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ORIGINAL ARTICLE

Increased cancer risk associated with the -607C/A polymorphism in interleukin-18 gene promoter: an updated meta-analysis including 12,502 subjects

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Summary

Purpose: *Increasing investigations have been performed on the association of -607C/A polymorphism in Interleukin-18 (IL-18) gene promoter with cancer risk and have yielded conflicting results. To derive a more precise estimation of the association, we performed an updated meta-analysis of all eligible studies.*

Methods: *We searched all eligible studies by using PubMed, MEDLINE, EMBASE, and China National Knowledge Infrastructure (CNKI) databases. The odds ratios (ORs) were pooled by the fixed-effects/random-effects model in STATA 12.0 software.*

Results: *This meta-analysis included 29 studies with 6,026 cases and 6,476 controls. Overall, significantly increased cancer risk was observed (A vs C: OR=1.10, 95% CI: 1.01,1.19, $P_{heterogeneity}$=0.001; AA vs CC: OR=1.17, 95% CI: 1.01,1.37, $P_{heterogeneity}$=0.007; CA vs CC: OR =1.15, 95% CI: 1.05,1.25, $P_{heterogeneity}$=0.152; AA/CA vs CC: OR =1.17, 95% CI: 1.06,1.31, $P_{heterogeneity}$=0.042). In subgroup analyses based on ethnicity, the results suggested a significantly increased risk of cancer in Asian population (CA vs CC: OR =1.11, 95% CI: 1.00-1.24, $P_{heterogeneity}$=0.353; AA/CA vs CC: OR =1.14, 95% CI: 1.02-1.29, $P_{heterogeneity}$=0.081) and in Mixed population (A vs C: OR=1.72, 95% CI: 1.22-2.43, $P_{heterogeneity}$=NA; AA vs CC: OR=2.84, 95% CI: 1.43-5.64, $P_{heterogeneity}$=NA; AA vs CC/CA: OR =2.43, 95% CI: 1.34-4.42, $P_{heterogeneity}$=NA; AA/CA vs CC: OR =1.69, 95% CI: 1.00-2.85, $P_{heterogeneity}$=NA); however, no significant association was found in Caucasian or African populations. In the subgroup analysis by cancer type we found a significantly increased susceptibility to breast cancer (A vs C: OR =1.33, 95% CI: 1.00-1.75, $P_{heterogeneity}$=0.155; AA vs CC: OR =1.80, 95% CI: 1.02-3.21, $P_{heterogeneity}$=0.162; AA/CA vs CC: OR =1.33, 95% CI: 1.00-1.78, $P_{heterogeneity}$=0.546), nasopharyngeal carcinoma (A vs C: OR =1.16, 95% CI: 1.01-1.32, $P_{heterogeneity}$=0.921; AA vs CC: OR =1.34, 95% CI: 1.02-1.75, $P_{heterogeneity}$=0.863; CA vs. CC: OR=1.36, 95% CI: 1.08-1.70, $P_{heterogeneity}$=0.824; AA/CA vs CC: OR =1.35, 95% CI: 1.09-1.68, $P_{heterogeneity}$=0.904), and esophageal cancer (CA vs CC: OR=1.37, 95% CI: 1.04-1.80, $P_{heterogeneity}$=0.528; AA/CA vs CC: OR =1.29, 95% CI: 1.00-1.66, $P_{heterogeneity}$=0.700).*

Conclusions: *The current meta-analysis suggests that the -607C/A polymorphism in IL-18 gene promoter is associated with a significantly increased risk of cancer, especially of breast cancer, nasopharyngeal carcinoma and esophageal cancer and in Asian and Mixed populations. More studies with diverse ethnic groups, larger sample size, and well controlled confounding factors are warranted to further investigate the association.*

Key words: *cancer risk, IL-18 polymorphism, meta-analysis*

Introduction

IL-18, a member of the IL-1 family, was originally observed as an inducer of interferon-gamma (IFN-γ) production [1]. It is produced by numerous immune and non-immune cells, which modulate both innate and adaptive immune responses [2,3]. Evidence has shown that IL-18 can exert both an-

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ti-cancerous and procancerous activities. On the one hand, IL-18 plays pleiotropic functions in activating natural killer cell cytotoxic effect and enhancing Th1 immune response mainly by stimulating the expression of IFN-γ and tumor necrosis factor-α (TNF-α), which result in the elimination of tumor cells in vivo [4-7]. On the other hand, recent evidence suggests a tumor stimulating activity of this multi-functional cytokine under some conditions [8]. Higher expression levels of IL-18 have been observed in various cancer cells [9,10]. It also has been shown that IL-18 has an important role in tumor progression. Evidence has suggested an increased level of IL-18 in the blood of metastatic patients compared to patients without metastasis and healthy controls [11].

The human IL-18 gene is composed of six exons and five introns located on chromosome 11q22.2–q22.3. Several single nucleotide polymorphisms (SNPs) have been identified in the IL-18 gene. A number of SNPs in the promoter region of IL-18 gene have been associated with differential levels of gene transcription and protein production. The G to C substitution at position -137 abolishes a histone 4 transcription factor-1-binding site and the C to A substitution at position -607 disrupts a cyclic AMP (cAMP) protein-binding site. The functional significance of these two SNPs has been shown by several researchers, and these two SNPs were attributed to the IL-18 higher transcription and protein production [8,12].

In the past decade, numerous studies have investigated the association between the -607C/A polymorphism of IL-18 gene and cancer risk [13-40], but the results are conflicting rather than conclusive. From 2010 to 2013, three meta-analyses were conducted with the aim to investigate the association between -607C/A polymorphism in IL-18 gene and cancer risk [41-43], demonstrating that it was associated with an increased overall cancer risk. However, to our knowledge, these three meta-analyses have some limitations and demerits such as relatively small numbers of included articles, including irrelevant studies, and not including all eligible studies; and from then on, a number of new case-control studies have been conducted [13-18]. Given the limitations and demerits mentioned above, we conducted an updated meta-analysis based on current evidence to further identify the precise association.

Methods

Publication search

To identify all the eligible studies, a comprehensive literature search was performed in PubMed, MEDLINE, EMBASE, and CNKI (China National Knowledge Infrastructure) databases until November 29th, 2014. The following search terms were used: ("Interleukin-18" or "IL-18" or "rs1946518") and ("polymorphism" or "SNP" or "mutation" or "genetic polymorphism" or "variation" or "single nucleotide polymorphism" or "variant") and ("carcinoma" or "cancer" or "tumor" or "neoplasm"). We also performed manual searches of references cited in the retrieved articles and previous reviews on the topic.

Inclusion and exclusion criteria

Studies that met the following criteria were included in this meta-analysis: (1) case-control studies which investigated the association between the -607 C/A polymorphism of IL-18 gene and cancer risk; (2) studies with genotype distribution information in cases and controls or odds ratio (OR) with its 95% confidence interval (CI) and p value; (3) cancers diagnosed by histopathology. Major reasons for exclusion of studies were as follows: (1) meta-analysis, reviews, abstracts, or comments; (2) not for cancer research; (3) studies that contained overlapping data.

Data extraction

Two authors (XNL and DLR) extracted data from all eligible publications independently and reached a consensus with other authors. The following information was abstracted: name of the first author, year of publication, country of origin, ethnicity, source of controls, cancer type, number of cases and controls, sample size, genotype frequencies of cases and controls, and Hardy-Weinberg equilibrium (HWE) in controls. Cancer types were classified as prostate cancer, esophageal cancer, nasopharyngeal carcinoma, colorectal cancer, breast cancer, cervical cancer, head and neck cancer and other types (renal cell carcinoma, lung cancer, gastric cancer, ovarian cancer, choriocarcinoma, hepatocellular carcinoma). Different ethnicities were categorized as Caucasian, Asian, African and Mixed. All eligible studies were defined as hospital-based (HB) or population-based (PB) according to the source of controls.

Statistics

The strength of the association between the -607C/A polymorphism of IL-18 gene and cancer risk was measured by ORs and the corresponding 95% CI in the allelic model (A vs C), homozygous model (AA vs CC), heterozygous model (CA vs CC), recessive model (AA vs CC/CA) and the dominant model (AA/CA vs CC). The significance of pooled ORs was valued by the Z test and was considered statistically significant when $p<0.05$.

Pearson's x^2 test was performed to examine HWE, and $p>0.05$ was considered to be in line with HWE. Chi-square-based Q statistic test was performed in order to evaluate possible between-study heterogeneity (het-

erogeneity was considered statistically significant if p<0.10) [44]. If there was no heterogeneity, the fixed-effect model was used according to the Mantel-Haenszel method; otherwise, the random-effects model (DerSimonian-Laird method) was applied [45]. Both subgroup analyses and meta-regression analyses were performed to explore the source of heterogeneity among variables such as ethnicity, cancer type, source of controls and sample size [46]. To evaluate the stability of the results, sensitivity analysis was conducted by deleting one single study each time [47]. Moreover, Begg funnel plots and Egger's regression test were undertaken to assess the potential publication bias, and a p<0.05 was considered significant [48,49]. It is worth mentioning that if a specific cancer type was evaluated in fewer than two case-control studies, it would fall into the "other types" group. All of the statistical analyses were conducted by STATA (version 12.0; Stata Corporation, College Station, Texas, USA).

Results

Characteristics of eligible studies

Based on our search strategy, a total of 1147 records were identified by initial search in the selected databases. After duplicates'removal, we screened the remaining 954 records by titles and abstracts. The screening process is shown in Figure 1. Eventually, 28 eligible articles were identified [13-40] (Table 1). Two separate case-control studies were included from the study conducted by Haghshenas et al. and were considered separately [28]. Thus, in all, we included 29 studies in this meta-analysis, with 6,026 cancer patients and 6,476 controls. Twenty-two eligible studies were conducted in Asia [13,15-22,24,26-36,38], 5 in Europe [23,25,37,39,40], one in Africa [35], and one in South America [14]. Studies including more than 500 participants were defined as "large sample size", otherwise as "small sample size". The distribution of genotypes in the controls were in agreement with HWE except for three studies (p=0.013 [23], p=0.012 [37], p=0.012 [39]).

Main results

The results of this meta-analysis are shown in Table 2. Overall, significant associations were detected between the -607C/A polymorphism of IL-18 gene and cancer susceptibility (allelic model, A vs C, OR=1.10, 95% CI: 1.01-1.19, $P_{heterogeneity}$=0.001, Figure 2; homozygous model, AA vs CC: OR=1.17, 95% CI: 1.01-1.37, $P_{heterogeneity}$=0.007, Figure 3; heterozygous model, CA vs CC: OR=1.15, 95% CI: 1.05-1.25, $P_{heterogeneity}$=0.152, Figure 4; dominant model, AA/CA vs CC: OR=1.17, 95% CI: 1.06-1.31, $P_{heterogeneity}$=0.042, Figure 5). No significant association was found in the recessive model (recessive model, AA vs CC/CA: OR=1.06, 95% CI: 0.93-1.20, $P_{heterogeneity}$=0.008).

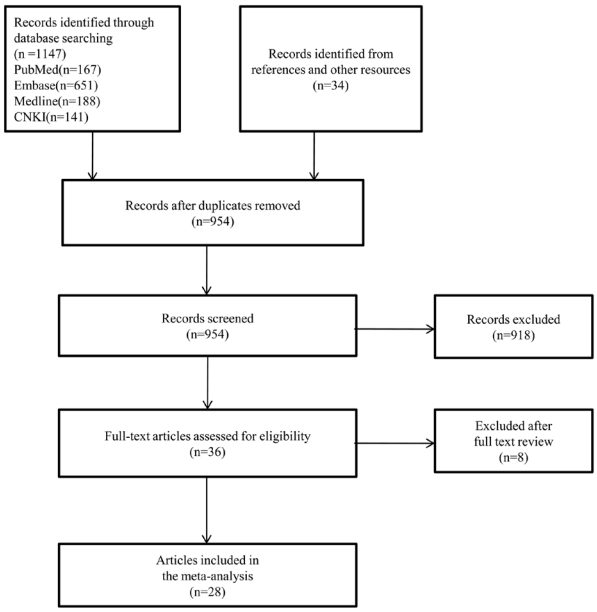

Figure 1. PRISMA flow chart for study selection. After comprehensive screening, 28 eligible articles were eventually included.

Subgroup analyses

Significant heterogeneity between studies was detected. Hence, subgroup analysis was conducted and is shown in Table 2. In the subgroup analyses by cancer type, increased risk was found in the allelic model, homozygous model, heterozygous model and dominant model for nasopharyngeal carcinoma (A vs C: OR=1.16, 95% CI: 1.01-1.32, $P_{heterogeneity}$=0.921; AA vs CC: OR=1.34, 95% CI: 1.02-1.75, $P_{heterogeneity}$=0.863; CA vs CC: OR=1.36, 95% CI: 1.08-1.70, $P_{heterogeneity}$=0.824; AA/CA vs CC: OR=1.35, 95% CI: 1.09-1.68, $P_{heterogeneity}$=0.904; Figure 3), in the heterozygous model and dominant model for esophageal cancer (CA vs CC: OR=1.37, 95% CI: 1.04-1.80, $P_{heterogeneity}$=0.528; AA/CA vs CC: OR=1.29, 95% CI: 1.00-1.66, $P_{heterogeneity}$=0.700; Figure 3), and in the allelic model, homozygous model and dominant model for breast cancer (A vs C: OR=1.33, 95% CI: 1.00-1.75, $P_{heterogeneity}$=0.155; AA vs CC: OR=1.80, 95% CI: 1.02-3.21, $P_{heterogeneity}$=0.162; AA/CA vs CC: OR=1.33, 95% CI: 1.00-1.78, $P_{heterogeneity}$=0.546; Figure 6). No significant results were observed in any genetic model of prostate cancer, colorectal cancer, cervical cancer, head

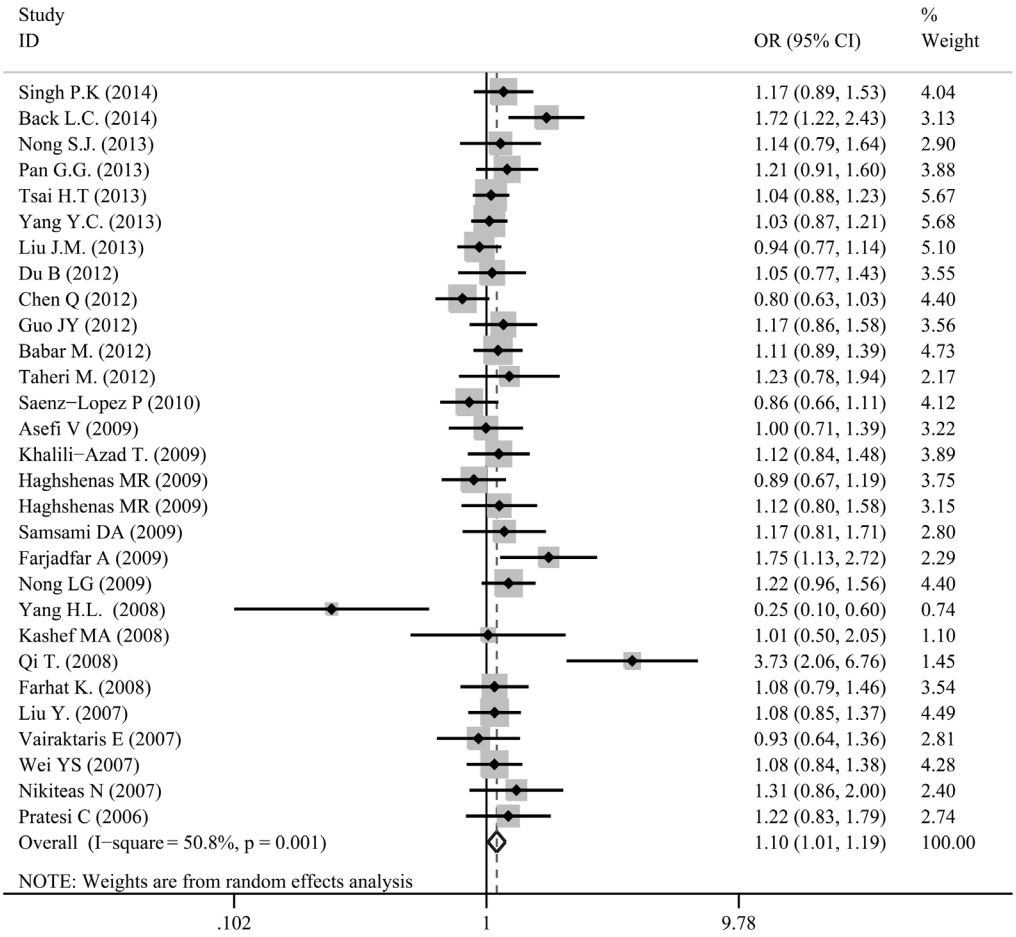

Figure 2. Forest plot for the association between the -607C/A polymorphism in interleukin-18 gene promoter and overall cancer risk under the allelic model (random-effects model).

and neck cancer, and other types.

In the subgroup analyses by ethnicity, significantly increased risk of cancer was detected among the Asian population in the heterozygous model and dominant model (CA vs CC: OR=1.11, 95% CI: 1.00-1.24, $P_{heterogeneity}$=0.353; AA/CA vs CC: OR=1.14, 95% CI: 1.02-1.29, $P_{heterogeneity}$=0.081; Figure 4), and among mixed population (A vs C, OR=1.72, 95% CI: 1.22-2.43, $P_{heterogeneity}$=NA; AA vs CC: OR=2.84, 95% CI: 1.43-5.64, $P_{heterogeneity}$=NA; AA vs CC/CA: OR=2.43, 95% CI: 1.34-4.42, $P_{heterogeneity}$=NA; AA/CA vs CC: OR =1.69, 95%CI: 1.00-2.85, $P_{heterogeneity}$=NA; Figure 7). However, no significant association was found in the Caucasian and African populations.

According to the source of controls, significantly increased risk of cancer was only observed in hospital-based controls in the allelic model, heterozygous model and dominant model (A vs C: OR=1.15, 95% CI: 1.00-1.31, $P_{heterogeneity}$=0.002; CA vs CC: OR=1.22, 95% CI: 1.03-1.45, $P_{heterogeneity}$=0.167; AA/CA vs CC: OR=1.25, 95% CI: 1.04-1.51, $P_{heterogeneity}$=0.034; Figure 8). However, among studies with population-based controls, no significant result was observed in any genetic model (Table 2).

In terms of the sample size, elevated cancer risk was only detected among studies with small sample size in the allelic model, homozygous model, heterozygous model and dominant model (A vs C: OR=1.16, 95% CI: 1.04-1.30, $P_{heterogeneity}$=0.003; AA vs CC: OR=1.32, 95%CI: 1.06-1.65, Pheterogeneity=0.015; CA vs CC: OR=1.28, 95%CI: 1.14-1.45, $P_{heterogeneity}$=0.749; Figure 9; AA/CA vs CC: OR=1.29, 95%CI: 1.14-1.46, $P_{heterogeneity}$=0.323), but among studies with large sample size no sig-

Table 1. Characteristics of eligible studies included in the meta-analysis

First author	*Year*	*country*	*Ethnicity*	*Control*	*Cancer type*	*No.of cases*	*No. of controls*	*Sample size*	*Cases*			*Controls*			*Phwe*
									CC	CA	AA	CC	CA	AA	
Singh [13]	2014	India	Asian	HB	Head and neck cancer	272	185	Small	79	154	39	65	96	24	0.214
Back [14]	2014	Brazil	Mixed	PB	Breast cancer	154	118	Small	39	66	49	43	56	19	0.914
Nong [15]	2013	China	Asian	PB	prostate cancer	126	125	Small	48	63	15	50	65	10	0.076
Pan [16]	2013	China	Asian	HB	Nasopharyngeal Carcinoma	190	200	Small	40	97	53	56	93	51	0.326
Tsai [17]	2013	China	Asian	PB	Head and neck cancer	567	559	Large	140	262	165	135	276	148	0.777
Yang [18]	2013	China	Asian	HB	Cervical cancer	470	722	Large	116	215	139	169	358	195	0.850
Liu [19]	2013	China	Asian	PB	prostate cancer	375	400	Large	100	172	103	94	196	110	0.712
Du [20]	2012	China	Asian	HB	Nasopharyngeal Carcinoma	150	180	Small	36	80	34	47	93	40	0.640
Chen [21]	2012	China	Asian	HB	Other types	228	300	Large	55	126	47	61	156	83	0.429
Guo [22]	2012	China	Asian	HB	Colorectal Cancer	170	160	Small	36	85	49	42	76	42	0.527
Babar [23]	2012	UK	Caucasian	PB	Esophageal Cancer	1070	194	Large	384	508	178	83	75	36	0.013*
Taheri [24]	2012	Iran	Asian	PB	Breast cancer	72	93	Small	29	32	11	40	45	8	0.346
Saenz-Lopez [25]	2010	Spain	Caucasian	PB	Other types	154	500	Large	59	76	19	166	261	73	0.069
Asefi [26]	2009	Iran	Asian	HB	Head and neck cancer	111	212	Small	43	53	15	82	101	29	0.812
Khalili-Azad [27]	2009	Iran	Asian	PB	Breast cancer	200	206	Small	64	103	33	76	97	33	0.826
Haghshenas [28]	2009	Iran	Asian	PB	Colorectal Cancer	142	311	Small	55	72	15	119	144	48	0.685
Haghshenas [28]	2009	Iran	Asian	PB	Other types	87	311	Small	31	40	16	119	144	48	0.685
Samsami [29]	2009	Iran	Asian	HB	Other types	85	158	Small	22	51	12	57	75	26	0.874
Farjadfar [30]	2009	Iran	Asian	HB	Other types	73	97	Small	15	45	13	40	46	11	0.682
Nong [31]	2009	China	Asian	PB	Nasopharyngeal Carcinoma	250	270	Large	47	132	71	69	133	68	0.808
Yang [32]	2008	China	Asian	PB	Cervical cancer	26	20	Small	9	15	2	3	6	11	0.201
Kashef [33]	2008	Iran	Asian	PB	Other types	19	103	Small	6	10	3	33	54	16	0.429
Tao [34]	2008	China	Asian	HB	Cervical cancer	50	50	Small	5	17	28	17	24	9	0.917
Farhat [35]	2008	Tunisia	African	PB	Nasopharyngeal Carcinoma Prostate	163	164	Small	41	94	28	53	77	34	0.537
Liu [36]	2007	China	Asian	HB	Cancer	265	280	Large	50	143	72	65	137	78	0.747
Vairaktaris [37]	2007	Germany	Caucasian	PB	Head and neck cancer	149	89	Small	55	66	28	35	32	22	0.012*
Wei [38]	2007	China	Asian	HB	Esophageal Cancer	235	250	Small	48	123	64	59	124	67	0.912
Nikiteas [39]	2007	Greece	Caucasian	PB	Colorectal Cancer	84	89	Small	19	47	18	35	32	22	0.012*
Pratesi [40]	2006	Italy	Caucasian	PB	Nasopharyngeal Carcinoma	89	130	Small	26	42	21	43	64	23	0.923

HB: hospital-based; PB: population-based; Other types:renal cell carcinoma ,lung cancer, gastric cancer, ovarian cancer, choriocarcinoma,hepatocellular carcinoma; large: studies with more than 500 participants; small: studies with less than 500 participants; Phwe: Hardy–Weinberg equilibrium; Phwe*<0.05;

Table 2. Meta-analysis results

	A vs C			*AA vs CC*			*CA vs CC*			*AA vs CC/CA*			*AA/CA vs CC*	
	Study	*OR (95% CI)*	*Ph*	*Study*	*OR (95% CI)*	*Ph*	*Study*	*OR (95% CI)*	*Ph*	*Study*	*OR (95% CI)*	*Ph*	*Study*	*OR (95% CI)*	*Ph*
Overall	29	1.10 (1.01-1.19)*	0.001	29	1.17 (1.01-1.37)*	0.007	29	1.15 (1.05-1.25)*[a]	0.152	29	1.06 (0.93-1.20)	0.008	29	1.17 (1.06 - 1.31)*	0.042
Ethnicity															
Asian	22	1.09 (0.99-1.19)	0.02	22	1.17 (0.98-1.40)	0.011	22	1.11 (1.00-1.24)*	0.353	22	1.07 (0.93-1.24)	0.022	22	1.14 (1.02-1.29)*	0.081
Caucasian	5	1.04 (0.90-1.21)	0.336	5	1.02 (0.78-1.34)	0.473	5	1.29 (0.91-1.85)	0.041	5	0.89 (0.70-1.14)	0.620	5	1.20 (0.89-1.62)	0.083
African	1	1.08 (0.79-1.46)	NA	1	1.06 (0.56-2.03)	NA	1	1.58 (0.95-2.62)	NA	1	0.79 (0.46-1.38)	NA	1	1.42 (0.88-2.30)	NA
Mixed	1	1.72 (1.22-2.43)*	NA	1	284 (1.43-5.64)*	NA	1	1.30 (0.74-2.28)	NA	1	2.43 (1.34-4.42)*	NA	1	1.69 (1.00-2.85)*	NA
Source of controls															
Hospital-based	12	1.15 (1.00-1.31)*	0.02	12	1.26 (0.98-1.62)	0.016	12	1.22 (1.03-1.45)*	0.167	12	1.09 (0.90-1.32)	0.043	12	1.25 (1.04-1.51)*	0.034
Population-based	17	1.07 (0.97-1.18)	0.034	17	1.12 (0.92-1.37)	0.050	17	1.13 (0.99-1.30)	0.222	17	1.03 (0.86-1.23)	0.025	17	1.13 (0.99-1.28)	0.189
Sample size															
Small	21	1.16 (1.04-1.30)*	0.03	21	1.32 (1.06-1.65)*	0.015	21	1.28 (1.14-1.45)*	0.749	21	1.12 (0.91-1.37)	0.004	21	1.29 (1.14-1.46)*	0.323
Large	8	1.01 (0.92-1.10)	0.264	8	1.01 (0.85-1.54)	0.301	8	1.03 (0.86-1.22)	0.071	8	1.01 (0.90-1.14)	0.434	8	1.02 (0.87-1.19)	0.101
Cancer type															
Prostate	3	1.01 (0.88-1.17)	0.550	3	1.04 (0.78-1.39)	0.400	3	1.02 (0.75-1.38)	0.218	3	1.02 (0.81-1.29)	0.589	3	1.03 (0.78-1.34)	0.265
Esophageal	2	1.09 (0.93-1.29)	0.852	2	1.11 (0.80-1.54)	0.783	2	1.37 (1.04-1.80)*	0.528	2	0.95 (0.71-1.25)	0.591	2	1.29 (1.00-1.66)*	0.700
Nasopharyngeal	5	1.16 (1.01-1.32)*	0.921	5	1.34 (1.02-1.75)*	0.863	5	136 (1.08-1.70)*	0.824	5	1.09 (0.88-1.36)	0.707	5	1.35 (1.09-1.68)*	0.904
Colorectal	3	1.08 (0.86-1.34)	0.262	3	1.09 (0.66-179)	0.213	3	1.46 (0.90-2.37)	0.097	3	0.90 (0.64-1.26)	0.359	3	1.34 (0.86-2.07)	0.118
Breast	3	1.33 (1.00-1.75)*	0.155	3	1.80 (1.02-3.21)*	0.162	3	1.21 (0.89-1.63)	0.784	3	1.63 (0.91-2.94)	0.099	3	1.33 (1.00-.178)*	0.546
Cervival	3	1.03 (0.34-3.16)	<0.001	3	1.01 (0.13-8.14)	<0.001	3	1.04 (0.60-1.81)	0.258	3	0.92 (0.17-4.86)	<0.001	3	1.19 (0.36-3.90)	0.007
Head and neck	4	1.05 (0.93-1.18)	0.797	4	1.07 (0.83-1.37)	0.759	4	1.06 (0.86-1.29)	0.460	4	1.06 (0.86-1.31)	0.577	4	1.06 (0.88-1.29)	0.643
Other types	6	1.05 (0.84-1.31)	0.038	6	1.04 (0.67-1.61)	0.080	6	1.18 (0.84-1.66)	0.054	6	0.88 (0.68-1.13)	0.417	6	1.16 (0.81-1.65)	0.024

OR: odds ratio, Ph: p value for heterogeneity, NA:not available. *OR with statistical significance; [a]OR:estimates for fixed-effects model; large: studies with more than 500 participants; small: studies with less than 500 participants; Other types: renal cell carcinoma ,lung cancer, gastric cancer, ovarian cancer, choriocarcinoma, hepatocellular carcinoma

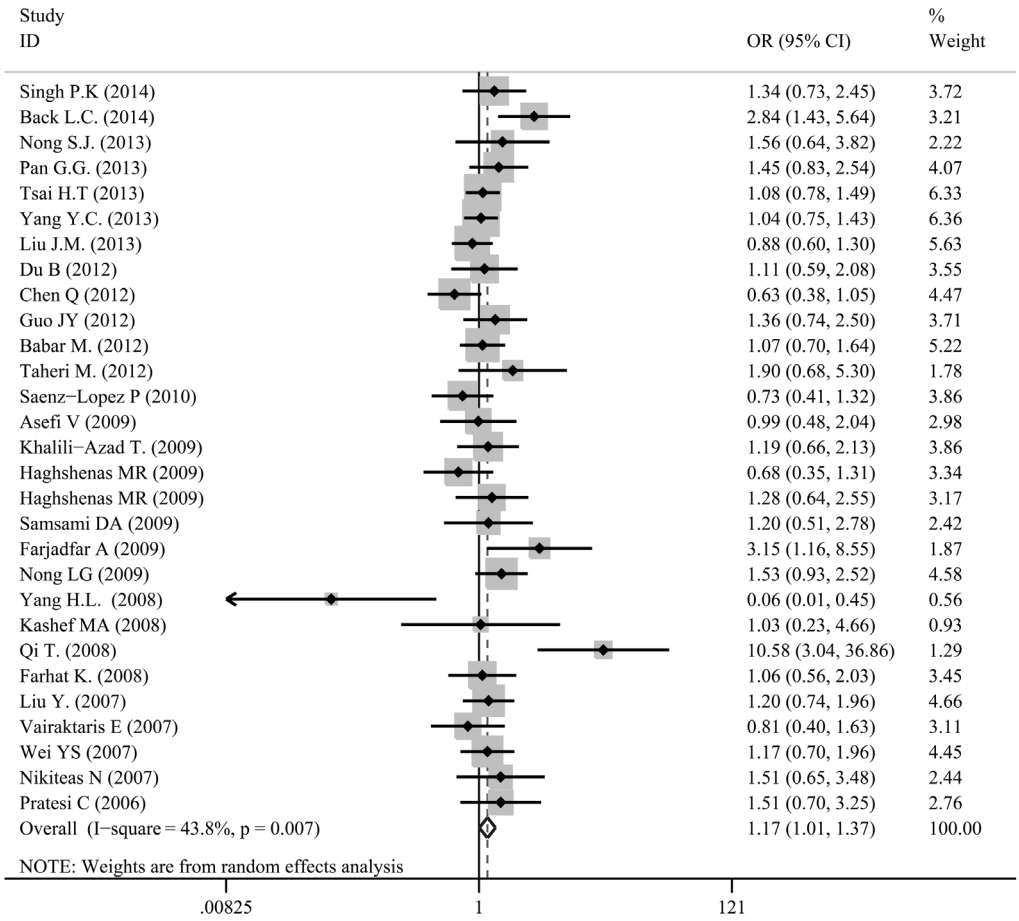

Figure 3. Forest plot for the association between the -607C/A polymorphism in interleukin-18 gene promoter and overall cancer risk under the homozygous model (random-effects model).

nificant results were observed in any genetic model.

Meta-regression and sensitivity analysis

Furthermore, we conducted a meta-regression analysis to explore the influence of ethnicity, source of controls, sample size and cancer type on the heterogeneity in the allelic, homozygous and dominant comparisons. The results suggested that in the allelic model (A vs C), homozygous model (AA vs CC), and dominant model (AA/CA vs CC), equally, sample size largely contributed to the source of heterogeneity (A vs C, p=0.004; AA vs CC , p<0.001; AA/CA vs CC, p=0.005). Sensitivity analysis was conducted to assess the robustness of the meta-analysis results by removing one single study in sequence. No significant change of the pooled ORs was found, which validated the stability of our results (Figure 10).

Publication bias

Begg's funnel plot and Egger's test were performed to evaluate the publication bias. The figure of the funnel plot did not show any evidence of obvious asymmetry (p=0.103 for A vs C; p=0.253 for AA vs CC; p=0.111 for CA vs CC; p=0.111 for AA/CA vs CC; Figure 11). Then, the Egger's test was performed and the results further suggested that publication bias did not exist (p=0.177 for A vs C; p=0.262 for AA vs CC; p=0.051 for CA vs CC; p=0.088 for AA/CA vs CC).

Discussion

The human IL-18 (hIL-18) gene is located on chromosome 11q22.2-q22.3, and consists of six exons and five introns. IL-18 is a pleiotropic, proinflammatory cytokine with dual effects on tumor development and progression [11]. IL-18 gene expression seems to be regulated at the

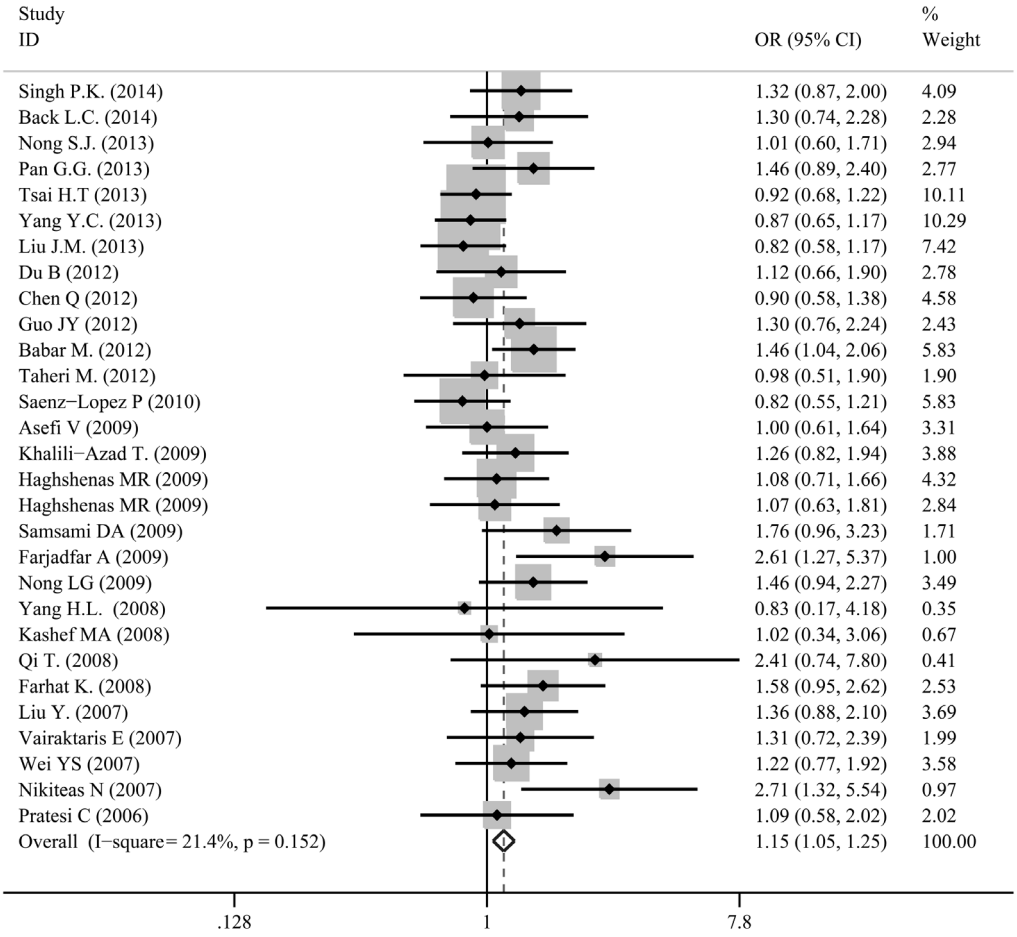

Figure 4. Forest plot for the association between the -607C/A polymorphism in interleukin-18 gene promoter and overall cancer risk under the heterozygous model (fixed-effects model).

transcriptional level by two SNPs at positions -607 (C/A) and -137 (G/C) in the promoter region of the gene. The functional significance of these two SNPs has been shown by several researchers, and the C allele at position -607 and the G allele at position -137 were attributed to the IL-18 higher transcription and protein production [8]. IL-18 and IFN-γ expression analysis by reverse transcription polymerase chain reaction (RT-PCR) showed that subjects homozygous for haplotype (-137 G/-607 C) had higher levels of IL-18 mRNA compared to other haplotypes [12]. Previous studies have suggested the pro-cancer effect of IL-18. It has been reported that IL-18 is associated with tumor growth [50,51]. Besides, IL-18, through vascular endothelial growth factor (VEGF), enhances the immune response stimulation that increases tumor metastasis [52,53]. Meanwhile, increased levels of IL-18 have been detected in various cancers [54-57]. All these findings indicate that there is an association between the -607C/A polymorphism in IL-18 gene promoter and oncogenesis.

In the past decade, numerous studies have investigated the association between the -607 C/A polymorphism of IL-18 gene and cancer risk [13-40]. However, the results are conflicting. In 2010, Mi et al. performed the first meta-analysis to investigate the association between -607C/A polymorphism in IL-18 gene and cancer risk [41], but considering that the number of included studies was relatively small, the results needed to be further confirmed. Later in 2013 two more meta-analyses were performed to investigate the association [42,43]. However, to our knowledge, these two meta-analyses have some limitations and demerits: Firstly, both authors included an irrelevant

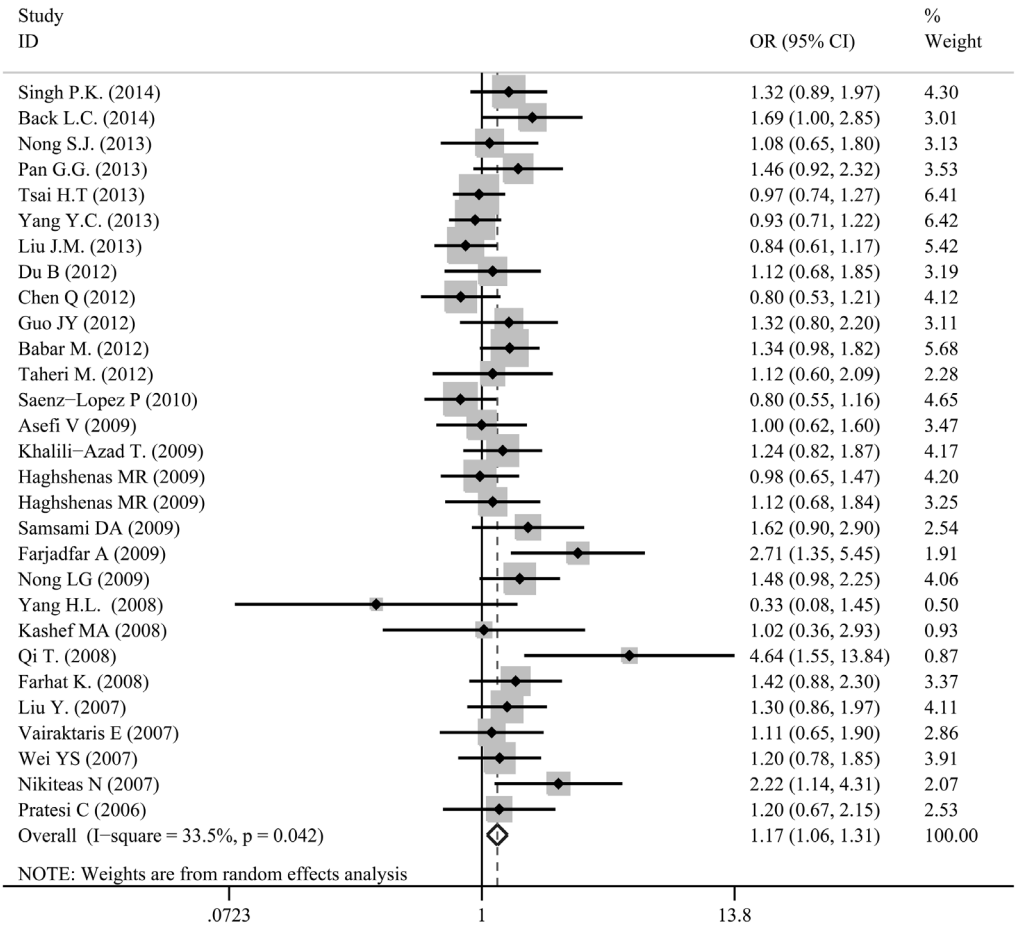

Figure 5. Forest plot for the association between the -607C/A polymorphism in interleukin-18 gene promoter and overall cancer risk under the dominant model (random-effects model).

study which dealt with IL-18 gene polymorphism -656G/T (rs1946519) with cancer risk [58], rather than the -607C/A polymorphism(rs1946518) in IL-18 gene promoter. Secondly, those two meta-analyses failed to retrieve all eligible studies [21,32]. Last but not least, a number of new case-control studies have been conducted after their publication [13-18]. Consequently, an updated meta-analysis of the association between -607 C/A polymorphism of IL-18 gene and overall cancer risk was of great value. To our knowledge, this is the most comprehensive meta-analysis investigating the impact of the -607C/A polymorphism in IL-18 gene promoter on cancer susceptibility.

In the present meta-analysis, 29 eligible studies with 6,026 cancer patients and 6,476 controls were identified and analyzed. Thus, a much larger sample size and improved statistical power could be achieved. All controls in the studies involved were mainly cancer-free. Our results suggested that the -607C/A polymorphism in IL-18 gene promoter is strongly associated with an increased risk of cancer under the allelic model, homozygous model, heterozygous model, and dominant model. In the recessive genetic models, no significant association was found.

In the former meta-analyses, no association between the -607C/A polymorphism of IL-18 gene and the risk of breast cancer was detected. However, in our subgroup analysis of cancer type, we found that the -607C/A polymorphism was statistically related with an increased risk of breast cancer. This is due to the newly published study conducted by Back et al. [14]. However, owing to the number of studies investigating the association of the -607C/A polymorphism and the risk of breast cancer was still small (only 3 in total), more studies are warranted to evaluate this association.

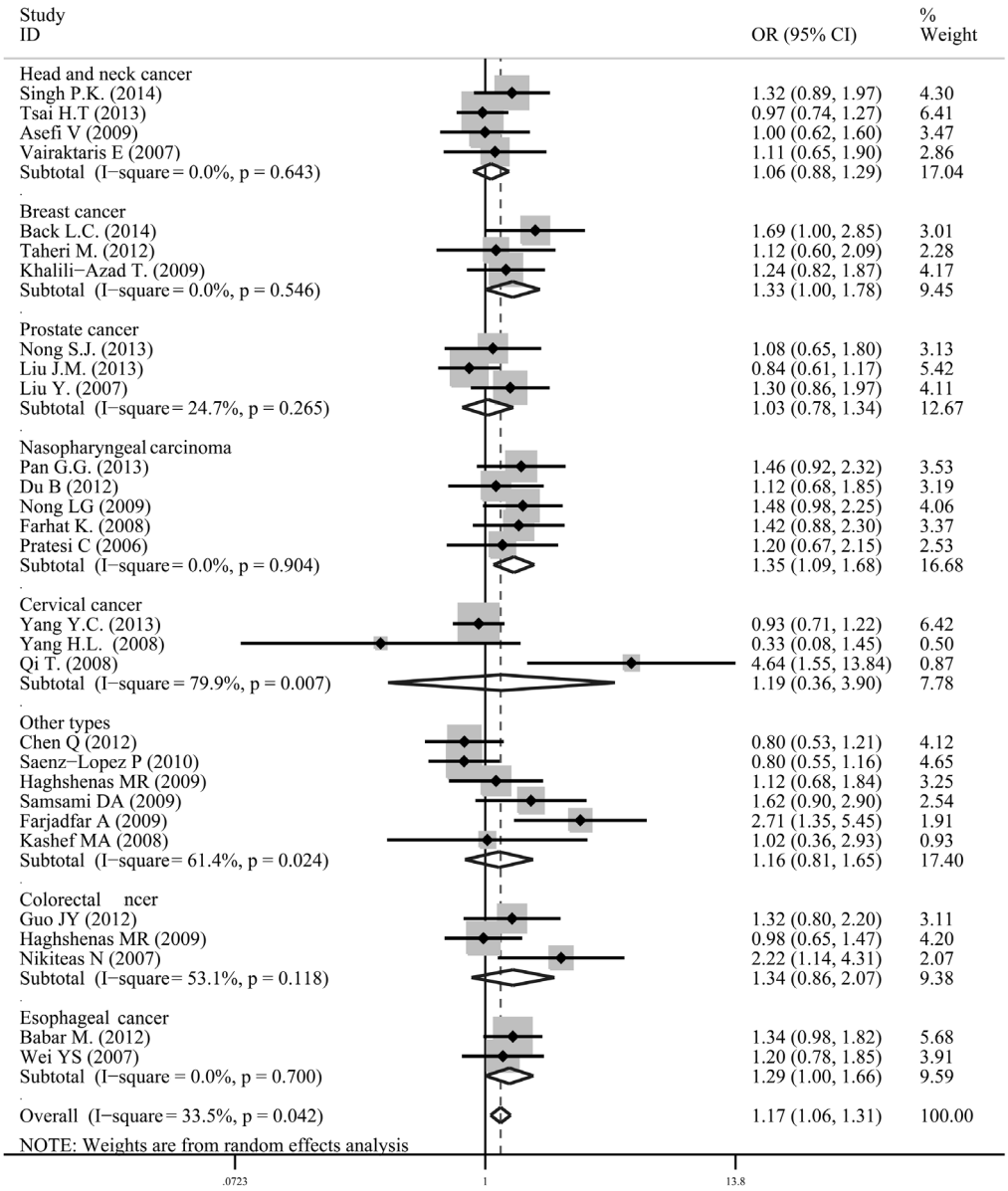

Figure 6. Forest plot for the subgroup analysis by cancer type under the dominant model (random-effects model).

Besides, we also found that the -607C/A polymorphism was related with an increased risk of nasopharyngeal carcinoma and esophageal cancer. However, no evidence of association was found in any genetic model of prostate cancer, colorectal cancer, cervical cancer, head and neck cancer, and other types. When stratified by ethnicity, a significantly increased risk of cancer was found in the Asian population and the mixed population, but not in the Caucasian or African populations, which may indicate that ethnic variation of genetic background would be modified by environmental factors [59], such as age, sex, diet, lifestyle, smoking, and so on.

There was relatively large heterogeneity in our results. Meta-regression was performed for the allelic model, homozygous model and dominant model according to ethnicity, source of controls, sample size, and cancer type. We found that sample size was the main source of heterogeneity in all three genetic models. This may be attributed to the small-study effect [43], which is produced by studies with small sample size. Small-study effect often reports larger effects and leads

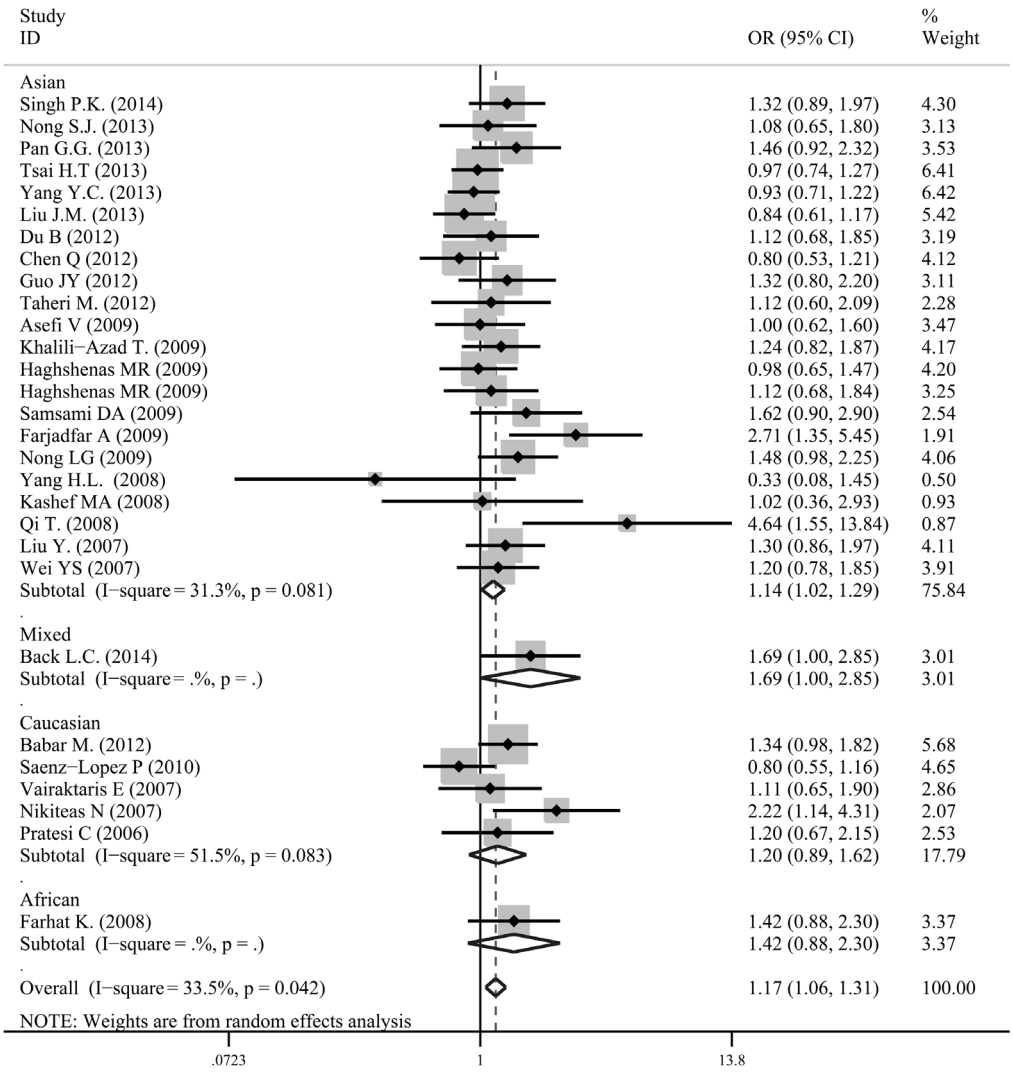

Figure 7. Forest plot for the subgroup analysis by ethnicity under the dominant model (random-effects model).

to between-study variance. However, it is hard to exclude this sort of heterogeneity, because recruitment of enough cases with specific cancer type is difficult.

Several limitations of our study should be taken into account. Firstly, due to insufficient information, stratified analysis could not be conducted by age, treatment, drinking status, smoking and other factors. Secondly, because heterogeneity was influenced by complicated factors, such as age, sex, genetic diversities, different lifestyle, and so on, together with the possible small-study effect mentioned above, it is difficult to exclude heterogeneity in our study. Last but not least, in our meta-analysis, most of the eligible case-control studies were conducted in Asians (22 of all the 29 studies). There were only 5 studies performed in Caucasians, 1 in African, and 1 in Brazil (Mixed population). Due to relatively fewer studies focusing on other ethnicities except for Asians, more studies focused on Caucasians, Africans, and other ethnic groups are imperative for further evaluating whether the genetic background of diverse ethnicities can modify the role of the -607C/A polymorphism in IL-18 gene promoter.

Despite these limitations, our meta-analysis had significantly higher statistical power than the previous studies that analyzed the association between the -607C/A polymorphism in IL-18 gene promoter and cancer risk, since the subjects involved in our meta-analysis were considerably increased compared with the previous studies.

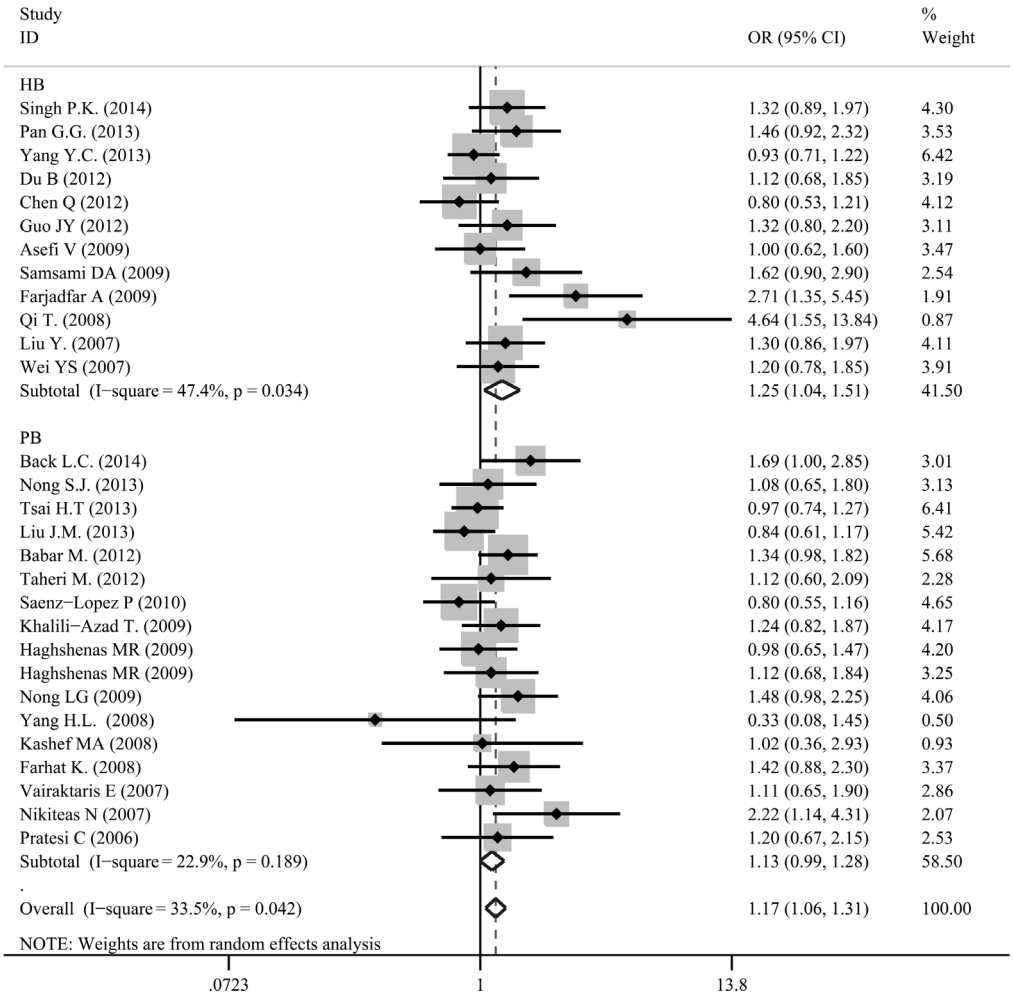

Figure 8. Forest plot for the subgroup analysis by source of controls under the dominant model (random-effects model) HB: hospital based, PB: population based.

Furthermore, a significant association was found in breast cancer, which was not detected in the previous studies. Above all, we modified some drawbacks and demerits of the previous studies, which will be helpful for future studies concerning this topic.

In conclusion, our meta-analysis suggests that the -607C/A polymorphism in IL-18 gene promoter is associated with a significantly increased risk of cancer, especially of breast cancer, nasopharyngeal carcinoma and esophageal cancer, and in Asian and Mixed populations. To verify the results, more studies with diverse ethnic groups, larger sample size, and well controlled confounding factors are warranted to further investigate the association.

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Author contributions

Conceived and designed the experiments: XNL YFZ. Performed the experiments: XNL DLR. Analyzed the data: XNL DLR CL. Contributed reagents/ materials/ analysis tools: XNL JCX YFZ. Wrote the paper: XNL DLR YYL. Revised manuscript: YFZ.

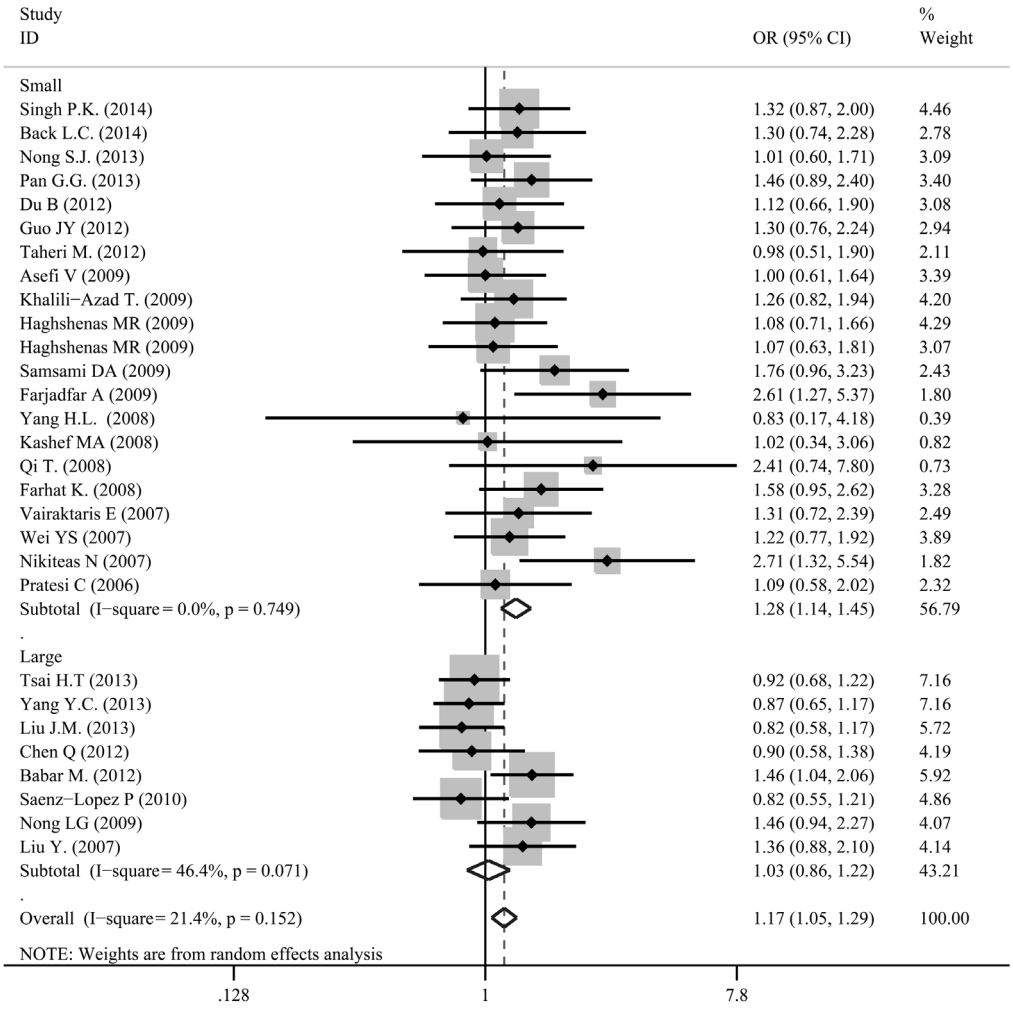

Figure 9. Forest plot for the subgroup analysis by sample size under the heterozygous model (random- effects model).

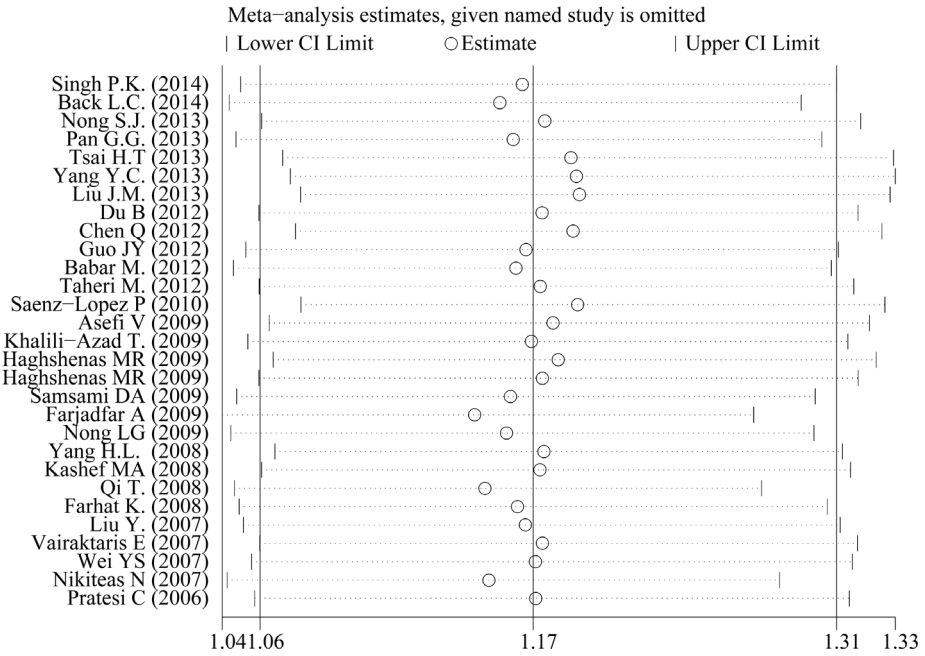

Figure 10. Sensitivity analysis on the association between the -607C/A polymorphism in interleukin-18 gene promoter and overall cancer risk under the dominant model. No statistically different results were observed when excluding every single study in sequence, indicating the stability of the results.

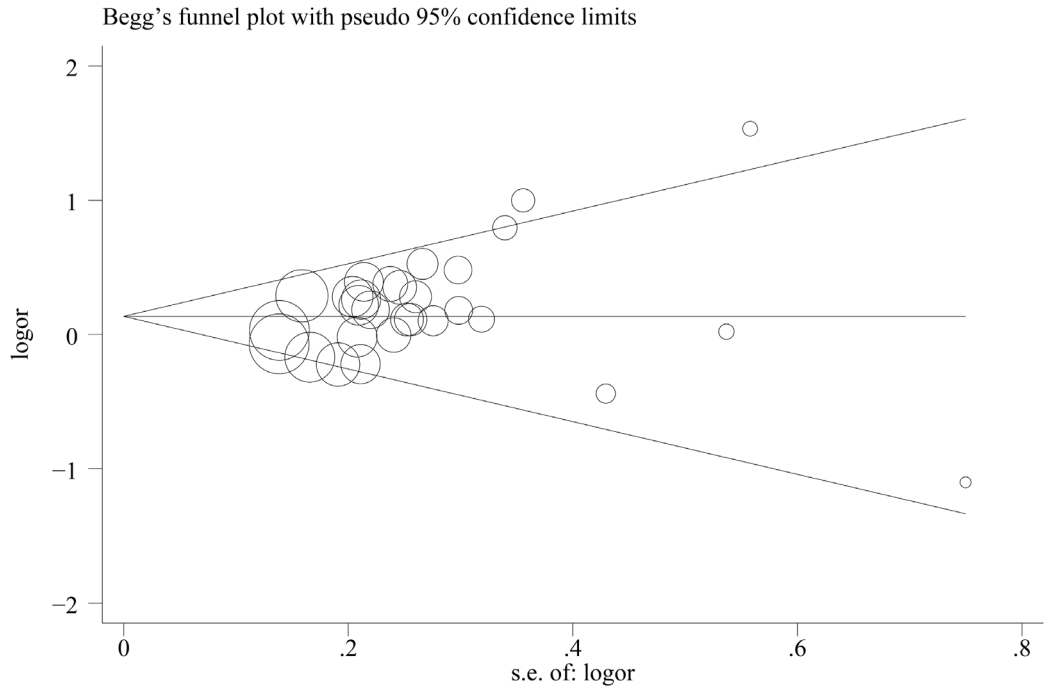

Figure 11. Begg's funnel plot on publication bias for overall data under the dominant model. The funnel plot seemed symmetrical, suggesting absence of publication bias.

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