might be due to the heterogeneity of cancer type, ethnicity, and sample size, considering that rs3757441 plays a protective role in lung cancer in a Korean population[21] but acts as a risk factor in CRC in a Han Chinese population.[24] Furthermore, we searched for articles related to *EZH2* rs41277434, and our results indicated that no significant association was found between rs41277343 and overall cancer risk or DSC risk.

Sensitivity analysis revealed that the results of our study were robust. Egger's and Begg's tests indicated a publication bias in homozygote and recessive models of rs3757441 and rs41277434. Future large-scale well-designed studies should be conducted to confirm the publication bias of the genetic models of rs375441 and rs41277434.

Several limitations of our meta-analysis should be considered. First, most of the eligible studies mainly focused on East Asian populations, whereas 2 studies involved Caucasians. Studies on other ethnicities were not included in this meta-analysis. Thus, our results were incomplete. The number of eligible studies and the sample size were relatively small and

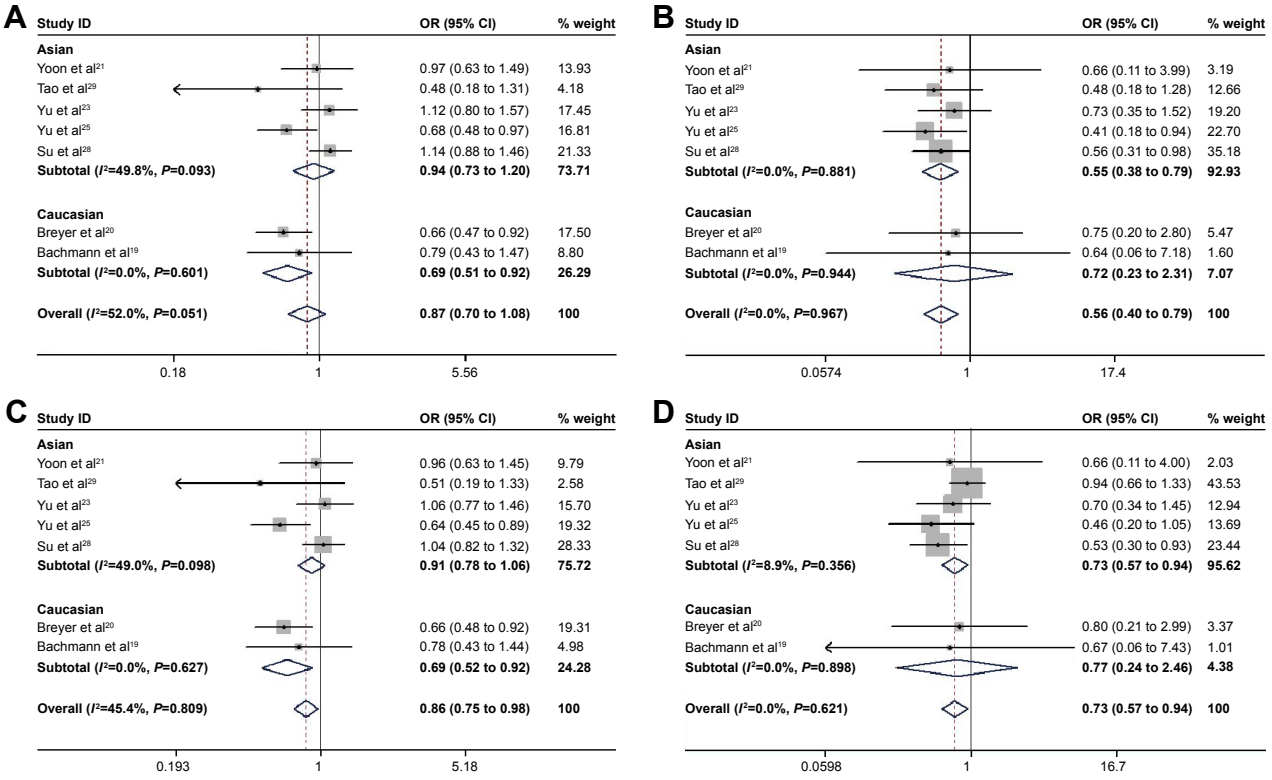

Figure 4 Forest plot for the relationship between rs2302427 and cancer risk: (**A**) CG/CC; (**B**) GG/CC; (**C**) CGGG/CC; (**D**) GG/CCCG.
Note: Weights are from random effects analysis.

might consequently cause a type II error. Second, our results were based on unadjusted estimates because of the lack of original data on age, gender, and smoking status. Potential bias caused by these factors might also persist. Third, differences among various cancers might lead to heterogeneity when all cancer types were pooled. Stratified analysis by specific cancer type was not conducted because of the insufficient number of studies on single cancer type. Finally, we only searched for publications in Chinese and English. As such, language restriction would limit our sample size.

Table 3 Publication bias in meta-analysis for each inheritance model

SNPs	Inheritance model	Studies	Begg's test		Egger's test	
			Z-value	*P*-value	95% CI	*P*-value
rs887569 C>T	Heterozygote genotype: CT/CC	5	0.73	0.462	(–0.54 to 5.08)	0.082
	Homozygote genotype: TT/CC	5	0.24	0.806	(–9.31 to 10.11)	0.904
	Dominant genetic model: CTTT/CC	5	0.73	0.462	(–1.75 to 6.47)	0.165
	Recessive genetic model: TT/CCCT	5	0.24	0.806	(–10.84 to 5.23)	0.328
rs2302427 C>G	Heterozygote genotype: CG/CC	7	0.60	0.548	(–5.94 to 1.95)	0.250
	Homozygote genotype: GG/CC	7	0.00	1.000	(–1.08 to 1.44)	0.729
	Dominant genetic model: CGGG/CC	7	1.20	0.230	(–5.41 to 2.10)	0.308
	Recessive genetic model: GG/CCCG	7	0.30	0.764	(–2.24 to 0.91)	0.328
rs3757441 T>C	Heterozygote genotype: CT/TT	9	1.98	0.048	(–11.99 to 3.87)	0.265
	Homozygote genotype: CC/TT	9	1.77	0.076	(–13.17 to –2.36)	0.012
	Dominant genetic model: CCCT/TT	9	1.77	0.076	(–17.25 to 5.26)	0.268
	Recessive genetic model: CC/CTTT	9	1.36	0.175	(–10.34 to –2.11)	0.009
rs41277434 A>C	Heterozygote genotype: AC/AA	7	0.90	0.368	(–1.66 to 0.47)	0.212
	Homozygote genotype: CC/AA	7	2.10	0.035	(–0.10 to 1.17)	0.083
	Dominant genetic model: ACCC/AA	7	1.20	0.230	(–1.56 to 0.54)	0.263
	Recessive genetic model: CC/AAAC	7	2.10	0.035	(–0.18 to 1.38)	0.106

Abbreviation: SNP, single nucleotide polymorphism.

Conclusion

Despite the limitations, our meta-analysis revealed that *EZH2* rs887569 and rs2302427 might be correlated with a decreased cancer risk in specific genetic models, whereas the association of *EZH2* rs3757441 and rs41277434 polymorphisms with overall cancer risk was not observed. To confirm our results and provide highly reliable evidence supporting these associations, we recommend future large-scale and well-designed studies on diverse ethnic populations and cancer types.

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Author contributions

ZL performed the experiments and wrote the paper. ZY performed the experiments, prepared figures, and/or tables. LH analyzed the data, prepared figures, and/or tables. LZ analyzed the data. YW reviewed drafts of the paper. MZ analyzed the data, contributed reagents/materials/analysis tools. GZ contributed reagents/materials/analysis tools and reviewed drafts of the paper. SC contributed reagents/materials/analysis tools. BX and MC conceived and designed the experiments, and reviewed drafts of the paper. All authors contributed toward data analysis, drafting and revising the paper and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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Supplementary materials

Table S1 Characteristics of eligible studies for each SNP in the meta-analysis

Gene	Reference	Years	Cancer type	Region	Ethnicity	Controls	NOS	Genotype-case			Genotype-control			Method	HWE *P*-value
EZH2	Rs887569 C>T							CC	CT	TT	CC	CT	TT		
	Huang et al[9]	2015	Colorectal cancer	China	Asian	PB	8	10	47	39	16	43	41	PCR-RFLP	0.41
	Wang et al[6]	2014	Colorectal cancer	China	Asian	HB	7	237	239	36	221	266	59	PCR-RFLP	0.11
	Ma et al[8]	2014	Esophageal squamous cell cancer	China	Asian	HB	7	126	253	97	129	264	99	PCR-RFLP	0.09
	Yoon et al[3]	2010	Lung cancer	Korea	Asian	PB	8	159	144	32	148	145	42	Illumina	0.49
	Chang et al[12]	2016	Bladder cancer	China	Asian	PB	8	180	171	24	150	182	43	PCR-RFLP	0.27
EZH2	Rs2302427 C>G							CC	CG	GG	CC	CG	GG		
	Yu et al[5]	2013	Hepatocellular carcinoma	Taiwan	Asian	HB	7	135	75	10	346	171	35	TaqMan	0.03
	Yoon et al[3]	2010	Lung cancer	Korea	Asian	PB	8	284	49	2	282	50	3	Illumina	0.64
	Yu et al[7]	2014	Urothelial cell carcinoma	Taiwan	Asian	HB	7	169	57	7	346	171	35	TaqMan	0.03
	Su et al[10]	2015	Oral squamous cell cancer	Taiwan	Asian	HB	7	356	200	20	346	171	35	TaqMan	0.03
	Breyer et al[2]	2009	Prostate cancer	America	Caucasian	PB	8	450	69	4	420	98	5	Illumina	0.79
	Tao et al[11]	2015	Breast cancer	China	Asian	PB	8	11	80	143	7	105	188	SNaPshot	0.08
	Bachmann et al[1]	2005	Prostate cancer	Germany	Caucasian	PB	8	243	42	2	78	17	1	SNaPshot	0.95
EZH2	Rs3757441T>C							TT	TC	CC	TT	TC	CC		
	Yu et al[5]	2013	Hepatocellular carcinoma	Taiwan	Asian	HB	7	131	80	9	271	223	58	TaqMan	0.23
	Wang et al[6]	2014	Colorectal cancer	China	Asian	HB	7	196	230	86	245	248	53	PCR-RFLP	0.39
	Yu et al[7]	2014	Urothelial cell carcinoma	Taiwan	Asian	HB	7	123	88	22	271	223	58	TaqMan	0.23
	Ma et al[8]	2014	Esophageal squamous cell cancer	China	Asian	HB	7	112	260	104	147	267	78	PCR-RFLP	0.43
	Su et al[10]	2015	Oral squamous cell cancer	Taiwan	Asian	HB	7	312	221	43	271	223	58	TaqMan	0.23
	Yoon et al[3]	2010	Lung cancer	Korea	Asian	PB	8	193	125	17	169	134	32	Illumina	0.47
	Zhou et al[4]	2012	Gastric cancer	China	Asian	PB	7	181	112	18	235	162	28	Sequenom	0.99
	Tao et al[11]	2015	Breast cancer	China	Asian	PB	8	127	91	16	144	129	27	SNaPshot	0.80
	Chang et al[12]	2016	Bladder cancer	China	Asian	PB	8	169	172	34	165	168	42	PCR-RFLP	0.94
EZH2	Rs41277434A>C							AA	AC	CC	AA	AC	CC		
	Yu et al[5]	2013	Hepatocellular carcinoma	Taiwan	Asian	HB	7	209	11	0	517	34	1	TaqMan	0.58
	Wang et al[6]	2014	Colorectal cancer	China	Asian	HB	7	193	236	83	212	248	86	PCR-RFLP	0.34
	Yu et al[7]	2014	Urothelial cell carcinoma	Taiwan	Asian	HB	7	218	15	0	517	34	1	TaqMan	0.58
	Ma et al[8]	2014	Esophageal squamous cell cancer	China	Asian	HB	7	133	242	101	141	231	120	PCR-RFLP	0.19
	Su et al[10]	2015	Oral squamous cell cancer	Taiwan	Asian	HB	7	540	35	1	517	34	1	TaqMan	0.58
	Yoon et al[3]	2010	Lung cancer	Korea	Asian	PB	8	293	40	2	298	36	1	Illumina	0.94
	Chang et al[12]	2016	Bladder cancer	China	Asian	PB	8	215	98	62	220	96	59	PCR-RFLP	5.6E-13

Abbreviations: HB, hospital-based controls; HWE, Hardy–Weinberg equilibrium; Illumina, Illumina GoldenGate platform; NOS, Newcastle–Ottawa Quality Assessment Scale; PB, population-based controls; PCR-RFPL, polymerase chain reaction-restriction fragment length polymorphism; SNaPshot, multiplex-PCR SNaPshot assay; SNP, single nucleotide polymorphism; TaqMan, TaqMan Real-Time PCR Assays.

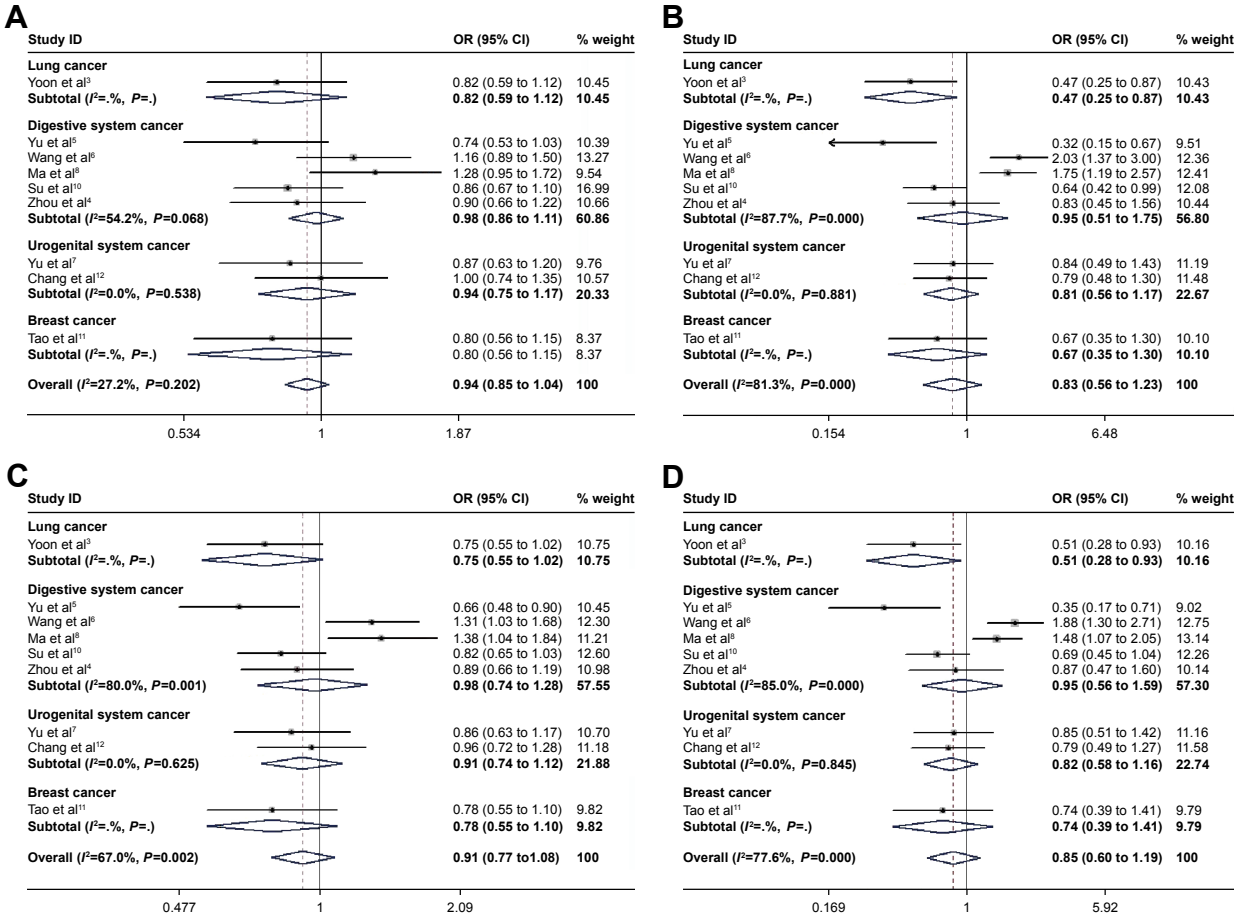

Figure S1 Forest plot for the relationship between rs3757441 and cancer risk: (**A**) CT/TT; (**B**) CC/TT; (**C**) CCCT/TT; (**D**) CC/CTTT.
Note: Weights are from random effects analysis.

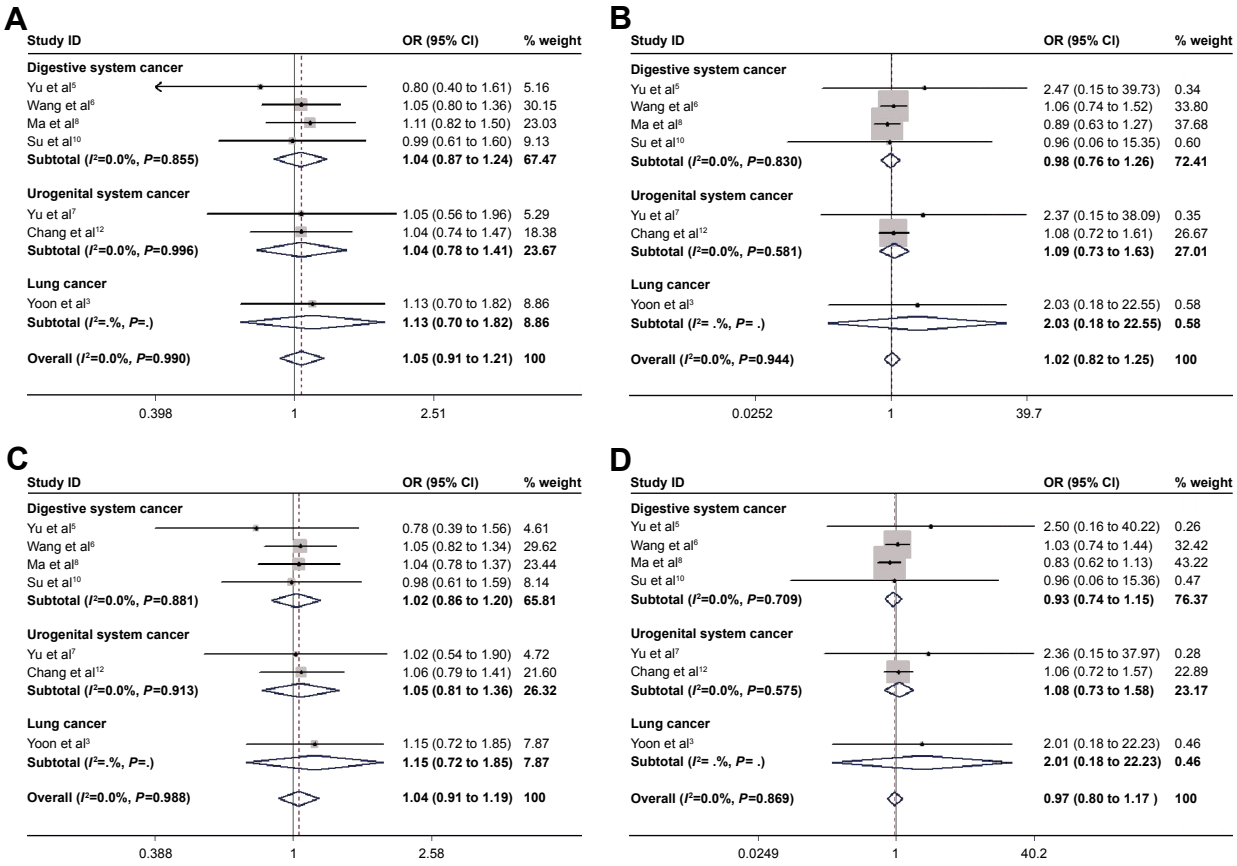

Figure S2 Forest plot for the relationship between rs41277434 and cancer risk: (**A**) AC/AA; (**B**) CC/AA; (**C**) ACCC/AA; (**D**) CC/AAAC.

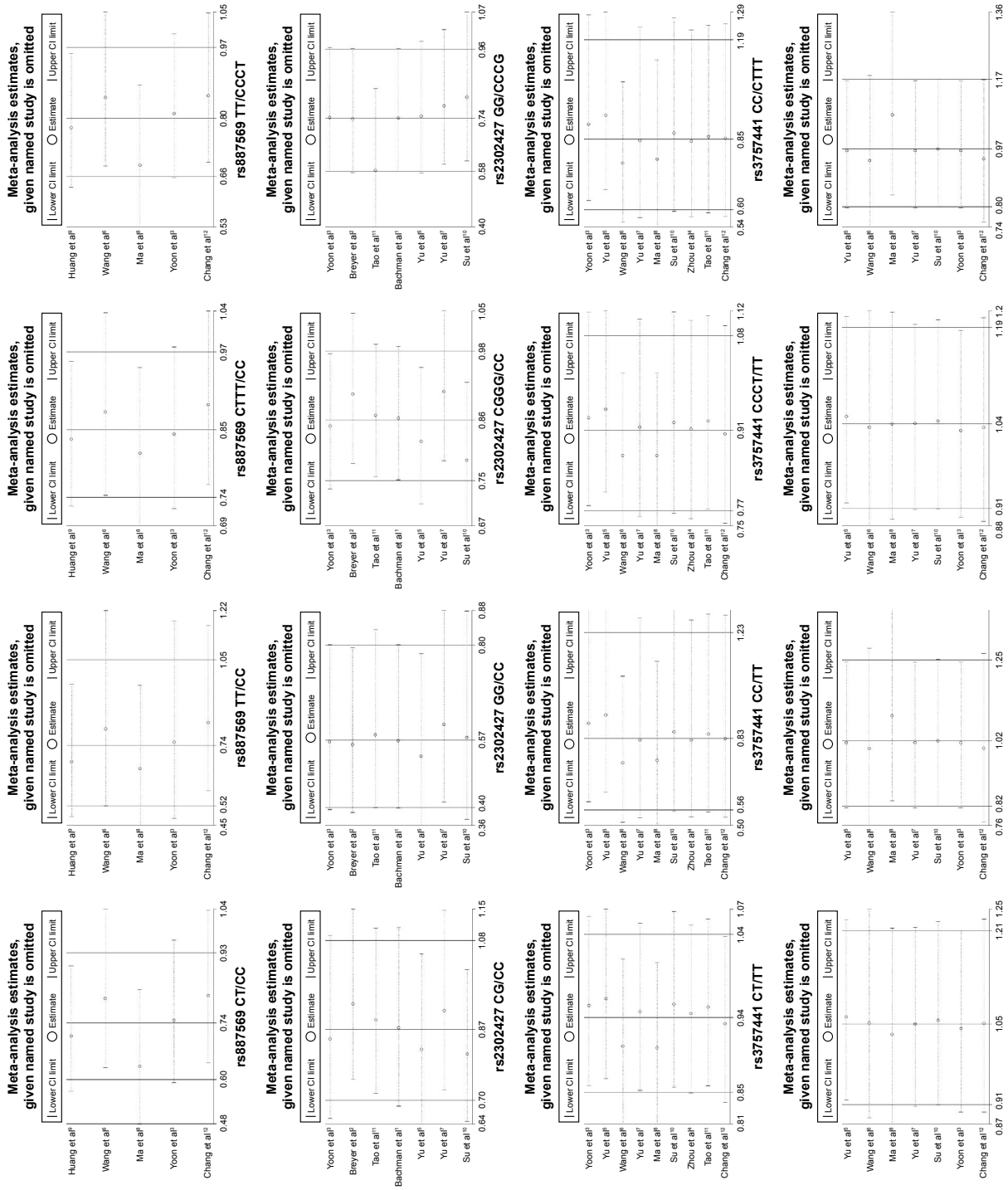

Figure S3 Forest plot of sensitivity analysis for EZH2 SNPs.
Abbreviation: SNP, single nucleotide polymorphism.

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