RESEARCH ARTICLE

Association of a Pre-miR-27a Polymorphism with Cancer Risk: an Updated Meta-analysis

Rong-Pan Bai[1,2&], Yu Weng[3&], Li-Ling Su[1], Ming-Juan Jin[1,4], Zheng-Ping Xu[1,2], Li-Qin Lu[5*], Guang-Di Chen[1,2*]

Abstract

MicroRNA-27a is highly expressed in cancers and has been identified as an oncogenic microRNA. A genetic variant in pre-miR-27a (rs895819) with a transition of A to G has been demonstrated to be associated with cancer risk; however, the results of these studies remain conflicting rather than conclusive. Therefore, we performed a meta-analysis to derive a more precise estimation. Through searching PubMed or other databases up to March 2014 using the following MeSH terms and keywords, "miR-27a", "polymorphism" and "cancer", seventeen case-control studies were identified in this meta-analysis, including 7,813 cases and 9,602. Crude odds ratios (ORs) and corresponding 95% confidence intervals (CIs) were calculated to investigate the association strength between rs895819 and the susceptibility of cancer. The results of the overall meta-analysis did not suggest any association between rs895819 polymorphism and cancer susceptibility, and this remained in Asians as a sub-group. In Caucasians, however, the rs895819 was associated with a reduced cancer risk in heterozygous (OR, 0.83; 95%CI, 0.75-0.93) and dominant models (OR, 0.84; 95%CI, 0.76-0.93), and the [G] allele of rs895819 showed a protective effect (OR, 0.90, 95%CI, 0.84-0.97). Further studies showed a significant association between the [G] allele of rs895819 and decreased risk of breast cancer (0.91; 95%CI, 0.85-0.98), and stratified analyses indicated a protective effect of the [G] allele in Caucasians (OR, 0.89; 95%CI, 0.82-0.98), younger breast cancer cases (OR, 0.87; 95%CI, 0.79-0.96), and in the group of unilateral breast cancer patients (OR, 0.90; 95%CI, 0.83-0.97). These findings suggest an association between pre-miR-27a polymorphism rs895819 and cancer risk in Caucasians. The protective effect of rs895819 [G] allele in younger breast cancer and in the group of unilateral breast cancer patients await further confirmation since the included studies in this meta-analysis were limited.

Keywords: microRNA-27a - meta-analysis - polymorphism - cancer

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Introduction

MicroRNAs (miRNAs) are a group of short noncoding, single-stranded RNAs with18-25 nucleotides in length, which post-transcriptionally inhibit gene expression by degradation of messenger RNA (mRNA) targets and (or) block protein translations of these targets (Pasquinelli, 2012). Since the initial discovery of miRNAs as essential regulators of development in the nematode Caenorhabditis elegans, thousands of miRNA genes have been identified in animal and plant genomes (Kozomara and Griffiths-Jones, 2011). It is well-known that miRNAs are implicated in many important biological processes such as cell proliferation, differentiation, migration, autophagy and apoptosis, etc., and disruption of miRNA function has been found to have relevance not only to tumorigenesis, but also to neurological, cardiovascular, developmental and other diseases (Esteller, 2011).

Single nucleotide polymorphisms (SNPs) are the most common form of variation present in the human genome. However, the sequencing has shown that rare SNPs are presented in miRNA coding genes, and specifically in miRNA seed regions (Chen and Rajewsky, 2006; Saunders et al., 2007). SNPs present in the miRNA gene regions can alter their expression and/or maturation through their transcripts (pri-miRNA, pre-miRNA) and lead to aberrant miRNA regulation (Ryan et al., 2010). SNPs in miRNA genes are thought to affect function in three ways, i.e., through the transcription of the primary transcript, through pri-miRNA and pre-miRNA processing or through effects on miRNA-mRNA interactions (Ryan et al., 2010). SNPs in miRNAs or their precursors are marked as novel genetic variations which may modify the cancer susceptibilities (Chen et al., 2008).

The oncogenic miR-27 is known to regulate components involved in numerous types of cancer,

*[1]Institute of Environmental Health, [2]Research Center of Molecular Medicine, [3]Department of Clinical Laboratory, Sir Run Run Shaw Hospital, Zhejiang Univerisity School of Medicine, [4]Department of Epidemiology and Biostatistics, Zhejiang Univerisity School of Public Health, [5]Department of Oncology, Zhejiang Provincial People's Hospital, Hangzhou, China [&]Equal contributors *For correspondence: chenguangdi@gmail.com or llq199@yahoo.com.cn*

including breast cancer (Mertens-Talcott et al., 2007), hepatocellular carcinoma (Wu et al., 2013), glioblastoma (Ge et al., 2013) and gastric cancer (Yang et al., 2014). Genetic variant in pre-miR-27a (rs895819) has been demonstrated to be associated with breast cancer risk and this association was mainly observed in the breast cancers with age of less than 50-year old group or in bilateral breast cancer group (Yang et al., 2010); however, later studies showed conflicting associations (Catucci et al., 2012; Zhang et al., 2012). Some other epidemiological studies have examined the association between rs895819 and cancer risk in other type of cancers, but showed an inconsistent conclusion (Sun et al., 2010; Hezova et al., 2012; Shi et al., 2012; Zhou et al., 2012). Meta-analysis studies revealed that rs895819 was a functional SNP and may have some relation to breast cancer susceptibility, or cancer development in Caucasian (Wang et al., 2012; Wang et al., 2013; Xu et al., 2013b; Zhong et al., 2013). Due to limited studies and data presented in original reports, however, these meta-analysis studies did not calculate the pooled odd ratio (OR) for different subgroups of breast cancers or other types of cancers. Furthermore, recent studies showed controversial results on the association of rs895819 with cancer risk (Wei et al., 2013; Zhang et al., 2013; Kupcinskas et al., 2014; Xiong et al., 2014; Yang et al., 2014). To clarify the association between rs895819 and cancer risk, in this study, we performed additional pooling analyses by including more recent publications to improve the efficiency of meta-analysis.

Materials and Methods

Identification and eligibility of relevant studies

To identify all articles that examined the association of miR-27a polymorphism with cancer, we conducted a literature search in the PubMed databases up to March 2014 using the following MeSH terms and keywords: "miR-27a", "polymorphism" and "cancer". Additional studies were identified by a manual search from other sources (e.g., Embase, Web of Knowledge, China National Knowledge Infrastructure), references of original studies or review articles on this topic. Eligible studies included in this meta-analysis had to meet the following criteria: (a) an unrelated case-control study, if studies had partly overlapped subjects, only the one with a larger sample size was selected, (b) available genotype frequency, (c) sufficient published data for estimating an odds ratio (OR) with 95% confidence interval (CI) and (d) the genotype frequencies in the control group were consistent with Hardy-Weinberg equilibrium.

Data extraction

Two investigators (R.B. and Y.W.) independently extracted data and reached a consensus on all of the items. The following information was extracted from each study: first author, year of publication, country of origin, ethnicity, cancer types, number of cases and controls, Hardy-Weinberg equilibrium test for the genotype frequencies of control groups, minor allele frequencies of the controls, sources of controls and genotyping methods. Different ethnicity descents were categorized as Asian and Caucasian.

Statistical analysis

Hardy-Weinberg equilibrium was tested by the chi-square goodness of fit test. Crude ORs with 95%CIs were used to assess the strength of association between the miR-27a polymorphism rs895819 and cancer risk. We first estimated the risks of the AG and GG genotypes on cancer, compared with the reference AA homozygote, and then evaluated the risks of (AG+GG *vs* AA) and (GG *vs* AA + AG) on cancer, assuming dominant and recessive effects of the variant GG allele, respectively. The effect of [G] allele on cancer risk was also evaluated by comparing with the reference [A] allele. Stratified analyses were also performed by types of cancer, ethnicities and sources of controls. For the breast cancer, subgroup analyses were performed by age of diagnosis and location of breast cancer. Potential heterogeneity was evaluated by the I^2-based Q-test. A random-effects (DerSimonian-Laird method) or fixed-effects (Mantel-Haenszel method) model was used to calculate pooled effect estimates in the presence ($P \leq 0.10$) or absence ($P > 0.10$) of heterogeneity. Publication bias was detected by Egger's test (Hayashino et al., 2005) and Begg's (Begg and Mazumdar, 1994) test for the overall pooled analysis of rs895819. Additionally, Begg's funnel plot was drawn, in which an asymmetry of the funnel plot suggests a potential publication bias. For the one-way sensitivity analysis, one single study was excluded each time, and the new pooled results could reflect the influence of that deleted study to the overall summary OR. All analyses were done with Stata software (version 11.0; StataCorp LP, College Station, TX), and all statistical tests were two-sided and considered statistically significant at P value < 0.05.

Results

Characteristics of studies

Twenty-five abstracts were retrieved through the search "miR-27a", "polymorphism" and "cancer", and eleven articles were identified as eligible studies (Sun et al., 2010; Yang et al., 2010; Catucci et al., 2012; Hezova et al., 2012; Shi et al., 2012; Zhang et al., 2012; Zhou et al., 2012; Wei et al., 2013; Zhang et al., 2013; Xiong et al., 2014; Yang et al., 2014). Out of the Twenty-five, seven articles were pooled analysis, commentary and review papers (Wang et al., 2012; Yang and Burwinkel, 2012; Ma et al., 2013; Wang et al., 2013; Xu et al., 2013b; Zhong et al., 2013; Chen et al., 2014), and four reports were cancer biology experimental studies (Hirota et al., 2012; Jahid et al., 2012; Zanetti et al., 2012; Yuan et al., 2013). Three studies were excluded given that they did not include controls (Yoon et al., 2012; Xu et al., 2013a), or reported non-cancer disease (Song et al., 2013). We also included five eligible articles by manual searching (Li, 2011; Zhang, 2011; Zhang, 2012; Xu et al., 2013b; Kupcinskas et al., 2014), in which the study by Li et al. included two independent case-control studies (Li, 2011) and the pooled analysis by Xu et al. included one unpublished paper with efficient case-control study data (Xu et al., 2013b). As a result, a total of seventeen studies

met the inclusion criteria and were identified as eligible studies (Figure 1).

Totally, 7,813 cases and 9,602 controls were included from seventeen studies, including five studies for breast cancer with 3,099 cases and 3,697 controls (Yang et al., 2010; Zhang, 2011; Catucci et al., 2012; Zhang et al., 2012; Zhang et al., 2013), five for gastric cancer with 1,776 cases and 2,350 controls (Sun et al., 2010; Zhou et al., 2012; Xu et al., 2013b; Kupcinskas et al., 2014; Yang et al., 2014), two for colorectal cancer with 660 cases and 680 controls (Hezova et al., 2012; Zhang, 2012), and one for esophageal cancer (Wei et al., 2013), live cancer (Li, 2011), nasopharyngeal cancer (Li, 2011), renal cancer (Shi et al., 2012) or cervical cancer (Xiong et al., 2014). For ethnic distribution, in addition to four studies on Caucasian descent, including two for breast cancer (Yang et al., 2010; Catucci et al., 2012), one for gastric cancer (Kupcinskas et al., 2014) and one for colorectal cancer (Hezova et al., 2012), there were thirteen studies of Asian origin. For the study design, the sources of controls from six studies were population-based, and the others were hospital-based. The genotype frequencies in the control group for each included study were consistent with Hardy-Weinberg equilibrium. The selected study characteristics were listed in Table 1.

Quantitative synthesis

Table 2 presents the results of the meta-analysis for all cancers. By pooling all the studies, the miR-27a polymorphism (rs895819) was not associated with a cancer risk in different models (Figure 2). In the subgroup analyses, we found that rs895819 polymorphism heterozygote (AG) was significantly correlated with reduced cancer risk in Caucasian (AG *vs* AA: OR, 0.83; 95%CI, 0.75-0.93), and this association was further confirmed in dominant model (AG+GG *vs* AA: OR, 0.84; 95%CI, 0.76-0.93) (Table 2). In addition, the [G] allele of rs895819 was associated with cancer risk in Caucasian ([G] *vs* [A]: OR, 0.90; 95%CI, 0.84-0.97). However, no association was found between rs895819 and cancer risk in Asian (Table 2). In stratified analysis by the sources of controls, we found that the rs895819 was not significantly correlated with cancer risk in any model from either population-based or hospital-based case-control studies.

When stratified by cancer types, the [G] allele of rs895819 was associated with reduced breast cancer risk ([G] *vs* [A]: OR, 0.91; 95%CI, 0.85-0.98) although no association was found in other four models. For cancers from digestive system, we did not find significant association of rs895819 with risk of all cancers from esophageal, stomach, colorectal and liver. For other cancers, the [G] allele was statistically associated with decreased risks of renal cell cancer and nasopharyngeal cancer compared with the A allele. Nevertheless, the comparison of [G] versus [A] in cervical cancer showed an opposite result. Since only one study was conducted in renal cell cancer, nasopharyngeal cancer and cervical cancer, we did not perform more analyses or pool these studies.

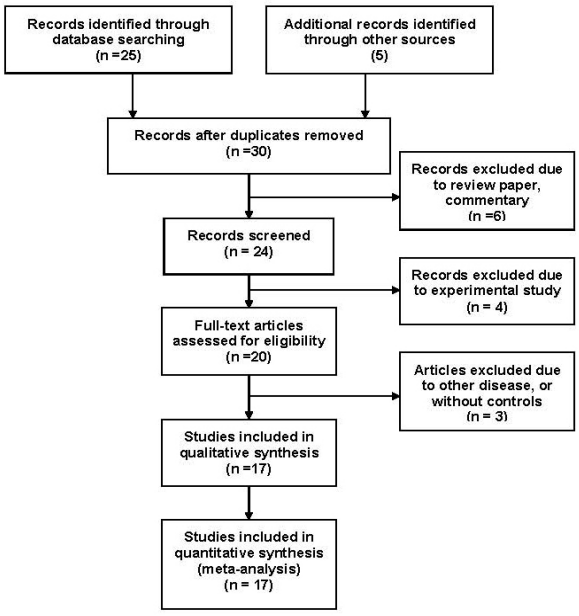

Figure 1. Flow Diagram of Studies Identification

Table 1. Characteristics of Literatures Included in the Meta-analysis

Author	Year	Origin	Ethnicity	Cancer type	Sample size (case/control)	HWE	MAF	Design	Genotyping method
Yang R	2010	Germany	Caucasian	Breast cancer	1189/1416	0.142	0.34	PB	DNA Sequencing
Zhang P	2011	China	Chinese	Breast cancer	376/190	0.605	0.26	PB	MassARRAY
Catucci I	2012	Italy	Caucasian	Breast cancer	1025/1593	0.051	0.3	PB	Taqman PCR
Zhang M	2012	China	Asian	Breast cancer	245/243	0.122	0.47	PB	PCR-RFLP
Zhang N	2013	China	Asian	Breast cancer	264/255	0.446	0.26	HB	Taqman PCR
Sun Q	2010	China	Asian	Gastric cancer	304/304	0.053	0.33	HB	PCR-RFLP
Zhou Y	2012	China	Asian	Gastric cancer	295/413	0.941	0.28	HB	MALDI-TOF
Xu Q	2013	China	Asian	Gastric cancer	222/305	0.437	0.25	HB	DNA Sequencing
Yang Q	2014	China	Asian	Gastric cancer	592/978	0.517	0.38	PB	Taqman PCR
Kupcinskas J	2014	Latvia	Caucasian	Gastric cancer	363/350	0.151	0.32	HB	Taqman PCR
Zhang M	2012	China	Asian	Colorectal Cancer	463/468	0.351	0.25	PB	PCR-RFLP
Hezova R	2012	Czech	Caucasian	Colorectal cancer	197/212	0.867	0.34	HB	Taqman PCR
Wei J	2013	China	Chinese	Esophageal cancer	379/377	0.322	0.26	HB	MALDI-TOF MS
Li P	2011	China	Chinese	Liver Cancer	401/459	0.751	0.29	HB	SNP stream
Li P	2011	China	Chinese	Nasopharyngeal Cancer	801/1022	0.658	0.3	HB	SNP stream
Shi D	2012	China	Asian	Renal cell cancer	594/600	0.373	0.3	HB	Taqman PCR
Xiong X	2014	China	Asian	Cervical cancer	103/417	0.255	0.26	HB	DNA Sequencing

Abbreviations: HWE, Hardy-Weinberg equilibrium; MAF, minor allele frequency; HB, Hospital based controls; PB, population based controls; PCR, Polymerase chain reaction; RFLP, Restriction fragment length polymorphism; MALDI-TOF, Matrix-assisted laser desorption/ ionization- time of flight

Table 2. Stratified Analysis of the Association between miR-27a Polymorphism and Cancer Risk

Groups	n[a]	AG *vs* AA		GG *vs* AA		AG + GG *vs* AA		GG *vs* AA + AG		G allele *vs* A allele	
		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]
All	17	0.93 (0.78-1.11)	<0.001	0.93 (0.79-1.10)	0.007	0.93 (0.80-1.08)	<0.001	0.98 (0.82-1.16)	0.002	0.95 (0.87-1.04)	<0.001
Ethnic											
Asian	13	0.96 (0.75-1.24)	<0.001	0.94 (0.74-1.19)	0.001	0.97 (0.78-1.20)	<0.001	0.95 (0.75-1.21)	<0.001	0.97 (0.85-1.10)	<0.001
Caucasian	4	0.83 (0.75-0.93)	0.478	0.89 (0.75-1.06)	0.847	0.84 (0.76-0.93)	0.669	0.98 (0.83-1.15)	0.68	0.90 (0.84-0.97)	0.905
Source of controls											
PB	6	0.87 (0.58-1.31)	<0.001	0.89 (0.77-1.02)	0.691	0.89 (0.65-1.22)	<0.001	1.00 (0.82-1.22)	0.095	0.92 (0.79-1.06)	0.001
HB	11	0.96 (0.83-1.10)	0.011	0.93 (0.69-1.25)	0.001	0.96 (0.83-1.10)	0.005	0.95 (0.72-1.25)	0.002	0.97 (0.87-1.09)	0.002
Cancer types											
Breast cancer	5	0.97 (0.78-1.20)	0.01	0.89 (0.75-1.05)	0.94	0.94 (0.79-1.11)	0.063	0.91 (0.77-1.06)	0.446	0.91 (0.85-0.98)	0.613
Cancers from digestive system	9	0.93 (0.65-1.33)	<0.001	0.90 (0.67-1.20)	0.008	0.94 (0.71-1.26)	<0.001	0.95 (0.72-1.26)	0.006	0.96 (0.82-1.13)	<0.001
Others											
Renal cell cancer	1	0.70 (0.55-0.89)		0.81 (0.53-1.24)		0.72 (0.57-0.90)		0.95 (0.62-1.43)		0.81 (0.67-0.97)	
Nasopharyngeal cancer	1	0.79 (0.65-0.96)		0.77 (0.54-1.01)		0.79 (0.66-0.95)		0.85 (0.60-1.20)		0.84 (0.72-0.97)	
Cervical cancer	1[c]	1.09 (0.69-1.74)		2.90 (1.42-5.94)		1.32 (0.85-2.03)		2.79 (1.41-5.54)		1.45 (1.05-2.02)	

[a] Number of comparisons; [b] *p* value of Q-test for heterogeneity analysis; [c] The crude OR and 95% CI were calculated based on the genotype frequencies.

Table 3. Stratified Analysis of the Association between miR-27a Polymorphism and Breast Cancer Risk

Groups	n[a]	AG *vs* AA		GG *vs* AA		AG + GG *vs* AA		GG *vs* AA + AG		G allele *vs* A allele	
		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]
All	5	0.97 (0.78-1.20)	0.01	0.89 (0.75-1.05)	0.94	0.94 (0.79-1.11)	0.063	0.91 (0.77-1.06)	0.446	0.91 (0.85-0.98)	0.613
Ethnic											
Asian	3	1.16 (0.80-1.68)	0.057	0.97 (0.68-1.39)	0.804	1.09 (0.88-1.34)	0.191	0.80 (0.58-1.10)	0.291	1.00 (0.85-1.16)	0.584
Caucasian	2	0.83 (0.74-0.94)	0.204	0.86 (0.71-1.05)	0.804	0.84 (0.75-0.94)	0.319	0.94 (0.79-1.13)	0.493	0.89 (0.82-0.97)	0.717
Source of controls											
PB	4	1.01 (0.78-1.30)	0.004	0.88 (0.74-1.05)	0.865	0.96 (0.79-1.18)	0.031	0.90 (0.76-1.06)	0.309	0.92 (0.85-0.99)	0.444
HB	1[c]	0.84 (0.59-1.21)		0.96 (0.46-2.02)		0.86 (0.60-1.21)		1.03 (0.50-2.13)		0.91 (0.69-1.20)	
Age											
< 50-year	4	0.87 (0.61-1.26)	0.001	0.85 (0.69-1.06)	0.912	0.84 (0.63-1.13)	0.011	0.90 (0.73-1.11)	0.46	0.87 (0.79-0.96)	0.274
≥ 50-year	4	0.99 (0.83-1.19)	0.591	0.87 (0.64-1.18)	0.441	0.97 (0.82-1.15)	0.557	0.86 (0.65-1.14)	0.382	0.95 (0.84-1.08)	0.453
Cancer locations											
Bilateral	2	0.86 (0.52-1.42)	0.066	0.85 (0.35-2.04)	0.062	0.86 (0.49-1.51)	0.027	0.95 (0.62-1.45)	0.158	0.90 (0.56-1.43)	0.02
Unilateral	4	0.93 (0.74-1.18)	0.016	0.86 (0.72-1.04)	0.852	0.86 (0.78-0.96)	0.12	0.89 (0.75-1.06)	0.235	0.90 (0.83-0.97)	0.892

[a] Number of comparisons; [b] *p* value of Q-test for heterogeneity analysis; [c] The crude OR and 95% CI were calculated based on the genotype frequencies.

Table 4. Stratified Analysis of the Association between miR-27a Polymorphism and Risk of Cancers from Digestive System

Groups	n[a]	AG *vs* AA		GG *vs* AA		AG + GG *vs* AA		GG *vs* AA + AG		G allele *vs* A allele	
		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]
All	9	0.93 (0.65-1.33)	<0.001	0.90 (0.67-1.20)	0.008	0.94 (0.71-1.26)	<0.001	0.95 (0.72-1.26)	0.006	0.96 (0.82-1.13)	<0.001
Cancers from digestive tracts	8	0.91 (0.61-1.35)	<0.001	0.86 (0.62-1.20)	0.005	0.92 (0.67-1.27)	<0.001	0.92 (0.67-1.28)	0.003	0.95 (0.79-1.13)	<0.001
UADT cancer	6	0.86 (0.52-1.43)	<0.001	0.78 (0.51-1.19)	0.003	0.88 (0.59-1.31)	<0.001	0.84 (0.54-1.31)	0.001	0.91 (0.74-1.13)	<0.001
Gastric cancer	5	0.84 (0.45-1.55)	<0.001	0.80 (0.48-1.32)	0.002	0.87 (0.54-1.41)	<0.001	0.89 (0.54-1.44)	0.001	0.92 (0.71-1.19)	<0.001
Corectal cancer	2	1.10 (0.88-1.38)	0.455	1.14 (0.77-1.69)	0.533	1.11 (0.90-1.38)	0.403	1.10(0.75-1.61)	0.651	1.09 (0.92-1.29)	0.384
Liver cancer	1[c]	1.15 (0.87-1.52)		1.12 (0.68-1.87)		1.14 (0.88-1.50)		1.05 (0.64-1.72)		1.09 (0.89-1.35)	

[a]Number of comparisons; [b]*P* value of Q-test for heterogeneity analysis; [c]The crude OR and 95% CI were calculated based on the genotype frequencies; Abbreviations: UADT, Upper aerodigestive tract

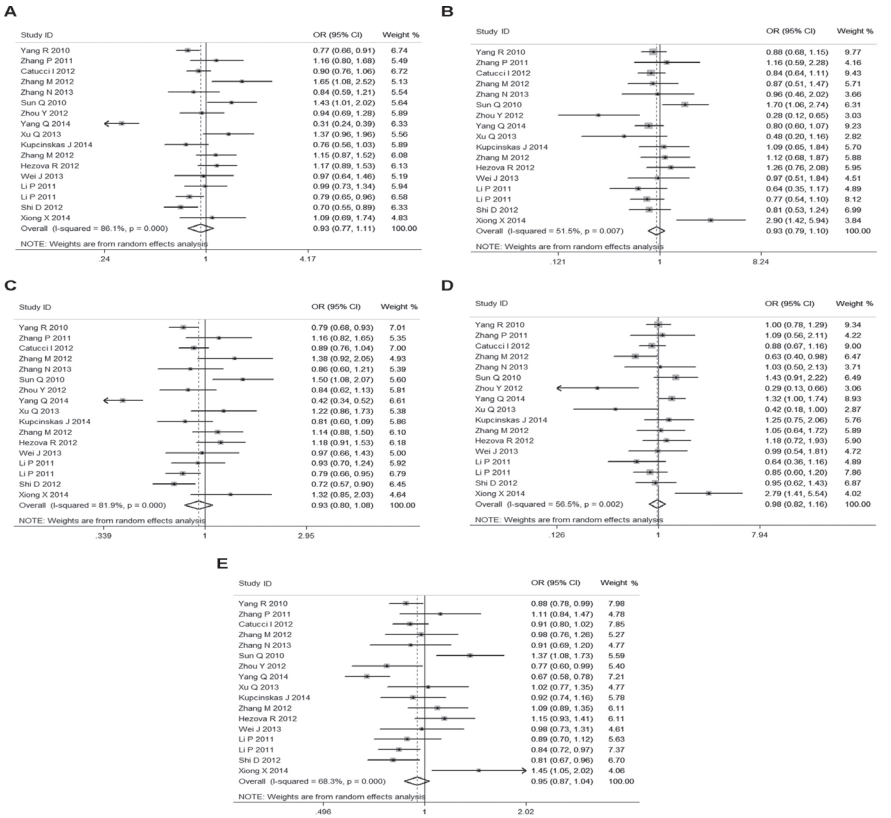

Figure 2. Forest Plots of Different Models for Meta-analysis on the Association of rs895819 with Cancer Risk. Heterozygote **(A)**, homozygote **(B)**, dominant **(C)**, recessive **(D)** and additive models **(E)** are presented. The squares and horizontal lines correspond to OR and 95% CI of specific study, and the area of squares reflects study weight (inverse of the variance). The diamond represents the pooled OR and its 95%CI

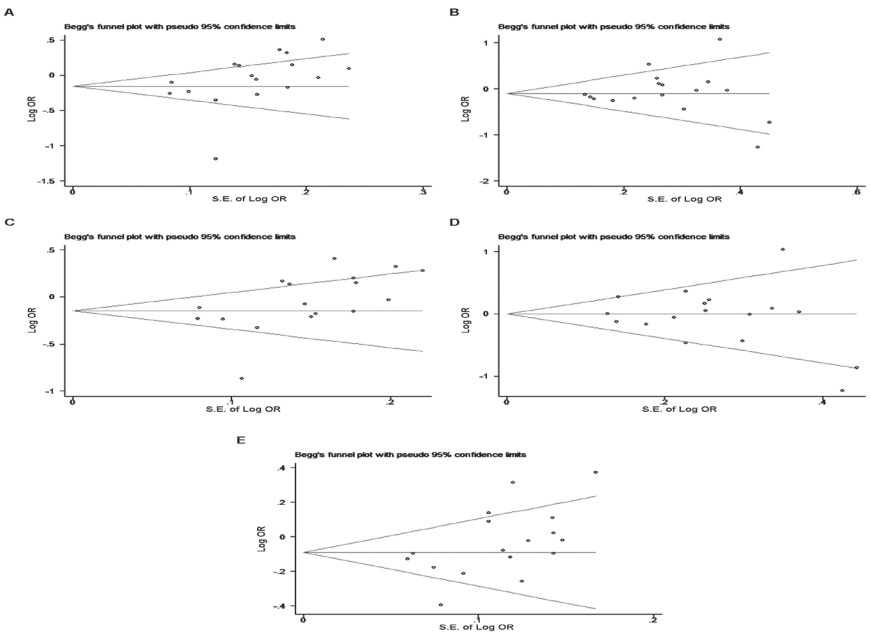

Figure 3. Funnel Plots Showed Symmetric or Asymmetric Distribution. Log OR was plotted against the standard error of log OR for studies on rs895819 in heterozygous **(A)**, homozygous **(B)**, dominant **(C)**, recessive **(D)** or additive model **(E)**. The dots represent specific studies for the indicated association

Next, we performed subgroup analyses for the breast cancer. We observed that the AG heterozygote (AG *vs* AA: OR, 0.83; 95%CI, 0.71-0.94) and AG/GG genotypes (AG+GG *vs* AA: OR, 0.84; 95%CI, 0.75-0.94) were associated with decreased risk of breast cancer when compared with the reference AA genotype in Caucasian, furthermore, the [G] allele was statistically associated with decreased

risk of breast cancer compared with the A allele ([G] *vs* [A]: OR, 0.89; 95%CI, 0.82-0.97) (Table 3). However, no association was found between rs895819 and breast cancer risk in Asian. For the source of control, [G] allele was statistically associated with decreased risk of breast cancer compared with the A allele ([G] *vs* [A]: OR, 0.92; 95%CI, 0.85-0.99). When the age was stratified as < 50-year old and ≥ 50-year, significant association was found between [G] allele of rs895819 and reduced breast cancer risk in younger ([G] *vs* [A]: OR, 0.87; 95%CI, 0.79-0.96) but not elder population. Interestingly, the [G] allele of rs895819 was associated with reduced breast cancer risk in the unilateral ([G] *vs* [A]: OR, 0.90; 95%CI, 0.83-0.97) but not bilateral group.

For cancers from digestive system, we did not find significant association between rs895819 and risk of cancers from digestive tracts when pooling studies on esophageal, gastric and colorectal cancers (Table 4). No association was found between rs895819 and risk of upper aero digestive cancers when pooling studies on esophageal and gastric cancers, or colorectal cancers. In addition, rs895819 was not associated with gastric cancer risk. We also conducted subgroup analyses for the cancers from digestive system, digestive tracts, upper aero digestive tracts or stomach, and the results showed no association between rs895819 and risk of cancers related to digestive system, when stratified by ethnicity, or the source of controls (Data not shown).

Publication bias

Begg's funnel plot and Egger's test were performed to assess the publication bias of literatures (Figure 3). The shape of the funnel plots for dominant model revealed asymmetry with a P value of 0.036, and the Egger's test showed a P value of 0.041, suggesting a publication bias. For additive model, although the funnel plots showed symmetric distribution with a P value of 0.077, the Egger's test indicated a publication bias with a P value of 0.040. These results suggest publication bias of this study.

Sensitivity analysis was conducted by deleting each study in turn from the pooled analysis to examine the influence of the removed data set to the overall ORs. Exclusion of each study did not influence the result in specific genotype comparison for rs895819, suggesting that the results of synthetic analysis were robust (Supplementary material).

Discussion

In the present study, we collected all available, published studies and performed a meta-analysis to examine the association between the miR-27a polymorphism and susceptibility to cancer. Seventeen studies were critically reviewed to clarify controversial results from previous reports. Our data showed that significantly reduced cancer risks were associated with [G] allele of rs895819 in Caucasian but not Asian subjects, and in breast cancer but not other types of cancers.

Previous meta-analysis by Wang et al. showed that significantly increased cancer risks were associated with rs895819 AG polymorphism heterozygote and dominant model in Asian based on two studies (549 cases and 547 controls) (Wang et al., 2012). By including one (Zhong et al., 2013), or two more studies (Wang et al., 2013), however, the later pooled analyses did not find this association in Asian. Recent meta-analysis based on nine studies showed no association between rs895819 and cancer risk in Asian (4,062 cases and 4,344 controls) (Xu et al., 2013b). In our meta-analysis, we collected thirteen studies on Asian subjects and confirmed that rs895819 was not associated with cancer risk in Asian (5,039 cases and 6,039 controls). For the Caucasian subjects, by pooling all four studies, we found a significant association between the [G] allele of rs895819 and reduced cancer risk, which was consistent with recent meta-analysis based on three studies (Xu et al., 2013b). Thus, [G] allele of rs895819 was associated with reduced cancer risk in Caucasian but not Asian subjects.

When stratified by the cancer type, our data was consistent with previous meta-analysis result that rs895819 was not associated with breast cancer risk in all four models while the [G] allele of rs895819 was associated with decreased risk in breast cancer (Xu et al., 2013b). Yang et al. for the first time reported that the protective effect of the [G] allele of rs895819 was mainly in the younger age group (<50-year of age) ([G] *vs* [A]: OR, 0.83; 95%CI, 0.70-0.98) (Yang et al., 2010). However, in the study by Catucci et al., the age stratification (<50-year and ≥50-year) did not show this correlation (Catucci et al., 2012). When pooling four studies, we found protective effect of [G] allele of rs895819 in the younger age group. Previous study indicated a protective effect of rs895819 [G] allele in the subgroup of bilateral breast cancer cases (Yang et al., 2010), but this association could not be confirmed by later study (Catucci et al., 2012), or pooled analysis on these two studies (Table 3). Interestingly, a protective effect of rs895819 [G] allele was seen in the subgroup of unilateral breast cancer cases in the present meta-analysis. These conflicting outcomes might be due to under-powered sample size of each study, and warrant the analysis of large cohorts to clearly establish the impact of rs895819 on breast cancer risk since the studies included in our pooled analysis were still limited.

The major limitation of this study is the heterogeneity for the rs895819 among these studies on different cancers, and different ethnic populations with same cancer (Table 2). The heterogeneity may be due to various factors, such as genetic susceptibility to different cancers, diversity in the population characteristics, differences in the number of cases and controls, genotyping methods and study design. Between-study heterogeneity was detected by restricted maximum likelihood-based random-effects meta-regression analysis. To eliminate heterogeneity, we carried out subgroup analysis and used a random-effects model to pool the results when the significant heterogeneity was present. In addition, our data suggest publication bias. Some unpublished eligible publications or papers in non-English or non-Chinese language were not available in the present meta-analysis, which might affect the results.

The SNP rs895819 is located at the loop of pre-miR-27a and involves an A>G nucleotide transition. Sun et

al. reported that this polymorphism could lead to process variation, increased serum miR-27a level and eventually predisposition of gastric cancer (Sun et al., 2010). But other study proposed that the [G] allele of rs895819 might impair the maturation of the miR-27a, thus, was associated with reduced familial breast cancer risk (Yang et al., 2010). The variation causing structural change in the crucial region of pre-miRNA could affect the maturation and the process of miRNA (Zeng and Cullen, 2003; Jazdzewski et al., 2008), e.g., impaired miRNA maturation process in the shortened loop of pre-miRNA by mutation or deletions (Zeng et al., 2005). However, the processing and maturation of miRNA is more complex and we currently do not know that the A>G transition of rs895819 in the center of the terminal loop affects structure of pre-miR-27a. The role of rs895819 in cancer development needs further investigations.

In conclusion, we found significant associations between the rs895819 and reduced cancer risk in Caucasian but not Asian. Protective effect of rs895819 [G] allele was seen in the younger age group, and in the subgroup of unilateral breast cancer cases. However, the number of studies included for our meta-analysis is still limited, and further studies based on larger well-designed populations are required to clarify the different effects of rs895819 on breast cancer risk with different stratification, e.g., age and cancer site.

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