Correlation of RASSF1A gene methylation with gastric cancer and its clinical features: a meta-analysis

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Abstract

Introduction: It has been reported that the development and progression of gastric cancer are strongly associated with Ras association domain family 1A (RASSF1A) gene methylation; however, some of the findings are contradictory. The aim of this study was to confirm the relationship between RASSF1A methylation and gastric cancer, and the relationship between gender, age, stage, differentiation, pathological type, metastasis of gastric cancer and RASSF1A methylation. We also explored the differences in RASSF1A gene methylation between Asian and non-Asian gastric cancer patients.

Material and methods: The database was searched for case-control studies of RASSF1A gene methylation associated with gastric cancer, and suitable literature was selected according to pre-defined inclusion and exclusion criteria. The quality of the included literature was evaluated, after which forest plots and funnel plots were performed to analyze sensitivity and publication bias.

Results: A total of 13 papers satisfied the inclusion criteria, and thus were included in this study. Meta-analysis showed that RASSF1A gene methylation was associated with gastric cancer (effect size (ES) = 17.13, 95% confidence interval (CI): 6.94–27.32, p = 0.001; p for heterogeneity = 0.183, I^2 = 25.8%). Age (OR = 0.67, 95% CI: 0.47–0.95, p = 0.025; p for heterogeneity = 0.257, I^2 = 22.5%), gastric cancer stage (OR = 0.62, 95% CI: 0.44–0.88, p = 0.008; p for heterogeneity = 0.615, I^2 = 0%), and gastric cancer metastasis (OR = 2.60, 95% CI: 1.04–6.46, p = 0.040; p for heterogeneity = 0.904, I^2 = 0%) were associated with RASSF1A gene methylation. Gender (OR = 1.16, 95% CI: 0.84–1.62, p = 0.369; p for heterogeneity = 0.704, I^2 = 0%), degree of gastric cancer differentiation (OR = 0.96, 95% CI: 0.60–1.52, p = 0.860; p for heterogeneity = 0.077, I^2 = 47.3%), and pathological type of gastric cancer (OR = 1.15, 95% CI: 0.64–2.09, p = 0.635; p for heterogeneity = 0.276, I^2 = 22.5%) were not associated with methylation of the RASSF1A gene. No significant publication bias was found in this study.

Conclusions: Gastric carcinogenesis was found to be associated with RASSF1A gene methylation. There were no significant differences in RASSF1A gene methylation among patients of different ages, different stages, and metastasis of gastric cancer. Yet, there were significant differences in RASSF1A gene methylation in patients of different gender, degree of gastric cancer differentiation, and type of gastric cancer pathology.

Key words: gastric cancer, RASSF1A, methylation, age, sex, metastasis, differentiation, stage.

Introduction

Gastric cancer is one of the most prevalent tumors globally [1]. Despite advances in chemotherapy and technology, gastric cancer is the third leading cause of cancer-related death worldwide [2]. It remains very high in some countries and regions due to the fact that most patients are usually diagnosed at advanced stages and the prognosis for advanced gastric cancer is poor [3, 4]. Gastric carcinogenesis is a complex accumulation of genetic epigenetic alterations, which occur in close association with the activation of proto-oncogenes and inactivation of oncogenes [5]. DNA methylation is a common epigenetic phenomenon, which usually occurs when DNA methyltransferase (DNMT) adds a methyl group to the carbon 5' position of CpG dinucleotide residues [6, 7]. DNA methylation maintains the normal conformation of chromosomes in cells. The RASSF1A gene, located at 3p21.3, is one of the tumor suppressor genes, whose full-length is 11000 bp; it contains 8 exons and 2 different promoters [8]. It also encodes the production of RASSF1A, which in turn regulates microtubules, stabilizes the genome, regulates the cell cycle, controls apoptosis and controls tumor infiltration [9, 10]. Numerous studies have verified the importance of RASSF1A for microtubule stability. The RASSF1A gene can encode RASSF1A, and the interaction between RASSF1A and microtubules can maintain the stability of microtubules [11]. RASSF1A inhibits tumors by regulating the stability of microtubules, the combination of spindles, and chromosome attachment. The loss of the RASSF1A gene in the microtubule region leads to the loss of tubulin stability, inhibition of death receptor dependent cell death, loss of the cell centrosome and structural changes of the mitotic spindle, thus weakening the stability of the genome and causing abnormal cell proliferation [12, 13]. Other studies have shown that RASSF1A can also regulate the process of cell mitosis [14]. The interaction between RASSF1A and RASSF1A gene binding protein 1 (RABP1) leads to the bipolar recruitment of RASSF1A to the spindle in the early and metaphase of mitosis, and interacts with CDC20 (one of the cell cycle related proteins), inhibits anaphase-promoting complex (APC), promotes the accumulation of cyclin A and cyclin B, and ultimately leads to the arrest of mitosis in the early and middle stages [15, 16]. The above results suggest that RASSF1A has an important role in normal cell growth, differentiation, and apoptosis. The RASSF1A gene is normally expressed in normal cells but rarely in cancer cells, which is due to the inactivation or silencing of the RASSF1A gene. The main mechanism of inactivation or silencing is the methylation of the RASSF1A gene. Previous studies have shown that RASSF1A gene methylation is a prevalent phenomenon of epigenetic alterations in gastric cancer cells [17]. However, some studies have reported that hypermethylation of the RASSF1A gene is not found in gastric cancer cells [18]. These contradictory results may be related to the complex pathological mechanisms of gastric cancer.

The aim of this study was to confirm the relationship between RASSF1A gene methylation and gastric cancer and further investigate the relationship between RASSF1A gene methylation and gender, age, gastric cancer stage, degree of gastric cancer differentiation, gastric cancer pathological type and gastric cancer metastasis.

Material and methods

Literature search strategy

The following databases were searched for relevant literature: PubMed, Embase, Cochrane Library, Web of Science and China National Knowledge Infrastructure databases. The search was conducted using Boolean logic operators in combination with search strategies and free words: [("RASSF1A" or "Ras-association domain family 1") and ("methylation") and ("Neoplasm, Stomach")]. As this study is an updated and more detailed elaboration of the study by Shi *et al.* [19], the timeframe was nearly ten years, and relevant studies included those published between January 2011 and January 2022, with no language restrictions. A manual search was also conducted for tentatively unpublished grey literature, with a grey literature count of 0.

Ethical approval was obtained for all participating sites for all included trials; written informed consent was provided by patients or their legal representatives in accordance with national and local regulations; the study protocol was pre-specified and PRISMA guidelines were followed for meta-analysis of individual patient data. The study was registered with PROSPERO under registration number CRD42021261585.

Study selection

The inclusion criteria were as follows: (1) case-control or cohort studies of gastric cancer; (2) all patients had pathologically confirmed gastric cancer; (3) methylation assays were limited to: methylation-specific PCR, (MSP), quantitative methylation-specific PCR (Q-MSP), pyrosequencing-based quantitative analysis (PSQ); (4) the frequency of RASSF1A methylation in gastric cancer tissues or patients' serum was reported.

The exclusion criteria were as follows: (1) duplicate literature from different databases; (2) patients with other tumors; (3) cellular or animal studies; (4) reviews, conference abstracts, case reports, letters.

Data extraction and quality assessment

The full texts of the included studies were carefully read by two authors who extracted the features of each study. Differences in extraction were evaluated by a third author and were eventually resolved through discussion between the three authors. Data extracted from these studies included the name of the first author, year of publication, country, gender, age, sample size, gastric cancer stage, degree of gastric cancer differentiation, and type of gastric cancer pathology. For case-control or cohort studies, the Newcastle-Ottawa Scale (NOS) quality evaluation tool was used, comprising eight dimensions with a total score of nine. An NOS score of ≥ 6 was considered to represent generally good quality; two of the included studies scored 9, six scored 8 and five scored 7.

Statistical analysis

Heterogeneity and publication bias of the included literature were evaluated using stata16. An inconsistency index (I^2) < 50% indicated no heterogeneity in the study, so a fixed-effects model was used; otherwise a random-effects model was used. Subgroup analysis was performed according to patient age, gender, gastric cancer stage, degree of cell differentiation, pathological type, and gastric cancer metastasis. Funnel plots were used to estimate publication bias.

Results

Search process

A total of 338 articles were retrieved from PubMed, Embase, Cochrane Library, Web of Science and China National Knowledge Infrastructure databases according to the search strategy. Among these, 207 duplicate articles were excluded, and after reading the titles or abstracts of the remaining 29 articles, 2 non-clinical studies, 9 studies with insufficient data and 5 reviews were excluded. Finally, the remaining 13 articles were included in the meta-analysis. Figure 1 shows a flow chart of the retrieval, inclusion and exclusion of studies, and the reasons for exclusion.

Characteristics of included studies

Table I shows the specific characteristics of the included studies [20–32]. The 13 included articles were all published in 2011–2020 and had sample sizes of 70–200. Nine of the included studies used gastric tissue to detect RASSF1A promoter methylation status, 4 were performed using serum samples, 4 were from European countries, 1 was from an African country and 8 were from Asian countries. Eight studies analyzed the relationship between gender and methylation of the RASSF1A gene, 7 analyzed the relationship between age (≤ 60 and > 60 years) and RASSF1A gene methylation, 8 analyzed the relationship between gastric cancer stage (early and advanced) and RASSF1A gene methylation, 7 analyzed the relationship between the degree of gastric cancer differentiation (good and poor) and RASSF1A gene methylation, 4 analyzed the relationship between gastric cancer metastasis (metastasis and no metastasis) and RASSF1A gene methylation, and 4 analyzed the relationship between gastric cancer pathology type (intestinal and diffuse) and RASSF1A gene methylation.

Results of quality assessment

The quality of the included studies is shown in Table II. The Newcastle–Ottawa Scale (NOS) table was used to evaluate the risk of patient selection in 13 studies. Of these 13 studies, 2 had 9 stars,

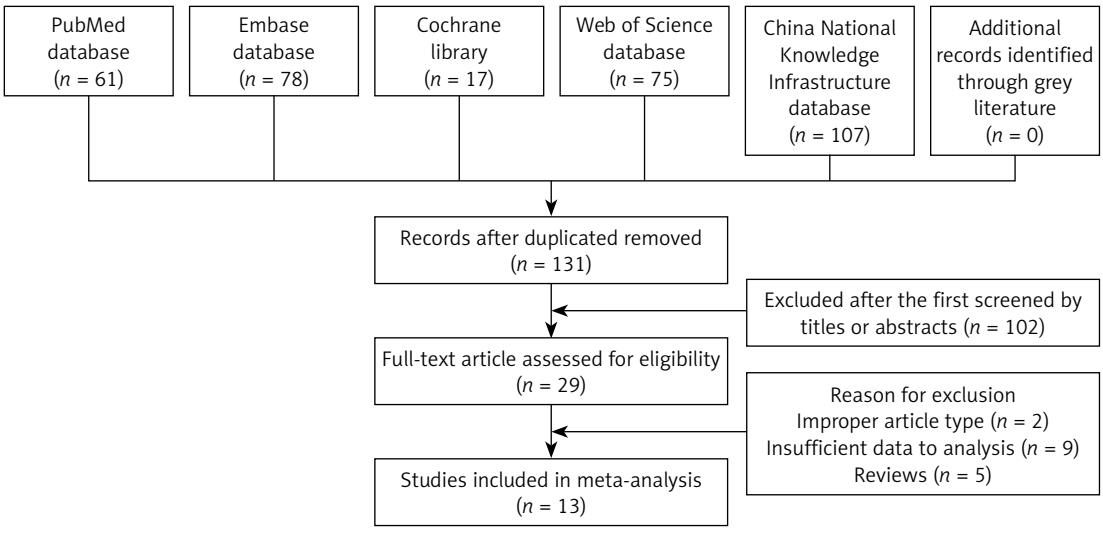

Figure 1. Flow diagram of study selection

Table I. Characteristics of studies included in the meta-analysis

Study	Year	Country	RASSF1A positive rate	*N*
Saliminejad [20]	2020	Iran	0.3333	96
Karamitrousis [21]	2020	Greece	0.7429	70
Nemtsova [22]	2017	Russia	0.1494	106
Bhat [23]	2016	India	0.4400	200
Pimson [24]	2016	Thailand	0.8317	101
Li [25]	2015	China	0.1275	102
Balgkouranidou [26]	2015	Greece	0.6849	73
Guo [27]	2014	China	0.0714	70
Yang [28]	2013	China	0.2830	113
Zhou [29]	2013	China	0.6522	92
Yao [30]	2012	China	0.2700	141
Balassiano [31]	2011	Denmark	0.3520	98
Ben Ayed-Guerfali [32]	2011	Tunisia	0.4557	79

Table II. Quality assessment using the Newcastle–Ottawa Scale

Research	Selection	Comparability	Exposure	Total points
Saliminejad	★★★★	★★	★★★	9
Karamitrousis	★★★★	★★	★★★	9
Nemtsova	★★★★	★★	★★	8
Bhat	★★★★	★	★★★	8
Pimson	★★★★	★★	★★	8
Li	★★★	★	★★★	7
Balgkouranidou	★★★★	★	★★★	8
Guo	★★★	★	★★★	7
Yang	★★★★	★	★★	7
Zhou	★★★★	★★	★★	8
Yao	★★★	★	★★★	7
Balassiano	★★★★	★★	★★	8
Ben Ayed-Guerfali	★★★	★	★★★	7

6 had 8 stars, and 5 had 7 stars. All the included studies had more than 6 stars, indicating that the quality of the included literature was good.

Results of meta-analysis

Figure 2 shows the clinical relationship between RASSF1A gene methylation and patients with gastric cancer (ES = 17.13, 95% CI: 6.94–27.32, p = 0.001; p for heterogeneity = 0.183, I^2 = 25.8%), with I^2 < 50% indicating no heterogeneity between studies. These 13 studies were all case-control studies, and the results indicated that the rate of RASSF1A gene methylation was higher in gastric cancer tissues or serum of gastric cancer patients than in normal tissues or normal individuals. The forest plot showed an effect size (ES) of 17.13, which was high, and also indicated the methylation rate of the RASSF1A gene in gastric cancer tissues or serum of gastric cancer patients.

Figure 3 shows whether RASSF1A gene methylation differs in gastric cancer patients by gender. A fixed-effects model was used as I^2 was < 50%. The results of subgroup analysis showed no significant difference in RASSF1A gene methylation among gastric cancer patients by gender (OR = 1.16, 95% CI: 0.84–1.62, p = 0.369; p for heterogeneity = 0.704, I^2 = 0%).

Figure 4 shows RASSF1A gene methylation differences among gastric cancer patients of different ages. A fixed-effects model was used as I^2 was < 50%. The results of the subgroup analysis showed that RASSF1A gene methylation differed in gastric cancer patients aged ≥ 60 years old versus < 60 years old (OR = 0.67, 95% CI: 0.47–0.95, p = 0.025; p for heterogeneity = 0.257, I^2 = 22.5%).

Figure 5 shows RASSF1A gene methylation differences among patients with different stages of gastric cancer. The subgroup results showed that

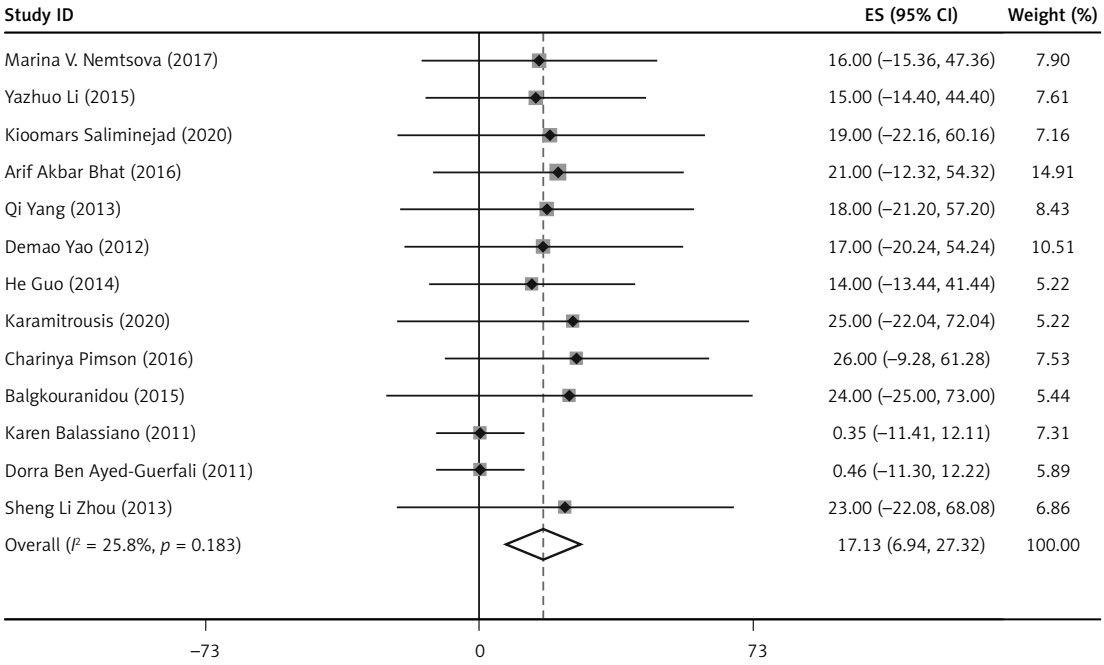

Figure 2. Forest plot of RASSF1A gene methylation in gastric cancer

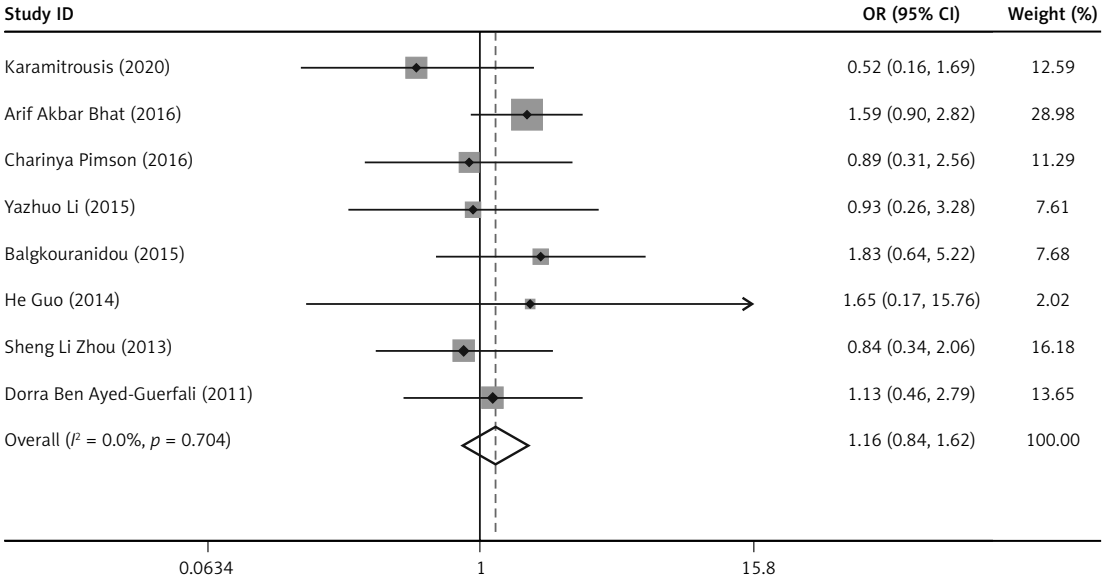

Figure 3. Forest plot of the relationship between gender and RASSF1A gene methylation in gastric cancer

RASSF1A gene methylation differed in patients with early versus advanced gastric cancer (OR = 0.62, 95% CI: 0.44–0.88, p = 0.008; p for heterogeneity = 0.615, I^2 = 0%).

Figure 6 shows RASSF1A gene methylation differences among patients with different degrees of differentiation of gastric cancer. The subgroup results showed no significant difference in RASSF1A gene methylation between well and poorly differentiated gastric cancer patients (OR = 0.96, 95% CI: 0.60–1.52, p = 0.860; p for heterogeneity = 0.077, I^2 = 47.3%).

Figure 7 shows RASSF1A gene methylation differences among patients with or without gastric cancer metastases. The subgroup results showed that RASSF1A gene methylation differed between patients with and without metastasis (OR = 2.60, 95% CI: 1.04–6.46, p = 0.040; p for heterogeneity = 0.904, I^2 = 0%).

Figure 8 shows RASSF1A gene methylation differences among patients with different pathological types of gastric cancer. The subgroup results showed no significant difference in RASSF1A gene methylation between patients with intestinal type and diffuse type of gastric cancer (OR = 1.15, 95% CI: 0.64–2.09, p = 0.635; p for heterogeneity = 0.276, I^2 = 22.5%).

Figure 9 illustrates a difference in RASSF1A gene methylation between Asian and non-Asian

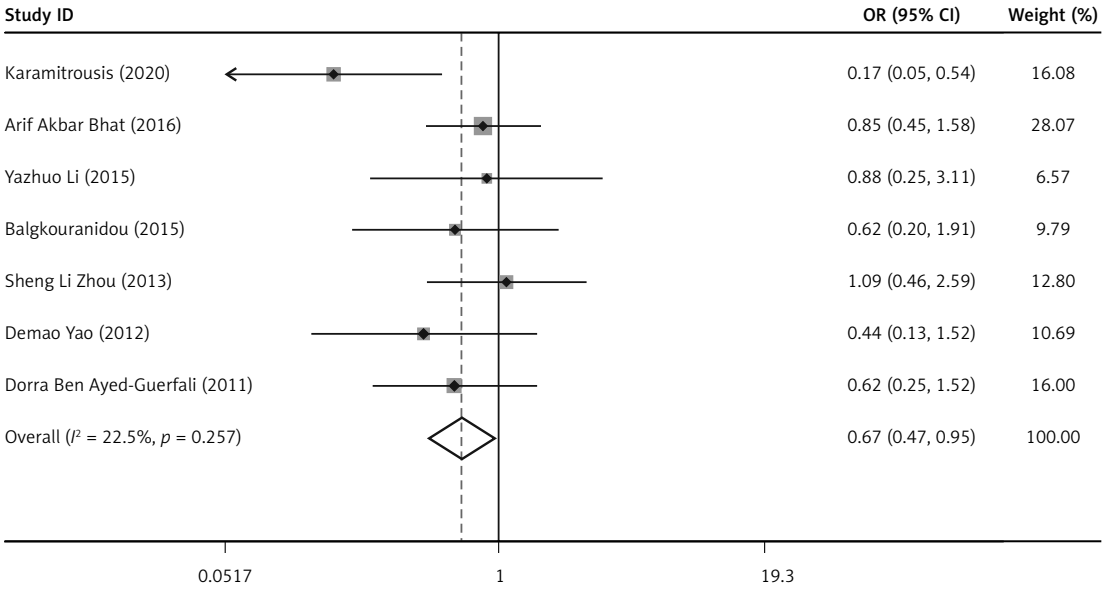

Figure 4. Forest plot of the relationship between age and RASSF1A gene methylation in gastric cancer

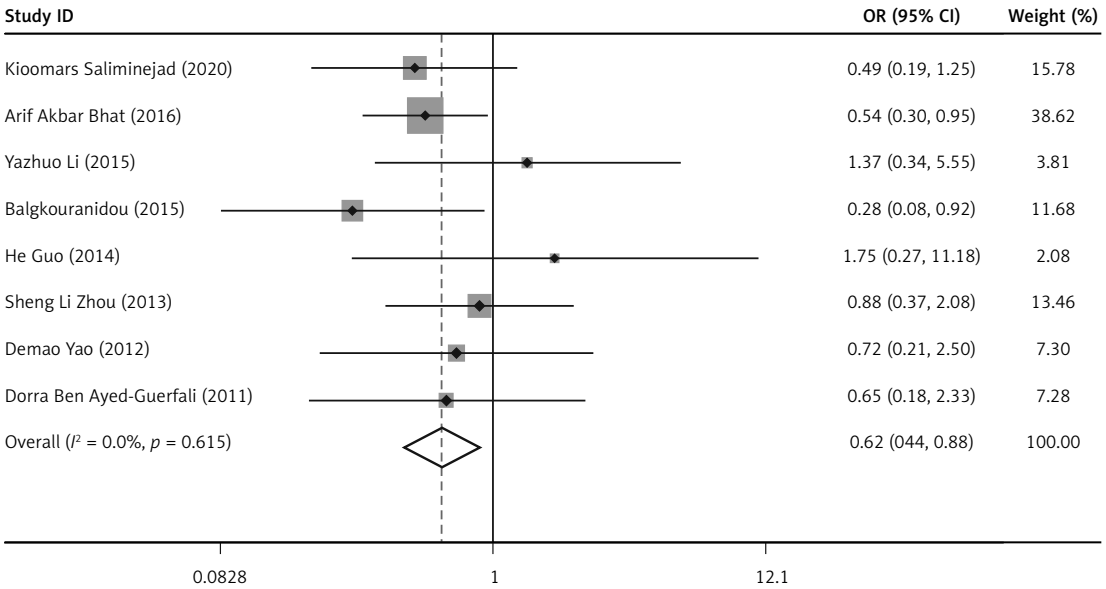

Figure 5. Forest plot of the relationship between gastric cancer stage and RASSF1A gene methylation in gastric cancer

gastric cancer patients. The results showed that the methylation rate of the RASSF1A gene in non-Asian gastric cancer patients was lower; yet, the difference was not statistically significant (ES = 12.37, 95% CI: –1.89–26.62). The methylation rate of the RASSF1A gene in Asian gastric cancer patients was higher, which was statistically significant (ES = 19.35, 95% CI: 5.98–32.73).

Results of sensitivity analysis and publication bias

Figure 10 shows the funnel plot of RASSF1A gene methylation in gastric cancer. Sensitivity analyses were conducted in this study after excluding the literature on a case-by-case basis, and the results were found to be relatively stable after testing. The results of Begg's test (z = 1.37, p = 0.123) and the Egger test (t = 1.24, p = 0.204) both indicated no potential publication bias.

Discussion

According to the results of the meta-analysis, the heterogeneity of RASSF1A gene methylation was low (I^2 = 25.8%); the experimental design and detection methods were basically the same in 13 studies. However, 9 studies tested gastric cancer tissues and 4 studies tested serum, which may be the main reason for the heterogeneity. In addition, as heterogeneity may also be related to gender, age, different stages of gastric cancer, different

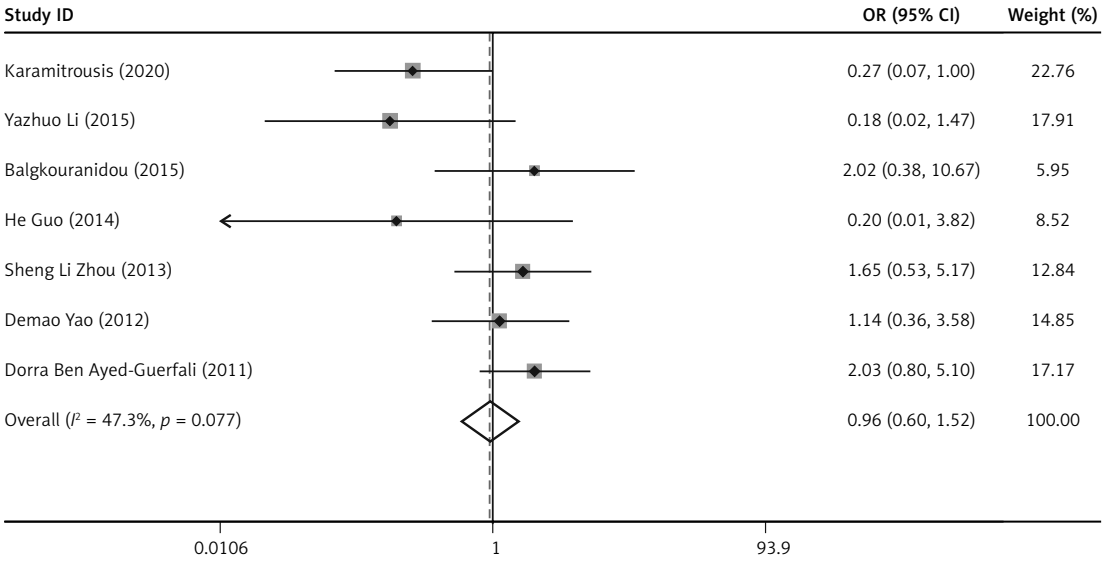

Figure 6. Forest plot of the relationship between the degree of differentiation of gastric cancer and the methylation of the RASSF1A gene in gastric cancer

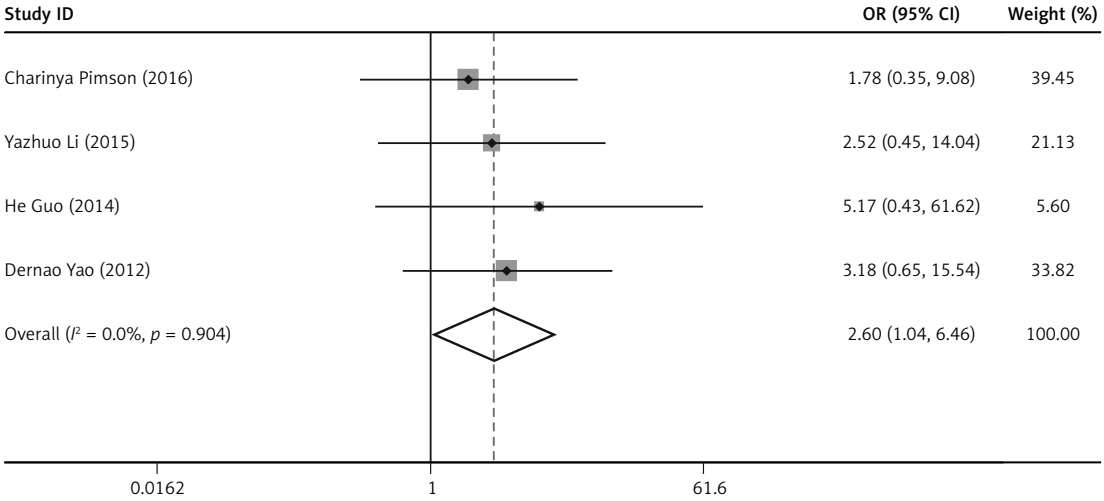

Figure 7. Forest plot of the relationship between gastric cancer metastasis and RASSF1A gene methylation in gastric cancer

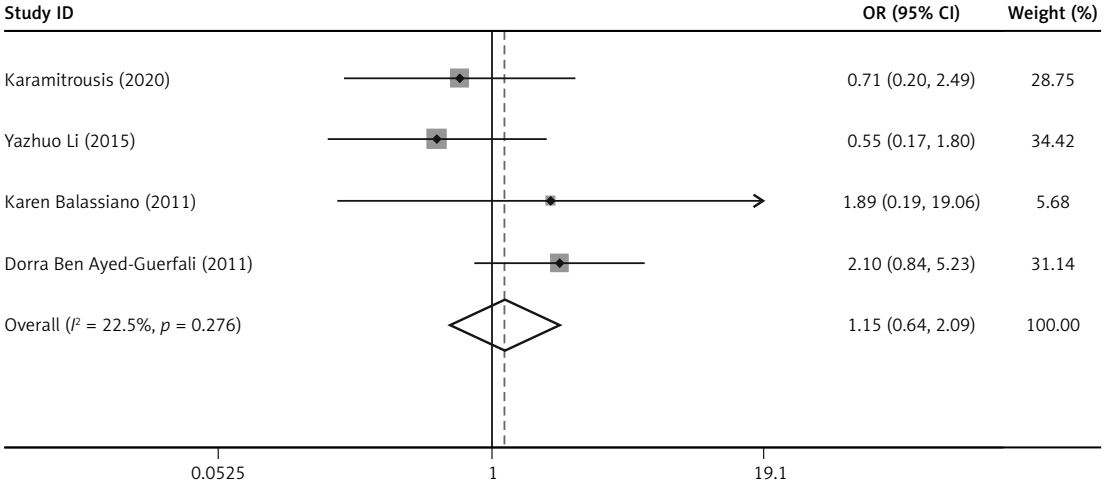

Figure 8. Forest plot of the relationship between the pathological type of gastric cancer and methylation of the RASSF1A gene in gastric cancer

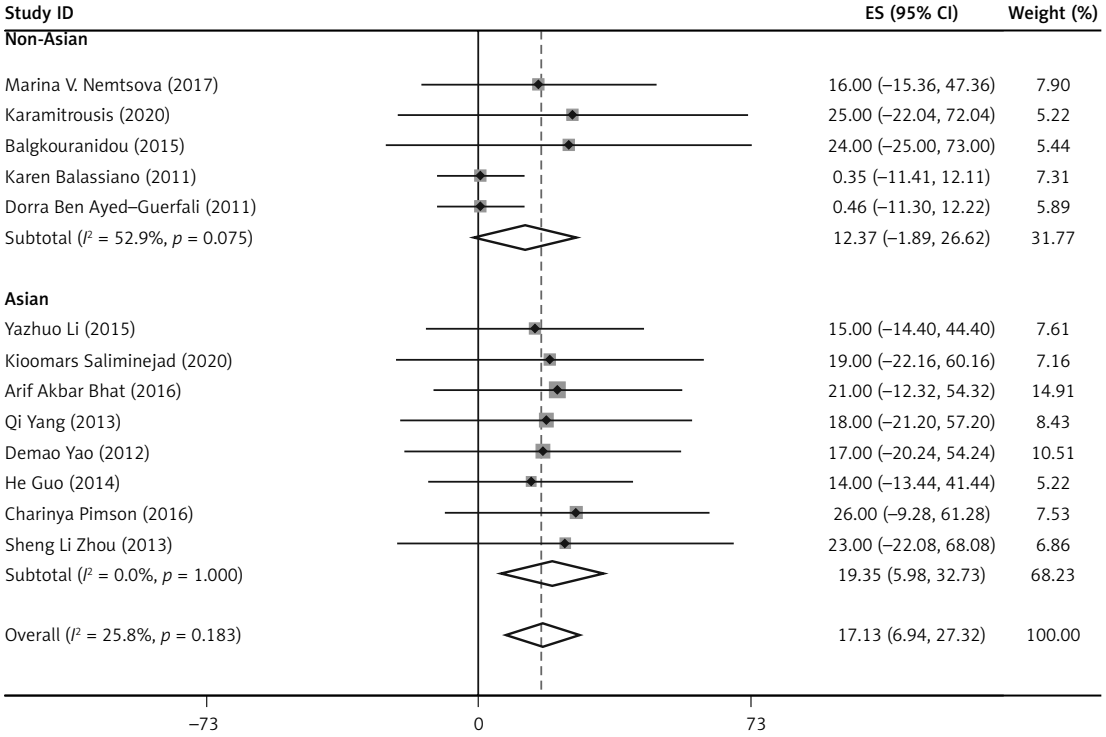

Figure 9. Subgroup of RASSF1A gene methylation in Asian and non-Asian gastric cancer patients

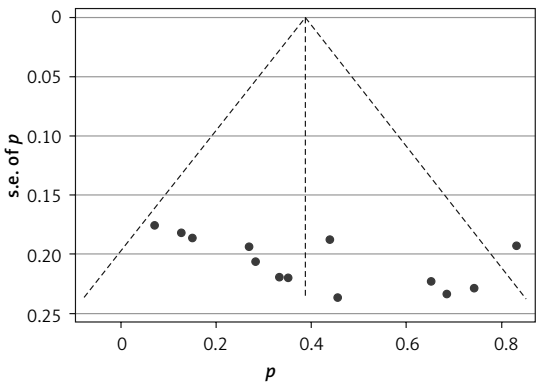

Figure 10. Funnel plot of RASSF1A gene methylation in gastric cancer

degrees of differentiation of gastric cancer, gastric cancer metastases, different pathological types of gastric cancer and race, we carried out subgroup analysis. The subgroup of gastric cancer with different degrees of differentiation had greater heterogeneity, which may be caused by the higher degree of methylation of the RASSF1A gene in poorly differentiated gastric cancer. Balgkouranidou *et al.* [26] proved that the frequency of DNA methylation in poorly differentiated gastric cancer patients was significantly higher than that in well-differentiated gastric cancer patients. At the same time, the age subgroup showed heterogeneity, which may be due to the decline in the body's ability to resist RASSF1A gene methylation as patients get older. Koch *et al.* [33] studied DNA methylation in many human tissues and confirmed that gene methylation is positively correlated with age. A comparison of Asian and non-Asian gastric cancer patients showed that Asian gastric cancer patients had higher RASSF1A gene methylation, so ethnicity may also be an important cause of heterogeneity. The final results suggested a link between RASSF1A methylation and gastric cancer.

Aberrant DNA methylation was found to be very common in human cancers and may be closely associated with the abnormal expression of oncogenes. Methylation of cytosine residues in the CpG island of the gene promoter inhibits transcription of tumor suppressors, and loss of gene function due to promoter methylation has a key role in promoting tumor formation [34]. The RASSF1A gene was found to have a direct role in the process of cell cycling, inducing apoptosis and regulating cell growth *in vitro* and *in vivo* [35, 36]. The Ras signaling pathway is a common signaling pathway, with Ras proteins shifting between an inactive GDP conformation and an active GTP conformation. The interaction between Ras protein and a range of downstream effector molecules is produced through the mitogen-activated protein kinase (MAPK) signaling pathway, including regulation of cell differentiation and growth, inhibition of cell growth, and promotion of cellular senescence and apoptosis, which function normally in healthy cells. The RASSF1A gene, an important regulatory molecule of the Ras protein, controls this regulation. The RASSF1A gene is a tumor sup-

pressor gene that is often silenced or inactivated by methylation of its promoter region in a variety of human tumors [37].

Li *et al.* [25] reported that RASSF1A gene methylation was not associated with gender, age, gastric cancer stage, degree of gastric cancer differentiation, gastric cancer metastasis or gastric cancer pathology type. Karamitrousis *et al.* [21] reported that RASSF1A gene methylation was associated with gender, age and gastric cancer stage. In response to these conflicting findings, 7 subgroup analyses were performed and the final results showed that only age, gastric cancer stage and gastric cancer metastasis were associated with RASSF1A gene methylation. At the same time, we found a difference in RASSF1A gene methylation between Asian and non-Asian patients with gastric cancer.

The main strength of this study is that it only analyzed data related to gastric cancer and RASSF1A methylation, excluding confounding factors caused by other tumors or genes. Despite this, there are still some weaknesses in this study, such as the small amount of literature included and the need to include more high quality studies for adequate evidence. The limitations of the literature data led to a meta-analysis of some other clinical features, such as: the degree of invasiveness, lymph node involvement and site of gastric cancer. Confirming the relationship between RASSF1A gene methylation and gastric cancer could provide a scientific basis for the mechanism of gastric carcinogenesis.

In conclusion, gastric carcinogenesis was found to be associated with RASSF1A gene methylation. There were no significant differences in RASSF1A gene methylation among patients of different age, different stage, and metastasis of gastric cancer. However, there were significant differences in RASSF1A gene methylation in patients of different gender, degree of gastric cancer differentiation, and type of gastric cancer pathology. At the same time, there was a difference in RASSF1A gene methylation between Asian and non-Asian patients with gastric cancer. Future studies should test whether RASSF1A gene methylation is associated with the development of other tumors.

Acknowledgments

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Conflict of interest

The authors declare no conflict of interest.

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