

Association between methylation of RASSF1A and breast cancer: a meta-analysis

Xingling DENG (✉ 1872438759@qq.com)

Affiliated tumor hospital of Guangxi medical university

Chaosi QIAN

Affiliated tumor hospital of Guangxi medical university

Guiting TAN

Affiliated tumor hospital of Guangxi medical university

Xueyan CHEN

Affiliated tumor hospital of Guangxi medical university

Liyong FENG

Affiliated tumor hospital of Guangxi medical university

Yinghua YU

Affiliated tumor hospital of Guangxi medical university

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Abstract

Objective: To systematically evaluate the relationship between methylation of RASSF1A and breast cancer. **Methods:** Screening the literatures that retrieved from PubMed, Cochrane Library and Ovid. Evaluate the quality of included studies. Data analysis performed by Revman5.3 and Stata12.0 software. **Results:** A total of 7 articles were included in this meta-analysis, including 1485 samples. The results indicate that the positive rate of RASSF1A methylation have conspicuous difference between breast cancer and normal group, breast cancer VS benign breast lesion, as well. Respectively (BC VS N, OR=4.52, 95%CI (2.02~10.10), P=0.0002; BC VS BBL, OR=3.63, 95%CI (2.50~5.43), P=0.0002; BBL VS N, OR=0.83, 95%CI (0.53~1.30), P=0.42). **Conclusion:** This meta-analysis confirms that hyper-methylation of RASSF1A is closely related to breast tumor. And it is of significance in differentiating benign breast lesions from malignant breast lesions.

Introduction

Breast cancer is the most common malignancy in women and is one of the three most frequently diagnosed cancers relating death in the world^[1]. Every year, approximately 1.7 million women are diagnosed with breast cancer and about 0.5 million die^[2]. In recent years, a lot of scholars have paid close attention to the molecular mechanism of breast cancer.

Genetic and epigenetic changes are closely related to malignant tumors, and have become a research hotspot in the diagnosis of breast cancer. Methylation of tumor suppressor genes, known as epigenetic alterations, have turned out to be a frequent event in development, differentiation and tissue homeostasis^[3, 4] of breast tumor. Methylation of particular genes is one of the earliest detectable changes in a tumor, and in some cases may occur even before the tumor is formed^[5].

Loss of genetic material on 3p21.3 chromosome is one of the most frequent and earliest events in the pathogenesis of many tumors^[6]. The RASSF1A gene, which located in chromosome 3p21.3, is composed of 8 exons and produces 7 transcripts^[5-7]. Initial studies suggested that RASSF1A would be a suppressor gene for breast cancers^[8].

Many studies have represented that RASSF1A methylation has remarkable effect on the biological characteristics of breast cancer. However, the methylation analysis techniques used in each study are different, as well as the CpG sites that studied, therefore, the methylation level of RASSF1A may different^[9]. All of these may have impact on the result of the correlation between RASSF1A methylation level and breast cancer, to some extent. To assess the association between RASSF1A methylation and breast cancer, as well as the difference between benign and malignant breast lesions, this meta-analysis and systematic review is performed.

Materials And Methods

Literature searching

Using "Breast cancer" including the synonyms likes "breast carcinoma", "breast neoplasm", "breast tumor", "breast tumour", "mammary cancer" and "RASSF1A" or "Ras association domain family 1A" to establish a retrieval model for integrated search in foreign databases, such as PubMed, Ovid, Cochrane Library. Furthermore, we scanned the reference and applied the related keywords and synonyms to search in additional publication. There were no restrictions on the design and publication status of the studies to make more sensitive. Relevant studies on methylation of RASSF1A in breast cancer were searched by computer, from the establishment of the database to January 31, 2019.

The inclusion criteria:

1. The source of the case is clear, and the diagnosis of breast carcinoma is definite,
2. Frequency of methylation of RASSF1A in breast cancer, benign breast lesions and normal groups all could be found or calculated.

The exclusion criteria:

1. Studies reported repeatedly or cannot find full text,
2. Studies that are letters, reviews, meta-analysis, conference abstracts, announcements, case reports or animal studies,
3. The research object is inconsistent or the data is incomplete.

Records screening:

First of all, screening the title and abstract of the literatures, the records about "Association between methylation of RASSF1A and breast tumor" would be selected to preliminary included group. Then, the documentations including reviews, meta-analysis, conference abstracts, announcements, case reports and animal experiments were excluded. Simultaneously, if the research object is inconsistent or the data is incomplete, they were be removed, as well. Remove the repeated records. Finally, the literatures, which the frequency of RASSF1A methylation in breast carcinoma, benign breast lesions and normal groups could not to be found or calculated were filtered out by reading the full text. And remove the studies that could not find full text. In this process of literature screening, two researchers (Xin Ling Deng and Chao Si Qian) screened independently, and in case of disagreement, they reached a consensus after discuss.

Quality assessment:

The Newcastle-Ottawa scale (NOS) table^[10], which is commonly used for assessing the quality of cohort studies and case-control studies, was applied to assess the quality of these included studies. The evaluation includes three aspects: the selection methods and comparability between the case and control group, and the assessment of exposure. The scores of quality assessment range from 0 to 9, and 0-3, 4-6, 7-9 represent low, medium, high quality, respectively. When two reviewers (Xin Ling Deng and Chao Si Qian) had different opinions on it, the two parties would negotiate or request the assistance of another researcher. In order to ensure the high quality of included literatures, the study got a score <5 was removed.

Data extraction:

The following information was extracted from the studies by two investigators (Xin Ling Deng and Chao Si Qian) on their own and crosscheck: The first author's name, publication year, material and method for methylation analysis. At the meantime, the frequency of methylation of RASSF1A in breast cancer, benign breast lesions and normal groups would be extracted into Excel table.

Statistical methods:

As assessing of the included literatures, we calculated Cochran's Q-test and Higgin's I^2 to analysis by Revman5.3. If $P > 0.05$, $I^2 < 50\%$, indicates that there is low or not statistical heterogeneity between the studies, therefore, fixed-effects model is applied. Otherwise, random-effects model is adopted ($P < 0.05$, $I^2 > 50\%$). In addition, we constructed funnel plot to evaluate publication bias intuitively. To assess the degree of asymmetry, we made Begg's test by Stata12.0. If Begg's test $P > 0.10$, funnel plot was symmetric, it suggest that no significant publication bias. As to sensitivity analysis, the combined effect size was calculated by using the fixed-effect model and the random-effect model respectively. And compare the consistency of results to reflect the reliability of the meta-analysis.

Results

The basic characteristics of the included studies

A total of 1288 literatures were found from the PubMed, Cochrane Library, Ovid. Finally, 7 studies^[11-17] were included in this meta-analysis after screening, with 1485 samples, including 642 breast tumor samples, 367 benign breast lesions and 476 normals. The specific screening process was shown in Fig 1. Basic information including the first author, publication year, samples and methods of methylation of the

included articles were extracted in table 1, and the results of methodological quality evaluation were displayed in table 2.

Heterogeneity test

Breast cancer VS Normal (BC VS N)

The result (in Fig2) reveal that there was moderate statistical heterogeneity between the records ($\chi^2=0.57$, $df=18.73$ ($P=0.005$), $I^2=68\%$), therefore, the random-effect model was applied. The combined effect OR=4.52, 95%CI :(2.02~10.10), 95%CI did not include 0, and the P value of Z test ($Z=3.67$, $P=0.0002$) was less than 0.1, indicating a statistical difference between the two groups.

Breast cancer VS Benign breast lesions (BC VS BBL)

The result (in Fig3) display that there was low statistical heterogeneity between the articles ($\chi^2=9.21$, $df=6$ ($P=0.16$), $I^2=35\%$), therefore, the fixed-effect model was used. The combined effect OR=3.63, 95%CI: (2.50~5.43), 95%CI did not include 0, and the P value of Z test ($Z=6.58$, $P<0.00001$) was less than 0.1, indicating a statistical difference between the two groups.

Benign breast lesions VS Normal (BBL VS N)

The result (in Fig4) show that there was no statistical heterogeneity between the studies ($\chi^2=5.31$, $df=5$ ($P=0.38$), $I^2=6\%$), therefore, the fixed-effect model was adopted. The combined effect OR=0.83, 95%CI:(0.53~1.30), 95%CI did not include 0, and the P value of Z test ($Z=0.81$, $P=0.42$) surpassed 0.1, indicating no statistical difference between the two groups.

Publication bias

There were absence of significant bias in the included studies, which could be seen from the symmetry of the funnel plot in Fig 5-7 and the p-value of Begg's tests (in table 3) that all exceed 0.1.

Sensitivity analysis

Applying fixed-effect model and random-effect model to calculate the combined effect size respectively. The consistency of results in table3 confirms that this meta-analysis was credible.

Discussion

The RASSF1A, whose official full name is ras association domain family member 1 A, is located at 3p21.31. The RASSF1A encodes a protein which resembles the RAS effector proteins. The protein can induce cell cycle arrest by inhibiting the cumulation of cyclin D1^[18]. The inactivation of RASSF1A was found to be related to the hyper-methylation of RASSF1A CpG-island promoter region^[19, 20]. It has been reported that the frequency of RASSF1A methylation in breast cancer patients is higher than in non-

cancer patients and healthy controls. However, at present, most studies only compare the cancer group with normal group and only a few studies involve benign groups. The methylation analysis techniques used and the CPG sites studied in each study are not exactly the same. These factors may have some effect on the consistency of the results. In order to get a more accurate result of the correlation between RASSF1A methylation and breast cancer, we performed this meta-analysis with 1485 cases to determine whether RASSF1A methylation can be used as an indicator in the diagnosis of breast cancer.

Our study showed that the comparison between the breast cancer group and the normal group had statistical significance, and the comparison between the benign lesion group and the malignant lesion group also had statistical significance, while the comparison between the benign lesion group and the normal control group had no statistical significance. It shows that RASSF1A hyper-methylation is positively correlated with the risk of breast cancer and suggests that RASSF1A hyper-methylation has potential value in the diagnosis of breast cancer.

A number of meta-analysis studies have shown that there is a correlation between hyper-methylation of RASSF1A promoter and different types of tumors, as well as the value of biomarkers for the methylation status of RASSF1A^[21-25]. In addition, an important implication from our findings is that hyper-methylation of the promoter of tumor suppressor loci in normal breast tissue may be a precursor to local recurrence. In addition, the detection of RASSF1A gene methylation level in breast tissue or blood may be practical for distinguishing benign lesions from malignant lesions to some extent.

However, there were some limitations in this study. Firstly, there were only three major databases which we identified studies. In addition, the sample size of included studies was small and some documents were failed to acquire the full text. They might affect the effectiveness of our analysis to some extent. Last but not least, publication bias was a concern, for the journals tend to publish positive results. Those omitting unpublished research, which had negative results, might lead to exaggeration of the results in our study.

In summary, the present study showed that hyper-methylation of RASSF1A in cfDNA circulating in plasma may be a potential biomarker for diagnosis of breast cancer. These findings could let us find a simple and accessible way to measure DNA methylation and help identify women in the early stages of breast cancer. But for these limitations existing, there is an urgently need to do large scale studies to determine whether hyper-methylation of RASSF1A can provide prognostic information in breast cancer.

Conclusion

This meta-analysis confirms that hyper-methylation of RASSF1A is closely related to breast tumor. And it is of significance in differentiating benign breast lesions from malignant breast lesions.

Abbreviations

RASSF1A: Ras association domain family 1A; BC: Breast cancer; N: Normal; BBL: Benign breast lesion.

Declarations

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Authors' contributions

Ying Hua Yu conceived the study. Xin Ling Deng and Xue Yan Chen designed and coordinated it. Xin Ling Deng and Chao Si Qian performed the statistical collection and analysis. Gui Ting Tang, Xin Ling Deng, Chao Si Qian and Xue Yan Chen drafted the manuscript. Li Ying Feng, Ying Hua Yu amended it. All authors participated in the review and revision of the manuscript. The final paper was composed, read and approved by all authors.

Competing interests

The authors declare that they have no competing interests.

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Availability of data and materials

Not applicable.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Author details

¹Affiliated tumor hospital of Guangxi medical university, Nanning 530021, Guangxi, China;

²Department of Breast Surgery, Affiliated tumor hospital of Guangxi medical university, Nanning 530021, Guangxi, China.

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Tables

Due to technical limitations, tables are only available as a download in the supplemental files section.

Figures

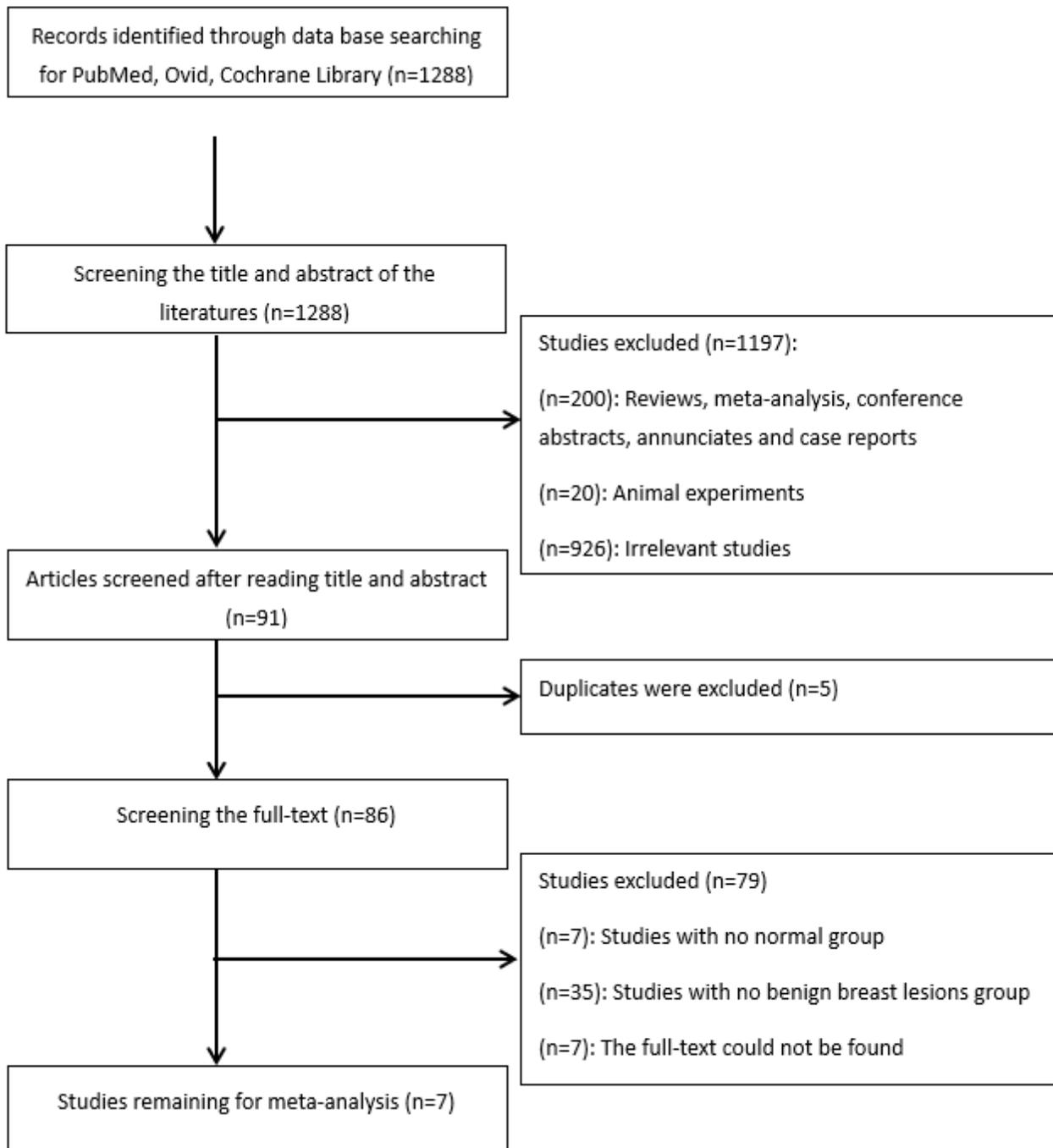


Figure 1

Flow chart of the study selection procedure

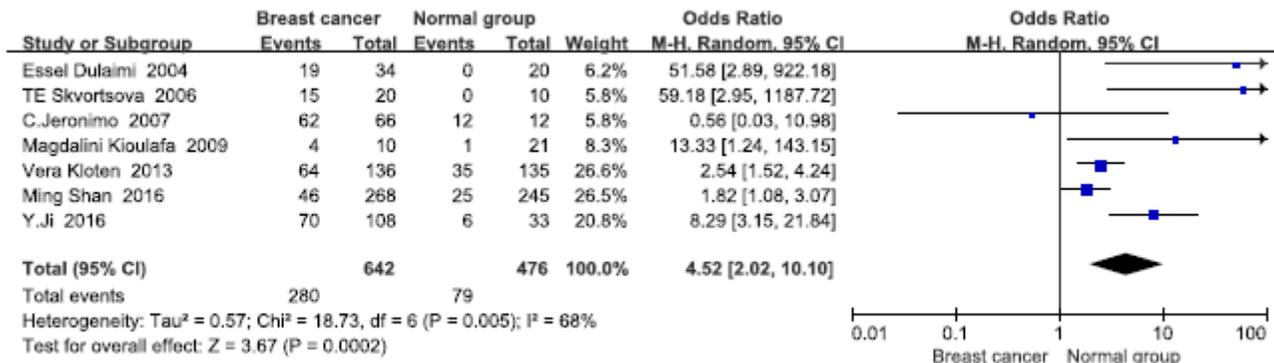


Figure 2

Forest plot of BC VS N

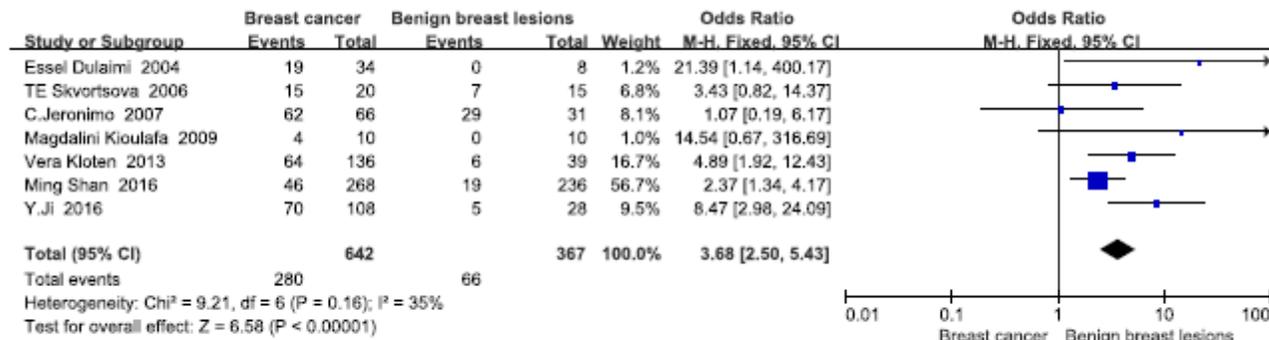


Figure 3

Forest plot of BC VS BBL

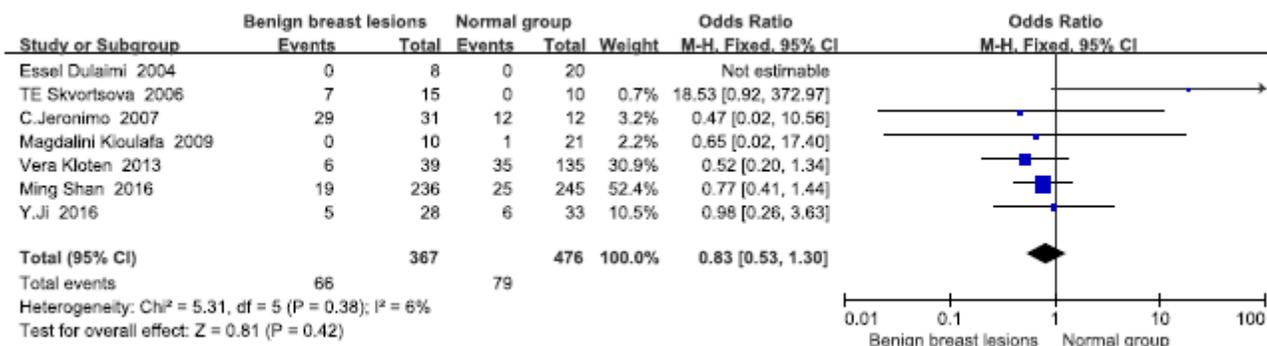


Figure 4

Forest plot of BC VS N

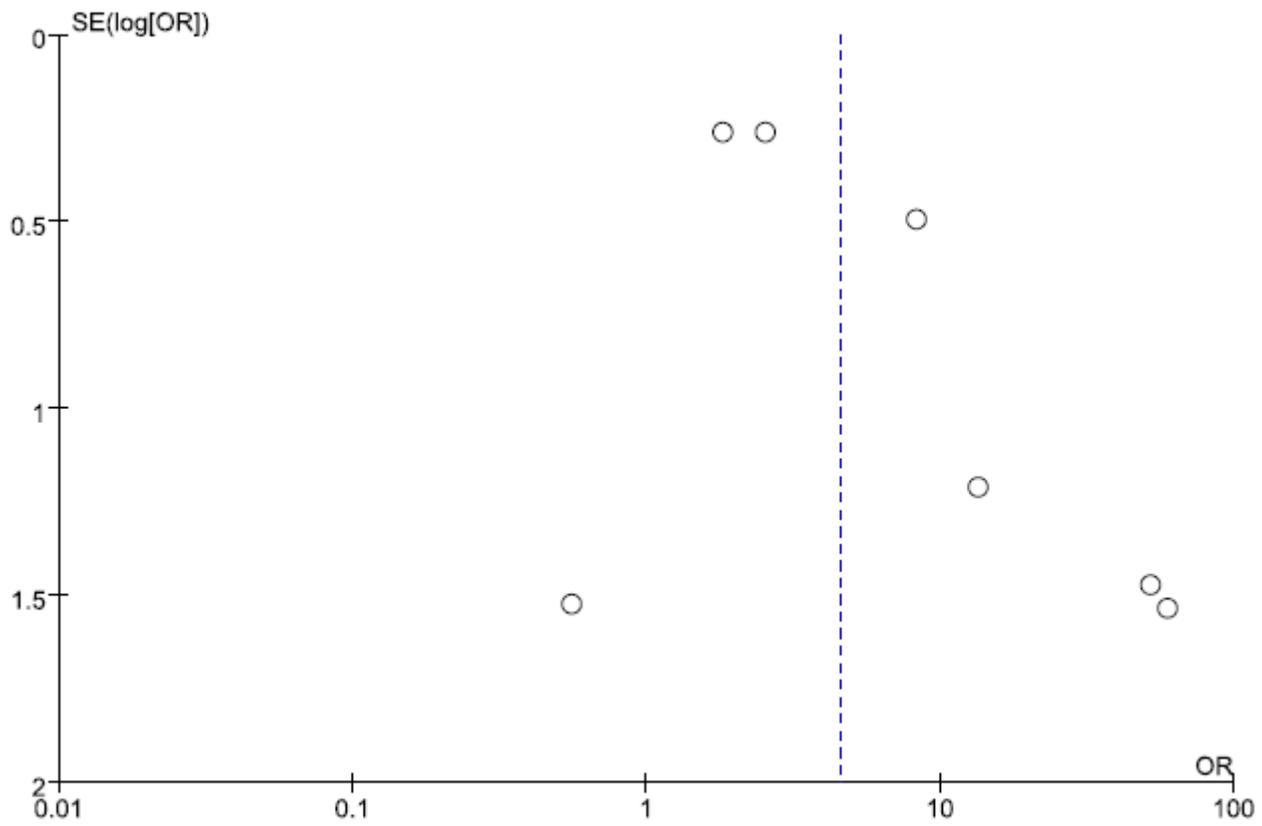


Figure 5

Funnel plot of BC VS N

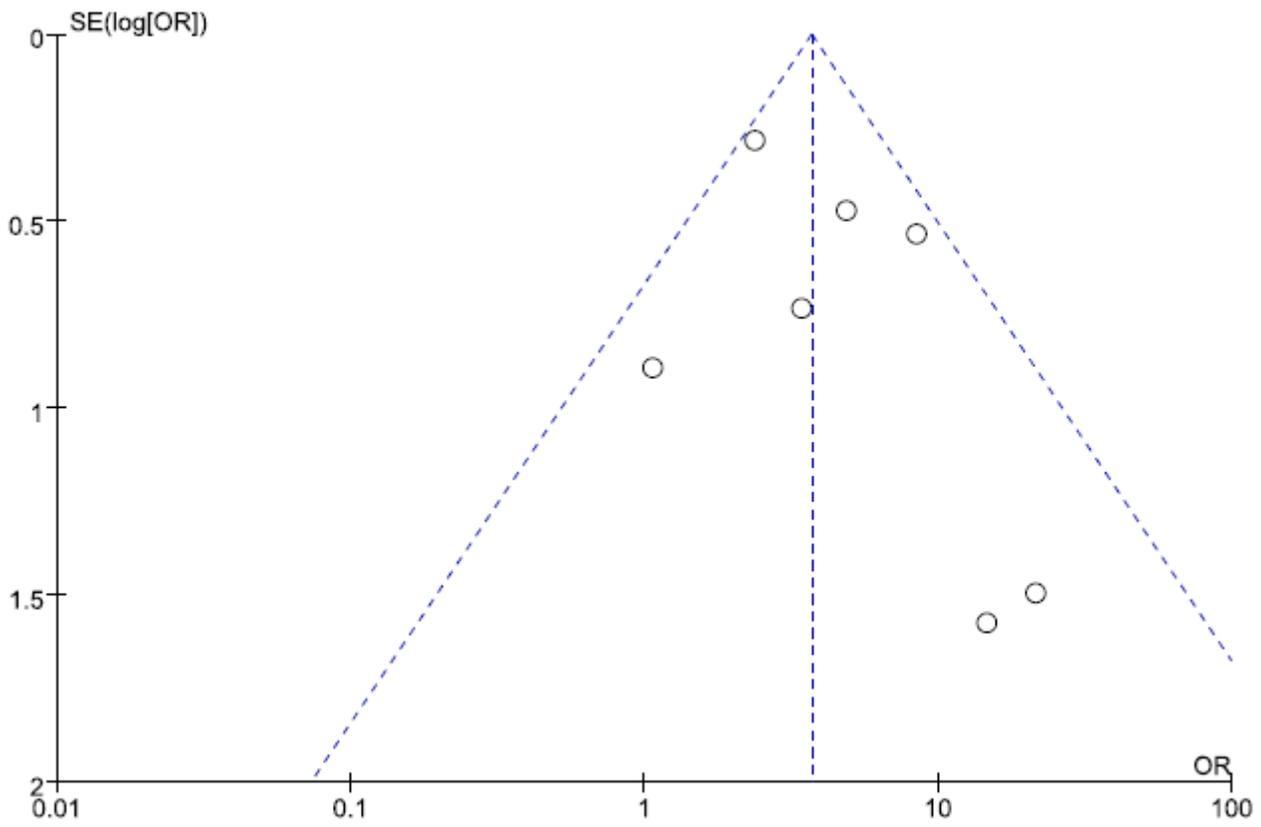


Figure 6

Funnel plot of BC VS BBL

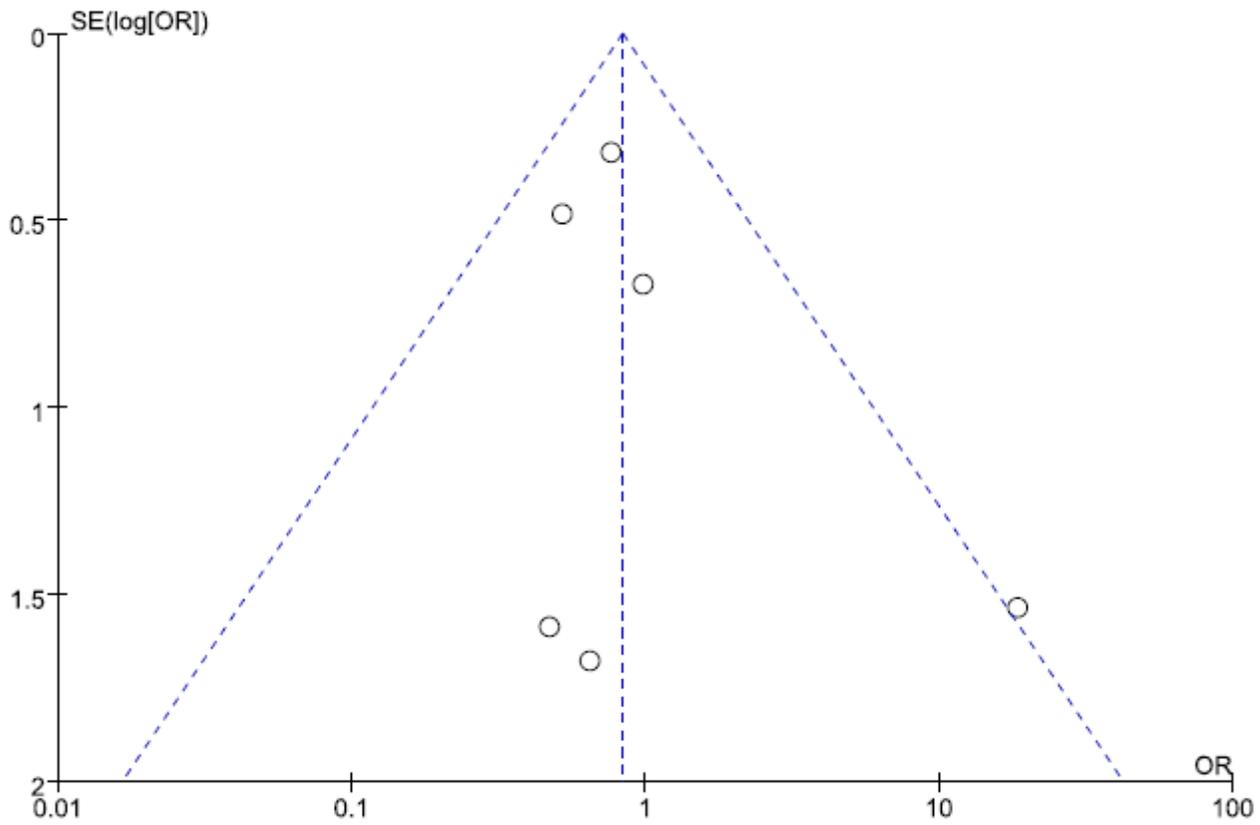


Figure 7

Funnel plot of BBL VS N

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