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Association between functional CD24 polymorphisms and susceptibility to autoimmune diseases: A meta-analysis

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Abstract

This study aimed to explore whether the functional *CD24* A57V and TG/del polymorphisms are associated with susceptibility to autoimmune diseases. A meta-analysis was conducted on the associations between the *CD24* A57V and TG/del polymorphisms and autoimmune diseases using (1) allele contrast, and (2) the recessive, (3) dominant, and (4) co-dominant models. Twenty-six comparative studies with 7,507 patients and 8,803 controls were included in the meta-analysis. The meta-analysis revealed a significant association between autoimmune disease and the *CD24* Val allele (OR = 1.285, 95% CI = 1.177–1.403, $p = 1.0 \times 10^{-9}$). Meta-analysis by autoimmune disease type showed a significant association between the *CD24* Val allele and multiple sclerosis (MS) (OR = 1.420, 95% CI = 1.239–1.628, $p = 4.7 \times 10^{-8}$) and systemic lupus erythematous (SLE) (OR = 1.282, 95% CI = 1.081–1.521, $p = 0.004$), but not Crohn's disease (CD) (OR = 1.003, 95% CI = 0.826–1.218, $p = 0.974$). Meta-analysis of the *CD24* Val/Val genotype showed an association with ulcerative colitis (OR = 1.778, 95% CI = 1.148–2.753, $p = 0.010$). In addition, meta-analysis by autoimmune disease type revealed a significant association between the *CD24* TG-deletion allele and MS (OR = 0.596, 95% CI = 0.415–0.856, $p = 0.005$) and CD (OR = 1.594, 95% CI = 1.175–2.161, $p = 0.003$). This meta-analysis indicates that the functional CD24 A57V and TG/del polymorphisms are associated with susceptibility to multiple autoimmune diseases including SLE, MS, UC and CD.

Key words: Autoimmune diseases, CD24, Polymorphism, Meta-analysis.

Introduction

Autoimmune diseases are a diverse group of complex diseases that affect up to 5% of the human population and are characterized by loss of self-tolerance leading to immune-mediated tissue destruction (25). These diseases are multifactorial and due to interactions between genetic and environmental factors. Furthermore, they share a number of characteristics that suggest common etiologic mechanisms. In particular, their pathophysiologies and their co-occurrence in families have prompted the hypothesis that autoimmune diseases share a genetic background (4). This hypothesis has been strengthened by meta-analyses of whole-genome scans showing that autoimmune diseases exhibit non-random clustering of disease susceptibility loci (3,22).

CD24 is a glycosylphosphatidylinositol (GPI)-anchored cell-surface protein on a variety of immune cells, including activated T cells, B cells, granulocytes, macrophages, and dendritic cells. CD24 plays a physiological role in the maturation of T and B cells and in the activation of CD4 and CD8 T cells by providing a CD28-independent co-stimulatory signal (17,24). CD24 interacts with very late antigen (VLA)-4 and vascular cell adhesion molecule (VCAM), which play an important role in lymphocyte migration to sites of inflammation (2). *CD24* is considered a genetic checkpoint in T-cell homoeostasis and pathogenesis of autoimmune diseases (24). The *CD24* A57V (rs8734) and TG/del (rs3838646) polymorphisms have been the best studied in autoimmune diseases. These two polymorphisms have functional relevance, altering CD24 expression levels (38) and *CD24* mRNA stability (34), respectively. The non-synonymous C to T coding *CD24* A57V polymorphism results in the substitution of an alanine (Ala) amino acid with valine (Val). This amino acid substitution precedes the presumed cleavage site for the GPI anchor, and T cells from *CD24* A57V Val carriers express CD24 at higher levels than those from individuals with the *CD24* Ala genotype (38). A TG deletion in the 3'-untranslated region (UTR) reduces the steady levels of *CD24* mRNA by more than 2-fold (34).

Studies have shown that these *CD24* polymorphisms are associated with several autoimmune diseases; on the other hand, other reports have found no such associations (7,10,11,14,16,23,26,27,29-33,36,39). These disparities are probably caused by small sample sizes, low statistical power, and/or clinical heterogeneity. Therefore, to overcome the limitations of individual studies, resolve inconsistencies, and reduce the likelihood that random errors are responsible for false-positive or false-negative associations, we performed a meta-analysis (18,20,21). Using the meta-analysis approach, the present study aimed to determine whether the *CD24* A57V and TG/del functional polymorphisms are associated with susceptibility to multiple autoimmune diseases.

Methods

Identification of eligible studies and data extraction

We performed a search of studies that examined associations between *CD24* polymorphisms and autoimmune diseases. The literature was searched using PubMed and Embase citation databases to identify available articles in which the *CD24* A57V and TG/del polymorphisms were analyzed in patients with autoimmune

diseases. In general, bibliographic searches for systematic reviews should include Pubmed, Embase, and the Cochrane Central Register of Controlled Trials. Additional databases are often useful to search. However, Cochrane register did not contain enough data on polymorphisms, data on polymorphisms can be retrieved from Pubmed and Embase with high precision by using search strategies. Combinations of keywords, such as "CD24," "polymorphism," "autoimmune diseases," and the names of individual diseases were entered as Medical Subject Heading (MeSH) and text words. References in cited studies were also investigated to identify additional studies not indexed by electronic databases from January 1990 to May 2005. No restrictions were placed on language, race, ethnicity, or geographic area. Autoimmune diseases were diagnosed according to classification criteria. Studies were included if they (1) were case-control studies reviewed by peers or independent scientists with relevant expertise, (2) contained original data, and (3) provided sufficient genotype data to calculate odds ratios (ORs). The following were excluded: (1) studies containing overlapping data, (2) studies in which numbers of null and wild genotypes could not be ascertained, (3) studies from meeting abstracts or unpublished sources, and (4) studies in which family members had been examined, for example those that used transmission disequilibrium tests, because the analyses conducted were based on linkage considerations. The following information was extracted from each study: author, year of publication, ethnicity of the study population, demographics of subjects, and numbers of cases and controls. Frequencies of alleles were calculated from genotype distributions. Data management resources were used to increase transparency and efficiency.

Evaluations of statistical associations

A chi-square test was used to determine whether observed genotype frequencies conformed to the Hardy-Weinberg equilibrium (HWE) (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl) to check the study quality based on the genotyping method. Meta-analyses were performed using (1) allelic contrast, (2) recessive, (3) dominant, and (4) co-dominant models for the *CD24* A57V and TG/del polymorphisms. Subgroup analyses were performed by ethnicity and autoimmune disease type to evaluate ethnic- and disease-specific effects. Point estimates of risks, ORs, and 95% confidence intervals (CIs) were estimated for each study. Cochran's Q-statistic was used to assess within- and between-study variations and heterogeneities. This heterogeneity test assesses the null hypothesis that all studies evaluated the same effect. I^2 values were used to quantify the effect of heterogeneity, with values ranging between 0% and 100% and representing the proportion of between-study variability attributable to heterogeneity rather than chance (13). I^2 values of 25%, 50%, and 75% were nominally defined as low, moderate, and high estimates. The fixed effects model assumes a genetic factor has the same effect on disease susceptibility across all studies investigated, and that observed variations between studies are caused by chance alone. The random effects model assumes that different studies show substantial diversity and assesses both within-study sampling error and between-study variance. When study groups are homogeneous, the two models are similar, but if this is not the case, the random effects model usually provides wider CIs than the fixed effects model. The random effects model, therefore, is used in the presence of significant between-study heterogeneity (6). Statistical manipulations were performed using the Comprehensive Meta-Analysis computer program (Biostat, Englewood, NJ, USA).

Evaluation of heterogeneity and publication bias

To examine a potential source of heterogeneity observed in the meta-analysis, a meta-regression was performed. A sensitivity analysis was also performed to assess the influence of each individual study on the pooled OR by sequentially omitting each individual study and to investigate statistically robust results from this meta-analysis. Funnel plots are often used to detect publication bias. However, due to the limitations of funnel plotting, which requires a range of studies of varying sizes that involve subjective judgments, publication bias was also evaluated using Egger's linear regression test (9), which measures funnel plot asymmetry using a natural logarithm scale of ORs. When asymmetry was indicated, we used the "trim and fill" method to adjust summary estimates for the observed bias (8). This method removes small studies until funnel plot symmetry is achieved and recalculates the center of the funnel before the eliminated studies are replaced with their missing mirror-image counterparts. A revised summary estimate was then calculated using all original studies and hypothetical "filled" studies.

Results

Studies included in the meta-analysis

One hundred and thirty reports were identified by electronic and manual searching, and 19 were selected for full-text review based on title and abstract details. Four reports were excluded due to a lack of genotype data or because they were review articles. Thus, 15 reports met the inclusion criteria (7,10,11,14,16,23,26,27,29-33,36,39). In addition, three of these reports contained data on two different groups (7,23,35), and one on three different groups (31), and we analyzed these studies independently. A total of 26 separate studies were considered in the meta-analysis, which in total contained 7,507 patients and 8,803 controls. Nineteen studies examined the *CD24* A57V polymorphism, corresponding to 4,933 patients and 5,390 controls, and seven studies examined the *CD24* TG/del polymorphism, corresponding to 2,574 patients and 3,413 controls. These studies encompassed multiple sclerosis (MS; n = 8), systemic lupus erythematosus (SLE; n = 6), Crohn's disease (CD; n = 4), ulcerative colitis (UC; n = 4), rheumatoid arthritis (RA; n = 2), giant cell arteritis (GCA; n = 2), autoimmune thyroid disease (AITD; n = 1), and sarcoidosis (n = 1). A disease-specific meta-analysis was performed on MS, SLE, CD, and UC, and ethnicity-specific meta-analysis was conducted in Caucasian and Asian populations. Selected characteristics of these studies related to the association between the *CD24* polymorphisms and diseases are summarized in Table 1. The statistical powers of these studies ranged between 21.4% and 99.0%, and five studies had a statistical power of more

Table 1. Characteristics of individual studies included in the meta-analyses.

A. CD24 A57V polymorphism

Author (Ref)	Ethnicity	Disease	Numbers		CD24 Val allele (%)		Allele association				Power (%)[a]
			Case	Control	Case	Control	OR	95% CI		p-value	
Lisiansky-1, 2014(23)	Jewish	CD	31	104	32.3	22.6	1.631	0.874	3.044	0.124	21.3
Lisiansky-2, 2014(23)	Jewish	UC	88	104	37.5	22.6	2.055	1.316	3.210	0.002	28.3
Gao, 2014(10)	Asian	SLE	54	70	44.4	27.1	2.147	1.262	3.655	0.005	19.9
Inoue, 2013(14)	Asian	AITD	338	103	41.6	33.5	1.412	1.018	1.959	0.039	55.5
Kollaee, 2011(16)	Middle Eastern	MS	120	120	36.7	27.1	1.559	1.058	2.296	0.025	34.0
Diaz-Gallo-1, 2011(7)	Caucasian	CD	366	628	27.0	28.0	0.952	0.776	1.168	0.639	88.3
Diaz-Gallo-2, 2011(7)	Caucasian	UC	322	628	30.4	28.0	1.124	0.912	1.384	0.273	86.9
Gonzalez, 2011(11)	Latin American	MS	102	205	33.3	31.0	1.114	0.778	1.595	0.555	41.7
Tanizawda, 2010(33)	Asian	Sarcoidosis	186	146	40.6	40.8	0.993	0.727	1.357	0.966	44.5
Piotrowsk, 2010(27)	Caucasian	SLE	250	350	38.6	30.7	1.418	1.114	1.805	0.005	68.7
Ronaghi, 2009(29)	Middle Eastern	MS	217	200	37.3	26.5	1.652	1.230	2.219	0.001	53.2
Sanchez, 2008(32)	Caucasian	RA	1015	842	28.7	24.8	1.220	1.054	1.413	0.008	99.0
Rueda, 2008(30)	Caucasian	GCA	120	185	32.1	25.4	1.387	0.970	1.984	0.073	41.5
Sanchez-1, 2007(31)	Caucasian	SLE	696	539	29.5	23.8	1.338	1.116	1.605	0.002	93.8
Sanchez-2, 2007(31)	Caucasian	SLE	257	317	29.4	27.4	1.100	0.850	1.423	0.470	66.8
Sanchez-3, 2007(31)	Caucasian	SLE	310	247	31.6	30.8	1.040	0.806	1.343	0.763	65.5
Otaegui, 2006(26)	Caucasian	MS	136	285	30.9	25.3	1.322	0.961	1.819	0.087	53.6
Cui, 2006(36)	Asian	MS	83	110	44.6	33.2	1.620	1.069	2.453	0.023	28.4
Zhou, 2003(39)	Unknown	MS	242	207	33.3	26.8	1.361	1.020	1.815	0.036	56.3

B. CD24 TG/del polymorphism

Author (Ref)	Ethnicity	Disease	Numbers		CD24 del allele (%)		Allele association				Power (%)[a]
			Case	Control	Case	Control	OR	95% CI		p-value	
Lisiansky-1, 2014(23)	Jewish	CD	31	105	8.1	5.7	1.447	0.490	4.279	0.504	21.4
Lisiansky-2, 2014(23)	Jewish	UC	87	105	5.2	5.7	0.900	0.370	2.189	0.816	28.3
Diaz-Gallo-1, 2011(7)	Caucasian	CD	371	629	10.8	7.0	1.607	1.170	2.207	0.003	88.5
Diaz-Gallo-2, 2011(7)	Caucasian	UC	310	629	6.5	7.0	0.917	0.623	1.350	0.660	86.5
Gonzalez, 2011(11)	Latin American	MS	102	205	4.4	5.6	0.777	0.353	1.710	0.530	41.7
Sanchez, 2008(32)	Caucasian	RA	1015	842	8.0	7.3	0.555	0.369	0.835	0.005	99.0
Rueda, 2008(30)	Caucasian	GCA	119	185	14.3	7.8	0.367	0.225	0.598	0.000	41.4
Wang-1, 2007(35)	Caucasian	MS	275	443	6.2	10.6	1.447	0.490	4.279	0.504	76.4
Wang-2, 2007(35)	Caucasian	SLE	264	270	4.5	11.5	0.900	0.370	2.189	0.816	63.7

Ref: references, OR: odds ratio, CI: confidence interval, [a]: Assuming a small effect size (convention $w = 0.1$) at a level of significance of 0.05, RA: rheumatoid arthritis, AITD: autoimmune thyroid disease, MS: multiple sclerosis, UC: ulcerative colitis, CD: Crohn's disease, GCA: giant cell arteritis, SLE: systemic lupus erythematosus.

than 80% (Table 1).

Meta-analysis of the CD24 A57V polymorphism in autoimmune diseases

A summary of meta-analyses findings on associations between the *CD24* A57V polymorphism and autoimmune diseases is provided in Table 2. The meta-analysis revealed a significant association between autoimmune disease and the *CD24* Val allele (OR = 1.285, 95% CI = 1.177–1.403, $p = 1.0 \times 10^{-9}$) (Fig. 1, Table 2), and stratification by ethnicity indicated an association between the *CD24* Val allele and autoimmune diseases in Caucasians (OR = 1.190, 95% CI = 1.107–1.280, $p = 2.6 \times 10^{-7}$) and Asians (OR = 1.421, 95% CI = 1.050–1.924, $p = 0.023$) (Table 2). Meta-analysis by autoimmune disease type showed a significant association between the *CD24* Val allele and MS (OR = 1.420, 95% CI = 1.239–1.628, $p = 4.7 \times 10^{-8}$) and SLE (OR = 1.282, 95% CI = 1.081–1.521, $p = 0.004$), but not CD (OR = 1.003, 95% CI

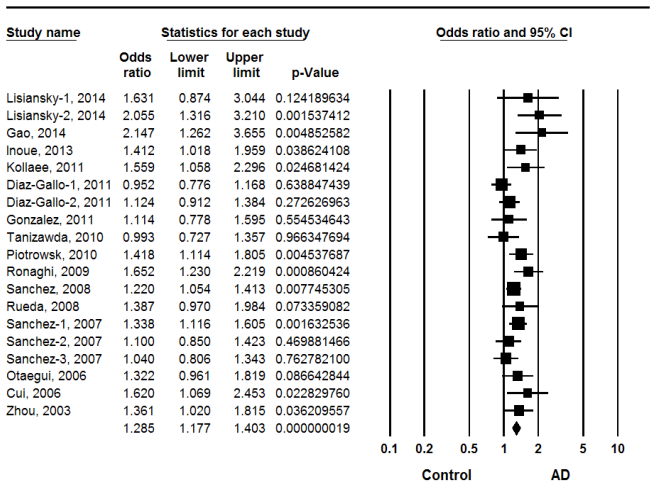

Figure 1. Odds ratios (ORs) and 95% confidence intervals (CIs) of individual studies and pooled data for the allelic association between the CD24 A57V polymorphism and autoimmune diseases.

Table 2. Analysis of the association between the CD24 A57V polymorphism and autoimmune diseases.

Polymorphism	Population	No. of studies	Numbers		Test of association			Test of heterogeneity		
			Case	Control	OR	95% CI	P-val	Model	p-val	I^2
CD24 Val vs. Ala	Overall	19	4,933	5,390	1.285	1.177–1.403	1.9×10^{-9}	R	0.023	43.5
	Caucasian	9	3,472	4,021	1.190	1.107–1.280	2.6×10^{-7}	F	0.192	28.3
	Asian	4	661	429	1.421	1.050–1.924	0.023	R	0.053	59.7
	MS	6	900	1,127	1.420	1.239–1.628	4.7×10^{-8}	F	0.600	0
	SLE	5	1,567	1,523	1.282	1.081–1.521	0.004	R	0.077	52.4
	CD	2	397	732	1.003	0.826–1.218	0.974	F	0.108	61.2
	UC	2	410	732	1.470	0.816–2.646	0.200	R	0.016	82.7
Val/Val vs. Val/Aal + Ala/Ala (Recessive)	Overall	19	4,933	5,390	1.812	1.487–2.207	$<1.0 \times 10^{-9}$	R	0.057	36.4
	Caucasian	9	3,472	4,021	1.903	1.427–2.537	1.1×10^{-6}	R	0.036	51.3
	Asian	4	661	429	1.336	0.939–1.901	0.107	F	0.527	0
	MS	6	900	1,127	2.268	1.791–4.628	2.4×10^{-8}	F	0.367	7.75
	SLE	5	1,567	1,523	1.686	1.295–2.196	1.0×10^{-5}	F	0.189	34.8
	CD	2	397	732	1.326	0.829–2.122	0.239	F	0.522	0
	UC	2	410	732	1.778	1.148–2.753	0.010	F	0.843	0
Val/Val + Val/Aal vs. Ala/Ala (Dominant)	Overall	19	4,933	5,390	1.288	1.134–1.463	1.0×10^{-5}	R	0.003	54.0
	Caucasian	9	3,472	4,021	1.125	1.025–1.234	0.013	F	0.183	29.5
	Asian	4	661	429	1.684	1.017–2.789	0.043	R	0.017	70.6
	MS	6	900	1,127	1.363	1.138–1.632	0.001	F	0.913	0
	SLE	5	1,567	1,523	1.313	1.032–1.675	0.028	R	0.043	59.5
	CD	2	397	732	1.301	0.486–3.480	0.601	R	0.021	81.2
	UC	2	410	732	1.647	0.602–4.508	0.331	R	0.002	89.6
Val/Val vs. Ala/Ala (Co-dominant)	Overall	19	4,933	5,390	1.975	1.608–2.427	$<1.0 \times 10^{-9}$	R	0.058	36.2
	Caucasian	9	3,472	4,021	1.962	1.445–2.614	1.5×10^{-6}	R	0.026	54.0
	Asian	4	661	429	1.634	1.112–2.400	0.012	F	0.204	34.6
	MS	6	900	1,127	2.461	1.776–3.410	6.0×10^{-9}	F	0.480	0
	SLE	5	1,567	1,523	1.883	1.432–2.480	6.6×10^{-7}	F	0.148	40.9
	CD	2	397	732	1.227	0.753–1.989	0.407	F	0.870	0
	UC	2	410	732	1.851	1.177–2.912	0.008	F	0.306	4.60
Val/Ala vs. Val/Val (Co-dominant)	Overall	19	4,933	5,390	1.183	1.032-1.356	0.016	R	0.002	55.8
	Caucasian	9	3,472	4,021	1.031	0.936-1.135	0.542	F	0.244	22.3
	Asian	4	661	429	1.632	0.948-2.808	0.077	R	0.015	71.2
	MS	6	900	1,127	1.175	0.970-1.422	0.098	F	0.866	0
	SLE	5	1,567	1,523	1.230	0.956-1.584	0.108	R	0.046	58.7
	CD	2	397	732	1.311	0.423-4.059	0.639	R	0.010	85.0
	UC	2	410	732	1.548	0.522-4.594	0.431	R	0.001	90.4

F: fixed effects model, R: random effects model, *p*-val: p-value., NA: not available, MS: multiple sclerosis, SLE: systemic lupus erythematosus, CD: Crohn's disease, UC: ulcerative colitis.

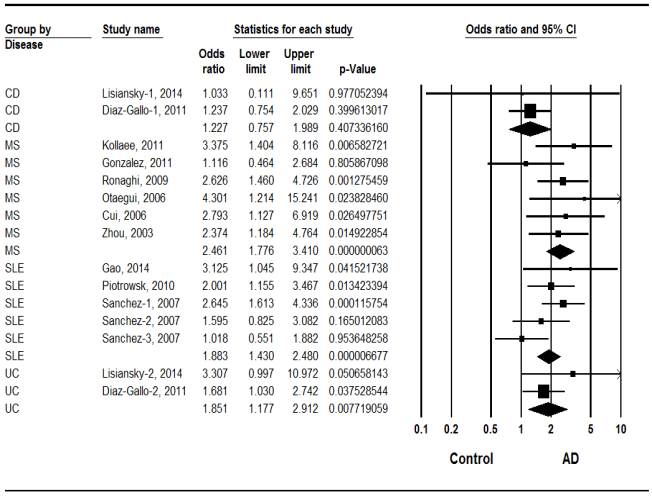

Figure 2. Odds ratios (ORs) and 95% confidence intervals (CIs) of individual studies and pooled data for the association between Val/Val vs. Ala/Ala of the CD24 A57V polymorphism and MS, SLE, CD, and UC.

= 0.826–1.218, p = 0.974) (Table 2). A similar pattern was noted for the *CD24* Val allele in the analysis using the dominant and homozygote contrast models (Fig. 2, Table 2). Meta-analysis of the *CD24* Val/Val genotype showed an association with UC (OR = 1.778, 95% CI = 1.148–2.753, p = 0.010) (Fig. 2, Table 2). Analysis using homozygote contrast also revealed an association between the *CD24* A57V polymorphism and UC (Table 2).

Meta-analysis of the CD24 TG/del polymorphism in autoimmune diseases

Our meta-analysis revealed no association between autoimmune disease and the *CD24* TG-deletion allele (OR = 0.945, 95% CI = 0.665–1.342, p = 0.751) (Table 3), and stratification by ethnicity indicated no association between the *CD24* del allele and autoimmune diseases in Caucasians (Table 3). However, meta-analysis by autoimmune disease type revealed a significant association between the *CD24* del allele and protection against MS (OR = 0.596, 95% CI = 0.415–0.856, p = 0.005), but not CD (OR = 1.594, 95% CI = 1.175–2.161, p = 0.003), or UC (OR = 0.914, 95% CI = 0.641–1.303, p = 0.620) (Fig. 3, Table 3). A single study showed a similar association between the *CD24* del allele and SLE (OR = 0.367, 95% CI = 0.225–0.598 $p = 5.6 \times 10^{-6}$) (Table 3). A similar pattern was noted for the *CD24* del allele in the analysis using the dominant and co-dominant models (Table 3).

Table 3. Analysis of the association between the CD24 TG/del polymorphism and autoimmune diseases.

Polymorphism	Population	No. of studies	Numbers		Test of association			Test of heterogeneity		
			Case	Control	OR	95% CI	P-val	Model	p-val	I^2
CD24 del vs. TG	Overall	9	2,574	3,413	0.945	0.665–1.342	0.751	R	0.000	80.3
	Caucasian	6	2,354	2,998	0.937	0.610–1.439	0.765	R	0.000	87.4
	MS	2	377	648	0.596	0.415–0.856	0.005	F	0.459	0
	SLE	1	264	270	0.367	0.225–0.598	5.6×10^{-6}	NA	NA	NA
	CD	2	402	734	1.594	1.175–2.161	0.003	F	0.856	0
	UC	2	397	734	0.914	0.641–1.303	0.620	F	0.970	0
del/del vs. del/TG + TG/TG (Recessive)	Overall	7	2,456	3,203	0.702	0.252–1.955	0.498	R	0.059	50.6
	Caucasian	6	2,354	2,998	0.585	0.201–1.703	0.325	R	0.055	53.7
	MS	2	377	648	0.797	0.133–4.762	0.803	F	0.135	55.1
	SLE	1	264	270	0.077	0.004–1.372	0.081	NA	NA	NA
	CD	1	371	629	2.876	1.037–7.980	0.042	NA	NA	NA
	UC	1	310	629	0.154	0.009–2.751	0.204	NA	NA	NA
del/del + del/TG vs. TG/TG (Dominant)	Overall	9	2,574	3,413	0.964	0.671–1.385	0.843	R	0.000	79.0
	Caucasian	6	2,354	2,998	0.975	0.627–1.516	0.912	R	0.000	86.3
	MS	2	377	648	0.576	0.386–0.833	0.004	F	0.654	0
	SLE	1	264	270	0.382	0.229–0.638	2.3×10^{-5}	NA	NA	NA
	CD	2	402	734	1.546	1.108–2.157	0.010	F	0.947	0
	UC	2	397	734	0.972	0.671–1.408	0.881	F	0.845	0
del/del vs. TG/TG (Co-dominant)	Overall	7	2,456	3,203	0.705	0.247–2.014	0.544	R	0.048	52.7
	Caucasian	6	2,354	2,998	0.584	0.194–1.761	0.340	R	0.043	56.4
	MS	2	377	648	0.739	0.124–4.418	0.740	F	0.130	56.3
	SLE	1	264	270	0.069	0.004–1.225	0.068	NA	NA	NA
	CD	1	371	629	3.029	1.090–8.415	0.034	NA	NA	NA
	UC	1	310	629	0.156	0.009–2.774	0.206	NA	NA	NA
del/TG vs. TG/TG (Co-dominant)	Overall	9	2,574	3,413	0.984	0.693–1.397	0.928	R	0.000	76.3
	Caucasian	6	2,354	2,998	1.014	0.665–1.546	0.948	R	0.000	84.2
	MS	2	377	648	0.563	0.380–0.835	0.004	F	0.912	0
	SLE	1	264	270	0.428	0.254–0.720	0.001	NA	NA	NA
	CD	2	402	734	1.440	1.016–2.041	0.040	F	0.950	0
	UC	2	397	734	1.035	0.712–1.505	0.855	F	0.731	0

F: fixed effects model, R: random effects model, *p*-val: p-value., NA: not available, MS: multiple sclerosis, SLE: systemic lupus erythematosus, CD: Crohn's disease, UC: ulcerative colitis.

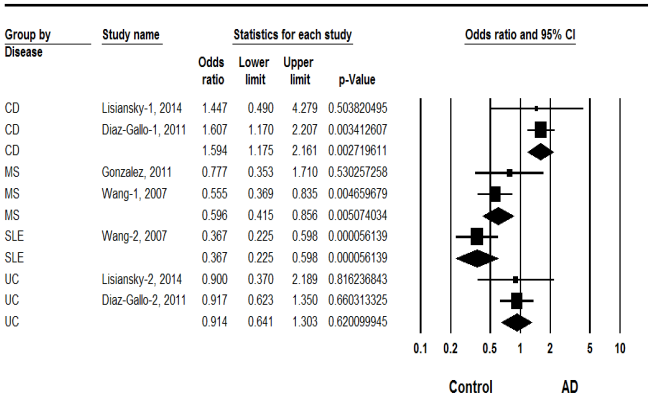

Figure 3. Odds ratios (ORs) and 95% confidence intervals (CIs) of individual studies and pooled data for the allelic association between the CD24 TG/del polymorphism and MS, SLE, CD, and UC.

Heterogeneity, study quality and publication bias

The distribution of genotypes in the normal control groups was not consistent with HWE in four studies (26,29,30,32). However, excluding these studies did not markedly affect our meta-analysis results on the association between the *CD24* A57V polymorphism and autoimmune diseases. Between-study heterogeneity in the meta-analysis of the *CD24* A57V polymorphism was found when all the subjects were considered (Tables 2). Meta-regression showed that ethnicity ($p = 0.042$), but not HWE ($p = 0.397$), had a significant impact on the heterogeneity in ORs associated with the *CD24* polymorphisms. No heterogeneity was found in the meta-analysis of the *CD24* TG/del polymorphism in subgroups by disease type (Table 3). The sensitivity analysis showed that no individual study significantly affected the pooled OR, indicating statistically robust results from this meta-analysis. The funnel plot showed asymmetry, and Egger's regression test showed evidence of publication bias in the meta-analysis of *CD24* A57V polymorphisms (Egger's regression test *p*-value = 0.023); thus, the "trim and fill" method was used to adjust for publication bias. However, after adjustment, previously significant ORs remained significant (OR = 1.197, 95% CI = 1.089–1.314) (Figure 4). In the meta-analysis of the *CD24* TG/del polymorphisms, there was no evidence of publication bias ((Egger's regression test *p*-value = 0.638).

Discussion

The *CD24* gene is located on chromosome 6q21, which is linked to autoimmune diseases (5,19,37). *CD24* has been considered a candidate gene for autoimmune disease because of its crucial role in the activation and differentiation of B cells and in the CD28-independent co-stimulatory pathway that can induce the activation of CD4 and CD8 T cells (17,24), as well as its location near linkage loci for autoimmune diseases (5,19,37). CD24 binds to P-selection and regulates VLA-4 binding to either VCAM-1 or fibronectin, which results in the recruitment of leukocytes to inflamed tissues (1,12).

Meta-analyses properly conducted with good metho-

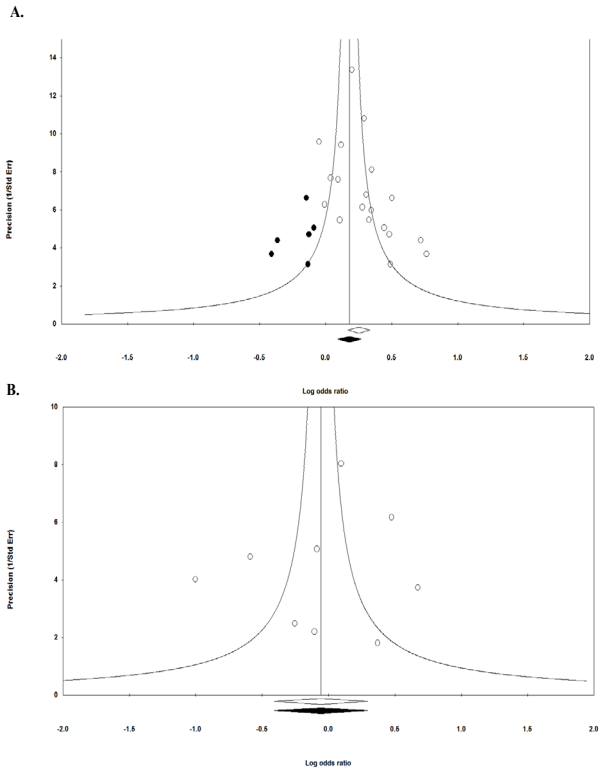

Figure 4. Funnel plot of studies that examined allelic associations between autoimmune disease and the CD24 A57V polymorphism (A) or the CD24 TG/del polymorphism (B) (Egger's regression *p*-value = 0.023, 0.638). The filled circles represent studies that showed a publication bias. The diamonds at the bottom of the figure show summary effect estimates before (open) and after (filled) publication bias adjustment (A).

dologies have been published (15,28), and the present study addresses the association between the *CD24* A57V and TG/del polymorphisms and susceptibility to autoimmune diseases. We found a significant association between autoimmune disease and the *CD24* A57V Val allele (OR = 1.285, 95% CI = 1.177–1.403, $p = 1.0 \times 10^{-9}$). After stratification by autoimmune disease, a subgroup meta-analysis indicated an association between the *CD24* A57V polymorphism and MS, SLE, and UC. In addition, meta-analysis by autoimmune disease type revealed a significant association between the *CD24* TG-deletion allele and MS (OR = 0.596, 95% CI = 0.415–0.856, $p = 0.005$), SLE (OR = 0.367, 95% CI = 0.225–0.598 $p = 5.6 \times 10^{-6}$), and CD (OR = 1.594, 95% CI = 1.175–2.161, $p = 0.003$).

The results of our study are consistent with the functional effects of the *CD24* A57V polymorphisms (38). The *CD24* A57V polymorphism leads to a non-conservative replacement of Ala with Val at the site that precedes the putative cleavage site for the GPI anchor (38). Because Ala and Val are substantially different in size, the replacement may increase the efficiency of cleavage and result in an increased expression of CD24 in the *CD24* Val/Val genotype. The *CD24* Val allele results in 30–40% more cell surface expression of CD24 than the *CD24* Ala allele (38). The enhanced induction of *CD24* may be an important checkpoint in the pathogenesis of autoimmune disease (24). It is possible that increased expression of CD24 could result in differentiation of B cells and stimulation of a CD28-independent co-stimulatory pathway that induces the activation of CD4 and CD8 T cells, thus contributing to the development of autoimmune diseases.

Our results are also consistent with those of functional studies on the *CD24* TG/del polymorphism (24,34). The *CD24* mRNA levels for the TG-deletion allele were 2.5-fold less than that of the wild-type allele, and the *CD24* del allele significantly reduced the stability of *CD24* mRNA (34). Our meta-analysis results demonstrate that a destabilizing TG deletion in the 3'-UTR of CD24 mRNA confers significant protection against the risk of MS and SLE. It is possible that decreased expression of CD24 results in reduced stability of *CD24* mRNA, thus contributing to the protection against autoimmune diseases including MS and SLE. However, the opposite effect has been reported for the *CD24* TG/del polymorphism in other autoimmune diseases. In this study, the *CD24* del allele is associated with reduced risk for MS and SLE; meanwhile, it is a risk factor for CD. This demonstrates that the same variant may influence diseases differently within the complex genetic component of autoimmunity (29). In MS and SLE, lower CD24 levels could act in a protective way, inhibiting T cell activation, whereas in CD, the lower *CD24* mRNA levels associated with the deletion allele might alter apoptosis in the B and T cell selection process, allowing the release of auto-reactive cells. Another possible explanation for this lack of concordance might be due to differences in the genetic background between these autoimmune diseases. The degree of linkage disequilibrium between *CD24* polymorphisms may differ across autoimmune diseases.

The present study has some limitations that require consideration. First, heterogeneity and confounding factors may have distorted the analysis. In particular, publication bias could have affected our findings, because studies that produced negative results may not have been published or may have been missed. Second, our ethnicity-specific meta-analysis included data only from Caucasian and Asian patients; thus, our results are applicable only to these ethnic groups. Further studies are required in different ethnic populations. Third, we did not stratify and analyze many factors, such as sex or clinical and environmental variables, because of a lack of data, and *CD24* polymorphisms may be associated with specific clinical manifestations in addition to disease susceptibility, as one example. Fourth, several diseases, such as RA, GCA, AITD, and sarcoidosis, were not analyzed in the present study. Thus, additional studies are warranted to explore the associations between these autoimmune diseases and the *CD24* polymorphisms.

This meta-analysis of published studies confirms that the functional CD24 A57V and TG/del polymorphisms are associated with susceptibility to multiple autoimmune diseases, including MS, SLE, CD, and UC. Variants of immune-mediated genes are believed to modulate a genetic predisposition to autoimmune disease. Our meta-analysis supports the notion that *CD24* is a checkpoint for homeostatic proliferation of T cells, which is implicated in the pathogenesis of autoimmune diseases, and that the *CD24* A57V and TG/del polymorphisms may modulate the development of autoimmune diseases. Further studies are required to clarify the role of the *CD24* gene in autoimmune pathogenesis.

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