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Review Article

Association between cytotoxic T lymphocyte-associated antigen 4 polymorphism and type 2 diabetes susceptibility: a meta-analysis

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Abstract: Background: The relationship between cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) exon-1 +49A/G polymorphism and Type 2 diabetes (T2D) susceptibility has been extensively researched for recent years, but the results are contradictory to some degree. Methods: We searched Pubmed, Embase, Chinese Biomedical and Chinese National Knowledge Infrastructure databases. The last search was performed on November 11, 2017. Finally, 11 case-control studies consist of 1870 cases and 2170 controls were included in this meta-analysis. Results: A significant relationship was found in the dominant genetic model (GG+AG versus AA: odds ratio (OR)=1.47, 95% confidence interval (CI) 1.23-1.75), recessive genetic model (GG versus AA+AG: OR=1.30, 95% CI 1.13-1.49), homozygote model (GG versus AA: OR=1.72, 95% CI 1.39-2.13), heterozygote model (AG versus AA: OR=1.38, 95% CI 1.15-1.65) and allele model (G versus A: OR=1.31, 95% CI 1.13-1.53). Additionally, subgroup analysis in different ethnicity showed that *CTLA-4* +49A/G polymorphism was associated with T2D susceptibility both in Asian population (GG+AG versus AA: OR=1.87, 95% CI 1.43-2.46) and Caucasian population (G versus A: OR=1.17, 95% CI 1.02-1.35). Conclusion: Our study indicated that *CTLA-4* +49A/G mutations are associated with increased risk of T2D.

Keywords: Type 2 diabetes, CTLA-4, polymorphism, meta-analysis

Introduction

Type 2 diabetes (T2D) characterized by deficient insulin secretion and insulin resistance is a complex endocrine disease that affects the function of various organs of the body [1]. T2D has become one of the greatest healthcare challenges throughout the world due to increasing energy intake and population aging in recent years [2, 3]. It is generally considered that T2D is triggered by the interaction among environmental factors and genetic predisposition even though the explicit etiology of T2D is still unclear. Numerous studies worldwide have proved that environmental factors are associated with the occurrence of T2D such as diet [4], leisure time physical activity [5, 6], sleep quality [7], depression and stress [8]. Environmental factors have been proved to be involved in the development of T2D, but genetic susceptibility to T2D development has also been realized. More than 100 genetic loci have been confirmed to be associated with the development of T2D [9], including cytotoxic T lymphocyte-associated antigen 4 (*CTLA-4*) gene [10], fat mass and obesity associated gene (*FTO*) gene [11], apolipoprotein E (*ApoE*) gene [12], ankyrin 1 gene (*ANK1*) [13], growth arrest-specific gene 6 (*Gas6*) and adapter-related protein complex 3 subunit sigma-2 (*Ap3s2*) [14]. Among various genes, *CTLA-4* has been one of the most widely studied genes [10, 15, 16].

CTLA-4 is a leukocyte differentiation antigen and a transmembrane receptor on T cells, sharing B7 molecule ligand with CD28 and taking part in the negative regulation of the immune response [17]. Additionally, the function of CTLA-4 can be regulated through extracellular and intracellular mechanisms [18, 19]. AS a negative regulatory molecule of the immune response, many studies have proved that *CTLA-*

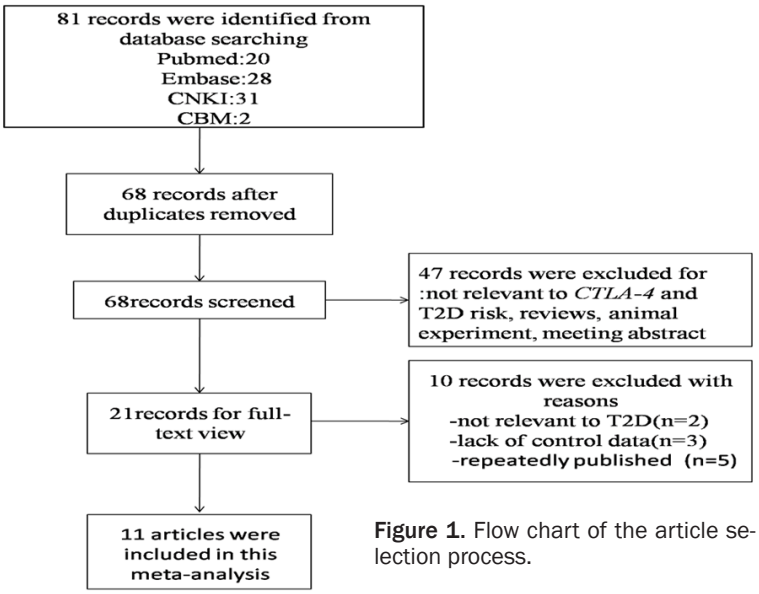

Figure 1. Flow chart of the article selection process.

4 polymorphism was related to the development of many autoimmune diseases, especially, T2D was one of the most widely researched diseases over the last few years [10, 15, 16, 20, 21]. The three most extensively investigated genetic polymorphisms of *CTLA-4* are +49A/G (rs231775), CT60G/A (rs3087243) and -318C/T (rs5742909). Among them, *CTLA-4* +49A/G polymorphism is the most frequently researched to date [16, 22-24]. In order to evaluate the relationship of *CTLA-4* +49A/G polymorphism and T2D, numerous researches have been conducted in recent years. Interestingly, the results of these researches were inconsistent. These controversial findings may be due to sample sizes, publication bias and racial differences. Therefore, we conducted this meta-analysis concerning the relationship between the polymorphism of *CTLA-4* +49A/G and T2D to solve the above-mentioned restrictions.

Materials and methods

Literature search

To identify the relevant articles, two investigators searched the PubMed, Embase, Chinese National Knowledge Infrastructure and Chinese biomedical databases respectively. The final search was performed on November 11, 2017 and the following keywords: “diabetes mellitus, type 2”, “type 2 diabetes”, “maturity-onset diabetes”, “non-insulin-dependent diabetes mellitus”, “cytotoxic T lymphocyte-associated antigen 4”, “CD152”, “CTLA-4”, “Polymorphism” were used as text words and Medical Subject Headings. No restriction on language was used and references of the inclusive articles were also searched.

Inclusion and exclusion criteria

On the one hand, the following inclusion criteria was applied to select the qualified articles: (1) concerning the relationship between *CTLA-4* +49A/G polymorphism and T2D; (2) case-control studies; (3) human subjects research; (4) appropriate data was provided from inclusion studies to calculate the odds ratio (OR) and 95% confidence interval (CI); (5) the genotype distributions of the subjects in the control group should be in accordance with Hardy-Weinberg equilibrium (HWE); (6) subjects in the control group should be free of T2D. On the other hand, the following exclusion strategies were applied to exclude the unqualified studies: (1) repeatedly published articles; (2) review, meta-analysis, animal study, case report, letter; (3) study without healthy control group; (4) study without suitable data to calculate the OR and 95% CI.

Data extraction

To screen out all the eligible articles, two reviewers carefully selected the eligible studies and collected data from them independently. Inconsistencies between the two investigators were settled by discussing with the third investigators. The following information about each eligible study was collected: (1) the name of the first author; (2) year of publication; (3) ethnicity of the study population; (4) gender and age for both groups (5) genotyping method; (6) sample size; (7) genotype frequency in the case and control groups.

Quality assessment

The quality of selected studies was evaluated carefully by two investigators according to the Newcastle-Ottawa Scale (NOS). The total score of one study was less than six indicated a low quality.

Table 1. Characteristics of the articles included in this meta-analysis

References	Years	Country	Ethnicity	Age mean ± SD (range)	Gender, n (Female/male)		Case/control	Genotype method	Quality score (NOS)
				Case/control	Case	Control			
Kiani et al	2016	Iran	Caucasians	55.8±6.7/34.4±9.6	46/65	41/59	111/100	ARMS-PCR	7
Jin et al	2015	China	Asians	52.4±11.2/48.1±10.8	142/188	185/297	330/482	PCR-RFLP	7
Chang et al	2014	Taiwanese	Asians	NA	NA	NA	449/432	PCR-RFLP	4
Shiau et al	2014	Taiwanese	Asians	NA	NA	NA	156/77	PCR-RFLP	4
Ahmadi et al	2013	Iran	Caucasians	NA	NA	63/44	56/107	PCR-RFLP	7
Haseda et al	2011	Japan	Asians	60 (32-74)/43 (27-61)	NA	NA	7/13	PCR-RFLP	6
Ding et al	2010	China	Asians	42±8/40±6	16/18	16/17	34/33	PCR-RFLP	8
Uzer et al	2010	Turkey	Caucasians	57.1±10.8/56.1±6.8	39/33	79/90	72/169	PCR-RFLP	8
Haller et al	2007	Estonia	Caucasians	64.5±10.1/45.5±14.2	150/94	151/101	244/252	PCR-RFLP	7
Gu et al	2007	China	Asians	62±10/60±9	NA	NA	111/39	PCR-RFLP	7
Rau et al	2001	Germany	Caucasians	59.6±18.4/NA	154/146	NA	300/466	PCR-RFLP	6

Abbreviations: NA, not available.

Table 2. Distribution of *CTLA4* polymorphism genotypes and alleles among T2D patients and controls

References	Case			Control			Case		Control		*P* for HWE
	AA	AG	GG	AA	AG	GG	G	A	G	A	
Kiani et al	9	42	60	20	39	41	162	60	121	79	0.0657
Jin et al	31	171	128	72	241	169	427	233	579	385	0.3541
Chang et al	40	201	208	64	208	160	617	281	528	336	0.7872
Shiau et al	13	76	67	13	46	18	210	102	72	82	0.0795
Ahmadi et al	35	18	3	67	36	4	24	88	44	170	0.7567
Haseda et al	1	3	3	3	3	7	9	5	17	9	0.0772
Ding et al	21	11	2	28	4	1	15	53	6	60	0.1255
Uzer et al	43	24	5	113	45	11	34	110	67	271	0.0348
Haller et al	76	122	46	77	135	40	214	274	215	289	0.1314
Gu et al	5	71	35	4	20	15	141	81	50	28	0.4754
Rau et al	34	140	126	68	215	183	392	208	581	351	0.707

Statistical analysis

The Stata 12.0 software (Stata, College Station, TX) was used to analyze the data. Firstly, the association of *CTLA-4* +49A/G polymorphism and T2D was evaluated by pooled OR with 95% CI in *Z*-test (statistically significant difference value of $P<0.05$). The pooled ORs were assessed in five genetic models: dominant genetic model, recessive genetic model, homozygote model, heterozygote model and allele model. Heterogeneity among all the including articles was evaluated by Cochran's Q statistic in combination with I^2 statistic (statistically significant difference value of $P<0.05$ or $I^2>50\%$). The pooled OR was evaluated by the fixed-effects model if $P\geq0.05$ or $I^2\leq50\%$; otherwise, random-effects model should be used. In addition, subgroup analysis was undertaken by ethnicity. Begg's funnel plot and Egger's test were performed to evaluate the publication bias (significant publication bias value of $P<0.05$) [25]. Sensitivity analysis was performed to evaluate the stability of the results by removing a study in turn. Lastly, the genotype distributions of control group were conducted by chi-square goodness-of-ft test and $P>0.01$ was considered that the genotype frequencies were in conformity to HWE.

Results

Characteristics of studies

In general, 81 articles were retrieved from online databases as mentioned above. Meta-analysis, meeting abstract, animal studies, reviews, repeatedly published study, study that was not relevant to *CTLA-4* +49A/G polymorphism and T2D risk and study without control data were excluded. Finally, 11 case-control studies consist of 1870 cases and 2170 controls were included in this study according to the inclusion and exclusion criteria (**Figure 1**) [10, 15, 16, 22-24, 26-30]. Among the 11 eligible articles, five studies' subjects were Caucasian [10, 16, 23, 26, 27] and the remaining six studies' subjects were from an Asian popu-

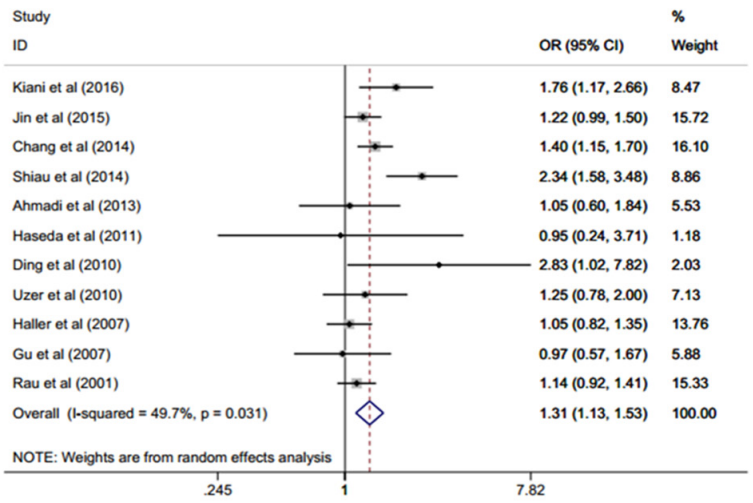

Figure 2. Forest plot for the association between *CTLA-4* +49A/G polymorphism and T2D (G vs. A).

Table 3. The results of the meta-analysis from various comparative genetic models

Genetic model	Population	OR (95% CI); *P*-value	Heterogeneity	
			P-value; I^2 (%)	Model
GG+AG vs. AA	All	1.47 (1.23-1.75); 0.000	0.218; 23.7	F
	Caucasian	1.23 (0.98-1.54); 0.079	0.210; 31.8	F
	Asian	1.87 (1.43-2.46); 0.000	0.899; 0.0	F
GG vs. AA+AG	All	1.30 (1.13-1.49); 0.000	0.414; 3.0	F
	Caucasian	1.23 (0.97-1.53); 0.067	0.771; 0.0	F
	Asian	1.35 (1.13-1.62); 0.001	0.150; 38.3	F
GG vs. AA	All	1.72 (1.39-2.13); 0.000	0.536; 0.0	F
	Caucasian	1.44 (1.06-1.95); 0.019	0.400; 1.0	F
	Asian	2.04 (1.52-2.75); 0.000	0.826; 0.0	F
AG vs. AA	All	1.38 (1.15-1.66); 0.001	0.347; 10.3	F
	Caucasian	1.17 (0.92-1.49); 0.206	0.316; 15.5	F
	Asian	1.72 (1.29-2.28); 0.000	0.812; 0.0	F
G vs. A	All	1.31 (1.13-1.53); 0.000	0.031; 49.7	R
	Caucasian	1.17 (1.02-1.35); 0.025	0.306; 17.1	F
	Asian	1.45 (1.12-1.87); 0.004	0.033; 58.9	R

Abbreviations: F, fixed-effects model; R, random-effects model.

lation [15, 22, 24, 28-30]. For genotyping, there were two genotyping methods used in the 11 eligible articles, one was polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and the other one was amplification refractory mutation system (ARMS-PCR). Furthermore, the genotype distributions of the subjects in the control group in the 11 eligible articles were in conformity to HWE. Characteristics of each eligible article were presented in **Table 1** and the genotype numbers were summarized in **Table 2**.

Quantitative synthesis

To assess the pooled OR, five genetic models were performed separately. For allele comparison, the random-effect model was applied in order to evaluate the pooled OR due to the presented heterogeneity (I^2=49.7%; P=0.031) in this model (**Figure 2**). Additionally, the fixed-effects model was conducted to assess the pooled OR in the remaining four genetic models owing to low heterogeneity (**Table 3**). Our results showed that *CTLA-4* +49A/G polymorphism was linked to the T2D susceptibility in the five genetic models (**Table 3**). Next, subgroup analysis was performed by ethnicity. For Asian population, significant association was found in the five genetic models (**Table 3**). However, statistically significant association was found only in homozygote model (GG versus AA: OR=1.44, 95% CI 1.06-1.95, P=0.019) (**Figure 3**) and allele model (G versus A: OR=1.17, 95% CI 1.02-1.35, P=0.025) (**Figure 4**) in Caucasians.

Sensitivity analysis and publication bias

To assess the stability of the pooled OR, sensitivity analysis was conducted in the five genetic models. No significant changes of pooled OR were found after sensitivity analysis (Figure S1). For publication bias, Begg's funnel plot and Egger's test was performed and the results showed that no presence of publication bias was detected in this study (P>|t|=0.103) (**Figure 5**).

Discussion

Diabetes mellitus is one of the most common complex metabolic diseases which characterized by hyperglycemia because of a deficient secretion of insulin and insulin resistance. In

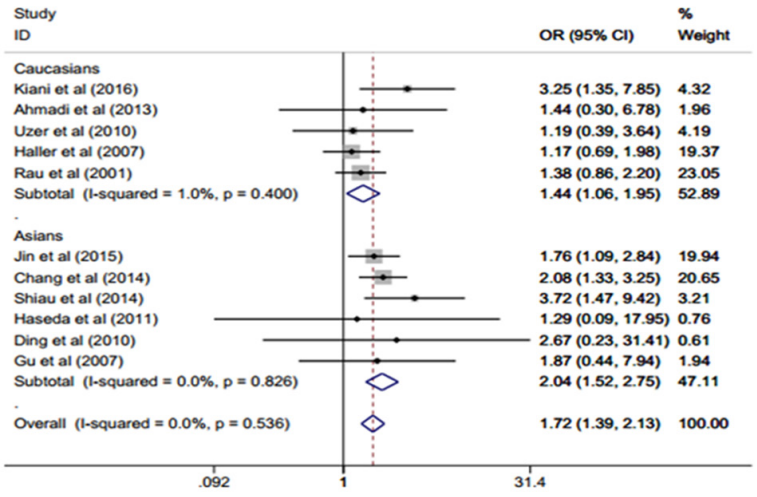

Figure 3. Forest plot for the association between *CTLA-4* +49A/G polymorphism and T2D (GG vs. AA). Subgroup analysis by ethnicity.

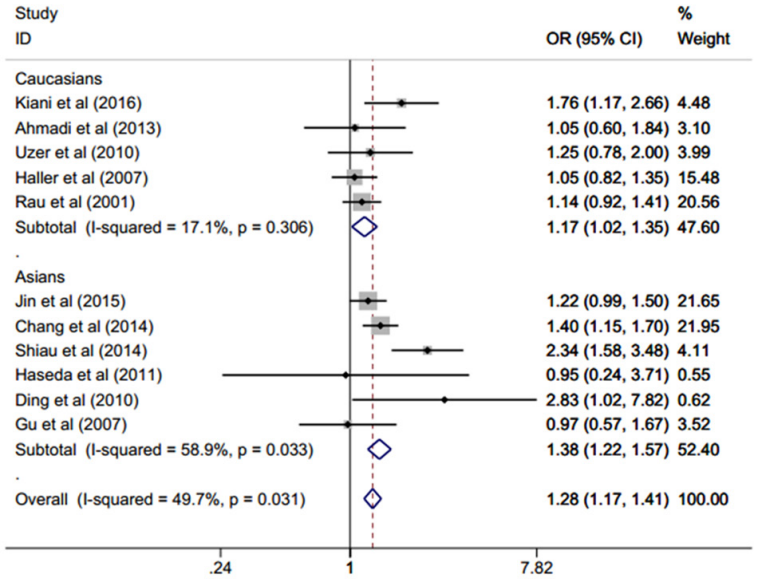

Figure 4. Forest plot for the association between *CTLA-4* +49A/G polymorphism and T2D (G vs. A). Subgroup analysis by ethnicity.

general, there are four types of diabetes mellitus, namely type 1 diabetes (T1D), T2D, gestational diabetes and other types of diabetes. Among them, T2D is one of the most widely studied diabetes mellitus in recent years. Although the exact cause of T2D is unclear until now, it is thought that the genetic susceptibility was associated with the occurrence of T2D. As a negative immune regulatory molecule, it has been found that CTLA-4 play a pivotal role in the occurrence and development of metabolic diseases [20, 31, 32]. Given its importance, many researches were conducted to evaluate the effect of the *CTLA-4* +49A/G polymorphism on T2D susceptibility for recent years. However, the results were not very consistent. Some of them found that *CTLA-4* +49A/G polymorphism was linked to the T2D susceptibility, but others showed that there was no association between them, mainly owing to the restriction of the individual studies. Therefore, we performed this meta-analysis to comprehensively evaluate the association between them through eliminating the restriction of individual studies.

A total of 4040 subjects from 11 case-control studies concerning the topic were included in this meta-analysis. To estimate the pooled ORs and 95% CI, five genetic models were performed separately and the results displayed that *CTLA-4* +49A/G polymorphism had a great contribution to the occurrence of overall T2D. For subgroup analysis, we found that *CTLA-4* +49A/G mutation was a risk factor for T2D both in Asian and Caucasian population. Interestingly, it seemed that the risk was much higher in Asian population than in Caucasian population, indicating that different genetic backgrounds and environmental factors may contribute to the occurrence of T2D. Although significant relationship was found only in allele model and homozygote model in Caucasians, trend of risk effect of *CTLA-4* +49A/G polymorphism was found in dominant genetic model, recessive genetic model and heterozygote model. The results might be significant in these three genetic models if more large-scale case-control studies in Caucasians were involved in this meta-analysis. Therefore, more studies in Caucasians were needed especially in the future.

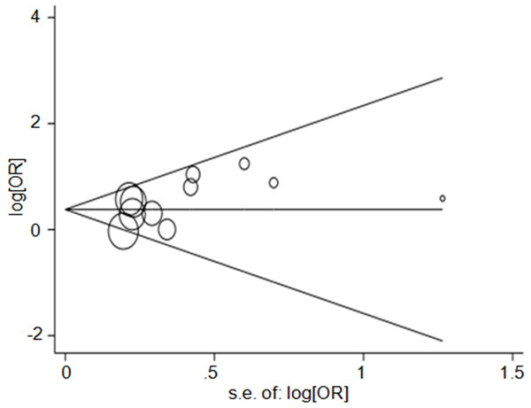

Figure 5. Begg's funnel plot for publication bias analysis of *CTLA-4* +49A/G polymorphism.

CTLA-4 +49A/G polymorphism has been extensively researched in diabetes mellitus for recent years. Jin et al showed that the *CTLA-4* +49A/G polymorphism contributed to the occurrence of latent autoimmune diabetes in adults (LADA) and T1D in Chinese adults [15]. A latest meta-analysis found that *CTLA-4* +49A/G polymorphism was a risk factor for T1D susceptibility [33]. Additionally, Si et al also found a positive relationship between *CTLA-4* +49A/G polymorphism and T1D susceptibility by performing a meta-analysis [34]. Interestingly, the results of these two meta-analysis studies were similar to those found in our study, implying that T2D and T1D might share some genetic background. As far as we know, many researches with respect to the relationship between *CTLA-4* +49A/G polymorphism and T2D were small sample size case-control studies. Thus, the results of these studies were far from certain. In this study, a total of 1870 T2D cases and 2170 controls from 11 case-control studies were included, so that our pooled results may provide more powerful evidence for the relationship between *CTLA-4* +49A/G polymorphism and T2D.

Both publication bias and heterogeneity are important impact factors that affect the outcome of meta-analysis. Therefore, Begg's funnel plot and Egger's test were conducted to evaluate the publication bias. The results showed no presence of publication bias in this study, suggesting the credible pooled results in our meta-analysis. In addition, heterogeneity was found only in the allele model in overall population. However, the heterogeneity increased in Asian population but decreased in Caucasian population in subgroup analysis by ethnicity, indicating genetic backgrounds contributed an important role to the source of heterogeneity.

To some degree, several limitations might have an effect on our pooled results. Firstly, the number of case-control studies included in our meta-analysis was relatively small, which impaired the power to evaluate the relationship between *CTLA-4* +49A/G polymorphism and T2D. Secondly, due to lacking of sufficient data in some studies, we could not get usable data on age, gender, blood sugar levels, physical activity and sleep quality so that we failed to perform subgroup analysis by these covariates. Thirdly, several case-control studies were excluded from this study owing to suspicious data which would lead to selection bias. Finally, all eligible articles included in this study were from Asians and Caucasians so that our results only apply to Asian and Caucasian population.

In conclusion, this meta-analysis suggested that *CTLA-4* +49A/G polymorphism contributed to the susceptibility to T2D. Moreover, the risk was higher in Asian population than in Caucasian population. Considering the limitations, more large-scale case-control studies about the association between *CTLA-4* +49A/G polymorphism and T2D are required to confirm our present findings in the future.

Disclosure of conflict of interest

None.

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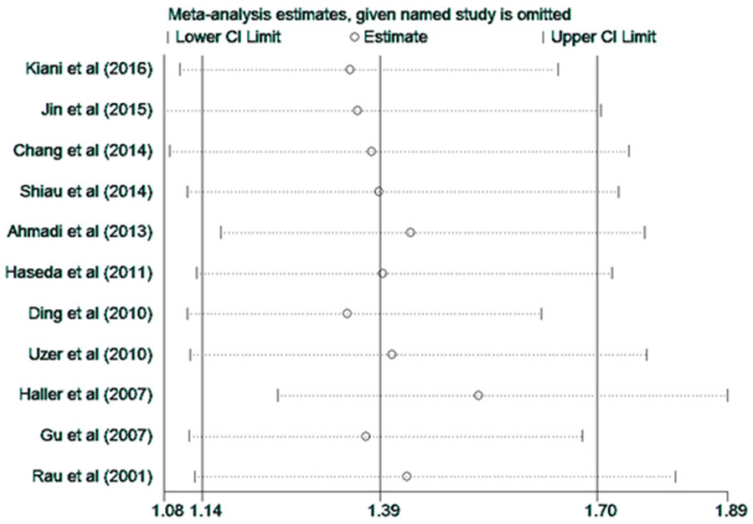

Figure S1. Sensitivity analysis for the *CTLA-4 +49A/G* polymorphism with T2D risk.