Research Article

Associations between cytotoxic T-lymphocyte-associated antigen 4 gene polymorphisms and diabetes mellitus: a meta-analysis of 76 case–control studies

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Background: Several genetic association studies already investigated potential roles of cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) gene polymorphisms in diabetes mellitus (DM), with inconsistent results. Therefore, we performed this meta-analysis to better assess the relationship between CTLA-4 gene polymorphisms and DM in a larger pooled population.

Methods: PubMed, Embase, Web of Science, and CNKI were systematically searched for eligible studies. Pooled odds ratios (ORs) with 95% confidence intervals (CIs) were calculated to estimate the strength of associations between CTLA-4 gene polymorphisms and DM in all possible genetic models.

Results: A total of 76 studies were finally included in our analyses. Significant associations with susceptibility to type 1 diabetes mellitus (T1DM) were detected for rs231775 (dominant model: \( P = 0.008, \ OR = 0.83, \ 95\% \ CI \ 0.73–0.95 \); recessive model: \( P = 0.003, \ OR = 1.27, \ 95\% \ CI \ 1.09–1.50 \); allele model: \( P = 0.004, \ OR = 0.85, \ 95\% \ CI \ 0.77–0.95 \)) and rs5742909 (recessive model: \( P = 0.02, \ OR = 1.50, \ 95\% \ CI \ 1.05–2.13 \)) polymorphisms in overall population. Further subgroup analyses revealed that rs231775 polymorphism was significantly associated with susceptibility to T1DM in Caucasians and South Asians, and rs5742909 polymorphism was significantly associated with susceptibility to T1DM in South Asians. Moreover, rs231775 polymorphism was also found to be significantly associated with susceptibility to type 2 diabetes mellitus (T2DM) in East Asians and South Asians.

Conclusions: Our findings indicated that rs231775 and rs5742909 polymorphisms may serve as genetic biomarkers of T1DM, and rs231775 polymorphism may also serve as a genetic biomarker of T2DM.

Introduction

Diabetes mellitus (DM), characterized by chronic hyperglycemia caused by deficiency in insulin secretion or resistance against insulin, is the most prevalent metabolic disorder worldwide, and it currently affects over 350 million people globally [1,2]. So far, the exact underlying pathogenic mechanism of DM is still not fully understood. Nevertheless, the fact that over 100 genetic loci were already found to be correlated with an increased susceptibility to DM by past genome-wide association studies suggested that genetic factors were crucial for the occurrence and development of DM [3,4].

Cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) is mainly expressed on activated T cells, and it serves a negative regulator of T cell activation and proliferation [5]. Previous studies showed that CTLA-4 could induce T cell tolerance and attenuate T cell mediated immune responses by binding with

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co-stimulating molecules, B7-1 (CD80) and B7-2 (CD86) [6], and dysfunction of CTLA-4 was demonstrated to be implicated in various autoimmune diseases including type 1 diabetes mellitus (T1DM) [7,8]. Consequently, CTLA-4 gene polymorphisms were intensively studied with regard to their associations with T1DM [9–12]. Recently, some pilot studies also analyzed potential associations between CTLA-4 gene polymorphisms and the much more prevalent type 2 diabetes mellitus (T2DM) [13,14]. Nevertheless, whether CTLA-4 gene polymorphisms were associated with T1DM and T2DM or not remain controversial, especially when they were conducted in different populations. Therefore, we performed the present meta-analysis to pool the data of all relevant studies, and obtain more conclusive results on associations of CTLA-4 gene polymorphisms with T1DM and T2DM.

Materials and methods

Literature search and inclusion criteria

The current meta-analysis was compiled with the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) statement [15]. Potentially relevant articles were searched in PubMed, Medline, Web of Science, and CNKI using the following key words: ‘Cytotoxic T-lymphocyte antigen 4’, ‘CTLA-4’, ‘polymorphism’, ‘variant’, ‘mutation’, ‘genotype’, ‘allele’, ‘diabetes mellitus’, ‘diabetes’, and ‘DM’. The initial literature search was conducted in October 2018 and the latest update was performed in January 2019. We also screened the reference lists of all retrieved articles to identify other potentially relevant studies.

Included studies should meet all the following criteria: (1) case–control study on associations between CTLA-4 gene polymorphisms and individual susceptibility to DM; (2) provide adequate data to calculate odds ratios (ORs) and 95% confidence intervals (CIs); (3) full text in English or Chinese available. For duplicate reports, only the most complete one was included. Family-based association studies, case reports, case series, reviews, comments, letters, and conference presentations were excluded.

Data extraction and quality assessment

The following data were extracted from included studies: (1) name of first author; (2) year of publication; (3) country and ethnicity of participants; (4) type of disease; (5) the number of cases and controls; and (6) genotypic distributions of CTLA-4 gene polymorphisms in cases and controls. The probability value ($p$ value) of Hardy–Weinberg equilibrium (HWE) test was also calculated.

The Newcastle–Ottawa scale (NOS) was used to assess the quality of eligible studies from three aspects: (1) selection of cases and controls; (2) comparability between cases and controls; and (3) exposure in cases and controls [16]. The NOS has a score range of 0–9, and studies with a score of more than 7 were assumed to be of high quality.

Two reviewers conducted data extraction and quality assessment independently. When necessary, the reviewers wrote to the corresponding authors for extra information. Any disagreement between two reviewers was solved by discussion until a consensus was reached.

Statistical analyses

All statistical analyses in the present study were conducted with Review Manager Version 5.3.3 (The Cochrane Collaboration, Software Update, Oxford, United Kingdom). ORs and 95% CIs were used to assess potential associations of CTLA-4 gene polymorphisms with the susceptibility to DM in dominant, recessive, over-dominant, and allele models, and a $p$ value of 0.05 or less was considered to be statistically significant. Between-study heterogeneity was evaluated by $I^2$ statistic. If $I^2$ was greater than 50%, random-effect models (REMs) would be used for analyses due to the existence of significant heterogeneities. Otherwise, fixed-effect models (FEMs) would be employed for analyses. Subgroup analyses by ethnicity of participants were subsequently performed. Sensitivity analyses were carried out to test the stability of the results. Funnel plots were applied to evaluate possible publication biases.

Results

Characteristics of included studies

Our systematic literature search yielded 842 results. After exclusion of irrelevant and duplicate articles by reading titles and abstracts, 135 potentially relevant articles were retrieved for further evaluation. Another 59 articles were subsequently excluded after reading the full text. Finally, a total of 76 studies that met the inclusion criteria of our meta-analysis were included (see Figure 1). Characteristics of included studies are shown in Table 1.
| First author, year | Country | Ethnicity | Type of disease | Sample size | Genotypes (wtwt/wtmt/mtmt) | P value for HWE | NOS score |
|-------------------|---------|-----------|-----------------|-------------|-----------------------------|----------------|-----------|
| Abe 1999          | Japan   | East Asian| T1DM            | 111/445     | 50/45/16                    | 0.969          | 7         |
| Ahmadi 2013       | Iran    | South Asian| T1DM            | 60/107      | 25/32/3                     | 0.757          | 7         |
| Ahmedov 2006      | Azerbaijan Republic | Caucasian | T1DM            | 160/271     | 80/58/22                    | 0.307          | 7         |
| Awata 1998        | Japan   | East Asian| T1DM            | 173/425     | 72/80/21                    | 0.938          | 7         |
| Balic 2009        | Chile   | Mixed     | T1DM            | 300/310     | 125/136/39                  | 0.267          | 7         |
| Baniasad 2006     | India   | South Asian| T1DM            | 130/180     | 50/62/18                    | 0.541          | 8         |
| Bennamansour 2010 | Tunisia | South Asian| T1DM            | 228/193     | 98/83/47                    | 0.102          | 7         |
| Bouzbis 2003      | Morocco | Caucasian | T1DM            | 118/114     | 59/52/7                     | 0.742          | 7         |
| Caputo 2005       | Argentina | Mixed     | T1DM            | 186/168     | 76/84/26                    | 0.924          | 7         |
| Çelmelı 2013      | Turkey  | Caucasian | T1DM            | 91/99       | 38/40/13                    | 0.161          | 7         |
| Chen 2011         | China   | East Asian| T1DM            | 360/728     | 199/136/25                  | 0.839          | 8         |
| Cinek 2002        | Czech Republic | Caucasian | T1DM            | 305/289     | 123/125/57                  | 0.458          | 8         |
| Cosentino 2002    | Italy   | Caucasian | T1DM            | 80/85       | 21/55/4                     | 0.219          | 7         |
| Dallos 2008       | Slovakia | Caucasian | T1DM            | 171/231     | 33/72/66                    | 0.164          | 8         |
| Ding 2010         | China   | East Asian| T1DM            | 23/33       | 2/14/7                      | 0.126          | 7         |
| Djilali-Saiah 1998 | France | Caucasian | T1DM            | 112/100     | 37/41/34                    | 0.070          | 7         |
| Donner 1997       | Germany | Caucasian | T1DM            | 293/325     | 91/147/55                   | 0.990          | 7         |
| Douroudis 2009    | Estonia | Caucasian | T1DM            | 170/230     | 45/79/46                    | 0.104          | 7         |
| Douroudis 2009    | Finland | Caucasian | T1DM            | 404/725     | 69/203/132                  | 0.232          | 7         |
| El Wafai 2011     | Saudi Arabia | South Asian| T1DM            | 39/46       | 9/21/9                      | 0.045          | 7         |
| Fajardy 2002      | France  | Caucasian | T1DM            | 134/273     | 41/76/17                    | 0.027          | 7         |
| Ferreira 2009     | Brazil  | Mixed     | T1DM            | 49/48       | 26/20/3                     | 0.997          | 7         |
| Genc 2004         | Turkey  | Caucasian | T1DM            | 48/80       | 24/20/4                     | 0.233          | 8         |
| Haller 2007       | Estonia | Caucasian | T1DM            | 131/252     | 27/62/42                    | 0.131          | 7         |
| Hauache 2005      | Brazil  | Mixed     | T1DM            | 124/75      | 42/63/19                    | 0.787          | 8         |
| Hayashi 1999      | Japan   | East Asian| T1DM            | 117/141     | 54/42/21                    | 0.005          | 7         |
| Ide 2004          | Japan   | East Asian| T1DM            | 116/114     | 56/49/11                    | 0.603          | 7         |
| Ihara 2001        | Japan   | East Asian| T1DM            | 160/200     | NA                         | NA             | NA        |
| Ikegami 2006      | Japan   | East Asian| T1DM            | 767/715     | 439/285/43                  | 0.131          | 7         |
| Jin 2015          | China   | East Asian| T1DM            | 402/482     | 182/194/26                  | 0.354          | 7         |
| Jung 2009         | Korea   | East Asian| T1DM            | 176/90      | 94/58/24                    | 0.053          | 7         |
| Kamoun 2001       | Tunisia | South Asian| T1DM            | 74/49       | 32/38/4                     | 0.110          | 7         |
| Kawasaki 2008     | Japan   | East Asian| T1DM            | 91/369      | 48/36/7                     | 0.484          | 7         |
| Khoshoo 2017      | Iran    | South Asian| T1DM            | 39/40       | 11/10/18                    | 0.114          | 7         |
| Kikuoka 2001      | Japan   | East Asian| T1DM            | 125/200     | 57/62/6                     | 0.287          | 8         |
| Klitz 2002        | USA     | Mixed     | T1DM            | 94/90       | NA                         | NA             | NA        |
| Korolija 2009     | Croatia | Caucasian | T1DM            | 102/193     | 48/36/18                    | 0.345          | 7         |
| Kumar 2015        | India   | South Asian| T1DM            | 232/305     | 95/101/36                   | 0.987          | 7         |
| Lee 2000          | Taiwan  | East Asian| T1DM            | 253/91      | 150/85/18                   | 0.378          | 7         |
| Lemos 2009        | Portugal | Caucasian | T1DM            | 207/249     | 82/95/30                    | 0.637          | 7         |
| Liang 2004        | Japan   | East Asian| T1DM            | 29/40       | 19/10/0                     | 0.013          | 7         |
| Ma 2002           | China   | East Asian| T1DM            | 31/36       | 5/11/15                    | 0.007          | 7         |
| McCormack 2001    | UK      | Caucasian | T1DM            | 144/307     | NA                         | NA             | NA        |
| Mochizuki 2003    | Japan   | East Asian| T1DM            | 97/60       | 44/36/17                    | 0.539          | 7         |
| Mojtahedi 2005    | Iran    | South Asian| T1DM            | 109/331     | 21/78/10                    | 0.826          | 7         |
| Monnin 2009       | USA     | Mixed     | T1DM            | 261/280     | 113/112/36                  | 0.702          | 7         |
| Mosad 2012        | Egypt   | South Asian| T1DM            | 104/78      | 37/59/8                     | 0.010          | 7         |
| Nistic 1996       | Italy   | Caucasian | T1DM            | 483/529     | 161/248/74                  | 0.329          | 8         |
| Ongagna 2002      | France  | Caucasian | T1DM            | 62/84       | 49/10/3                     | 0.013          | 7         |

Continued over
Table 1 The characteristics of included studies (Continued)

| First author, year, Country, Ethnicity | Type of disease | Sample size | Genotypes (wt/wt/mt/mtm) | P value for HWE | NOS score |
|----------------------------------------|----------------|-------------|--------------------------|----------------|-----------|
| Osei-Hyiaman 2001 Japan East Asian T1DM | 350/420        | 110/166/74  | 201/177/42               | 0.741          | 8         |
| Padma-Malini 2018 India South Asian T1DM | 196/196        | 78/93/25    | 128/61/7                 | 0.936          | 8         |
| Pérez 2009 Chile Mixed T1DM | 260/255        | 116/110/34  | 110/106/39               | 0.115          | 7         |
| Philip 2011 India South Asian T1DM | 53/53          | 5/30/18     | 32/15/6                  | 0.064          | 7         |
| Ranjouri 2016 Iran South Asian T1DM | 50/50          | 36/12/2     | 41/7/2                   | 0.044          | 8         |
| Saleh 2009 Egypt South Asian T1DM | 396/396        | 166/175/55  | 215/150/31               | 0.501          | 7         |
| Song 2012 China East Asian T1DM | 108/100        | 73/25/10    | 45/39/16                 | 0.138          | 7         |
| Steck 2005 USA Mixed T1DM | 102/198         | NA          | NA                       | NA             | 7         |
| Takara 2000 Japan East Asian T1DM | 74/107          | 16/25/33    | 34/43/30                 | 0.044          | 7         |
| Tavares 2015 Brazil Mixed T1DM | 204/305        | 82/91/31    | 127/140/38               | 0.952          | 7         |
| Van der Auwera 1997 Belgium Caucasian T1DM | 525/530        | NA          | NA                       | NA             | 7         |
| Wang 2002 China East Asian T1DM | 90/84          | 13/54/23    | 32/42/10                 | 0.500          | 7         |
| Wang 2008 China East Asian T1DM | 48/192         | 4/29/15     | 124/52/18                | 0.004          | 8         |
| Wood 2002 Germany Caucasian T1DM | 176/220        | 59/84/33    | 99/95/26                 | 0.662          | 7         |
| Xiang 2006 China East Asian T1DM | 179/290        | 79/86/14    | 87/153/50                | 0.216          | 7         |
| Yanagawa 1999 Japan East Asian T1DM | 110/200        | 45/46/19    | 78/88/34                 | 0.287          | 7         |
| Yang 2006 China East Asian T1DM | 34/71          | 23/8/3      | 32/28/11                 | 0.253          | 7         |
| Zalloua 2004 USA Mixed T1DM | 190/102        | 91/75/24    | 53/45/4                  | 0.137          | 7         |
| Ahmadi 2013 Iran South Asian T1DM | 56/107         | 35/18/3     | 67/36/4                  | 0.757          | 7         |
| Ding 2010 China East Asian T1DM | 34/33          | 21/11/2     | 28/4/1                   | 0.126          | 7         |
| Gu 2007 China East Asian T1DM | 111/39         | 35/71/5     | 15/20/4                  | 0.475          | 7         |
| Haller 2007 Estonia Caucasian T1DM | 224/252        | 76/122/46   | 77/135/40                | 0.131          | 7         |
| Jin 2015 China East Asian T1DM | 330/482        | 128/171/31  | 169/241/72               | 0.354          | 7         |
| Khosrosho 2017 Iran South Asian T1DM | 71/40          | 39/17/18    | 13/15/12                 | 0.114          | 7         |
| Kiani 2016 Iran South Asian T1DM | 111/100        | 60/42/9     | 41/39/20                 | 0.066          | 7         |
| Ma 2002 China East Asian T1DM | 31/36          | 7/17/7      | 19/9/8                   | 0.007          | 7         |
| Rau 2001 Germany Caucasian T1DM | 300/466        | 126/140/34  | 183/215/68               | 0.707          | 8         |
| Shih 2018 Taiwan East Asian T1DM | 278/287        | 118/127/33  | 101/150/36               | 0.084          | 7         |
| Uzer 2010 Turkey Caucasian T1DM | 72/169         | 43/24/5     | 113/45/11                | 0.035          | 7         |
| Wang 2008 China East Asian T1DM | 192/192        | 59/102/31   | 124/52/16                | 0.004          | 8         |
| Yu 2006 China East Asian T1DM | 121/39         | 35/71/5     | 15/20/4                  | 0.475          | 7         |
| Almasi 2015 Iran South Asian T2DM | 153/189        | 143/10/0    | 174/14/1                 | 0.235          | 7         |
| Balli 2009 Chile Mixed T2DM | 300/310        | 243/50/7    | 253/47/10                | <0.001         | 7         |
| Baniasadi 2006 India South Asian T2DM | 130/180        | 113/15/2    | 170/10/0                 | 0.701          | 8         |
| Benmansour Tunisia South Asian T2DM | 228/193        | 159/52/17   | 156/28/9                 | <0.001         | 7         |
| Bouqbi 2003 Morocco Caucasian T1DM | 118/114        | 106/12/0    | 110/4/0                  | 0.849          | 7         |
| Caputo 2007 Argentina Mixed T2DM | 178/136        | 149/28/1    | 110/26/0                 | 0.218          | 7         |
| Chen 2011 China East Asian T1DM | 359/728        | 281/71/7    | 550/164/14               | 0.664          | 8         |
| Douroudis 2009 Estonia Caucasian T1DM | 61/230         | 52/8/1      | 178/49/3                 | 0.857          | 7         |
| Ihrara 2001 Japan East Asian T1DM | 160/200        | NA          | NA                       | NA             | 7         |
| Lee 2001 Taiwan East Asian T1DM | 347/260        | 303/42/2    | 201/56/3                 | 0.681          | 7         |
| Saleh 2008 Egypt South Asian T1DM | 396/396        | 180/178/36  | 214/164/18               | 0.053          | 7         |
| Steck 2005 USA Mixed T1DM | 102/198         | NA          | NA                       | NA             | 7         |
| Wang 2008 China East Asian T1DM | 48/189         | 30/18/0     | 155/34/0                 | 0.174          | 8         |
| Zouidi 2014 Tunisia South Asian T1DM | 76/162         | 68/7/1      | 145/15/2                 | 0.040          | 7         |
| Kiani 2016 Iran South Asian T2DM | 111/100        | 75/26/10    | 88/10/2                  | 0.020          | 7         |
| Shih 2018 Taiwan East Asian T2DM | 278/287        | 227/49/2    | 215/67/5                 | 0.933          | 7         |
| Uzer 2010 Turkey Caucasian T2DM | 72/169         | 55/14/3     | 116/43/10                | 0.036          | 7         |
| Wang 2008 China East Asian T2DM | 192/189        | 157/35/0    | 155/34/0                 | 0.174          | 8         |

Abbreviations: wt, wild type; mt, mutant type; NA, not available.
CTLA-4 gene polymorphisms and the susceptibility to DM

Significant associations with susceptibility to T1DM were detected for rs231775 (dominant model: \( P=0.008, OR = 0.83, 95\%CI 0.73–0.95 \); recessive model: \( P=0.003, OR = 1.27, 95\%CI 1.09–1.50 \); allele model: \( P=0.004, OR = 0.85, 95\%CI 0.77–0.95 \)) and rs5742909 (recessive model: \( P=0.02, OR = 1.50, 95\%CI 1.05–2.13 \)) polymorphisms in overall population. Nevertheless, no any positive results were detected for T2DM in overall population. Furthermore, no any positive results were detected for T2DM in overall population.

Further subgroup analyses revealed that rs231775 polymorphism was significantly associated with susceptibility to T1DM in Caucasians (dominant, recessive, and allele models) and South Asians (dominant, recessive, over-dominant, and allele models), but not in East Asians. Moreover, rs231775 polymorphism was also significantly associated with susceptibility to T2DM in East Asians (over-dominant model) and South Asians (recessive and allele models), but not in Caucasians. Additionally, we also found that rs5742909 polymorphism was significantly associated with susceptibility to T1DM in South Asians (dominant, recessive, over-dominant, and allele models), but not in East Asians and Caucasians (see Table 2).
| Variables | Sample size | Dominant comparison | Recessive comparison | Over-dominant comparison | Allele comparison |
|-----------|-------------|---------------------|----------------------|--------------------------|------------------|
|           |             | P value | OR (95%CI) | P value | OR (95%CI) | P value | OR (95%CI) | P value | OR (95%CI) |
| rs231775 A/G |             |     |     |     |     |     |     |     |     |
| T1DM      |             |     |     |     |     |     |     |     |     |
| Overall   | 11420/14674 | 0.008* | 0.83 (0.73–0.95) | 0.008* | 1.27 (1.09–1.50) | 0.59 | 1.03 (0.93–1.13) | 0.004* | 0.85 (0.77–0.95) |
| Caucasian | 3854/5102   | <0.0001* | 0.74 (0.67–0.81) | <0.0001* | 1.61 (1.42–1.83) | 0.76 | 0.99 (0.90–1.08) | <0.0001* | 0.77 (0.72–0.82) |
| East Asian| 4024/5633   | 0.73 | 1.05 (0.89–1.37) | 0.78 | 0.95 (0.69–1.32) | 0.32 | 0.92 (0.77–1.09) | 0.79 | 1.03 (0.83–1.28) |
| South Asian| 1710/2024 | <0.0001* | 0.52 (0.38–0.70) | 0.005* | 1.79 (1.19–2.70) | 0.001* | 1.47 (1.17–1.86) | <0.0001* | 0.60 (0.48–0.75) |
| T2DM      |             |     |     |     |     |     |     |     |     |
| Overall   | 1951/2242   | 0.34 | 0.85 (0.61–1.19) | 0.12 | 1.16 (0.96–1.40) | 0.14 | 1.22 (0.94–1.59) | 0.58 | 0.94 (0.74–1.19) |
| Caucasian | 616/887     | 0.82 | 1.03 (0.83–1.27) | 0.75 | 0.95 (0.70–1.29) | 0.99 | 1.00 (0.81–1.23) | 0.75 | 1.03 (0.88–1.20) |
| East Asian| 1097/1108   | 0.08 | 0.58 (0.32–1.07) | 0.59 | 0.88 (0.54–1.42) | 0.04† | 1.66 (1.03–2.68) | 0.15 | 0.74 (0.49–1.12) |
| South Asian| 238/247    | 0.06 | 0.59 (0.34–1.02) | 0.02‡ | 1.56 (1.08–2.27) | 0.36 | 0.84 (0.57–1.23) | 0.003§ | 0.65 (0.49–0.87) |
| rs5742909 C/T |            |     |     |     |     |     |     |     |     |
| T1DM      |             |     |     |     |     |     |     |     |     |
| Overall   | 2656/3485   | 0.37 | 0.87 (0.65–1.18) | 0.02‡ | 1.50 (1.05–2.13) | 0.51 | 1.10 (0.83–1.45) | 0.36 | 0.89 (0.70–1.13) |
| Caucasian | 179/344     | 0.77 | 0.78 (0.15–3.96) | 0.84 | 1.26 (1.34–12.30) | 0.80 | 1.25 (0.23–6.72) | 0.72 | 0.76 (0.17–3.36) |
| East Asian| 914/1377    | 0.99 | 1.00 (0.47–2.14) | 0.74 | 0.87 (0.38–1.98) | 1.00 | 1.00 (0.47–2.13) | 0.80 | 1.07 (0.65–1.73) |
| South Asian| 983/1120   | 0.0004† | 0.68 (0.55–0.84) | 0.002‡ | 2.05 (1.30–3.23) | 0.04† | 1.27 (1.02–1.58) | <0.0001† | 0.69 (0.58–0.82) |
| T2DM      |             |     |     |     |     |     |     |     |     |
| Overall   | 653/745     | 0.80 | 0.92 (0.48–1.77) | 0.89 | 1.11 (0.27–4.65) | 0.93 | 1.03 (0.61–1.73) | 0.76 | 0.90 (0.47–1.74) |
| East Asian| 470/476     | 0.13 | 1.28 (0.93–1.75) | 0.29 | 0.41 (0.08–2.12) | 0.20 | 0.81 (0.59–1.12) | 0.11 | 1.27 (0.95–1.71) |

*S *P < 0.01, †P < 0.0001, ‡P < 0.05, §P < 0.001.

**Sensitivity analyses**

Sensitivity analyses were carried out to test the stability of meta-analysis results by eliminating studies that deviated from HWE. No changes of results were detected for investigated CTLA-4 gene polymorphisms in any comparisons, which indicated that our findings were quite statistically reliable.

**Publication biases**

Potential publication biases in the present study were evaluated with funnel plots. No obvious asymmetry of funnel plots was observed in any comparisons, which suggested that our findings were unlikely to be impacted by severe publication biases.

**Discussion**

Despite enormous advancements in pharmacotherapy over the past few decades, DM and its associated vascular complications are still leading causes of death and disability all over the world [17,18]. To date, the exact cause of DM is still largely unclear in spite of extensive investigations. However, the obvious familial aggregation tendency of
DM indicated that genetic factors may significantly contribute to its occurrence and development [19]. Thus, identify potential genetic biomarkers is of particularly importance for an early diagnosis and a better prognosis of DM patients.

Previous studies showed that interferon α and its associated pathways could induce autoantigen presentation, active autoactive monocytes, cytotoxic T-lymphocytes and NK cells, elicit endoplasmic reticulum stress of human islet B cells, and impair insulin production [20,21]. These results indicated that autoimmunity might result in destruction of islet B cells, contribute to less insulin production, and give rise to the development of DM. As far as we know, this is so far the most comprehensive meta-analysis about CTLA-4 gene polymorphisms and DM, and our pooled analyses revealed that rs231775 and rs5742909 polymorphisms may serve as genetic biomarkers of T1DM, and rs231775 polymorphism may also serve as a genetic biomarker of T2DM. The stabilities of synthetic results were evaluated by sensitivity analyses, and no alterations of results were observed in any comparisons, which suggested that our findings were statistically stable. As for evaluation of heterogeneities, significant heterogeneities were detected for rs231775 polymorphism in every comparison of overall analyses for T1DM, and thus all analyses were performed with REMs. But in further subgroup analyses, a reduction tendency of heterogeneity was found in South Asians, which suggested that differences in ethnicity could partially explain observed heterogeneities between studies.

There are several points that need to be addressed about the present study. First, our findings indicated that rs231775 and rs5742909 polymorphisms could be used to identify individuals at higher risk of developing T1DM, and rs231775 polymorphism could also be used to identify individuals at higher risk of developing T2DM. There are two possible explanations for our positive findings. First, rs231775 and rs5742909 polymorphisms of the CTLA-4 gene may lead to alternations in gene expression or changes in CTLA-4 protein structure, which may subsequently affect biological functions of CTLA-4, result in immune dysfunction and ultimately impact individual susceptibility to DM, especially T1DM. Second, it is noteworthy that several analyses were still based on limited number of studies, and therefore, further replication studies, especially in T2DM are still warranted to confirm these findings. Third, the pathogenic mechanism of DM is extremely complex, and hence despite our positive findings, it is unlikely that a single genetic polymorphism could significantly contribute to its development [22,23]. Fourth, due to lack of raw data, we failed to explore possible interactions of investigated CTLA-4 gene polymorphisms. But to better illustrate the potential associations of CTLA-4 gene polymorphisms with DM, we strongly recommend further studies to perform haplotype analyses and explore potential gene–gene interactions.

Our meta-analysis certainly has some limitations. First, although the general methodology qualities of included studies were good, it should be noted that we did not have access to genotypic distributions of investigated polymorphisms according to base characteristics of study subjects. Therefore, our results were derived from unadjusted estimations, and failure to conduct further adjusted analyses for baseline characteristics of participants such as age, gender, and co-morbidity conditions may influence the authenticity of our findings [24]. Second, significant heterogeneities were detected in certain subgroup comparisons, which indicated that the inconsistent results of included studies could not be fully explained by differences in ethnic background, and other unmeasured characteristics of participants may also partially attribute to between-study heterogeneities [25]. Third, associations between CTLA-4 gene polymorphisms and DM may also be influenced by gene–environmental interactions. However, the majority of studies did not consider these potential interactions, which impeded us to perform relevant analyses accordingly [26]. Fourth, since only published articles were eligible for analyses, although funnel plots revealed no obvious publication biases, we still could not rule out the possibility of potential publication biases. Taken these limitations into consideration, the results of the present study should be interpreted with caution.

In conclusion, our findings indicated that rs231775 and rs5742909 polymorphisms may serve as genetic biomarkers of T1DM, and rs231775 polymorphism may also serve as a genetic biomarker of T2DM. Further well-designed studies, especially in T2DM are still warranted to confirm our findings, and future investigations also need to explore possible roles of other CTLA-4 gene polymorphisms in DM.

Author Contribution

Min Chen and ShuMin Li conceived of the study, participated in its design, conducted the systematic literature review, performed data analyses, and drafted the manuscript. Both the authors have read and approved the final manuscript.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Abbreviations
Cl, confidence interval; CTLA-4, cytotoxic T-lymphocyte-associated antigen 4; DM, diabetes mellitus; HWE, Hardy–Weinberg equilibrium; NOS, Newcastle–Ottawa scale; OR, odds ratio; REM, random-effect model; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus.

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