CTLA-4 polymorphisms associate with breast cancer susceptibility in Asians: a meta-analysis

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Previous studies have investigated the association between cytotoxic T-lymphocyte antigen-4 (*CTLA-4*) polymorphisms and breast cancer susceptibility, but the results remained inconsistent. Therefore, we evaluated the relationship between four common *CTLA-4* polymorphisms and breast cancer risk by a meta-analysis, aiming to derive a comprehensive and precise conclusion. We searched EMBASE, Pubmed, Web of Science, CNKI, and Wanfang databases until July 18th, 2016. Finally, ten eligible studies involving 4544 breast cancer patients and 4515 cancer-free controls were included and all these studies were from Asian. Odds ratio (OR) and 95% confidence interval (CI) were used to evaluate the breast cancer risk in five genetic models. The results indicated that *CTLA-4* +49A>G (rs231775) polymorphism had a significant association with decreased breast cancer risk in allelic, homozygous, dominant and recessive models. And +6230G>A (rs3087243) polymorphism reduced breast cancer risk specially in Chinese population under homozygous and recessive models. In contrast, -1661A>G (rs4553808) polymorphism increased breast cancer risk in allelic, heterozygous and dominant models. Whereas -1722 T>C (rs733618) did not relate to breast cancer risk. In conclusion, *CTLA-4* polymorphisms significantly associate with breast cancer susceptibility in Asian population and different gene loci may have different effects on breast cancer development. Further large-scale studies including multi-racial population are required to confirm our findings.

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Abstract

Previous studies have investigated the association between cytotoxic T-lymphocyte antigen-4 (*CTLA-4*) polymorphisms and breast cancer susceptibility, but the results remained inconsistent. Therefore, we evaluated the relationship between four common *CTLA-4* polymorphisms and breast cancer risk by a meta-analysis, aiming to derive a comprehensive and precise conclusion. We searched EMBASE, Pubmed, Web of Science, CNKI, and Wanfang databases until July 18th, 2016. Finally, ten eligible studies involving 4544 breast cancer patients and 4515 cancer-free controls were included and all these studies were from Asian. Odds ratio (OR) and 95% confidence interval (CI) were used to evaluate the breast cancer risk in five genetic models. The results indicated that *CTLA-4* +49A>G (rs231775) polymorphism had a significant association with decreased breast cancer risk in allelic, homozygous, dominant and recessive models. And +6230G>A (rs3087243) polymorphism reduced breast cancer risk specially in Chinese population under homozygous and recessive models. In contrast, -1661A>G (rs4553808) polymorphism increased breast cancer risk in allelic, heterozygous and dominant models. Whereas -1722 T>C (rs733618) did not relate to breast cancer risk. In conclusion, *CTLA-4* polymorphisms significantly associate with breast cancer susceptibility in Asian population and different gene loci may have different effects on breast cancer development. Further large-scale studies including multi-racial population are required to confirm our findings.

Introduction

Breast cancer has been the most common type of cancer and the main cause of cancer death among women in the world, which was estimated to have 1.7 million new cases in 2012(Torre et

47 al. 2015). Breast cancer is an extremely heterogeneous disease in the clinic and the potential
48 molecular mechanism of carcinogenesis has not been clearly understood so far. In recent years,
49 inherited factors were identified to play a critical role in the development of breast cancer
50 (Reeves et al. 2012).

51 Researches on the field of tumour immunology found that the immune system can influence
52 tumour occurence during the period of elimination, equilibrium and escape (Dunn et al. 2004).
53 Cytotoxic T-lymphocyte antigen-4 (CTLA-4), which was also designated as CD152, expressed
54 mainly on activated T cells. As an immunosuppressive cytokine, it can inhibit T-lymphocyte
55 proliferation and activation (Sun et al. 2008). Numerous researches have demonstrated that
56 blockage of CTLA-4 function can improve antitumor immunity (Leach et al. 1996; Ribas et al.
57 2004; Vandenborre et al. 1999). This indicates CTLA-4 may exert positive effects on
58 carcinogenesis. The human *CTLA-4* gene, which locates in human chromosome 2q33, is one of
59 the most important genes involved in immune responses to a variety of antigens (Walunas et al.
60 2011). *CTLA-4* gene comprises four exons and has several important polymorphisms in the entire
61 region, including the +49G>A (rs231775) in exon 1 (Donner et al. 1997), the +6230G>A
62 (rs3087243) in 3'-untranslated region (Hughes 2006), the -1661A>G (rs4553808) and -1722
63 T>C (rs733618) in the promoter region (Johnson et al. 2001) , which are the most commonly
64 studied single nucleotide polymorphisms (SNPs). These SNPs are important because they can
65 alter the host immune response by affecting the transcription of *CTLA-4* gene, the expression of
66 CTLA-4 protein, and the interaction of CTLA-4 and CD80 ligand (Anjos et al. 2002; Sun et al.
67 2008; Wang et al. 2002).

Numerous investigations have demonstrated that *CTLA-4* genetic polymorphisms may have association with human breast cancer susceptibility (Erfani et al. 2006; Ghaderi et al. 2004; Li et al. 2012; Li et al. 2008; Minhas et al. 2014; Sun et al. 2008; Wang et al. 2007; Zhifu et al. 2015; Kong 2010). The results showed that some of the polymorphisms such as rs733618 and rs4553808 may increase the breast cancer risk while other polymorphisms such as rs231775 and rs3087243 may reduce the risk of breast cancer. Considering a single study does not have enough power to detect the overall effects, we conducted a meta-analysis which is a statistical analysis of the data from some collection of studies in order to synthesize the results to obtain a more reliable evaluation of the relationship between the four common SNPs in *CTLA-4* gene and breast cancer susceptibility.

Materials and methods

Our meta-analysis was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines(Moher et al. 2010).

Search strategy

We searched the databases of EMBASE, PubMed, Web of Science, Wanfang, as well as Chinese National Knowledge Infrastructure (CNKI) to identify all the relevant articles up to July 18th, 2016. Keywords for search were: “CTLA-4 or Cytotoxic T-lymphocyte antigen 4 or CD152”, “polymorphism or variation or SNP or rs231775 or rs308724 or rs4553808 or rs733618”, and “breast cancer”. The eligible article must be published in English or Chinese. References in retrieved articles were also searched manually.

Criteria for selection

All studies selected for further meta-analysis must conform to the included criteria: 1) case-control study conducted in human and investigated the association of SNPs in *CTLA-4* with breast cancer susceptibility; 2) All the breast cancer patients were diagnosed by pathology or histology; 3) detailed data of the allele and genotype distributions are available; 4) the controls were cancer-free individuals. In addition, articles meet the following criteria were excluded: 1) articles that were reviews, conference abstracts, or repeat publications; 2) study design were based on family; 3) studies with no control groups. The quality of each included study was assessed by Newcastle-Ottawa Scale for case-control studies (Wells, et al. 2014).

Data extraction

According to the selection criteria, two authors (Zhiming Dai and Tian Tian) reviewed the literature independently and extracted the raw data and information from each eligible study including: first author, publication year, country of origin, racial ancestry, source of control, genotype method, total number of case and control, allele frequency and genotype distribution in case and control, and *P* value of HWE in control. Any discrepancy was discussed between authors and refereed by Zhijun Dai to reach a consensus.

Statistical analysis

For each study, ORs and 95% CIs were computed to estimate the breast cancer risk associated with *CTLA-4* polymorphisms. Pooled ORs were calculated under the following genetic models: allele comparison of B *vs.* A, homozygote of BB *vs.* AA, heterozygote of AB *vs.* AA, dominant model of (BB+AB) *vs.* AA and recessive model of BB *vs.* (AA+AB). Heterogeneity among studies were estimated by I^2 test and chi^2-based Q statistic and significance

was considered at I^2>50% (Higgins & Thompson 2002). We adopted the random-effects model to analyze the combined ORs if I^2 value was greater than 50%. Otherwise, a fixed-effects model should be exerted (Petitti 2001). We carried out subgroup analysis to estimate the specific effects of ethnicity and source of control. We also conducted a sensitivity analysis to assess the consistency and stability of our meta-analysis by omitting individual study in turn. Additionally, Begg's funnel plot and Egger's test were used to assess publication bias, and significance was identified as P<0.05 (Begg & Mazumdar 1994; Egger et al. 1997). All the statistical analyses were implemented with the Review Manager (Version 5.3; Cochrane Collaboration, London, UK) and STATA software (Version 12.0; Stata Corp, College Station, TX).

Results

Characteristics of included studies

Finally, 9 articles comprising 10 studies investigating *CTLA-4* +49A>G (rs231775) and/or +6230G>A (rs3087243) and/or -1722 T>C (rs733618) and/or -1661A/G (rs4553808) polymorphisms were identified for further analysis (Fig.1). Table 1 presented the characteristics of selected studies. Of the 10 studies, 7 were from China, 2 were from Iran, and 1 was from India. Additionally, 7 studies were based on population and 3 based on hospital. Moreover, genotype distributions in control group of each included study complied with Hardy-Weinberg equilibriums (HWE) (P>0.05) except only one study for one SNP (Erfani et al. 2006). The detailed data of the allele frequency and genotype distribution as well as HWE from each study were showed in Table 2.

Meta-analysis results

Seven studies containing 3,613 cases and 3,608 controls focused on breast cancer risk with *CTLA-4* rs231775 polymorphism. As presented in Table 3, significantly decreased risk was observed in the overall population in all the models except heterozygote (G *vs.* A: OR = 0.86, 95% CI = 0.80-0.92, *P*=0.000, Fig.2A; GG *vs.* AA: OR = 0.68, 95% CI = 0.57-0.81, *P*=0.000; GG *vs.* AA+AG: OR = 0.79, 95% CI = 0.71-0.87, *P*=0.000; AG+GG *vs.* AA: OR = 0.85, 95% CI = 0.74-0.97, *P*=0.02;). In subgroup analyses, rs231775 was also found to significantly reduce the breast cancer risk in Chinese and subgroup based on population under allelic, homozygous and recessive models.

There were 4 studies all of which were from China with 1,402 cases and 1,407 controls investigating the relationship between and breast cancer susceptibility and *CTLA-4* rs3087243 polymorphism. The results presented a significantly lower breast cancer risk in homozygous and recessive genetic models in Chinese women (AA *vs.* GG: OR = 0.68, 95% CI = 0.49-0.95, *P*=0.02, Fig.2B; AA *vs.* GG+GA: OR = 0.77, 95% CI = 0.61-0.97, *P*=0.02,).

For *CTLA-4* rs733618 polymorphism, we aseessed 4 studies containing 1,560 cases and 1,489 controls. Overall, our analysis did not suggest any association between rs733618 and breast cancer susceptibility. However, when stratifying by source of control, rs733618 was observed to increase breast cancer risk based on population in three genetic models (C *vs.* T: OR = 1.19, 95% CI = 1.05-1.34, *P*= 0.007; CC *vs.* TT: OR = 1.37, 95% CI = 1.05-1.78, *P*= 0.02; CT *vs.* TT: OR = 1.22, 95% CI = 1.02-1.47, *P*= 0.03).

Five studies involving 1656 cases and 1,625 controls investigated the breast cancer risk with *CTLA-4* rs4553808 polymorphism. We observed a higher risk in overall analysis under three

models (G *vs.* A: OR = 1.34, 95% CI = 1.16-1.53, *P*= 0.000; AG *vs.* AA: OR = 1.45, 95% CI = 1.23-1.70, *P*= 0.000; AG+GG *vs.* AA: OR = 1.43, 95% CI = 1.22-1.67, *P*= 0.000, Fig.2C). And the results were similar in Chinese subgroup. When stratifying by source of control, rs4553808 was also noted to increase breast cancer risk in allelic and heterozygous models based on population.

Heterogeneity analysis and sensitivity analysis

As presented in Table 4, no obvious heterogeneity was detected for the four *CTLA-4* polymorphisms in most of the genetic models. For the few in which existed significant heterogeneity (I^2>50%), random-effects model was applied.

Each study was sequentially removed to assess the impact of single study on the combined ORs. The result showed that the omission of any study didn't alter the overall estimations substantially, indicating that our meta-analysis results were robust (Fig.3).

Publication bias

We implemened Begg's funnel plot and Egger's test to asesess the publication bias. As shown in Fig.4, funnel plot failed to display obvious asymmetry. The Egger's test result didn't reveal any publication bias for the four SNPs in *CTLA-4* gene and breast cancer risk either (Table 5, *P*>0.05).

Discussion

It was reported that mutation in human *CTLA-4* gene resulted in quantitative reduction of CTLA-4 expression and led to a severe immunoregulatory disorder (Kuehn et al. 2014). Several investigations have suggested that particular *CTLA-4* gene polymorphisms are linked to cancer

development or progression (Erfani et al. 2006; Tang et al. 2014; Wang et al. 2007). However, the results from those studies remained conflicting. In one previous study, the author found that rs733618 and rs4553808 polymorphisms in *CTLA-4* increased the breast cancer risk whereas rs231775 and rs3087243 polymorphisms did not have significant associations with breast cancer risk(Li et al. 2012). However, in other studies, rs3087243 and rs 231775 polymorphisms were found to reduce the risk of breast cancer while rs733618 did not associated with breast cancer risk(Sun et al. 2008; Wang et al. 2007). Since CTLA-4 is important in carcinogenesis and single study does not have enough statistical power to detect the effects, we carried out this meta-analysis which synthesized the results of the included studies with a statistical analysis of the data from these studies to draw a more reliable conclusion about the association between *CTLA-4* SNPs and breast cancer susceptibility.

In present meta-analysis, we identified that *CTLA-4* rs231775 had an association with breast cancer susceptibility. We observed a significantly decreased risk in both overall and subgroup analysis in different genetic models. Some previous meta-analyses have also involved the relationship between rs231775 polymorphism and several tumor sites including breast cancer (Gao et al. 2014; Geng et al. 2014; Wang et al. 2015; Zhang et al. 2011). The results suggested the A allele of rs231775 may contribute to breast cancer susceptibility. Our result confirmed that the A allele of *CTLA-4* rs231775 polymorphism has more possibility to increase breast cancer risk than G allele. Nevertheless, our study differs from theirs because we specifically focused on breast cancer and our meta-analysis included more studies than theirs. So, our results are more reliable.

194 *CTLA-4* rs3087243 polymorphism was found to decrease breast cancer risk under
195 homozygous and recessive genetic models in Chinese. Our results implied that individuals carry
196 AA genotype are less susceptible to breast cancer than those carry GG or (GG+GA) genotypes.
197 Previous studies also found that rs3087243 was associated with breast cancer susceptibility as a
198 subgroup of several cancer sites (Yan et al. 2013; Zhao et al. 2014). The results were similar with
199 our research, but our our meta-analysis included one more study and have more statistical power.
200 We didn't find any relationship between *CTLA-4* rs733618 and breast cancer risk in the
201 overall analysis under any genetic model. However, there was a higher risk in the population-
202 based group under all the genetic models except recessive model. Considering that we selected
203 only four eligible studies and most of them were small-size sample (<500), these results need to
204 be taken with caution. One previous study found a positive signal of rs733618 polymorphism
205 with breast cancer (Li et al. 2012) while other two studies showed negative signal (Erfani et al.
206 2006; Tang et al. 2014). So further researches with larger sample size should be designed and
207 implemented to validate or refute these conclusions.
208 In contrast, *CTLA-4* rs4553808 polymorphism was realated to an increased breast cancer risk
209 in both overall and Chinese population in allelic, heterozygous and dominant models. This
210 suggested that *CTLA-4* -1661G allele is more likely to be a risk factor of breast cancer than A
211 allele. Previous meta-analysis also found rs4553808 may increase cancer risk especially for
212 breast cancer (Geng et al. 2014; Yan et al. 2013; Zhao et al. 2014). But these studies investigated
213 the association of this single SNP with various types of cancer while our study specifically
214 focused on breast cancer and investigated several SNPs. Notably, for this SNP, the *P*-value of

HWE in control of one study was less than 0.05(Erfani et al. 2006), suggesting that the study population was not representative of a broad population. Nevertheless, we decided to keep this study because deleting it did not affect the pooled ORs significantly.

Several limitations of our research should be noticed. Firstly, the sample size in this meta-analysis was relatively small, especially for rs3087243, rs733618 and rs4553808 polymorphisms. Secondly, our results need to be interpreted with caution since we did not find any studies from Europe, Africa or America and most of the included studies were from China. Therefore, more studies with large population and more ethnic groups are needed to provide sufficient statistical power. Thirdly, other factors such as environmental variants, age, and living habit are generally considered to contribute to cancer susceptibility. Lacking data of these factors for adjustment may impact the estimation of breast cancer risk. Lastly, bias may still exist because we failed to find any studies of other races and we did not have access to gray literature.

Conclusion

In summary, our meta-analysis suggests that rs231775, rs3087243 and rs4553808 polymorphisms in human *CTLA-4* gene significantly associated with breast cancer susceptibility in Asians, particularly in Chinese population. In consideration of the limitations of our work, further large-scale studies including multi-racial population are required to confirm our findings.

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Table 1(on next page)

Characteristics of the studies included in the meta-analysis.

1

First author	Year	Country	Ethnicity	Genotyping medthod	Source of control	Total sample size (case/control)	SNP
Yu	2015	China	Chinese	PCR-RFLP	PB	376/366	1;2;3;4
Minhas	2014	India	Indian	PCR-RFLP	PB	250/250	1
Li D	2012	China	Chinese	PCR-RFLP	PB	581/566	1;2;3;4
Kong	2010	China	Chinese	PCR-RFLP	HB	315/322	4
Sun	2008a	China	Chinese	PCR-RFLP	PB	1060/1070	1
Sun	2008b	China	Chinese	PCR-RFLP	PB	1037/1070	1
Li H	2008	China	Chinese	PCR-RFLP	HB	328/327	2;3
Wang	2007	China	Chinese	PCR-RFLP	PB	117/148	1;2;4
Erfani	2006	Iran	Iranian	PCR-CTPP	PB	283/245	3;4
Ghaderi	2004	Iran	Iranian	PCR-RFLP	HB	197/151	1

2 PCR: polymerase chain reaction; RFLP: restriction fragment length polymorphism; CTPP: confronting two
3 pairs primers; PB: population based; HB: hospital based; SNP: single-nucleotide polymorphism; SNP No.1:
4 +49A>G (rs231775), 2: +6230G>A (rs3087243), 3: -1722T>C (rs733618), 4: -1661A>G (rs4553808)
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Table 2(on next page)

Genotype distributions and allele frequencies of *CTLA-4* polymorphisms in cases and controls.

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Study	Genotype (N)								Allele Frequency (N)				*P* of HWE
	Case				Control				Case		Control		
	total	AA	AB	BB	total	AA	AB	BB	A	B	A	B	
+49A>G (rs231775)													
Yu 2015	376	174	175	27	366	174	157	35	523	229	505	227	0.96
Minhas2014	250	111	113	26	250	105	121	24	335	165	331	169	0.20
Li D 2012	576	49	281	246	553	54	243	256	379	773	351	755	0.74
Sun 2008a	1060	101	485	474	1070	65	446	559	660	1406	576	1564	0.15
Sun 2008b	1037	100	455	482	1070	73	451	546	655	1419	597	1543	0.12
Wang 2007	117	48	59	10	148	55	70	23	155	79	180	116	0.93
Ghaderi2004	197	84	104	9	151	60	72	19	272	122	192	110	0.72
+6230G>A (rs3087243)													
Yu 2015	376	257	110	9	366	252	103	11	624	128	607	125	0.90
Li D 2012	581	361	197	23	566	361	182	23	919	243	904	228	0.99
Li H 2008	328	32	124	172	327	20	114	193	188	468	154	500	0.57
Wang 2007	117	24	47	46	148	18	56	74	95	139	92	204	0.16
-1722T>C (rs733618)													
Yu 2015	376	123	186	67	366	137	166	63	432	320	440	292	0.30
Li D 2012	574	184	276	114	551	207	256	88	644	504	670	432	0.55
Li H 2008	328	125	163	40	327	111	168	48	413	243	390	264	0.22
Erfani 2006	282	225	54	3	245	204	41	0	504	60	449	41	0.15
-1661A>G (rs4553808)													
Yu 2015	376	273	91	12	366	281	78	7	637	115	640	92	0.56
Li D 2012	574	405	153	16	551	425	115	11	963	185	965	137	0.33
Kong 2010	315	204	105	6	322	241	76	5	513	117	558	86	0.72
Wang 2007	109	62	45	2	148	111	35	2	169	49	257	39	0.68
Erfani 2006	282	211	65	6	238	184	43	11	487	77	411	65	0.001

2 A: the major allele; B: the minor allele; HWE: Hardy-Weinberg equilibrium

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Table 3(on next page)

Meta-analysis results of *CTLA-4* polymorphisms and BC risk

SNP	B vs A		BB vs AA		AB vs AA		BB vs AA+AB		AB+BB vs AA	
	OR (95%CI)	*P*	OR (95%CI)	*P*	OR (95%CI)	*P*	OR (95%CI)	*P*	OR (95%CI)	*P*
+49A>G (rs231775)										
Overall	0.86 (0.80-0.92)	**0.000**	0.68 (0.57-0.81)	**0.000**	0.92 (0.80-1.06)	0.23	0.79 (0.71-0.87)	**0.000**	0.85 (0.74-0.97)	**0.02**
Chinese	0.85 (0.79-0.92)	**0.000**	0.68 (0.56-0.82)	**0.000**	0.92 (0.73-1.16)	0.49	0.79 (0.71-0.88)	**0.000**	0.84 (0.65-1.08)	0.17
PB	0.86 (0.80-0.93)	**0.000**	0.70 (0.59-0.84)	**0.000**	0.91 (0.78-1.05)	0.19	0.80 (0.72-0.89)	**0.000**	0.85 (0.69-1.04)	0.12
+6230G>A (rs3087243)										
Chinese	0.87 (0.71-1.07)	0.20	0.68 (0.49-0.95)	**0.02**	0.99 (0.83-1.19)	0.94	0.77 (0.61-0.97)	**0.02**	0.87 (0.65-1.17)	0.36
PB	0.91 (0.71-1.17)	0.48	0.75 (0.50-1.12)	0.15	1.03 (0.85-1.25)	0.76	0.77 (0.54-1.09)	0.14	1.00 (0.82-1.20)	0.99
-1722T>C (rs733618)										
Overall	1.09 (0.93-1.29)	0.29	1.15 (0.79-1.68)	0.47	1.13 (0.96-1.32)	0.15	1.11 (0.90-1.37)	0.32	1.14 (0.98-1.33)	0.09
Chinese	1.07 (0.88-1.29)	0.51	1.12 (0.77-1.63)	0.55	1.12 0.94-1.33()	0.22	1.10 (0.89-1.35)	0.39	1.11 (0.86-1.43)	0.43
PB	1.19 (1.05-1.34)	**0.007**	1.37 (1.05-1.78)	**0.02**	1.22 (1.02-1.47)	**0.03**	1.21 (0.96-1.54)	0.11	1.26 (1.06-1.50)	**0.01**
-1661A>G (rs4553808)										
Overall	1.34 (1.16-1.53)	**0.000**	1.22 (0.78-1.92)	0.38	1.45 (1.23-1.70)	**0.000**	1.12 (0.72-1.76)	0.61	1.43 (1.22-1.67)	**0.000**
Chinese	1.41 (1.21-1.63)	**0.000**	1.59 (0.95-2.67)	0.08	1.47 (1.24-1.75)	**0.000**	1.45 (0.86-2.43)	0.16	1.48 (1.25-1.75)	**0.000**
PB	1.30 (1.11-1.52)	**0.001**	1.19 (0.73-1.94)	0.48	1.40 (1.17-1.68)	**0.000**	1.11 (0.68-1.80)	0.68	1.38 (1.16-1.64)	**0.000**
HWE	1.41 (1.21-1.63)	**0.000**	1.59 (0.95-2.67)	0.08	1.47 (1.24-1.75)	**0.000**	1.45 (0.86-2.43)	0.16	1.48 (1.25-1.75)	**0.000**

A: the major allele; B: the minor allele; CI: confidence interval; OR: odds ratio; PB: population based; HB: hospital based; SNP: single-nucleotide polymorphism; HWE: subgroup excluding the study departing from HWE

Table 4(on next page)

Heterogeneity-analysis results of *CTLA-4* polymorphisms and BC risk

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SNP	B vs A			BB vs AA			AB vs AA			BB vs AA+AB			AB+BB vs AA		
	I^2	*P*	EM	I^2	*P*	EM	I^2	*P*	EM	I^2	*P*	EM	I^2	*P*	EM
+49A>G (rs231775)															
Overall	0%	0.46	F	45%	0.09	F	29%	0.21	F	27%	0.22	F	41%	0.12	F
Chinese	12%	0.34	F	40%	0.15	F	51%	0.09	R	0%	0.59	F	60%	0.04	R
PB	6%	0.38	F	39%	0.14	F	39%	0.15	F	0%	0.56	F	51%	0.07	R
+6230G>A (rs3087243)															
Chinese	58%	0.07	R	8%	0.36	F	0%	0.38	F	0%	0.78	F	53%	0.09	R
PB	60%	0.08	R	24%	0.27	F	16%	0.31	F	0%	0.58	F	46%	0.16	F
-1722T>C (rs733618)															
Overall	52%	0.10	R	51%	0.10	R	7%	0.36	F	35%	0.22	F	39%	0.18	F
Chinese	64%	0.06	R	59%	0.09	R	37%	0.21	F	31%	0.23	F	57%	0.10	R
PB	0%	0.74	F	0%	0.45	F	0%	0.99	F	0%	0.37	F	0%	0.98	F
-1661A>G (rs4553808)															
Overall	27%	0.24	F	9%	0.35	F	14%	0.32	F	6%	0.37	F	24%	0.26	F
Chinese	0%	0.48	F	0%	0.99	F	32%	0.22	F	0%	0.98	F	24%	0.27	F
PB	39%	0.18	F	31%	0.23	F	27%	0.25	F	30%	0.24	F	34%	0.21	F
HWE	0%	0.48	F	0%	0.99	F	32%	0.22	F	0%	0.98	F	24%	0.27	F

2 EM: Effects model; F: fixed effects model; R: random effects model; PB: population based; HB: hospital
3 based; SNP: single-nucleotide polymorphism; HWE: subgroup excluding the study departing from HWE

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Table 5(on next page)

Egger's test result of *CTLA-4* polymorphisms and BC risk based on allele frequency

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SNP	Coefficient	SE	t	*P*	95% CI
rs231775	0.71	1.13	0.63	0.557	-2.19 - 3.61
rs3087243	-5.19	3.20	-1.62	0.246	-18.94 - 0.56
rs733618	-0.03	3.17	-0.01	0.993	-13.67 - 13.61
rs4553808	1.26	2.82	0.45	0.686	-7.70 - 10.21

2 SNP: single-nucleotide polymorphism; 95% CI: 95% confidence interval;
3 SE: standard error
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Figure 1

Flow chart of the studies selection

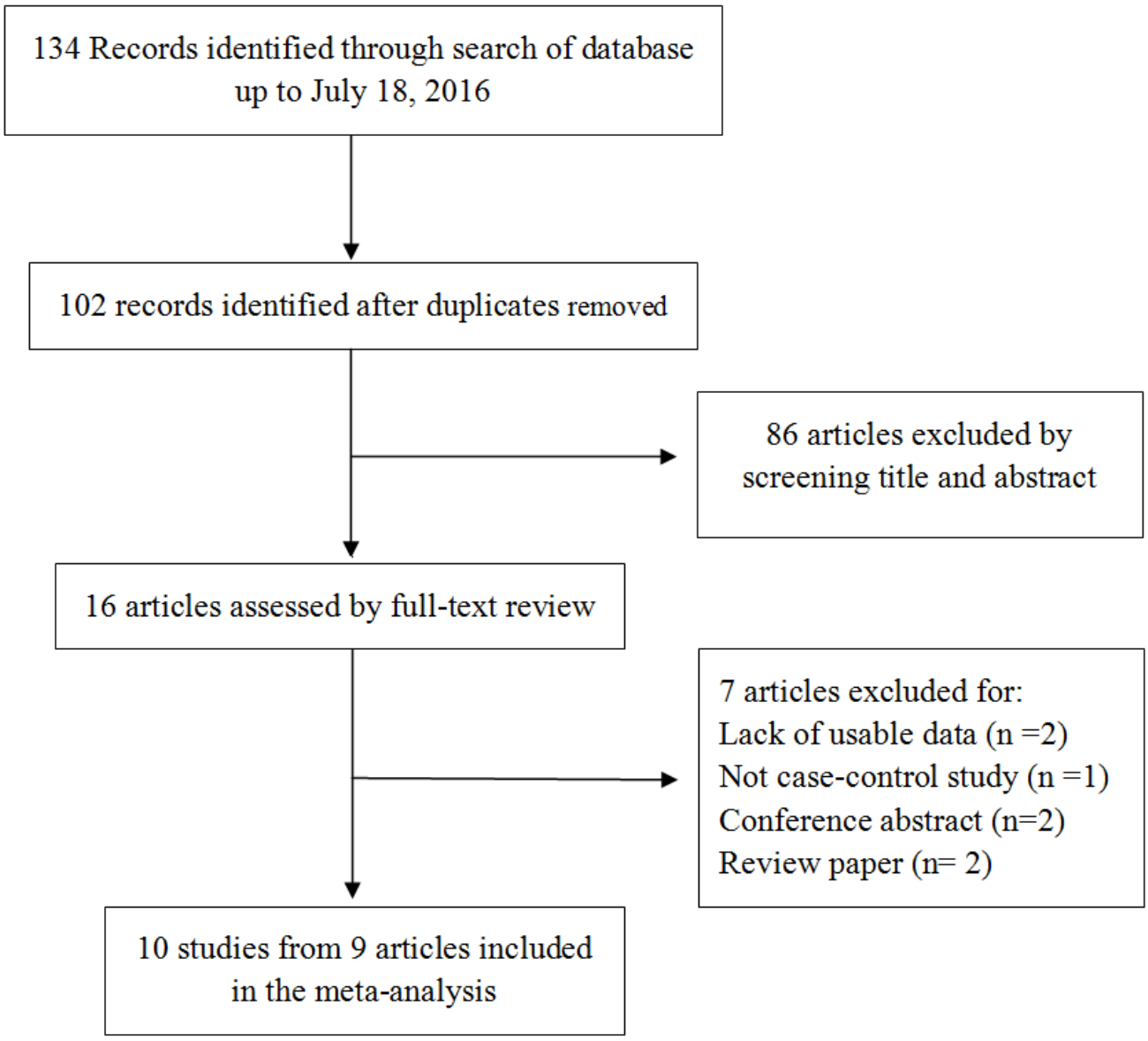

Figure 2

Forest plots of *CTLA-4* polymorphisms and breast cancer risk

A) rs231775 under G *vs.* A; B) rs3087243 under AA *vs.* GG; C) rs4553808 under AG+GG *vs.* AA. The squares and horizontal lines correspond to the study-specific OR and 95% CI. The area of the squares reflects the weight (inverse of the variance). The diamond represents the summary OR and 95% CI.

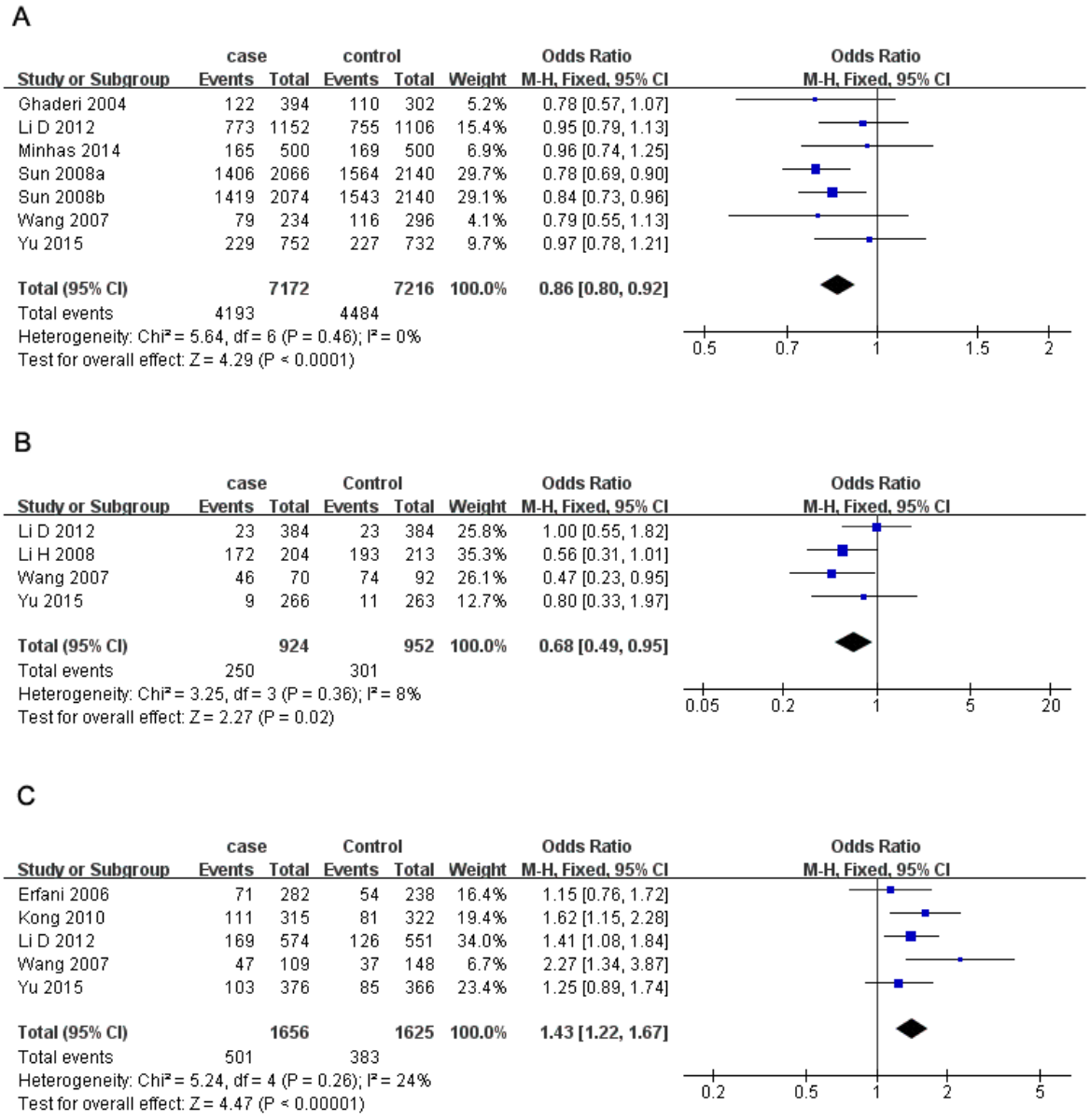

Figure 3

Sensitivity analysis of *CTLA-4* polymorphisms and breast cancer risk

A) rs231775 under G *vs.* A; B) rs3087243 under AA *vs.* GG+GA; C) rs733618 under TC *vs.* TT; D) rs4553808 under G *vs.* A. Each point represents the pooled OR after omitting single study in left column. The two ends of the dotted lines represent the 95% CI.

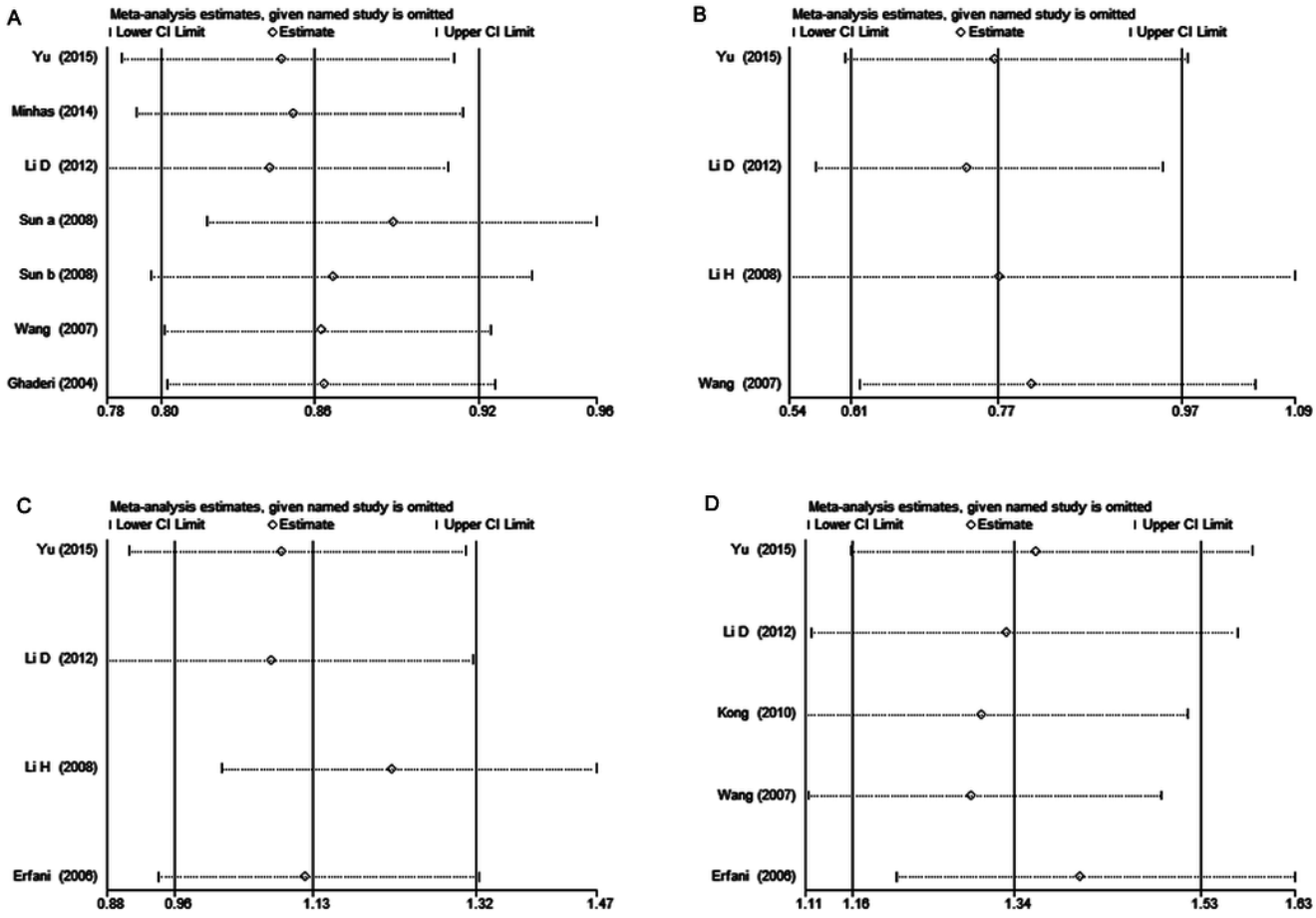

Figure 4

Begg's funnel plots of publication bias for the association of *CTLA-4* polymorphisms and breast cancer risk

A) rs231775, B) rs3087243, C) rs733618, D) rs4553808 under allelic model. Each point represents a single study for the indicated association.