Tumor Necrosis Factor-α Promoter –308 A/G Polymorphism and Rheumatoid Arthritis Susceptibility: A Metaanalysis

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ABSTRACT. ***Objective.*** Tumor necrosis factor-α (TNF-α) promoter –308 A/G polymorphism has been reported to be associated with rheumatoid arthritis (RA) with inconsistent results. We investigated whether TNF-α –308 A/G polymorphism confers susceptibility to RA.

Methods. We conducted a random effect metaanalysis on the association of genotypes A/A (recessive effect), A/A + A/G (dominant effect), A allele, and A/A vs G/G genotypes of the TNF-α –308 polymorphisms with RA overall and within different ethnic populations.

Results. Fourteen studies, 10 of Europeans, 3 of Latin Americans, and one Asian, were included in this metaanalysis. An association between RA and the TNF-α –308 A allele was not found in the overall population (OR 1.005, 95% CI 0.715–1.412, p = 0.976). However, stratification by ethnicity indicated that the TNF-α A allele was significantly associated with RA in Latin Americans (OR 2.004, 95% CI 1.158–3.467, p = 0.013). Conversely, there was no association detected for the TNF-α A allele with RA patients from the European samples (OR 0.911, 95% CI 0.684–1.212, p = 0.520). The OR for the A/A + A/G genotype, the A/A genotypes, and the A/A vs G/G genotypes in samples overall and in each ethnic group showed a similar trend to those for the TNF-α A allele.

Conclusion. This metaanalysis demonstrates that the TNF-α –308 A/G polymorphism may represent a significant risk factor for RA in Latin Americans, but not in Europeans. (First Release Dec 1, 2006; J Rheumatol 2007;34:43–9)

Key Indexing Terms:
RHEUMATOID ARTHRITIS | TUMOR NECROSIS FACTOR-α | POLYMORPHISMS | METAANALYSIS | SUSCEPTIBILITY

Rheumatoid arthritis (RA) is a chronic inflammatory disease involving predominantly synovial joints and affecting up to 1% of the population worldwide[1]. Although the etiology of RA remains unsolved, a genetic component to RA susceptibility has been established. Familial aggregation and evidence of multiple genetic linkage and associations with RA demonstrate an underlying genetic basis of the disease[2,3]. Multiple genetic associations have been identified, suggesting involvement of human leukocyte antigen (HLA) in susceptibility to RA. The HLA loci are the most powerful recognized genetic factors for RA[4].

Tumor necrosis factor-α (TNF-α) is a potent proinflammatory cytokine that plays an important role in inflammatory and immune responses, including in RA[5]. TNF-α stimulates cytokine production, enhancing expression of adhesion molecules and increasing neutrophil activation. It acts as a costimulator for T cell activation and antibody production[6]. The *TNF-α* gene is located on chromosome 6, within the class III region of HLA. Several single-nucleotide polymorphisms have been identified in the TNF-α promoter[7]. Among these, a common polymorphism in the promoter, a G to A substitution at position –308, has been studied intensively. It is not clear whether the TNF-α promoter –308 A/G polymorphism has a functional significance, but there is some suggestion that there may be a small but significant effect of TNF-α promoter –308 A/G polymorphism, with the A allele being associated with greater levels of TNF transcription[8,9].

Several studies have examined the potential contribution of TNF-α promoter –308 A/G polymorphism to RA susceptibility[10-23]. However, these studies have shown mixed results due to small sample sizes and low statistical power. Individual studies with small sample sizes do not have sufficient statistical power to detect a positive association, nor to confirm an absence of association. The low statistical power of individual studies to detect small differences between cases and controls is one factor to explain the lack of conclusive results. To detect a small or moderate genetic effect of polymorphisms, metaanalyses or larger sample sizes are required.

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Metaanalysis integrates previous research, providing increased statistical power and resolution by pooling the results of independent analyses[24]. Metaanalysis is a powerful method to overcome the problem of small sample size and inadequate statistical power of genetic studies of complex traits. We investigated whether the TNF-α promoter –308 A/G polymorphism contributes to susceptibility to RA, using a metaanalysis with published data.

MATERIALS AND METHODS

Identification of eligible studies and data extraction. We performed an exhaustive search on studies that examined the association of the TNF-α promoter –308 A/G polymorphism with RA. A search of the literature was made using Medline citations to identify available articles in which the TNF-α promoter –308 A/G polymorphism was determined in patients with RA and controls (most recent study May 2006). References in the studies were reviewed to identify additional studies not indexed by Medline. "Tumor necrosis factor," "TNF-α," "polymorphism," "RA," and "rheumatoid arthritis" were entered as both medical subject heading (MeSH) terms and text words. No language or country restrictions were applied. A study was included in the analysis if (1) it was a case control study, (2) it was original data (independence among studies), and (3) it provided enough data to calculate odds ratios. We excluded the following: (1) studies that contained overlapping data, (2) studies in which the number of null and wild genotypes could not be ascertained, and (3) studies in which family members had been studied because their analysis is based on linkage considerations. From each study, the following information was extracted: first author, year of publication, racial descent of study population, the number of cases and controls, and the available genotype and allele frequency information from the TNF-α promoter –308 A/G polymorphism.

Evaluation of publication bias. The funnel plot method was used to detect publication bias. However, due to the limitation that a funnel plot needs a range of studies with varying size and subjective judgment, we have evaluated publication bias using Egger's linear regression test[25]. The Egger test measures a funnel plot asymmetry on the natural logarithm scale of the odds ratio.

Evaluation of the statistical association. Allele frequencies at the TNF-α promoter –308 A/G polymorphism from the respective study were determined by the allele counting method. A chi-square test was used to determine if observed frequencies of genotypes conformed to Hardy-Weinberg equilibrium (HWE). Studies of the controls found not to be in HWE were subjected to a sensitivity analysis.

We examined the contrast of the allelic effect of A (minor allele) versus G (common allele), and also examined the contrast of A/A versus A/G + G/G genotypes, as well as the contrast of A/A + A/G versus G/G genotypes. These contrasts correspond to the recessive and dominant effects of the A allele, respectively. And we also examined the contrast of the genotypic-based effect of A/A versus G/G. The point estimates of the risk, the odds ratio, and its 95% confidence interval were estimated for each study. We assessed the within- and between-study variation or heterogeneity by testing Cochran's Q-statistic. This heterogeneity test assessed the null hypothesis that all studies were evaluating the same effect. We also quantified the effect of heterogeneity by using a recently developed measure, $I^2 = 100\% \times (Q - df)/Q$[26]. I^2 ranges between 0% and 100% and represents the proportion of between-study variability that can be attributed to heterogeneity rather than chance. I^2 values of 25%, 50%, and 75% were assigned as low, moderate, and high estimates. The random effect model was used for this metaanalysis. The random effect model assumes that different studies may estimate different underlying effects and considers both within-study (intra-study) variation and between-study variation. Finally, the overall or pooled estimate of OR was obtained by the DerSimonian and Laird method in the random effect model[27]. The pooled OR in the metaanalysis was calculated by weighting individual OR by the inverse of their variance. Statistical manipulations were undertaken using the Comprehensive Meta-analysis computer program (Biostat, Englewood, NJ, USA).

We estimated the expected power of each individual study as determined by the probability of detecting a true association between TNF-α –308 A/G polymorphism and RA at the 0.05 level of significance, assuming OR of 1.5 and 2.0 for differences in allele frequency, and the minor (disease) allele frequency is of 0.2. The power analysis was performed at http://calculators.stat.ucla.edu/powercalc/.

RESULTS

Studies included in the metaanalysis. A total of 14 relevant studies with TNF-α promoter –308 A/G polymorphism and RA met the inclusion criteria[10-23]. Eleven studies were excluded for the following reasons: data duplication (n = 4)[28-31], no allele or genotyping data (n = 4)[32-35], family based study design (n = 2)[36,37], and possible reversed description of the genotypes (n = 1)[38].

These 14 studies consisted of 10 European[10-19], 3 Latin American[20-22], and 1 Asian population samples[23] (Table 1). The allele frequency of the TNF-α promoter –308 A/G polymorphism was extracted from all of the 14 studies, but the genotype frequency was available from 12 studies[10,11,13,14,16-23] because 2 studies did not give the genotype data[12,15]. Because of the inadequate sample populations available for the Asian group, we performed ethnicity-specific metaanalysis only in the European and Latin American populations.

Evaluation of study quality and sensitivity analysis. The distribution of the genotype of the TNF-α promoter –308 A/G polymorphism in control groups was consistent with the HWE, except for data from 2[13,14]. Deviation from HWE among controls could imply some potential biases in the selection of controls, or genotyping errors, but excluding the studies with an absence of HWE in the controls did not materially affect the overall results (data not shown). There was no evidence of publication bias in this metaanalysis (Egger's regression test p values > 0.1).

Evaluation of TNF-α promoter –318 A/G polymorphism and association with RA. Selected characteristics of 14 case control studies for TNF-α –308 A/G polymorphism and the risk of RA are summarized in Table 1. Table 1 also shows the expected power of each individual study to reveal an association between this polymorphism and RA. Only 5 of the 14 studies used in the metaanalysis had more than 80% power to detect an effect of this size (Table 1). The OR with 95% CI of individual studies for the association of the A allele at the TNF-α locus and RA are shown in Table 1. One study from Europe and 2 from Latin America showed significantly increased OR of the A allele, but one study from Asia showed significantly decreased OR for the A allele.

A summary of metaanalysis for the TNF-α promoter –308 polymorphism with RA is shown in Table 2. From the metaanalysis, an association between RA and the TNF-α A allele was not found in the overall population (OR 1.005, 95% CI 0.715–1.412, p = 0.976; Figure 1A). However, stratification by ethnicity indicates that the TNF-α A allele was significant-

Table 1. Characteristics of individual studies included in metaanalysis.

Study	Population	RA, n	Control, n	OR (95% CI) for A vs G Allele	p*	Expected Power,**, % OR 1.5	OR 2.0
Pawlik[10]	European (Poland)	91	105	0.59 (0.32–1.12)	0.58	22.3	56.1
Balog[11]	European (Hungary)	23	75	1.16 (0.50–2.69)	0.84	10.8	24.6
Miterski[12]	European (German)	151	312	0.95 (0.65–1.40)	NA	41.0	86.5
Maury[13]	European (Finland)	110	400	0.54 (0.33–0.89)	0.04	35.7	80.5
Cvetkovic[14]	European (Sweden)	154	324	0.62 (0.43–0.87)	< 0.05	41.8	87.3
Lacki[15]	European (Poland)	68	28	1.12 (0.48–2.59)	NA	11.6	27.2
van Krugten[16]	European (Netherlands)	283	138	0.68 (0.48–0.97)	0.68	36.1	81.0
Vinasco[17]	European (Spain)	60	102	1.84 (0.95–3.58)	0.25	18.3	46.3
Danis[18]	European (Australia)	34	57	3.47 (1.60–7.52)	0.33	12.1	28.8
Fugger[19]	European (Denmark)	24	131	0.82 (0.40–1.64)	0.12	11.8	27.7
Rodriguez[20]	Latin American (Mexico)	133	162	2.10 (1.06–4.16)	0.56	31.1	73.7
Correa[21]	Latin American (Colombia)	165	430	1.75 (1.23–2.48)	0.82	46.6	91.2
Cuenca[22]	Latin American (Chile)	92	42	2.72 (0.91–8.15)	0.75	15.0	37.1
Yen[23]	Asian (Taiwan)	97	97	0.10 (0.03–0.33)	0.92	22.2	56.2

* Hardy-Weinberg equilibrium of genotypes of controls. ** Frequency of A = 0.2, α = 0.05. A: disease allele; NS: not significant; NA: not available.

Table 2. Metaanalysis of the TNF-α promoter –308 A/G polymorphism and RA association.

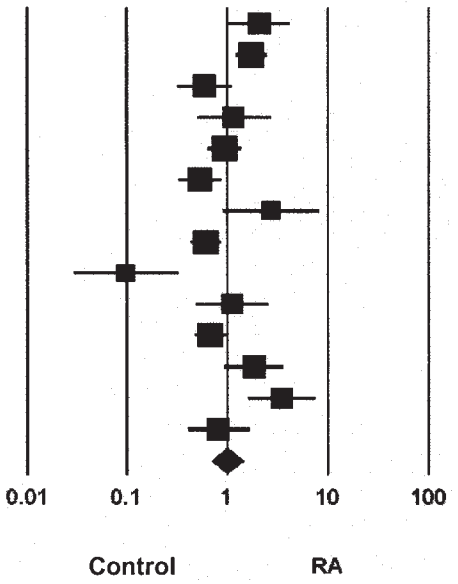
Polymorphism	Population	No of Studies	Test of Association OR (95% CI)	p	Test of Heterogeneity Q	p	I^2
Promoter –318 A vs G	Overall	14	1.005 (0.715–1.412)	0.976	65.473	< 0.0001	80.144
	European	10	0.911 (0.684–1.212)	0.520	28.141	0.001	68.019
	LA	3	2.004 (1.158–3.467)	0.013	0.692	0.707	0
Promoter –318 A/A + A/G vs G/G (recessive)	Overall	12	0.996 (0.653–1.520)	0.985	55.364	< 0.0001	80.131
	European	8	0.890 (0.625–1.267)	0.517	21.053	0.004	66.751
	LA	3	2.028 (1.126–3.652)	0.019	0.433	0.805	0
Promoter –318 A/A vs A/G + G/A	Overall	12	1.025 (0.277–3.787)	0.970	22.081	0.024	50.184
	European	8	0.796 (0.283–2.237)	0.665	17.504	0.014	60.648
	LA	3	2.725 (0.463–16.03)	0.268	0.866	0.648	0
Promoter –318 A/A vs G/G	Overall	12	1.000 (0.405–2.470)	1.000	18.9	0.064	41.7
	European	8	0.823 (0.280–2.419)	0.723	17.4	0.015	59.7
	LA	3	1.650 (0.2623–10.41)	0.594	1.02	0.602	0

LA: Latin American

ly associated with RA in Latin Americans (OR 2.004, 95% CI 1.158–3.467, p = 0.013). Conversely, no association was detected for the TNF-α A allele with RA patients from the European samples (OR 0.911, 95% CI 0.684–1.212, p = 0.520; Figure 1B). The overall OR for the A/A + A/G genotype of the TNF-α promoter –308 was 0.692 in the overall population (95% CI 0.225–2.128, p = 0.520; Table 2). Stratification by ethnicity indicates that the A/A + A/G geno-

A.

Study name	Ethnicity	Odds ratio	Lower limit	Upper limit	Z-Value	p-Value
Rodriguez, 2005	Latin American	2.096	1.056	4.159	2.116	0.034
Correa, 2005	Latin American	1.748	1.231	2.482	3.122	0.002
Pawlik, 2005	European	0.595	0.317	1.115	-1.620	0.105
Balog, 2004	European	1.160	0.500	2.693	0.346	0.730
Miterski, 2004	European	0.951	0.648	1.396	-0.255	0.799
Maury, 2003	European	0.544	0.334	0.885	-2.449	0.014
Cunea, 2003	Latin American	2.716	0.905	8.147	1.783	0.075
Cvetkovic, 2002	European	0.615	0.434	0.873	-2.719	0.007
Yen, 2001	Asian	0.097	0.029	0.326	-3.774	0.000
Lacki, 2000	European	1.119	0.484	2.588	0.263	0.793
van Kugten, 1999	European	0.683	0.481	0.970	-2.131	0.033
Vinasco, 1997	European	1.840	0.945	3.581	1.795	0.073
Danis, 1995	European	3.471	1.602	7.523	3.154	0.002
Fugger, 1989	European	0.816	0.403	1.652	-0.565	0.572
		1.005	0.715	1.412	0.030	0.976



B.

Group by Subgroup within study	Study name	Ethnicity	Odds ratio	Lower limit	Upper limit	Z-Value	p-Value
European	Pawlik, 2005	European	0.595	0.317	1.115	-1.620	0.105
European	Balog, 2004	European	1.160	0.500	2.693	0.346	0.730
European	Miterski, 2004	European	0.951	0.648	1.396	-0.255	0.799
European	Maury, 2003	European	0.544	0.334	0.885	-2.449	0.014
European	Cvetkovic, 2002	European	0.615	0.434	0.873	-2.719	0.007
European	Lacki, 2000	European	1.119	0.484	2.588	0.263	0.793
European	van Kugten, 1999	European	0.683	0.481	0.970	-2.131	0.033
European	Vinasco, 1997	European	1.840	0.945	3.581	1.795	0.073
European	Danis, 1995	European	3.471	1.602	7.523	3.154	0.002
European	Fugger, 1989	European	0.816	0.403	1.652	-0.565	0.572
European			0.911	0.684	1.212	-0.643	0.520
Latin American	Rodriguez, 2005	Latin American	2.096	1.056	4.159	2.116	0.034
Latin American	Correa, 2005	Latin American	1.748	1.231	2.482	3.122	0.002
Latin American	Cunea, 2003	Latin American	2.716	0.905	8.147	1.783	0.075
Latin American			2.004	1.158	3.467	2.485	0.013

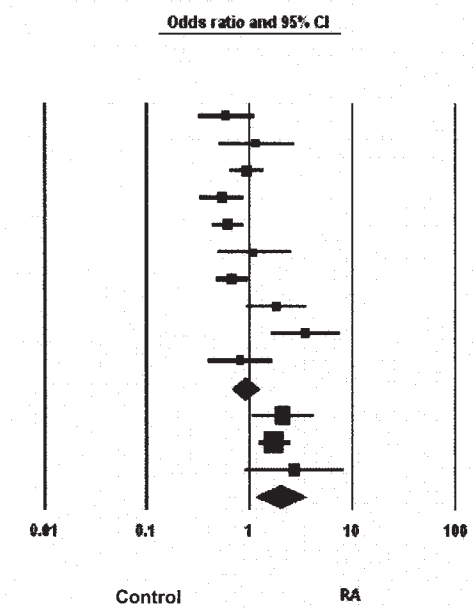


Figure 1. OR and 95% CI of individual studies and pooled data for the association of TNF-α –308 risk allele A and RA in overall (A) and subgroup populations (B) from Europe and Latin America. The TNF-α allele A was a risk factor for RA in Latin Americans, but not in Europeans.

type was a risk factor for RA in Latin Americans (OR 2.028, 95% CI 1.126–3.652, p = 0.019) but not in Europeans (OR 0.890, 95% CI 0.625–1.267, p = 0.517). There was no heterogeneity among studies in metaanalyses for Latin Americans (I^2 = 0, p values = 0.602 to 0.805), but there was heterogeneity among studies in the European populations and overall (Table 2). The significance of association in Latin Americans remained after correction for multiple testing.

The OR for the A/A genotype and the A/A vs G/G genotype overall and in each ethnic group showed a trend similar to those for the A allele and the A/A and A/G genotype, but they did not reach statistical significance (Table 2).

Evaluation of TNF-α promoter –238 A/G polymorphism and association with RA. Six studies consisting of 3 European[16,17,39], 2 Latin American[20,21], and one Asian[23] were included in this metaanalysis. No association between RA and the TNF-α –238 A allele was found in the overall population (OR 0.560, 95% CI 0.236–1.196, p = 0.134). However, stratification by ethnicity indicated that the TNF-α –238 A allele was significantly associated with RA in Latin Americans (OR 0.189, 95% CI 0.058–0.614, p = 0.006). TNF-α –238 A allele was protective for RA in Latin Americans. Conversely, no association was detected for the TNF-α –238 A allele with RA patients from the European samples (OR 0.785, 95% CI 0.380–1.622, p = 0.513).

DISCUSSION

TNF-α is located within the HLA class III region in chromosome 6p21.3, which is known as the strongest linkage locus for RA[4], and TNF-α –308 A/G polymorphism has been reported to be associated with several autoimmune disorders including RA[40,41]. The reports comparing TNF production from cells of individuals bearing the TNF-α –308 A allele versus individuals with other alleles have generated conflicting results[42,43]. And studies of an association of TNF-α –308A/G polymorphism with RA have also reported conflicting results[10-23]. This discrepancy among results is not surprising. Persistent difficulties in obtaining robust and replicable results in genetic association studies are almost certainly encountered because genetic effects are small, requiring studies with many thousands of subjects or metaanalysis to be detected.

Our metaanalysis showed no significant RA susceptibility from the TNF-α –308 promoter A/G polymorphism in the overall population samples. Subsequently, a metaanalysis after stratification by ethnicity detected a significant association with the TNF-α polymorphism in the Latin American-derived samples, but not in European samples. The available data support TNF-α –308 A/G polymorphism being an ethnic population-specific risk factor for RA, although the small number of Latin American-derived studies available to date reduces our confidence in this conclusion. The most powerful study to date came from Latin America[21], and 3 studies from Latin America have shown the same direction of the association; and there was no heterogeneity among the studies, suggesting strong association of the TNF-α –308 A/G polymorphism with RA in this ethnic population.

Metaanalysis of the A allele and A/A +A/G genotype revealed a significant association with RA in Latin American data, but metaanalyses of the A/A genotype and the A/A vs G/G genotype showed no significant association in the same groups. The OR of the A/A genotype and the A/A vs G/G genotype increased in Latin American data, although they did not reach statistical significance. This finding may be explained due to low statistical power based on the rare frequency of the A/A genotype. The metaanalyses consistently showed no heterogeneity among studies from Latin America. Taken together, these findings suggest that TNF-α –308 A/G polymorphism may confer a risk for RA in Latin Americans. Since only 3 studies were included, this result must be interpreted with caution. More Latin American studies should be recruited to clarify this possible association.

Interestingly, the metaanalysis did not reveal any association of the TNF-α –308 A/G polymorphism with RA in Europeans. The finding of different associations according to ethnicity is somewhat surprising. An explanation is needed to explain why the TNF-α –308 A/G polymorphism is not associated with RA in European populations and plays a different role in different ethnic populations. First, genetic heterogeneity for RA may exist in different populations. Whole-genome linkage studies on RA have shown genetic heterogeneity[4]. Second, clinical heterogeneity may explain the discrepancy. The contribution of differences in patient populations may cause different results. Third, different linkage disequilibrium (LD) patterns may contribute to the discrepancy. This polymorphism may be in LD with a nearby causal variant in one ethnic group, but not in another. The TNF-α –308 A allele associated with RA in Mexicans and Colombians was HLA-DRB1-independent, since no LD was found between TNF and HLA-DRB1 loci[20,21]. Fourth, the difference might arise from chance, such as type I error, or because of multiple testing that inflates the type I error. Fifth, TNF polymorphisms may occur with distinct patterns in ethnically distinct populations[44].

The region spanning the TNF cluster has been implicated in susceptibility to numerous immunopathological diseases, including RA. The class III region of HLA including TNF-α lies between the class I and II regions and contains genes important for the innate immune system, including the complement components C2 and C4[45]. Possible LD across the HLA region has hampered the identification of the precise genes involved.

Some limitations should be discussed in this metaanalysis. First, publication bias and heterogeneity may be present, distorting the metaanalysis. We could not construct a funnel plot for each metaanalysis due to small numbers of studies, and there was significant heterogeneity among studies in persons of European descent. Second, the numbers of subjects and studies included in the ethnicity-specific metaanalysis were small. There were only 3 studies for Latin Americans. This

study may not have enough power to expose an association between TNF-α –308 A/G polymorphism and RA in each ethnic group. Third, although the available genetic data may implicate the TNF-α –308 A/G polymorphism as a determinant of RA susceptibility in Latin Americans, studies from other ethnic groups are needed to see if the TNF-α –308 A/G polymorphism confers a risk for RA in other populations. We could not do metaanalyses in Asian ethnic groups due to no or a small number of studies. Further studies on the association of the TNF-α –308 A/G polymorphism and RA are needed in other ethnic populations.

Our metaanalysis demonstrates that the TNF-α –308 A/G polymorphism may represent a significant risk factor for RA in Latin Americans, but it is not likely to confer susceptibility to RA in Europeans. More studies are needed to clarify its role in RA in various ethnic groups.

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