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Meta-analysis confirms that a common G/C variant in the *pre-miR-146a* gene contributes to cancer susceptibility and that ethnicity, gender and smoking status are risk factors

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ABSTRACT. Evidence has shown that miR-146a is involved in carcinogenesis, and a common G/C variant (rs2910164) in the *pre-miR-146a* gene has been associated with various types of cancer. We summarized the data from 22 published case-control studies on the association between rs2910164 and cancer risk and performed subgroup analyses by ethnicity, gender and smoking status. We found a significant association between the *pre-miR-146a* polymorphism and cancer risk in Caucasian populations (odds ratio (OR) = 0.93, 95%

confidence interval (CI) = 0.88-0.99 for G- *vs* C-allele), while the significance was borderline in Asian populations (OR = 1.11, 95%CI = 1.00-1.23 for G- *vs* C-allele). A significantly increased risk of cancer was found in males with GG/GC genotypes (OR = 1.23, 95%CI = 1.10-1.37), and the significance was more pronounced in smokers (OR = 1.82, 95%CI = 1.32-2.51) than in non-smokers (OR = 1.24, 95%CI = 1.01-1.53). We conclude that there is evidence that the *pre-miR-146a* polymorphism contributes to cancer susceptibilities and that gender and smoking status affect the probability of cancer in individuals with this polymorphism.

Key words: miR-146a; Polymorphism; Cancer susceptibility; Gender; Smoking

INTRODUCTION

MicroRNAs (miRNAs) represent a class of small RNA that were first described in *Caenorhabditis elegans* in 1993. They are conserved, small (about 17-27 nucleotides in length), endogenous, non-coding molecules that negatively regulate gene expression by suppressing translation or degradation of mRNAs (Bartel, 2004). It is estimated that there are about 1000 miRNAs in the human genome and that they regulate nearly 30% of human genes by non-stringent binding to mRNA 3'-untranslated regions (Bentwich et al., 2005). Previous studies have documented that miRNAs affect not only biologic processes, such as metabolism, proliferation, tissue differentiation, maintenance of cell identity, apoptosis, cell signal regulation, organ development, and aging, but also tumorigenesis (Ambros, 2004).

In recent times, miR-146a, an miRNA important to the negative regulation of acute responses during the activation of the innate immune system, has attracted considerable attention (Taganov et al., 2006). Further, up- or downregulation of miR-146a is observed in human disorders, such as inflammatory diseases (Perry et al., 2008) and cancers (Reis et al., 2010). Evidence has shown that miR-146a can directly inhibit the expression of IRAK1 and TRAF6, impair nuclear factor (NF)-κB activity, and suppress the expression of NF-κB-target genes, such as *IL-6*, *IL-8*, *IL-1β*, and *TNF-α* (Bhaumik et al., 2008).

However, the expression levels of miR-146a vary with the cancer type. In papillary thyroid carcinoma (Pallante et al., 2006) and cervical cancer (Wang et al., 2008), the levels are increased, but reduced in prostate cancer (Volinia et al., 2006). Moreover, reports indicate that changes in miR-146a expression observed in these solid tumors are probably related to a common G/C polymorphism located in the stem-loop of *pre-miR-146a* (rs2910164) (Xu et al., 2010). However, association studies with small sample sizes lack statistical power and may produce conflicting conclusions. During the preparation of this manuscript, several meta-analyses concerning rs2910164 polymorphism and cancer risk were published (Gao et al., 2011; Qiu et al., 2011; Xu et al., 2011; Wang et al., 2012). However, these studies have not addressed the interaction of compounding factors. Moreover, several well-designed studies were published after their studies. Hence, we updated the meta-analysis in the light of the newly published studies and took into consideration interactions of possible compounding factors to generate more valuable results.

MATERIAL AND METHODS

Identification and selection of relevant studies

Studies were identified by searching the PubMed and Embase databases for reports published up to October 10, 2011, by using the following key words: "*pre-miR-146a*", "*microRNA-146a*", "*miR-146a*", "polymorphism", "variant", or "rs2910164" along with "cancer", "neoplasm", or "carcinoma". All studies matching the eligible criteria were retrieved. Additional studies were identified by a hand search of the references of original studies. Eligibility of papers for the present study was defined by the following criteria: i) an unrelated case-control study and ii) availability of genotype frequency. Major reasons for the exclusion of studies were i) no control population, ii) no available genotype frequency, and iii) duplication of previous publication.

Data extraction

Two investigators extracted the information independently according to the inclusion criteria listed above. The following information was extracted from each study: first author's last name, year of publication, country of origin, ethnicity, cancer type, and frequencies of genotypes in cases and controls. For studies that included individuals from different countries, data were extracted separately. In gene-environment interaction analyses, since some studies presented data only for GG/GC and CC, we calculated the odds ratios (ORs) for GG/GC *vs* CC according to the smoking status and gender.

Statistical analysis

Crude ORs with 95% confidence intervals (CIs) were calculated to confirm the strength of the association between the *pre-miR-146a* polymorphism and cancer susceptibility. Stratified analyses were also performed by cancer type, ethnicity, gender, and smoking status.

A chi-square-based *Q*-test was performed to check the heterogeneity. A P value of over 0.05 was considered to suggest a lack of heterogeneity among the studies. Thus, the combined OR was calculated by the fixed-effects model (the Mantel-Haenszel method) or the random-effects model (the DerSimonian and Laird method). Publication bias was assessed using inverted funnel plots. Funnel plot asymmetry was assessed using the Egger linear regression test ($P < 0.05$ was considered representative of statistically significant publication bias). Hardy-Weinberg equilibrium (HWE) was tested using the goodness-of-fit chi-square test. All statistical analyses were performed with the Stata software (version 10.0; StataCorp LP, College Station, TX, USA).

RESULTS

Selection of studies

Characteristics of eligible studies are presented in Supplementary Table 1. Four hundred and sixty-five papers were retrieved by using the search terms (PubMed: 195 and Embase:

270). The study selection process is shown in Figure 1. Finally, 22 articles (Jazdzewski et al., 2008; Xu et al., 2008; Hoffman et al., 2009; Hu et al., 2009; Tian et al., 2009; Catucci et al., 2010; Guo et al., 2010; Liu et al., 2010; Okubo et al., 2010; Pastrello et al., 2010; Srivastava et al., 2010; Xu et al., 2010; Zeng et al., 2010; Garcia et al., 2011; George et al., 2011; Akkiz et al., 2011; Hishida et al., 2011; Mittal et al., 2011; Permuth-Wey et al., 2011; Yue et al., 2011; Zhou et al., 2011; Zhou et al., 2012) with 11,901 cases and 14,200 controls were included in the present meta-analysis. An article by Catucci et al. (2010) reported a case-control study of a German population and an Italian population separately, and one by Jazdzewski et al. (2008) reported studies on populations of Finland, Poland, and USA separately. Pastrello et al. (2010) explored *pre-miR-146a* polymorphism in both breast and ovarian cancer risk with the same control group; therefore, the investigation was considered as a single study and was categorized into the "other cancer type". In all, 22 articles (25 studies) were finally included in our analysis. The articles cited 11 studies on Caucasian populations and 14 studies on Asian populations. As shown in Figure 2, the G-allele of rs2910164 had a higher representation in cases and controls of Caucasian populations (75.07 and 76.58%, respectively) than in those of Asian populations (50.42 and 47.88%, respectively). The distribution of all the genotypes in the controls was consistent with the HWE in all studies, except for 3 studies (Catucci et al., 2010; George et al., 2011; Mittal et al., 2011).

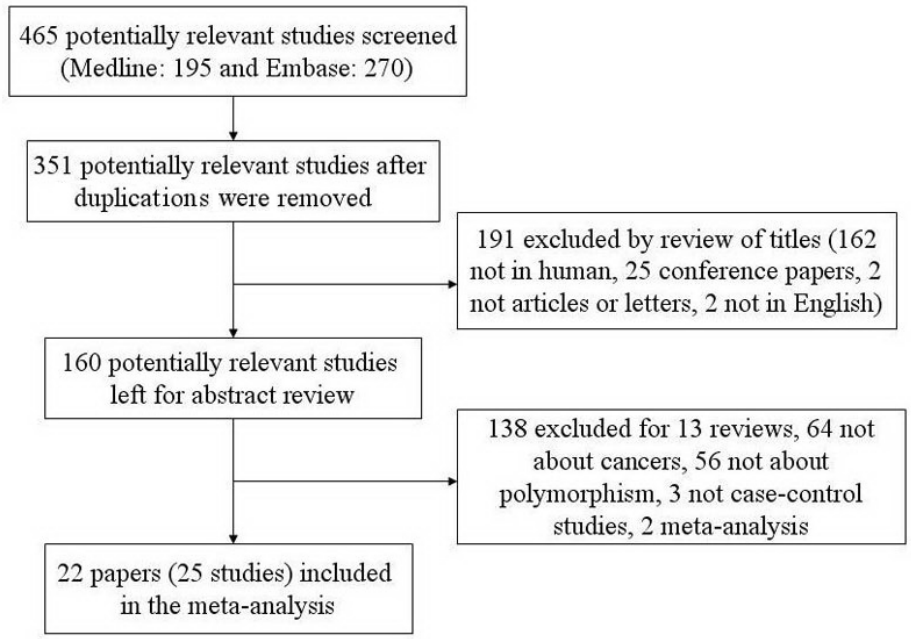

Figure 1. Studies identified with inclusion and exclusion criteria.

Several studies collected information on possible confounding factors, such as smoking status (Guo et al., 2010; Xu et al., 2010; Zeng et al., 2010) and gender (Xu et al., 2008; Zeng et al., 2010; Akkiz et al., 2011), and the gene-environment interaction effect was analyzed in subgroups.

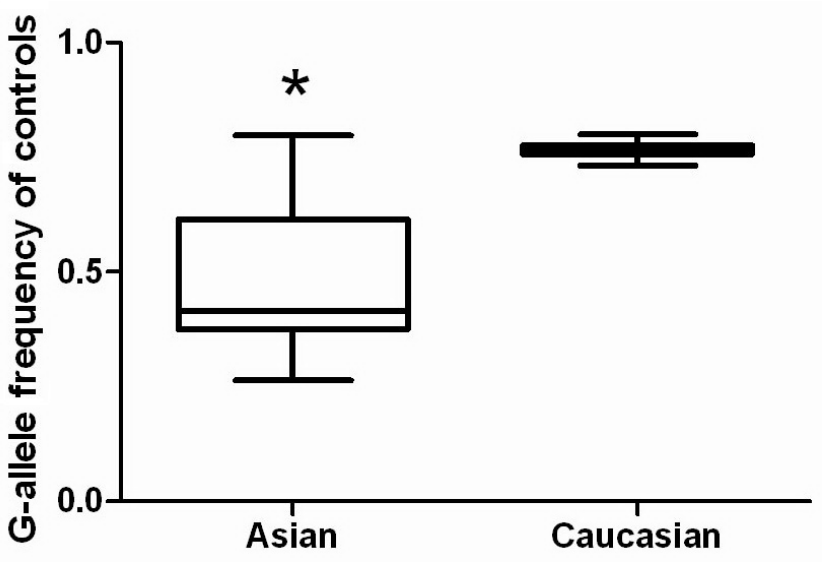

Figure 2. Frequencies of the G-allele among controls stratified by ethnicity. *P < 0.001.

Main results

As shown in Table 1 and Figure 3A, when comparing the G-allele *vs* C-allele, a reduced effect of the *pre-miR-146a* polymorphism was found in the Caucasian population (OR = 0.93, 95%CI = 0.88-0.99), while a borderline risk effect was observed in the Asian population (OR = 1.11, 95%CI = 1.00-1.23).

Table 1. Stratified analyses of the miR-146a polymorphism and cancer susceptibility.

Variables	N	Cases/controls	G-allele *vs* C-allele		GG/GC *vs* CC	
			OR (95%CI)	P§/P†	OR (95%CI)	P§/P†
Total	25	11901/14200	1.03 (0.96-1.10)‡	0.474/<0.001	1.12 (0.96-1.31)‡	0.420/0.010
Cancer types						
Breast cancer	5	4137/4314	0.96 (0.90-1.03)	0.299/0.691	0.89 (0.76-1.05)	0.162/0.336
Gastric cancer	3	1439/2638	1.00 (0.78-1.27)‡	0.980/0.003	1.03 (0.73-1.44)‡	0.884/0.003
Papillary thyroid cancer	3	608/901	0.92 (0.77-1.10)	0.353/0.332	2.55 (1.28-5.06)	0.008/0.283
Prostate cancer	2	410/510	1.23 (0.96-1.59)	0.109/0.200	1.32 (0.94-1.86)	0.111/0.235
Cervical cancer	2	673/752	1.39 (1.20-1.61)	<0.001/0.796	1.53 (1.22-1.92)	<0.001/0.359
Liver cancer	3	887/1209	1.08 (0.89-1.32)	0.440/0.128	1.09 (0.81-1.48)	0.565/0.186
Others	7	3747/3876	0.99 (0.87-1.14)‡	0.930/0.007	0.89 (0.62-1.27)‡	0.523/0.006
Ethnicities						
Caucasian	11	5761/6243	0.93 (0.88-0.99)	0.024/0.796	0.93 (0.71-1.21)‡	0.575/0.010
Asian	14	6140/7957	1.11 (1.00-1.23)‡	0.051/<0.001	1.14 (0.98-1.33)‡	0.094/<0.001
Gender						
Female	11	5401/5071	-		1.02 (0.83-1.25)‡	0.848/0.014
Male	5	1360/1249	-		1.37 (1.15-1.64)	<0.001/0.766
Smoking status						
Smoker	3	520/457	-		1.82 (1.32-2.51)	<0.001/0.153
Non-smoker	3	479/592	-		1.24 (1.01-1.53)	0.042/0.792

N = number of comparisons; §P value of the *Z*-test; †P value of the *Q*-test for heterogeneity test; ‡random-effects model was used when P value for heterogeneity test was <0.05; otherwise, fixed-effects model was used.

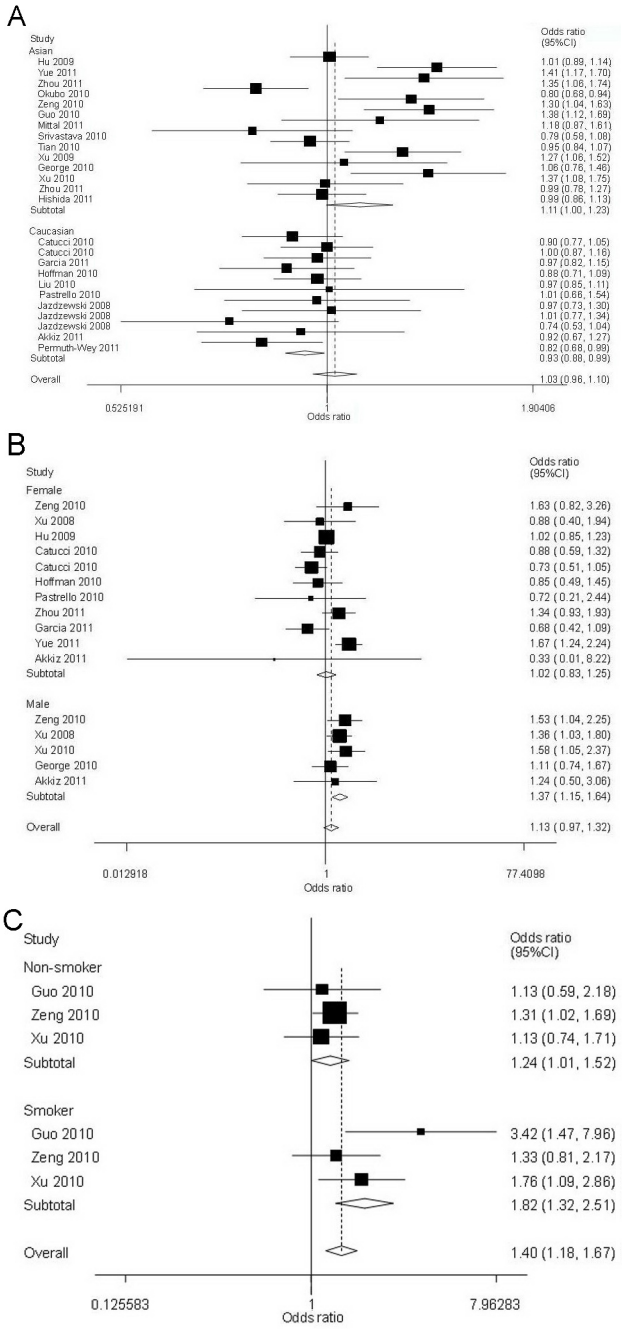

Figure 3. Forest plot of cancer risk associated with miR-146a rs2910164 polymorphism (G-allele *vs* C-allele) in the stratified analyses by ethnicity (**A**), gender (**B**) and smoking status (**C**). The squares and horizontal lines correspond to the study-specific odds ratio (ORs) and 95% confidence interval (95%CI). The area of the squares reflects the weight (inverse of the variance). The diamond represents the summary OR and 95%CI.

Moreover, the GG and GC genotypes showed a higher risk effect on papillary thyroid cancer and cervical cancer than the CC genotype (OR = 2.55, 95%CI = 1.28-5.06 and OR = 1.53, 95%CI = 1.22-1.92, respectively, also shown in Table 1). Then, we divided these studies into 2 subgroups according to gender and smoking status. Interestingly, a significant association between rs2910164 and cancer risk was observed among male patients (OR = 1.37, 95%CI = 1.15-1.64 for GG/GC *vs* CC), but not among female patients (OR = 1.02, 95%CI = 0.83-1.25 for GG/GC *vs* CC; Figure 3B). Moreover, the significance of the association was more pronounced in smokers (OR = 1.82, 95%CI = 1.32-2.51 for GG/GC *vs* CC) than in non-smokers (OR = 1.24, 95%CI = 1.01-1.53 for GG/GC *vs* CC; Figure 3C).

Sensitivity analysis was further conducted to ascertain whether modification of the inclusion criteria affected the final results. However, no individual study affected the overall OR since omission of any study made no material difference (Figure 4).

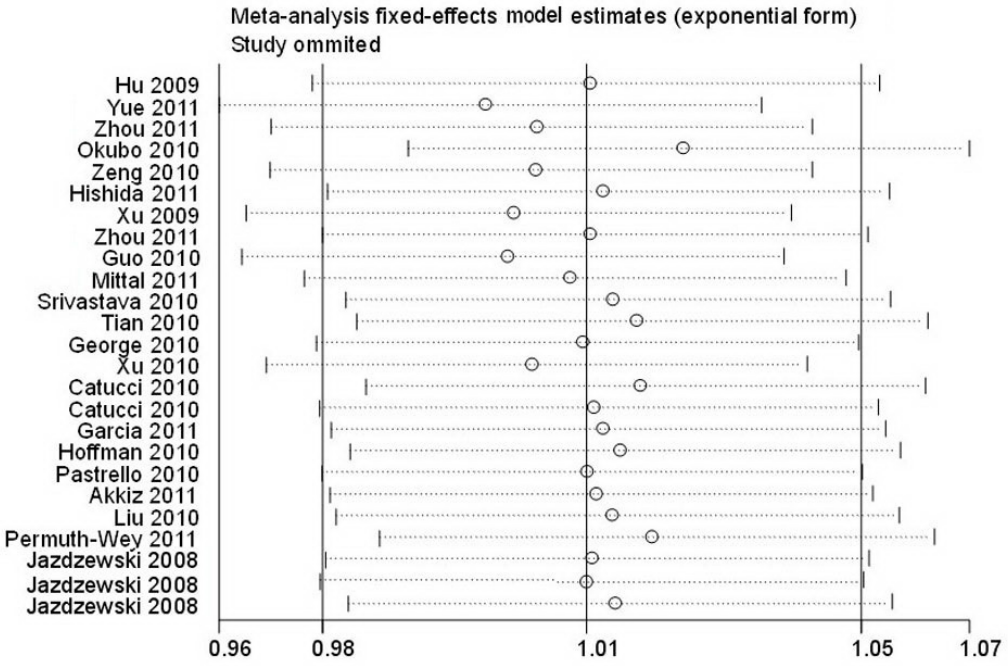

Figure 4. Sensitivity analysis conducted to assess the influence of each study on the pooled odd ratio (OR) by omission of individual studies.

Publication bias

Funnel plots and the Egger test (Figure 5) were used to check for publication bias. The assessment revealed that there was no evident publication bias (t = 0.69, P = 0.494 for G-allele *vs* C-allele).

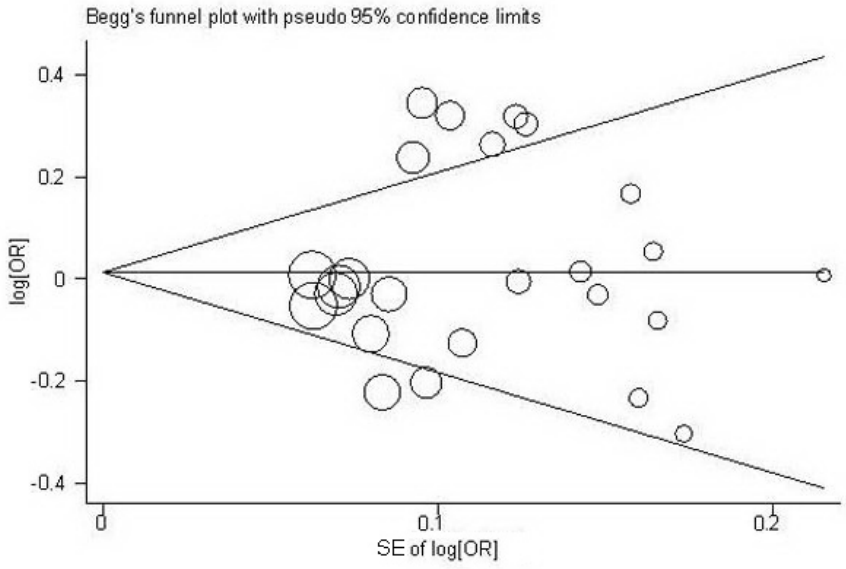

Figure 5. Begg funnel plot for publication bias test (t = 0.69, P = 0.494 for G-allele *vs* C-allele). Each point represents a separate study for the indicated association. Log[OR], natural logarithm of odd ratio (OR). Horizontal line, mean effect size. SE = Standard error of the mean.

DISCUSSION

As described above, miR-146a functions as a cancer-related microRNA by targeting several key genes in different pathways. The G/C variation located in the stem-loop of *pre-miR-146a* was reported to impair the functions of mature miR-146a (Jazdzewski et al., 2008). However, the exact mechanisms of how the *pre-miR-146a* rs2910164 polymorphism acts in diseases at the molecular level remain unclear. Since miR-146a and its targets play a critical role in different cancers and the rs2910164 polymorphism in the *pre-miR-146a* gene has been reported to be associated with mature miR-146a expression or risk of cancers, rs2910164 can be considered a potential biomarker for detecting cancers. As expected, the results from our study indicated that the G-allele of *pre-miR-146a* rs2910164 might be a protective factor against cancer development in Caucasians but a risk factor in the case of Asians, male patients, and smokers.

The main message of this meta-analysis was that the association between *pre-miR-146a* polymorphism and cancer susceptibility was modified by ethnicity. Although we failed to detect a significant association of this polymorphism on cancer susceptibility in the pooled analysis, we found a reduced effect of the *pre-miR-146a* polymorphism in the Caucasian population, but a borderline risk effect in Asian populations; this implied that the effect of the rs2910164 polymorphism on cancer susceptibility was probably associated with ethnic differences in genetic backgrounds and the residential environment (Hirschhorn et al., 2002). Moreover, the G-allele of rs2910164 had a higher representation in controls of Caucasians than in those of Asians (76.58 *vs* 47.88%). The large differences between the Caucasians and Asians in the mean allele frequency and also the different effects of *pre-miR-146a* polymorphism on

the 2 races may be the result of natural selection pressures or imbalance due to other related genetic variants.

Moreover, when stratifying the studies by gender, the GG and GC genotypes of rs2910164 represented a risk factor for cancers in male patients but not in female patients. Hormones have been reported to be related with cancer formation and progression. The observed differences in the cancer susceptibility of male and female patients in the present study may be attributed to the interactions between the polymorphism and the sex hormones during carcinogenesis (Xu et al., 2008), which was exemplified by the case of MDM2 SNP309 (Bond and Levine, 2007). Tobacco smoke contains multiple carcinogens, such as polycyclic aromatic hydrocarbons and *N*-nitroso compounds, and smoking has proven to be an independent risk factor in several cancers, such as lung cancer (Hecht, 2002) and esophageal squamous cell carcinoma (Guo et al., 2010). The increased risk of cigarette smoking on cancer has been reported by many studies, and a positive association exhibited a dose-response relationship for intensity, duration, and cumulative consumption of cigarettes (Liang et al., 2009; Ji et al., 2011). The findings that the miR-146a polymorphism and cancer risk exert a joint effect in smokers reflected the additive effect of the smoking-induced increase in the levels of reactive oxygen species production and DNA adducts.

The present study has several limitations. First of all, some of the included studies had problems with methodological issues. For example, the source and selection criteria of the controls were not clearly stated in several studies (Jazdzewski et al., 2008), and the controls might not be uniform. Misclassification bias results in deviation of the genotype distribution in the controls. Further, the genetic distributions of the controls in the 2 studies did not meet the HWE, due to a selection bias in the controls and/or population stratification or genotyping errors since some of them used polymerase chain reaction-restriction fragment length polymorphism as their genotyping methods. In addition, not all studies collected information on possible confounding factors, such as smoking, which also prevented further evaluation of the potential effect of this polymorphism. Last, the stratification by gender may be misleading since a majority of studies concerning the female strata are cancers that are not all gender specific; furthermore, the interpretation of these results may be misleading due to a potential confounding effect of smoking.

Despite these limitations, this was the first meta-analysis about microRNA polymorphism on cancer susceptibility that takes into consideration possible confounding factors, such as smoking status and gender; pooling the results of all published independent studies greatly enhanced the statistical power of this analysis. Further, the quality of the studies included in our meta-analysis was satisfactory and was in strict accordance with our inclusion criterion.

CONCLUSION

Taken together, this study, despite some limitations, suggests that the *pre-miR-146a* rs2910164 polymorphism has a significant association with reduced cancer risk in Caucasians and increased cancer susceptibility in Asians, male patients, and smokers. Further prospective studies on larger samples comprising participants from different races are essential to confirm this conclusion, along with studies on the exact mechanisms of interaction between rs2910164 genotypes and gender and smoking.

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Competing interests

The authors declare that they have no conflict of interest related to the publication of this manuscript.

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SUPPLEMENTARY MATERIAL

Supplementary Table 1. Main characteristics of all studies included in the meta-analysis.

First author	Year	Country	Ethnicity	Cancer type	Case	Control	Cases			Controls			OR (95%CI) (GG/GC *vs* CC)	HWE
							GG	GC	CC	GG	GC	CC		
Hu	2009	China	Asian	Breast cancer	1009	1093	165	515	329	180	551	362	1.02 (0.85-1.23)	Y
Catucci	2010	German	Caucasian	Breast cancer	805	904	451	304	50	536	318	50	0.88 (0.59-1.32)	Y
		Italy			754	1243	409	286	59	650	520	73	0.73 (0.51-1.05)	N
Hoffman	2009	USA	Caucasian	Breast cancer	439	478	234	176	29	273	178	27	0.85 (0.49-1.45)	Y
Pastrello	2010	Italy	Caucasian	Breast cancer	101	155	60	36	5	90	59	6	0.77 (0.23-2.60)	Y
Okubo	2010	Japan	Asian	Gastric cancer	552	697	73	243	236	121	322	254	0.77 (0.61-0.96)	Y
Zeng	2010	China	Asian	Gastric cancer	304	304	62	153	89	53	132	119	1.55 (1.11-2.18)	Y
George	2011	India	Asian	Prostate cancer	159	230	4	79	76	7	107	116	1.11 (0.74-1.67)	N
Xu	2010	China	Asian	Prostate cancer	251	280	68	135	48	54	150	76	1.58 (1.05-2.37)	Y
Liu	2010	USA	Caucasian	Head and neck cancer	1109	1130	630	411	68	655	405	70	1.01 (0.72-1.43)	Y
Xu	2008	China	Asian	Liver cancer	479	504	80	241	158	58	249	197	1.30 (1.00-1.69)	Y
Jazdzewski	2008	Finland Poland USA	Caucasian	Papillary thyroid cancer	206	274	99	104	3	150	105	19	5.04 (1.47-17.28)	Y
					201	475	115	82	4	286	163	26	2.85 (0.98-8.28)	Y
					201	152	91	101	9	90	52	10	1.50 (0.59-3.79)	Y
Guo	2010	China	Asian	Esophageal squamous cell carcinoma	444	468	234	190	20	206	220	42	2.09 (1.21-3.62)	Y
Tian	2009	China	Asian	Lung cancer	1058	1035	360	510	188	364	502	169	0.90 (0.72-1.13)	Y
Zhou	2011	China	Asian	Cervical cancer	226	309	43	113	70	34	159	116	1.34 (0.93-1.93)	Y
Srivastava	2010	India	Asian	Gallbladder cancer	230	224	129	90	11	138	81	5	0.46 (0.16-1.33)	Y
Mittal	2011	India	Asian	Bladder cancer	212	250	127	79	6	135	108	7	0.99 (0.33-2.98)	N
Yue	2011	China	Asian	Cervical cancer	447	443	118	224	105	87	206	150	1.67 (1.24-2.24)	Y
Garcia	2011	French	Caucasian	Breast cancer	1130	596	676	388	66	352	220	24	0.68 (0.42-1.08)	Y
Zhou	2012	China	Asian	Liver cancer	186	483	33	86	67	71	254	158	0.86 (0.61-1.23)	Y
Hishida	2011	Japan	Asian	Gastric cancer	583	1637	82	271	230	229	775	633	0.97 (0.80-1.17)	Y
Akkiz	2011	Turkey	Caucasian	Liver cancer	222	222	137	75	10	144	67	11	1.11 (0.46-2.66)	Y
Permuth-Wey	2011	USA	Caucasian	Glioma	593	614	345	198	50	375	214	25	0.46 (0.28-0.76)	Y

HWE = Hardy-Weinberg equilibrium.