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Effects of EZH2 Polymorphisms on Susceptibility to Various Carcinomas: Evidence from 6 Publications

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Abstract

Enhancer of zeste homolog2 (EZH2) is a polycomb group protein, playing a key role in epigenetic chromatin modifying, cell-cycle regulating, and has been reported associated with a variety of malignancies. Published research on *EZH2* gene polymorphism and cancer risk are inconsistent, therefore, this study aimed to evaluate the association between *EZH2* gene polymorphism and several types of cancer risks. Computerized literature search was carried out in the PubMed, Web of Science, EMBASE and China National Knowledge Infrastructure (CNKI) before the date of July 20, 2017. Each gene was estimated by odds ratios(ORs) and 95% confidence intervals (CIs) and I^2 value was used to assess heterogeneity. Overall analysis were carried out with RevMan5.3 software. The result showed the individuals carrying at least one T allele at EZH2 rs3757441 had a 1.52 fold higher risk of developing cancer and those carrying at least one C allele at EZH2 rs2302427 had a 1.68 fold higher risk of developing cancer. In particular, EZH2 rs6950683 polymorphism and cancer risk was significantly observed in each comparison model. In conclusion, our meta-analysis indicated that the EZH2 polymorphism was associated with risks of developing carcinomas and larger sample sizes of studies are suggested performed to further validate our results.

Keywords: *EZH2*; Cancer; Polymorphism; Meta-analysis

Introduction

Cancer is a common and major public health burden all over the world. It is estimated that the annual number of cancer diagnosis will increase from 14 million (in 2012) to 22 million by 2032, worldwide. Thus, it is urgent neceseary to help prevent the occurrence and developmeng of cancer.

The polycomb group protein enhancer of zeste homolog 2 (EZH2) is a subunit of the multi-enzyme complex polycomb repressive complex 2 and is reported to serve as a histone methyl transferase, playing a key role in recruiting of protein regulator of cytokinesis [1], leading to initiation of gene silencing [2-5]. An increasing number of studies have reported high-expression of EZH2 predicts advanced stages of human cancer progression and poor prognosis and may promote the epithelial-mesenchymal transition process [6,7].

Single-nucleotide polymorphisms (SNPs) are the most common type among all the DNA sequence variation, impacting many genetic diseases' progression and play a critical role in regulating susceptibility to different kinds of carcinoma [8,9]. Previous studies have demonstrated that EZH2 has emerged as a putative oncological therapy target such as RUNX3, PSP94 and further influence the progression of carcinoma [10-12].

To date, previous researches revealed that the most widely studied SNPs in EZH2 (rs3757441, rs2302427, rs6950683 and rs41277434) have been reported to be functional and may be related with cancer risks, but the conclusions remain to be inconsistent [13-16]. Therefore, the objective of this meta-analysis was to evaluate the effect of *EZH2 gene* polymorphisms with susceptibility to cancer and provide a more comprehensive result.

Materials and Methods

Data sources and searching

We searched PubMed, Web of Science, EMBASE, and China National Knowledge Infrastructure (CNKI) for eligible studies assessing the association of EZH2 polymorphisms and cancer risk before the date of July 20, 2017. The search terms were"cancer" in combination with "EZH2 or enhancer of homolog 2" in combination with "polymorphism or variant or mutation" at first. Then rs3757441, rs2302427, rs6950683, and rs41277434 were searched as the keyword for the results, respectively.

Inclusion and exclusion criteria

All relevant studies have to meet the following requirements: 1) research methods must be case-control or cohort studies, 2) studies investigating the association between *EZH2* gene polymorphisms and cancer risk, and 3) sufficient data available to calculate an odds ratio (OR) with 95% confidence interval (CI). The exclusion criteria of the meta-analysis were: 1) studies not focusing on the correlation between EZH2 polymorphisms and cancer risk; 2) insufficient original data was available for data extraction ;and 3) letters, reviews,meta-analysis and editorial articles.

Data extraction and quality assessment

The data of eligible studies were extracted in duplicate by two investigator independently (WWT and HDS). The following items were recorded in detail: name of first author, year of publication,

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type of cancer, number of cases and controls, location, variations and genotyping method. Ethnicity was simply categorized as Chinese or Korean.

The quality of the studies was modified from previous meta-analysis and independently assessed by 2 authors (Tables 1 and 2) [17,18]. Quality scores ranged from 0 points to 13 points and 0 points means the while 13 points means the best. Studies scoring less than 9 points were regarded as low quality, and those scoring 9 points or higher were regarded as high quality. To avoid bias in the data extraction process, any disagreement between these two investigators was resolved by consensus or by consultation with additional authors.

Statistical analysis

Crude ORs with their corresponding 95% CIs were used to assess the strength of association between EZH2 polymorphisms and cancer risk. The Hardy–Weinberg equilibrium (HWE) was peformed, and a P<0.05 was considered as significant disequilibrium. RevMan5.3software was used to perform this meta-analysis and I^2 statistic test was used to examine the heterogeneity. When I^2>50%, severe heterogeneity was considered and the random-effects model would be applied and while I^2<50%, fixed-effects model would be applied. The potential publication bias was assessed by using a "funnel plot" and Begg's test.

Criteria	Score
Source of cases	
Selected from population or cancer registry	3
Selected from hospital	2
Selected from pathology archives, but without description	1
Not described	0
Source of controls	
Population-based	3
Blood donors or volunteers	2
Hospital-based	2
Not described	0
Genotyping examination	
Genotyping done under "blind" conditions	2
Unblinded or not mentioned	1
Hardy–Weinberg equilibrium	
Hardy–Weinberg equilibrium in control group	2
Hardy–Weinberg disequilibrium in control group	1
Total sample size	
>500	3
>200 but <500	2
<200	1

Table 1: Scale for quality assessment.

Results

Study characteristics

A total of 6 articles were included in the meta-analysis after full-text review as shown in (Figure 1) [19-24]. Table 1 presented characteristics of the chosen studies. Among these 6 case-control studies, 5 studies investigated the EZH2 rs3757441 polymorphism , 4 studies investigated the EZH2 rs2302427 polymorphism, 4 studies investigated the EZH2 rs6950683 polymorphism, and 3 studies investigated the EZH2 rs41277434 polymorphism. 5 studies were performed in Chinese populations and one study was performed in Korean populations.

Association between *EZH2* gene polymorphism and susceptibility to cancers

The detailed results of this meta-analysis were shown in Table 3. The comparisons of four SNP (rs3757441, rs2302427, rs6950683, and rs41277434) were all listed as follows.

Association between *EZH2* gene rs3757441 polymorphism and cancers: For the *EZH2* gene rs3757441 polymorphism, a total of 5 studies were included in the meta-analysis (Figure 2). In the overall analysis, no significant difference between the EZH2 rs3757441 polymorphism and cancer risk was found in the recessive model (TT *vs.* CT+CC, OR 0.87, P=0.71). However, an obvious difference between the EZH2 rs3757441 polymorphism and cancer risk was observed under the dominant model(CT+TT *vs.* CC, OR 1.52, P=0.0003) and homozygous comparison model (TT *vs.* CC, OR 1.62, P<0.0001).

Association between *EZH2* gene rs2302427 polymorphism and cancers: For the *EZH2* gene rs2302427 (Figure 3), no obviously difference between rs2302427 polymorphism and cancer risk was found in comparison model (CC *vs.* CC+CG, OR 0.98, P=0.089). However, the result revealed a significant association between the

First author	Year	Ethnicity	Sample size		Cancer type	location	variations	Genotyping method	HWE in controls	Quality scores
			cases	controls						
Kyong-Ah Yoon	2010	Korean	335	335	lung cancer	rs3757441	C/T	unclear	0.775	10
Yung-Luen Yu	2014	Chinese (Taiwan)	233	552	urothelial cell carcinoma	rs3757441	C/T	Real-time PCR	0.112	10
						rs2302427	G/C		0.013*	9
						rs41277434	A/C		0.845	10
						rs6950683	C/T		0.011*	9
WEN-SHIN CHANG	2016	Chinese (Taiwan)	375	375	bladder cancer	rs3757441,	C/T	PCR-RFLP	0.437	10
						rs41277434	A/C		1.84E-24*	9
Yung-Luen Yu	2013	Chinese (Taiwan)	220	552	hepatocellular carcinoma	rs3757441	C/T	Real-time PCR	0.360	10
						rs2302427	G/C		0.076	10
						rs41277434	A/C		0.728	10
						rs6950683	C/T		0.038*	9
Shu-Bin Gao	2015	Chinese	110	289	hepatocellular carcinoma	rs2302427	G/C	Real-time PCR	0.721	10
Kuo-Jung Su	2015	Chinese (Taiwan)	576	552	oral squamous cell carcinoma	rs3757441	C/T	Real-time PCR	0.215	10
						rs2302427	G/C		0.508	10
						rs41277434	A/C		0.436	10
						rs6950683	C/T		0.030	9

Table 2: Characteristics of the 6 eligible studies included in the meta-analysis.

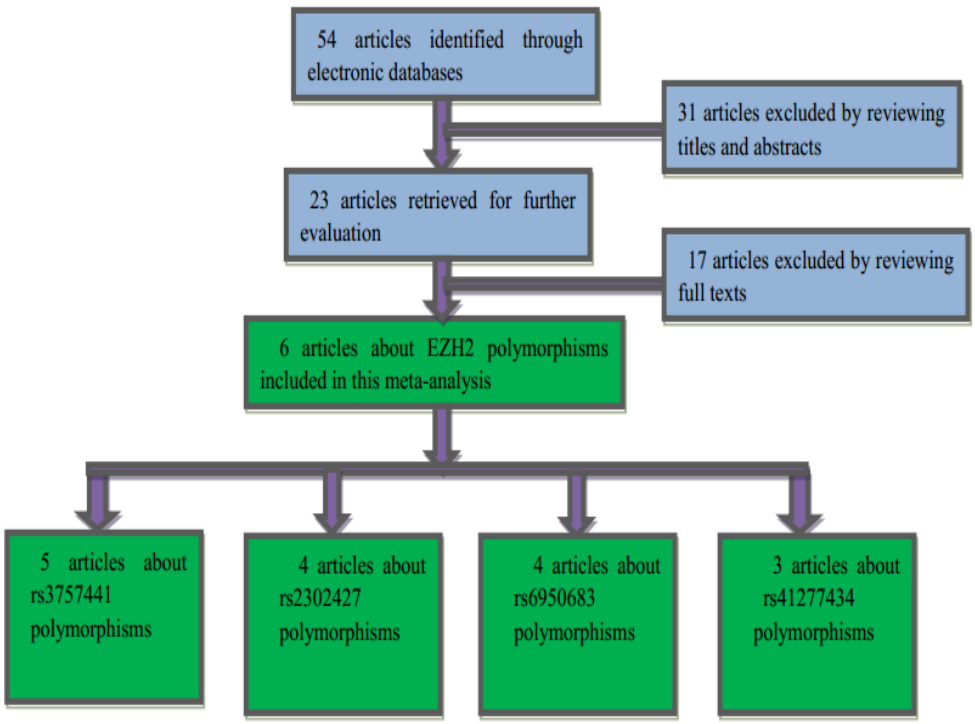

Figure 1: Flowchart of study selection.

Genes SNP	Comparisons	Traditional meta-analysis		
		OR (95% CI)	I^2 (%)	P value
rs3757441	TT *vs.* CT+CC	0.87 (0.41,1.84)	97	0.71
	CT+TT *vs.* CC	1.52 (1.21,1.91)	24	0.0003*
	TT *vs.* CC	1.62 (1.28,2.06)	34	<0.0001*
rs2302427	CC *vs.* CG+GG	0.98 (0.70,1.37)	76	0.89
	CC+CG *vs.* GG	1.68 (1.15,2.44)	7	0.007*
	CC *vs.* GG	1.66 (1.14,2.43)	28	0.009*
rs41277434	AC+ CC *vs.* AA	1.00 (0.81,1.25)	0	0.97
	CC *vs.* AA+AC	1.05 (0.27,1.54)	0	0.80
	CC *vs.* AA	1.06 (0.72,1.57)	0	0.76
rs 6950683	TT *vs.* CT+CC	1.38 (1.17,1.62)	0	<0.0001*
	CT+TT *vs.* CC	2.67 (1.38,5.15)	8	0.0001*
	TT *vs.* CC	1.93 (1.44,2.58)	22	<0.0001*

Table 3: Odds Ratios and 95% Confidence Intervals of the association between four SNP and cancer.

EZH2 rs2302427 polymorphism and cancer risk under the dominant model(CC+CG *vs.* GG, OR 1.68, P=0.007) and homozygous comparison model (CC *vs.* GG, OR1.66, P=0.009). Moreover, a subgroup for *EZH2* gene rs2302427 polymorphism and hepatocellular carcinoma risk was analyzed (Figure 4) but no obviously relationship was found (CC *vs.* CC+CG, OR 1.20, P=0.58, CC+CG *vs.* GG, OR 0.76, P=0.27, CC *vs.* GG, OR 1.13, P=0.71) .

Association between *EZH2 gene* rs41277434 polymorphism and cancers: For the *EZH2* gene rs41277434 polymorphism, a total of 4 studies were included (Figure 5). In the overall analysis, it showed no significant association between the EZH2 rs41277434 polymorphism and cancer risk in comparison model (AC+ CC *vs.* AA, OR 1.00, P=0.97, CC *vs.* AA+ AC, OR 1.05, P=0.80; CC *vs.* AA, OR 1.06, P=0.76).

Association between *EZH2 gene* rs6950683 polymorphism and cancers: For the *EZH2* gene rs6950683 (Figure 6), the result showed an obvious difference between the EZH2 rs6950683 polymorphism and cancer risk in each comparison model (TT *vs.* CC+CT, OR 01.38, P<0.0001; CT+TT *vs.* CC, OR 1.75, P=0.0001; TT *vs.* CC, OR 1.93, P<0.0001) .

Publication bias: The publication bias was evaluated by Begg's test. The heterogeneity was significantly observed only under EZH2 rs3757441 polymorphism TT *vs.* CT+CC model and rs2302427 polymorphism CC *vs.* CC+CG model, resulting from ethnic differences or country differences, thus the random-effects model was peformed. For other polymorphisms models, no significant publication bias was found .

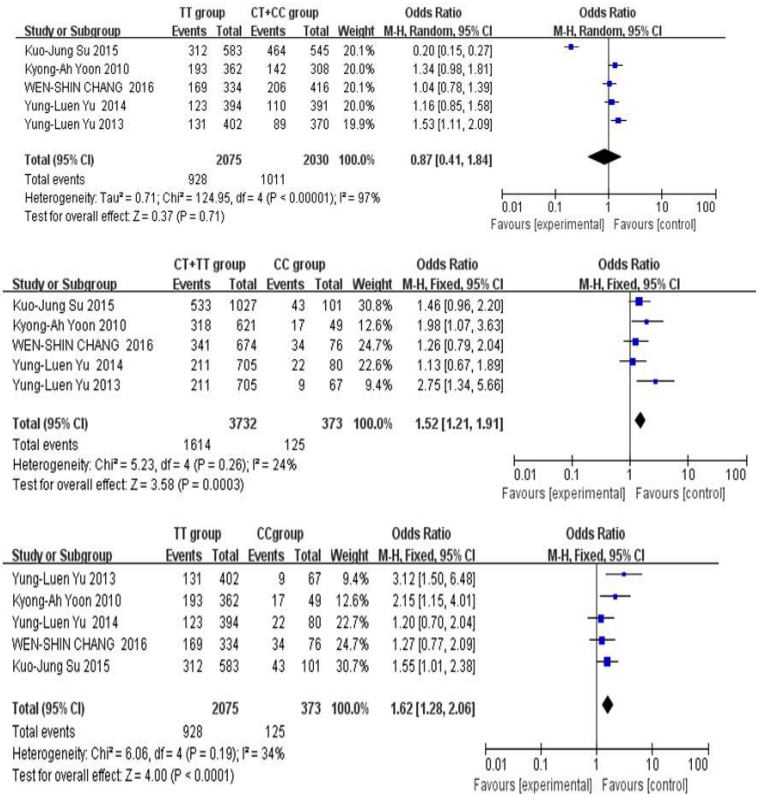

Figure 2: Forest plot for *EZH2* gene rs3757441 polymorphism and cancer risk.

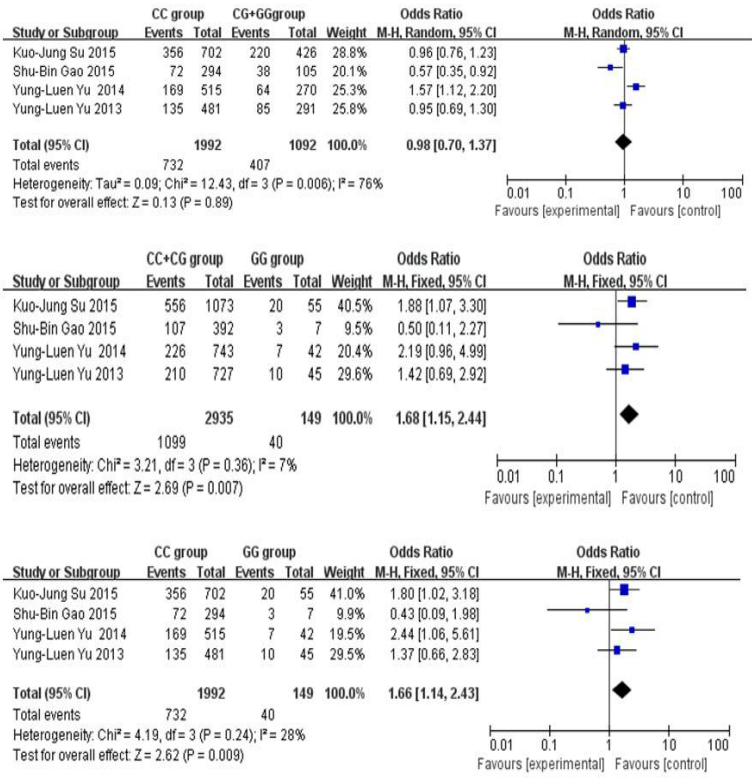

Figure 3: Forest plot for *EZH2* gene rs2302427 polymorphism and cancer risk.

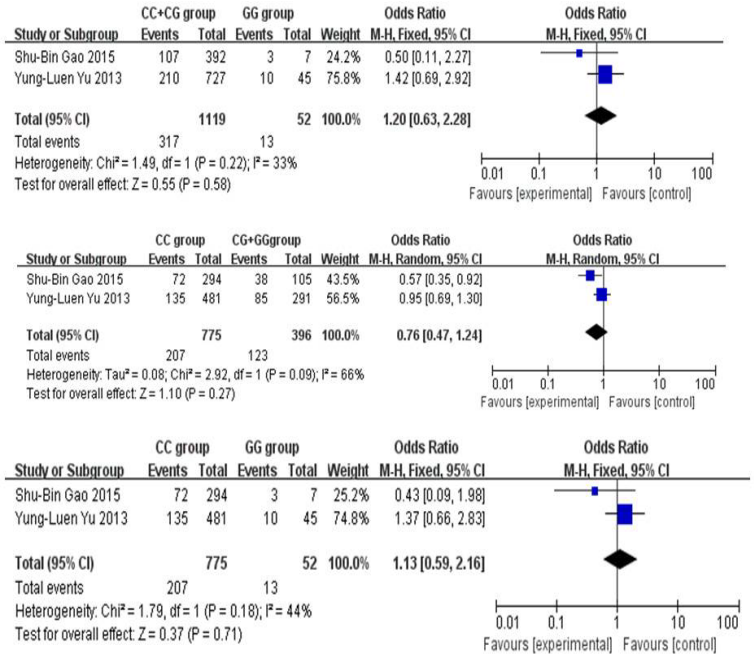

Figure 4: Forest plot for *EZH2* gene rs2302427 polymorphism and hepatocellular carcinoma risk.

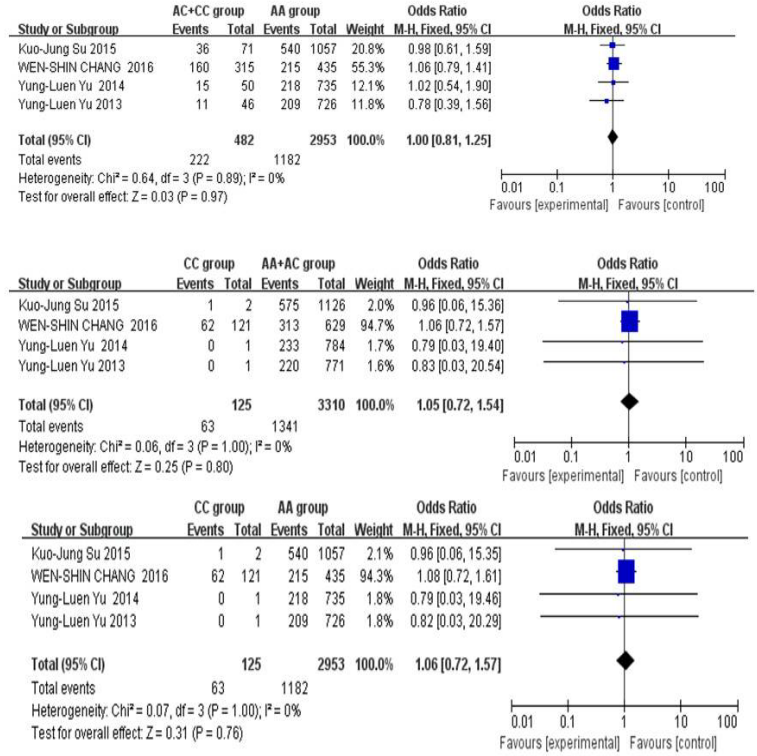

Figure 5: Forest plot for *EZH2* gene rs41277434 polymorphism and cancer risk.

Discussion

Epidemiological studies suggest single nucleotide polymorphisms are important in mediating an individual's susceptibility to varous types of cancers [25]. Based on testability that can be analyzed from blood samples, these SNPs are attractive molecular markers for translational studies. EZH2 plays a significant role in regulating cell cycle and its gene has been considered as a new oncogene and potential anti-tumor therapeutic target. In recent years, an increasing number of studies have reported the polymorphism of EZH2 to be associated with

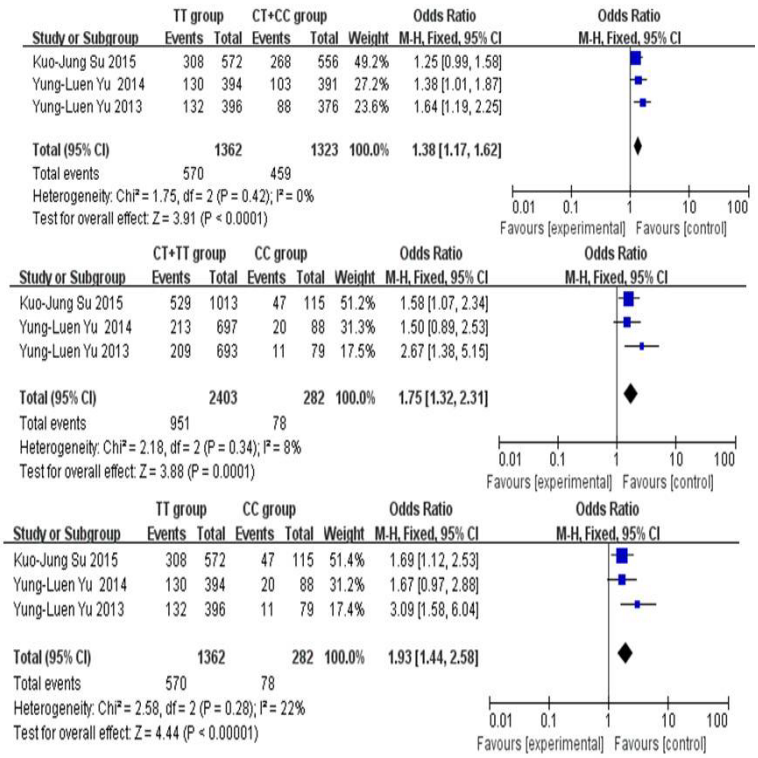

Figure 6: Forest plot for *EZH2* gene rs6950683 polymorphism and cancer risk.

several types of cancers. Tao R, et al. discovered that the G allele of rs10274701 significantly increased the EZH2 expression level in triple-negative breast cancer and demonstrated a significant association between TNBC risk and the polymorphisms of EZH2 [26]. Similarly, in a study of esophageal squamous cell carcinoma (ESCC), researchers have confirmed that *EZH2* gene 626-394T>C genotype increased risks of occurence of ESCCs [27]. However, the results of studies have not always been consistent; therefore, we performed this meta-analysis to provide a more reliable analysis result of the association between *EZH2* gene polymorphisms and susceptibility to cancer.

In this overall meta-analysis, a total of four SNP for the *EZH2* gene was studied. A significant association was observed between the EZH2 rs3757441 polymorphism and cancer risk under the dominant model and homozygous comparison model, indicating the individuals carrying at least one T allele at EZH2 rs3757441 had a 1.52 fold higher risk of developing cancer than did wild-type carriers. EZH2 rs3757441, an intronic SNP, may affect gene expression through several mechanisms, including splicing variants, microRNA-targeting sequences changing and binding transcription–factor sites [28-30]. A study from English population showed EZH2 rs3757441 C/C genotype was associated with stronger EZH2 and H3K27me3 immunoreactivity in primary colorectal cancer and this SNP may serve as a promising biomarker for EZH2-targeting agents [31]. Besides, Crea F's study found one allelic variant (rs3757441 C/C versus C/T or T/T) was significantly associated with shorter PFS and OS in Italy population and an EZH2 SNP may be useful to predict clinical outcome in metastatic colorectal cancer patients [32]. These results revealed that there may be mutations in EZH2 rs3757441 between different races.

EZH2 rs2302427, located in exon 6, has been reported to cause aspartic acid changing to histidine, affecting the function of protein by this non-synonymous amino-acid change [22]. In our study, there was a significant association between the EZH2 rs2302427 polymorphism and cancer risk under the dominant modeland homozygous comparison model, revealing that the individuals carrying at least one C allele at EZH2 rs2302427 had a 1.68 fold higher risk of developing cancer than did wild-type carriers. For the *EZH2* gene rs41277434 polymorphism, we did not find any significant association between rs41277434 polymorphisms and cancer risk in comparison model. EZH2 rs6950683, located near exon 1, may impact gene expression by affecting the promoter region and our result showed a significant association between the EZH2 rs6950683polymorphisms and cancer risk in each comparison model, which revealed that the individuals carrying at least one T allele at EZH2 rs6950683 had a 1.75 fold higher risk of developing cancer than did wild-type carriers.

In summary, our meta-analysis indicated that the EZH2 polymorphism was associated with risks of developing carcinomas. However, several limitations cannot be overlooked when interpreting this article. Firstly, our results were limited to two populations, which are Korean and Chinese and more studies from different countries with different ethnic groups are needed for further study. Secondly, due to data size and source limitations, we did not perform further subgroup analyzes including gender, age, and different TNM stages of cancers. Last but not least, because of the limited number of articles and data available for research, bias may have occurred in the course of the research, so we need more data and more research to validate our conclusion.

Conclusion

In summary, this meta-analysis indicated that the EZH2 rs3757441,

rs2302427, rs6950683 polymorphism may contribute to cancer susceptibility. More biological researches of microenvironment are needed to confirm this point of view and to illuminate the mechanisms.

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