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Updated analysis of studies on the cytotoxic T-lymphocyte-associated antigen-4 gene A49G polymorphism and Hashimoto's thyroiditis risk

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ABSTRACT. Published data on the association between the cytotoxic T-lymphocyte-associated antigen-4 gene A49G polymorphism and the risk for Hashimoto's thyroiditis (HT) are inconclusive. A meta-analysis was performed to derive a more precise estimation. Published case-control studies in English or Chinese were identified. In total, 24 studies with 2295 cases and 4521 controls were investigated. A random-effect model was performed irrespective of between-study heterogeneity. Study quality was assessed in duplicate, and subgroup analyses were conducted by ethnicity or age. Overall, the 49G allele was associated with an increase in HT risk [odds ratio (OR) = 1.31; 95% confidence interval (95%CI) = 1.17-1.47; P < 0.00001]. In a subgroup analysis by ethnicity, comparison of allele 49G with 49A generated a 27% increased risk among East Asians (OR = 1.48; 95%CI = 1.24-1.76; P < 0.00001) and whites (OR = 1.27; 95%CI = 1.12-1.44; P = 0.0002). We also found an increased risk among adults (OR = 1.31; 95%CI = 1.17-1.47; P < 0.00001) but not among children (OR = 1.44; 95%CI = 0.75-2.79; P = 0.27), possibly owing to the small sample sizes in children. No publication

biases were observed. This meta-analysis suggested that the cytotoxic T-lymphocyte-associated antigen-4 gene 49G allele was associated with an increased HT risk, especially in adults.

Key words: Meta-analysis; Hashimoto's thyroiditis; *CTLA-4*; A49G polymorphism

INTRODUCTION

Hashimoto's thyroiditis (HT) is one of the most common human autoimmune thyroid diseases. In HT, the antibodies against thyroid peroxidase or thyroglobulin appear characteristically in the sera of patients, and tissue damage owing to T-cell-mediated cytotoxicity usually contributes to the gradual development of hypothyroidism (Weetman, 2003). Several reports have demonstrated that multiple genetic factors are responsible for the development of HT, and the cytotoxic T-lymphocyte-associated antigen-4 (*CTLA-4*) gene has been extensively studied.

CTLA-4 (CD152), which is expressed on the surface of activated T lymphocytes, is a negative regulator of T-cell activation (Scheipers and Reiser, 1998). Evidence is accumulating that the *CTLA-4* gene is associated with a variety of autoimmune conditions (Tomer, 2001), such as autoimmune thyroid diseases (Graves' disease, HT, and thyroid antibodies) (Ban et al., 2003), Addison's disease (Donner et al., 1997), insulin-dependent diabetes mellitus (Kavvoura and Ioannidis, 2005; Baniasadi et al., 2006), systemic lupus erythematosus (Lee et al., 2005), and systemic sclerosis (Almasi et al., 2006). The *CTLA-4* gene is located on 2q33, where an A/G single nucleotide polymorphism at position 49 (exon 1, codon 17) has been identified.

Several case-control studies have investigated the association between the A49G polymorphism and HT, but they had low statistical power and their results were often not reproducible. To address this issue systematically, we performed a meta-analysis of all available case-control studies reported in English or Chinese to explore the association between the *CTLA-4* gene A49G polymorphism and the risk for HT.

MATERIAL AND METHODS

Publication search

The electronic databases Medline (Ovid), PubMed, China National Knowledge Infrastructure, Wanfang, and Weipu were used to search for electronic publications that were published as of November 2011. The key words used for the search were "CTLA4 or CTLA-4 or cytotoxic T-cell lymphocyte associated antigen 4 or CD152" and "Hashimoto*" and "49 or A49G", combined with "gene or variant or polymorphism or allele". If multiple publications were available from the same study group, the most complete and recent results were abstracted. Search results were limited to articles published in English or Chinese and studies performed in human subjects.

Inclusion/exclusion criteria

Case-control studies were included in this meta-analysis, regardless of sample size,

1) if they explored the association between the *CTLA-4* gene A49G polymorphism and HT among unrelated subjects, 2) if genotyping was performed using validated methods, and 3) if they provided sufficient information on genotype or allele frequencies to allow an estimation of odds ratio (OR) and its corresponding 95% confidence interval (95%CI). HT was diagnosed by the presence of goiter, hypothyroidism, and elevated microsomal or thyroid peroxidase autoantibodies. Thyroid ultrasound showed reduced echogenicity.

Data extraction

Two reviewers (Y.F. and R.J.) independently checked all potentially relevant studies. The following data were collected from each study: first author, year of publication, ethnicity, study design, diagnostic criteria, baseline characteristics of the study population, total number of cases and controls, and genotype distributions in cases and controls. Information on the Hardy-Weinberg equilibrium (HWE) test was also tracked or calculated if unavailable. After data extraction, discrepancies were adjudicated by discussion and a consensus was reached.

Statistical methods

The meta-analysis was calculated using the Review Manager version 5.0.19 software [http://www.cc-ims.net/revman/download (accessed October 12, 2012)]. HWE was assessed with the Pearson chi-squared test or the Fisher exact test (SAS version 9.1.3, Institute Inc., Cary, NC, USA). No assumptions were required for genetic models of inheritance for the polymorphism under study.

Generally, the inconsistency index was used to examine the presence of between-study heterogeneity with a statistical significance of 0.1. In this study, we applied the random-effect model for all comparisons because it accommodates the possibility that the underlying effect differs across studies. For practical use, the random-effect model is more conservative and has a wider 95%CI than the fixed-effect model.

In addition, sensitivity analysis or subgroup analysis was performed to look at more narrowly drawn subsets of the studies by removing an individual study each time or studies with similar features such as deviation from HWE to assess their separate influence. Finally, we assessed publication bias using the fail-safe number (N_{fs}) with the significance set at 0.05 for each meta-comparison. Specifically, if the calculated N_{fs} value was smaller than the number of studies observed, the meta-analysis results might have publication bias. We calculated the $N_{fs0.05}$ according to the formula $N_{fs0.05} = (\sum Z / 1.64)\ 2 - k$, where k is the number of articles included.

RESULTS

Study inclusion and characteristics

Based on our search strategy, the primary screening produced 33 potentially relevant articles, of which 24 studies met the inclusion criteria after evaluating the association of the A49G polymorphism with HT [Donner et al., 1997; Awata et al., 1998; Kouki et al., 2000; Park et al., 2000; Petrone et al., 2001; Wang et al., 2001; Tomoyose et al., 2002; Terauchi et al., 2003; Ueda et al., 2003; Zhou et al., 2003; Balbi et al., 2007; Kavvoura et

al., 2007 (a meta-analysis, which included 3 unpublished studies: Ban et al.; Akazumi et al.; Ghaderi et al.); Dallos et al., 2008; Yesilkaya et al., 2008; Yu et al., 2008; Bicek et al., 2009; Kucharska et al., 2009; Sahin et al., 2009; Shi et al., 2010; Benhatchi et al., 2011; Yang et al., 2012; Farra et al., 2012]. In total, 2165 HT patients and 4284 controls were examined. Of these 24 articles, 17 were published in English (Donner et al., 1997; Awata et al., 1998; Kouki et al., 2000; Petrone et al., 2001; Tomoyose et al., 2002; Terauchi et al., 2003; Ueda et al., 2003; Balbi et al., 2007; Kavvoura et al., 2007; Dallos et al., 2008; Yesilkaya et al., 2008; Bicek et al., 2009; Kucharska et al., 2009; Sahin et al., 2009; Benhatchi et al., 2011; Yang et al., 2012; Farra et al., 2012), and 4 were in Chinese (Wang et al., 2001; Zhou et al., 2003; Yu et al., 2008; Shi et al., 2010). Three unpublished articles contained results of a study by Kavvoura et al. (2007). Additionally, the genotypes in controls for one case-control study were inconsistent with HWE (Shi et al., 2010). The detailed selection process is presented in Figure 1.

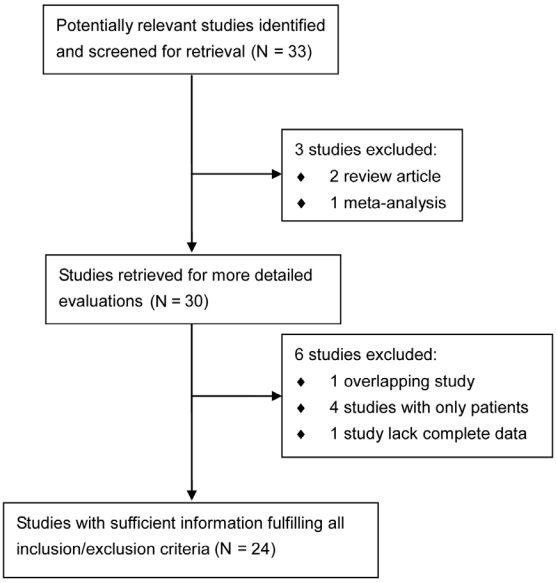

Figure 1. Flow diagram of search strategy and study selection.

The baseline characteristics of qualified studies are presented in Table 1. Of the 24 case-controls studies examining the association between the *CTLA-4* A49G polymorphism and HT risk, 11 included East Asians [Awata et al., 1998; Park et al., 2000; Wang et al., 2001; Tomoyose et al., 2002; Terauchi et al., 2003; Zhou et al., 2003; Kavvoura et al., 2007 (a meta-analysis, which included 2 unpublished studies: Ban et al. and Ghaderi et al.); Yu et al., 2008; Shi et al., 2010; Yang et al., 2012]; 9 included whites (Donner et al., 1997; Kouki et al., 2000; Petrone et al., 2001; Ueda et al., 2003; Balbi et al., 2007; Dallos et al., 2008; Bicek et al., 2009; Kucharska et al., 2009; Benhatchi et al., 2011); 4 included the Middle Easterners: two studies that included Turks (Yesilkaya et al., 2008; Sahin et al., 2009), one that included Iranians (Kavvoura et al., 2007), and one that included Lebanese (Farra et al., 2012). Twenty-one studies were performed in adults, whereas 3 were in children (Dallos et al., 2008; Yesilkaya et al., 2008; Kucharska et al., 2009).

Table 1. Baseline characteristics of all study populations in the meta-analysis.

Reference	Ethnicity	Sample size, N (male, %)		49 G allele frequency (%)		Characteristics
		Cases	Controls	Cases	Controls	
Donner et al., 1997	Canadian German	73	466	48.6	37.7	The controls were gender- and age-matched healthy volunteers from Germany and Canada
Awata et al., 1998	Japanese	88	425	72.2	63.2	Case (average age: 43.1); the controls were healthy volunteers
Kouki et al., 2000	American	18	43	38.9	38.4	Healthy controls with no clinical evidence or a family history of autoimmune diseases
Park et al., 2000	Korean	110 (0.9)	199 (34.2)	64.5	68.1	Case (age: 37.4 ± 12.0); control (age: 38.1 ± 15.0)
Petrone et al., 2001	Italian	126 (6.3)	301 (52.2)	33.3	31.2	Healthy controls with no clinical evidence or a family history of autoimmune diseases
Wang et al., 2001	Chinese	35 (17.1)	84 (46.4)	58.6	37.1	Control (age: 29 ± 9)
Tomoyose et al., 2002	Japanese	143 (11.9)	199 (26.1)	67.5	53.0	Healthy controls with no clinical evidence or a family history of autoimmune diseases
Terauchi et al., 2003	Japanese	70	105	69.3	57.1	Healthy controls with no clinical evidence or a family history of autoimmune diseases
Zhou et al., 2003	Chinese	46 (32.6)	50 (30.0)	67.4	52.0	Case (age: 34.64 ± 10.32); control (age: 33.35 ± 9.78); adult
Ueda et al., 2003	English	210	842	44.0	37.1	Ethnically matched controls with no history
Ban et al., 2006*	Japanese	183	179	65.6	62.3	Healthy controls with no clinical evidence or a family history of autoimmune diseases
Akamizu et al., 2006*	Japanese	139	234	71.6	66.2	Healthy controls with no clinical evidence or a family history of autoimmune diseases
Ghaderi et al., 2006*	Iranians	37	98	32.4	31.6	Healthy controls with no clinical evidence or a family history of autoimmune diseases
Balbi et al., 2007	Italian	20 (0)	113 (0)	30.0	26.5	Cases (HT with SSc patients), controls (healthy volunteers and SSc without HT)
Yeşilkaya et al., 2008	Turkish	88 (11.4)	112 (45.6)	5.7	1.34	Case (age: 14.5 ± 3.2); control (age: 14.1 ± 2.9); children
Dallos et al., 2008	Slovaks	119 (24)	136 (50)	45.4	35.7	Case (age: 11.1 ± 4.9); children
Yu et al., 2008	Chinese	80 (26.3)	126 (31.0)	72.5	60.3	Control (age: 34.5 ± 11.3)
Sahin et al., 2009	Turkish	197 (36.0)	98 (35.7)	33.8	31.6	Case (age: 42.92 ± 13.40); control (age: 41.92 ± 14.40)
Kucharska et al., 2009	Pole	45	55	55.6	60.2	Case (age: 15 ± 2); the controls were age-matched healthy volunteers; children
Bicek et al., 2009	Slovenia	112 (8.9)	117 (7.69)	33.9	32.5	Case (average age: 52.6); control (average age: 40.2)
Shi et al., 2010	Chinese	18 (27.8)	87 (17.2)	27.8	24.7	Control (age: 38.93 ± 11.7)
Yang et al., 2012	Chinese	208 (5.8)	215 (25.6)	73.3	64.0	Case (age: 33.11 ± 12.06); control (age: 34.76 ± 11.39)
Benhatchi et al., 2011	Slovaks	57	51	42.1	34.3	Case (age: 46.96 ± 12.73); control (age: 42.81 ± 15.96)
Farra et al., 2012	Lebanese	73 (6.85)	186 (29.0)	29.5	26.6	Case (age: 44.6 ± 13.4); control (age: 44.5 ± 12.9)

HT = Hashimoto's thyroiditis; SSc = systemic sclerosis. *These unpublished articles are included in the meta-analysis of Kavvoura et al., 2007.

Main meta-results

We analyzed the 49G allele (relative to the 49A allele) and HT risk for all 24 studies (Figure 2). The inconsistency index was 44% and P was 0.01, suggesting moderate heterogeneity. Thus, we chose the random-effect model to synthesize the data. Overall, comparison of alleles 49G and 49A generated a 31% increased risk for HT (95%CI = 1.17-1.47; P < 0.00001). Sensitivity analyses were performed by excluding studies with cases or controls not in HWE from the overall analysis (Shi et al., 2010), and the results showed that the A49G and HT association was not significantly altered.

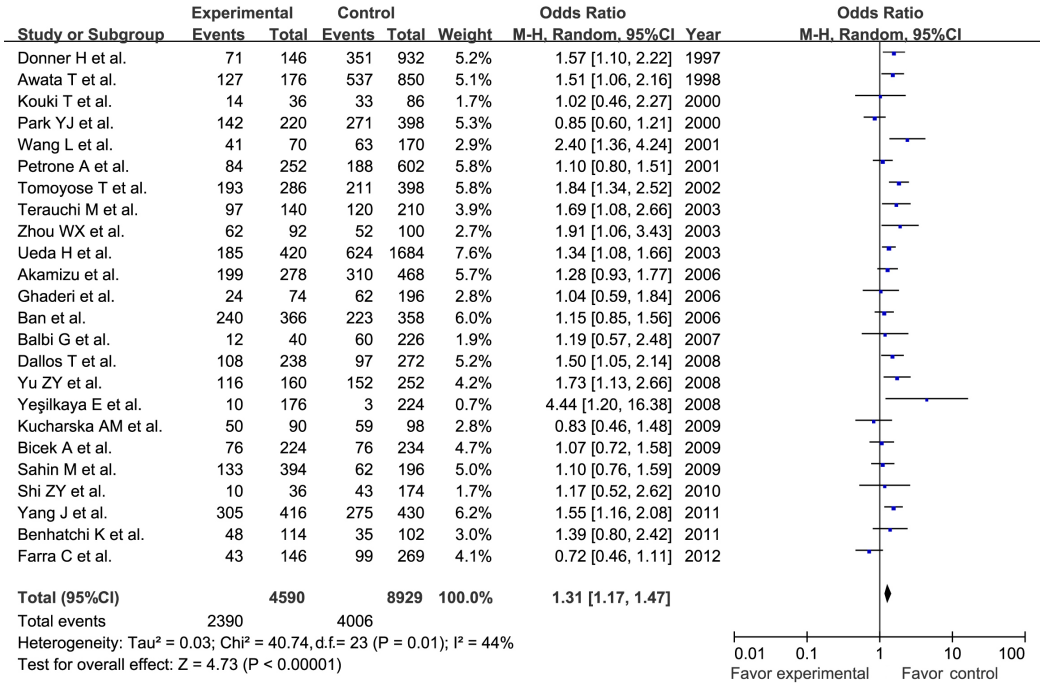

Figure 2. Pooled random-effect model based on odds ratio of Hashimoto's thyroiditis and *CTLA-4* for contrasts of 49G *vs* 49A. M.-H. = Mantel-Haenszel estimator; 95%CI = 95% confidence interval; d.f. = degrees of freedom.

We performed codominant model (GG *vs* AA and GA *vs* AA), dominant model (GG + GA *vs* AA), and recessive model (GG *vs* GA + AA) analyses. Compared with the 49AA genotype, the 49GG and 49GA genotypes both conferred a significantly increased risk of HT. Under the dominant and recessive models, 49G allele carriers still had increased HT risk. Summary results of other genetic comparisons are listed in Table 2.

Subgroup analyses

In the subgroup analysis by ethnicity, significantly increased risks were found among East Asians (OR = 1.48; 95%CI = 1.24-1.76; P < 0.00001) and whites (OR = 1.27; 95%CI =

1.12-1.44; P = 0.0002) but not among Middle Easterners, including Turks, Iranians, and Lebanese (OR = 1.01; 95%CI = 0.79-1.29; P = 0.95), under an allele comparison (see Table 2). In the subgroup analysis by age, significantly increased risks were found among adults (OR = 1.31; 95%CI = 1.17-1.47; P < 0.00001) but not among children (OR = 1.44; 95%CI = 0.75-2.79; P = 0.27). Summary results of other genetic comparisons are listed in Table 2.

Table 2. Summary of different comparative results.

Genetic model	Overall or subgroup	Study number (N)	Participant (N)	OR (95%CI)	Z	P	I^2 (%)	P_{het}
G *vs* A	All	24	13519	1.31 (1.17, 1.47)	4.73	<0.00001	44	0.01
	All in HWE	23	13309	1.35 (1.21, 1.51)	5.25	<0.00001	39	0.04
	Caucasians	9	5796	1.27 (1.12, 1.44)	3.75	0.0002	0	0.59
	The East Asians	10	5838	1.48 (1.24, 1.76)	4.40	<0.0001	53	0.02
	Middle Easterns	4	1675	1.01 (0.79, 1.29)	0.07	0.95	60	0.06
	Children	3	1098	1.44 (0.75, 2.79)	1.10	0.27	68	0.04
	Adults	20	12421	1.31 (1.17, 1.47)	4.61	<0.00001	42	0.02
GG *vs* AA	All	24	3817	1.94 (1.60, 2.36)	6.65	<0.00001	11	0.32
	All in HWE	23	3733	1.96 (1.60, 2.40)	6.48	<0.00001	14	0.27
	Caucasians	9	1545	1.72 (1.31, 2.26)	3.91	0.0001	0	0.82
	The East Asians	10	1607	2.48 (1.71, 3.60)	4.79	<0.00001	43	0.07
	Middle Easterns	4	581	1.39 (0.77, 2.52)	1.10	0.27	0	0.81
	Children	3	398	1.30 (0.52, 3.26)	0.56	0.57	57	0.13
	Adults	20	3419	2.01 (1.64, 2.45)	6.87	<0.00001	5	0.39
GA *vs* AA	All	24	5110	1.41 (1.14, 1.74)	3.23	0.001	46	0.007
	All in HWE	23	5021	1.43 (1.16, 1.76)	3.37	0.0008	47	0.008
	Caucasians	9	2397	1.21 (0.88, 1.65)	1.18	0.24	51	0.05
	The East Asians	10	1707	1.97 (1.47, 2.64)	4.52	<0.00001	14	0.32
	Middle Easterns	4	588	1.19 (0.49, 2.87)	0.38	0.71	70	0.04
	Children	3	461	1.77 (0.84, 3.72)	1.49	0.14	47	0.15
	Adults	20	4231	1.43 (1.12, 1.83)	2.82	0.005	53	0.004
GG+GA *vs* AA	All	24	6444	1.56 (1.25, 1.93)	4.00	<0.00001	51	0.003
	All in HWE	23	6339	1.58 (1.27, 1.97)	4.07	<0.00001	52	0.003
	Caucasians	9	2790	1.28 (0.98, 1.69)	1.80	0.07	44	0.09
	The East Asians	10	2919	2.19 (1.61, 2.99)	4.95	<0.00001	29	0.18
	Middle Easterns	4	630	1.27 (0.59, 2.71)	0.61	0.54	64	0.06
	Children	3	549	1.71 (0.78, 3.76)	1.33	0.18	59	0.09
	Adults	20	5790	1.57 (1.24, 1.99)	3.72	0.0002	54	0.004
GG *vs* GA+AA	All	24	6444	1.42 (1.24, 1.62)	5.12	<0.00001	3	0.42
	All in HWE	23	6339	1.42 (1.23, 1.63)	4.93	<0.00001	7	0.36
	Caucasians	9	2696	1.48 (1.14, 1.91)	2.93	0.003	0	0.83
	The East Asians	10	2919	1.42 (1.15, 1.76)	3.27	0.001	41	0.08
	Middle Easterns	4	630	1.77 (0.88, 3.56)	1.59	0.11	0	0.64
	Children	3	549	1.16 (0.67, 2.00)	0.53	0.60	15	0.28
	Adults	20	5790	1.44 (1.24, 1.66)	4.91	<0.00001	10	0.34

HWE = Hardy-Weinberg equilibrium; OR = odds ratio; 95%CI = 95% confidence interval.

Publication bias

To assess publication bias, we calculated N_{fs} at a significance level of 0.05 for each comparison. The $N_{fs0.05}$ values for all contrasts were greater than the number of studies included in this meta-analysis.

DISCUSSION

Previously, Kavvoura et al. (2007) had performed a meta-analysis to investigate *CTLA-4* polymorphism and autoimmune thyroid diseases, and found that the A49G polymorphism of

CTLA-4 was linked to the occurrence of HT (Kavvoura et al., 2007). Several additional studies that have investigated the association between the *CTLA-4* gene A49G polymorphism and HT found no link between them. The goal of this meta-analysis was to combine the results of previous studies to achieve summary conclusions about the association between the *CTLA-4* gene A49G polymorphism and HT. Our meta-analysis, including 2295 patients with HT and 4521 controls, investigated the association of A49G polymorphism with HT in all studies and in ethnicity and age subgroups from the 24 studies. We included 11 novel case-control studies (Balbi et al., 2007; Dallos et al., 2008; Yesilkaya et al., 2008; Yu et al., 2008; Bicek et al., 2009; Kucharska et al., 2009; Sahin et al., 2009; Shi et al., 2010; Benhatchi et al., 2011; Yang et al., 2012; Farra et al., 2012). Furthermore, we excluded studies when the genotype distribution in controls was not in HWE (Shi et al., 2010). Although all statistical biases could not be eliminated, our results suggested that the 49G allele of *CTLA-4* was associated with an increased HT risk.

We also carried out subgroup analysis by ethnicity and age. For ethnicity, our results showed that the 49G allele had a significant 27% increase for HT risk in whites and a 48% increase in East Asians after excluding studies not in HWE. Similar trends were also found under other models, with the exception of the codominant model in Caucasians. We also found no association between A49G and HT in Middle Easterners. Considering the wider confidence intervals of estimates and the small sample sizes in population-based studies, more studies are required to quantify this effect size reliably.

With respect to age, allele frequency comparison showed that the risks of HT in 49G allele carriers are higher in adults than in children. Other genetic model comparisons also showed a risk for HT that was higher in adults than in children.

Our study had some notable limitations. First, only published studies in English and Chinese were included, and some relevant published studies or unpublished studies with null results may have been missed, which might have biased the results. Second, owing to the relatively small number of certain eligible studies, such as the Arabs, we were unable to perform further subgroup analyses - for example by gender or other ethnicity - because of limited data. Third, some of the individual studies included a small number of cases, which may have affected the statistical power of the publication bias. Last but not the least, the potential role of the *CTLA-4* A49G polymorphism may be diluted or masked by other gene-gene or gene-environment interactions. Therefore, we must refrain from drawing definitive conclusions until additional studies can confirm our results. We minimized the likelihood of bias by creating a detailed protocol before initiating our study, performing a meticulous search for publications, and using explicit methods for publication selection, data extraction, and data analysis.

Taken together, the results of this meta-analysis extended previous findings on the association between the *CTLA-4* gene A49G polymorphism and HT by showing that the polymorphism was associated with an increased HT risk among whites and East Asians. Further studies should investigate adjacent markers to *CTLA-4* A49G to confirm whether this association is causal or due to linkage disequilibrium. Moreover, studies of the biological mechanism and function of *CTLA-4* A49G polymorphism in HT are warranted.

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