

# Association between CD40 rs1883832 and immune-related diseases susceptibility: A meta-analysis

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## ABSTRACT

**Background/objective:** It has been reported that CD40 rs1883832 might be associated with immune-related diseases susceptibility. Owing to mixed and inconclusive results, we conducted a meta-analysis of case-control studies to summarize and clarify this association.

**Methods/main results:** A systematic search of studies on the association between CD40 rs1883832 and immune-related diseases susceptibility was conducted in databases. Odds ratios and 95% confidence intervals were used to pool the effect size. 40 articles were included in our meta-analysis.

**Conclusions:** CD40 rs1883832 is associated with decreased risk of Graves' disease, especially in Asian; CD40 rs1883832 is associated with increased risk of multiple sclerosis; CD40 -1C>T (rs1883832) is not associated with the susceptibility of Hashimoto's thyroiditis, systemic sclerosis or Asthma; there is insufficient data to fully confirm the association between CD40 rs1883832 and systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), Behçet's disease (BD), myasthenia gravis (MG), Crohn's disease (CD), ulcerative colitis (UC), Sarcoidosis, Fuch uveitis syndrome (FUS), Vogt-Koyanagi-Harada syndrome (VKH), Kawasaki disease (KD), giant cell arteritis (GCA) or Immune thrombocytopenia (ITP).

## INTRODUCTION

CD40, expressed by some immune and non-immune cells, is a tumor necrosis factor receptor that plays a critical role in adaptive immunity. CD40 involves in B cell proliferation, the generation of plasma cells, B cell memory and the development of immature dendritic cells. [1-3] CD40 is an attractive candidate receptor for contributing to a variety of immune-related processes in which B and T cell activation play a role in pathogenesis [4].

CD40 -1C>T (rs1883832) is a functional single nucleotide polymorphism (SNP) located at -1 from the start codon of CD40 gene. The rs1883832T decreased the translational efficiency of CD40 transcripts, resulting in less CD40 protein level [5].

Association between CD40 rs1883832 and immune-related diseases susceptibility has been studied in several populations. Sample sizes in these studies are relatively small. In Graves' disease and Hashimoto's thyroiditis, meta-analysis has been performed before, [6, 7] however new studies and more immune-related diseases need to be involved. Therefore, we decided to perform a meta-analysis of case-control studies to estimate it.

## MATERIALS AND METHODS

### Identification of eligible studies

A systematic search in PubMed, Embase, Cochrane Library, clinicaltrials.gov, CNKI (China National

Knowledge Infrastructure), WanFangData (one China database) and CQVIP (one China database) databases were carried out by two independent investigators. The following terms were used: “CD40 OR TNFRSF5 OR Bp50” AND “rs1883832 OR C/T-1 OR C>T-1 OR -1C/T OR -1C>T OR C-1T OR Kozak OR 5'-untranslated region OR 5'-utr”, without any limitation applied. The last search update was performed on August 4, 2016. References of related studies and reviews were also manually searched for additional studies. GWAS were searched in Immunobase.

## Inclusion and exclusion criteria

Studies selected in this meta-analysis must meet the following inclusion criteria: (1) evaluation of the association between CD40 rs1883832 and immune-related diseases; (2) case-control study; (3) studies focusing on tissues of human beings; (4) detailed genotype data could be acquired to calculate the odds ratios (ORs) and 95% confidence intervals (95% CIs). Exclusion criteria: (1) duplication of previous publications (When there were multiple publications from the same population, only the largest study was included); (2) comment, review and editorial; (3) study without detailed genotype data; (4) GWAS; (5) studies focusing on cell lines. Dissertation thesis were included in the analysis.

Study selection was achieved by two investigators independently, according to the inclusion and exclusion criteria by screening the title, abstract and full-text. Any dispute was solved by discussion.

## Data extraction

Two investigators extracted data of the eligible studies independently. In the case of a conflict, an agreement was reached by discussion. If the dissent still existed, the third investigator would be involved to adjudicate the disagreements. Try to contact the author by email for detailed genotype data.

The following contents were collected: first author's surname, year of publication, disease type, the characteristics of cases and controls, source of control groups, country of origin, the detective sample, ethnicity, genotyping method, Hardy-Weinberg equilibrium, number of cases and controls for each genotype.

## Methodological quality assessment

The qualities of included studies were evaluated independently by two investigators according to Newcastle-Ottawa Scale (NOS) [8] and the most important factor was “age, gender and country”. Quality scores range from 0 to 9, and higher scores means better quality of the study. Disagreement was resolved through discussion.

## Statistics analysis

Our meta-analysis was conducted according to the PRISMA checklists. [9] Hardy-Weinberg equilibrium (HWE) was evaluated for each study by Chi-square test in control groups, and  $P < 0.05$  was considered as a significant departure from HWE. OR and 95% CIs were calculated to evaluate the strength of the association between CD40 rs1883832 and immune-related diseases. Pooled ORs were obtained from combination of single studies by allelic comparison (T vs C), dominant model (CT+TT vs CC), recessive model (TT vs CC+CT), homozygote comparison (TT vs CC) and heterozygote comparison (CT vs CC), respectively. The statistical significant level was determined by Z-test with  $P$  value less than 0.05.

Heterogeneity was evaluated by  $Q$ -test and  $I^2$  index. [10] When  $Q$ -test's  $P$ -value was less than 0.10 and/or  $I^2$  index was more than 50%, the random-effects model (DerSimonian and Laird method) was used; otherwise, the fixed-effects model (Mantel and Haenszel method) was conducted. [11] Sensitivity analyses were performed towards each genetic model to evaluate effect of each study on combined ORs by sequentially excluding each study in total and in any subgroup including more than two studies. Besides, subgroup analyses were stratified by disease, ethnicity (Caucasian and Asian), control type (population-based and hospital-based) and HWE ( $P < 0.05$  and  $P \geq 0.05$ ). Potential publication bias was checked by Begg's funnel [12] plots and Egger's test. [13] An asymmetric plot, the  $P$  value of Begg's test ( $P_B$ ) less than 0.05, and the  $P$  value of Egger's test ( $P_E$ ) less than 0.05 was considered a significant publication bias. All statistical analyses were performed with Stata 12.0 software (StataCorp, College Station, Texas, USA). A two-tailed  $P < 0.05$  was considered significant except for specified conditions, where a certain  $P$  value was declared.

## RESULTS

### Characteristics of studies

A total of 388 articles were acquired from databases (PubMed = 78, Embase = 98, Cochrane = 2, clinicaltrials.gov = 0, CNKI = 118, WanFangData = 79, CQVIP = 12, other sources (from manually search) = 1 [14]). The selection process was shown in Figure 1. 11 full-text articles were excluded (2 duplicate study [63, 64]; 3 detailed genotype data could not be extracted and might from the same population of another study [14–16]; 4 improper full text [17–20]; 2 might from the same population of a larger study [21, 22]). Finally, 40 articles [23–62] were included in our meta-analysis. The characteristics of each study were shown in Supplementary Table 1. Different genotyping methods were utilized including sequencing, PCR-RFLP, PCR-

**Table 1: Summary of pooled ORs in the meta-analysis**

	Number (cases/controls)	T vs C		TT vs CC		CT vs CC		CT+TT vs CC		TT vs CC+CT	
		OR* (95%CI)	I <sup>2</sup> (%)	OR (95%CI)	I <sup>2</sup> (%)	OR (95%CI)	I <sup>2</sup> (%)	OR (95%CI)	I <sup>2</sup> (%)	OR (95%CI)	I <sup>2</sup> (%)
GD*	5006/4537	<b>0.738 (0.664–0.820)</b>	60.1	0.537 (0.424–0.679)	57.3	0.786 (0.719–0.859)	29.9	0.706 (0.619–0.806)	51.9	0.657 (0.577–0.748)	46.2
HWE*	4560/4171	<b>0.775 (0.697–0.861)</b>	55.8	0.623 (0.535–0.727)	49.7	<b>0.809 (0.738–0.886)</b>	24.2	<b>0.774 (0.709–0.845)</b>	46.4	<b>0.696 (0.604–0.801)</b>	35.2
PB*	4746/4289	<b>0.744 (0.666–0.830)</b>	60.7	0.541 (0.420–0.696)	58.7	<b>0.788 (0.720–0.863)</b>	33.5	<b>0.712 (0.620–0.817)</b>	53.2	<b>0.670 (0.584–0.769)</b>	48.0
Caucasian	2247/2297	<b>0.832 (0.697–0.992)</b>	60.4	0.745 (0.462–1.201)	53.5	<b>0.816 (0.721–0.923)</b>	0.8	<b>0.814 (0.722–0.917)</b>	43.0	0.863 (0.662–1.125)	45.3
Asian	2759/2240	<b>0.700 (0.619–0.791)</b>	53.5	0.507 (0.428–0.600)	48.0	<b>0.755 (0.665–0.858)</b>	39.9	<b>0.653 (0.546–0.781)</b>	51.5	<b>0.602 (0.518–0.699)</b>	34.6
HWE	2313/1874	<b>0.743 (0.652–0.847)</b>	50.7	0.553 (0.459–0.667)	34.8	<b>0.800 (0.698–0.917)</b>	38.8	<b>0.731 (0.642–0.831)</b>	49.6	<b>0.638 (0.540–0.754)</b>	9.7
PB	2499/1992	<b>0.703 (0.615–0.804)</b>	56.2	0.482 (0.367–0.633)	51.9	<b>0.758 (0.663–0.866)</b>	44.8	<b>0.654 (0.538–0.796)</b>	54.9	<b>0.609 (0.518–0.716)</b>	39.5
HT*	576/604	1.079 (0.914–1.274)	0.0	1.171 (0.825–1.661)	0.0	1.121 (0.870–1.445)	0.0	1.129 (0.888–1.435)	0.0	1.069 (0.781–1.463)	0.0
MS*	3851/4368	<b>1.175 (1.093–1.263)</b>	23.0	<b>1.329 (1.117–1.582)</b>	29.3	<b>1.189 (1.081–1.309)</b>	0.0	<b>1.213 (1.107–1.329)</b>	0.0	<b>1.239 (1.047–1.467)</b>	20.9
HWE	2321/1471	<b>1.249 (1.120–1.393)</b>	0.0	<b>1.573 (1.195–2.071)</b>	4.3	<b>1.227 (1.066–1.414)</b>	0.0	<b>1.277 (1.116–1.460)</b>	0.0	<b>1.458 (1.114–1.908)</b>	0.0
PB	2172/3489	<b>1.162 (1.067–1.265)</b>	39.7	<b>1.302 (1.066–1.591)</b>	45.3	<b>1.178 (1.050–1.321)</b>	0.0	<b>1.199 (1.075–1.338)</b>	0.0	<b>1.217 (1.002–1.477)</b>	38.8
SSc*	2605/3138	1.022 (0.940–1.111)	0.0	1.086 (0.888–1.328)	40.8	0.994 (0.890–1.110)	27.1	1.009 (0.908–1.120)	0.0	1.099 (0.793–1.523)	<b>56.9</b>
SLE*	905/1307	1.115 (0.808–1.537)	<b>78.5</b>	1.249 (0.695–2.245)	<b>72.5</b>	<i>1.042 (0.570–1.903)</i>	84.3	1.093 (0.611–1.956)	<b>85.1</b>	1.183 (0.927–1.509)	0.2
PB	700/1087	0.950 (0.620–1.455)	<b>77.4</b>	0.963 (0.534–1.734)	<b>54.0</b>	0.779 (0.284–2.139)	89.6	0.817 (0.333–2.004)	<b>88.4</b>	1.059 (0.793–1.413)	0.0
Asthma	675/333	0.980 (0.807–1.189)	2.1	0.903 (0.602–1.354)	0.0	1.007 (0.555–1.826)	71.5	0.978 (0.592–1.614)	<b>64.6</b>	0.911 (0.630–1.319)	0.0
RA*	1722/2021	0.949 (0.792–1.138)	<b>52.5</b>	0.887 (0.563–1.397)	<b>60.2</b>	0.942 (0.821–1.081)	0.0	0.918 (0.805–1.046)	11.6	0.890 (0.604–1.313)	52.9
BD*	658/627	1.062 (0.796–1.416)	<b>67.1</b>	1.189 (0.640–2.209)	<b>66.5</b>	0.857 (0.673–1.091)	0.0	0.959 (0.765–1.203)	0.0	1.284 (0.661–2.492)	<b>74.3</b>
Overall	19482/24998	<b>0.932 (0.873–0.994)</b>	<b>74.6</b>	<i>0.873 (0.760–1.002)</i>	<b>70.1</b>	0.944 (0.875–1.017)	<b>59.9</b>	<i>0.924 (0.852–1.001)</i>	<b>69.7</b>	0.918 (0.821–1.027)	<b>60.6</b>
HWE	15471/15941	<i>0.943 (0.880–1.010)</i>	<b>70.1</b>	0.905 (0.781–1.047)	<b>65.3</b>	0.940 (0.869–1.017)	<b>52.7</b>	<i>0.929 (0.853–1.012)</i>	<b>64.2</b>	0.947 (0.841–1.068)	<b>55.0</b>
PB	17126/23175	<b>0.921 (0.861–0.984)</b>	<b>72.6</b>	<b>0.851 (0.739–0.981)</b>	<b>68.0</b>	<i>0.927 (0.858–1.002)</i>	<b>58.8</b>	<b>0.908 (0.836–0.986)</b>	<b>67.9</b>	<i>0.905 (0.805–1.017)</i>	<b>58.9</b>
Caucasian	12919/18426	1.019 (0.948–1.095)	<b>66.9</b>	1.073 (0.918–1.254)	<b>55.4</b>	1.010 (0.931–1.095)	<b>54.5</b>	1.015 (0.932–1.106)	<b>62.7</b>	<i>1.057 (0.968–1.154)</i>	48.3
HWE	9354/9735	0.993 (0.910–1.085)	<b>65.3</b>	1.051 (0.860–1.286)	<b>57.0</b>	0.968 (0.910–1.029)	42.1	0.975 (0.883–1.075)	<b>56.7</b>	1.070 (0.891–1.284)	<b>50.7</b>
PB	11240/17547	1.007 (0.935–1.085)	<b>65.6</b>	1.051 (0.893–1.237)	<b>55.3</b>	0.996 (0.916–1.083)	<b>53.1</b>	1.001 (0.917–1.093)	<b>61.2</b>	1.041 (0.950–1.140)	48.9
Asian	6563/6572	<b>0.863 (0.775–0.961)</b>	<b>76.0</b>	<b>0.747 (0.604–0.923)</b>	<b>73.2</b>	<b>0.870 (0.762–0.994)</b>	<b>61.1</b>	<b>0.834 (0.722–0.963)</b>	<b>71.1</b>	<b>0.820 (0.696–0.965)</b>	<b>63.6</b>
HWE	6117/6206	<i>0.904 (0.814–1.004)</i>	<b>72.9</b>	<i>0.819 (0.667–1.006)</i>	<b>68.8</b>	0.911 (0.798–1.040)	<b>59.1</b>	<i>0.884 (0.767–1.019)</i>	<b>68.6</b>	<i>0.878 (0.750–1.027)</i>	<b>56.8</b>
PB	5886/5628	<b>0.846 (0.758–0.945)</b>	<b>73.5</b>	<b>0.717 (0.577–0.891)</b>	<b>70.3</b>	<b>0.845 (0.735–0.972)</b>	<b>59.9</b>	<b>0.810 (0.698–0.940)</b>	<b>69.0</b>	<b>0.801 (0.674–0.951)</b>	<b>61.4</b>

\*OR: Odds ratio; CI: confidence interval; PB: population-based; HWE: in all studies of this subgroup, the HWE's P value ≥ 0.05. GD: Graves' disease; HT: Hashimoto's thyroiditis; MS: multiple sclerosis; SSc: systemic sclerosis; SLE: systemic lupus erythematosus; RA: rheumatoid arthritis; BD: Behçet's disease. \*Results with statistical significant difference were marked as bold. Unstable results in sensitivity analyses were marked as italic.

HRM, TaqMan PCR, MassARRAY. Blood samples were used for genotyping in all studies.

immune-related diseases risk was found in other analyses. (Detailed in Table 1).

### Overall analyses and subgroup analyses

Summary results of each genetic model were listed in Table 1. Significantly decreased risk of immune-related diseases was found in almost all genetic models of GD (Graves' disease) and its subgroups except for homozygote comparison (TT vs CC) and recessive model (TT vs CC+CT) of GD's Caucasian subgroup. Significantly increased risk was found in all genetic models of MS (multiple sclerosis) and its subgroups. Overall, significantly decreased risk of immune-related diseases was found in allelic comparison (T vs C) and in several subgroups. No statistically significant changes of

### Sensitivity analyses

Sensitivity analyses were performed in any comparison and any subgroup including more than two studies. When study Tomer Y [23], Heward JM [25], Kurylowicz A [28] or Jacobson E [32] was excluded, statistically different results were obtained in allelic comparison (T vs C) of GD's Caucasian subgroup. Statistically different results were obtained in recessive model (TT vs CC+CT) of MS and in several genetic models of MS's HWE and PB subgroup. Statistically different results were obtained in heterozygote comparison (CT vs CC) of SLE (systemic lupus erythematosus).

Overall, statistically different results were obtained in several genetic models and in some subgroups. (Table 1 and Supplementary Data).

Because of the limited number of included studies, sensitivity analyses could not be performed in RA (rheumatoid arthritis), BD (Behçet's disease) and PB subgroup of SLE.

Other results showed stability in sensitivity analyses. (Supplementary data).

### Publication bias

Begg's funnel plot and Egger's test were used to assess the publication bias. Symmetry of funnel plot,  $P$  value of Begg's test ( $P_B$ ) and  $P$  value of Egger's test ( $P_E$ )

were evaluated in every genetic model overall and in MS, GD, GD's Asian subgroup, GD's Caucasian subgroup. Significant publication bias was found in heterozygote comparison (CT vs CC) overall ( $P_B = 0.043$ ), dominant model (CT+TT vs CC) overall ( $P_B = 0.000$ ), recessive model (TT vs CC+CT) overall ( $P_E = 0.021$ ), recessive model (TT vs CC+CT) of MS ( $P_E = 0.033$ ), homozygote comparison (TT vs CC) of GD's Asian subgroup ( $P_E = 0.016$ ), dominant model (CT+TT vs CC) of GD's Caucasian subgroup ( $P_E = 0.044$ ). (Supplementary data).

### DISCUSSION

In GD, 5006 cases/4537 controls were involved, and we found CD40 -1C>T was associated with decreased

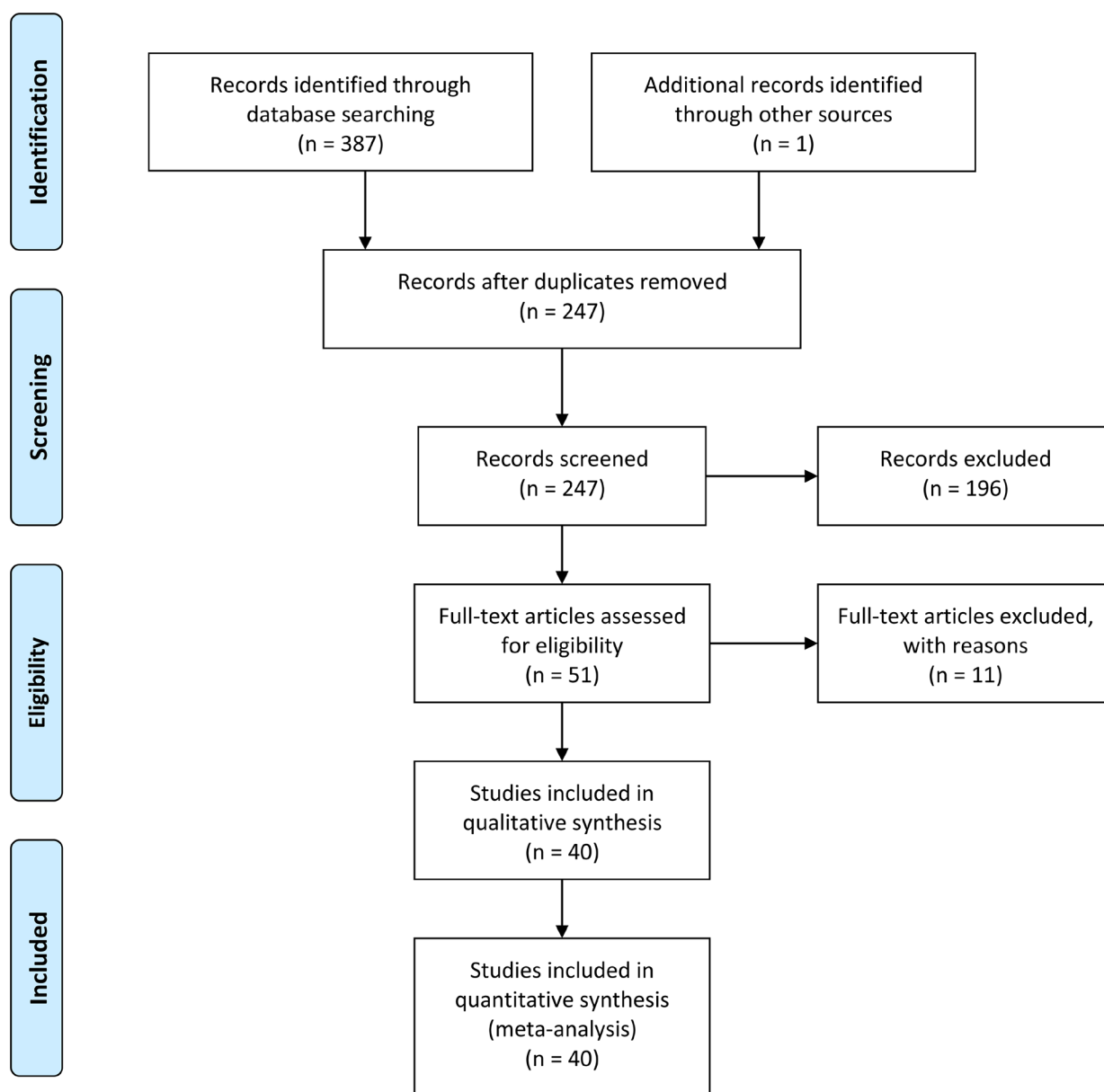


Figure 1: Flow chart of study selection.



risk of GD in any genetic model and the results showed stability in sensitivity analyses and no publication bias. Similar stable results were shown in GD's Asian subgroup in any genetic model, but significant publication bias was found in homozygote comparison (TT vs CC). Similar stable results were shown in GD's Caucasian subgroup in heterozygote comparison (CT vs CC) and dominant model (CT+TT vs CC), but significant publication bias was found in dominant model (CT+TT vs CC). This may reveal the difference in ethnicity.

In MS, 3851 cases/4368 controls were involved, and we found CD40 -1C>T was associated with increased risk of MS in 4 genetic models and the results showed stability and no publication bias. Similar stable results were found in its HWE and PB subgroups in allelic comparison (T vs C) and dominant model (CT+TT vs CC), however, publication bias analyses could not be performed. Our results in MS was consistent with the GWAS (OR: 1.0740; 95%CI: 1.0360–1.1140; *P* value: 1.19E-04) performed by IMSGC et al. [65] in Caucasian.

In HT (576 cases/604 controls), SSc (2605 cases/3138 controls) and Asthma (675 cases/333 controls), no association was found in any genetic model and the results showed stability in sensitivity analyses.

In SLE (905 cases/1307 controls), no evidence was found for the association between CD40 -1C>T and SLE susceptibility in any genetic model, however, the results in heterozygote comparison (CT vs CC) lacked stability. In three studies of SLE, increased risk of SLE was found in dominant model (CT+TT vs CC: OR (95%CI) = 1.27 (1.02, 1.57), *P* = 0.03) of Joo YB [48] (593 cases/978 controls; Asian), however it lost significance after correction by age and sex. Similar results were found in dominant model (CT+TT vs CC: OR (95%CI) = 1.88 (1.23, 2.89), *P* = 0.004) of Wu C [50] (205 cases/220 controls; Asian), but no correction was done further. In Zhu Q [49] (107 cases/109 controls; Asian), decreased risk of SLE was found in dominant model (CT+TT vs CC: OR (95%CI) = 0.495 (0.278, 0.881), *P* = 0.017) and no correction was done. Thus no certain conclusion can be drawn about the association between CD40 -1C>T and SLE susceptibility, and more studies were needed.

In RA, BD, MG, CD, UC, Sarcoidosis, FUS, VKH, KD, GCA and ITP, less than three studies were included so that sensitivity analyses could not be performed. In two studies of RA, decreased risk of RA was found in allelic comparison (T vs C: OR (95%CI) = 0.89 (0.79, 0.99), *P* = 0.038) and homozygote comparison (TT vs CC: OR (95%CI) = 0.735 (0.553, 0.978), *P* = 0.035) of García BM [55] (1510 cases/1545 controls; Caucasian), but no association was found in any genetic model of Liu R [54] (212 cases/476 controls; Asian). In two studies of BD, increased risk of BD was found in recessive model (TT vs TC+CC: OR (95%CI) = 1.73 (1.22, 2.46), *P* = 0.002) and homozygote comparison (TT vs CC: OR (95%CI) = 1.565 (1.057, 2.317), *P* = 0.025) of Chen F [56] (373 cases/402 controls; Asian), but no association was found in

any genetic model of Inal EE [57] (285 cases/225 controls; Caucasian).

Overall, because the association between CD40 -1C>T and GD conflicted with MS, the importance and scientific significance of the results decreased. Furthermore, 15 results were found unstable in sensitivity analyses and significant publication bias was found in 3 genetic models.

CD40 is expressed by some immune and non-immune cells. Thyroid follicular cells and orbital fibroblasts also express CD40 [66, 67]. Orbital fibroblasts are the target cells in Graves ophthalmopathy. *In vitro* studies demonstrated that the activation of CD40 on orbital fibroblasts leads to increased glycosaminoglycan productions, suggesting an important role in the pathogenesis of Graves' ophthalmopathy [68]. The association between CD40 overactivation and GD has been firmly established. Transgenic mouse models constitutively overexpressing thyroidal CD40 develop more severe experimental autoimmune GD and thyrotoxicosis, whereas blockade of CD40 stimulation in experimental animal models suppresses progression to overt thyroiditis [69]. Similarly, functional blockade of CD40 with a murine antibody effectively prevents clinical expression in an animal model of multiple sclerosis. [70] CD40 -1C>T decreased the translational efficiency of CD40 transcripts, resulting in less CD40 protein level. However, our meta-analysis result and GWAS by IMSGC et al. [65] indicated that CD40 -1C>T is associated with increased risk of MS. Recently research showed that CD4(+) and CD8(+) Treg, which can be induced by CD40-activated B cells, play important roles in the maintenance of immune tolerance. The immune function of CD4(+)CD25(high) Tregs in MS patients significantly decreases as compared with normal controls. Adoptive transfer of CD8(+) Treg in rodents or induction of CD8(+) Treg in humans can prevent or treat autoimmune diseases. [71–73] It seems that different immune-related disease involves in different kind of disruption of immune balance.

Meanwhile, the limitations of this meta-analysis need to be addressed. To date, the number of available studies which can be included in this meta-analysis were small, especially available studies about HT, MS, SSc, SLE, Asthma, RA, BD, MG, CD, UC, Sarcoidosis, FUS, VKH, KD, GCA and ITP. Data for subgroup analyses were scanty. For example, gender subgroup analyses can not be done. For another example, the ethnicity in all studies of HT, SLE and Asthma were Asian, and in all studies of MS and SSc were Caucasian. Sensitivity analyses and publication bias analyses could not be performed in all subgroups. Some studies shared there controls with each other, like study NO.2 shared controls with NO.20, which were counted redundantly. (Detailed in Supplementary Table 1) Related studies published in other languages or unpublished were possibly missed.

In conclusion, our results suggested that: (1) CD40 -1C>T (rs1883832) is associated with decreased risk

of Graves' disease (GD), especially in Asian; (2) CD40 -1C>T (rs1883832) is associated with increased risk of MS (multiple sclerosis); (3) CD40 -1C>T (rs1883832) is not associated with the susceptibility of Hashimoto's thyroiditis (HT), systemic sclerosis (SSc) or Asthma; (4) there is insufficient data to fully confirm the association between CD40 -1C>T (rs1883832) and SLE, RA, BD, MG, CD, UC, Sarcoidosis, FUS, VKH, KD, GCA or ITP, and the results should be interpreted with caution. Well-designed studies with larger sample size and more subgroups are required to validate the risk identified in the current meta-analysis.

## CONFLICTS OF INTEREST

None.

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