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Original Article

Relationship between CTLA4 + 49 A/G polymorphism and susceptibility to type 1 diabetes mellitus: a meta-analysis

Muhadasi Tuerxunyiming[1,2], Muhetaer Muhemaiti[3], Weiya Niu[2], Qifeng Li[1,2]

[1]Xinjiang Institute of Pediatrics, People's Hospital of Xinjiang Uygur Autonomous Region, Urumqi 830001, Xinjiang, China. [2]Children's Hospital of Xinjiang Uygur Autonomous Region, Urumqi 830054, Xinjiang, China; [3]Department of Pediatrics, People's Hospital of Xinjiang Uygur Autonomous Region, Urumqi 830001, Xinjiang, China

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Abstract: Background: This study aims to investigate the relationship between G/A polymorphism of CTLA4 rs231775 gene and susceptibility to type 1 diabetes (T1DM) in children through Meta-analysis. Material and Methods: We systematically searched the databases of PubMed, Web of Science, CNKI, Wanfang database and VIP database from 1999 to 2017 and analyzed the odds ratio (OR) and 95% confidence interval (CI). RevMan5.3 software was used for meta-analysis. Results: In Total, 19 studies were included in this meta-analysis. We found that rs231775 G/A polymorphisms of the CTLA4 gene was associated with T1DM. However, different ethnic groups had different degrees of correlation under different genetic models. The combined analysis showed that, in all the populations of the T1DM group, the recessive (GG vs GA + AA), the dominant (GG + GA vs AA), the homozygous (GG vs AA) and the heterozygous (GG vs GA) genetic models were significantly different from the control group. However, in the allele genetic model (G vs A), there was no statistically significant difference. Besides, the subgroup analysis showed that the rs231775 G/A polymorphism of the CTLA4 gene in the Asian population was significantly different between T1DM and control groups, including the allele, dominant, recessive, and homozygous genetic models, but not the heterozygous genetic model. However, there was no significant difference in the rs231775 G/A polymorphism of the CTLA4 gene between the European and South American populations. Conclusion: The risk ratio in T1DM of the G allele is significantly higher than that of the A allele in the Asian population, suggesting that the rs231775 G allele of the CTLA4 gene may increase the susceptibility of Asian people to T1DM.

Keywords: CTLA4, Meta-analysis, polymorphism, type 1 diabetes, susceptibility

Introduction

Globally, the incidence of type 1 diabetes mellitus (T1DM) continues to increase. According to a European survey, the incidence of T1DM in children has increased at an average rate of 3.9% per year in recent years, and younger children have the highest growth rate [1]. It is estimated that the number of newly diagnosed children with T1DM will double by 2020 [2]. Till now, the main treatment of T1DM is insulin therapy, which emphasizes intensive treatment to reduce the risk of related microvascular complications [3]. Insulin therapy, however, cannot maintain normal glucose levels in patients and multiple daily injections often leads to poor compliance in children, and hypoglycemia [4]. In China, T1DM mainly manifests as short survival time and poor glycemic control [5]. The lack of knowledge of diabetes and poor economic conditions are the main reasons for shorter life expectancy in children with T1DM in China [6].

T1DM is an autoimmune disease caused by T-lymphocyte-mediated islet B cell-specific damage, leading to insufficient insulin secretion [7]. The etiology and mechanism is not yet fully understood. The significant physiological and pathological features of T1DM include the marked reduction or loss of islet B cells, resulting in a significant decrease or loss of insulin secretion [8]. It is shown that the development of T1DM is determined by the balance between pathogenic T cells and regulatory T cells [9]. There may be auto-reactive T lym-

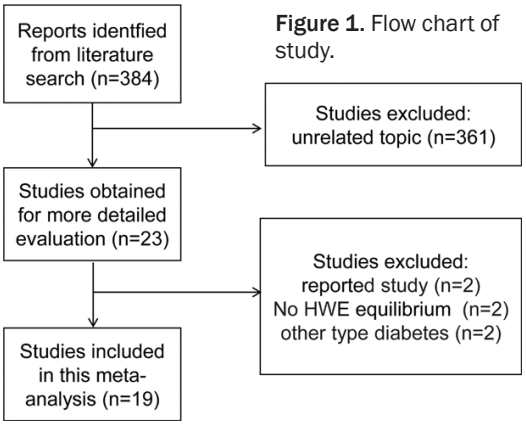

Figure 1. Flow chart of study.

phocytes that target one of the islet auto-antigens [10].

Cytotoxic T lymphocyte associated antigen 4 (CTLA4) is an important T cell regulator, which is mainly involved in T cell mediated immune homeostasis and immune tolerance [11]. It has been reported that CTLA4 gene polymorphisms may affect T cell activity in a number of ways. For example, A/G of CTLA4 rs231775 is a missense mutation identified in recent decades, in which adenine 49 in the first exon region of CTLA4 is replaced by guanine (hereinafter referred to as CTLA4 A49G) [12]. As a result, threonine 17 is changed to alanine and the protein activity is changed, which may increase the risk of T1DM [13]. So far, no consensus has been reached on the relationship of CTLA4 A49G with T1DM among different ethnic groups in different regions or among different ethnic groups within the same region.

In 2012, there was a meta-analysis of the association between the A/G mutation at CTLA4 rs231775 and the susceptibility to T1DM [14], which demonstrated an association between CTLA4 rs231775 polymorphisms and T1DM among children. The CTLA4 A/G allele is a risk gene for T1DM in children.

In this study, we investigated the association of the CTLA4 rs231775 site A/G mutation with susceptibility to T1DM in children more comprehensively. A subgroup analysis of different ethnic groups was conducted.

Materials and methods

Literature search

We searched PubMed, Web of Science, CNKI, Wanfang Database, VIP and other databases, as well as hand-searched dissertations to screen the literature on association between the A/G polymorphism of rs231775 in the CTLA4 gene and T1DM. Literature published from 1999 to 2017 were comprehensively searched. The key words for the literature search included type 1 diabetes, CTLA4, rs231775, genetic polymorphism and susceptibility.

Inclusion criteria

1) Literature on the relationship between CTLA4 genetic polymorphisms and susceptibility to T1DM. 2) The languages were Chinese or English. 3) The study design was a case-control study with T1DM patients and healthy controls. 4) Literature with a CTLA4 genotype distribution and related information, and a comprehensive statistical indicator (OR value) directly or indirectly. 5) DNA sequencing method was reasonable. 6) T1DM diagnostic criteria was in line with the World Health Organization diabetes diagnostic criteria or the American Diabetes Association medical treatment standards. 7) Control population had no family history of diabetes and was selected randomly. 8) The gender and ages in each group had no statistical difference.

Exclusion criteria

1) Repeated reports with the same population. 2) Diabetes combined with other diseases. 3) Literature on other gene polymorphisms. 4) Literature with small sample sizes. 5) Reviews. 6) Data to calculate the OR value was not available. 7) Literature without control groups or without a case-control study. 8) Hardy-Weinberg equilibrium was not obtained.

Literature quality evaluation

Literature quality was independently evaluated by two investigators using the Newcastle-Ottawa Scale *(NOS)* (with a total score of 9) [15]. If the two investigators could not reach a consensus, discussion with a third investigator was performed. The evaluations included population selection, comparability and exposure evaluation, which were all based on the *NOS* assessment scale.

Data extraction

The main information extracted from each of the articles included the first author, the year of

Table 1: The basic characteristics of the included literature [16-34]

Authors	Year	Study design	Subgroup	Source of controls	NOS Score	T1DM			Control			Case/control	
						AA	AG	GG	AA	AG	GG		
Hayashi et al [24]	1999	Case-control	Japanese	Hospital	7	21	42	54	22	47	72	117	141
Kikuoka et al [28]	2001	Case-control	Japanese	Hospital	8	6	62	57	34	88	78	125	200
Cinek et al [16]	2001	Case-control	Czech	Hospital	8	123	125	57	106	133	50	305	289
Fajardy et al [20]	2002	Case-control	French	Hospital	8	41	76	17	96	146	31	134	273
Mochizuki et al [31]	2003	Case-control	Japanese	Hospital	6	17	36	44	12	27	21	97	60
Haller et al [22]	2004	Case-control	Estonia	Hospital	6	18	29	22	50	85	23	69	158
Liang et al [30]	2004	Case-control	Japanese	Hospital	6	0	10	19	3	27	10	29	40
Mojtahedi et al [32]	2005	Case-control	Iranian	Hospital	6	21	78	10	146	149	36	109	331
Hauache et al [23]	2005	Case-control	Brazilian	Hospital	5	42	63	19	30	34	11	124	75
AhmedovG et al [18]	2006	Case-control	Azerbaijan Ian	Hospital	5	80	58	22	143	103	25	160	271
Baniasadi et al [19]	2006	Case-control	North Indians	Hospital	6	50	62	18	76	79	25	130	180
Ferreira et al [21]	2009	Case-control	Brazilians	Hospital	6	52	40	6	44	42	10	98	96
Jung et al [27]	2009	Case-control	Korean	Hospital	6	24	58	94	13	31	46	176	90
Lemos et al [29]	2009	Case-control	Portuguese	Hospital	5	82	95	30	111	108	30	207	249
Jin et al [26]	2009	Case-control	China	Hospital	5	20	155	157	70	239	167	332	476
Philip et al [34]	2011	Case-control	Southern India	Hospital	5	5	30	18	32	15	6	53	53
Ahmadi et al [17]	2013	Case-control	Iranian	Hospital	6	25	32	3	67	36	4	60	107
Jin et al [25]	2014	Case-control	China	Hospital	6	26	194	182	72	241	169	402	482
Mosaad et al [33]	2017	Case-control	Egyptian	Hospital	7	17	21	2	12	8	0	40	20

Table 2. Meta-analysis results

Genetic model	Pooled OR (95% CI)	Z value	*P* value	Study number	Total size
Allelic genetic model	1.32 [0.98, 1.78]	1.80	0.07	19	6755
Asia	1.51 [0.99, 2.29]	1.94	0.05	13	4383
Europe	1.10 [0.67, 1.79]	0.37	0.71	4	1857
South America	0.95 [0.40, 2.25]	0.11	0.91	2	515
Recessive genetic model	1.55 [1.12, 2.15]	3.63	0.008	19	4749
Asia	1.73 [1.10, 2.73]	2.36	0.02	13	2978
Europe	1.56 [0.91, 2.67]	1.63	0.10	4	1424
South America	0.82 [0.42, 1.59]	0.60	0.55	2	347
Dominant genetic model	0.52[0.34, 0.79]	3.06	0.002	20	4056
Asia	0.42 [0.24,0.76]	2.89	0.004	13	2774
Europe	0.75 [0.43,1.32]	0.99	0.32	4	627
South America	0.52 [0.34, 0.79]	1.67	0.09	2	168
Homozygous genetic model	1.35 [1.04, 1.77]	2.22	0.03	19	1809
Asia	1.75 [1.15, 2.65]	2.63	0.008	13	1014
Europe	1.24 [0.87, 1.76]	1.20	0.23	4	260
South America	0.82 [0.26, 2.58]	0.33	0.74	2	46
Heterozygous genetic mode	1.60 [1.05, 2.46]	2.17	0.03	19	2820
Asia	1.77 [0.93, 3.34]	1.74	0.08	13	1814
Europe	1.80 [0.89, 3.64]	1.64	0.1	4	797
South America	0.75 [0.37, 1.51]	0.80	0.42	2	179

Notes: OR: odds ratio. CI: confidence interval. Total size: the total number of T1DM cases and the control group. Allelic genetic model: G allele distribution frequency; Recessive genetic model: GG versus GA + AA. Dominant genetic model: GC + CC versus GG. Homozygous genetic model: GG versus AA. Heterozygous genetic mode: GG versus GA.

publication, the type of study design, the ethnicity in the study, the sample size of each group, the number of cases of the three genotypes (including AA, AG and GG) in the T1DM group and the control group and the distribution of the control genotypes. If the opinions were inconsistent, the two investigators discussed to solve the inconsistencies or consulted a third investigator.

Outcome indicators

In this paper, the odds ratio (OR) and 95% confidence interval (CI) of the allele genetic model (G vs A), recessive genetic model (GG vs GA + AA), dominant genetic model (GG + GA vs AA), homozygous genetic model (GG vs AA), and heterozygous genetic model (GG vs GA) were used as outcome indicators to compare the distribution of the allele and genotypes between T1DM group and control group. At the same time, a

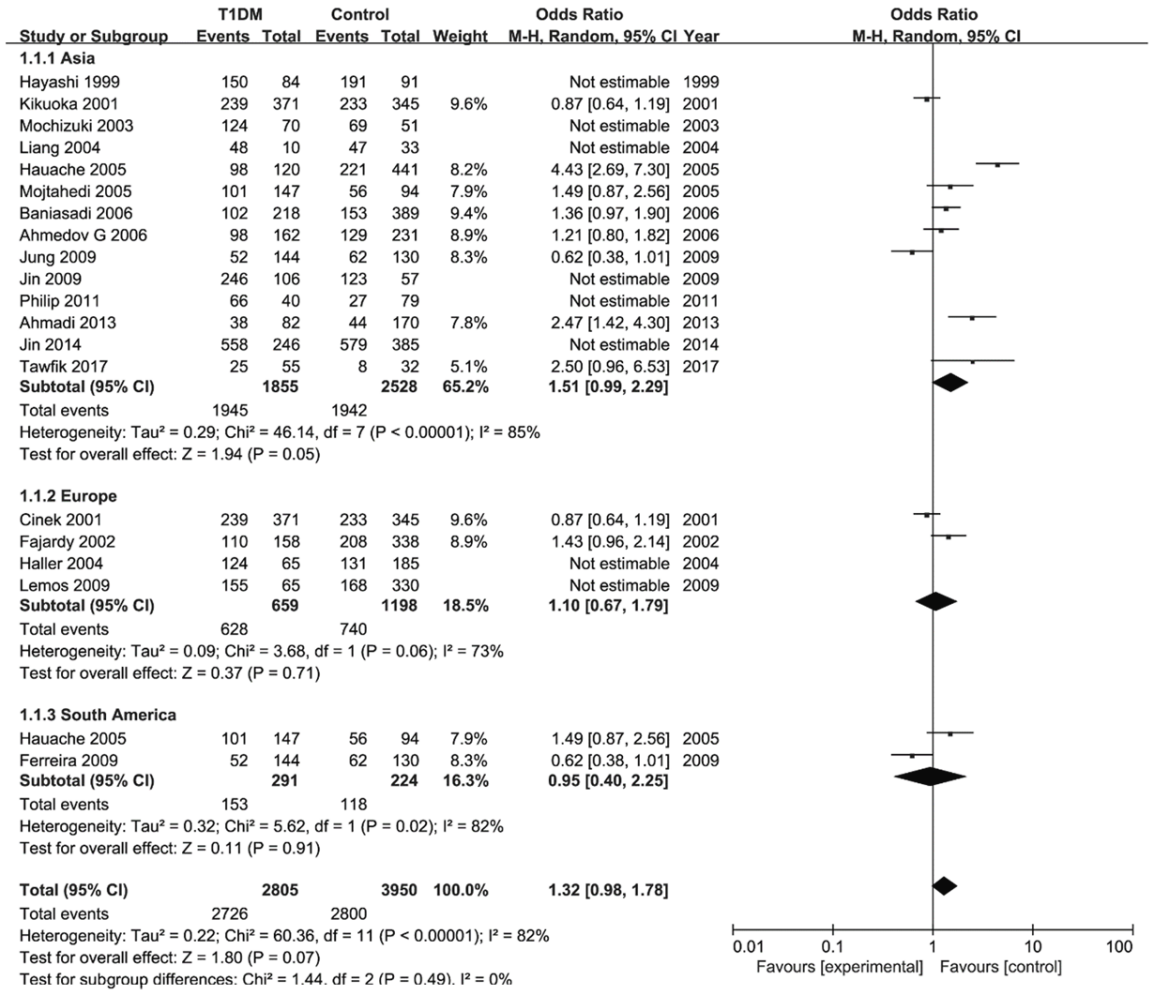

Figure 2. Forest plot of the T1DM associated gene rs231775 site A/G polymorphism under the allelic genetic model (distribution of G allelic frequency of CTLA4 rs231775 gene).

subgroup analysis of different ethnic groups was performed.

Statistical analysis

Review Manager 5.3 software (Version: 5.3.5, Sun Microsystems Inc.) was used to analyze the original data of the literature. Heterogeneity tests were performed on the included data, and heterogeneity was assessed using I^2 (I^2 = 0-24%: no heterogeneity; I^2 = 25%-49%: moderate heterogeneity; I^2 = 50%-74%: high heterogeneity: I^2 = 75%-100%: very high heterogeneity). If $I^2 < 50\%$, the fixed effect model was used, and if $I^2 \geq 50\%$, the random effect model was used. A funnel plot was used to evaluate potential publication bias. $P < 0.05$ was determined as statistically significant difference.

Results

Literature screening process

A total of 384 articles were screened. Among them, 361 articles were excluded after reading the title or the abstract. After reading the full texts, 7 articles were excluded, including 2 articles published repeatedly, 3 articles that were not in Hardy-Weinberg equilibrium and 2 articles of cohort research (see the prism flowchart, **Figure 1**). Finally, we included 19 articles [16-34] for the Meta-analysis (**Table 1**).

The basic characteristics of the included literatures

The study included 19 articles, with a total of 6358 case subjects (**Table 1**). There were 2767

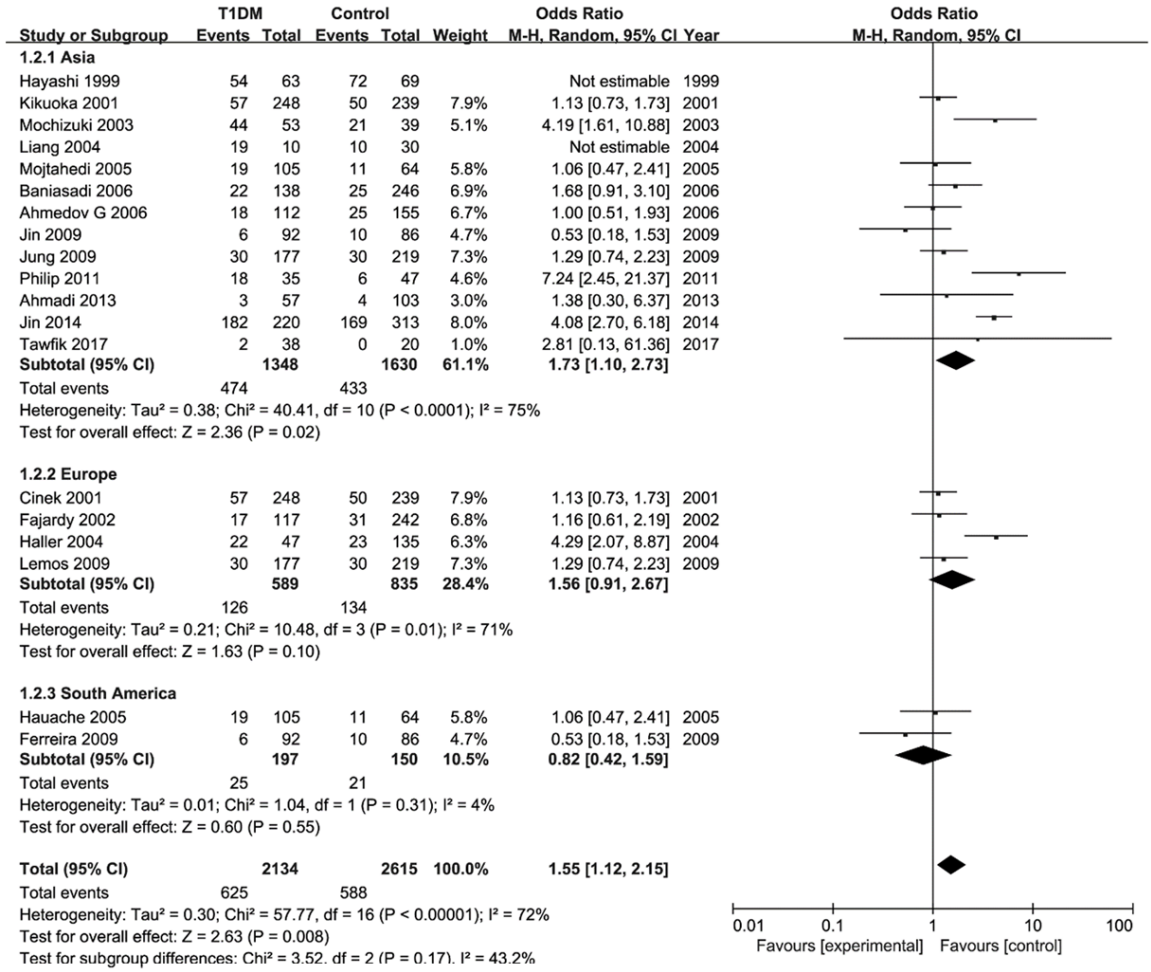

Figure 3. Forest plot of the T1DM associated gene rs231775 site A/G polymorphism under the recessive genetic model (distribution of G allelic frequency of CTLA4 rs231775 gene).

T1DM cases and 3591 control cases. The distribution of allele A/G genotype of CTLA4 rs231775 in T1DM and control group is shown in **Table 1**. These articles were of good quality as assessed by the Newcastle-Ottawa Scale. Among the 19 articles, 13 were data from Asian populations, 4 were data from the European population, and 2 were data from South America populations. The genotypes in all 19 articles met the Hardy-Weinberg equilibrium.

Random effect models for all populations

We used random effect models to analyze the results for all populations. The distribution of CTLA4 rs231775 polymorphism between T1DM group and control group was analyzed (**Table 2**). The results showed that the distribution of the recessive genetic model (OR: 1.55, 95% CI: 1.12-2.15), the dominant genetic model (OR: 0.52, 95%CI: 0.34~0.79) and the homozygous genetic model (OR: 1.35, 95% CI: 1.04-1.77) were significantly different between T1DM group and control group ($P < 0.05$). However, there was no statistically significant difference in the distribution of allele genetic model (OR: 1.32, 95% CI: 0.98-1.78).

Subgroup analysis from Asia

In this study, we also conducted a subgroup analysis according to different ethnic populations. In Mongolian populations of East Asia, there were significant differences between T1DM group and control group in the allele genetic model (OR: 1.51, 95% CI: 0.99-2.29) (**Figure 2**) (**Table 2**), the recessive genetic model (OR: 1.73, 95% CI: 1.10-2.73) (**Figure 3**) (**Table 2**), the dominant genetic model (OR: 0.42, 95% CI: 0.24-0.76) (**Figure 4**) (**Table 2**)

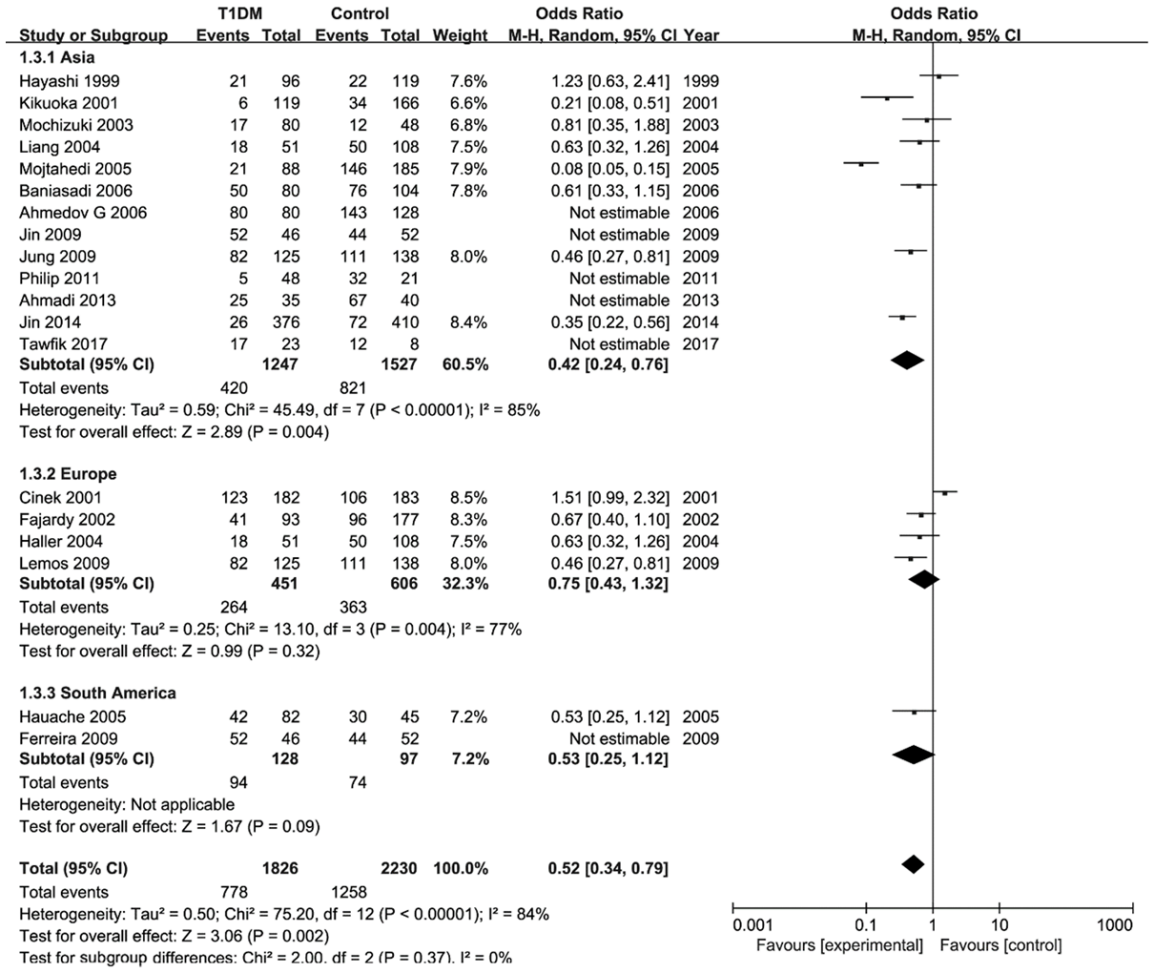

Figure 4. Forest plot of the T1DM associated gene rs231775site A/G polymorphism under the dominant genetic model (distribution of G allelic frequency of CTLA4 rs231775 gene).

and homozygous genetic model (OR: 1.75, 95% CI: 1.15-2.65) (**Figure 5**) (**Table 2**). However, the heterozygous genetic model (OR: 1.77, 95% CI: 0.93~3.34) showed no significant difference (**Figure 6**) (**Table 2**). Subgroup analysis showed that the rs231775 A/G polymorphism of the CTLA4 gene in the Asian population was associated with the susceptibility to T1DM.

Subgroup analysis from Europe

In the Caucasian population of Europe, the rs231775 A/G polymorphism of the CTLA4 gene had no relationship with the susceptibility to T1DM. The difference was not statistically significant in all genetic models, including the allele genetic model (OR: 1.10, 95% CI: 0.67 - 1.79) (**Figure 2**) (**Table 2**), recessive genetic model (OR: 1.56, 95% CI: 0.91-2.67) (**Figure 3**) (**Table 2**), dominant genetic model (OR: 0.75, 95% CI: 0.43-1.32) (**Figure 4**) (**Table 2**), homozygous genetic model (OR: 1.24, 95% CI: 0.87-1.76) (**Figure 5**) (**Table 2**), and heterozygous genetic model (OR: 1.80, 95% CI: 0.89-3.64) (**Figure 6**) (**Table 2**).

Subgroup analysis from South America

Similarly, in Caucasian populations of South America, there was no significant difference in the rs231775 A/G polymorphism of the CTLA4 gene between T1DM and control group. The analyzed gene polymorphisms included the allelic genetic model (OR: 0.95, 95% CI: 0.40-2.25) (**Figure 2**) (**Table 2**), recessive genetic model (OR: 0.82, 95% CI: 0.42-1.59) (**Figure 3**) (**Table 2**), dominant genetic model (OR: 0.52, 95% CI: 0.34-0.79) (**Figure 4**) (**Table 2**), homozygous genetic model (OR: 0.82, 95% CI: 0.26-2.58) (**Figure 5**) (**Table 2**) and heterozygous genetic model (OR: 0.75, 95% CI: 0.37-1.51) (**Figure 6**) (**Table 2**). Thus, the rs231775 A/G

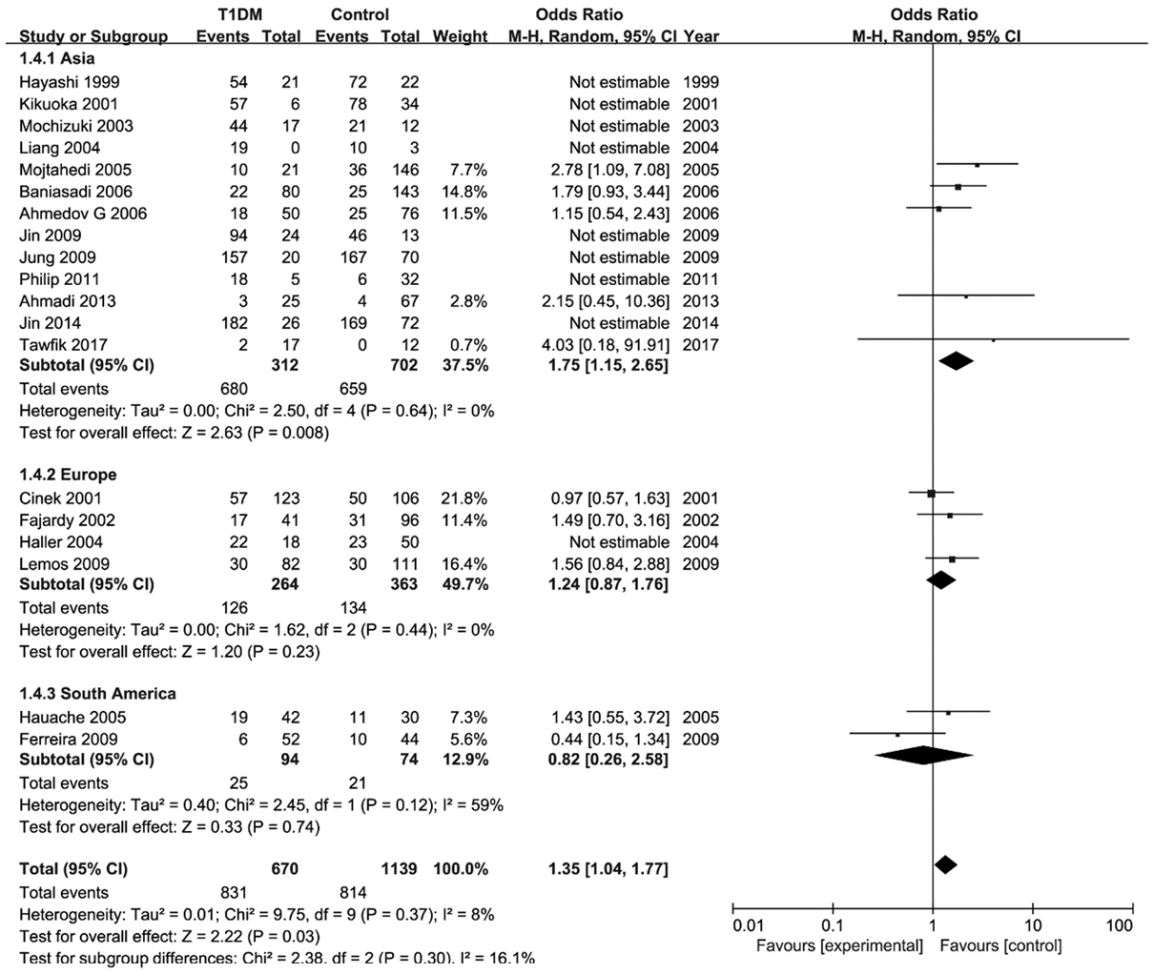

Figure 5. Forest plot of the T1DM associated gene rs231775 site A/G polymorphism under the homozygous genetic model (distribution of G allelic frequency of CTLA4 rs231775 gene).

polymorphism of the CTLA4 gene was not related to the susceptibility of T1DM in the Caucasian population of South America.

Analysis of publication bias

Using Revman5.3 Meta-analysis software, a funnel plot was generated to evaluate the potential publication bias. If the funnel diagram is asymmetric, it indicates a publication bias, otherwise, there is no publication bias. As shown in **Figure 7**, the points in the figure were symmetrically distributed on both sides of the midline, which were basically within a 95% CI, indicating no obvious publication bias.

Discussion

T1DM is an autoimmune disease [35]. The classic evolutionary model of T1DM, namely the Eisenbart H model, is constantly being updated. There is evidence that islet autoimmunity and dysfunction often persist for years before the diagnosis of T1DM [36]. T1DM in children is quite different from T1DM in adults in the occurrence, progression speed and prognosis [37]. Besides, in children, the impact of genetic factors in the development of T1DM is more severe than that in adults.

CTLA4 is located on chromosome 2q33, in which there are many T lymphocyte regulatory genes such as CTLA4, CD28 and ICOS [38]. Since 1996, studies in different countries such as Belgium, Poland, Japan and Africa have reported the widespread existence of CTLA4 rs231775 polymorphisms and found that it may be related to the increased incidence of T1DM [39-41].

At present, the relationship between rs231775 A/G polymorphism of CTLA4 and susceptibility

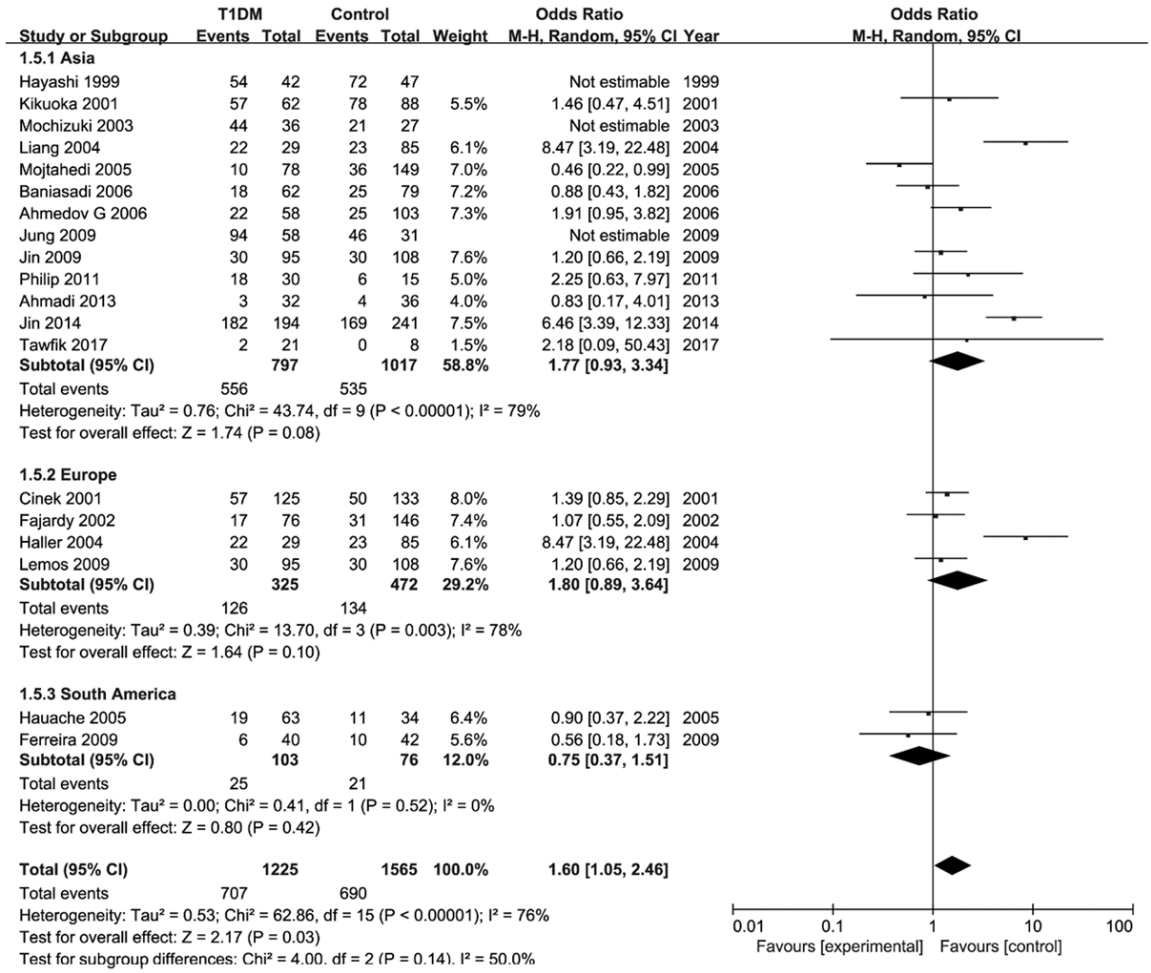

Figure 6. Forest plot of the T1DM associated gene rs231775 site A/G polymorphism under the heterozygous genetic model (distribution of G allelic frequency of CTLA4 rs231775 gene).

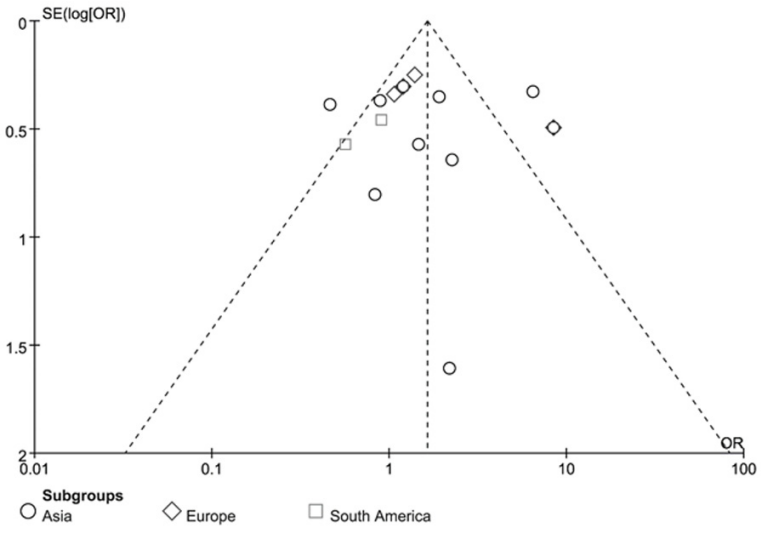

Figure 7. Funnel plot for publication bias.

to T1DM in different ethnic groups has been widely reported [42]. The meta-analysis in this study involved 19 articles on rs231775 polymorphisms of the CTLA4 gene and susceptibility to T1DM in different ethnic groups, including 2767 cases of T1DM and 3591 cases of control. The results showed that the rs231775 A/G polymorphism locus of the CTLA4 gene was associated with T1DM in different ethnic groups, and the correlation degree was different under different genetic models. For all the populations, the genetic models of

the recessive genetic model, the dominant genetic model, the homozygous genetic model and the heterozygous genetic model were significantly different between the T1DM group and control group. However, in the allele genetic model, the difference was not statistically significant. The risk ratio of allele G was significantly higher than that of allele A in the whole population, suggesting that allele G may be the risky allele of T1DM.

We then analyzed the distribution of these genetic models in sub-populations of Asia, Europe and South America, respectively. It was found that the rs231775 G/A polymorphism of the CTLA4 gene in the Asian population was significantly different between T1DM and control groups, including the allele genetic model, dominant genetic model, recessive genetic model, and homozygous genetic model, but not the heterozygous genetic model. This indicates that the risk ratio of allele G is significantly higher than that of allele A, suggesting that allele G may be a risky allele of T1DM in Asian populations. However, there was no significant difference in rs231775 G/A polymorphism of the CTLA4 gene in the European and South American Caucasian populations. This may be caused by racial differences. Further studies are warranted.

This study has several limitations. First, there were only 4 articles on the European population and 2 articles on the South American population. Compared with the Asian population, the sample size of the European population and South American population was relatively small. Therefore, the relationship of the CTLA4 gene with T1DM in European and South American populations remains to be further validated by large, case-by-case, case-control or prospective studies. Second, the sample size was relatively small.

In conclusion, the rs231775 G allele mutation of the CTLA4 gene may increase the susceptibility to T1DM in Asian populations.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Qifeng Li, Xinjiang Institute of Pediatrics, People's Hospital of Xinjiang Uygur Autonomous Region, No.91 Tianchi Road, Tianshan District, Urumqi 830001, Xinjiang China. Tel: 86-0991-8564289; Fax: 86-0991-8564289; E-mail: liqiqi521@sina.com

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