Association of rs712 polymorphism in Kras gene 3'-untranslated region and cancer risk: A meta-analysis

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Summary

Purpose: Mutation and polymorphism of Kras oncogene are considered as candidate risk factor and drug response predictor for cancer. However, the conclusions of accumulating reports related to the relationship of rs712 of Kras gene and risk of cancer remain nuclear.

Methods: We performed a meta-analysis including 6 eligible studies containing 1661 cases and 2139 controls to explore the role of rs712 in the risk of cancer development.

Results: Meta-analysis results showed that rs712 allele T (P_H=0.08, odds ratio/OR=1.35, 95% confidence inter-val/CI=1.17-1.55) and genotype TT (P_H=0.174, OR=2.32, 95% CI=1.60-3.37), and allele T carrier genotype (GT/TT) (P_H=0.14, OR=1.30, 95% CI=1.10-1.55) were strongly associated with cancer in Chinese population. No evidence of association was observed between rs712 and risk of cancer in overall population.

Conclusion: The findings suggested that allele T, genotype TT and allele T carrier (GT/TT) of rs712 may increase susceptibility to cancer risk in Chinese population, and can be used as a genetic factor for evaluating risk of cancer.

Key words: cancer, Kras, polymorphism, rs712

Introduction

Notwithstanding the mounting of data facilitating the understanding of the carcinogenetic mechanisms and exploring cancer preventing strategies, hundreds of thousands of people are diagnosed or die of cancer worldwide. According to the 2013 report of the National Center for Health Statistics of USA, a total of 1,660,290 new cancer cases and 580,350 cancer deaths were projected to occur in the United States in 2013 [1]. Many factors have been demonstrated to associate with carcinogenesis, such as diet, smoking, inflammation, and genetic variation of a series of essential genes that play an important role in carcinogenesis. Mutation, single nucleotide polymorphisms (SNPs), copy number variations (CNV) and epigenetic dysregulation of oncogenes or tumor suppressor genes were found, leading to activation or silencing of related genes which, in turn, could lead to carcinogenesis [2]. Hence, investigation of genetic variations of related genes is an important research field to explore their role in carcinogenesis.

Kras gene is an important oncogene which belongs to Ras gene family [3]. It is located on 12p12.1 chromosome, including 6 exons and 5 introns. It encodes a GDP/GTP-binding protein that belongs to the small GTPase superfamily and it acts as an intracellular signal transducer in Kras-related RAF/MEK/MAPK, AKT and ERK pathways to regulate cell proliferation and differentiation [4-6]. It has been demonstrated that Kras mutations in codons 12, 13, 61 play an essential role in the pathogenesis of various human solid tumors and the response of metastatic colorectal cancer (mCRC) patients treated with anti-epidermal growth factor receptor monoclonal antibod-

ies (cetuximab and panitumumab) (anti-EGFR) [7,8]. More than 30% of CRC patients bear Kras mutation [9]. Moreover, small interference RNAs (siRNA) or microRNA (miRNA) could bind to Kras gene and repress its expression, contributing to inhibition of tumor growth and invasion [10]. For instance, there are several Let-7 complementary binding sites (LCS) in 3'-untranslated region (3'-UTR) of Kras and Let-7 miRNA could bind to Kras messenger RNA (mRNA), contributing to degradation or translation repression [11]. In addition, rs712, a SNP located in LCS1 of Kras 3'-UTR, was supposed to modulate the binding ability with Let-7, leading to Kras aberrant expression [12].

Recently, a study reported by Li et al. indicated that rs712 was associated with gastric cancer [13]. However, several other studies showed no association between rs712 and nasopharyngeal and CRC [14,15], and therefore, the precise conclusion concerning rs712 and risk of cancer remains unknown.

With the purpose to possibly acquiring more information on this issue, we conducted a comprehensive meta-analysis of published studies to evaluate the association of rs712 and risk of cancer.

Methods

Relevant studies were searched in Wangfang and Pubmed databases dating up to May of 2014 in English and Chinese languages, and manual retrieval was used to obtain additional substantial relevant articles. The terms rs712 and cancer, Kras polymorphism and cancer were used to search these databases. Firstly, we identified each retrieval literature title and abstract to obtain a relevant study. Secondly, we identified each relevant full-text study as eligible study for meta-analysis in accordance with the following inclusion criteria: 1) cases were patients with solid tumors and controls were healthy individuals; 2) case-control study concerning rs712 and cancer risk; 3) study provided sufficient genotype frequency data and OR and 95% CI; 4) genotype distribution of control group was consistent with Hardy-Weinberg equilibrium (HWE).

Two investigators (Wei-Hai Zhao and Xiao-Fei Qu) independently extracted data from each eligible study. Any disagreement was solved by a third investigator and discussion to reach consensus. Baseline characteristics data, the first author's name and year of publication, country or region, ethnicity, cases and controls, genotype assay, genotype data, OR and 95% CI, were extracted from each eligible study.

Statistics

OR and 95% CI were commonly used to assess the
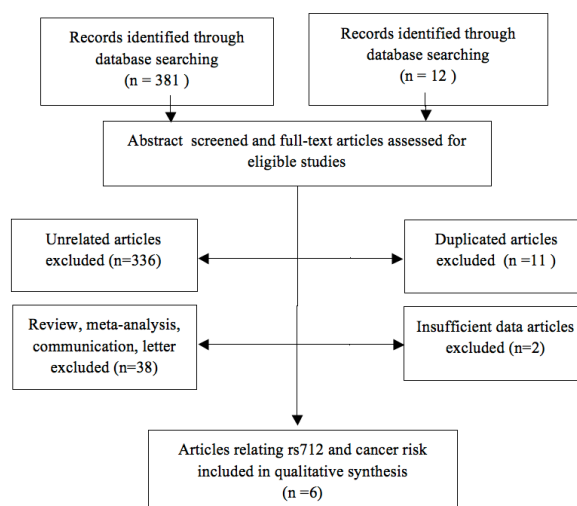
Figure 1. Flow chart of literature retrieval and eligible studies selected.

strength between rs712 and cancer risk. Cochran Q test and I2 were used to assess the heterogeneity between each eligible study [16], and $P_H<0.10$ was considered as significant. The random model was chosen to evaluate the combined data when $P_H<0.10$; otherwise, the fixed model was used to estimate the overall effect [17]. Sensitivity analysis was used to increase the stability of meta-analysis by changing the estimated model or omitting each eligible study. Possible publication bias was commonly estimated by both Begg's funnel plot and Egger's test [18,19], and symmetry of funnel plot and $P_E>0.05$ were considered as non-existence of publication bias. All calculations were performed using Stata (Version11.0, Stata Corporation, College Station, TX).

Results

The flow chart of retrieval and identification of eligible studies is shown in Figure 1. A total of 393 articles were obtained from the databases: 336 unrelated articles, 11 duplicated articles, 38 reviews, meta-analysis, communication and letters and 2 studies with insufficient data were excluded in accordance with the inclusion and exclusion criteria. In the end, only 6 case-control studies concerning rs712 and cancer risk were enrolled as eligible studies [13-15,20-22]. The baseline characteristics of the eligible studies are listed in Table 1.

The results of heterogeneity test and overall effect of meta-analysis as well as Egger's test are shown in Table 2. Table 2 shows no significant association of T allele (P_H=0.002, OR=1.24, 95% CI=0.99-1.56), GT genotype (P_H=0.22, OR=1.11, 95% CI=0.96-1.29), TT genotype (P_H=0.001,

Table 1. Baseline characteristics of each included study of rs712 and cancer risk

Author	Year	Country	Ethnicity	Cancer type	Number of cases and controls	Source of controls	Detection	HWE
Landi	2012	Czech	Caucasian	Colorectal cancer	717/1171	Hospital based	AS-PCR	Yes
Li	2013	China	Chinese	Gastric cancer	181/674	Hospital based	PCR-RFLP	Yes
Pan	2013	China	Chinese	Colorectal cancer	339/313	Hospital based	PCR-RFLP	Yes
Pan	2013	China	Chinese	Nasopharyngeal carcinoma	188/356	Hospital based	PCR-RFLP	Yes
Peng	2010	China	Chinese	Non-small cell lung cancer	83/80	Hospital based	PCR-RFLP	Yes
Yan	2013	China	Chinese	Glioma	153/204	Hospital based	PCR-RFLP	Yes

AS-PCR: allele specific polymerase chain reaction, PCR-RFLP: polymerase chain reaction restriction fragment length polymorphism, HWE: Hardy-Weinberg equilibrium

Table 2. Results of meta-analysis of rs712 and cancer risk

Comparison	Race	I^2	p values			OR and 95%CI	
		%	P_1	P_2	P_3	Fixed model	Random model
GT vs GG	Overall	28.6	0.22	0.16	0.39	1.11(0.96-1.29)	1.13(0.94-1.35)
	Chinese	22.9	0.27	0.06	-	1.19(0.99-1.42)	1.19(0.97-1.47)
	Caucasian	-	-	0.75	-	0.96(0.74-1.24)	0.96(0.74-1.24)
TT vs GG	Overall	76.4	0.001	0.06	0.193	1.33(0.99-1.70)	1.79(0.97-3.31)
	Chinese	37.0	0.174	<0.001		2.32(1.60-3.37)	2.28(1.39-3.74)
	Caucasian	-	-	0.45		0.88(0.64-1.22)	0.88(0.64-1.22)
GT/TT vs GG	Overall	57.8	0.04	0.10	0.41	1.17(1.02-1.34)	1.21(0.97-1.52)
	Chinese	42.9	0.14	0.002	-	1.30(1.10-1.55)	1.31(1.02-1.34)
	Caucasian	-	-	0.59	-	0.94(0.73-1.19)	0.94(0.73-1.19)
T vs G	Overall	73.7	0.002	0.06	0.27	1.16(1.04-1.29)	1.24(0.99-1.56)
	Chinese	53.5	0.08	0.007	-	1.35(1.17-1.55)	1.34(1.08-1.66)
	Caucasian	-	-	0.50	-	0.95(0.80-1.11)	0.95(0.80-1.11)

P_1: P-value of heterogeneity test, P_2: P-value of Z test, P_3: P-value of Egger's test

OR=1.79, 95% CI=0.97-3.31), and T allele carrier (GT/TT) (P_H=0.04, OR=1.21, 95% CI=0.97-1.52) of rs712 with risk of cancer in overall population. Stratification according to ethnicity showed significant associations between rs712 and cancer risk when comparing T vs G (P_H=0.08, OR=1.34, 95% CI=1.08-1.66) and TT vs GG (P_H=0.174, OR=2.32, 95% CI=1.60-3.37), GT/TT vs GG (P_H=0.14, OR=1.30, 95% CI=1.10-1.55) in Chinese population (Figure 2A-C).

The results of sensitivity analysis showed that the corresponding pooled ORs did not change substantially in all comparisons when omitting each study successively each time or changing the evaluated model. The shape of Begg's funnel plot in Figure 3 was symmetrical and p values of Egger's test in Table 2 were > 0.05, suggesting that there was no publication bias in each comparison.

Discussion

miRNAs are endogenous small non-coding RNAs of 17-24 nucleotides. A miRNA can bind to target mRNAs 3'-UTR and negatively regulate relevant gene expression, predominantly at the post-transcriptional level [23]. In humans it is extensively involved in a series of physiological and pathological processes, such as cell proliferation and apoptosis [24], tissue and organ growth and development [25], as well as invasion and metastasis of cancer [26]. Let-7 is a tumor suppressed miRNA family including Let-7a-g and I [27]. Kras mRNA expression can be easily influenced through 10 Let-7 LCSs in Kras 3'-UTR [28]. Rs712, the alternation from allele T to G, was supposed to modulate the binding ability of Let-7 and Kras mRNA [29]. Some authors supported that rs712 was associated with cancer [13], but others claimed the opposite [14,15].
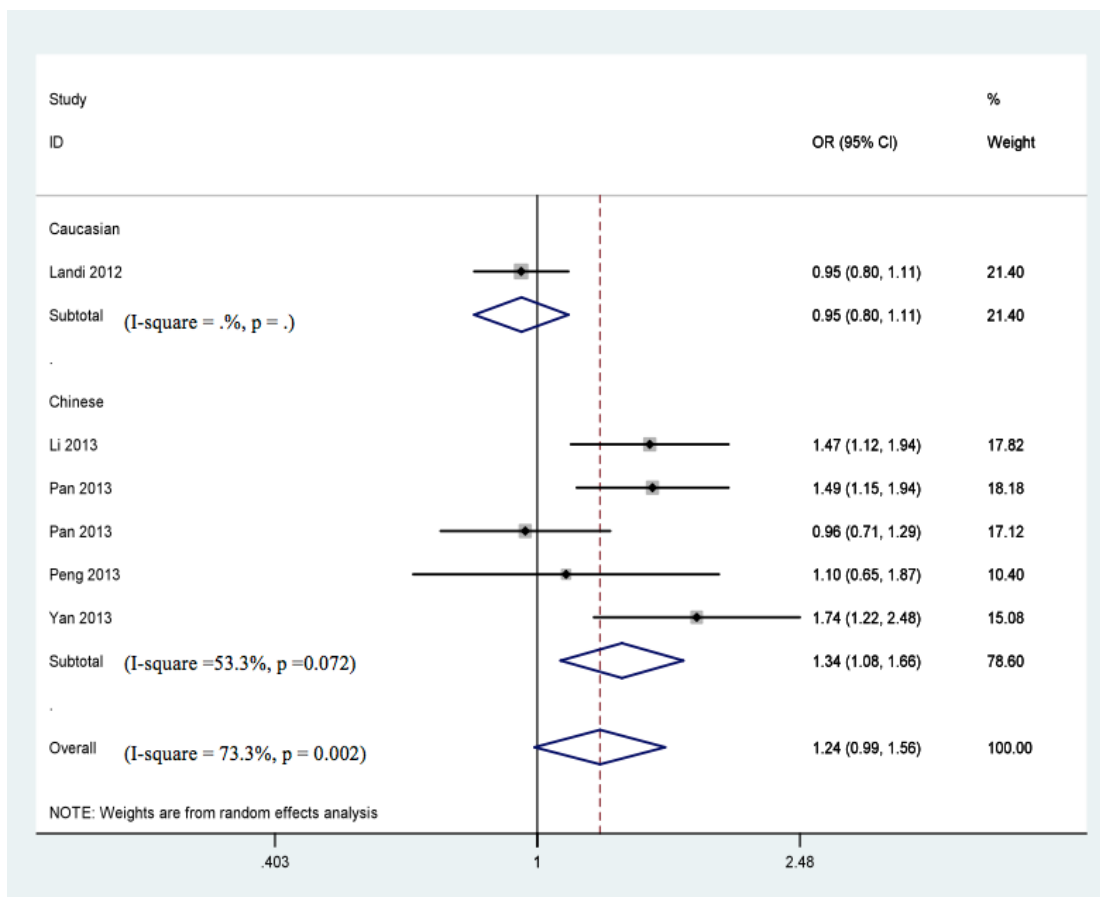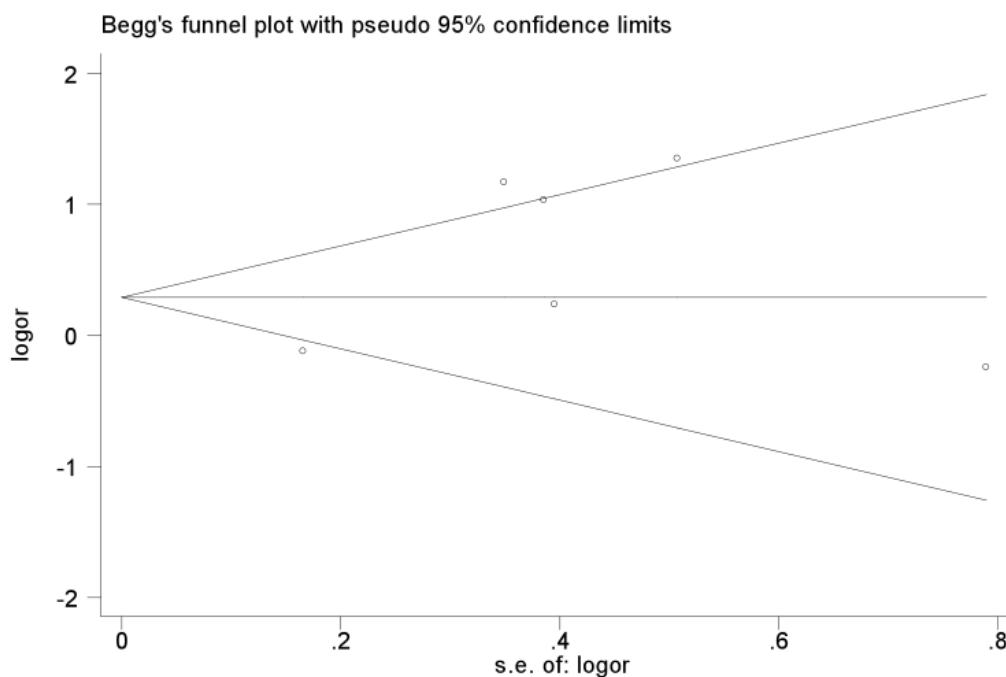
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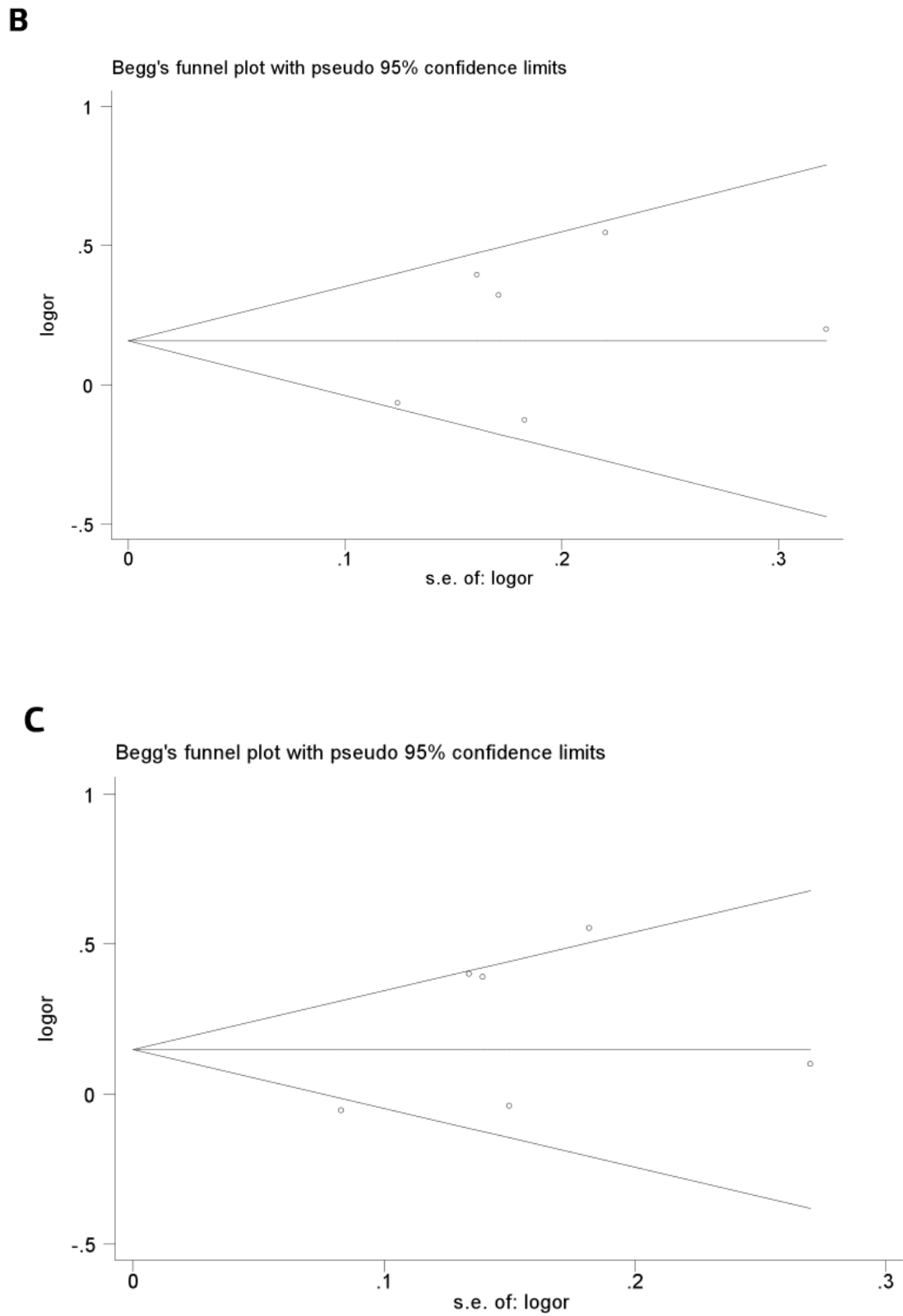

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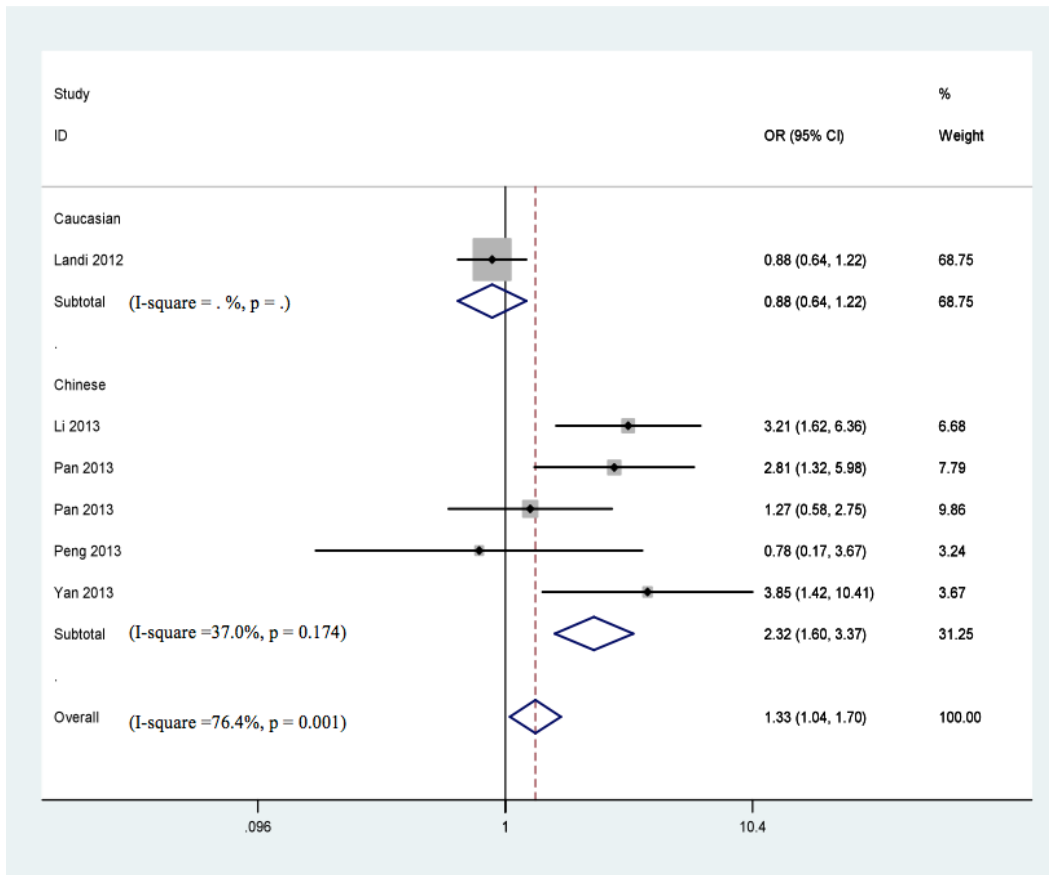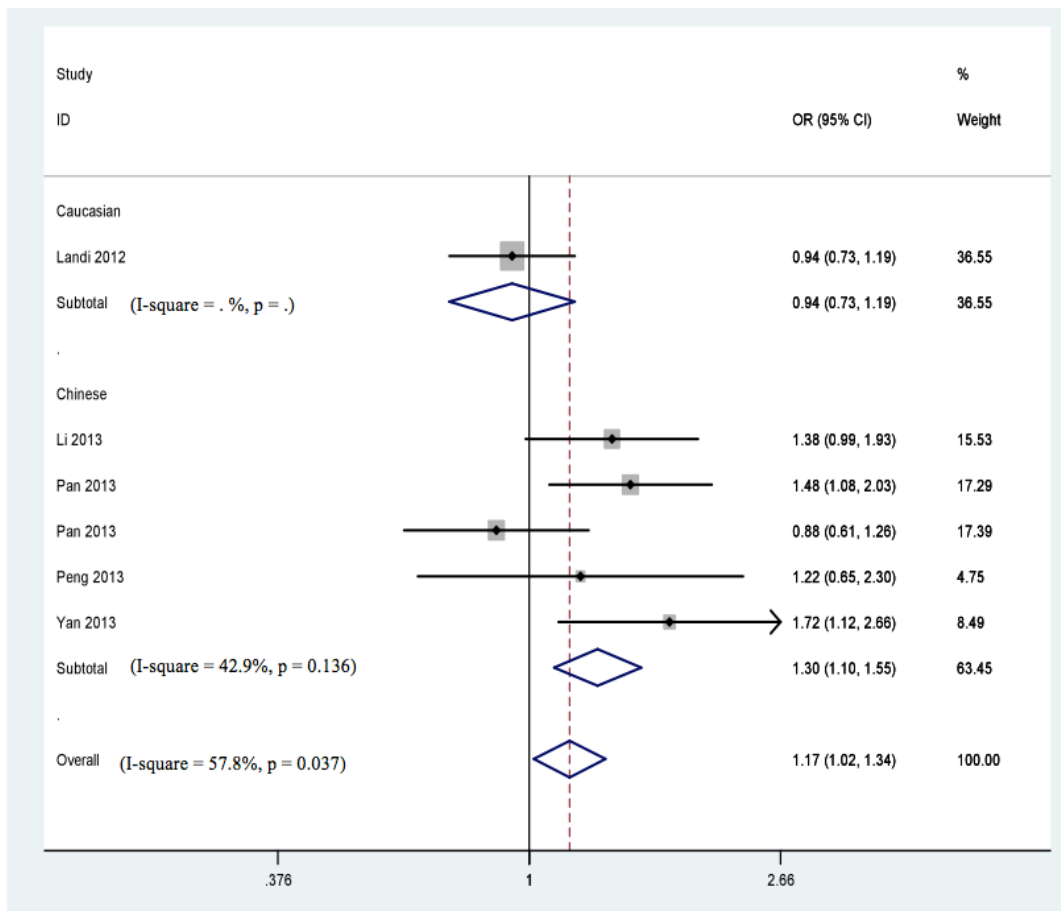
Figure 2. The meta-analysis results of rs712 and cancer risk. **A:** TT vs GG; **B:** GT/TT vs GG; **C:** T vs G.

B

C

Figure 3. Begg's funnel plot of rs712 and cancer risk. **A:** TT vs GG; **B:** GT/TT vs GG; **C:** T vs G.

In our study, the association between rs712 and risk of cancer was investigated using meta-analysis. The results showed that T alleles, GT, GT/TT of rs712 were not associated with cancer, suggesting T allele, GT and GT/TT of rs712 might not increase susceptibility to cancer in the overall population. Because of few published studies concerning rs712 and risk of cancer in Caucasian populations, there was only one eligible study enrolled in the present meta-analysis. The small sample size of case and control groups in this study doesn't allow for a reliable conclusion in Caucasian subjects. Therefore, we did not observe positive association in Caucasian populations. However, T allele and GT/TT genotype were significantly associated with cancer risk in Chinese, indicating that rs712 was a susceptible locus for risk of cancer only in Chinese subjects. These results were consistent with the results reported by Ying et al. [30] and can be interpreted as follows: the larger sample size of cases and controls in eligible studies concerning rs712 and cancer risk in Chinese might have increased the power to get a relatively precise result. Furthermore, the alternation from G to T allele of rs712 disrupts the binding site in 3'-UTR of Kras and influences the combining capacity between Let-7 and Kras mRNA [28,29]. In addition, low circulating Let-7 levels or reduced activity of Let-7 might lead to upregulated Kras expression [29], eventually contributing to carcinogenesis, invasion and metastasis [15,31].

To our knowledge, this meta-analysis is the first to comprehensively evaluate the association between Kras rs712 and cancer risk, providing a more precise assessment in comparison with single studies with small samples. Nevertheless, several limitations should be addressed. The search for eligible studies was limited to Pubmed and Wangfang databases only and manual retrieval in English and Chinese, which might lose other-language published studies and consequently result in selection bias. The majority of eligible studies in our meta-analysis, including less than 1000 cases and controls, attenuated the statistical power and the sample size of the present study is not large enough to offer more precise results. OR and 95% CI of this study are crude, for we cannot obtain substantial data, such as age, sex, smoking and drinking to calculate an adjusted OR and 95% CI.

In summary, T allele and genotype GT/TT rs712 may be cancer-susceptible factors for Chinese population. Due to the limitations of this study, larger sample size and well-designed studies and function analysis are warranted to further validate our findings.

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