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ORIGINAL RESEARCH

The involvement of Kras gene 3′-UTR polymorphisms in risk of cancer and influence on patient response to anti-EGFR therapy in metastatic colorectal cancer: a meta-analysis

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Background: Genetic variation of the *Kras* oncogene is a candidate factor for increasing susceptibility to carcinoma and modulating response of metastatic colorectal cancer (mCRC) patients treated with anti-epidermal growth factor receptor monoclonal antibody (anti-EGFR). However, results from an increasing number of studies concerning the association of Kras gene rs712 and rs61764370 polymorphisms with risk of cancer and treatment of mCRC using anti-EGFR remain equivocal.

Methods: Risk associations were evaluated in 1,661 cases and 2,139 controls from six studies concerning rs712 and 14,796 cases and 14,985 controls from 29 studies concerning rs61764370. Response association was also examined in a subset of four studies pertaining to rs61764370 and anti-EGFR treatment in mCRC.

Results: Results of a meta-analysis showed that allele T (*P*-value of heterogeneity test [P_H] =0.08, odds ratio [OR] =1.33, 95% confidence interval [CI]: 1.08–1.64) and genotype GT/TT (P_H=0.14, OR =1.30, 95% CI: 1.10–1.55) in rs712 were strongly associated with cancer in Chinese subjects. No evidence of association was observed between rs712 and risk of cancer in the overall population or between rs61764370 and ovarian, breast, colorectal, or non-small-cell lung cancer risk in the Caucasian population. No significant association was found between rs61764370 and patient response to anti-EGFR therapy in mCRC.

Conclusion: The findings not only provide further evidence that allele T of rs712 increases genetic predisposition to cancer in Chinese population, but also no significant association between rs61764370 and cancer risk in Caucasian population, and suggest that genotype GT/TT of rs61764370 may not be a biomarker for predicting clinical outcome of anti-EGFR therapy in mCRC.

Keywords: rs712, rs61764370, single nuclear polymorphism

Introduction

In spite of abundant emerging data contributing to understanding of the molecular mechanisms of carcinogenesis and cancer prevention, the number of new diagnoses and death rates, especially in developing countries, continue to rise. In the People's Republic of China, cancer morbidity and mortality rates in 2009 were 285.91/100,000 and 180.54/100,000, respectively, which were higher than the rates of 250.03/100,000 and 166.22/100,000, respectively, in 2004.[1–3] Further, a 2012 US cancer report showed that approximately 1.6 million new cancer cases and 0.58 million cancer deaths were projected to occur in 2013.[4] Many factors, such as mutation, single nucleotide polymorphism (SNP), and epigenetic dysregulation of oncogene or tumor suppressor

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gene, have been found to lead to activation of oncogene or expressed silence of tumor suppressor gene and eventually give rise to carcinogenesis.[5]

Kras gene, a member of the Ras gene family, is one of the most important oncogenes in carcinogenesis and acts as an intracellular signal transducer.[6] It encodes a guanosine diphosphate (GDP)/GTP guanosine triphosphate (GTP)-binding protein that belongs to the small GTPase superfamily, regulates signal transduction, and is involved in cell proliferation and differentiation through Kras-related RAF/MEK/MAPK, AKT, and ERK pathways.[6–8] Mutation of the *Kras* oncogene plays a pivotal role in the pathogenesis of various solid tumors in humans,[9] with a 30%–60% mutation frequency detected in colorectal adenocarcinomas.[10] On the other hand, repression of Kras expression could inhibit tumor growth and invasion by small interfering RNA (siRNA) or microRNA (miRNA).[11] Let-7 miRNA posttranscriptionally regulates Kras oncogene expression by targeting the 3′-untranslated region (3′-UTR) of messenger RNA (mRNA) for degradation or translation repression.[12] Let-7 complementary binding site (LCS) SNPs, located in Kras gene 3′-URT, have been found to modulate the binding ability with let-7,[12] consequently resulting in aberrant expression of Kras gene. Thus, these loci are considered candidate genetic susceptibility factors for carcinogenesis.

Recently, emerging studies concerning let-7 LCS polymorphisms in *Kras* 3′-UTR, rs712 and rs61764370, reported that these SNPs increased risk of cancer and affected the survival of patients with malignant cancer using anti-epidermal growth factor receptor monoclonal antibody (EGFR) therapy in metastatic colorectal cancer (mCRC).[13,14] However, other studies pertaining to these loci had conflicting conclusions.[15,16]

On the basis of accumulating evidence, a comprehensive meta-analysis of retrospective and prospective studies was conducted for the following purposes: 1) to evaluate the association of rs712 and rs61764370 with risk of cancer; and 2) to estimate the influence of rs61764370 genotypes on anti-EGFR treatment in mCRC.

Materials and methods

Study identification and selection

In this meta-analysis, relevant studies dating to November 2013 were searched for in the PubMed, Google Scholar, Embase, and Wanfang Data in accordance with the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) guidelines.[17] Additional studies were identified by manual retrieval in order to obtain substantial articles. The following search terms were used: 1) "rs712, rs61764370 or LCS6 and tumor, cancer or carcinoma"; 2) "Kras polymorphism and tumor, cancer or carcinoma"; 3) "Let-7, Kras and tumor, cancer or carcinoma"; 4) "Let-7, Kras, LCS6 and cancer, EGFR". Relevant studies were first identified through review of each retrieved title and abstract. Then, relevant full-text studies were identified as eligible for meta-analysis according to the following inclusion criteria: 1) case control study concerning rs712, rs61764370, and cancer risk, or anti-EGFR therapy in mCRC, in English or Chinese; 2) cases were solid cancer patients and controls were cancer-free healthy individuals; 3) sufficient genotype frequency data were provided for calculating odds ratio (OR) and 95% confidence interval (CI); and 4) genotype distribution of the control group was consistent with Hardy–Weinberg equilibrium. Non-case control studies, reviews, comments, communications, meta-analyses, single-group design studies, and case control studies with duplicated data were excluded from this study.

Data extraction

Two investigators (Hou-Qun Ying and Feng Wang) independently extracted data from each study identified as eligible per the inclusion and exclusion criteria. A consensus was required for the inclusion of studies. From each eligible study, baseline characteristic data were extracted, which comprised the following: author name or abbreviated study name; year of publication; country; ethnicity; cases and controls; detection

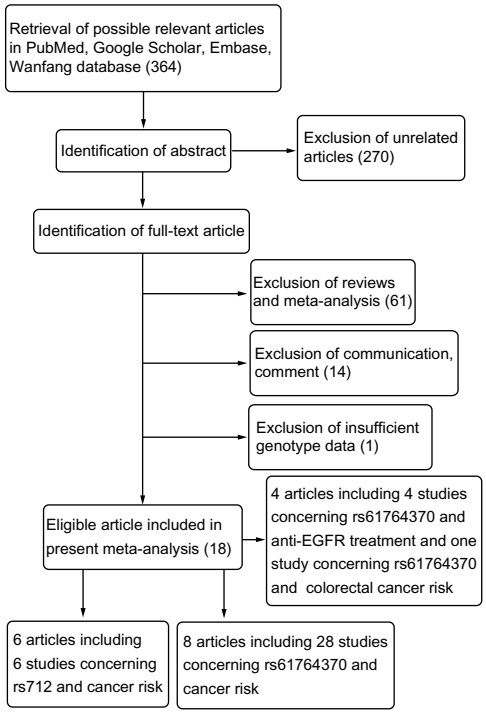


Figure 1 Flowchart of retrieval and identification of eligible studies.
Abbreviation: EGFR, epidermal growth factor receptor monoclonal antibody.

Table 1 Baseline characteristics of each eligible study concerning Kras polymorphisms and risk of cancer

Study and year	Country	Ethnicity	Cases	Controls	Analysis assay
BEL 2011[28]	Belgium	Caucasian	173 invasive epithelial ovarian cancer patients	253 healthy controls	Fluidigm
BWH 2011[28]	USA	Caucasian	137 invasive epithelial ovarian cancer patients	142 healthy controls	Illumina Hap317
Chin et al, 2008[30]	USA	Caucasian	325 non-small-cell lung cancer patients	325 healthy controls	TaqMan®-PCR
Chin et al, 2008 (2)[30]	USA	Caucasian	2,205 non-small-cell lung cancer patients	1,497 healthy controls	TaqMan®-PCR
Christensen et al, 2009[33]	USA	Caucasian	513 head and neck squamous cell cancer patients	597 healthy controls	TaqMan®-PCR
Cerne et al, 2012[29]	Slovenia	Caucasian	530 sporadic and 165 familial breast cancer cases	270 cancer-free controls	TaqMan®n-PCR
DOV 2011[28]	USA	Caucasian	698 invasive epithelial ovarian cancer patients	721 healthy controls	TaqMan®-PCR
GER 2011[28]	Germany	Caucasian	213 invasive epithelial ovarian cancer patients	265 healthy controls	Fluidigm
HJO 2011[28]	Germany	Caucasian	195 invasive epithelial ovarian cancer patients	151 healthy controls	Fluidigm
HMO 2011[28]	Belarus	Caucasian	259 invasive epithelial ovarian cancer patients	426 healthy controls	Fluidigm
HOC 2011[28]	Finland	Caucasian	350 invasive epithelial ovarian cancer patients	434 healthy controls	Fluidigm
Hollestelle et al, 2011[27]	the Netherlands	Caucasian	1,042 breast cancer	797 cancer-free controls	TaqMan®-PCR
HOP 2011[28]	USA	Caucasian	365 invasive epithelial ovarian cancer patients	368 healthy controls	TaqMan®-PCR
Kjersem et al, 2012[35]	Norway	Caucasian	197 colorectal cancer patients	358 healthy controls	TaqMan®-PCR
Landi et al, 2012[15]	Czech Republic	Caucasian	717 colorectal cancer patients	1,171 healthy volunteers	AS-PCR
Li et al, 2013[23]	People's Republic of China	Chinese	181 gastric cancer patients	674 cancer free controls	PCR-RFLP
MAY 2011[28]	USA	Caucasian	358 invasive epithelial ovarian cancer patients	520 healthy controls	Illumina 610 Quad
NCO 2011[28]	USA	Caucasian	494 invasive epithelial ovarian cancer patients	655 healthy controls	Illumina 610 Quad
NTH 2011[28]	the Netherlands	Caucasian	296 invasive epithelial ovarian cancer patients	327 healthy controls	Fluidigm
OVA 2011[28]	Canada	Caucasian	494 invasive epithelial ovarian cancer patients	416 healthy controls	Fluidigm
Paranjape et al, 2011[31]	USA	Caucasian	415 breast cancer patients	457 healthy controls	TaqMan® PCR
Pan et al, 2014[13]	People's Republic of China	Chinese	339 colorectal cancer patients	313 healthy controls	PCR-RFLP
Pan et al, 2014[25]	People's Republic of China	Chinese	188 nasopharyngeal carcinoma patients	356 healthy controls	PCR-RFLP
Peng et al, 2010[26]	People's Republic of China	Chinese	83 non-small-cell lung cancer patients	80 healthy volunteers	PCR-RFLP
PVM 2011[28]	Denmark	Caucasian	201 invasive epithelial ovarian cancer patients	215 healthy controls	Fluidigm
Ratner et al, 2010[32]	USA	Caucasian	100 ovarian cancer patients	101 healthy controls	TaqMan®-PCR
Ratner et al, 2010 (2)[32]	USA	Caucasian	320 ovarian cancer patients	322 healthy controls	TaqMan®-PCR
Ryan et al, 2012[34]	USA	Caucasian	375 colorectal cancer patients	202 healthy controls	No data
TBO 2011[28]	USA	Caucasian	227 invasive epithelial ovarian cancer patients	168 healthy controls	Illumina 610 Quad
TOR 2011[28]	Canada	Caucasian	734 invasive epithelial ovarian cancer patients	556 healthy controls	Illumina 610 Quad
UCI 2011[28]	USA	Caucasian	192 invasive epithelial ovarian cancer patients	372 healthy controls	Fluidigm
UK-GWAS 2011[28]	UK	Caucasian	1,325 invasive epithelial ovarian cancer patients	1,325 healthy controls	Fluidigm

(*Continued*)

Table 1 (*Continued*)

Study and year	Country	Ethnicity	Cases	Controls	Analysis assay
UK2 2011[28]	UK	Caucasian	1,778 invasive epithelial ovarian cancer patients	2,355 healthy controls	Illumina 610 Quad
USC 2011[28]	USA	Caucasian	260 invasive epithelial ovarian cancer patients	343 healthy controls	TaqMan®-PCR
Yan et al, 2013[24]	People's Republic of China	Chinese	153 glioma patients	204 healthy controls	PCR-RFLP

Abbreviations: AS-PCR, allele-specific PCR; PCR, polymerase chain reaction; PCR-RFLP; PCR–restriction fragment length polymorphism; BEL, Belgium Ovarian Cancer Study; BWH, Brigham Women's Hospital Study; DOV, Diseases of the Ovary and their Evaluation Study; GER, German Ovarian Cancer Study; HJO, Hannover–Jena Ovarian Cancer Study; HMO, Hannover–Minsk Ovarian Cancer Study; HOC, Helsinki Ovarian Cancer Study; HOP, Hormones and Ovarian Cancer Prediction Study; MAY, Mayo Clinic Ovarian Cancer Study; NCO, North Carolina Ovarian Cancer Study; NTH, Nijmegen Ovarian Cancer Study; OVA, Ovarian Cancer Study; PVM, Pelvic Mass Study and Malignant Ovarian Cancer Study; TBO, Tampa Bay Ovarian Cancer Study; TOR, Familial Ovarian Tumour Study; UCI, UC Irvine Ovarian Cancer Study; UK2, SEARCH, Southampton Ovarian Cancer Study, Scottish Randomized Trial in Ovarian Cancer, United Kingdom Ovarian Cancer Population Study; USC; Los Angeles County Case–Control Studies of Ovarian Cancer; UK-GWAS, SEARCH, United Kingdom Ovarian Cancer Population Study, Cancer Research UK Familial Ovarian Cancer Register, Royal Marsden Hospital Study, UK 1958 Birth cohort, UK Colorectal control..

method; genotype data; number of total and part responses as well as nonresponses; ORs; and 95% CIs.

Statistical analysis

Crude ORs and 95% CIs were used as common measurements for assessing the strength between Kras polymorphism and cancer risk as well as response to anti-EGFR therapy in mCRC patients. Heterogeneity was assessed by Cochran's Q test and I^2,[18,19] and a *P*-value of heterogeneity test (P_H) <0.10 was considered significant heterogeneity. The fixed model was chosen to evaluate the combined data when the heterogeneity test was assumed to be homogenous; otherwise, the random model was used to estimate the overall effect.[20,21] Stability of meta-analysis was estimated using sensitivity analysis by omitting each eligible study successively. Both Begg's funnel plot and Egger's test were used to establish possible publication bias,[21,22] and asymmetry of funnel plot and *P*-value of Egger's test <0.05 were considered to indicate the existence of publication bias. All calculations were performed using Stata (v 11.0; StataCorp LP, College Station, TX, USA) and RevMan (v 5.2; The Nordic Cochrane Centre, The Cochrane Collaboration, Copenhagen, Denmark) software.

Results

Eligible studies

The flowchart of the eligible study search is shown in Figure 1. In total, 364 articles were obtained from the databases and by manual retrieval. According to the inclusion and exclusion criteria, 270 unrelated articles, 61 reviews and meta-analyses, 14 comments or communications, and one study with insufficient genotype data were excluded from the present study. As a result, a total of six case control studies[13,15,23–26] concerning rs712 and cancer risk, 29 case control studies[27–35] relating to rs61764370 and cancer, and four studies[14,16,35,36] concerning rs61764370 and anti-EGFR treatment in mCRC were enrolled as eligible studies. The baseline characteristics of eligible studies are listed in Tables 1 and 2.

rs712 and cancer risk

The results of heterogeneity testing and overall effects of meta-analysis and Egger's test are listed in Table 3. As shown in Table 3 and Figure 2, no significant association was found between rs712 and risk of cancer in the overall population (P_H=0.27, OR =1.10, 95% CI: 0.95–1.28 for genotype GT versus genotype GG; P_H=0.04, OR =1.21, 95% CI: 0.90–1.50 for genotype

Table 2 Baseline characteristics of each eligible study of rs61764370 and clinical outcome of metastatic colorectal cancer patients treated with anti-EGFR

Study and year	Country	Ethnicity	Cases	Anti-EGFR antibody	CR + PR		SD + PD		*P*-value
					TT genotype	TG/GG genotype	TT genotype	TG/GG genotype	
Graziano et al, 2010[36]	Italy	Caucasian	121 metastatic colorectal cancer patients	Cetuximab	20	6	67	28	>0.05
Sebio et al, 2013[16]	Spain	Caucasian	92 metastatic colorectal cancer patients	Cetuximab and panitumumab	23	0	49	20	<0.01
Kjersem et al, 2012[35]	Norway	Caucasian	355 metastatic colorectal cancer patients	Cetuximab	140	33	157	25	>0.05
Zhang et al, 2011[14]	USA	Caucasian	98 metastatic colorectal cancer patients	Cetuximab	5	5	78	10	<0.01

Abbreviations: CR, complete response; EGFR, epidermal growth factor receptor monoclonal antibody; PD, progressive disease; PR, partial response; SD, stable disease.

GT/TT versus genotype GG; P_H=0.002, OR =1.23, 95% CI: 0.98–1.54 for T versus G). After stratifying the population into Chinese and Caucasian subgroups, significant associations were observed in comparisons of GT/TT and GG (P_H=0.14, OR =1.30, 95% CI: 1.10–1.55) and T and G (P_H=0.08, OR =1.33, 95% CI: 1.08–1.64) in the Chinese population.

rs61764370 and cancer risk

Because of the low frequency of genotype GG in rs61764370, the majority of studies did not provide data for genotype GG, but, combining GG and GT, one single comparison (GT/GG versus TT) was evaluated in this locus. The comparison was analyzed in 29 studies, which included 14,796 cases and 147,985 controls. As shown in Table 3 and Figure 2, the GT/GG genotype of rs61764370 was not significantly associated with cancer risk in the overall population (P_H=0.03, OR =1.06, 95% CI: 0.97–1.15). After stratification analyses in accordance with cancer type, the GT/GG genotype was not observed to be associated with ovarian cancer (P_H=0.008, OR =1.06, 95% CI: 0.95–1.19), breast cancer (P_H=0.97, OR =0.99, 95% CI: 0.83–1.19), colorectal cancer (P_H=0.50, OR =1.13, 95% CI: 0.83–1.54), or non-small-cell lung cancer (P_H=0.05, OR =0.93, 95% CI: 0.60–1.43).

rs61764370 and response of anti-EGFR treatment in mCRC

The association of rs61764370 and influence of anti-EGFR treatment in mCRC patients were estimated in combining with four original studies. Result in overall population showed that no statistically significant association was found between GT/GG genotype and response of mCRC treated with anti-EGFR (P_H=0.003, OR =1.18, 95% CI =0.34–4.71) (Figure 3).

Sensitivity analysis

The stability of this meta-analysis was examined to establish the influence of each eligible study on the pooled ORs by omitting a single study successively each time, and the corresponding pooled ORs were not materially changed in any comparison.

Publication bias

Possible publication bias was assessed using Begg's funnel plot and Egger's test. As shown in Table 3 and Figure 3, the shapes of the funnel plots were symmetrical, and the *P*-values from the Egger's test indicated that no publication bias was found in any comparison.

Discussion

miRNA is an endogenous small non-coding RNA of 17–24 nucleotides that negatively regulates gene expression at the posttranscriptional level, predominantly by binding to the 3′-UTR of target mRNAs through nucleotide pairing.[37] It provides a wide range of functions in various physiological and pathological processes, including organ growth and development, cell proliferation and differentiation, and carcinogenesis and metastasis.[38–41] Let-7, the first discovered miRNA family, which includes let-7a–g and i, has been verified as a tumor suppressor factor in various kinds of cancer.[12,42,43] Expression of Kras was downregulated through ten let-7 LCSs, which

Table 3 Meta-analysis results of rs712, rs61764370, and cancer risks as well as response of anti-EGFR therapy in metastatic colorectal cancer patients

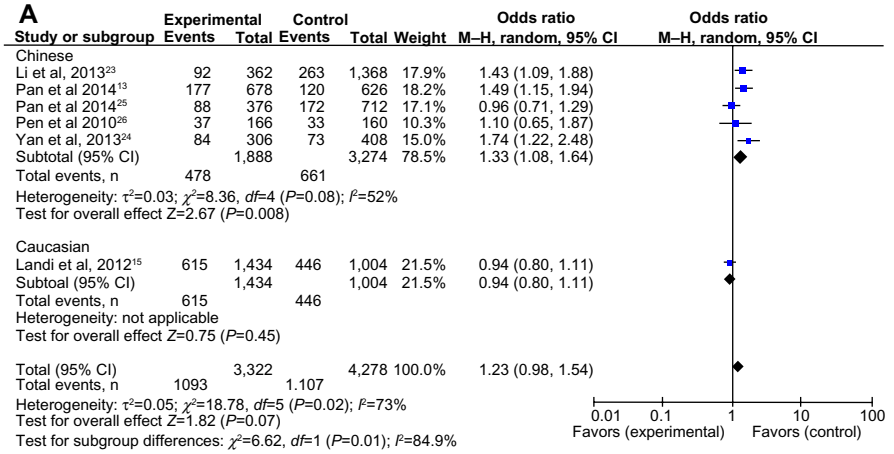
Locus	Comparison	Population/Subgroup	P_H	I^2	P_Z	P_E	OR and 95% CI
rs712	Genotype GT vs genotype GG	Overall	0.23	27%	0.19	0.39	1.10 (0.95–1.28)
		Chinese	0.27	23%	0.07	NA	1.18 (0.98–1.41)
		Caucasian	NA	NA	0.75	NA	0.96 (0.74–1.24)
	Genotype GT/TT vs genotype GG	Overall	0.04	58%	0.10	0.41	1.21 (0.90–1.50)
		Chinese	0.14	43%	0.002	NA	1.30 (1.10–1.55)
		Caucasian	NA	NA	0.59	NA	0.94 (0.73–1.19)
	T vs G	Overall	0.002	73%	0.07	0.27	1.23 (0.98–1.54)
		Chinese	0.08	52%	0.008	NA	1.33 (1.08–1.64)
		Caucasian	NA	NA	0.45	NA	0.94 (0.80–1.11)
rs61764370	Genotype GT/GG vs genotype TT	Overall	0.03	37%	0.20	0.32	1.06 (0.97–1.15)
		Ovarian cancer	0.008	48%	0.28	NA	1.06 (0.95–1.19)
		Breast cancer	0.97	0%	0.95	NA	0.99 (0.83–1.19)
		Colorectal cancer	0.50	0%	0.42	NA	1.13 (0.83–1.54)
		Non-small-cell lung cancer	0.05	73%	0.73	NA	0.93 (0.60–1.43)
rs61764370[a]	Genotype GT/GG vs genotype TT	Overall	0.003	78%	0.79	NA	1.18 (0.34–4.17)

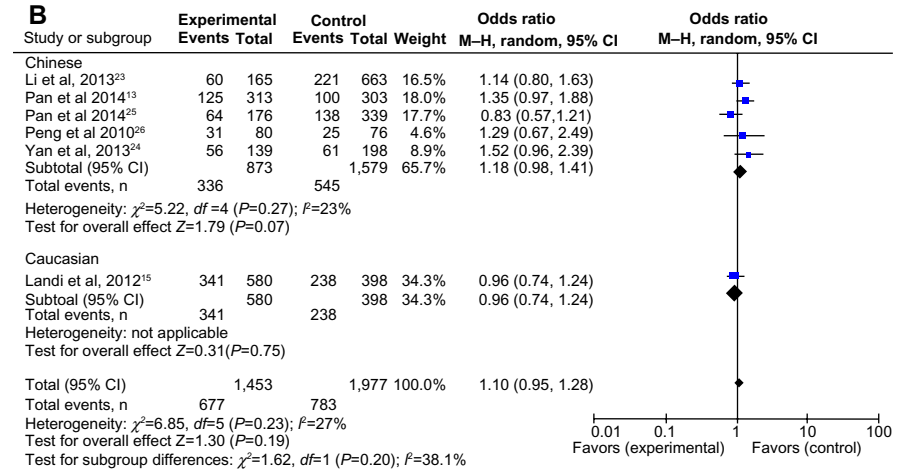
Note: [a]Meta-analysis result of rs61764370 and response of anti-EGFR therapy in metastatic colorectal cancer.

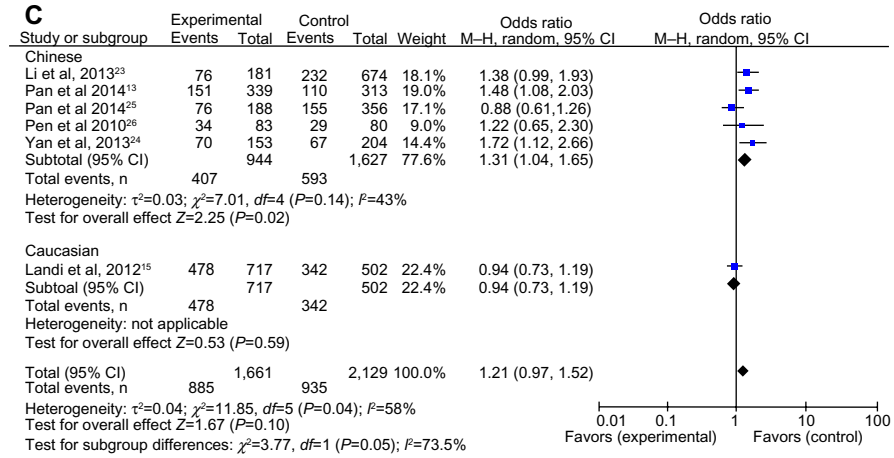
Abbreviations: CI, confidence interval; EGFR, epidermal growth factor receptor monoclonal antibody; NA, not applicable; OR, odds ratio; P_H, *P*-value of heterogeneity test; P_Z, *P*-value of *Z*-test; P_E, *P*-value of Egger's test; vs, versus.

were found in *Kras* 3′-UTR.[30] SNPs of rs712 in LCS1 and rs61764370 in LCS6 can disrupt the let-7 binding site and decrease the combining capacity between them, contributing to aberrant Kras expression.[30] Increasing evidence shows two SNPs (rs712 and rs61764370) not only are associated with cancer, but also rs61764370 can modulate the anti-EGFR treatment response in mCRC. Meanwhile, contradictory results have been observed in other studies.[13,14,16]

In the current study, the possible associations of rs712 and rs61764370 with risk of cancer and anti-EGFR therapy efficacy in mCRC were investigated by meta-analysis. The results showed that genotypes GT and GT/TT and allele T of rs712,







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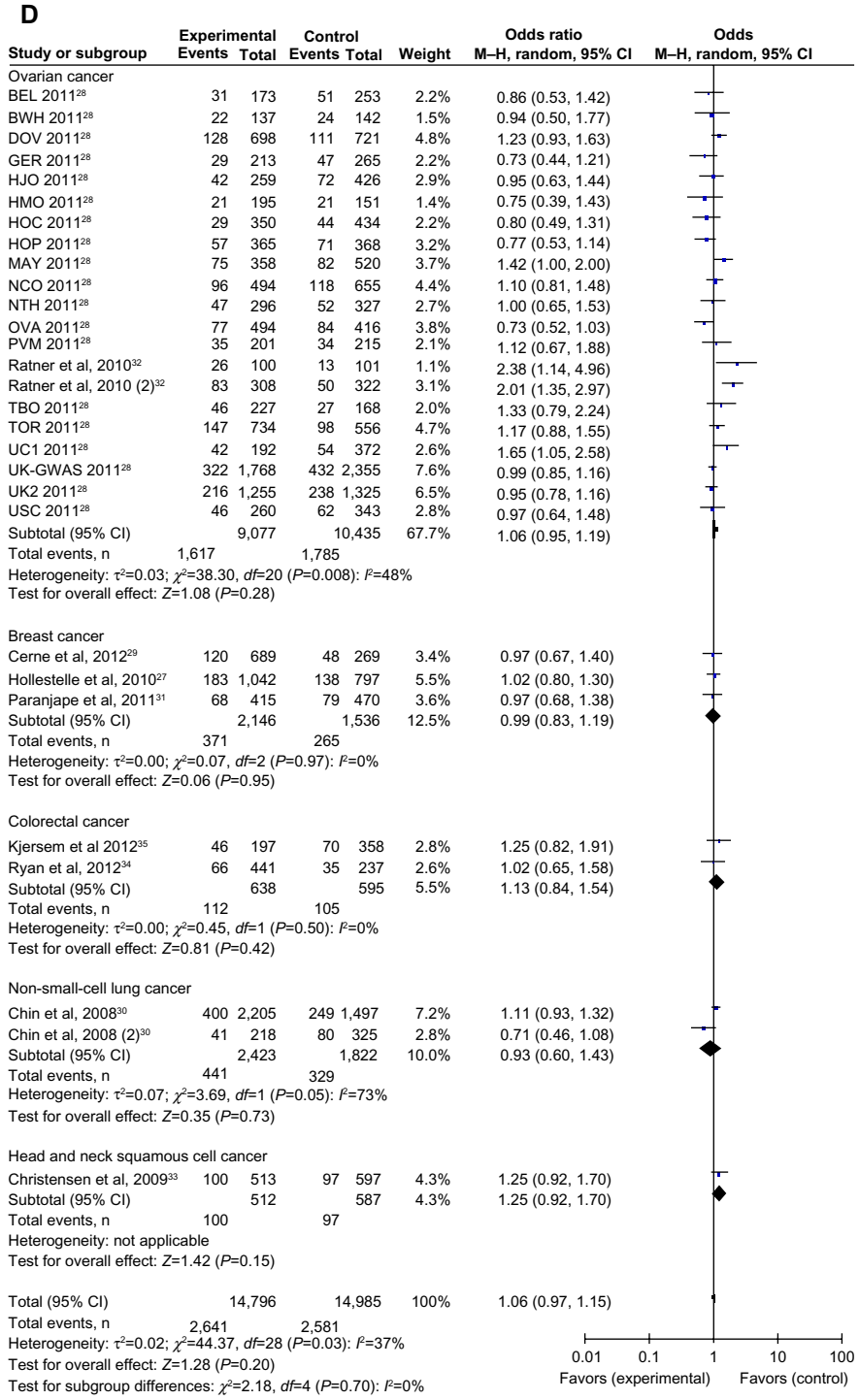

Figure 2 Results of meta-analysis of rs712 and rs61764370 polymorphism loci and cancer risk.

Notes: (**A**) T versus G of rs712. (**B**) Genotype GT versus genotype GG of rs712. (**C**) Genotype GT/TT versus genotype GG of rs712. (**D**) Genotype GT/GG versus genotype TT of rs61764370.

Abbreviations: CI, confidence interval; M–H, Mantel–Haenszel; BEL, Belgium Ovarian Cancer Study; BWH, Brigham Women's Hospital Study; DOV:, Diseases of the Ovary and their Evaluation Study; GER, German Ovarian Cancer Study; HJO, Hannover–Jena Ovarian Cancer Study; HMO, Hannover–Minsk Ovarian Cancer Study; HOC, Helsinki Ovarian Cancer Study; HOP, Hormones and Ovarian Cancer Prediction Study; MAY, Mayo Clinic Ovarian Cancer Study; NCO, North Carolina Ovarian Cancer Study; NTH, Nijmegen Ovarian Cancer Study; OVA, Ovarian Cancer Study; PVM, Pelvic Mass Study and Malignant Ovarian Cancer Study; TBO, Tampa Bay Ovarian Cancer Study; TOR, Familial Ovarian Tumour Study; UCI, UC Irvine Ovarian Cancer Study; UK2, SEARCH, Southampton Ovarian Cancer Study, Scottish Randomized Trial in Ovarian Cancer, United Kingdom Ovarian Cancer Population Study; USC; Los Angeles County Case–Control Studies of Ovarian Cancer; UK-GWAS, SEARCH, United Kingdom Ovarian Cancer Population Study, Cancer Research UK Familial Ovarian Cancer Register, Royal Marsden Hospital Study, UK 1958 Birth cohort, UK Colorectal control.

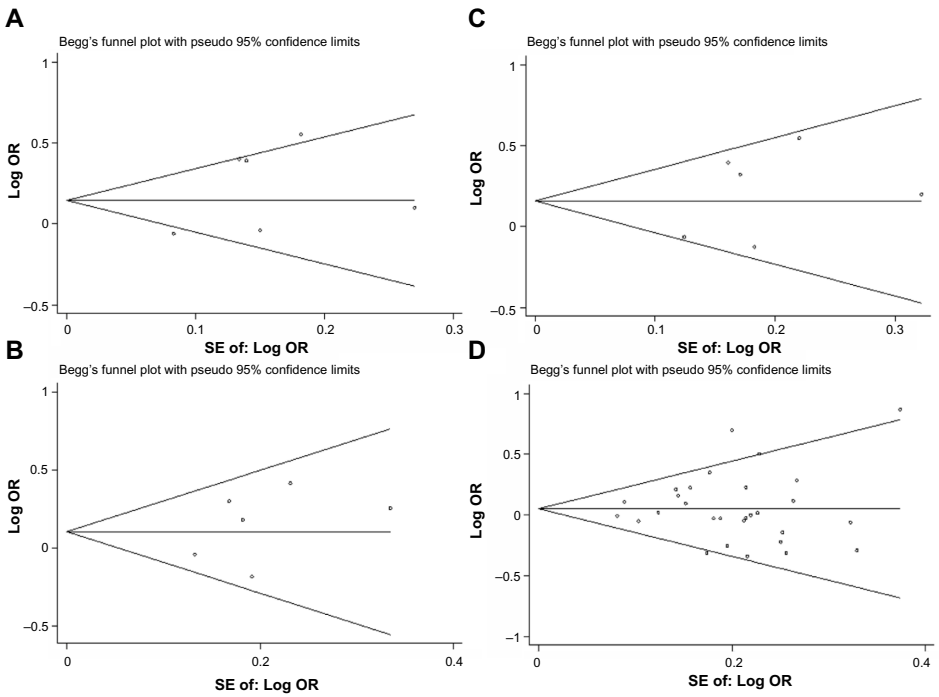

Figure 3 Begg's funnel plots of rs712, rs61764370, and cancer risk.
Notes: (**A**) T versus G of rs712. (**B**) Genotype GT versus genotype GG of rs712. (**C**) Genotype GT/TT versus genotype GG of rs712. (**D**) Genotype GT/GG versus genotype TT of rs61764370.
Abbreviations: Log, logarithm; OR, odds ratio; SE, standard error.

and genotype GT/GG of rs61764370, were not associated with cancer, revealing that appearance of genotypes GT and GT/TT and the T allele of rs712 might not increase predisposition to cancer in the overall population and that genotype GT/GG of rs61764370 was not a genetic susceptibility factor for cancer in the Caucasian population. Significant associations were observed between genotype GT/TT and allele T of rs712 and risk of cancer in Chinese populations. The findings suggest that genotype GT/TT and allele T of rs712 could increase cancer risk and might be genetic susceptibility factors for cancer, only in the Chinese population. The following possible reasons might account for our findings.

Due to differences in ethnic genetic backgrounds in Caucasian and Chinese populations, frequency of the G allele of rs61764370 in the Chinese population is less than 1%, and no study reported an association of this locus with cancer risk in the Chinese population. Although rs712 allele frequency in the Caucasian population is higher than 5%, only one eligible study[15] reported the association between rs712 and cancer risk in this population; therefore, small sample sizes of cases and controls in eligible studies may limit the power to reach a more precise result in Caucasian populations, for only one eligible study with sample size of cases and controls were less than 1000 concerning rs712 and cancer risk in Caucasian population. Moreover, on the basis of capability of let-7 regulating *Kras* expression, we deduced that the allele T of rs712 might disrupt and interfere with the combining capacity between let-7 and the 3′-URT of *Kras* mRNA and somehow lower the level of cellular let-7 concentration or reduce its activity.[30,44] Due to loss of inhibition, expression of Kras is upregulated. Consequently, lower concentration or activity of let-7 and higher Kras-expressed p21 protein are involved in promoting cell proliferation and division, leading to carcinogenesis and metastases.[45,46]

Biological target treatment is an effective measure for malignant cancer therapy. Anti-EGFR monoclonal antibodies, cetuximab and panitumumab, are extensively used in mCRC therapy until now. Both mutation and SNP of Kras gene has been reported to affect response rates of mCRC treated with anti-EGFR.[47] Combining each including study, our meta-analysis results showed no statistically significant effect of genotype GT/GG of rs61764370 on response rates of mCRC patients treated with anti-EGFR, suggesting that genotype GT/GG does not influence the anti-EGFR therapy response in mCRC, thus should not be considered a predictor of the efficacy of anti-EGFR therapy in mCRC.

The current meta-analysis is, to our knowledge, the first assessment of the relationship between Kras polymorphism and risk of cancer, as well as the first assessment of treatment of anti-EGFR in mCRC, and provides a more reliable

estimation of the association between rs712, rs61764370 and cancer risk as well as response to anti-EGFR therapy in mCRC patients when compared with any single study with small samples. However, there are several limitations of the meta-analysis, which should be addressed. First, retrieval of eligible studies was only performed in PubMed, Google Scholar, Embase, and Wanfang databases in English and Chinese, which means eligible studies published in other languages may have been overlooked, which could have led to selection bias. Second, small numbers of cases (<1,000) in the majority of eligible studies decreased the statistical power. Third, the sample size of this meta-analysis is the largest of sample size in the Meta-analysis so far, but it was neither large nor comprehensive enough to allow for a precise conclusion to be reached, especially in Chinese or Caucasian population. Finally, due to unavailable data in some included studies, we could not perform a meta-analysis based on adjustments for age, diet, smoking, or other environmental factors.

Conclusion

Genotype GT/TT and allele T of rs712 may be potential risk factors for developing cancer in the Chinese population, while GT/GG of rs61764370 neither increases predisposition to cancer in Caucasian people nor predicts clinical outcome of anti-EGFR therapy in mCRC. Given the limitations of the current study, a larger sample size and functional analysis are warranted to further validate the results.

Acknowledgments

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Disclosure

The authors report no conflicts of interest in this work.

References

1. Chen WQ, Zhang SW, Zheng RS, et al. [Report of cancer incidence and mortality in China, 2009]. *China Cancer*. 2013;22:2–12. Chinese.
2. Zhang SW, Chen WQ, Lei ZL, Zou XN, Zhao P. [A report of cancer incidence from 37 cancer registries in China, 2004]. *China Cancer*. 2008;17:909–912. Chinese.
3. Chen WQ, Zhang SW, Kong LZ, Lei ZL, Zhao P. [Cancer mortality report of 34 cancer registries in China, 2004]. *China Cancer*. 2008;17:913–916. Chinese.
4. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2013. *CA Cancer J Clin*. 2013;63:11–30.
5. Garraway LA, Lander ES. Lessons from the cancer genome. *Cell*. 2013;153:17–37.
6. Kranenburg O. The KRAS oncogene: past, present, and future. *Biochim Biophys Acta*. 2005;1756:81–82.
7. Kent OA, Fox-Talbot K, Halushka MK. RREB1 repressed miR-143/145 modulates KRAS signaling through downregulation of multiple targets. *Oncogene*. 2013;32:2576–2585.
8. Deng M, Tang H, Zhou Y, et al. miR-216b suppresses tumor growth and invasion by targeting KRAS in nasopharyngeal carcinoma. *J Cell Sci*. 2011;124:2997–3005.
9. Rodenhuis S, van de Wetering ML, Mooi WJ, Evers SG, van Zandwijk N, Bos JL. Mutational activation of the K-ras oncogene. A possible pathogenetic factor in adenocarcinoma of the lung. *N Engl J Med*. 1987;317:929–935.
10. Brink M, de Goeij AF, Weijenberg MP, et al. K-ras oncogene mutations in sporadic colorectal cancer in The Netherlands Cohort Study. *Carcinogenesis*. 2003;24:703–710.
11. Dassow H, Aigner A. MicroRNAs (miRNAs) in colorectal cancer: from aberrant expression towards therapy. *Curr Pharm Des*. 2013;19:1242–1252.
12. Johnson SM, Grosshans H, Shingara J, et al. RAS is regulated by the let-7 microRNA family. *Cell*. 2005;120:635–647.
13. Pan XM, Sun RF, Li ZH, et al. A let-7 KRAS rs712 polymorphism increases colorectal cancer risk. *Tumour Biol*. 2014;35:831–835.
14. Zhang W, Winder T, Ning Y, et al. A let-7 microRNA-binding site polymorphism in 3′-untranslated region of KRAS gene predicts response in wild-type KRAS patients with metastatic colorectal cancer treated with cetuximab monotherapy. *Ann Oncol*. 2011;22:104–109.
15. Landi D, Gemignani F, Landi S. Role of variations within microRNA-binding sites in cancer. *Mutagenesis*. 2012;27:205–210.
16. Sebio A, Paré L, Páez D, et al. The LCS6 polymorphism in the binding site of let-7 microRNA to the KRAS 3′-untranslated region: its role in the efficacy of anti-EGFR-based therapy in metastatic colorectal cancer patients. *Pharmacogenet Genomics*. 2013;23:142–147.
17. Stroup DF, Berlin JA, Morton SC, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. *JAMA*. 2000;283:2008–2012.
18. Biggerstaff BJ, Jackson D. The exact distribution of Cochran's heterogeneity statistic in one-way random effects meta-analysis. *Stat Med*. 2008;27:6093–6110.
19. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. *Stat Med*. 2002;21:1539–1558.
20. Hedges LV, Vevea JL. Fixed- and random-effects models in meta-analysis. *Psychol Methods*. 1998;3:486–504.
21. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ*. 1997;315:629–634.
22. Begg CB, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. *Biometrics*. 1994;50:1088–1101.
23. Li ZH, Pan XM, Han BW, et al. A let-7 binding site polymorphism rs712 in the KRAS 3′ UTR is associated with an increased risk of gastric cancer. *Tumour Biol*. 2013;34:3159–3163.
24. Yan L, Wang QW, Tian KL. Association between the single nucleotide polymorphism of let-7 target gene KRAS-binding site rs712 and risk of glioma. *Chin J Cancer Prev Treat*. 2013;20:811–814. Chinese.
25. Pan XM, Jia J, Guo XM, et al. Lack of association between let-7 binding site polymorphism rs712 and risk of nasopharyngeal carcinoma. *Fam Cancer*. 2014;13:93–97.
26. Peng XB, Zhao J, Lei Z, Liu RY, Liu ZY, Jiang XF, Zhang HT. [Association of a SNP in microRNA let-7 complementary region in KRAS 3′UTR with non-small cell lung cancer]. *Soochow Univ J Med Sci*. 2010;4:786–790. Chinese.
27. Hollestelle A, Pelletier C, Hooning M, et al. Prevalence of the variant allele rs61764370 T>G in the 3′UTR of KRAS among Dutch BRCA1, BRCA2 and non-BRCA1/BRCA2 breast cancer families. *Breast Cancer Res Treat*. 2011;128:79–84.

28. Pharoah PD, Palmieri RT, Ramus SJ, et al. The role of KRAS rs61764370 in invasive epithelial ovarian cancer: implications for clinical testing. *Clin Cancer Res*. 2011;17:3742–3750.
29. Cerne JZ, Stegel V, Gersak K, Novakovic S. KRAS rs61764370 is associated with HER2-overexpressed and poorly-differentiated breast cancer in hormone replacement therapy users: a case control study. *BMC Cancer*. 2012;12:105.
30. Chin LJ, Ratner E, Leng S, et al. A SNP in a let-7 microRNA complementary site in the KRAS 3′ untranslated region increases non-small cell lung cancer risk. *Cancer Res*. 2008;68:8535–8540.
31. Paranjape T, Heneghan H, Lindner R, et al. A 3′-untranslated region KRAS variant and triple-negative breast cancer: a case-control and genetic analysis. *Lancet Oncol*. 2011;12:377–386.
32. Ratner E, Lu L, Boeke M, et al. A KRAS-variant in ovarian cancer acts as a genetic marker of cancer risk. *Cancer Res*. 2010;70:6509–6515.
33. Christensen BC, Moyer BJ, Avissar M, et al. A let-7 microRNA-binding site polymorphism in the KRAS 3′ UTR is associated with reduced survival in oral cancers. *Carcinogenesis*. 2009;30:1003–1007.
34. Ryan BM, Robles AI, Harris CC. KRAS-LCS6 genotype as a prognostic marker in early-stage CRC – letter. *Clin Cancer Res*. 2012;18:3487–3488; author reply 3489.
35. Kjersem JB, Ikdahl T, Guren T, et al. Let-7 miRNA-binding site polymorphism in the KRAS 3′UTR; colorectal cancer screening population prevalence and influence on clinical outcome in patients with metastatic colorectal cancer treated with 5-fluorouracil and oxaliplatin ± cetuximab. *BMC Cancer*. 2012;12:534.
36. Graziano F, Canestrari E, Loupakis F, et al. Genetic modulation of the Let-7 microRNA binding to KRAS 3′-untranslated region and survival of metastatic colorectal cancer patients treated with salvage cetuximab-irinotecan. *Pharmacogenomics J*. 2010;10:458–464.
37. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell*. 2004;116:281–297.
38. Alvarez-Garcia I, Miska EA. MicroRNA functions in animal development and human disease. *Development*. 2005;132:4653–4662.
39. Zhao C, Sun G, Li S, et al. MicroRNA let-7b regulates neural stem cell proliferation and differentiation by targeting nuclear receptor TLX signaling. *Proc Natl Acad Sci U S A*. 2010;107:1876–1881.
40. Mirnezami AH, Pickard K, Zhang L, Primrose JN, Packham G. MicroRNAs: key players in carcinogenesis and novel therapeutic targets. *Eur J Surg Oncol*. 2009;35:339–347.
41. Asangani IA, Rasheed SA, Nikolova DA, et al. MicroRNA-21 (miR-21) post-transcriptionally downregulates tumor suppressor Pdcd4 and stimulates invasion, intravasation and metastasis in colorectal cancer. *Oncogene*. 2008;27:2128–2136.
42. Kumar MS, Erkeland SJ, Pester RE, et al. Suppression of non-small cell lung tumor development by the let-7 microRNA family. *Proc Natl Acad Sci U S A*. 2008;105:3903–3908.
43. Park SM, Shell S, Radjabi AR, et al. Let-7 prevents early cancer progression by suppressing expression of the embryonic gene HMGA2. *Cell Cycle*. 2007;6:2585–2590.
44. Landi D, Gemignani F, Naccarati A, et al. Polymorphisms within micro-RNA-binding sites and risk of sporadic colorectal cancer. *Carcinogenesis*. 2008;29:579–584.
45. Ruzzo A, Graziano F, Vincenzi B, et al. High let-7a microRNA levels in KRAS-mutated colorectal carcinomas may rescue anti-EGFR therapy effects in patients with chemotherapy-refractory metastatic disease. *Oncologist*. 2012;17:823–829.
46. Scharovsky OG, Rozados VR, Gervasoni SI, Matar P. Inhibition of ras oncogene: a novel approach to antineoplastic therapy. *J Biomed Sci*. 2000;7:292–298.
47. Di Fiore F, Blanchard F, Charbonnier F, et al. Clinical relevance of KRAS mutation detection in metastatic colorectal cancer treated by Cetuximab plus chemotherapy. *Br J Cancer*. 2007;96:1166–1169.