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Original Article

A meta-analysis of association between CTLA-4 -1722T/C polymorphisms and systemic lupus erythematosus susceptibility

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Abstract: Number of studies assessed the association between Cytotoxic T lymphocyte antigen-4 (CTLA-4) -1722T/C polymorphisms and systemic lupus erythematosus (SLE) was increasing. However, these studies have provided conflicting results. In this study, meta-analysis was performed to invest the correlation between CTLA-4 -1722T/C polymorphisms and SLE susceptibility. Eligible case-control studies published up to June 2015 were selected from PubMed, EMBASE, MEDLINE, CNKI and WanFang databases. Meta-analysis was performed using Stata12.0 software to analyze the data according to the inclusion and exclusion criteria. A total of 11 studies comprising 1722 SLE cases and 1829 controls were included. Odds ratios (ORs) with 95% confidence intervals (CIs) were used to assess the strength of associations. In addition, Begg's test was used to measure publication bias. The meta-analysis results demonstrated that there was significant association between CTLA-4 -1722T/C polymorphisms and SLE susceptibility (TT+TC vs. CC: OR=1.80, 95% CI=1.15-2.80, P<0.05; TT vs. TC+CC: OR=1.39, 95% CI=1.04-1.85, P<0.05; TT vs. CC: OR=2.05, 95% CI=1.24-3.38; TC vs. CC, OR=1.62, 95% CI=1.06-2.49, P<0.05). When stratified by ethnicity, the association still existed for Asian population (T vs. C: OR=1.50, 95% CI=1.15-1.95, P<0.01), but no significant correlation existed for Europeans and Africans groups. In suumary, there is a significant association between CTLA-4 -1722T/C gene polymorphisms and SLE susceptibility.

Keywords: Cytotoxic T lymphocyte antigen-4, systemic lupus erythematosus, meta-analysis, susceptibility

Introduction

Cytotoxic T-lymphocyte antigen-4 (CTLA-4) is a lymphocyte surface molecule that is related to immune signal transmission. It is mainly expressed on activated T lymphocytes, controlling peripheral T lymphocyte tolerance. CTLA-4 competes with CD28 and combines with antigen-presenting cell surface B7 molecules to inhibit the proliferation and activation of T-cells [1]. A growing number of studies have confirmed that CTLA-4 is a major molecule in the regulation of T cell proliferation and cytokine production [2, 3]. Abnormal expression of CTLA-4 on T cells may alter the activation of T cells, thereby affecting the immune process. CTLA-4 is a vital biomarker and plays an important role in many autoimmune diseases, such as insulin-dependent diabetes mellitus (IDDM), Hashimoto's disease (HT), rheumatoid arthritis, etc. [4].

Systemic lupus erythematosus (SLE) is a typical immunological disease which results from the production of autoantibodies and affects multiple organs. It is common in women, but ethnicity also influences the age of onset and severity of its manifestations [5]. The precise aetiopathogenesis of SLE is still unclear, but current studies suggests that environmental and genetic factors play an important role in the development of SLE [6, 7]. One potential susceptibility region for human SLE is located in 2q33-35, an interval that includes the CTLA-4 gene [8]. Polymorphism -1722T/C is located in the promoter region of CTLA-4, and its contribution to ESL susceptibility varies within different regions and groups according to different SLE study reports. This study aimed to evaluate whether CTLA-4 gene polymorphism -1722T/C contributes to SLE susceptibility. This was done by conducting a meta-analysis from all eligible case-control studies published until June 2015.

Table 1. Summary of eligible studies considered in the meta-analysis

First author	Year	Area	N	Genotyping method	Case			Control			Case		Control		HWE
					TT	TC	CC	TT	TC	CC	T	C	T	C	
Hudson [14]	2002	Korea	330	PCR-RFLP	73	48	9	66	103	31	194	66	235	165	0.376
Aguilar [15]	2003	Spain	470	PCR-RFLP	221	55	0	150	44	0	497	55	344	44	0.075
Fernandez [16]	2004	Spain	449	PCR-RFLP							380	48	447	23	NA
Xu [17]	2004	China	213	PCR-RFLP	28	60	15	36	46	28	116	90	118	102	0.095
Parks [18]	2004	USA	216	PCR-RFLP	109	34	1	48	23	1	251	37	120	24	0.336
Parks [18]	2004	USA	287	PCR-RFLP	67	16	2	175	24	3	150	20	376	28	0.054
Takeuchi [19]	2007	Japan	165	PCR-RFLP	19	25	16	34	52	18	63	57	120	88	0.805
Gao [20]	2007	China	297	PCR-RFLP	51	36	10	66	92	42	138	56	224	176	0.347
Qi [21]	2009	China	575	PCR-RFLP	111	125	37	94	146	62	347	199	334	270	0.701
Khalaf [22]	2011	China	449	PCR-RFLP	42	87	19	29	80	61	171	125	138	202	0.752
Guindy [23]	2015	Egyptian	100	PCR-RFLP	50	10	0	31	9	0					0.422

Materials and methods

Search strategy

A literature search was performed in the Pubmed, EMBASE, Medline, Chinese National Knowledge Infrastructure (CNKI) and Wanfang database. The following key words and search terms were applied: "systemic lupus erythematosus" or "SLE", "CTLA-4" or "T lymphocyte antigen-4", "polymorphism''. The last update was performed in June 2015. We only used data from full-published papers. Unpublished reports and meeting or conference abstracts were excluded from the study.

Inclusion criteria

Studies included in the current meta-analysis had to meet the following criteria: (1) evaluate the association between CTLA-4 gene -1722T/C polymorphisms and SLE susceptibility; (2) case-control design; (3) sufficient data for estimating an odds ratio (OR) with 95% confidence interval (CI) and consistent with Hardy-Weinberg equilibrium (HWE); (4) studies with full text articles. Review articles, editorials, conference abstracts, case reports and letters were excluded. When a study included subjects of different ethnicities or countries, the related data were extracted separately.

Data extraction and assessment of study quality

Two investigators extracted the data in accordance with the inclusion criteria. For each study, the following characteristics were extracted: the name of the first author, year of publication, ethnicity, method of genotyping, total sample size, number of genotypes and alleles in both case and control groups. Different ethnic descents were categorized as Asian, European or African. When a study reported results on different subpopulations, we separated the comparisons in the meta-analysis.

Statistical analysis

The analysis of data was performed with STATA12.0 software. Four genetic models, (1) allele contrast (T versus C), (2) additive genetic model (TT versus CC; TC vs. CC), (3) dominant model (TT+TC versus CC), and (4) recessive model (TT versus TC+CC), were measured in this meta-analysis, and association values of CTLA-4 genetic polymorphisms and SLE susceptibility were estimated by odds ratios (ORs) and 95% confidence intervals (CIs). The statistical significance of pooled ORs was measured by the Z-test and $P<0.05$ was considered statistically significant. The heterogeneity across all studies was tested by the I^2 index and chi-square-based-test. When there was significant heterogeneity ($P<0.10$ and $I^2>50\%$), we checked by the random-effects model (Der Simonian and Laird method) [9]. A lack of heterogeneity ($P>0.10$ and $I^2<50\%$) allowed for the use of the fixed-effects model (Mantel-Haenszel method) [10]. Subgroup meta-analyses were conducted according to different ethnicities. In addition, publication bias was measured by funnel plots. When $P<0.05$, it meant that there was a publication bias in the meta-analysis.

Table 2. Meta-analysis of the association between the CTLA-4 -1722T/C polymorphism and SLE

Polymorphism	Population	No. of studies	Test of heterogeneity			Test of association	
			P	Model	I^2 (%)	OR 95% CI	*P*
TT vs. CC	All	11	0.011	R	61.7	2.05 (1.24-3.38)	0.005
	Asian	6	0.006	R	69.0	2.21 (1.30-3.76)	0.003
	European	3				0.57 (0.09-3.51)	0.548
	African	2				2.27 (0.14-36.07)	0.565
TC vs. CC	All	11	0.040	R	52.4	1.62 (1.06-2.49)	0.026
	Asian	6	0.013	R	65.3	1.66 (1.03-2.67)	0.037
	European	3				1.00 (0.15-6.67)	1
	African	2				1.48 (0.09-24.85)	0.786
TT vs. TC	All	11	0.015	R	55.9	1.28 (0.98-1.68)	0.072
	Asian	6	0.018	R	63.4	1.39 (0.97-1.99)	0.07
	European	3	0.087	R	65.8	0.87 (0.43-1.74)	0.69
	African	2	0.925	R	0	1.51 (0.89-2.56)	0.13
TT+TC vs. CC	All	11	0.014	R	60.0	1.80 (1.15-2.80)	0.009
	Asian	6	0.007	R	68.8	1.89 (1.18-3.03)	0.008
	European	3				0.63 (0.10-3.81)	0.611
	African	2				2.01 (0.12-32.67)	0.622
TT vs. TC+CC	All	11	0.003	R	63.8	1.39 (1.04-1.85)	0.025
	Asian	6	0.011	R	66.4	1.58 (1.11-2.24)	0.011
	European	3	0.077	R	68.0	0.86 (0.43-1.73)	0.669
	African	2	0.907	R	0	1.53 (0.90-2.60)	0.116
T vs. C	All	11	0	R	82.4	1.18 (0.88-1.58)	0.268
	Asian	6	0.003	R	72.2	1.50 (1.15-1.95)	0.002
	European	3	0.006	R	80.5	0.65 (0.34-1.27)	0.208
	African	2				1.36 (0.78-2.37)	0.284

Results

Identification of eligible studies

Through database search, 72 articles were identified after an initial search. We reviewed all titles or abstracts according to the inclusion criteria, and selected 14 studies for detailed evaluation. The studies of Chua [11], Yu [12] and Wang [13] were further excluded after full-text review because the distribution of genotypes in the controls was not consistent with the Hardy-Weinberg equilibrium. Finally, a total of 11 case-control studies concerning the association between the CTLA-4 -1722T/C polymorphisms and SLE were included in this meta-analysis (10 articles). As summarized in **Table 1**, 11 eligible studies were selected for this meta-analysis, including 1722 SLE cases and 1829 controls [14-23].

Table 1 displays the essential information including first author, year of publication, area, ethnicity, genotyping method and numbers of cases and controls of CTLA-4 -1722T/C polymorphisms. A subgroup analysis was performed for groups of different ethnicity. Cases from Spain and the United States were categorized as Europeans. Cases from China, Japan and Korea were merged into the Asian group and cases from Egypt were categorized as Africans. All the studies indicated that the genotypic distribution of the controls was in agreement with the Hardy-Weinberg equilibrium.

Quantitative synthesis

The results of the association between CTLA-4 -1722T/C polymorphisms and SLE and the heterogeneity test are shown in **Table 2**. A total of 1722 SLE cases and 1829 controls from 11 studies were included. The strength of the association was measured by ORs with 95% CIs. All studies that followed HWE were pooled. We detected significant associations between -1722T/C polymorphisms and SLE susceptibili-

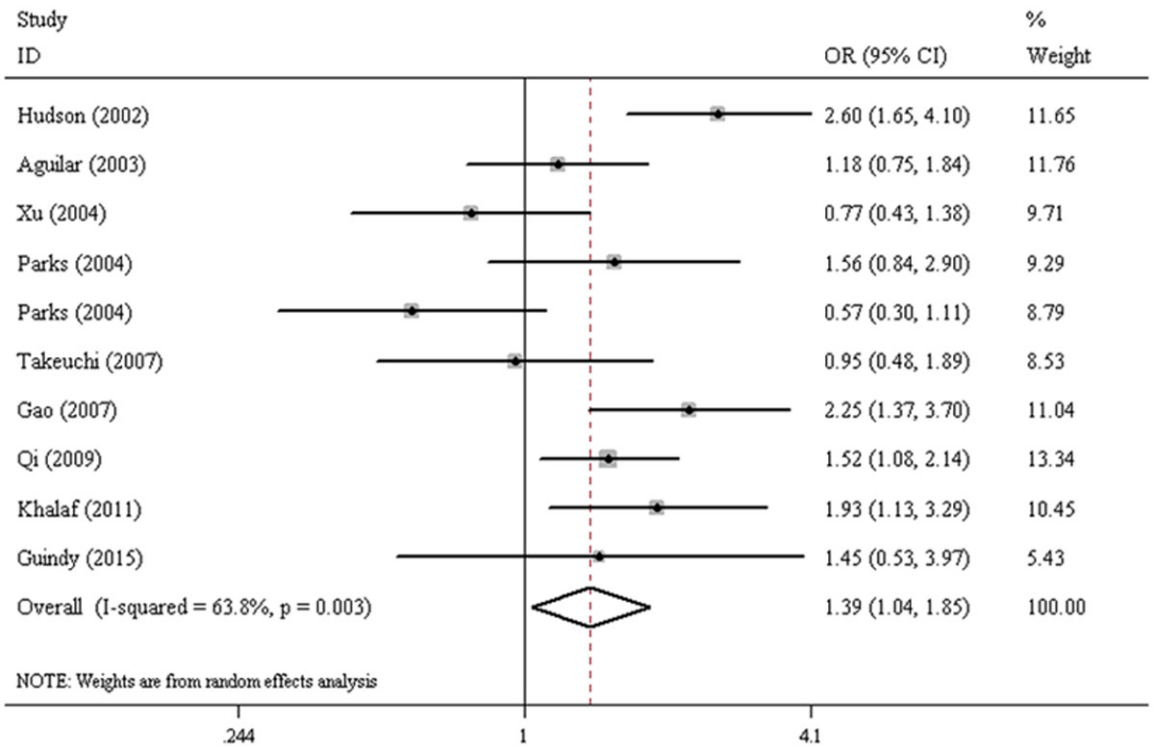

Figure 1. Meta-analysis for CTLA-4 -1722T/C polymorphisms variant genotypes TT vs. TC+CC in all populations. OR, indicates odds ratio; CI, confidence interval; I^2, measure to quantify the degree of heterogeneity in meta-analyses.

ty in the overall population when examining the contrast of TT+TC vs. CC (OR=1.80, 95% CI=1.15-2.80, P<0.01), TT vs. TC+CC (OR=1.39, 95% CI=1.04-1.85, P<0.05) (**Figure 1**), TT vs. CC (OR=2.05, 95% CI=1.24-3.38) and TC vs. CC (OR=1.62, 95% CI=1.06-2.49, P<0.05). However, we did not detect an association between T vs. C (OR=1.18, 95% CI=0.88-1.58, P>0.05).

Significant association between the -1722T/C polymorphisms and SLE susceptibility in the subgroup analysis based on ethnicity was observed. In the ethnicity subgroup, 6 case-control studies were from Asia, 4 case-control studies were from Europe, and 2 case-control studies were from Africa. Our result indicated that there was significant association in the Asian population when the contrast of TT+TC vs. CC (OR=1.89, 95% CI=1.18-3.03, P<0.05), TT vs. TC+CC (OR=1.58, 95% CI=1.11-2.24, P<0.05), TT vs. CC (OR=2.21, 95% CI=1.30-3.76), TC vs. CC (OR=1.66, 95% CI=1.03-2.67, P<0.05) was examined (**Figure 2**). There is also association in the Asian population for T vs. C (OR=1.50, 95% CI=1.15-1.95, P<0.05). In the African and European population, statistically significant results between -1722T/C polymorphisms and SLE susceptibility were not found.

Publication bias test

In order to evaluate the publication bias, Begg's Funnel plots were performed, and the results showed that no obvious asymmetry existed for the meta-analyses of CTLA-4 -1722T/C polymorphisms. The Egger test showed no publication bias for TT+TC vs. CC, P=0.603; TT vs. TC+CC, P=0.311; TT vs. CC, P=0.567; TC vs. CC, P=0.631 (**Figure 3**). These results indicated that biases from publications or other factors did not affect the final results of our meta-analysis on the association between CTLA-4 -1722T/C polymorphisms and SLE susceptibility.

Discussion

SLE is a complex chronic autoimmune disease of unknown etiology and pathogenesis which causes the deposition of immune complexes in different organs. The occurrence of SLE depends on complex multifactorial interactions

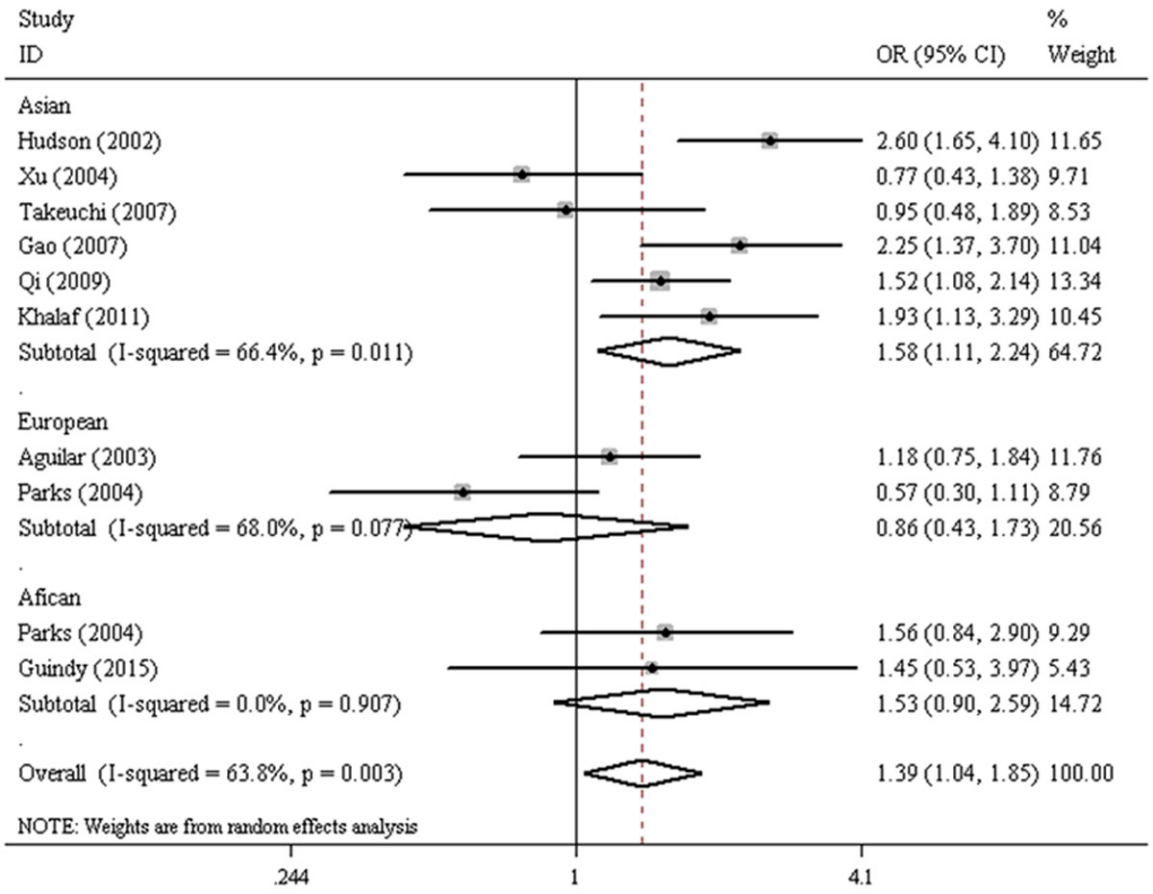

Figure 2. Meta-analysis for CTLA-4 -1722T/C polymorphisms variant genotypes TT vs. TC+CC in ethnicity subgroups. OR, indicates odds ratio; CI, confidence interval; I^2, measure to quantify the degree of heterogeneity in meta-analyses.

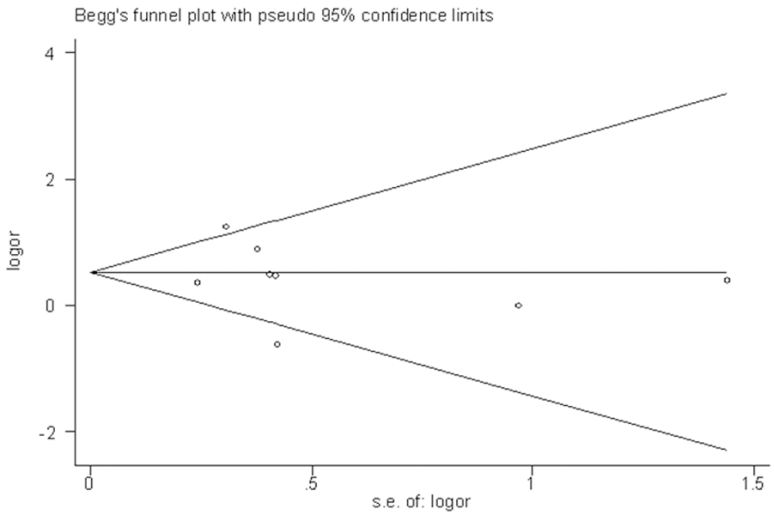

Figure 3. Begg's funnel plot for publication bias of studies on the CTLA-4 -1722T/C variant genotypes TC vs. CC.

between various genetic and environmental factors. The characteristics of SLE include the production of auto antibodies directed at nuclear, cytoplasmic and cell surface auto antigens. Several studies have reported that many genes encoding proteins and multiple polymorphisms in genes with particular functions in the immune system may be associated with SLE development [24, 25].

Several observations suggest that CTLA-4 plays an important role in the maintenance of immune tolerance. CTLA-4 signaling mediates a negative regulator in both the cellular and the humoral immune responses. Negative signaling via CTLA-4 plays an active role in regulating autoreactive T cells [26]. Several observations suggest that CTLA-4

+49A/G, -318C/T polymorphisms are associated with some autoimmune disorders [27, 28]. A number of studies have tested the association of CTLA-4 -1722C/T polymorphic markers with SLE, but report conflicting results.

Our meta-analysis included 11 studies, including 1722 SLE cases and 1829 healthy controls. Meta-analysis showed that for the whole population the association between CTLA-4 polymorphism -1722C/T and SLE susceptibility was statistically significant for genotype TT vs. CC and TT + TC vs. CC. Stratification by ethnicity displayed that for Asians the frequency of allele T vs. C, TT vs. CC and genotype TT + TC vs. CC was higher in the case group than the control group, and statistically significant. However, we have found that there was a lack of association between CTLA-4 -1722C/T polymorphisms and SLE susceptibility in the European and African subgroups. On the one hand, because of the limited number of samples included in each study, a valid statistical evidence could not be provided. Further studies are required to identify the causal alleles within these loci. On the other hand, we found a significant heterogeneity in the analysis of -1722T/C polymorphisms and there were several reasons such as age, sex, genetic background, environmental exposures and genes of tumor necrosis factor (TNF), interleukin-Fcγ receptors (FcγRs) that may explain the association between gene polymorphisms and different ethnicity. Our findings are inconsistent with Lee's [29], who showed that there was no association between the CTLA-4 -1722C/T polymorphisms and SLE. However, our study included more studies and more accurate inclusion criteria than Lee's, there were still some limitations in our study. The result showed that the CTLA-4 -1722T/C polymorphisms were associated with SLE susceptibility, especially in the Asian subgroup. Although there was a lack of association between CTLA-4 -1722C/T polymorphisms with SLE susceptibility in European and African group, a larger sample size should be analyzed to further verify this.

Disclosure of conflict of interest

None.

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