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Association of four CTLA-4 gene polymorphisms with pemphigus risk: a systematic review, meta-analysis, and meta-regression

Tarak Dhaouadi, Awatef Riahi, Taïeb Ben Abdallah, Yousr Gorgi and Imen Sfar

Abstract

Objectives: This review aimed to summarize the existing data on the contribution of four single nucleotide polymorphisms (SNPs) in the cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) genes to pemphigus susceptibility.

Methods: An electronic literature search for eligible studies among those published prior to 30 April 2024 was conducted through the PubMed, EMBASE, Web of Science, and Scopus databases. To minimize publication bias, an additional search was performed via the Google Scholar and Semantic Scholar search engines. Meta-analyses, together with subgroup analyses and meta-regressions, were performed for the following four CTLA-4 SNPs: rs231775, rs5742909, rs3087243, and rs733618.

Results: Combined analyses revealed a significant increase in pemphigus risk conferred by the CTLA-4 rs5742909*C and rs733618*C alleles. Conversely, there was no evidence of any significant association between the rs231775*G and rs3087243*G alleles and susceptibility to pemphigus. Subgroup analyses by ethnicity and pemphigus type (vulgaris or foliaceus) and meta-regressions did not reveal any significant difference.

Conclusion: This meta-analysis suggested that two of the four investigated CTLA-4 SNPs were significantly associated with increased pemphigus risk.

Registration: This review has been registered on PROSPERO: CRD42024550668; available from: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42024550668

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Introduction

Pemphigus refers to a group of life-threatening autoimmune vesiculobullous blistering diseases that mainly affect the skin and mucous membranes.[1] The hallmark of pemphigus is epidermis clefting and debonding from surface-close epithelia associated with acantholysis, which is defined as the separation of keratinocytes from each other.[1] Pemphigus diseases originate from the production of IgG autoantibodies directed against two epidermal desmosome structural proteins: desmoglein (Dsg)1 and Dsg3.[1,2]

The most frequently observed pemphigus disease is pemphigus vulgaris (PV), in which blisters form within the deepest layers of the epidermis.[3] The second pemphigus disease group includes those causing blisters confined to the superficial epidermis, of which pemphigus foliaceus (PF) is by far the most common.[1,3] PF mainly affects the skin with no apparent mucous membrane involvement. However, PV can lead to the development of both cutaneous and mucosal lesions, making it a more serious disease.[3]

The exact etiology and pathogenesis of pemphigus are not fully clear. Nevertheless, increasing data have suggested that genetic factors play a critical role in pemphigus pathogenesis and could influence treatment responses.[3] In fact, many observational studies have provided strong evidence for genetic susceptibility to pemphigus development, such as familial aggregation, the disparate disease incidence among various populations, and the increased prevalence of autoimmune disorders in relatives of pemphigus patients.[2] For other multifactorial autoimmune conditions, it is believed that both genetic and environmental factors can trigger aberrant immune responses against Dsg proteins, along with impaired regulatory responses in pemphigus.[3]

In this regard, the cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) receptor plays a critical role in immune response regulation. Indeed, CTLA-4 is a coinhibitory receptor that counteracts CD28-mediated T cell activation through binding to B7-1 and B7-2 molecules (CD80 and CD86, respectively) with a significantly higher avidity.[4] In humans, CTLA-4 is involved in numerous autoimmune diseases through genetic association, including systemic lupus erythematosus, rheumatoid arthritis, Grave's disease, autoimmune hypothyroidism, and type 1 diabetes.[5] The human gene encoding CTLA-4 is 6.2 kb in length and consists of four exons: exons 1 and 2 encode the leader sequence peptide and extracellular Ig like domain, respectively, while exons 3 and 4 are respectively responsible for producing the transmembrane and cytoplasmic domains.[5] This gene exhibits several single nucleotide polymorphisms (SNPs) that can influence its expression levels. For example, the rs5742909 (-318 C > T) SNP was found to be associated with a roughly 30% increase in CTLA-4 production.[6] Additionally, a study by Mäurer et al.[7] revealed that the rs231775 ($+49$ A > G) SNP, which results in a threonine-to-alanine amino acid change at position 17, altered T cell proliferation by changing intracellular CTLA-4 circulation. Furthermore, the rs3087243 (CT60) SNP was associated with lower expression levels

of the mRNA transcript encoding the soluble form of CTLA-4.[8] In addition, the rs3087243 SNP could alter regulatory T cell (Treg) levels.[9] Four SNPs in the CTLA-4 gene, namely rs231775, rs5742909, rs3087243, and rs733618, have been investigated previously in pemphigus patients. However, the published data were mostly conflicting and require further clarification.

In this study, we aimed to review the existing data on how these four CTLA-4 gene SNPs contribute to pemphigus susceptibility, as well as to investigate the between-study heterogeneity using subgroup analyses and meta-regressions.

Materials and methods

Search strategy

This study was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines for systematic reviews and meta-analyses.[10] An electronic literature search for eligible studies among those published prior to 30 April 2024 was conducted through the PubMed, EMBASE, Web of Science, and Scopus databases. To minimize publication bias, an additional search was carried out via the Google Scholar and Semantic Scholar search engines. The following search string was used: (((CTLA-4) OR (2q33)) AND ((Polymorphism) OR (SNP) OR (Variant) OR (Mutation) OR (Allele) OR (Allelic) OR (Genotype) OR (Genotypic) OR (rs231775) OR (+49 A/G) OR (rs5742909) OR (−318 C/T) OR (rs3087243) OR (CT60) OR (+6230 G > A) OR (rs733618) OR (−1722 T > C)) AND ((pemphigus) OR (PV) OR (PF))). The literature search was performed without any language restriction. A detailed search strategy for each database is available within Supplemental File 1.

Selection criteria

All studies were independently assessed and evaluated by two reviewers (TD and IS) for eligibility.

For selection, the following inclusion criteria were used: studies of case-control (retrospective) or cohort (prospective) design that included pemphigus patients and healthy control subjects; studies assessing the association between CTLA-4 gene polymorphisms and pemphigus risk; studies providing precise results with genotypes or alleles frequencies.

Additionally, the following exclusion criteria were used: genotype frequencies are not consistent with Hardy–Weinberg Equilibrium (HWE) as recommended by Trikalinos et al.;[11] studies carried out in patients with other bullous skin diseases; case series of subjects, narrative or systematic review, comments, or meta-analysis. If many studies were performed using duplicate cases, then only the study with complete data and the largest sample size was included.

Definition of a pemphigus case

A pemphigus case was defined as a biopsy-proven epidermis separation with positive direct and/or indirect immunofluorescence microscopy and/or detection with anti-Dsg1 and/or anti-Dsg3 antibodies.

Data extraction

Data were extracted using a predeveloped form and entered into an Excel datasheet. Two investigators (TD and IS) independently extracted the following information: first author, year of publication, study design, country, ethnicity, mean or median age, gender ratio (M/F), number of patients, number of controls, pemphigus disease area index (PDAI), treatment, follow-up, treatment response, and CTLA-4 SNP genotyping method (Table 1). The genotype frequencies for the rs231775,

Table 1. Characteristics of the included studies.

Study	Country	Pemphigus type	Age (years)	Sex ratio (F/M)	Genotyping method	Patients	Controls	NOS score
Abida 2020	Tunisia	PF*	35 [18–84]	14 (99/7)	PCR-RFLP	106	205	8
Bacanli 2017	Turkey	NS†	51.7 ± 13.4	1.4 (69/49)	PCR-RFLP	118	108	6
Dalla Costa 2010	Brazil	PF	NS	NS	PCR-RFLP/ PCR-SSOP	269	395	8
Fernandez Mestre 2009	Venezuela	PF + PV‡	NS	NS	PCR-RFLP	48	98	7
Narbutt 2010	Poland	PF + PV	27–61	1.25 (30/24)	PCR-RFLP	54	176	7
Pavoni 2006	Brazil	PF	NS	NS	Nested PCR	118	291	7
Pincerati 2010	Brazil	PF	0–84	NS	PCR-RFLP	248	367	7
Tanasilovic 2017	Serbia	PF + PV	NS	NS	Real-Time PCR	61	486	7

*PF, pemphigus foliaceus; †NS: not specified; ‡PV, pemphigus vulgaris; NOS, Newcastle–Ottawa Scale; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; PCR-SSOP, sequence-specific oligonucleotide probes.

rs5742909, rs3087243, and rs733618 SNPs are shown in Table 2. A third investigator (AR) examined the results of the extracted data for potential discrepancies.

Quality assessment

The quality of eligible studies was independently assessed by two reviewers (TD and IS) using the Newcastle–Ottawa Scale (NOS),[12] which is based on the following three general categories: selection (4 points), comparability of the study groups (2 points), and ascertainment of outcome (3 points). Studies with a score ≥ 7 were classified as high-quality reports. Additionally, the risk of bias was assessed for each included study using a generic form (Excel spreadsheet) and visualized via the Cochrane ROBVIS online tool (https://mcguinlu.shinyapps.io/robvis/). Two additional independent reviewers (TBA and YG) examined the quality assessment results.

Study endpoints

The primary endpoint of this meta-analysis was to estimate the strength of the association between CTLA-4 SNPs and pemphigus risk. The secondary endpoint was to evaluate the potential confounding factors that might influence the impact of the aforementioned SNPs on pemphigus risk to identify the sources of heterogeneity.

Statistical analysis

Statistical analyses were performed using the Cochrane Review Manager 5.4 software, OpenMeta-Analyst software, and MetaGenyo online available software (https://metagenyo.genyo.es/). The associations of CTLA-4 SNPs with pemphigus risk were assessed using pooled odds ratios (ORs) with the 95% confidence interval (95% CI). The statistical significance of pooled ORs was tested by Z-test, with the significance threshold set at 0.05. Random effects models (DerSimonian–Laird) were used as recommended by Borenstein et al.[13] Indeed, the random effects model applies when the eligible studies were performed in genetically diverse populations.[13] Forest plots were generated to display the distribution of effect sizes (ORs) across included studies. Sensitivity analyses were carried out to examine the stability of the results by sequentially omitting each individual study if there were at least three included studies. The between-study heterogeneity was tested by Q tests (significance threshold: 0.1), quantified via the I^2 calculation (proportion of true effects variance),

Table 2. Cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) genotyping data in pemphigus patients and controls.

SNP	Patients			Controls		
rs231775 +49 A > G	A/A	A/G	G/G	A/A	A/G	G/G
Abida 2020	37	43	5	76	66	9
Bacanli 2017	50	47	21	43	50	15
Dalla Costa 2010	110	80	57	165	160	57
Fernandez Mestre 2009 (PV)	7	16	14	32	47	19
Fernandez Mestre 2009 (PF)	4	5	2	32	47	19
Narbutt 2010 (PV)	14	18	8	53	95	28
Narbutt 2010 (PF)	4	9	1	53	95	28
Pavoni 2006	55	48	15	121	135	35
rs5742909 −318 C > T	C/C	C/T	T/T	C/C	C/T	T/T
Bacanli 2017	96	22	0	86	19	2
Dalla Costa 2010	200	29	0	303	64	7
Pavoni 2006	95	23	0	232	55	4
Tanasilovic 2017	51	10	0	392	86	8
rs3087243 (CT60) +6230 G > A	G/G	G/A	A/A	G/G	G/A	A/A
Abida 2020	29	47	21	54	81	49
Bacanli 2017	24	65	25	19	53	33
Pincerati 2010	94	107	47	122	182	63
rs733618 −1722 T > C	T/T	T/C	C/C	T/T	T/C	C/C
Dalla Costa 2010	184	33	7	297	39	2
Tanasilovic 2017	48	13	0	396	85	5

SNP, single nucleotide polymorphism; PF, pemphigus foliaceus; PV, pemphigus vulgaris.

and analyzed by determining the 95% prediction intervals (PIs). PIs were obtained through the Comprehensive Meta-Analysis (CMA) Prediction Intervals free software (https://meta-analysis-workshops.com/pages/predictionintervals), with the calculation based on the following four items: OR, upper bound of the 95% CI, Tau^2, and number of included studies. Of note, the 95% PI calculation requires at least three included studies in a meta-analysis. Subsequently, the heterogeneity was explored for potential sources by subgroup analyses and meta-regressions. Briefly, studies were stratified by ethnicity as follows: 1) North African, 2) Caucasian, and 3) South American. Meta-regressions were performed using the patient/control ratio and risk allele frequency in the control group as independent variables. Both univariate and multivariate models of meta-regression were generated to assess the presence of potential confounding factors. Publication bias was assessed by Egger's test and visualized by generating funnel plots. The Egger's test *P*-value computation requires at least three included studies in a meta-analysis. HWE was examined for each study and for every SNP by assessing both the individual univariate and multivariate-adjusted *P*-values. The HWE adjusted *P*-values for the control groups are shown for each SNP in Table 3.

Table 3. Cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) single nucleotide polymorphism (SNP) Hardy–Weinberg equilibrium (HWE) assessment in the control groups.

	HWE *P*-value			
Study	rs231775	rs5742909	rs3087243	rs733618
Abida 2020	0.277	_	0.1067	_
Bacanli 2017	0.9391	0.4413	0.7758	_
Dalla Costa 2010	0.0802	0.1044	_	0.5636
Fernandez Mestre 2009	0.815	_	_	_
Narbutt 2010	0.1769	_	_	_
Pavoni 2006	0.7767	0.72	_	_
Pincerati 2010	_	_	0.7282	_
Tanasilovic 2017	_	0.2009	_	0.8541

In this study, the codominant genetic model (allele contrast) was applied. Additionally, the results of recessive and dominant genetic models are displayed.

Supplementary File 1 contains additional relevant tables and figures. A PRISMA checklist is available as Supplementary File 2.

Systematic review registration

This review has been registered on PROSPERO (CRD42024550668) and is available from: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42024550668

Results

Search results and study characteristics

A PRISMA flow diagram was generated to depict the study selection process (Figure 1). Overall, eight studies with a total of 1022 pemphigus cases and 2126 controls were included in the present study.[14–21] The characteristics of the included studies are summarized in Table 1. The genotyping data of the cases and controls are shown in Table 2. Six studies were included for the rs231775 SNP,[14–19] four for the rs5742909 SNP,[15,16,19,21] three for the rs3087243 SNP,[14,15,20] and two for the rs733618 SNP.[16,21] The NOS quality score results for each included study are shown in Table 1. The characteristics of the included SNPs are described in Table S1. The risks of bias are summarized in Figure 2. The two reviewers/investigators (TD and IS) had 100% agreement for quality assessment and extracted data. Three additional reviewers/investigators (AR, TBA, and YG) examined and compared the quality assessment and extracted data results.

CTLA-4 rs231775 SNP meta-analysis

Combined analysis did not reveal any significant association between the rs231775*G allele and pemphigus risk (OR [95% CI] = 1.09 [0.95–1.26], $P = 0.2032$) (Table 4, Figure 3). Likewise, the dominant genetic model did not show any influence of rs231775 on the susceptibility to pemphigus (OR [95% CI] = 0.98 [0.8–1.18], $P = 0.8017$) (Table 4, Figure 3). However, under the recessive model, the rs231775*G/G homozygous mutant genotype was significantly associated with pemphigus risk (OR [95% CI] = 1.45 [1.12–1.89], $P = 0.0055$) (Table 4, Figure 3). Additionally, there was no between-study heterogeneity with each of the three genetic models (all $I^2 = 0\%$; codominant model $P = 0.4797$, recessive model $P = 0.5985$, dominant model $P = 0.6707$) (Table 4,

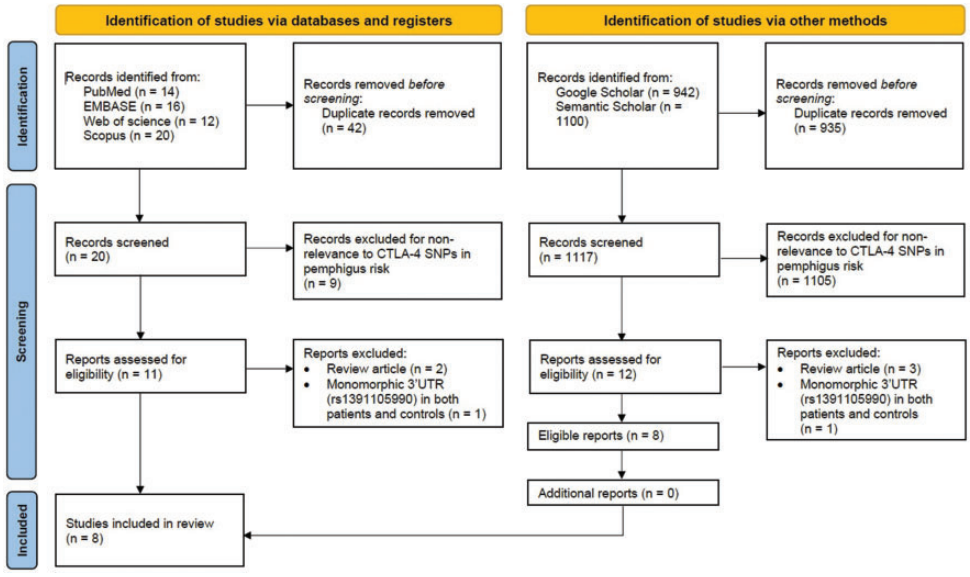

Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram for study selection.

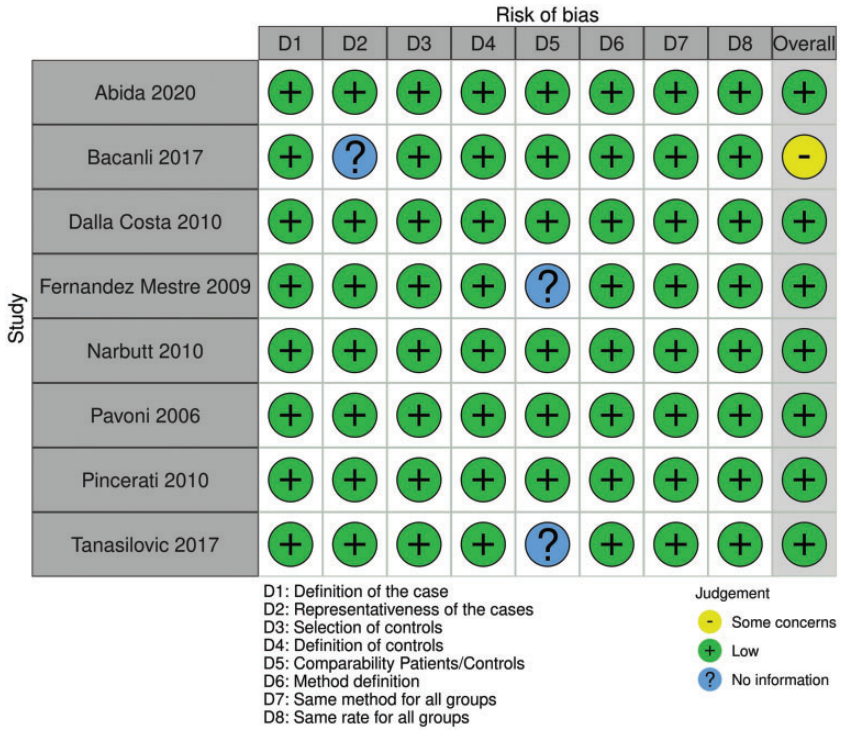

Figure 2. Summary of study risk of bias.

Table 4. Main results of the association of cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) single nucleotide polymorphisms (SNPs) with pemphigus risk.

			Pemphigus risk		Heterogeneity	
SNP	Genetic model	Contrast	OR [95% CI]	*P*-value	I^2	*P*-value
rs231775	Codominant	G vs. A	1.09 [0.95–1.26]	0.2032	0%	0.4797
+49 A > G	Recessive	GG vs. AG + AA	1.45 [1.12–1.89]	0.0055	0%	0.5985
	Dominant	GG + AG vs. AA	0.98 [0.8–1.18]	0.8017	0%	0.6707
rs5742909	Codominant	C vs. T	1.36 [1.04–1.78]	0.0256	0%	0.5985
−318 C > T	Recessive	CC vs. CT + TT	1.27 [0.95–1.69]	0.1039	0%	0.6197
	Dominant	CC + CT vs. TT	4.52 [1.05–19.52]	0.0431	0%	0.4225
rs3087243 (CT60)	Codominant	G vs. A	1.12 [0.94–1.33]	0.2026	0%	0.6978
+6230 G > A	Recessive	GG vs. AG + AA	1.17 [0.9–1.52]	0.2346	0%	0.8572
	Dominant	GG + AG vs. AA	1.17 [0.81–1.7]	0.4013	33%	0.2266
rs733618	Codominant	C vs. T	1.5 [1.03–2.18]	0.0334	9%	0.2945
−1722 T > C	Recessive	CC vs. CT + TT	3.83 [0.95–15.5]	0.0592	0%	0.3599
	Dominant	CC + CT vs. TT	1.44 [0.98–2.12]	0.0599	0%	0.5421

OR, odds ratio; CI, confidence interval.

Figure 3). Although there was no inconsistency between the included studies, we performed subgroup analyses and meta-regressions. The subgroup analyses by ethnicity and pemphigus type (PV or PF) did not show any significant difference (Figure S1/S2). Similarly, the meta-regressions did not show any correlation between the pemphigus risk and the risk allele frequency or patient/control ratio (Table S2, Figure S3/S4).

CTLA-4 rs5742909 SNP meta-analysis

The integrated analysis revealed a significant association between the rs5742909*C wild-type allele and susceptibility to pemphigus (OR [95% CI] = 1.36 [1.04–1.78], $P = 0.0256$) (Table 4, Figure 4). Likewise, the dominant genetic model exhibited a significant association with pemphigus risk (OR [95% CI] = 4.52 [1.05–19.52], $P = 0.0431$) (Table 4, Figure 4). Conversely, under the recessive model, the rs5742909*C/C genotype was not associated with pemphigus risk (OR [95% CI] = 1.27 [0.95–1.69], $P = 0.1039$). Additionally, there was no between-study heterogeneity for all genetic models (Table 4). The subgroup analysis by ethnicity did not reveal any significant difference (Figure S5), while the meta-regressions did not show any correlation between the effect size and C allele frequency in the controls or the patient/control ratio (Table S3, Figure S6/S7).

CTLA-4 rs3087243 SNP meta-analysis

There were only three included studies[14,15,20] in the rs3087243 SNP meta-analysis. The rs3087243*G wild-type allele was not associated with pemphigus susceptibility (OR [95% CI] = 1.12 [0.94–1.33], $P = 0.2026$) (Table 4, Figure 5). Similarly, both the recessive and dominant genetic models did not show any association with pemphigus risk (OR [95% CI] = 1.17 [0.9–1.52], $P = 0.2346$; OR [95% CI] = 1.17 [0.81–1.7], $P = 0.4013$, respectively) (Table 4, Figure 5). There was also little to no between-study heterogeneity with the three genetic models (Table 4).

CTLA-4 rs733618 SNP meta-analysis

Only two studies[16,21] were included in the rs733618 meta-analysis. The combined

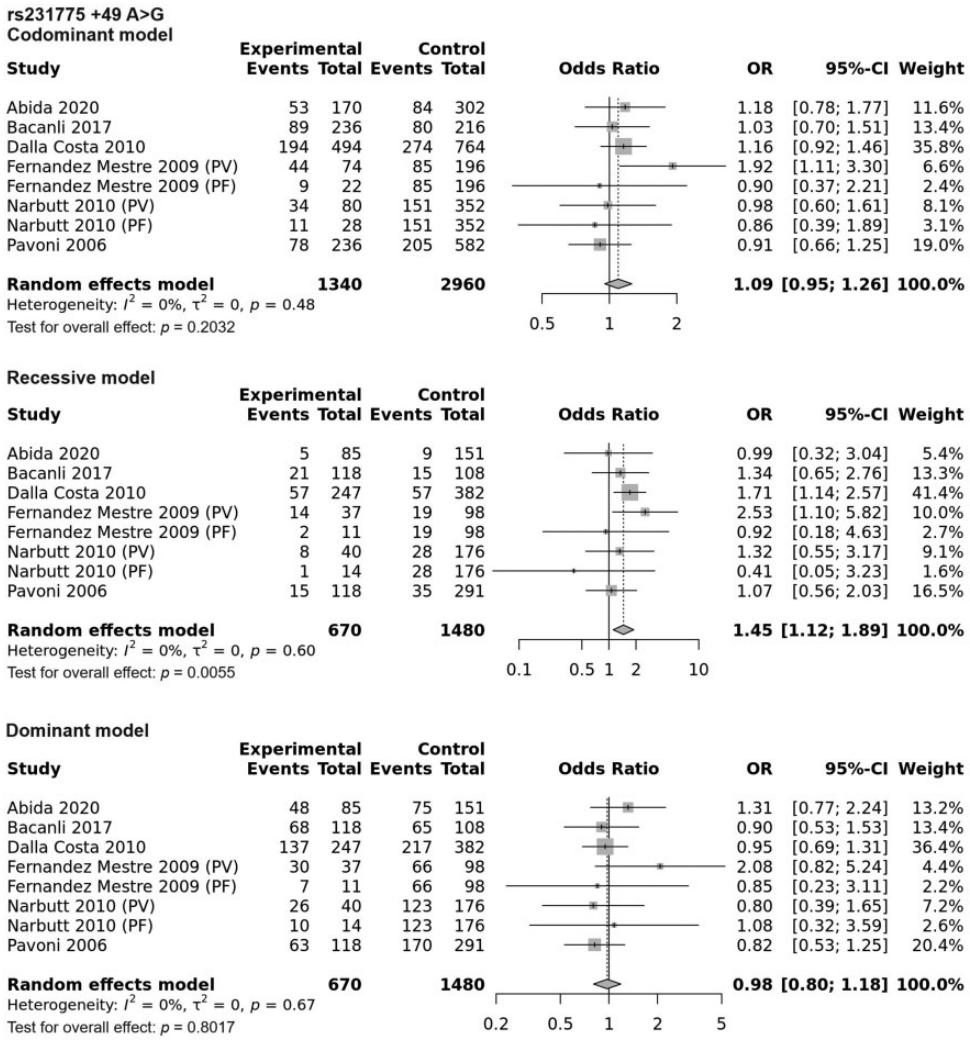

Figure 3. Forest plots for the association between the cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) rs2304365 single nucleotide polymorphism (SNP) and pemphigus risk by genetic model.

analysis showed a significant association of the rs733618*C allele with pemphigus risk (OR [95% CI] = 1.5 [1.03–2.18], $P = 0.0334$) (Table 4, Figure 6). In addition, there was a very low level of between-study heterogeneity ($I^2 = 9\%$, $Tau^2 = 0.0073$, $P = 0.2945$) (Table 4). The recessive and dominant genetic models revealed nearly significant associations with susceptibility to pemphigus ($P = 0.0592$ and $P = 0.0599$, respectively) (Table 4, Figure 6). Because only two studies were included, subgroup analysis and meta-regressions could not be performed.

Sensitivity analysis

The sensitivity analysis revealed that the observed association results were stable (Figures S10–S12), suggesting a high level of integrity with reliable results.

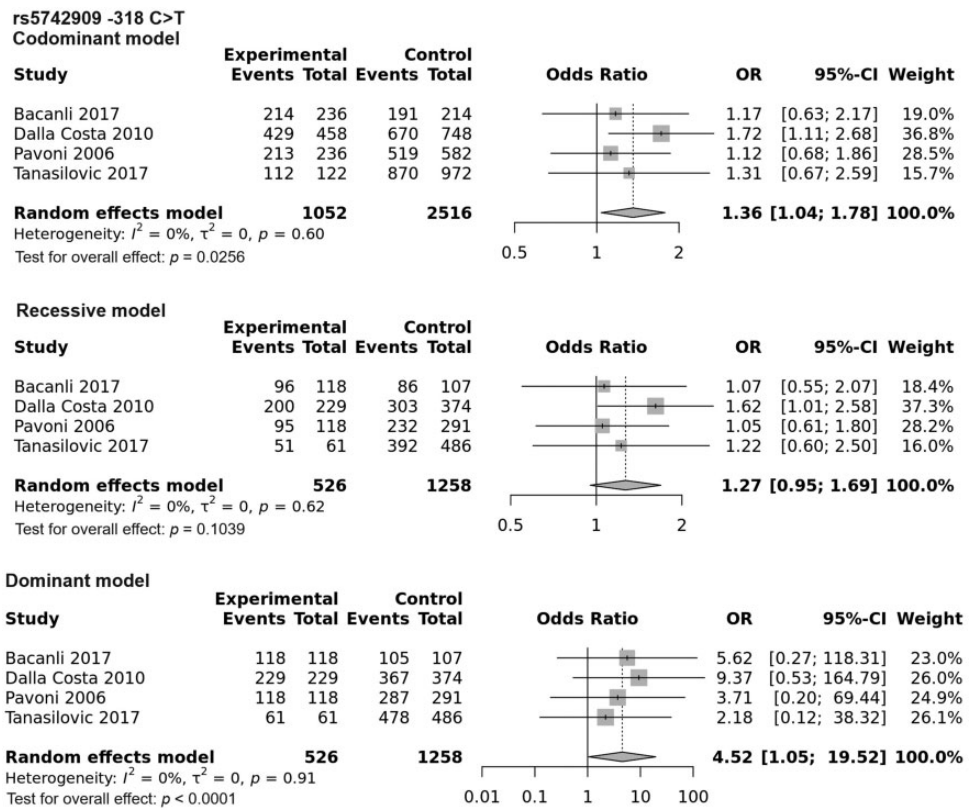

Figure 4. Forest plot for the association between the cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) rs5742909 single nucleotide polymorphism (SNP) and pemphigus risk by genetic model.

Publication bias

The generated funnel plots (Figure 7) were found to be symmetrical overall, with Egger's tests confirming the findings with non-significant *P*-values for the rs231775, rs5742909, and rs3087243 SNPs ($P = 0.9385$, $P = 0.4374$ and $P = 0.3414$, respectively). This indicated that the results were not weakened by publication biases. Because only two studies were included for the rs733618 SNP meta-analysis, Egger's test could not be conducted.

Overall, the rs5742909 (−318 C > T) and rs733618 (−1722 T > C) promoter SNPs were found to be significantly associated with pemphigus risk. Conversely, the association with pemphigus susceptibility of the rs231775 (+49 A/G) SNP was noted only when the recessive model was applied, while the rs3087243 SNP was not associated with pemphigus risk under any of the genetic models.

Discussion

Pemphigus is an antibody-mediated bullous skin disease caused by IgG autoantibody binding to desmosomes directed against the Dsg1 and/or Dsg3 proteins.[1–3] Like other autoimmune diseases, pemphigus has a complex etiology that includes multigenetic background and environmental factors.[2,3] Among the genetic factors, human leukocyte antigen (HLA) class II genes were the most associated with pemphigus risk. In fact, the HLA-DRB1*04, DRB1*08,

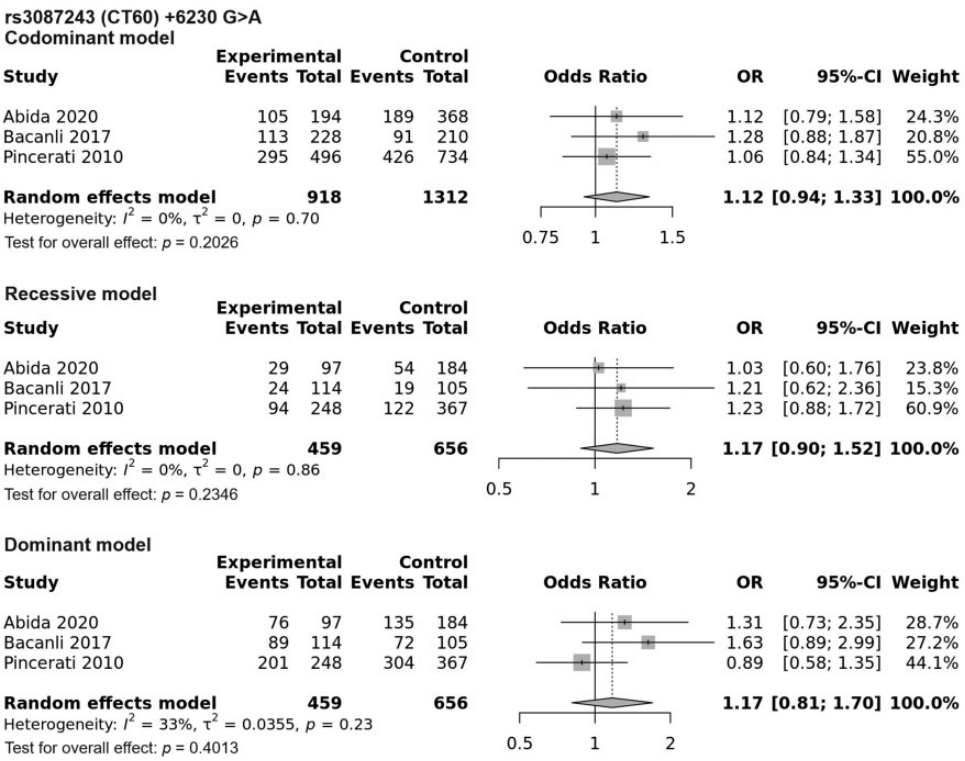

Figure 5. Forest plot for the association between the cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) rs3087243 single nucleotide polymorphism (SNP) and pemphigus risk by genetic model.

DRB1*14, DQB1*0503, and DQB1*0302 alleles were found to be significant pemphigus susceptibility factors.[22,23] However, HLA genes are only part of the genetic component of pemphigus. Other non-HLA pemphigus susceptibility genes have been identified, including immunologically relevant genes and skin-related genes.[3] For the genes involved in immune responses, some studies have examined the impact of costimulatory and coinhibitory molecules, such as CD28, inducible T cell costimulator (ICOS), and CTLA-4, on the risk of pemphigus.[14–21] Most notably, investigations of certain CTLA-4 gene SNPs, which have previously been associated with several autoimmune conditions, have revealed significant associations with pemphigus risk, albeit with conflicting results.[14–21] To the best of our knowledge, this study was the first to summarize the published results on pemphigus risk conferred by the following CTLA-4 SNPs: rs231775, rs5742909, rs3087243, and rs733618.

The present study revealed that the CTLA-4 rs231775*G allele was not associated with an increased pemphigus risk, with no between-study heterogeneity ($I^2 = 0\%$). Among all the included studies, only one report in a subgroup of Venezuelan patients with PV noted a significant increase in risk conferred by the rs231775*G allele.[17] Examination of the dominant genetic model did not provide the study with any additional significant finding. Under the recessive model, the rs231775*G/G genotype was found to be significantly

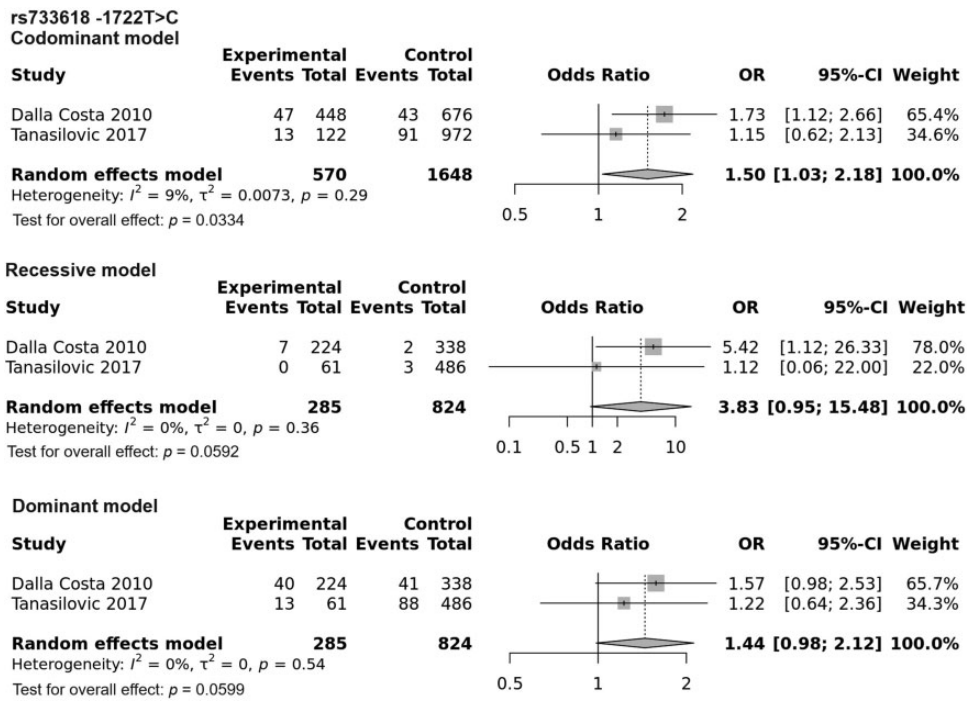

Figure 6. Forest plot for the association between the cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) rs733618 single nucleotide polymorphism (SNP) and pemphigus risk by genetic model.

associated with an approximately 45% increase in pemphigus risk. Taken together, these data indicate that the impact of the rs231775 SNP on pemphigus risk is potentially weak and could therefore be missed in many association studies. Furthermore, the subgroup analyses by ethnicity and pemphigus type, as well as the meta-regressions, failed to indicate any significant association.

This study showed that the CTLA-4 rs5742909*C allele was associated with an approximately 36% increased pemphigus risk. There was also no between-study heterogeneity. The effect size could fall between a 1.04-fold and 1.78-fold increased pemphigus risk in 95% of all comparable populations. Moreover, while the recessive model did not show any significant association, the dominant model displayed an even greater association value, with the rs17315309*C/C and *C/T genotypes conferring a 4.5-fold increase in pemphigus risk. The subgroup analysis by ethnicity and meta-regressions did not reveal any significant association.

The current study did not show any significant association between the CTLA-4 rs3087243*G allele and susceptibility to pemphigus. Similarly, both the recessive and dominant genetic models did not reveal an increase in pemphigus risk. In addition, there was little to no between-study heterogeneity across all assessed genetic models. Moreover, the meta-regression using the risk allele frequency in the controls and the patient/control ratio revealed no correlation with the effect size. Thus, the data suggest that the rs3087243 SNP has no influence on pemphigus risk. Nevertheless, because there were only three included reports, further studies in large independent cohorts are required.

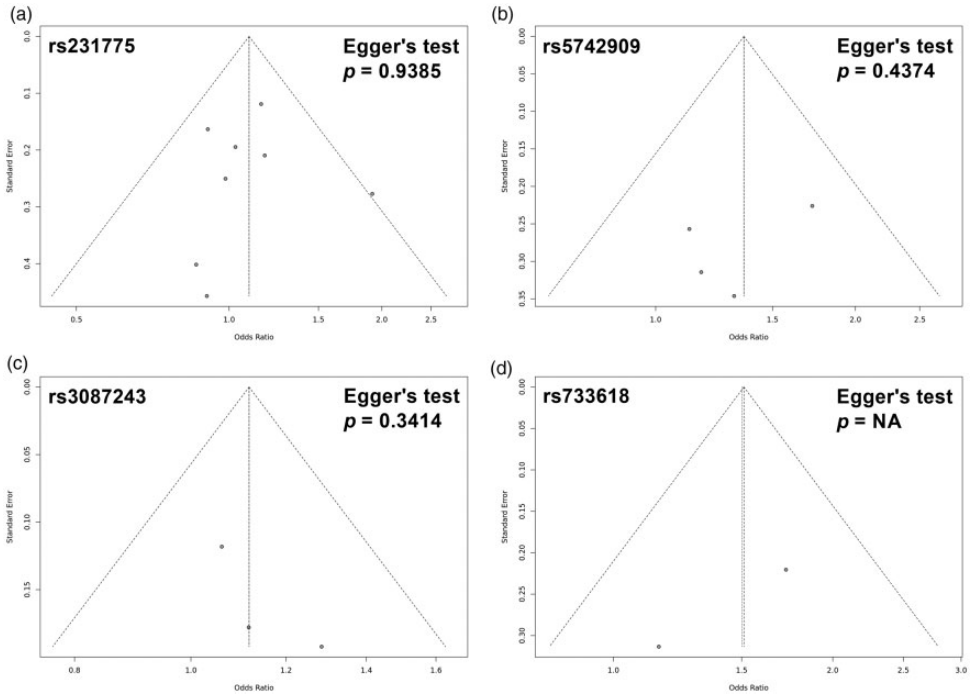

Figure 7. Funnel plots assessing publication bias: symmetrical funnel plots with no evidence of publication biases.

In the present study, the CTLA-4 rs733618*C allele was found to be associated with an approximately 50% increase in pemphigus risk, with a very small amount of between-study heterogeneity ($I^2 = 9\%$). Therefore, the true effect in 95% of all comparable populations could fall between a 1.12-fold and 2.66-fold increase in pemphigus risk. Conversely, both the recessive and dominant genetic models did not harbor any significant association with the susceptibility to pemphigus. It is important to note that only two studies were included in the rs733618 SNP meta-analysis. This issue prevented a precise quantification of both the effect size and between-study heterogeneity. Therefore, future studies are needed to better investigate the role of the rs733618 SNP in susceptibility to pemphigus.

In summary, the present meta-analysis revealed that the CTLA-4 rs5742909 (-318 C > T) and rs733618 (-1722 T > C) SNPs could play significant roles in susceptibility to pemphigus, along with no evidence of between-study heterogeneity. However, there was a small number of included studies in both meta-analyses. Additionally, if the recessive model is applied, then the rs231775 SNP could potentially influence pemphigus risk. Both the rs5742909 and rs733618 SNPs are located in the promoter region of the CTLA-4 gene. These two SNPs are associated with lower CTLA-4 expression levels, which could alter immune response regulation and increase the risk of autoimmunity.[6] Indeed, one study examined peripheral blood mononuclear cells from pemphigus patients, which revealed significantly downregulated CTLA-4 expression levels compared with healthy subjects.[24] Moreover, a higher prednisolone dosage, PDAI score,

and anti-DSG3 levels were significantly negatively correlated with CTLA-4 expression levels.[24] Taken together, these data indicate that genetically-determined CTLA-4 expression patterns could play a major role in susceptibility to pemphigus and potentially influence its severity and treatment response.

To our knowledge, this study was the first to perform several meta-analyses for associations between CTLA-4 gene SNPs and pemphigus risk. However, there are some limitations to this work. First, like other autoimmune diseases, pemphigus risk depends on several factors, such as environmental, infectious, psychological, and genetic factors. Because the analyses in this study involved pooling CTLA-4 SNP aggregate findings without any access to raw data, there was a lack of further adjustments for baseline characteristics. Second, most of the included studies were performed in South American populations, with only two studies in Caucasian populations and one study in North African populations. Hence, the present meta-analysis results cannot be generalized to Sub-Saharan African, Asian, or North American populations, causing future studies in these populations to be required. Third, there was a small number of included studies. For example, only two studies investigated how the rs733618 SNP influences pemphigus risk, which prevented us from accurately analyzing the between-study heterogeneity and suitably quantifying Tau^2. Therefore, our findings require replication in independent large cohorts. Fourth, only 1022 pemphigus cases and 2126 controls were included in the current meta-analysis, which may have underpowered the yielded results. Fifthly, most of the included studies did not specify demographic data, such as the gender ratio, onset age, and family history of pemphigus or other autoimmune diseases. Therefore, we could not adjust the combined results on the aforementioned parameters through extensive meta-regressions. Lastly, the included studies did not specify the PDAI in patients or response to treatment, which prevented us from analyzing the impact of the CTLA-4 SNPs on disease severity and progression under treatment.

Conclusions

This meta-analysis showed that two of the four investigated CTLA-4 SNPs, rs5742909 (-318 C > T) and rs733618 (-1722 T > C), were significantly associated with increased pemphigus risk. However, additional studies in independent cohorts, mainly in Sub-Saharan African, Asian, and North American populations, are needed to further validate the results of the present meta-analysis.

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Author contributions

Study conceptualization: TD, AR, YG, TBA, IS; Data curation: TD and AR; Formal analysis: TD and IS; Investigation: TD, AR, IS; Methodology: TD, IS, TBA, YG; Project administration: YG, TBA, IS; Supervision: YG, TBA, IS; Writing – original draft: TD; Writing – review & editing: TD.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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Supplementary material

Supplemental material for this article is available online.

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