Research Paper

DNMT1, DNMT3A and DNMT3B Polymorphisms Associated With Gastric Cancer Risk: A Systematic Review and Meta-analysis

Hongjia Li [a,1], Wen Li [a,1], Shanshan Liu [a], Shaoqi Zong [a], Weibing Wang [b], Jianlin Ren [a], Qi Li [c], Fenggang Hou [a,*], Qi Shi [a,*]

[a] Oncology Department of Shanghai Municipal Hospital of Traditional Chinese Medicine affiliated to Shanghai University of Traditional Chinese Medicine, Shanghai 200071, China
[b] Fudan University School of Public Health, Shanghai 200032, China
[c] Department of Medical Oncology, Shuguang Hospital, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China

ABSTRACT

Background: Increasing studies showed that abnormal changes in single nucleotide polymorphisms (SNPs) of DNMTs (DNMT1, DNMT3A and DNMT3B) were associated with occurrence or decrease of various tumors. However, the associations between DNMTs variations and gastric cancer (GC) risk were still conflicting. We aimed to assess the effect of DNMTs polymorphisms on the susceptibility to GC.
Methods: Firstly, we did a meta-analysis for 7 SNPs (rs16999593, rs2228611, rs8101866 in DNMT1, rs1550117, rs13420827 in DNMT3A, rs1569686, rs2424913 in DNMT3B). Four genetic models (homozygote, heterozygote, dominant and recessive model) were used. Moreover, a meta-sensitivity and subgroup analysis was performed to clarify heterogeneity source. Lastly, 17 SNPs that couldn't be meta-analyzed were presented in a systematic review.
Findings: 20 studies were included, 13 studies could be meta-analyzed and 7 ones could not. Firstly, a meta-analysis on 13 studies (3959 GC cases and 5992 controls) for 7 SNPs showed that GC risk increased in rs16999593 (heterozygote model: OR 1.36, 95%CI 1.14–1.61; dominant model: OR 1.36, 95%CI 1.15–1.60) and rs1550117 (homozygote model: OR 2.03, 95%CI 1.38–3.00; dominant model: OR 1.20, 95%CI 1.01–1.42; recessive model: OR 1.96, 95%CI 1.33–2.89) but decreased in rs1569686 (dominant model: OR 0.74, 95%CI 0.61–0.90). The remaining SNPs were not found associated with GC risk. Furthermore, the subgroup analysis indicated that for rs1550117 and rs1569686, the significant associations were particularly found in people from Chinese Jiangsu province (rs1550117, OR 1.77, 95%CI 1.25–2.51; rs1569686, OR 0.48, 95%CI 0.36–0.64) and that PCR-RFLP was a sensitive method to discover significant associations (rs1550117, OR 1.77, 95%CI 1.25–2.51; rs1569686, OR 0.49, 95%CI 0.37–0.65). Lastly, a systematic review on 7 studies for 17 SNPs suggested that rs36012910, rs7560488 and rs6087990 might have a potential effect on GC initiation.
Conclusion: This meta-analysis demonstrated that rs16999593 and rs1550117 could contribute to GC risk and that rs1569686 might be a protective factor against gastric carcinogenesis. By using these SNPs as biomarkers, it is feasible to estimate the risk of acquiring GC and thus formulate timely preventive strategy.

1. Introduction

In 2012, 951,000 new gastric cancer (GC) cases and 723,000 deaths were estimated worldwide, making it the fifth most common tumor (Ferlay et al., 2015; Torre et al., 2015). GC is a complex disease arising from environmental and genetic factors. However in individuals infected with H. pylori, defined as a definite gastric carcinogen (Yang, 2006), only a few eventually develop into GC, which suggested that host genetic factors may play a crucial role in the susceptibility of GC (Saeki et al., 2013).

The epigenetics is believed to be important in the development of cancers, which was defined as a stably heritable changes through modifying gene expression without DNA sequence alterations (Esteller, 2008). The most common epigenetic phenomenon is DNA methylation that refers to a methyl group is conferred to the 5′ carbon of a cytosine in a CpG dinucleotide. It is catalyzed by a family of DNA methyltransferases (DNMTs) mainly consisting of three activated forms: DNMT1, DNMT3A and DNMT3B. DNMT1 is thought to be a maintenance DNA methyltransferase which principally maintains CpG methylation, involving in embryonic development and somatic cells survival (Brown and Robertson, 2007) and it is encoded by DNMT1 gene which locates on
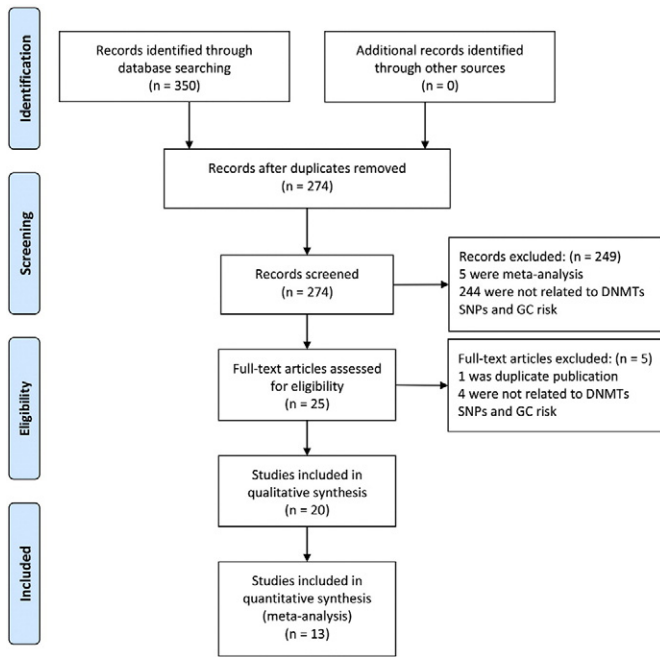
Fig. 1. Flow chart of study selection process.

chromosome 19p13.2 (Jiang et al., 2012a). DNMT3A and DNMT3B are considered as de novo methyltransferases which are required for the establishment of embryonic methylation patterns, mainly occurring during gametogenesis and early development (Okano et al., 1999) and they are encoded by *DNMT3A* and *DNMT3B* genes locating on chromosome 2p23 and 20q11.2 respectively (Yang et al., 2012).

There is considerable evidence that a number of abnormal changes in single nucleotide polymorphisms (SNPs) of *DNMTs* (*DNMT1*, *DNMT3A* and *DNMT3B*), which could cause DNA hypo-methylation or hyper-methylation (Gao et al., 2011; Fu et al., 2010; Harder et al.,

2008; Zhao and Bu, 2012), are correlated to tumor occurrence or decrease (Luo et al., 2015; Chang et al., 2014; Mostowska et al., 2013; Kullmann et al., 2013; Sun et al., 2012; Xiang et al., 2010; Kanai et al., 2003) such as head and neck cancer, and colorectal cancer (Zhu et al., 2015; Duan et al., 2015). However, the associations between *DNMTs* SNPs and GC risk were still conflicting (Jiang et al., 2012a; Yang et al., 2012). Therefore, for the first time, the effects of *DNMTs* polymorphisms on the susceptibility to GC were systematically and comprehensively estimated.

2. Materials and Methods

2.1. Search

We did a literature search of PubMed, MEDLINE, Embase, Sinomed, CNKI, and WanFang databases to identify relevant studies up to June 1, 2016, using the search strategy: (stomach OR gastric) AND (neoplasms OR tumors OR cancers OR carcinomas) AND (DNMT1 OR DNMT3A OR DNMT3B OR DNMTs OR DNA methyltransferases). The languages were limited to English and Chinese. The search strategy for PubMed was listed in Appendix A.

2.2. Selection Criteria

All studies included in the meta-analysis were accorded with the following inclusion criteria: (a). study focused on the association of *DNMTs* polymorphisms and GC risk; (b). case-control or cohort studies. In addition, exclusion criteria were as follows: (a). reviews or meta-analysis; (b). overlapped articles or studies with overlapping data.

2.3. Data Extraction

Two investigators independently extracted the following data: first author, year of publication, province/country of origin, ascertainment of cases, source of controls, genotyping methods, *DNMT* genes, SNPs,

Table 1
Characteristics of 13 studies included in the meta-analysis.

Study	Province/Country	Ascertainment of cases	Source of controls	Genotyping methods	Gene	SNPs	Sample size (cases/controls)	HWE (controls)	Score
Yan et al., 2015	Shandong/China	Histological	HB	Sequencing	*DNMT1*	rs16999593 rs2228611	310/420	0.469 0.423	9
Yang et al., 2012	Jiangxi/China	Histological	HB	MassArray	*DNMT1*	rs16999593 rs2228611 rs8101866	242/294	0.120 0.068 0.747	9
					DNMT3A	rs1550117 rs13420827		0.444	
Jiang et al., 2012a, b	Jilin/China	Histological	HB	TaqMan	*DNMT1*	rs16999593 rs8101866	447/961	0.910	9
Khatami et al., 2009	Fars/Iran, Tork/Iran	Histological	HB	PCR-RFLP	*DNMT1*	rs2228611	200/200	0.187	9
Cao et al., 2013	Jilin/China	Histological	HB	TaqMan	*DNMT3A*	rs1550117 rs13420827	447/961	0.658 0.833	9
Fan et al., 2010	Jiangsu/China	Histological	HB/PB	PCR-RFLP	*DNMT3A*	rs1550117	208/346	0.205	12
Liu, 2009	Jiangsu/China	NA	NA	PCR-RFLP	*DNMT3B*	rs2424913 rs1569686	308/189 313/350	0.942 >0.05	6
Wang et al., 2015a, b	Jilin/China	Histological	HB	TaqMan	*DNMT3B*	rs1569686	447/961	0.001	7
Zhang et al., 2014	Heilongjiang/China	NA	NA	PCR-RFLP	*DNMT3B*	rs1569686	50/60	0.389	4
Hu et al., 2010	Jiangsu/China	Histological	HB/PB	PCR-RFLP	*DNMT3B*	rs2424913 rs1569686	259/262	0.926 0.901	12
Zhang, 2008	Jiangsu/China	NA	HB	PCR-RFLP	*DNMT3B*	rs2424913 rs1569686	156/156	0.968 0.001	6
Wang et al., 2005	Hebei/China	Histological	HB/PB	PCR-RFLP	*DNMT3B*	rs2424913	212/294	0.654	12
Aung et al., 2005	Hiroshima/Japan, Yamaguchi/Japan	Histological	HB	PCR-RFLP	*DNMT3B*	rs2424913	152/247	1.000	6

NA, not available; HB, hospital based; PB, population based; PCR-RFLP, polymorphism chain reaction-restriction fragment length polymorphism; *DNMT genes*, deoxyribonucleic acid methyltransferase genes; SNPs, single nucleotide polymorphisms; HWE, Hardy-Weinberg equilibrium.

number of cases and controls, and value of HWE. To ensure accuracy of the data, inconsistencies were discussed with another reviewer until reach a consensus.

2.4. Quality Assessment

The quality of each study was assessed according to the quality assessment criteria (Table S1) (Thakkinstian et al., 2011; Xue et al., 2015), in which the overall quality scores ranged from 0 to 15. Studies with scores ≥ 9 were regarded as high quality studies; otherwise, studies were considered to have a low quality.

2.5. Data Analysis

Stata software (version 12.0; Stata Corporation, College Station, TX) was used to perform all analysis. We used four types of genetic models (Lieb et al., 2006): homozygote model (homozygous rare vs. homozygous frequent allele), heterozygote model (heterozygous vs. homozygous frequent allele), dominant model (homozygous rare + heterozygous vs. homozygous frequent allele) and recessive model (homozygous rare vs. heterozygous + homozygous frequent allele). Association between $DNMTs$ polymorphisms and the GC risk was evaluated by pooled odds ratios (OR), 95% confidence interval (95% CI) and P value of Z test (P_{OR}). If 95%IC across 1 or $P_{OR} < 0.05$, a significant association existed. Then if OR or 95%IC < 1, the mutant gene was a protective factor; otherwise, it was a risk factor. Heterogeneity was analyzed using the P value of Q test (P_{het}) and I^2. If $P_{het} < 0.1$ or $I^2 > 50\%$, a significant heterogeneity existed. And then a sensitivity analysis and a subgroup analysis were performed. Sensitivity analysis was conducted through omitting one study by turns (Lu et al., 2016), if the 95%CI markedly deviated from the original interval or the I^2 largely decreased, this study was an originator of heterogeneity.

3. Results

3.1. Literature Search and Study Characteristics

A total of 350 records were identified through database searching. After removing duplicates, 274 records were screened on details of the abstracts. In those 249 publications were excluded because 5 were meta-analysis and the other 244 were not related to $DNMTs$ SNPs and GC risk. Then 25 full-text articles were obtained to be assessed, in which 5 articles were excluded because 1 was duplicate publication and 4 did not contain information on $DNMTs$ SNPs and GC risk. Ultimately, 20 eligible studies (Jiang et al., 2012a; Yang et al., 2012; Yan et al., 2015; Khatami et al., 2009; Wu et al., 2014; Cao et al., 2013; Wu et al., 2012; Fan et al., 2010; Liu, 2009; Zhang et al., 2014; Hu et al., 2010; Zhang, 2008; Liu, 2008; Wang et al., 2005; Aung et al., 2005; Wang et al., 2015a; Jiang et al., 2013; Jiang et al., 2012b; Cao et al., 2012; Chang et al., 2010)were included in the qualitative synthesis, and 7 of them could not be quantitatively synthesized (3 studies respectively reported a different SNP (Wu et al., 2014; Wu et al., 2012; Liu, 2008), 4 studies were conference abstracts (Jiang et al., 2013; Jiang et al., 2012b; Cao et al., 2012; Chang et al., 2010)), so 13 studies involving 3959 GC cases and 5992 healthy controls were finally included in the meta-analysis (Fig. 1). Among the 20 studies, 18 studies were for Chinese population (respectively from Jiangsu, Jiangxi, Hebei, Shandong, Jilin and Heilongjiang provinces of China), 1 study was for Iranian population (from Fars and Tork) and another one was for Japanese population (from Hiroshima and Yamaguchi). According to the quality assessment criteria (Table S1), scores of the 13 studies (included in the meta-analysis) were 4–12 and 8 studies were with high quality scores (Xue et al., 2015). The main characteristics of the 13 studies were listed in Table 1.

3.2. Meta-analysis and Systematic Review

The associations between $DNMTs$ polymorphisms and gastric carcinogenesis were shown in Table 2 and the statistically significant associations (only Chinese population were discovered in significant associations) were represented in Fig. 2. In terms of $DNMT1$ and $DNMT3A$, GC risk increased. For rs16999593, there was an association under heterozygote and dominant models (TC vs. TT: OR 1.36, 95%CI 1.14–1.61; TC/CC vs. TT: OR 1.36, 95%CI 1.15–1.60) but not homozygote and recessive models (CC vs. TT: OR 1.36, 95%CI 0.93–1.99; CC vs. TC/TT: OR 1.22, 95%CI 0.84–1.78). For rs1550117, the increased GC risk was discovered under homozygote, dominant and recessive models (AA vs. GG: OR 2.03, 95%CI 1.38–3.00; GA/AA vs. GG: OR 1.20, 95%CI 1.01–1.42; AA vs. GA/GG: OR 1.96, 95%CI 1.33–2.89) but not heterozygote model (GA vs. GG: OR 1.12, 95%CI 0.93–1.33). Conversely, GC risk decreased in $DNMT3B$. For rs1569686, the association was found under dominant model (GT/GG vs. TT: OR 0.74, 95%CI 0.61–0.90) but not heterozygote, homozygote and recessive models (GT vs. TT: OR 0.88, 95%CI 0.69–1.13; GG vs. TT: OR 0.96, 95%CI 0.46–2.01; GG vs. GT/TT: OR 0.97, 95%CI 0.46–2.02). Except all of the above, for rs2228611, rs8101866, rs13420827 and rs2424913, no significant associations were observed among all of the genetic models. Lastly, for SNPs not able to be

Table 2
Meta-analysis of association between $DNMTs$ SNPs and gastric cancer risk.

SNPs	N (cases/controls)	OR (95%CI)	P_{OR}[a]	I^2	P_{het}[b]
DNMT1 rs16999593					
TC vs. TT[c]	949/1609	**1.36 (1.14,1.61)**	**0.001**	**0.0%**	**0.540**
CC vs. TT[d]	654/1202	1.36 (0.93,1.99)	0.117	0.0%	0.743
TC/CC vs. TT[e]	999/1675	**1.36 (1.15,1.60)**	**0.000**	**0.0%**	**0.720**
CC vs. TC/TT[f]	999/1675	1.22 (0.84,1.78)	0.303	0.0%	0.635
DNMT1 rs2228611					
GA vs. GG[c]	656/804	1.09 (0.88,1.36)	0.408	0.0%	0.732
AA vs. GG[d]	427/537	0.87 (0.60,1.27)	0.478	11.0%	0.325
GA/AA vs. GG[e]	752/912	1.05 (0.86,1.29)	0.622	0.0%	0.987
AA vs. GA/GG[f]	752/912	0.97 (0.71,1.32)	0.829	56.9%	0.098
DNMT1 rs8101866					
TC vs. TT[c]	643/1159	0.99 (0.81, 1.21)	0.926	48.2%	0.165
CC vs. TT[d]	411/751	0.80 (0.55,1.17)	0.252	0.0%	0.452
TC/CC vs. TT[e]	686/1255	0.96 (0.80, 1.16)	0.662	0.0%	0.324
CC vs. TC/TT[f]	686/1255	0.80 (0.55,1.17)	0.252	13.1%	0.283
DNMT3A rs1550117					
GA vs. GG[c]	839/1548	1.12 (0.93,1.33)	0.229	0.0%	0.436
AA vs. GG[d]	605/1102	**2.03 (1.38,3.00)**	**0.000**	**86.9%**	**0.000**
GA/AA vs. GG[e]	1104/1892	**1.20 (1.01,1.42)**	**0.038**	**69.0%**	**0.040**
AA vs. GA/GG[f]	896/1601	**1.96 (1.33,2.89)**	**0.001**	**85.8%**	**0.001**
DNMT3A rs13420827					
CG vs. CC[c]	656/1206	0.84 (0.68,1.03)	0.090	44.3%	0.180
GG vs. CC[d]	495/851	1.16 (0.73,1.85)	0.523	0.0%	0.423
CG/GG vs. CC[e]	689/1255	0.87 (0.72,1.06)	0.171	0.0%	0.336
GG vs. CG/CC[f]	689/1255	1.23 (0.78,1.95)	0.371	0.0%	0.320
DNMT3B rs2424913					
CT vs. TT[c]	1086/1053	0.66 (0.32,1.36)	0.258	0.0%	0.992
CC vs. TT[d]	1075/1032	3.02 (0.12,74.69)	0.500	–	–
CT/CC vs. TT[e]	1087/1053	0.71 (0.35,1.44)	0.346	0.0%	0.849
CC vs. CT/TT[f]	1087/1053	3.02 (0.12,74.69)	0.500	–	–
DNMT3B rs1569686					
GT vs. TT[c]	745/1262	0.88 (0.69,1.13)	0.320	83.7%	0.002
GG vs. TT[d]	644/1072	0.96 (0.46,2.01)	0.923	3.1%	0.310
GT/GG vs. TT[e]	1225/1789	**0.74 (0.61,0.90)**	**0.003**	**80.1%**	**0.000**
GG vs. GT/TT[f]	756/1283	0.97 (0.46,2.02)	0.930	0.0%	0.394

The bolds pointed to models that had statistically significant associations with gastric cancer.

[a] P value of the Z-test for odds ration test.
[b] P value of the Q-test for heterogeneity test.
[c] Heterozygote model (heterozygous vs. homozygous frequent allele).
[d] Homozygote model (homozygous rare vs. homozygous frequent allele).
[e] Dominant model (homozygous rare + heterozygous vs. homozygous frequent allele).
[f] Recessive model (homozygous rare vs. heterozygous + homozygous frequent allele).

quantitatively synthesized, the systematic review presented their associations with GC (Table 3). Three SNPs rs36012910, rs7560488 and rs6087990 (Wu et al., 2014; Wu et al., 2012; Liu, 2008) were reported associated with GC and others not.

3.3. Heterogeneity Analysis (Sensitivity and Subgroup Analysis)

There was obvious heterogeneity in rs1550117 (AA vs. GG I^2 86.9%, P_{het} 0.000; GA/AA vs. GG: I^2 69.0%, P_{het} 0.040; AA vs. GA/GG: I^2 85.8%, P_{het} 0.001) and rs1569686 (GT vs. TT: I^2 83.7%, P_{het} 0.002; GT/GG vs. TT: I^2 80.1%, P_{het} 0.000). A sensitivity analysis was conducted to explore which study primarily influenced the pooled ORs (Table S2, Fig. S1–S2). For rs1550117, the heterogeneity was mostly caused by a study (Fan et al., 2010), since when it was removed, 95%IC changed in direction of association (OR 1.06, 95%CI 0.87–1.29) and heterogeneity went to zero (I^2 0%, P_{het} 0.73). Likewise, for rs1569686, Wang et al. (2015b) was found to be the major originator after excluded (95%IC didn't change in direction but heterogeneity went to zero: OR 0.49, 95%CI 0.37–0.65, I^2 0%, P_{het} 0.88). We compared characteristics of the two studies to the other's. Two factors were screened out to explain the heterogeneity: population areas (Jiangsu province or others) and genotyping methods (PCR-RFLP or others). Then a subgroup analysis was performed (Fig. 3). Population areas: for Jiangsu population, rs1550117 and rs1569686 were associated with GC (OR 1.77, 95%CI 1.25–2.51; OR 0.48, 95%CI 0.36–0.64), but for others (Jiangxi, Jilin and Heilongjiang provinces) no associations were found (OR 1.06, 95%CI 0.87–1.29; OR 1.15, 95%CI 0.87–1.52). Genotyping methods: by PCR-RFLP, rs1550117 and rs1569686 were detected associated with GC (OR 1.77, 95%CI 1.25–2.51; OR 0.49, 95%CI 0.37–0.65) but by others (TaqMan and MassArray) significant

associations were not discovered (OR 1.06, 95%CI 0.87–1.29; OR 1.20, 95%CI 0.90–1.60).

4. Discussion

Of the seven SNPs, two (rs16999593 and rs1550117) and one (rs1569686) were significantly associated with GC risk indicating a range of effects from the increased (DNMT1 and DNMT3A) to the reduced (DNMT3B).

4.1. DNMT1

Our results proved rs16999593 as a potential biomarker for GC susceptibility which was exactly consistent with the results on other types of cancers, such as breast cancer and prostate cancer (Tao et al., 2015; He et al., 2014). In addition, we did not find rs2228611 associated with GC, but it was recently reported that patients carrying the mutant genotypes significantly lived longer than those bearing the wild, indicating that rs2228611 might be a positive prognostic marker for GC survival (Jia et al., 2016).

4.2. DNMT3A and DNMT3B

In terms of rs1550117, our findings opposed a previous meta-analysis and we could attribute this contradiction to differences in using homozygote models (Liu et al., 2015). For rs1569686, we consider it as a protective factor for gastric carcinogenesis and similar results were discovered in head and neck cancer, lung cancer and colorectal cancer (Duan et al., 2015; Zhang et al., 2015; Xia et al., 2015; Zhu et al., 2012). However, another study argued it was associated with poor
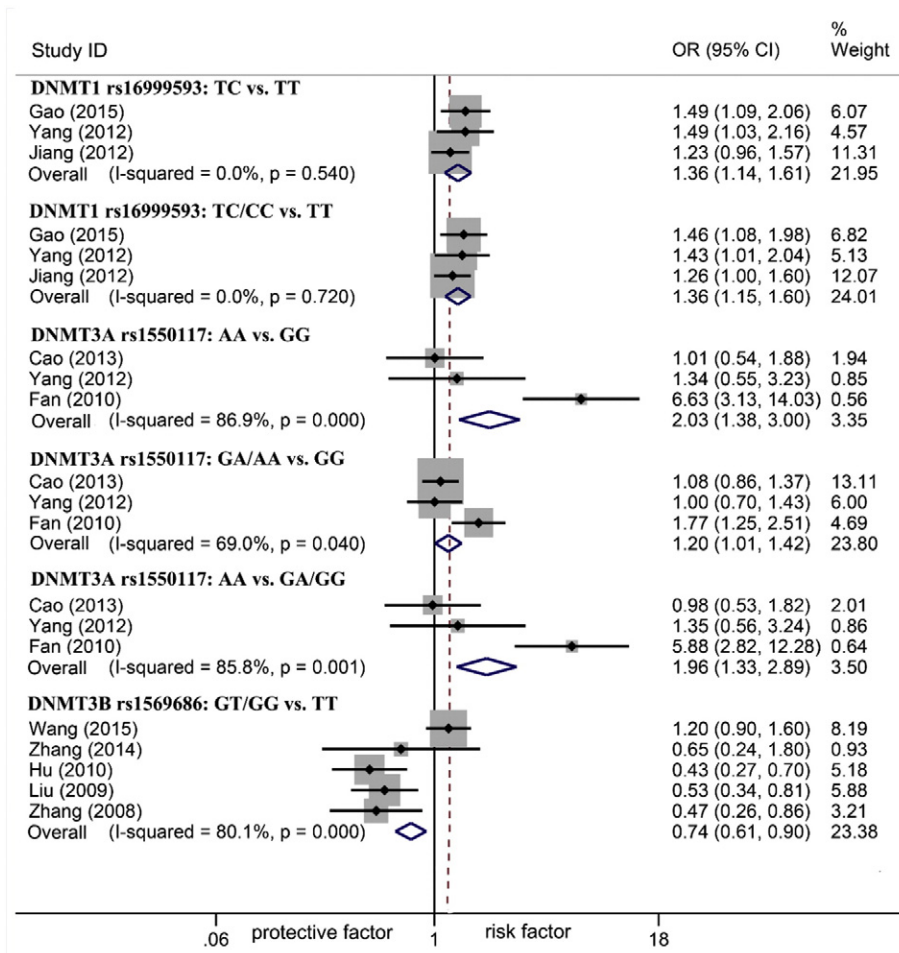

Fig. 2. Forest plot of DNMT1, DNMT3A and DNMT3B polymorphisms associated with GC risk.

Table 3
Systematic review of associations between *DNMTs* SNPs and gastric cancer risk.

Study	Country	Sample size (cases/controls)	Gene	SNPs	OR (95%CI)	
					Heterozygote model	Homozygote model
Yang et al., 2012	China	242/294	*DNMT1*	rs2114724 C > T	1.16 (0.81, 1.68)	0.62 (0.30, 1.27)
Jiang et al., 2012a, b	China	447/961	*DNMT1*	rs10420321 A > G	0.96 (0.66, 1.41)	1.17 (1.88,1.55)
Jiang et al., 2012a, b	China	447/961	*DNMT1*	rs8111085 T > C	1.08 (0.88, 1.43)	1.18 (0.82, 1.69)
Jiang et al., 2012a, b	China	447/961	*DNMT1*	rs2288349 G > A	0.93 (0.71, 1.22)	0.81 (0.50, 1.33)
Khatami et al., 2009	Iran	200/200	*DNMT1*	rs721186 G > A	1.12 (0.06, 16.0)	–
Khatami et al., 2009	Iran	200/200	*DNMT1*	rs13784 G > A	–	–
Khatami et al., 2009	Iran	200/200	*DNMT1*	rs11488 A > T	–	–
Wu et al., 2012	China	340/251	*DNMT3A*	**rs36012910 A > G**	**2.44 (1.37, 4.33)**	1.00 (0.98, 1.01)
Yang et al., 2012	China	242/294	*DNMT3A*	rs13428812 A > G	0.93 (0.64, 1.35)	1.11 (0.58, 2.12)
Yang et al., 2012	China	242/294	*DNMT3A*	rs11887120 T > C	0.96 (0.63, 1.47)	1.26 (0.76, 2.07)
Wu et al., 2014	China	405/408	*DNMT3A*	**rs7560488 T > C**	**1.73 (1.24, 2.41)**	**2.50 (1.01, 6.23)**
Wang et al., 2015a, b	China	447/961	*DNMT3B*	rs6119954 G > A	1.00 (0.76, 1.31)	1.37 (0.88, 2.13)
Wang et al., 2015a, b	China	447/961	*DNMT3B*	rs4911107 A > G	0.86 (0.26, 2.88)	0.76 (0.23, 2.46)
Wang et al., 2015a, b	China	447/961	*DNMT3B*	rs4911259 G > T	0.86 (0.26, 2.89)	0.76 (0.23, 2.45)
Wang et al., 2015a, b	China	447/961	*DNMT3B*	rs8118663 A > G	1.28 (0.95, 1.72)	1.32 (0.91, 1.91)
Yang et al., 2012	China	242/294	*DNMT3B*	rs2424908 T > C	0.98 (0.66, 1.45)	1.05 (0.64, 1.71)
Liu, 2008	China	313/350	*DNMT3B*	**rs6087990 C > T**	–	**1.46 (1.07, 2.01)**

SNPs, single nucleotide polymorphisms; heterozygote model (heterozygous vs. homozygous frequent allele); homozygote model (homozygous rare vs. homozygous frequent allele). The bolds pointed to SNPs that had statistically significant associations with gastric cancer.

prognosis in GC cases (Wang et al., 2015a). Maybe it played different roles in pathogenesis and prognosis. Particularly, we found in Jiangsu, a high GC incidence area of China (Liu et al., 2007), mutant rs1550117 doubled the risk and mutant rs1569686 lowered by a half of it. Also, even though some studies discovered TaqMan was more specific and

sensitive than PCR-RFLP to detect polymorphisms or virus (Martinez-Trevino et al., 2016; Campsall et al., 2004), we found PCR-RFLP was so far a best method for risk detection in GC. Regarding rs2424913, we didn't find it associated with GC in Chinese. A review reported it could significantly decrease cancers in African but not Asian
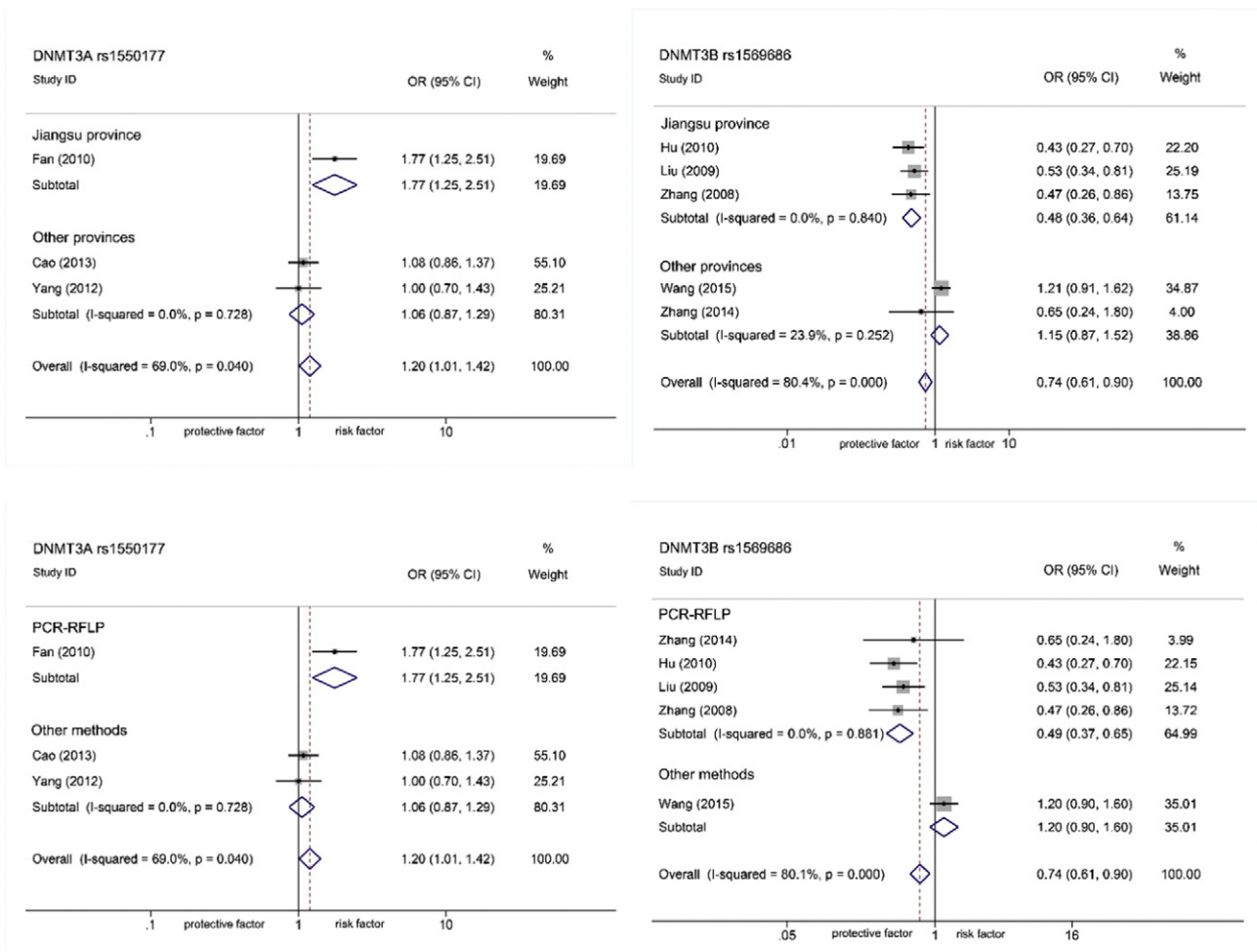
Fig. 3. Forest plot of subgroup analysis on *DNMT3A* rs1550117 and *DNMT3B* rs1569686 polymorphisms (dominant model) by population area and genetic methods. Population area (Jiangsu province and other provinces: Jiangxi, Jilin and Heilong Jiang provinces, in China) (A); Genetic methods (PCR-RFLP and other methods: TaqMan and MassArray) (B).

(Duan et al., 2015). It was speculated whether rs2424913 enabled African to catch GC rather than other populations. Although some meta-analysis studies demonstrated that rs6087990 might confer protection against overall cancers (Duan et al., 2015; Zhang et al., 2015), but it represented an opposite effect on GC as our systematic review showed (Liu, 2008).

4.3. Strengths and Limitations

Previous meta-analysis studies primarily evaluated associations between a few SNPs and cancers without classification, such as GC (Zhu et al., 2015; Duan et al., 2015; Liu et al., 2015; Zhang et al., 2015; Xia et al., 2015). The major strengths of our study was its comprehensive and systematic focus on GC and SNPs from three main types of *DNMTs*, 17 SNPs in total. Also, some mistakes in previous results were corrected in our study (Liu et al., 2015). At the same time, there were some limitations. Firstly, significant heterogeneities were observed for a few genetic models. Although a sensitivity analysis and a subgroup analysis were performed to clarify sources, we cannot find all potential factors. Second the meta-analysis findings were currently restricted to Chinese population pending results from other populations in future studies.

5. Conclusion

Our meta-analysis suggested that *DNMT1* rs16999593 and *DNMT3A* rs1550117 could contribute to GC and that *DNMT3B* rs1569686 might function as a protective factor against gastric carcinogenesis. By using these significant SNPs as biomarkers, it is feasible to estimate the risk of catching GC and thus formulate timely preventive strategy.

Author Contributions

F.G.H., Q.S. and H.J.L conceived and designed the study. H.J.L., W.L., S.S.L. and S.Q.Z. took full responsibility for data collecting and accuracy. H.J.L. and W.L. performed the meta-analysis and systematic review, and drafted the manuscript. W.B.W., J.L.R., and Q.L. helped revise the manuscript.

Funding

Conflicts of Interest

The authors have no conflicts of interest.

Appendix A. The Search Strategy for PubMed Comprised the Following

1. Colorectal neoplasms[mesh]
2. DNMT1[tiab]
3. DNMT3A [tiab]
4. DNMT3B[tiab]
5. DNMTs[tiab]
6. DNA methyltransferases[tiab]
7. (2 or 3 or 4 or 5 or 6)
8. 1 and 7

Appendix A. Supplementary Data

Supplementary data to this article can be found online at doi:10.1016/j.ebiom.2016.10.028.

References

Aung, P.P., et al., 2005. No evidence of correlation between the single nucleotide polymorphism of DNMT3B promoter and gastric cancer risk in a Japanese population. Oncol. Rep. 14 (5), 1151–1154.

Brown, K.D., Robertson, K.D., 2007. DNMT1 knockout delivers a strong blow to genome stability and cell viability. Nat. Genet. 39 (3), 289–290.

Campsall, P.A., et al., 2004. Detection and genotyping of *Varicella-zoster* virus by TaqMan allelic discrimination real-time PCR. J. Clin. Microbiol. 42 (4), 1409–1413.

Cao, X., et al., 2012. Single nucleotide polymorphisms of DNA methyltransferase 1 gene is associated with the risk of gastric atrophy and *Helicobacter pylori* infection. J. Gastroenterol. Hepatol. 27, 143.

Cao, X.Y., et al., 2013. DNMT3a rs1550117 polymorphism association with increased risk of *Helicobacter pylori* infection. Asian Pac. J. Cancer Prev. 14 (10), 5713–5718.

Chang, S.C., et al., 2010. One-carbon metabolism related gene polymorphisms and the risk of stomach cancer in a Chinese population. Am. J. Epidemiol. 171, S67.

Chang, S.C., et al., 2014. Single nucleotide polymorphisms of one-carbon metabolism and cancers of the esophagus, stomach, and liver in a Chinese population. PLoS One 9(10).

Duan, F., et al., 2015. Systematic evaluation of cancer risk associated with DNMT3B polymorphisms. J. Cancer Res. Clin. Oncol. 141 (7), 1205–1220.

Esteller, M., 2008. Epigenetics in cancer. N. Engl. J. Med. 358 (11), 1148–1159.

Fan, H., et al., 2010. A functional polymorphism in the DNA methyltransferase-3A promoter modifies the susceptibility in gastric cancer but not in esophageal carcinoma. BMC Med. 8, 12.

Ferlay, J., et al., 2015. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int. J. Cancer 136 (5), E359–E386.

Fu, H.Y., et al., 2010. Arsenic trioxide inhibits DNA methyltransferase and restores expression of methylation-silenced CDKN2B/CDKN2A genes in human hematologic malignant cells. Oncol. Rep. 24 (2), 335–343.

Gao, Q., et al., 2011. Deletion of the de novo DNA methyltransferase Dnmt3a promotes lung tumor progression. Proc. Natl. Acad. Sci. U. S. A. 108 (44), 18061–18066.

Harder, J., et al., 2008. Quantitative promoter methylation analysis of hepatocellular carcinoma, cirrhotic and normal liver. Int. J. Cancer 122 (12), 2800–2804.

He, B.S., Pan, Y.Q., Zhu, C.B., 2014. Polymorphisms of DNA methyltransferases and the risk of prostate cancer. Zhonghua Nan Ke Xue 20 (12), 1077–1081.

Hu, J., et al., 2010. DNMT3B promoter polymorphism and risk of gastric cancer. Dig. Dis. Sci. 55 (4), 1011–1016.

Jia, Z., et al., 2016. Polymorphisms of the DNA methyltransferase 1 gene predict survival of gastric cancer patients receiving tumorectomy. Dis. Markers 2016, 8578064.

Jiang, J., et al., 2012a. Polymorphisms of the DNA methyltransferase 1 associated with reduced risks of *Helicobacter pylori* infection and increased risks of gastric atrophy. PLoS One 7 (9), e46058.

Jiang, J., et al., 2012b. Role of polymorphisms of DNA methyltransferases in risks of gastric cancer and atrophic gastritis. Eur. J. Cancer 48, S3.

Jiang, J., et al., 2013. Polymorphisms of DNA methyltransferase 3a associated with risk of helicobacter pylori infection in gastric cancer. Eur. J. Cancer 49, S18.

Kanai, Y., et al., 2003. Mutation of the DNA methyltransferase (DNMT) 1 gene in human colorectal cancers. Cancer Lett. 192 (1), 75–82.

Khatami, F., et al., 2009. Lack of effects of single nucleotide polymorphisms of the DNA methyltransferase 1 gene on gastric cancer in Iranian patients: a case control study. Asian Pac. J. Cancer Prev. 10 (6), 1177–1182.

Kullmann, K., et al., 2013. DNMT1 genetic polymorphisms affect breast cancer risk in the central European Caucasian population. Clin. Epigenetics 5 (1).

Lieb, W., et al., 2006. Association of angiotensin-converting enzyme 2 (ACE2) gene polymorphisms with parameters of left ventricular hypertrophy in men. Results of the MONICA Augsburg echocardiographic substudy. J. Mol. Med. (Berl.) 84 (1), 88–96.

Liu, D., 2008. Correlation analysis of DNMT3B −283 SNP and the susceptibility of gastric cancer. Inner Mongolia J. Tradit. Chin. Med. 27 (11), 187–188.

Liu, D., 2009. Associated Study on the Promoter SNPs of DNMT3A/3B With Genetic Susceptibility to Gastric Cancer and Esophageal Cancer. Southeast University.

Liu, A., et al., 2007. Case-control analysis on stomach cancer in a high cancer incidence area of Jiangsu province. Chin. J. Public Health 23 (5), 575–576.

Liu, C.H., et al., 2015. DNMT3A −448A > G polymorphism and cancer risk: a meta-analysis. Genet. Mol. Res. 14 (2), 3640–3649.

Lu, L., et al., 2016. Prognostic and clinicopathological value of Gli-1 expression in gastric cancer: a meta-analysis. Oncotarget.

Luo, Y., et al., 2015. The association of DNA methyltransferase 1 gene polymorphisms with susceptibility to childhood acute lymphoblastic leukemia. Biomed. Pharmacother. 73, 35–39.

Martinez-Trevino, D.A., et al., 2016. Rapid detection of the GSTM3 A/B polymorphism using real-time PCR with TaqMan probes. Arch. Med. Res.

Mostowska, A., et al., 2013. DNMT1, DNMT3A and DNMT3B gene variants in relation to ovarian cancer risk in the Polish population. Mol. Biol. Rep. 40 (8), 4893–4899.

Okano, M., et al., 1999. DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. Cell 99 (3), 247–257.

Saeki, N., et al., 2013. Genetic factors related to gastric cancer susceptibility identified using a genome-wide association study. Cancer Sci. 104 (1), 1–8.

Sun, M.Y., et al., 2012. Association of DNMT1 and DNMT3B polymorphisms with breast cancer risk in Han Chinese women from South China. Genet. Mol. Res. 11 (4), 4330–4341.

Tao, R., et al., 2015. The possible role of EZH2 and DNMT1 polymorphisms in sporadic triple-negative breast carcinoma in southern Chinese females. Tumor Biol. 36 (12), 9849–9855.

Thakkinstian, A., et al., 2011. Systematic review and meta-analysis of the association between complement component 3 and age-related macular degeneration: a HuGE review and meta-analysis. Am. J. Epidemiol. 173 (12), 1365–1379.

Torre, L.A., et al., 2015. Global cancer statistics, 2012. CA Cancer J. Clin. 65 (2), 87–108.

Wang, Y.M., et al., 2005. Single nucleotide polymorphism in DNA methyltransferase 3B promoter and its association with gastric cardiac adenocarcinoma in North China. World J. Gastroenterol. 11 (23), 3623–3627.

Wang, C., et al., 2015a. Polymorphism of DNA methyltransferase 3b and association with development and prognosis in gastric cancer. PLoS One 10 (8), e0134059.

Wang, C., et al., 2015b. Association between polymorphisms of DNMT3b gene and its expression level in gastic cancer tissue and gastric cancer. J. Jilin Univ. (Med. Ed.) 41 (2), 368–373.

Wu, Q., et al., 2012. DNMT3A rs36012910 A > G polymorphism and gastric cancer susceptibility in a Chinese population. Mol. Biol. Rep. 39 (12), 10949–10955.

Wu, H., et al., 2014. A novel functional TagSNP Rs7560488 in the DNMT3A1 promoter is associated with susceptibility to gastric cancer by modulating promoter activity. PLoS One 9 (3), e92911.

Xia, Z., et al., 2015. Quantitative assessment of the association between DNMT3B-579G > T polymorphism and cancer risk. Cancer Biomark. 15 (5), 707–716.

Xiang, G., et al., 2010. Association of DNMT1 gene polymorphisms in exons with sporadic infiltrating ductal breast carcinoma among Chinese Han women in the Heilongjiang Province. Clin. Breast Cancer 10 (5), 373–377.

Xue, W., et al., 2015. Association between PLCE1 rs2274223 A > G polymorphism and cancer risk: proof from a meta-analysis. Sci. Rep. 5, 7986.

Yan, G., Yanxin, C., Xiangjun, J., 2015. Association of Dnmt1 single-nucleotide polymorphisms and risk of gastric cancer. Chi. J. Oncol. Prev. Treat. 7 (6), 394–397.

Yang, L., 2006. Incidence and mortality of gastric cancer in China. World J. Gastroenterol. 12 (1), 17–20.

Yang, X.X., et al., 2012. Risk-association of DNA methyltransferases polymorphisms with gastric cancer in the Southern Chinese population. Int. J. Mol. Sci. 13 (7), 8364–8378.

Zhang, S., 2008. Correlation Analysis of SNP in DNMT3B Promoter Region and Gastric Cancer Risk. Jiamusi University.

Zhang, S., et al., 2014. Association of DNMT3B SNP (−579G/T) with the risk of gastric cancer in Jiamusi. Heilongjiang Med. Pharm. 37 (5), 82–83.

Zhang, Y., et al., 2015. Association of DNMT3B −283 T > C and −579 G > T polymorphisms with decreased cancer risk: evidence from a meta-analysis. Int. J. Clin. Exp. Med. 8 (8), 13028–13038.

Zhao, C., Bu, X., 2012. Promoter methylation of tumor-related genes in gastric carcinogenesis. Histol. Histopathol. 27 (10), 1271–1282.

Zhu, S., et al., 2012. DNMT3B polymorphisms and cancer risk: a meta analysis of 24 case-control studies. Mol. Biol. Rep. 39 (4), 4429–4437.

Zhu, J., et al., 2015. Polymorphism of DNA methyltransferase 3B −149C/T and cancer risk: a meta-analysis. Med. Oncol. 32 (1), 399.