J Chin Med Assoc

ORIGINAL ARTICLE

Cytotoxic T-lymphocyte antigen 4 polymorphisms and breast cancer susceptibility: Evidence from a meta-analysis

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Abstract

Background: Cytotoxic T-lymphocyte antigen 4 (CTLA-4) is an immune checkpoint and regulates the immune function of T cells. However, previous findings regarding the association of CTLA-4 polymorphisms and breast cancer remain inconclusive. Therefore, we performed a meta-analysis to investigate the potential effects of five polymorphisms (–1722 T/C, –1661 A/G –318 C/T, +49 A/G, and CT60 A/G) in the *CTLA-4* gene on breast cancer susceptibility.

Methods: Relevant literatures were systematically searched through electronic databases including PubMed, EMBASE, and Web of Science up to October 10, 2021. Available data were extracted and odds ratios (ORs) with 95% confidence intervals were used to estimate the pooling effect size. The Newcastle-Ottawa Scale was applied for assessing the quality of included studies. We conducted subgroup analyses based on ethnicity and control sources to explore levels of heterogeneity. Moreover, sensitivity analysis and publication bias were assessed.

Results: Finally, a total of 12 eligible studies regarding CTLA-4 polymorphisms and breast cancer were included. For overall analyses, only the +49 A/G polymorphism was significantly associated with breast cancer under allelic (OR = 1.19), dominant (OR = 1.27), and recessive (OR = 1.27) models. Ethnicity-based subgroup analysis found that the +49 A/G polymorphism has a significant risk (OR = 2.03) of breast cancer under the recessive model in the non-Asian population. Studies with hospital-based controls showed that the +49 A/G polymorphism has significant breast cancer risks under allelic (OR = 1.44), dominant (OR = 1.86), and recessive (OR = 1.60) models. In addition, those with population-based controls found that –1722 T/C polymorphism has a significant breast cancer risk under allelic (OR = 1.19) and dominant (OR = 1.26) models.

Conclusion: This meta-analysis suggested that CTLA-4 + 49 A/G polymorphism may significantly associate with breast cancer susceptibility. Future studies containing various populations are helpful for evaluating the impacts of CTLA-4 polymorphisms on breast cancer susceptibility.

Keywords: Breast cancer; Cytotoxic T-lymphocyte antigen 4; Immune checkpoint; Meta-analysis; Polymorphism

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Conflicts of interest: Dr. Shih-Hwa Chiou, an editorial board member at the Journal of the Chinese Medical Association, had no role in the peer review process of or decision to publish this article. The other authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article

Journal of Chinese Medical Association. (2023) 86: 207-219.

Received February 21, 2022; accepted June 9, 2022.

doi: 10.1097/JCMA.0000000000000851.

1. INTRODUCTION

Breast cancer is the leading cancer in women globally, with an estimated 2.3 million new cases in 2020.[1] The increased incidence of breast cancer indicates a high prevalence of reproductive and hormonal risk factors, lifestyle risk factors, as well as many genetic variants.[2,3] Breast cancer treatment often consists of a combination of surgery, radiation, hormone therapy, and chemotherapy. The aim of an adjuvant systemic therapy is to improve disease-free survival and overall survival,[4] whereas neoadjuvant systemic therapies aim to not only increase breast conservation but also improve prognosis if the tumor shows a complete pathologic response.[5] However, studies have shown that after 5 years of adjuvant endocrine therapy, a 10% to 41% persistent risk of distant recurrence exists for at least 20 years after the initial diagnosis.[6] Therefore, new treatment

combinations are in demand to prolong the survival of patients and reduce the recurrence rate.

Researchers in the field of cancer immunotherapy found that the immune system, in particular T cells, is capable of attacking cancer cells.[7] Cytotoxic T-lymphocyte antigen 4 (CTLA-4), also called CD152, is expressed mainly on activated T cells. As one of the most fundamental immunosuppressive cytokines, it acts as an immune checkpoint to inhibit T-cell proliferation and activation.[8] Structurally, both CTLA-4 and CD28 form membrane-bound homodimers, and therefore, they bind to the same ligands, namely B7-1 (CD80) and B7-2 (CD86), which are expressed by antigen-presenting cells.[9] However, CTLA-4 has a higher affinity than CD28 for the B7 ligands of T cells, representing its role in maintaining immunological self-tolerance and immune homeostasis.[10] Numerous studies have demonstrated that an immune checkpoint blockade of CTLA-4 can unleash a therapeutic response of T cells against cancer.[11,12] This indicates that the blockade of the immune checkpoint CTLA-4 may be a valuable cancer immunotherapeutic approach.

The *CTLA-4* gene is located on chromosome 2q33 and is composed of four exons that possess several vital single-nucleotide polymorphisms (SNPs).[13] Among the CTLA-4 polymorphisms, +49A>G (rs231775) in exon 1; −1661A>G (rs4553808), −318C>T (rs5742909), and −1722T>C (rs733618) in the promoter region; and CT60A>G (rs3087243) in the 3′-untranslated region were widely studied and have been reported to be associated with susceptibility to autoimmune disease and various cancers.[14] Numerous epidemiological studies have been performed to assess the possible interaction between the *CTLA-4* gene polymorphism and breast cancer susceptibility.[15–26] In one previous study (Li et al, 2012), rs733618 and rs4553808 polymorphisms in CTLA-4 increased the breast cancer risk. In other studies (Sun et al, 2008; Wang et al, 2007), rs3087243 and rs231775 polymorphisms were found to reduce the risk of breast cancer. However, studies with small sample sizes do not have enough statistical power to detect a true effect at all.

Recent studies have focused on the association between CTLA-4 polymorphisms and the risk of breast cancer. A meta-analysis of 10 studies[27] concluded that the rs231775, rs3087243, and rs4553808 polymorphisms in CTLA-4 are significantly associated with breast cancer. However, the results of the relationship between multiple CTLA-4 genetic polymorphisms and breast cancer are still inconclusive. Therefore, we performed this updated meta-analysis on all published case-control studies to derive a reliable evaluation of the relationship between CTLA-4 polymorphisms and breast cancer susceptibility.

2. METHODS

2.1. Search strategy

This systematic review and meta-analysis were conducted in accordance with the 2020 Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines. Our review protocol was registered on PROSPERO (registration number CRD 42022291552). We searched the electronic databases PubMed, EMBASE, and Cochrane Library without any language restriction from their inception date until August 2021. For selecting eligible studies, the keywords "Cytotoxic T lymphocyte antigen 4," "CTLA-4" or "CD152," "breast cancer," and "polymorphism" were searched in the title and abstract.

2.2. Inclusion and exclusion criteria

The inclusion criteria were as follows: (1) a case-control study evaluating the association between CTLA-4 polymorphisms and breast cancer risk; (2) studies included healthy subjects as controls; and (3) genotype data for cases and controls were available. We excluded articles that met at least one of the following criteria: (1) study types other than case-control studies; (2) animal research; and (3) insufficient genotype data. Titles and abstracts of potentially eligible studies were screened, and their full texts were retrieved and reviewed based on the eligibility criteria.

2.3. Data extraction and quality assessment

All relevant data were independently extracted from the included studies by two authors (H.Y. Chang and Y.L. Lo). The extracted data included the first author, publication year, country, ethnicity, sources of controls, number of cases and controls, genotype data of cases and controls, and genotyping method. Any disagreement was resolved through discussion or further consultation with a senior author (Y.H. Wang) to achieve a final decision.

The methodological quality of included studies was assessed using the Newcastle-Ottawa Scale. All included studies were judged on three perspectives: selection of the study groups; comparability of the groups; and ascertainment of exposure and outcomes of interest.

2.4. Statistical analysis

The strength of the association between CTLA-4 polymorphisms and breast cancer susceptibility was estimated using an odds ratio (OR) with a 95% confidence interval (CI). The statistical significance of the pooled OR was estimated using the Z test. Pooled ORs were calculated using the following genetic models: allelic comparison, dominant model, and recessive model. A goodness-of-fit χ^2 test with Hardy-Weinberg equilibrium was conducted to determine whether genotype frequencies deviated for each SNP of CTLA-4 in the controls. Between-study heterogeneity was evaluated using the Cochran Q-test. A random-effects model was used to calculate a pooled OR when significant heterogeneity was observed (p-value of Q-test < 0.05 or I^2 > 50%), otherwise, a fixed-effects model was selected. In case of concerns regarding heterogeneity, sensitivity analyses were performed with exclusion of one study in turns to assess the consistency and stability of the meta-analysis. In addition, a subgroup analysis according to ethnicity and source of control was conducted to assess the possible causes of heterogeneity. Both the funnel plot and Egger's test were used to assess publication bias. All statistical analyses were implemented using Review Manager Version 5.4 (Cochrane Collaboration, London, United Kingdom).

3. RESULTS

3.1. Characteristics of included studies

The initial keyword-based literature search of PubMed, Embase, and Cochrane Library yielded 126 results (Fig. 1). After duplicates and studies that did not meet our inclusion criteria were removed, 12 studies with 4786 cases and 4833 controls were finally included in the present meta-analysis. The baseline characteristics of the included studies are summarized in Table 1, which lists 13 studies. However, two of the 13 studies (Sun-North and South) were from the same article discussing two areas of the Chinese population. Although we extracted the data separately, we still describe a total of 12 studies in the later description of our manuscript. All the included studies were published between 2004 and 2020. The number of study participants ranged from a minimum of 155 to a maximum of 2130 across studies. Among the 12 studies, 10 studies involved Asian participants (five studies from East Asia, three from West Asia, and two from South Asia), one involved African participants (Babteen 2020),[20] and one involved European participants (Isitmangil 2016).

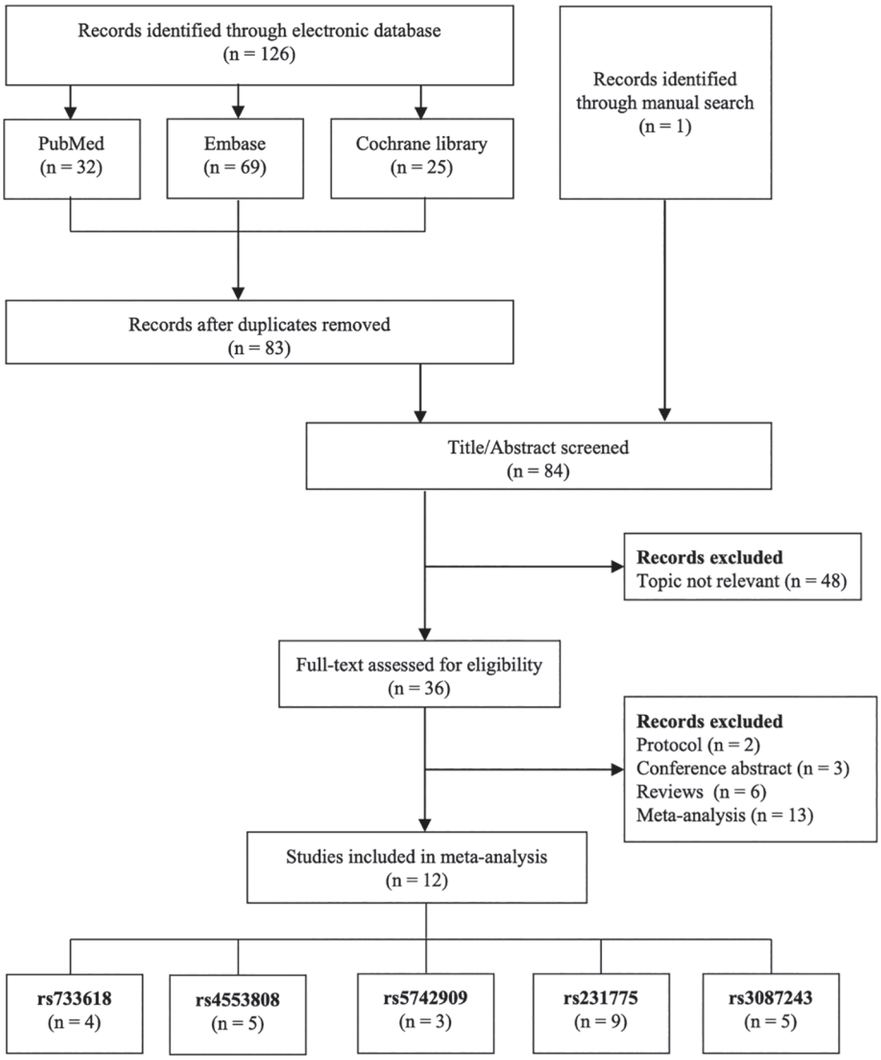

Fig. 1 Flowchart of the study selection process.

Table 1

Characteristics of studies included in the meta-analysis

Author (Year)	Country	Ethnicity	Sample Size (Case/Control)	Source of Control	Genotyping Method	Included SNPs
Babteen (2020)[20]	Egypt	Africa	93/179	HB	TaqMan	rs231775
Erfani (2006)[15]	Iran	West Asia	283/245	PB	PCR-RFLP/PCR-ARMS	rs733618, rs4553808, rs5742909
Farbod (2015)[16]	Iran	West Asia	100/100	PB	PCR-RFLP	rs4553808
Ghaderi (2004)[17]	Iran	West Asia	197/151	HB	PCR-RFLP	rs231775
Goske (2017)[18]	India	South Asia	285/285	HB	PCR-RFLP	rs3087243
Isitmangil (2016)[19]	Turkey	Europe	79/76	HB	PCR-RFLP	rs5742909, rs231775
Li H (2008)[21]	China	East Asia	328/327	HB	PCR-RFLP	rs733618, rs3087243
Li D (2012)[22]	China	East Asia	581/566	PB	PCR-RFLP	rs733618, rs4553808, rs231775, rs3087243
Minhas (2014)[23]	India	South Asia	250/250	PB	PCR-RFLP	rs231775
Sun (2008)—North[24]	China	East Asia	1060/1070	PB	PCR-RFLP	rs231775
Sun (2008)—South[24]	China	East Asia	1037/1070	PB	PCR-RFLP	rs231775
Wang (2007)[25]	China	East Asia	117/148	PB	PCR-RFLP	rs4553808, rs5742909, rs231775, rs3087243
Yu (2015)[26]	China	East Asia	376/366	PB	PCR-RFLP	rs733618, rs4553808, rs231775, rs3087243

ARMS = amplification-refractory mutation system; HB = hospital based; PB = population based; PCR = polymerase chain reaction; RFLP = restriction fragment length polymorphism; SNP = single-nucleotide polymorphism.

Population-based (PB) controls were enrolled in seven case-control studies, whereas hospital-based (HB) controls were enrolled in five studies. Most of the studies performed the classic polymerase chain reaction–restriction fragment length polymorphism assay for SNP genotyping. Five CTLA-4 polymorphisms, namely, –1772T>C (rs733618), –1661A>G (rs4553808), –318C>T (rs5742909), +49A>G (rs231775), and CT60G>A (rs3087243), genotype distributions, and allele frequencies are listed in Table 2. Among the 12 studies, 3 presented a mild deviation from Hardy-Weinberg equilibrium (HWE) (two studies on rs4553808 [Erfani 2006, Farbod 2015] and one study on rs3087243 [Goske 2017]), which we further performed sensitivity analysis and decided to keep them in our later meta-analysis due to compatible results. Methodological quality assessment of eligible studies with a Newcastle-Ottawa Scale score is detailed in Table 3. In terms of quality scores, all studies were of high quality.

3.2. Meta-analysis for the associations of CTLA-4 polymorphisms and breast cancer

The pooled ORs of the association between CTLA-4 polymorphisms and the risk of breast cancer are summarized in Table 4. A random-effects model was applied because of the significant heterogeneity in genetic comparison models. To assess the potential influence of these study characteristics on the results, we performed a subgroup analysis stratified based on ethnicity.

3.3. CTLA-4 rs733618 (–1722 T/C) polymorphism

The association between the rs733618 polymorphism and breast cancer risk was analyzed in four studies with 1568 cases and 1504 controls. No significantly increased risk of breast cancer was observed in any genetic comparison (Table 4 and Supplementary Fig. S1, http://links.lww.com/JCMA/A170). No significant association was observed between the rs733618 polymorphism and breast cancer risk among any specific ethnic group. However, subgroup analysis of control sources revealed that three studies based on PB showed an increased risk of breast cancer (allelic model OR = 1.19, 95% CI, 1.05-1.34; and dominant model OR = 1.26, 95% CI, 1.06-1.50) but not in studies based on HB (Table 4 and Supplementary Fig. S2, http://links.lww.com/JCMA/A170).

3.4. CTLA-4 rs4553808 (–1661 A/G) polymorphism

The association between the rs4553808 polymorphism and breast cancer risk was analyzed in five studies with 1457 cases and 1425 controls. No statistical evidence was observed between the rs4553808 polymorphism and breast cancer risk in any genetic model (Table 4 and Supplementary Fig. S3, http://links.lww.com/JCMA/A170). When data were stratified based on ethnicity, associations of the rs4553808 polymorphism with increased risk of breast cancer were found to be significant in the East Asian group (allelic model: OR = 1.39, 95% CI, 1.15-1.68) but not in the West Asian group. A subgroup analysis based on the source of controls was not performed due to insufficient studies (Table 4 and Supplementary Fig. S4, http://links.lww.com/JCMA/A170).

3.5. CTLA-4 rs5742909 (–318 C/T) polymorphism

The association between the rs5742909 polymorphism and breast cancer risk was analyzed in three studies with 479 cases and 469 controls. No significant association was found between the rs5742909 polymorphism and breast cancer risk in any genetic model (Table 4 and Supplementary Fig. S5, http://links.lww.com/JCMA/A170). Subgroup analyses were not performed due to insufficient studies.

3.6. CTLA-4 rs231775 (+49 A/G) polymorphism

For the CTLA-4 rs231775 polymorphism, nine studies involving 3790 cases and 3876 controls were considered. The pooled analysis showed that the rs231775 polymorphism increased the risk of breast cancer under the allelic (OR = 1.19, 95% CI, 1.10-1.30), dominant (OR = 1.27, 95% CI, 1.14-1.42), and recessive models (OR = 1.27, 95% CI, 1.05-1.53; Fig. 2). In the subgroup analysis, the associations between the CTLA-4 rs231775 polymorphism and breast cancer susceptibility were found to be significant in the Asian group (allelic model: OR = 1.17, 95% CI, 1.09-1.26; and dominant model: OR = 1.28, 95% CI, 1.11-1.47) and in the non-Asian group (allelic model: OR = 1.61, 95% CI, 1.18-2.19; and dominant model: OR = 2.03, 95% CI, 1.36-3.02). Individuals had a significantly increased risk of breast cancer in the PB control group (allelic model: OR = 1.16, 95% CI, 1.07-1.26; and dominant model: OR = 1.25, 95% CI, 1.13-1.39) and in the HB control group (allelic model: OR = 1.44, 95% CI, 1.15-1.79; dominant model: OR = 1.86, 95% CI, 1.03-3.38; and recessive model: OR = 1.60, 95% CI, 1.06–2.43; Table 4 and Fig. 3).

3.7. CTLA-4 rs3087243 (CT60 A/G) polymorphism

The association between the rs3087243 polymorphism and breast cancer risk was analyzed in five studies with 1687 cases and 1692 controls. The pooled results showed no significant association between the CTLA-4 rs3087243 polymorphism and breast cancer risk (Table 4 and Supplementary Fig. S6, http://links.lww.com/JCMA/A170). In the subgroup analysis, associations with breast cancer risk were found to be significant in the East Asian group (dominant model OR = 1.30, 95% CI, 1.03-1.64) but not in the West Asian group. No statistical evidence of association was observed in the stratification based on either PB or HB controls (Table 4 and Supplementary Fig. S7, http://links.lww.com/JCMA/A170).

3.8. Sensitivity analysis and publication bias

Significant heterogeneity was observed in genetic comparison models. We performed sensitivity analyses using the leave-one-out approach, and the results were similar to those of studies in which controls violating the HWE were excluded. All the results of sensitivity analyses indicated that the pooled ORs for CTLA-4 polymorphisms and lung cancer susceptibility were not essentially changed with the exclusion of each study in turn by using various genetic models, which suggests that our overall results were robust.

Regarding the evaluation of potential publication bias of selected studies in the present study, we observed that the shapes of funnel plots displayed no evidence of asymmetry among all genetic models (Supplementary Fig. S8, http://links.lww.com/JCMA/A170). In addition, we performed the Egger's test and found no significant publication bias ($p = 0.582$).

4. DISCUSSION

CTLA-4 acts as an immune checkpoint that suppresses the immune response, and this characteristic may prevent cancer cells from being attacked by the immune system in cancer. SNPs are the most common forms of genetic variations, and their mutations modulate cancer predisposition through the alteration of the expression level or function of a certain gene.

CTLA-4 polymorphisms are associated with susceptibility to various cancers,[28] that is, particular *CTLA-4* gene variants are associated with cancer development. Previous meta-analysis by Feng et al.[29] included evidence from 67 studies have indicated that three SNPs, namely, rs231775(49 A>G), rs4553808(–1661 A>G), and rs5742909(–318 C>T), are significantly related to

Table 2

Genotype distribution and allele frequencies of CTLA-4 polymorphisms in the included literatures

First Author (Year)	Cases				Controls				HWE[a]
	Genotypes (n)			Allele (%)	Genotypes (n)			Allele (%)	(*p*)
−1722 T>C (rs733618)	T/T	T/C	C/C	C	T/T	T/C	C/C	C	
Erfani (2006)	225	54	3	10.64	204	41	0	8.37	0.360
Li H (2008)	125	163	40	37.04	111	168	48	40.37	0.479
Li D (2012)	184	276	114	43.90	207	256	88	39.20	0.838
Yu (2015)	123	186	67	42.55	137	166	63	39.89	0.584
−1661 A>G (rs4553808)	A/A	A/G	G/G	G	A/A	A/G	G/G	G	
Erfani (2006)	211	65	6	13.65	184	43	11	13.66	0.001
Farbod (2015)	43	51	6	31.50	28	69	3	37.50	<0.001
Li D (2012)	405	153	16	16.11	425	115	11	12.43	0.623
Wang (2007)	62	45	2	22.48	111	35	2	13.18	0.920
Yu (2015)	273	91	12	15.29	281	78	7	12.57	0.845
−318 C>T (rs5742909)	C/C	C/T	T/T	T	C/C	C/T	T/T	T	
Erfani (2006)	244	38	1	7.07	206	31	4	8.09	0.111
Isitmangil (2016)	68	9	2	8.23	63	11	2	9.87	0.267
Wang (2007)	84	33	0	14.10	129	19	0	6.42	0.706
49 A>G (rs231775)	A/A	A/G	G/G	G	A/A	A/G	G/G	G	
Babteen (2020)	51	35	7	26.34	67	92	20	36.87	0.379
Ghaderi (2004)	84	104	9	30.96	60	72	19	36.42	0.936
Isitmangil (2016)	49	24	6	22.78	34	36	6	31.58	0.704
Li D (2012)	49	281	246	67.10	54	243	256	68.26	0.946
Minhas (2014)	111	113	26	33.00	105	121	24	33.80	0.436
Sun (2008) (North)	101	485	474	67.59	65	446	559	73.08	0.151
Sun (2008) (South)	100	455	482	68.42	73	451	546	72.10	0.296
Wang (2007)	48	59	10	33.76	55	70	23	39.19	0.996
Yu (2015)	174	175	27	30.45	174	157	35	31.01	0.999
CT60 G>A (rs3087243)	G/G	G/A	A/A	A	G/G	G/A	A/A	A	
Goske (2017)	71	197	17	40.53	74	202	9	38.60	<0.001
Li H (2008)	32	124	172	71.34	20	114	193	76.45	0.848
Li D (2012)	361	197	23	20.91	361	182	23	20.14	1.000
Wang (2007)	24	47	46	59.40	18	56	74	68.92	0.364
Yu (2015)	257	110	9	17.02	252	103	11	17.08	0.993

HWE = Hardy-Weinberg equilibrium.

[a]*p*-value of Hardy-Weinberg equilibrium for the control group.

Table 3

Quality assessment of the included case-control studies with Newcastle-Ottawa Scale

First Author (Year)	Selection				Comparability		Exposure			Total score
	a	b	c	d	e	f	g	h	i	
Babteen (2020)	*	*	-	*	*	*	*	*	*	8
Erfani (2006)	*	*	*	*	*	*	*	*	*	9
Farbod (2015)	-	*	*	*	-	*	*	*	*	7
Ghaderi (2004)	*	*	-	*	*	*	*	*	*	8
Goske (2017)	*	*	-	*	*	*	*	*	*	8
Isitmangil (2016)	*	*	-	*	-	*	*	*	*	7
Li H (2008)	*	*	-	*	-	*	*	*	*	7
Li D (2012)	*	*	*	*	*	*	*	*	*	9
Minhas (2014)	-	*	*	-	*	*	-	*	*	6
Sun (2008) (N)	-	*	*	*	*	*	*	*	*	8
Sun (2008) (S)	-	*	*	*	*	*	*	*	*	8
Wang (2007)	*	*	*	*	*	*	*	*	*	9
Yu (2015)	*	*	*	*	-	*	*	*	*	8

a.Adequacy of the case definition.
b.Representativeness of the cases.
c.Selection of controls.
d.Definition of controls.
e.Study controls for ethnicity.
f.Study controls for any additional factor.
g.Ascertainment of exposure.
h.Same method of ascertainment for cases and controls.
i.Non-response rate.

Table 4

Overall and subgroup analyses for *CTLA-4* gene polymorphisms and breast cancer

			Allele Model		Dominant Model		Recessive Model	
Polymorphism	**Subgroup**	**No. of Studies**	**OR (95%CI)**	***p***	**OR (95%CI)**	***p***	**OR (95%CI)**	***p***
–1722 T>C (rs733618)			C vs T		CC + TC vs TT		CC vs TC + TT	
	Overall ethnicity	4	1.09 (0.93-1.29)	0.29	1.14 (0.93-1.39)	0.22	1.09 (0.82-1.43)	0.56
	East Asia	3	1.07 (0.88-1.29)	0.51	1.11 (0.86-1.43)	0.43	1.07 (0.82-1.40)	0.60
	West Asia	1	1.30 (0.86-1.98)	0.21	1.26 (0.81-1.96)	0.31	6.15 (0.32-119.62)	0.23
	Controls							
	PB	3	**1.19 (1.05-1.34)**	**0.008**	**1.26 (1.06-1.50)**	**0.01**	1.21 (0.95-1.53)	0.12
	HB	1	0.87 (0.70-1.09)	0.22	0.83 (0.61-1.15)	0.27	0.81 (0.51-1.27)	0.35
-1661 A>G (rs4553808)			G vs A		GG + AG vs AA		GG vs AG + AA	
	Overall Ethnicity	5	1.20 (0.94-1.53)	0.15	1.10 (0.74-1.64)	0.64	1.19 (0.70-2.04)	0.52
	East Asia	3	**1.39 (1.15-1.68)**	**0.0006**	1.33 (0.79-2.23)	0.28	1.50 (0.85-2.67)	0.16
	West Asia	2	0.89 (0.68-1.17)	0.41	0.79 (0.36-1.73)	0.56	0.89 (0.20-3.91)	0.87
-318 C>T (rs5742909)			C vs T		CC + TC vs TT		CC vs TC + TT	
	Overall	3	0.83 (0.42-1.67)	0.61	2.06 (0.46-9.17)	0.34	0.78 (0.37-1.65)	0.52
+49 A>G (rs231775)			A vs G		AA + AG vsGG		AA vs AG + GG	
	Overall Ethnicity	9	**1.19 (1.10-1.30)**	**<.0001**	**1.27 (1.14-1.42)**	**<.0001**	**1.27 (1.05-1.53)**	**0.01**
	Asia	7	**1.17 (1.09-1.26)**	**<.0001**	**1.28 (1.11-1.47)**	**0.0005**	1.17 (0.98-1.40)	0.08
	Non-Asia	2	**1.61 (1.18-2.19)**	**0.003**	1.34 (0.65-2.73)	0.43	**2.03 (1.36-3.02)**	**0.0005**
	Controls							
	PB	6	**1.16 (1.07-1.26)**	**0.0005**	**1.25 (1.13-1.39)**	**<.0001**	1.18 (0.96-1.45)	0.12
	HB	3	**1.44 (1.15-1.79)**	**0.001**	**1.86 (1.03-3.38)**	**0.04**	**1.60 (1.06-2.43)**	**0.03**
CT60 G>A (rs3087243)			G vs A		GG + AG vs AA		GG vs AG + AA	
	Overall Ethnicity	5	0.95 (0.99-1.38)	0.80	1.18 (0.88-1.57)	0.28	1.08 (0.86-1.34)	0.51
	East Asia	4	0.96 (0.60-1.56)	0.88	**1.30 (1.03-1.64)**	**0.02**	1.15 (0.86-1.53)	0.36
	South Asia	1	0.92 (0.73-1.17)	0.51	0.51 (0.23-1.17)	0.11	0.95 (0.65-1.38)	0.77
	Controls							
	PB	3	0.87 (0.48-1.60)	0.66	1.30 (0.92-1.84)	0.14	1.05 (0.79-1.38)	0.75
	HB	2	1.09 (0.78-1.54)	0.60	0.89 (0.36-2.19)	0.80	1.20 (0.70-2.06)	0.52

CI = confidence interval; HB = hospital based; PB = population based.

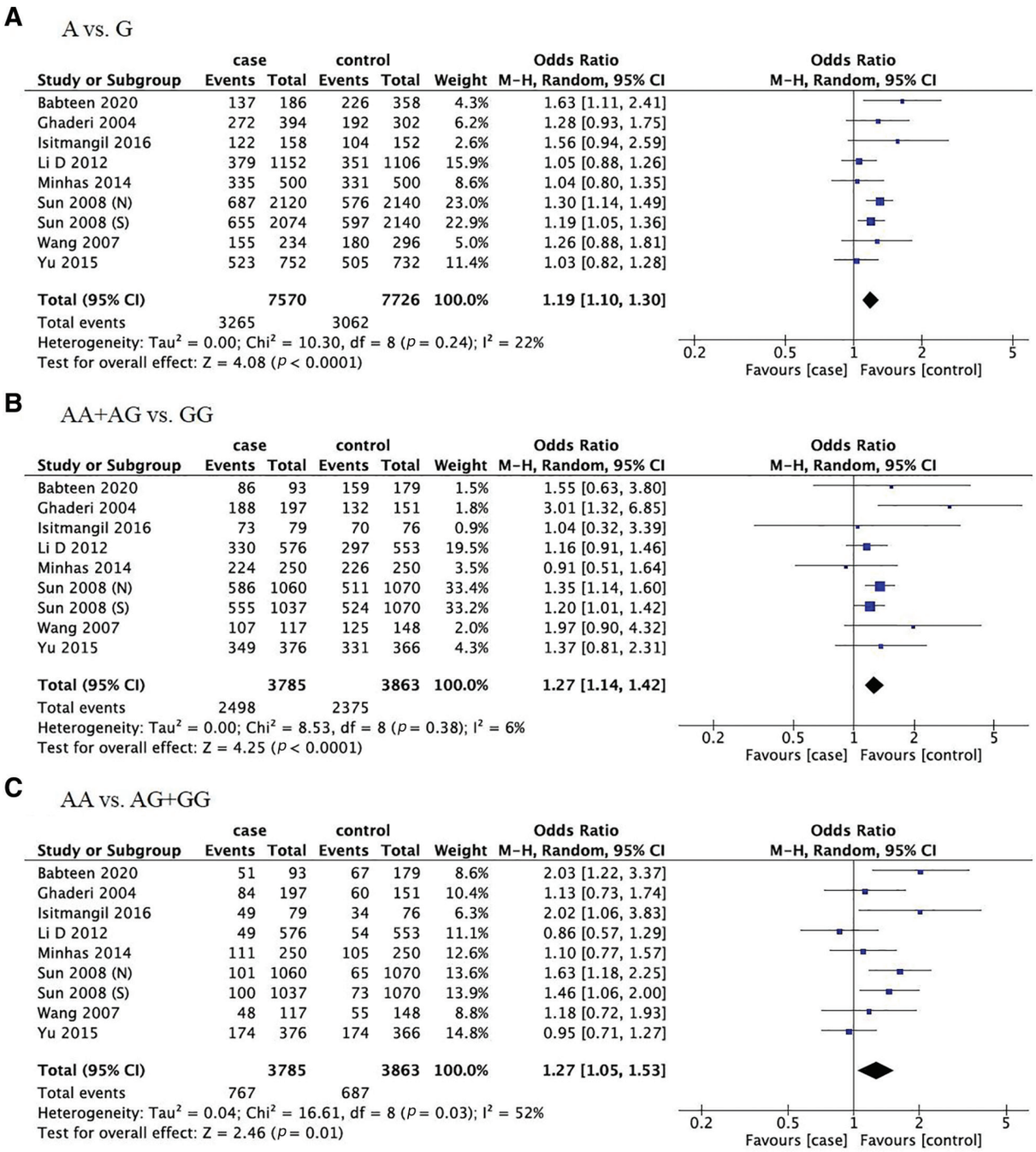

Fig. 2 Forest plots for the association between CTLA-4 rs231775 polymorphism and breast cancer risk under the allele (A), dominant (B), and recessive (C) models. CTLA-4 = cytotoxic T-lymphocyte antigen 4.

the risk of various cancers, while rs3087243 (CT60 G>A) and rs733618 (-1722 T>C) were not associated with overall cancer risk. In the stratified analysis based on cancer types and ethnicity, both rs231775 and rs4553808 conferred an increased risk of breast cancer on the Asian population.[29] From a considerable number of meta-analyses, it might be concluded that the CTLA rs231775 A allele is associated with an increased risk of cancers, including breast, bone, and cervical cancers.[28] However, the associations between other CTLA-4 polymorphisms and cancer remained inconclusive regarding their functional relevance with tumors or differences in distribution of genotypes between populations of different ethnic groups and limited number of studies. Therefore, we performed this meta-analysis to discuss the associations between CTLA-4 polymorphisms and breast cancer risk. In the present meta-analysis, we included 12 studies with 4786 cases and 4833 controls. We focused on breast cancer, investigated data from updated studies, and analyzed potential factors that affect the outcomes through subgroup analyses.

The main findings of this comprehensive study suggested that the A allele in rs231775 (49 A>G) is associated with significantly

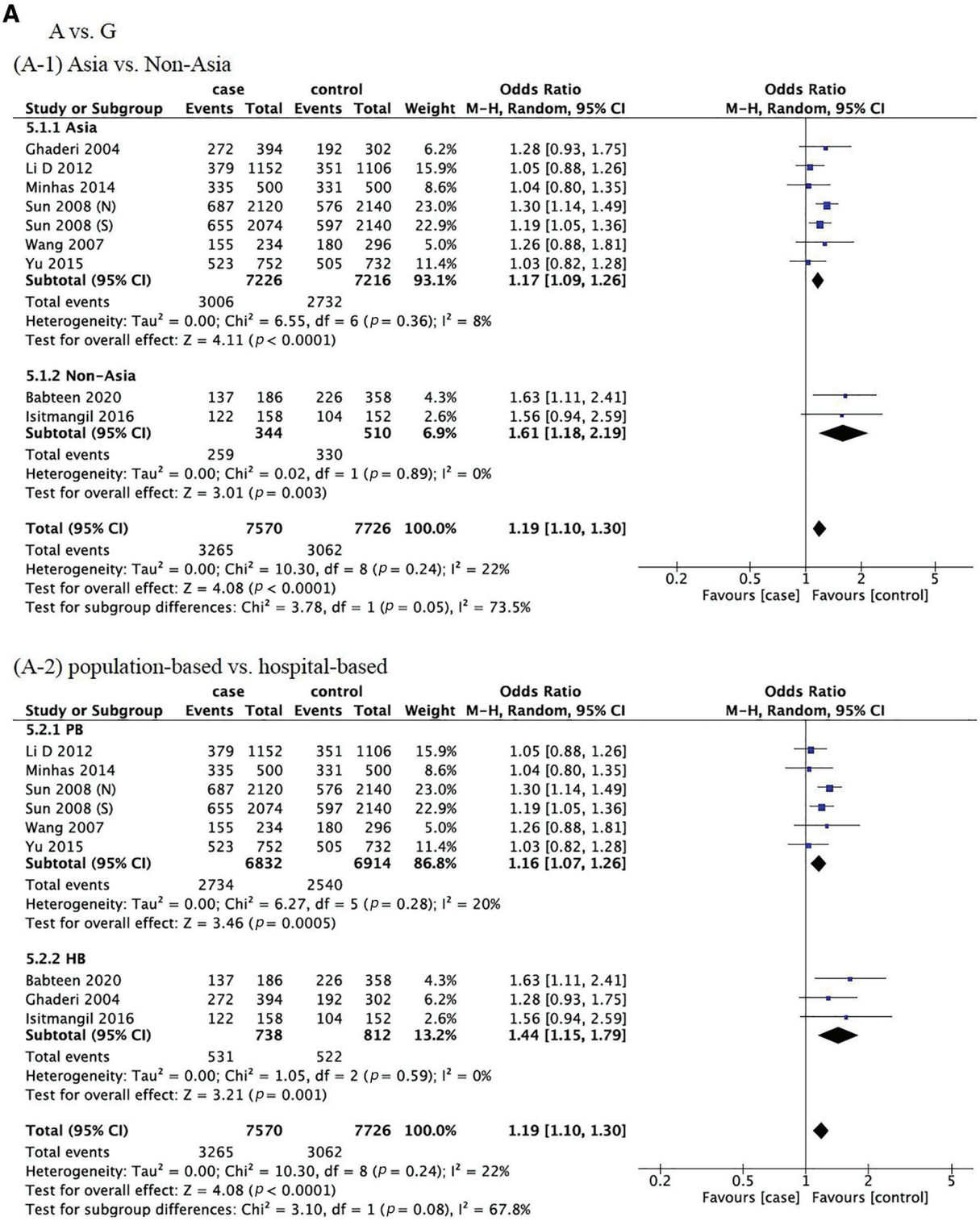

Fig. 3 Subgroup analysis of CTLA-4 rs231775 polymorphism under different genetic models. A, Allele model: (A-1) Asia vs Non-Asia; (A-2) population-based vs hospital-based. B, Dominant model: (B-1) Asia vs Non-Asia; (B-2) population-based vs hospital-based; recessive model. C, (C-1) Asia vs Non-Asia; (C-2) population-based vs hospital-based. CTLA-4 = cytotoxic T-lymphocyte antigen 4.

high risks of breast cancer. This result was synthesized from nine studies with 3790 cases and 3876 controls and is compatible with a previous meta-analysis that stated that the G allele is a protective factor for breast cancer (G vs A, OR = 0.83, 95% CI, 0.77-0.89, p = 0.000; GG + AG vs AA, OR = 0.78, 95% CI, 0.66-0.92, p = 0.003; and GG vs AG + AA, OR = 0.78, 95%

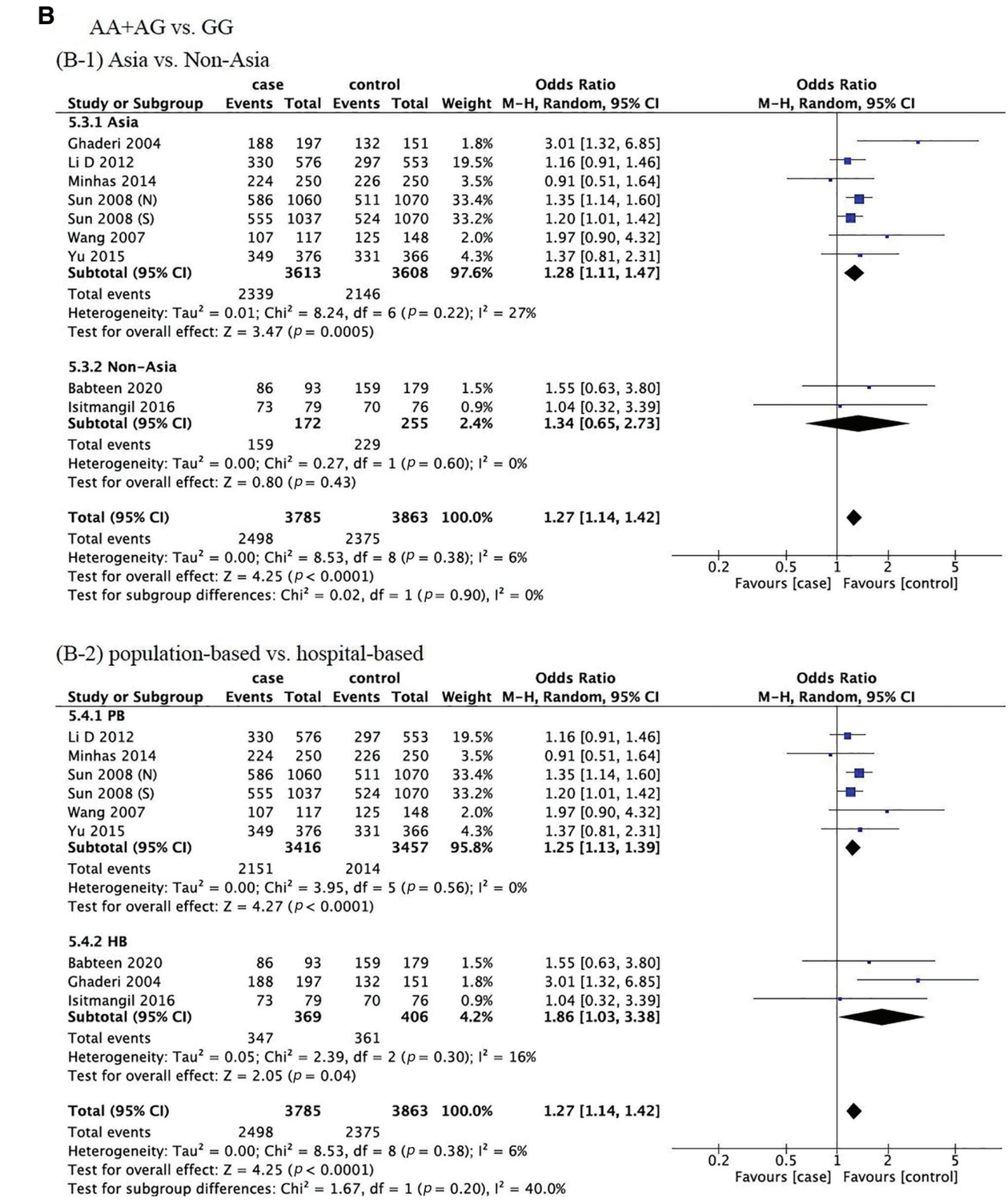

Fig. 3 Continued.

CI, 0.70-0.87, p = 0.000).[30] Our results are more reliable than previous studies because we included relevant articles that are not limited to Asian studies. Breast cancer susceptibility was indicated in the Asian population under the allele and dominant models (A vs G, OR = 1.17, 95% CI = 1.09–1.26, p < 0.0001; AA + AG vs GG, OR = 1.28, 95% CI = 1.11–1.47, p = 0.0005) and in the non-Asian population under the allele and recessive models (A vs G, OR = 1.61, 95% CI = 1.18–2.19, p = 0.003; AA

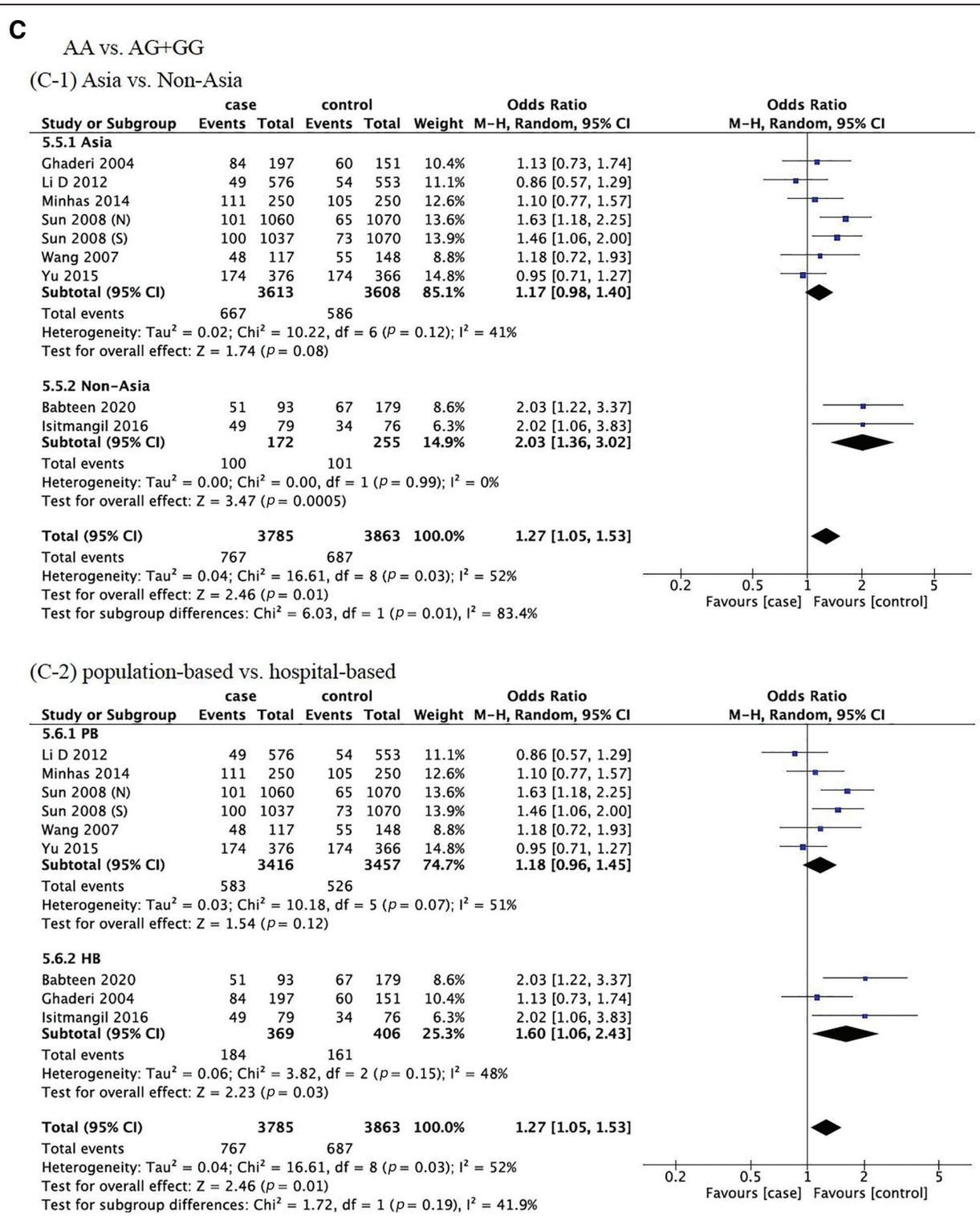

Fig. 3 Continued.

vs AG + GG, OR = 2.03, 95% CI = 1.36–3.02, p = 0.0005) in our study. The obtained results demonstrated that the carriage of the CTLA-4 rs231775 A allele increased the risk of breast cancer, which was in agreement with previous analyses.

Further results were interpreted from the rest of the four SNPs. Three *CTLA-4* gene variations are T/C changes at –1722 (rs733618), A/G transition at -1661 (rs4553808), and –318 C/T (rs5742909) within the promoter region. A variant

of rs733618 may affect the binding sites of transcription factors, whereas the CTLA-4 rs4553808 SNP may alter the potential response element for myocyte enhancer factor 2.[31,32] Also, the high promoter activity of CTLA-4 was associated with an allelic variant of rs5742909 in the promoter region.[33] Although no significant associations were observed in the overall analysis between rs733618(-1722 T>C), rs4553808(–1661 A>G), rs5742909(–318 C>T), and rs3087243(CT60 G>A), subgroup analyses were performed. They revealed that in rs733618, the variant T allele is more likely than the C allele to be a risk factor for breast cancer in the PB group. When the results were stratified based on ethnicity, we observed that the rs4553808 G allele may confer breast cancer risk in East Asians. The involvement of the T allele of rs733618 and the G allele of rs4553808 in the susceptibility to breast cancer is unclear. CTLA-4 + 6230 CT60A/G (rs3087243) SNP is located in the 3′UTR region, which contains regulatory elements that may affect mRNA stability and induce degradation and nuclear transportation.[34] Regarding rs3087243, we observed that the fact that those with an AA genotype are less susceptible to breast cancer is applicable only to East Asians. The role of the rs3087243 A allele as a protective factor against breast cancer remains unclear.

CTLA-4 rs231775 (49 A>G) is the most extensively studied polymorphism in relation to cancer. It is a non-synonymous SNP located on exon 1, which leads to an amino acid change from threonine (Thr) to alanine (Ala), and the A allele is known to be correlated with an increased expression of CTLA-4 mRNA and protein.[30] This amino acid substitution results in an enhanced interaction between the CTLA-4 molecule and the costimulatory receptor B7.1 that consequently reduces the activation and proliferation of T lymphocytes. The interaction between CTLA-4-Thr, B7.1, and CTLA-4-Ala was observed in reciprocal coimmunoprecipitation assays. It was presumed that AA homozygotes may express less CTLA-4 than GG homozygotes on the T-cell surface. In line with previous observations, the 49 AA genotype was associated with significantly lower T-cell proliferation in PBMC studies.[24] It has also been postulated that the CTLA-4 rs231775 polymorphism in the leader sequence may influence the rates of endocytosis or surface trafficking.[35,36] It is therefore reasonable to consider the CTLA-4 rs231775 A allele as a risk factor for the development of cancer.

The prognostic value of CTLA-4 varies in different cancer types. Inherited genetic markers, such as SNPs, could be useful in cancer risk prediction and the selection of patients who may benefit from immunotherapy. A meta-analysis revealed no significant association between CTLA-4 expression level and overall survival related to several cancer types as a whole but with high heterogeneity (HR = 1.25, 95% CI = 0.98–1.56, I^2 = 71.7%, p = 0.000). However, the SNP subgroup analysis demonstrated that the pooled HR for overall survival was 1.47 (95% CI, 1.14-1.89) in the 49 AA genotype and that it was an independent factor for poor cancer prognosis. Furthermore, Babteen et al, through in silico and laboratory experiments, demonstrated that AG and GG genotypes in rs231775 (49 A>G) polymorphism are markers of poor cancer prognosis.[20] Moreover, CTLA-4 genetic variants may have a role in breast cancer progression. Erfani et al. mentioned an association between the –1661 AA genotype and lesser lymph nodes involvement (p = 0.017) with higher ER expression (p = 0.004), whereas the –318 CC genotype is associated with lesser lymph nodes involvement (p = 0.007).[15] Along with CTLA-4 expression, the clinical prediction of breast cancer prognosis also relies on clinicopathological factors. Patients with breast cancer who had a conjunction of stage N2-3 tumors, a high CTLA-4 grade, and the Ki-67 index showed significantly poor survival.[10]

Cancer immunotherapy has become an important part of tumor therapy. There is increasing evidence to suggest that the immune checkpoints within the tumor microenvironment appear to play a crucial role in modulating tumor survival and progression.[37] Among them, CTLA-4, programmed death 1 (PD-1), and programmed death-ligand 1 (PD-L1) are primarily and broadly studied as targets for immune checkpoint blocking therapy in a wide spectrum of cancers.[38] Recently, the FDA approved the use of atezolizumab, an anti-PD-L1 monoclonal antibody, to treat patients with unresectable locally advanced or metastatic triple-negative breast cancer.[39] Furthermore, combined treatment with trastuzumab emtansine (T-DM1) and CTLA-4/PD-1 blocking antibodies was curative because it enhanced T-cell infiltration and promoted tumor rejection.[40] In addition, Christmas et al showed that combining a histone deacetylase inhibitor with anti-PD-1, anti-CTLA-4, or both significantly improved tumor-free survival in HER2/neu transgenic breast cancer mouse models.[41] These findings provide a rationale for combination therapy in patients with breast cancer. In this review, we focused specifically on the potential influences of *CTLA-4* gene polymorphisms on breast cancer risk. SNPs may be considered as biomarkers of cancer risk that lead to differences in individual susceptibility. Further studies are needed to examine the effects of gene-gene interactions, particularly in the context of CTLA-4 and PD-1/PD-L1 pathways. Moreover, the examination of potential interactions between SNPs may provide new interesting data of clinical significance.

Our study had a few limitations. First, the sample size and the articles included for each SNP were imbalanced, ranging from 948 people in rs5742909 to 7666 people in rs231775. We may have observed a greater number of significant results if more study data were available. Second, because most studies were conducted in Asia, we could gather only two non-Asian datasets from the electronic database; therefore, generalization of the study results to the entire non-Asian population should be done with caution. Third, the haplotype analysis was not performed due to limited data, and further studies are required to observe the pooling effect.

In conclusion, the meta-analysis suggested that the A allele in rs231775 (49 A>G) showed a significantly high risk of breast cancer in both Asian and non-Asian populations. East Asian individuals with breast cancer have a stronger correlation with CTLA-4 genetic polymorphism than the other populations in terms of the other four SNPs. CTLA-4 variants could be an indicator to provide patients with genomics-based precision medicine and to provide physicians with an adequate immunotherapy strategy for managing breast cancer.

ACKNOWLEDGMENTS

This work was supported by grants from the Ministry of Science and Technology College Student Research Program (110-2813-C-038-132-B), Far Eastern Memorial Hospital National Yang Ming Chiao Tung University Joint Research Program (FEMH-NYCU 108DN13 and 107DN09), and Cheng-Hsin General Hospital (CY11012, CY10934, and CY10813).

APPENDIX A. SUPPLEMENTARY DATA

Supplementary data related to this article can be found at http://links.lww.com/JCMA/A170.

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