The Association of CTLA-4 rs231775 and rs3087243 Polymorphisms With Latent Autoimmune Diabetes in Adults: A Meta-analysis

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Research

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Abstract

Background

Polymorphisms rs231775 and rs3087243 of cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) gene have been associated with risk of latent autoimmune diabetes in adults (LADA). However, the results were inconsistent. The purpose of this study was to quantitatively assess the relationship between polymorphisms rs231775 and rs3087243 of CTLA-4 and LADA in a larger pooled population by performing a meta-analysis.

Methods

Systematic search for eligible studies was conducted in PubMed, Web of Science, and Embase. Case-control studies containing genotype frequencies of polymorphisms rs231775 or rs3087243 of CTLA-4 gene were selected, and pooled odds ratios (ORs) with 95% confidence intervals (CIs) were used to assess the associations between polymorphisms of CTLA-4 and LADA in allelic genetic model, dominant genetic model, and recessive genetic model.

Results

A total of eleven studies, in which five studies reported rs231775, two studies reported rs3087342, and four studies reported both rs231775 and rs3087243, were identified. Among them, one study wasn't included in the following meta-analysis because the distribution of genotypes in the control group didn't comply with Hardy-Weinberg equilibrium. Significant associations with susceptibility to LADA were detected for rs231775 (785 cases and 3435 controls, allelic genetic model OR 1.53, 95%CI 1.22-1.92; dominant genetic model OR 2.16, 95%CI 1.43-3.28; recessive genetic model OR 1.49, 95%CI 1.13-1.97) and for rs3087243 (820 cases and 4824 controls, allelic genetic model OR 1.26, 95%CI 1.03-1.55; dominant genetic model OR 1.11, 95%CI 0.88-1.40; recessive genetic model OR 1.41, 95%CI 1.07-1.86) in overall population. Further subgroup analyses for ethnicity (Asian, Caucasian, and African) have also indicated the positive association between rs231775 and LADA. As for rs3087243, subgroup analyses detected the association between polymorphism and LADA in Caucasian population under recessive model.

Conclusions

Polymorphisms rs231775 and rs3087243 of CTLA-4 gene are potential risk factors for LADA and may serve as novel genetic biomarkers of LADA.

Background

Latent autoimmune diabetes in adults (LADA), also termed as “type 1.5 diabetes mellitus”, “slowly progressive insulin-dependent diabetes mellitus”, or “latent type 1 diabetes”, is defined as an autoimmune disease(1). Patients with LADA are always initially misdiagnosed as type 2 diabetes mellitus (T2DM) because of the lack of ketoacidosis and requirement of insulin treatment but exhibit positive diabetic-associated autoantibodies and faster deterioration of beta-cell function, which are characteristics of type 1 diabetes mellitus (T1DM). Results from multiple large multicenter studies have indicated the prevalence of LADA accounts for 2–10% of all patients with diabetes(2–4). The ethnic and geographical characteristics of LADA was similar to T1DM. For example, studies have demonstrated that the positivity of diabetic-associated autoantibodies in phenotypic T2DM were higher in Caucasians than in Asians(5). Furthermore, in the Europe, the frequency of GADA in T2DM was higher in the northern countries than in the south(6–8). A similar trend was also reported in the LADA China Study(4). These findings indicate that LADA may share common pathogenic mechanisms with T1DM.

However, the exact pathogenesis of LADA was still unclear. Whether LADA is a distinct disease or just intermediate condition between T1DM and T2DM remains controversial(9, 10). In addition, the diagnostic criteria of LADA are not categorical. Determining the phenotypic and immunogenetic features of LADA is rather challenging because LADA is a clinical and metabolic hybrid of T1DM and T2DM(11–14). Therefore, efforts on revealing the underlying pathogenic mechanisms of LADA are critical for applying targeted treatment and exploring early detection.

It has been clearly indicated that genetic factors contribute to development of diabetes and genome-wide association studies (GWAS) have identified a great many of risk loci to T1DM and T2DM. The known susceptible loci even can explain 80–85% of heritability of T1DM(15). By contrast, the genetic-associated study is relatively lacking with regard to LADA. However, some recent available studies indicate a genetic overlap between LADA and T1DM (such as *HLA-DRB1-DQB1*, *PTPN22*, *CTLA-4*, and *SH2B3*) as well as T2DM (such as *TCF7L2*, *FTO*, and *KCNQ1*)(16, 17). Cytotoxic T-lymphocyte antigen-4 (*CTLA-4*) is an important candidate gene of T1DM and has been confirmed by several studies(18, 19). CTLA-4 protein, as a co-stimulatory molecule, can inhibit T cell activation, and therefore repress autoimmune response(20). Previous studies have indicated that the single nucleotide polymorphisms (SNPs) rs231775 and rs3087243 of *CTLA-4* were potential risk factors for T1DM(21, 22). In addition, many studies have evaluated the association between polymorphisms (rs231775 and rs3087243) and LADA in multiple populations(23–33). However, the results are inconclusive. Therefore, we performed this meta-analysis to further assess their associations based on existing relevant studies and aimed to improve the acknowledgement of pathogenesis of LADA.

Methods

Publication search

This meta-analysis was complied with the PRISMA guideline(34). Systematic literature searches (up to May 2021) were performed by using PubMed, Web of Science, and Embase. The searching strategy was as follows: (“LADA” OR “latent autoimmune diabetes in adults”) and (“*CTLA-4*” OR “cytotoxic T-lymphocyte antigen-4”) and (“polymorphism” OR “SNP” OR “allele” OR “variant” OR “genotype”). In addition, the reference list of original studies was manually screened to identify potentially relevant articles.

Inclusion and exclusion criteria

The included studies must meet the following criteria: (1) case-control study written in English; (2) report the associations between polymorphisms (rs231775 and rs3087243) of *CTLA-4* and LADA; (3) provide odds ratios (ORs) and 95% confidential intervals (CIs) or detailed data to calculate ORs and 95% CIs. Family-based association studies, letters, conference abstracts, case reports, reviews, and commentary were excluded.

Data extraction and quality assessment

The following data was collected from the included articles: (1) name of the first author; (2) publication year; (3) region and ethnicity of participants; (4) the genotyping methods (5) the number of cases and controls; (6) genotype frequencies of investigated polymorphisms of cases and controls; (7) OR value and 95% CI. The Hardy-Weinberg equilibrium (HWE) of control group was tested by online software (<http://ihg.gsf.de/cgi-bin/hw/hwa1.pl>). The Newcastle-Ottawa Scale (NOS) was applied to assess the quality of included studies from three aspects: the selection of case and control (0–4 points), comparability (0–2 points), and exposure assessment (0–3 points). Studies with at least 6 points were assumed to be of high quality.

Statistical analysis

All statistical analyses were performed by the software STATA version 12.0 (STATA, College Station, TX). Higgins I-squared statistic and Cochran's Q test were used to evaluate the heterogeneity of included studies. If $P_{heterogeneity} > 0.1$ and $I^2 < 50\%$, the fixed-effects model was used to calculate the pooled ORs and 95% CIs, otherwise the random-effects model was applied. The subgroup analysis, sensitivity analysis, and meta-regression were used to seek the source of heterogeneity. Begg's test and Egger's test were used to assess the publication bias. P value less than 0.05 was considered as statistically significant.

Results

Study characteristics

Totally, eleven studies in which five studies reported rs231775, two studies reported rs3087342, and four studies reported both rs231775 and rs3087243, were identified (Fig. 1)(23–33). Characteristics of identified articles were shown in Table 1. Among them, one study wasn't included in the following meta-analysis because the distribution of genotypes in the control group wasn't in accordance in Hardy-Weinberg equilibrium.

Table 1 Characteristics of included studies															
Author	Year	SNP	alleles	Region	Ethnicity	Genotyping method	Case			Control			OR	95% CI	Nc Ca
							AA	AG	GG	AA	AG	GG			
Cosentino	2002	rs231775	G/A	Italy	Caucasian	PCR	21	55	4	40	40	5	1.559	0.986–2.463	80
Caputo	2005	rs231775	G/A	Argentina	Caucasian	PCR	22	35	6	71	76	21	1.099	0.719–1.681	63
Haller	2007	rs231775	G/A	Estonia	Caucasian	PCR-RFLP	8	33	20	77	135	40	2.003	1.339–2.996	61
Douroudis	2009	rs3087243	G/A	Estonia	Caucasian	PCR	3	22	36	29	119	82	2.100	1.323–3.332	61
Pettersen	2010	rs231775	G/A	Norway	Caucasian	Taqman	24	52	48	263	715	499	1.072	0.823–1.396	12
		rs3087243	G/A				23	56	37	284	722	485	0.972	0.742–1.272	
Jin	2011	rs231775	G/A	China	Asian	PCR-RFLP	11	73	51	70	239	167	1.218	0.920–1.615	13
		rs3087243	G/A				5	52	78	23	183	270	1.063	0.771–1.464	
Kisan	2012	rs231775	G/A	Estonia	Caucasian	PCR-RFLP/Taqman	9	35	21	68	124	37	1.908	1.284–2.834	65
		rs3087243	G/A				4	24	37	29	118	82	1.911	1.229–2.971	
Qi	2014	rs231775	G/A	China	Asian	PCR-RFLP	5	29	14	124	52	16	5.22	3.250–8.383	48
Delitala	2015	rs3087243	G/A	Italy	Caucasian	Taqman	60	90	52	563	966	375	1.126	0.917–1.383	20
Jin	2015	rs231775	G/A	China	Asian	PCR-RFLP	15	114	102	72	241	169	1.468	1.161–1.858	23
		rs3087243	G/A				8	80	143	23	187	272	1.215	0.929–1.590	
Alshareef	2019	rs231775	G/A	Eastern Sudan	African	PCR-RFLP	5	16	3	162	70	8	3.877	2.098–7.162	24

Associations between polymorphisms of CTLA-4 and LADA

The results of meta-analysis were shown in Table 2. Significant associations with susceptibility to LADA were detected in the meta-analysis for rs231775 polymorphism (Allelic model: OR 1.53, 95% CI 1.22–1.92, $P_H=0.002$; Dominant model: OR 2.16, 95% CI 1.43–3.28, $P_H=0.003$; Recessive model: OR 1.49, 95% CI 1.13–1.97, $P_H=0.073$) (Fig. 2). Under allelic and recessive model, the polymorphism rs3087243 was associated with LADA susceptibility (Allelic model: OR 1.26, 95%CI 1.03–1.55, $P_H=0.022$; Recessive model: OR 1.41, 95% CI 1.07–1.86, $P_H=0.021$) (Fig. 3). Further subgroup analysis indicated that polymorphism rs231775 was associated with LADA susceptibility in Asians (Allelic model, Dominant model, and Recessive model), Caucasians (Allelic model, and Dominant model), and Africans (Allelic model, Dominant model, Recessive model). As for polymorphism rs3087243, subgroup analysis revealed an association between rs3087243 and LADA in Caucasians (Recessive model) but not in Asians.

Table 2
The results of meta-analysis

SNP	Allelic model G/A			Dominant model GG + GA/AA			Recessive model GG/GA + AA		
	OR (95%CI)	I^2	P_H	OR (95%CI)	I^2	P_H	OR (95%CI)	I^2	P_H
rs231775									
Overall	1.53(1.22–1.92)	68.70%	0.002	2.16(1.43–3.28)	68%	0.003	1.49(1.13–1.97)	46%	0.073
Ethnicity									
Asian	1.36(1.14–1.63)	0	0.318	2.26(1.46–3.50)	0	0.560	1.32(1.02–1.70)	4%	0.307
Caucasian	1.45(1.10–1.92)	62.80%	0.030	1.78(1.09–2.90)	65.80%	0.020	1.53(0.96–2.44)	56.10%	0.059
African	3.88(2.10–7.16)	NA	NA	7.89(2.84–21.92)	NA	NA	4.14(1.02–16.8)	NA	NA
rs3087243									
Overall	1.26(1.03–1.55)	62.10%	0.022	1.11(0.88–1.40)*	0.40%	0.413	1.41(1.07–1.86)	62.50%	0.021
Ethnicity									
Asian	1.15(0.94–1.41)	0	0.531	1.37(0.73–2.56)	0	0.931	1.16(0.91–1.49)	0	0.473
Caucasian	1.37(0.99–1.91)	76.20%	0.006	1.07(0.83–1.38)	33.60%	0.211	1.63(1.06–2.50)	71.70%	0.014
Note: * present the OR value and 95% CI were obtained based on fixed-effects model, while others were obtained based on random-effects model.									

To explore the potential source of heterogeneity among included studies, we performed sensitivity analysis to evaluate the influence of each study. No changes of results were detected for both rs231775 (Fig. 4) and rs3087243 (Fig. 5) of *CTLA-4* in allelic, dominant, and recessive models. Meta-regression analysis was also applied to try to find the source of heterogeneity. However, the ethnicity (rs231775: Allelic model, $P = 0.218$; Dominant model, $P = 0.096$; Recessive model, $P = 0.757$; rs3087243: Allelic model, $P = 0.546$; Dominant model, $P = 0.545$; Recessive model, $P = 0.358$;) and publication year (before and after year 2010) (rs231775: Allelic model, $P = 0.399$; Dominant model, $P = 0.188$; Recessive model, $P = 0.569$) were not the source of heterogeneity. The statistical method or other unknown factors may contribute to the heterogeneity. The random-effects model was appropriate under this condition to obtain the pooled OR value.

Publication bias

Begg’s test and Egger’s test were used to assess the publication bias. As shown in Table 3, Begg’s and Egger’s test showed statistically significant ($P < 0.05$) under dominant model for both rs231775 and rs3087243. Therefore, we performed a trim and fill method to estimate the number of missing studies and recalculate the pooled OR value after combining the missing hypothetical studies (rs231775: OR 1.797, 95% CI 1.188–2.719, random-effect model; rs3087243: OR 1.000, 95% CI 0.806–1.242, fixed-effect model). The funnel plots after addition of two missing hypothetical studies for rs231775 and three miss hypothetical studies for rs3087243 were basically symmetrical, respectively (Fig. 6).

Table 3
The results of publication bias

SNP	Begg's test		Egger's test	
	Z	P	t	P
rs231775				
Allelic model	1.36	0.174	2.08	0.082
Dominant model	2.10	0.035	5.11	0.002
Recessive model	0.12	0.902	0.58	0.582
rs3087243				
Allelic model	1.13	0.26	2.48	0.068
Dominant model	2.25	0.024	4.09	0.015
Recessive model	0.75	0.452	1.93	0.126

Discussion

We performed a meta-analysis to determine the effects of polymorphisms rs231775 and rs3087243 of *CTLA-4* on the LADA. In this meta-analysis, ten eligible studies including 785 cases and 3435 controls for rs231775 and 820 cases and 4828 controls for rs3087243 were analyzed. The present study revealed a significant association between polymorphism rs231775 and LADA under allelic, dominant, and recessive models. Similar positive associations were obtained in the following subgroup analysis by ethnicity except under recessive model in Caucasians. As for polymorphism rs3087243, significant association with LADA was identified in overall populations under allelic and recessive models. Subgroup analysis reported an association between rs3087243 and LADA in Caucasians under recessive model. Asian-derived populations indicated an unrelated result. Different genetic background and limited study may partially explain the difference.

The *CTLA-4* gene is located on human chromosome 2q33.2. Its encoding product expressed on the cell surface of T lymphocytes and served as a negative regulator of T-cell activation(35). Therefore, dysfunction of *CTLA-4* can increase self-reactivity of T lymphocyte and may contribute to autoimmune response(19, 36). Previous studies have also indicated associations between *CTLA-4* and multiple autoimmune diseases, such as T1DM(37), systemic lupus erythematosus(38), and rheumatoid arthritis(39). The rs231775 polymorphism located in exon 1 of *CTLA-4* and led to substitution of alanine with threonine. The mutant protein caused the reduction of *CTLA-4* cell surface expression(20). Polymorphism rs3087243 located in downstream of the poly (A) termination site and might play an important role in the mRNA stability of soluble *CTLA-4* (sCTLA-4). Previous study has indicated that individuals with potential risk genotype G/G exhibited lower production of sCTLA-4 than those with A/A genotype(19). Therefore, these two polymorphisms may lead to increased T-cell activation and higher probability of autoimmunity because of decreased function of *CTLA-4*. Latent autoimmune diabetes in adults, as an autoimmune disease, has also been associated with the polymorphisms of *CTLA-4*. However, the results are inconclusive. Our meta-analysis revealed significant associations between two investigated polymorphisms and susceptibility to LADA. This finding may contribute to early diagnosis and better prognosis of LADA patients, and identification of the underlying pathogenic mechanisms of LADA.

There were some limitations in this meta-analysis. First, heterogeneity was detected for polymorphisms rs231775 and rs3087243 in the overall population analyses for LADA, thus the random-effects model was used. In subsequent subgroup analysis, reduced tendency of heterogeneity was observed in Asians, which indicated the ethnicity might partially explain the heterogeneity. However, other unidentified factors may still contribute to the heterogeneity of included studies. Second, the number of relevant studies was relatively limited, especially in Asians and Africans, therefore, more replication studies are warranted. Third, our results were drawn based on unadjusted data and failed to adjust baseline parameters of participants. Although the methodology quality of included studies was good, the confounding factors may influence the authenticity of our results. Finally, publication bias was detected in this meta-analysis. Although we performed trim and fill method to evaluate the potential missing studies, the results may still be influenced to some extent. Taken together, the present results should be interpreted with caution.

Conclusion

In conclusion, our results indicated the polymorphisms rs231775 and rs3087243 may serve as genetic biomarkers of LADA. Further studies, especially in Asians and Africans are needed to confirm these findings.

Declarations

Ethics approval and consent to participate: Not applicable

Consent for publication: Not applicable

Availability of data and materials: All data generated or analyzed during this study are included in this published article.

Competing interests: The authors declare that they have no competing interests.

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Author's contributions: ZX and ZZ participated in research design. HP, SL, GH, and XL selected the articles. HP performed the data analysis. The manuscript was drafted by HP and critically reviewed and discussed with other co-authors. All the authors read and approved the final manuscript.

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Figures

Figure 1
Flowchart of study selection

a

b

c

Figure 2

Forest plot for rs231775 polymorphism and the risk of LADA under Allelic genetic model (a), Dominant genetic model (b), and Recessive genetic model (c).

a

b

c

Figure 3

Forest plot for rs3087243 polymorphism and the risk of LADA under Allelic genetic model (a), Dominant genetic model (b), and Recessive genetic model (c).

Figure 4

The sensitivity analysis results of rs231775 with LADA under Allelic genetic model (a), Dominant genetic model (b), and Recessive genetic model (c).

Figure 5

The sensitivity analysis results of rs3087243 with LADA under Allelic genetic model (a), Dominant genetic model (b), and Recessive genetic model (c).

Figure 6

Filled funnel plot for rs231775 under dominant genetic model (a) and for rs3087243 under dominant genetic model (b).