



The association between let-7 microRNA-binding site polymorphism rs712 and cancer risk: a meta-analysis and trial sequential analysis

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Background: Previous studies have investigated the relationship between let-7 microRNA-binding site polymorphism rs712 and cancer susceptibility. However, the available conclusions remained inconsistent. The present meta-analysis was thus performed to clarify such associations.

Methods: A meta-analysis including 11 studies was performed with 3,572 cases and 4,749 controls. Relevant studies were searched in the databases EMBASE, PubMed and Web of Science, covering relevant papers published until September 1st, 2016. We pooled data with odds ratios (ORs) and 95% confidence intervals (CIs) to assess the strength of the associations. Besides, Begg's funnel plots and Egger's regression test were utilized to evaluate publication bias. Furthermore, we took advantage of trial sequential analysis to evaluate whether the evidence of the results was sufficient.

Results: Overall, the results showed that significant cancer risk was associated with rs712 for heterozygote model OR =1.10 (95% CI: 1.002–1.22), homozygote model OR =1.71 (95% CI: 1.18–2.49), dominant model OR =1.19 (95% CI: 1.04–1.35), recessive model OR=1.64 (95% CI: 1.17–2.31) and allele model OR =1.21 (95% CI: 1.06–1.39). Moreover, trial sequential analyses for the first time were performed to confirm such associations, demonstrating that the results of our study were based on sufficient evidence.

Conclusions: This results of the meta-analysis suggested that rs712 polymorphism was associated with cancer susceptibility, which might act as a potential biomarker for evaluating cancer risk.

Keywords: let-7; gene polymorphism; rs712; cancer risk; meta-analysis

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Introduction

MicroRNAs (miRNAs) are short sequences of small non-protein coding mRNA, which could bind to specific regions of target mRNA transcripts. MiRNAs post-transcriptionally regulate the expression level of the target gene in the way of inducing mRNA degradation or inhibiting mRNA translation, which depends on the matching degree between the miRNA and its target mRNA (1). Disregulation of miRNAs may function as both tumor suppressors and oncogenes (2,3). As a polymorphism at or near a miRNA binding site of functional genes, MiRNA-related polymorphism could influence gene expression by interfering with miRNA function (4). Recent studies have shown that single nucleotide polymorphisms (SNPs) residing on miRNA and/or miRNA-binding sites might be associated with human cancers (5,6).

As a member of ras gene family, The KRAS gene plays important roles in nosogenesis of cancer (7). Studies found the let-7 family of miRNAs binding to the 3' untranslated region (UTR) of human KRAS could affect the binding ability and impact the following KRAS transcription (8). Previous studies have reported that let-7 binding site polymorphism rs712 increased cancer risk involving gastric cancer (GC), colorectal cancer (CRC), non-small cell carcinoma (NSCLC), oral squamous cell carcinoma (OSCC) and others, but some other studies observed insignificant or opposite results (5,9-13).

In order to drive a more precise relationship between rs712 and the risk of cancer, we thereby performed such meta-analysis.

Methods

Studies were searched in the electronic databases EMBASE, PubMed and Web of Science up to September 1st, 2016. Available publications were identified using the following keywords or text words: "rs712", "let-7", or "KRAS", and "single nucleotide polymorphism", or "polymorphism" and "cancer" or "carcinoma". All studies were assessed to retrieve the most eligible literatures and the search was restricted on human subjects only. Besides, we also performed a manual search for relevant researches from reference lists by internet search engines. We also inspected review articles to search other eligible studies.

The included studies must met the inclusion criteria: (I) a case-control, nested case-control, or cohort design; (II) evaluate the rs712 polymorphism among various

cancer patients and controls; (III) available genotype data for extraction to calculate ORs with 95% CIs; (IV) when duplications or the same patients used in several publications existed, we only chose the most recent or complete study in the meta-analysis. Additionally, the major exclusion criterion was as follows: (I) no available genotype frequency data; (II) non case-control studies, case reports, letters, reviewed editorial articles; (III) duplicated publications with previous studies.

Data extraction

Two researchers (B Yu and X Wang) inspected studies independently and extracted appropriate information and data with a standard protocol, to ensure the reliability and the accuracy of the results. Moreover, the results were reviewed by a third investigator. We extracted the following information form from each study: name of first author, publication date, type of carcinoma, ethnicity, source of controls, methods of genotyping, and total number and genotype frequencies (GG, GT or TT respectively) of cases and controls of rs712. Moreover, the Hardy-Weinberg equilibrium (HWE) test results were also recorded.

Statistical analysis

The following contrasts for rs712 polymorphism and cancer risk were evaluated, according to five genetic comparison models: *homozygous model* (GG vs. TT), *heterozygous model* (GT vs. TT), *dominant model* (GT + GG vs. TT), *recessive model* (GG vs. TT + GT) and *allele model* (G vs. T). The pooled ORs with 95% CIs were presented to assess the strength of association between rs712 and susceptibility to cancer. Based on the detection of heterogeneity, the fixed-effects model or the random-effects model was selected in the present meta-analysis. The χ^2 -based Q-statistic was utilized to assess the Between-study heterogeneity, and $P < 0.05$ was deemed statistically significant. The ORs were pooled according to the fixed-effects model, if the result of the heterogeneity test was $P > 0.05$. Moreover, we also tested whether genotype frequencies of controls were in HWE by the goodness-of-fit chi-square test and $P < 0.05$ was regarded significant disequilibrium (14). Sensitivity analysis was performed by calculating the stability of results to assess the influence of a single study in this meta-analysis once at a time. Additionally, publication bias was investigated using Begg's funnel plot, and funnel plot asymmetry was further assessed by the Egger linear regression test. When the P

value of the Egger test was <0.05 , statistical significance was considered (15). All statistical analysis was conducted by Stata software (version 12.0; StataCorp LP, College Station, TX, USA).

Trial sequential analysis

Because of limited data and repetitive calculating of accumulating data, cumulative meta-analyses may increase the risks of producing type I and type II errors (16-18). Thus, the trial sequential analysis software (TSA, version 0.9; Copenhagen Trial Unit, Copenhagen, Denmark, 2011) was applied to assess the risk of type I errors in the cumulative meta-analysis, which combined an estimation of required information size with an adjusted threshold for statistical significance with dispersed data (18,19). Besides, the trial sequential monitoring boundaries were used to decide whether a trial could be terminated early and adjusted the CIs and reduces type I errors (20). If the cumulative Z-curve exceeds the required information size or crosses the trial sequential monitoring boundary, the results suggest a sufficient level of evidence in this meta-analysis is reached and no further studies are required. Otherwise, the obtained results are insufficient (21). In the present meta-analysis, TSA was performed to calculate the required information size and construct the trial sequential monitoring boundaries, based on an overall type-I error of 5%, a relative risk reduction of 20% in mortality and overall morbidity and a statistical test power of 80%.

Results

Studies characteristics

As shown in *Figure 1*, 662 records of rs712 and cancer were retrieved. After screening titles and abstracts of relevant articles, 643 articles were excluded because they were not related to the inclusion criteria. Furthermore, nineteen candidate studies were excluded after complete reading. Finally, eleven case-control studies were included in the meta-analysis, published between 2008 and 2015. All of them were retrospective in design.

The present meta-analysis including 3,572 cases of patients with various carcinomas and 4,749 controls from a total of eleven case-control studies on rs712 polymorphism and cancer risk (9,10,22-30), and the detailed data of each study is listed in *Table 1*. Besides, the distribution of genotypes in the controls of all studies was consistent with

HWE. The flowchart of literature search and selection process is shown in *Figure 1*. The types of cancers in these studies included breast cancer (BC), CRC, cervical squamous cell carcinoma (CSCC), papillary thyroid cancer (PTC), nasopharyngeal carcinoma (NPC), glioma, NSCLC and GC (9-14). In these studies, three genotyping methods were applied, such as iMLDR, PCR-RFLP and AS-PCR to assay the polymorphism in rs712. Besides, ten studies were conducted on Asian population, and only one study was carried out on Caucasian population. Furthermore, we divided them into population-based group or hospital-based group in all studies, to distinguish between different sources of control group.

3Quantitative synthesis results

In this meta-analysis, the random-effect model was selected to calculate the pooled OR with corresponding 95% CIs except *heterozygote model*, because the between-study heterogeneity among those studies was obvious ($P<0.05$). The combined results showed that rs712 polymorphism was significantly associated with cancer risk. Overall, the main results of this meta-analysis about the associations between rs712 polymorphism and the risk of cancer were listed in *Table 2*. The meta-analytic results showed that significant cancer risk was associated with rs712 for *heterozygote model* OR =1.10 (95% CI: 1.002–1.22), *homozygote model* OR =1.71 (95% CI: 1.18–2.49), *dominant model* OR =1.19 (95% CI: 1.04–1.35), *recessive model* OR =1.64 (95% CI: 1.17–2.31) and *allele model* OR =1.21 (95% CI: 1.06–1.39) (*Figure 2*). Moreover, for the first time, TSA were performed to confirm such associations, demonstrating that the findings in the current study were proved to be solid with sufficient evidence. In total, with the effect of rs712 gene polymorphism, the carriers of T allele held higher cancer risk than carriers of G allele.

Test of heterogeneity

Because of the heterogeneity, a Galbraith radial plot was utilized under the dominant model (*Figure 3*), which also indicated that no significant heterogeneity between studies was observed.

Publication bias

The potential publication bias for the all available data was assessed by the Begg's funnel plot and Egger's test.

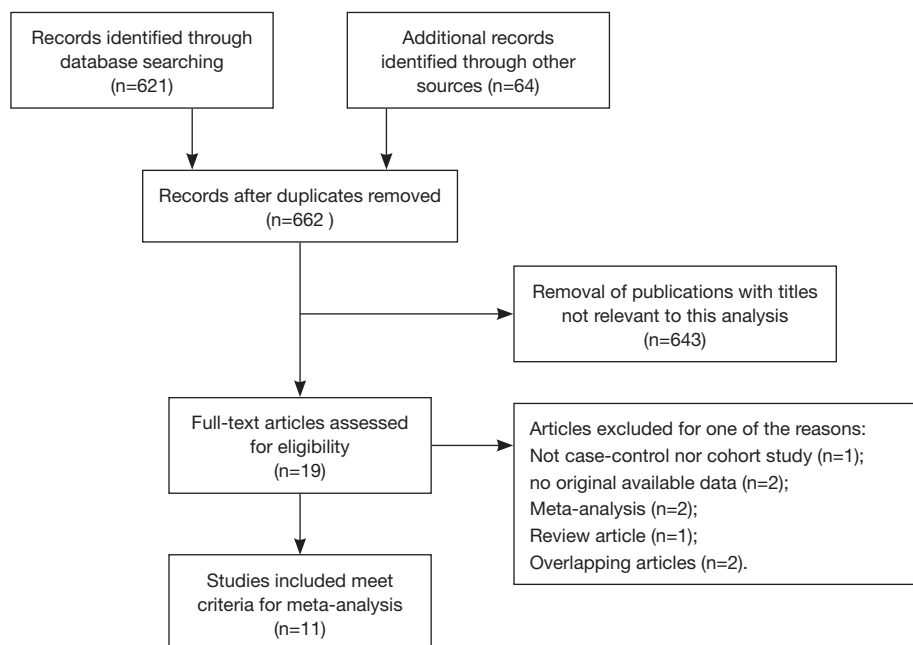


Figure 1 Flow diagram of selection process.

The shape of the funnel plot seemed symmetrical in the funnel plot analysis (*Figure 4*). No publication bias was demonstrated in the Begg's test ($P=0.679$) and Egger's test in the dominant model ($P=0.584$).

Sensitivity analysis

Sensitivity analysis was performed to evaluate the influence of each study on the pooled OR by omitting each individual studies in turn. *Figure 5* showed the sensitivity analysis under the dominant model of the association of rs712 and cancer risk, demonstrating that our results were reliable and robust.

Trial sequential analysis results

In our current study, the cumulative Z-curve exceeded the trial sequential monitoring boundary (*Figure 6*), suggesting sufficient evidence of the association between rs712 polymorphism and risk of cancer.

Discussion

In recent years, increasing studies in cancer research focused on the field of miRNAs, which form an important class of regulators and participate in various functions including

apoptosis, development and cell proliferation (30,31). Considering miRNAs play important roles in the pathogenesis of cancer, it might be regarded as potential biomarkers for cancer risk or development. The KRAS gene was confirmed to promote tumorigenesis through the pathway of RAF/MEK/MAPK (32). KRAS mutation with a single amino acid substitution could lead to an activating mutation or increasing KRAS expression, and might have connections with cancers. Let-7 could bind to specific site in 3'UTR of KRAS mRNA so as to down-regulate RAS and then function as tumor-suppressing gene (8). Additionally, rs712, as one of the 8–10 putative let-7 miRNA complementary sites, might adjust KRAS expression (5).

Recently, a growing number of studies involved the relationship between rs712 and risk of various carcinomas, but the results were inconclusive. Li *et al.* found that there was significant difference in the TT genotype of rs712 polymorphism between the GC patients and the controls group, when GG genotyping was taken as a reference (OR =3.05, 95% CI: 1.53–6.08). Thus, we suggested that the KRAS rs712 polymorphism might be a genetic marker in the development of GC (9). In addition, Pan *et al.* showed that the genotype and allele frequencies of the rs712 polymorphism was associated with an increased risk of NPC, compared to the controls group (GT *vs.* GG, OR =0.83, 95% CI: 0.51–1.21; TT *vs.* GG, OR =1.27,

Table 1 Characteristics of individual studies included in the meta-analysis

Year	Surname	Ethnicity	SOC	Genotyping	Cancer type	Case	Control	Case (n)			Control(n)			HWE
								GG	GT	TT	GG	GT	TT	
2015	Huang	Asian	HB	PCR-RFLP	BC	228	251	155	65	8	173	71	7	Yes
2015	Jiang	Asian	PB	PCR-RFLP	CRC	586	476	372	176	38	331	133	12	Yes
2015	Dai	Asian	PB	iMLDR	CRC	430	430	253	145	32	283	130	17	Yes
2015	Liang	Asian	HB	PCR-RFLP	CSCC	415	504	257	144	14	327	163	14	Yes
2014	Jin	Asian	HB	PCR-RFLP	PTC	252	290	154	84	14	183	92	15	Yes
2014	Pan	Asian	HB	PCR-RFLP	NPC	188	356	112	64	12	201	138	17	Yes
2014	Pan	Asian	HB	PCR-RFLP	CRC	339	313	188	125	26	203	100	10	Yes
2013	Li	Asian	HB	PCR-RFLP	GC	181	674	105	60	16	442	211	21	Yes
2013	Yan	Asian	HB	PCR-RFLP	Glioma	153	204	83	56	14	137	61	6	Yes
2010	Peng	Asian	HB	PCR-RFLP	NSCLC	83	80	49	31	3	51	25	4	Yes
2008	Landi	Caucasian	HB	AS-PCR	CRC	717	1171	239	341	137	364	571	236	Yes

SOC, source of controls; PB, population-based controls; HB, hospital-based controls; BC, breast cancer; CRC, colorectal cancer; CSCC, cervical squamous cell carcinoma; HCC, hepatocellular carcinoma; PTC, papillary thyroid cancer; NPC, nasopharyngeal carcinoma; NSCLC, non-small cell carcinoma; GC, gastric cancer; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; AS-PCR, allele specific polymerase chain reaction; iMLDR, improved multi-plex ligation detection reaction.

Table 2 Meta-analysis results of association between rs712 polymorphism and cancer risk

N ^a	Sample size	GT vs. GG		TT vs. GG		GT/TT vs. GG		TT vs. GT/GG		T vs. G	
		OR (95% CI)*	P ^b	OR (95% CI)*	P ^b	OR (95% CI)*	P ^b	OR (95% CI)*	P ^b	OR (95% CI)*	P ^b
11	8,321	1.10 (1.002-1.22)	0.425	1.71 (1.18-2.49)	0.001	1.19 (1.04-1.35)	0.064	1.64 (1.17-2.31)	0.002	1.21 (1.06-1.39)	0.002

^a, number of studies; ^b, P value of Q test for heterogeneity; *, random-effects model was used when P value for heterogeneity test <0.1; otherwise, fixed-effects model was used.

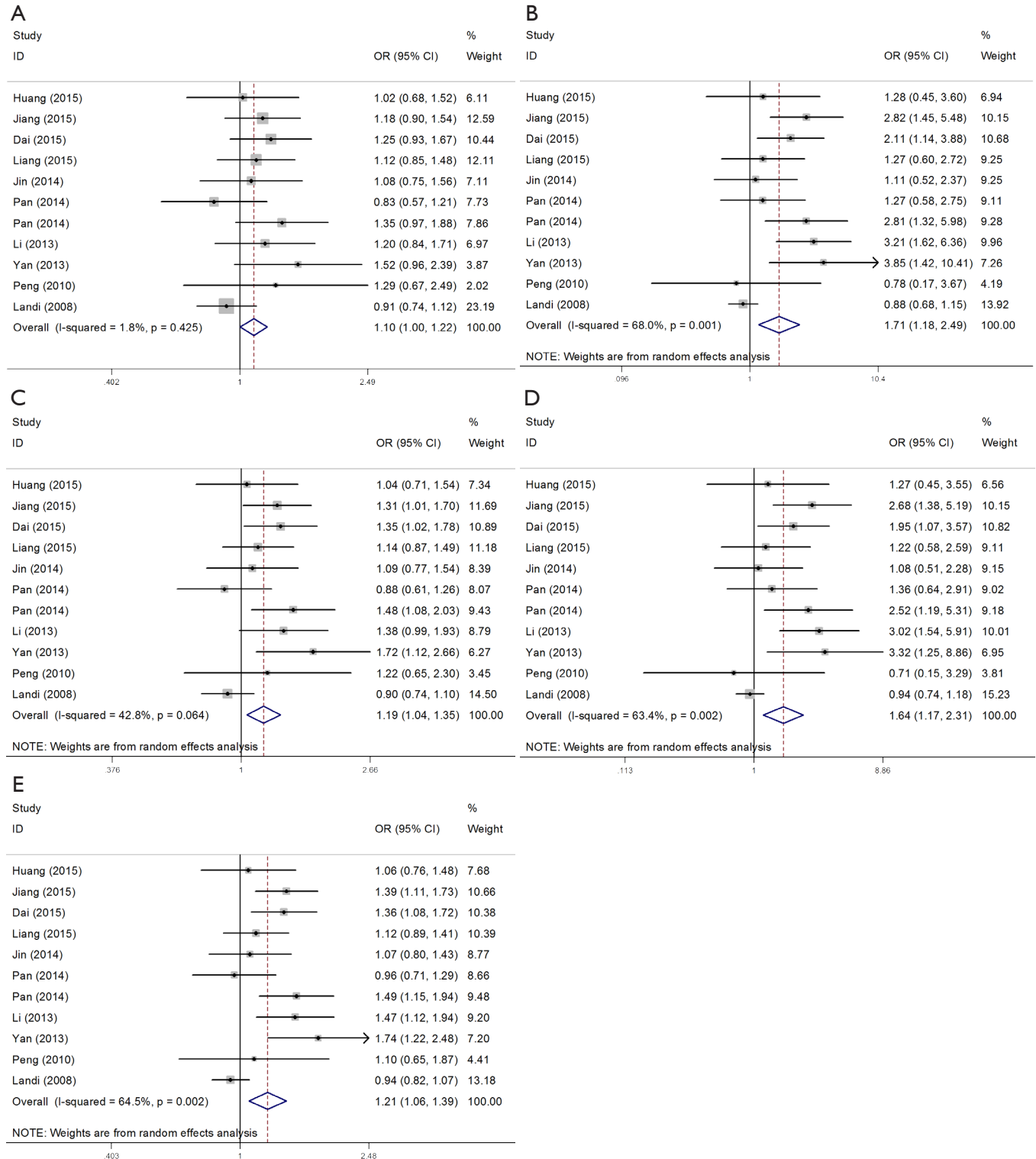


Figure 2 Forest plots of cancer risk associated with the rs712 polymorphism. (A) In the heterozygote model; (B) in the homozygote model; (C) in the dominant model; (D) in the recessive model; (E) in the allele model.

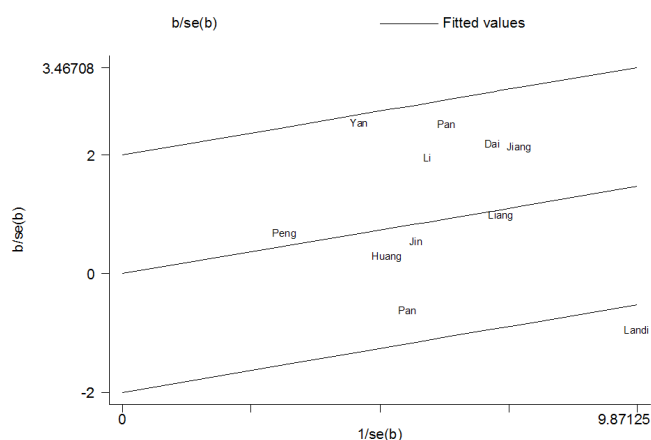


Figure 3 Galbraith plot of the relationship between rs712 polymorphism and cancer susceptibility (dominant model).

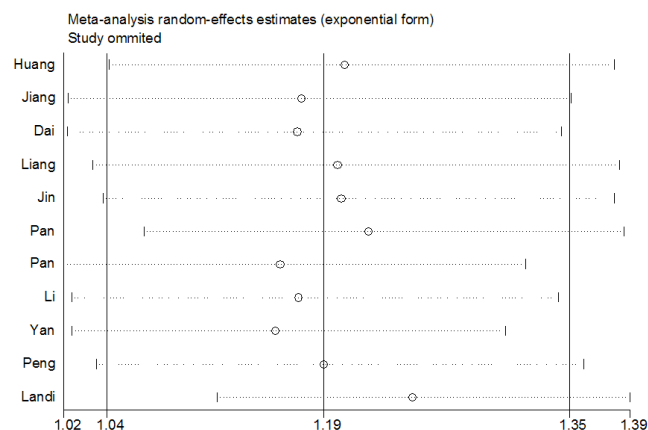


Figure 5 Sensitivity analysis (dominant model).

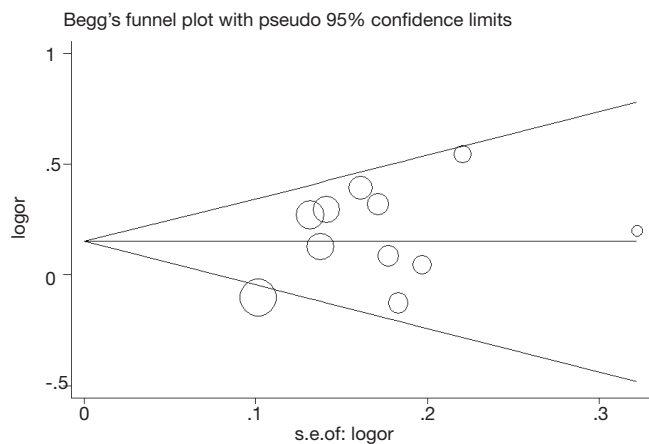


Figure 4 Publication bias test with Begg's funnel plot.

95% CI: 0.58–2.75) (28). However, studies from Wang *et al.* made the opposite results, which showed that GT or TT genotyping were protecting factors for OSCC (OR =0.26, 95% CI: 0.10–0.60) (11). Thus, we conducted this meta-analysis to investigate the relationship between rs712 polymorphism and cancer risk. The results of this meta-analysis indicated that G>T variant of rs712 increase cancer risk mainly in Asians.

Compared to a single study especially in analyzing unexplained studies, meta-analysis is a powerful tool and can provide more sufficient results (33). As a result, we suggested there existed a much stronger advantage to prove the association between rs712 with cancer susceptibility. The present meta-analysis deemed that the let-7 genetic

polymorphism might influence the risk of cancer. In these previous studies, these outcomes remained unclear. Therefore, we need a better method to analyze and understand the association between let-7 polymorphism and susceptibility to cancer. In addition, for the first time, TSA was adopted to reduce the risk of type I error and testify whether our results were established on firm evidence of effect. In the current meta-analysis, our results revealed the T allele of let-7 genetic polymorphism rs712 increases cancer susceptibility, mainly among Asian ethnicity.

As a powerful and useful approach, TSA is introduced to calculate the required information size for the meta-analysis with the adaptation of monitoring boundaries and to reduce the risk of type I error and estimate whether further trials are needed (20). Compare to the traditional meta-analysis, more reliable evidence is obtained by TSA. Here, when an additional study was involved, an updated meta-analysis was performed and the cumulative Z-curve of each meta-analysis was constructed, so as to dictate whether a sufficient level of evidence has been reached or the futility boundary is reached (21). In the present meta-analysis, the cumulative Z-curve exceeded the trial sequential monitoring boundary, which meant that our results were established on firm evidence of effect.

Overall, sufficient statistical evidence including the large sample size and the implementation of TSA were used to estimate such association. However, there are several limitations were involved in our study. Firstly, the studies included in this meta-analysis were mainly conducted on Asians and only a study was Caucasian populations. Besides, there are no reports on the other population available.

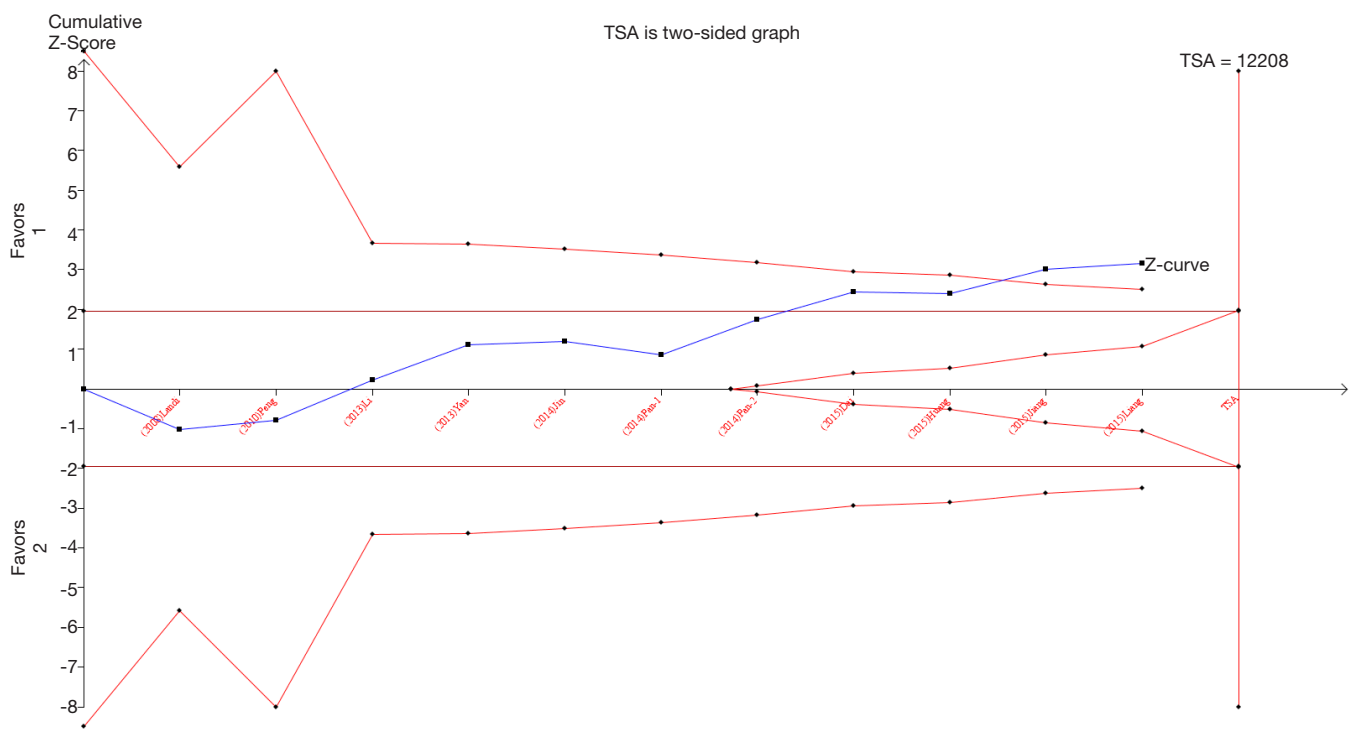


Figure 6 Trial sequential analysis of relationship between rs712 polymorphism and the risk of cancer. The required information size was calculated according to a two side $\alpha=5\%$, $\beta=20\%$, and a 95% confidence intervals.

Thus, more researches should pay attention to the influence of ethnicity factors in the future. Secondly, only eleven studies were included in this study, which might weaken the reliability of our meta-analysis. Therefore, more well-designed studies with large cases of each specific cancer should be conducted to validate the relationship between let-7 polymorphism rs712 and cancer risk. Besides, the results of this meta-analysis were that based on unadjusted estimates, so we should take into consideration the effect of multiple confounders such as life-style, age, environmental factors and so on. Moreover, the obvious heterogeneity existed in our study probably due to the difference in the tumor types. Hence, to improve reliability of the meta-analysis, more studies should need to investigate rs712 and risk of cancer in the subsequent years.

Conclusions

This meta-analysis suggested that a let-7 binding site polymorphism rs712 in the KRAS 3'UTR increased the risk of cancer in Asians and it has the potential to be a prognostic factor in various carcinoma in the future.

To further evaluate the relationship between genetic polymorphism rs712 and susceptibility for every specific carcinoma, large and better-designed studies are needed.

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Footnote

Conflicts of Interest: The authors have completed the ICMJE uniform disclosure form (available at <http://dx.doi.org/10.21037/amj.2017.05.07>). Dr. Xiao Li serves as an unpaid section editor of AME Medical Journal. The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This article does not contain any studies with human participants or animals performed by any of the authors.

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