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ARTICLE

Meta-analysis of TNF-α promoter −308 A/G polymorphism and SLE susceptibility

Young Ho Lee[1,2], John B Harley[2,3,4,5] and Swapan K Nath*[,2,3,4]

[1]*Division of Rheumatology, Korea University, Seoul, Korea;* [2]*Arthritis and Immunology Research Program, Oklahoma Medical Research Foundation, Oklahoma City, OK, USA;* [3]*Genetic Epidemiology Unit, Oklahoma Medical Research Foundation, Oklahoma City, OK, USA;* [4]*University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA;* [5]*US Department of Veterans Affairs Medical Center, Oklahoma City, OK, USA*

Alleles of tumor necrosis factor-α (TNF-α) gene have been inconsistently associated with systemic lupus erythematosus (SLE), particularly the 308-A/G functional promoter polymorphism. To generate large-scale evidence on whether 308-A/G promoter polymorphism is associated with SLE susceptibility we have conducted a meta-analysis. We have identified 21 studies of this polymorphism and SLE using MEDLINE search. Meta-analysis was performed for genotypes A/A (recessive effect), A/A + A/G (dominant effect), and A allele in fixed or random effects models. All control samples were in Hardy–Weinberg proportion. The overall odds ratio (OR) of the A/A genotype was 3.2 (95% CI = 2.0–5.3, $P<0.001$). Stratification by ethnicity indicated that the A/A genotype was associated with SLE in European-derived population (OR = 4.0, CI = 2.5–6.4, $P<0.001$). No association was detected in Asian-derived population (OR, 1.3, CI = 0.3–6.3, $P=0.76$). The overall OR for the risk genotypes (A/A and A/G) was 2.0 (CI = 1.3–3.1, $P<0.001$). Similar results were found between the risk allele A and SLE where a significant association was found in European population (OR = 2.1, CI = 1.6–2.7, $P<0.001$), but not in Asian (OR = 1.4, CI = 0.8–2.3, $P=0.2$) or African (OR = 1.2, CI = 0.6–2.5, $P=0.59$) populations. In summary, this meta-analysis demonstrates that the TNF-α promoter −308 A/G polymorphism may confer susceptibility to SLE, especially in European-derived population.

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Introduction

Systemic lupus erythematosus (SLE) is a disorder of generalized autoimmunity, characterized by multisystemic organ involvement, polyclonal B-cell activation and the production of autoantibodies. Significant familial aggregation, convincing demonstration of multiple genetic linkage and associations with SLE demonstrate an underlying genetic basis of the disease.[1,2] However, many genes probably interact with each other and with the environment in a multifaceted way, making identification of any gene difficult.[3] Genes involved in potentially disease generating biological mechanisms are ideal candidates for genetic etiological involvement.

Multiple genetic associations have been identified, suggesting involvement of major histocompatibility complex (MHC) in susceptibility to SLE. Tumor necrosis factor-α (TNF-α) gene is located on chromosome 6, within the class III region of MHC.[4] TNF-α is a potential pro-inflammatory cytokine that plays an important role in inflammatory and immune responses.[5] TNF-α stimulates cytokine production, enhancing expression of adhesion

*Correspondence: Dr SK Nath, Arthritis and Immunology Research Program, Oklahoma Medical Research Foundation, 825 N.E. 13th Street, Oklahoma City, OK 73104, USA. Tel: +1 405 271 7765;
Fax: +1 405 271 4110;
E-mail: swapan-nath@omrf.ouhsc.edu
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molecules and neutrophil activation and acts as a co-stimulator for T-cell activation and antibody production. It is unclear as to whether the role of TNF-α as a mediator of inflammation is a beneficial or deleterious one in the susceptibility to SLE. Involvement in SLE has been suggested by findings from the experiments on human SLE and animal models. For example, TNF-α levels are increased in SLE patients compared with controls and strongly correlate with SLE activity.[6] The minor allele at −308 base pairs from the transcription initiation site in the promoter of TNF-α (TNF-α −308 A or TNF-α2) has been associated with production of TNF-α and susceptibility to SLE.[7–13] However, many studies have shown inconclusive or contradictory results. This inconsistency may be due to studies with inadequate statistical power, racial and ethnic differences, publication bias, or uncorrected multiple hypothesis testing. Although it is unclear whether the TNF-α promoter −308 A/G polymorphism has a functional significance, several evidences suggest that there may be a small but significant effect of the TNF-α promoter −308 A/G polymorphism, with the A allele being associated with slightly greater levels of TNF-α transcription.[14]

Meta-analysis is a statistical procedure for combining results from several studies to produce a single estimate of the major effect with enhanced precision.[15] One of the major advantages of meta-analysis is to increase sample size, which may reduce the probability that random error will produce false-positive or false-negative associations. Therefore, meta-analysis is an ideal and powerful tool for summarizing the inconsistent results from different studies. The aim of the present study was to investigate whether the TNF-α promoter −308 A/G polymorphism is a risk factor to the SLE susceptibility, using a meta-analysis.

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 SLE. A search of the literature was made using MEDLINE citation to identify available articles in which the TNF-α promoter −308 A/G polymorphism was determined in SLE patients and control (most recent one was May 2005). References in the studies were reviewed to identify additional studies not indexed by MEDLINE. 'Tumor necrosis factor', 'TNF-α', 'polymorphism', SLE, and 'systemic lupus erythematosus' were entered as both medical subject heading (MeSH) terms and text words. No language restrictions were applied. A study was included in the analysis if (1) it was published up to March 2004, (2) it was original data (independence among studies), and (3) it provided enough data to calculate odds ratio (OR). When a study reported the results on different subpopulation, we treated them as a separate study in the meta-analysis. 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, we extracted the available genotype and allele frequency information from the TNF-α promoter −308 A/G polymorphism.

Evaluation of publication bias

A funnel plot has been used in order to assess the publication bias.[16] However, the funnel plot is no longer considered strictly as a test of publication bias. All studies were of relatively small sample size with no large differences in the standard errors of the lnOR estimates, therefore it would not make much sense to examine whether larger studies differed from smaller ones.

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 χ^2-test was used to determine if observed frequencies of genotypes conformed to Hardy–Weinberg (H–W) expectations. We also tested for H–W proportion separately for each race-specific pools.

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. The point estimates of the risk, the OR, and its 95% confidence interval (CI) were estimated for each study. We assessed the within- and between-study variation or heterogeneity by testing Cochran's *Q*-statistic.[17] This heterogeneity test assessed the null hypothesis that all studies were evaluating the same effect. A significant *Q*-statistic ($P<0.10$) indicated heterogeneity across studies, and then the random effect model was used for meta-analysis. Otherwise, the fixed effect model was used. Fixed effect model assumes all of the studies are estimating the same underlying effect and considers only within-study variation. We also quantified the effect of heterogeneity using a recently developed measure, $I^2 = 100\% \times (Q-\mathrm{df})/Q$.[18] The I^2-statistic measures the degree of inconsistency in the studies by calculating what percentage of the total variation across studies is due to heterogeneity rather than by chance.

Finally, the overall or pooled estimate of risk (OR) was obtained by using Peto's method in the fixed effect model[19] and by DerSimonian and Laid method in the random effect model.[20] Pooled OR in the meta-analysis was performed weighting individual OR by the inverse of their variance. We also 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 SLE 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 was estimated on the basis of the method described earlier.[21] Statistical manipulations for the meta-analysis were undertaken using program EasyMA (http://www.spc.univ-lyon1.fr/easyma/download.htm).

Results

Studies included in the meta-analysis

The literature search identified 59 potentially relevant publications. Among them, 40 studies were excluded because they did not meet the inclusion criteria. They were studies on other TNF polymorphism such as TNF receptor genes (19 publications), studies on other diseases (16 publications) or animal studies (four publications) or family study (one publication). There were no studies in which the number of null and wild genotypes could not be ascertained. A total of 19 relevant studies with TNF-α promoter −308 A/G polymorphism and SLE were selected for the meta-analysis.[7,8,10–12,22–35] Among them, two of the eligible studies contained data on two different ethnic groups[30,31] and we treated them independently. Therefore, a total of 21 separate comparisons were considered in our meta-analysis. These 21 studies consisted of 10 European, seven Asian, three African and one Mexican population samples (Table 1). Although the allele frequency of the TNF-α promoter −308 A/G polymorphism was extracted from 21 studies, the genotype frequency was available from 13 studies. Therefore, the meta-analysis was performed with 13 studies overall and 11 (seven European and four Asian) when divided by ethnical origin for genotype-based analysis. Because of the inadequate sample populations available for Mexican groups, we have performed group-specific meta-analysis in European, Asian and African-derived populations. As the genotype data on TNF-α −308 A/G polymorphism was available for one of three studies in African population,[30] meta-analysis was performed only on A allele of TNF-α −308 in African population.

Evaluation of study quality

The distribution of the genotype in control group in Europeans, Asians and Africans was consistent with H–W equilibrium (European, $P=0.59$; Asian, $P=0.76$; African, $P=0.55$).

Evaluation of A/G polymorphism and SLE association

Selected characteristics of 21 case–control studies for TNF-α −308-A/G polymorphism and the risk of SLE are summarized in Table 1. Also, Table 1 shows the expected power of each individual study to demonstrate an association between this polymorphism and SLE. Interestingly, none of the 21 individual studies demonstrated a reasonable statistical power to detect the association (Table 1). The ORs with 95% CI of individual studies for the association of the A allele at the TNF-α locus and SLE are

Table 1 Characteristics of individual studies included in meta-analysis

		Subject numbers (frequency of A allele, %)					*Expected power (%) (Frequency. of A allele = 0.2, α = 0.05)*	
Study	*Population*	*Control*	*SLE*	*OR for A versus G allele*	*95% CI*	*HWE*	*OR = 1.5*	*OR = 2.0*
Parks *et al*[30]	European	203 (17)	86 (28)	2.0	1.3–3.0	0.44	26.6	65.5
May *et al*[29]	European	57 (12)	47 (28)	2.7	1.3–5.6	0.33	13.8	33.8
Van der Linden *et al*[11]	European	253 (13)	91 (29)	2.6	1.7–4.0	0.22	28.9	70.0
Rood *et al*[8]	European	177 (12)	99 (35)	4.1	2.6–6.3	0.65	27.7	67.7
Rudwaleit *et al*[31]	European	96 (16)	49 (26)	1.8	1.0–3.2	NA	16.3	40.9
D'Alfonso *et al*[25]	European	174 (14)	123 (15)	1.1	0.7–1.7	NA	30.8	73.2
Danis *et al*[7]	European	57 (11)	40 (30)	3.3	1.6–7.0	0.33	13.0	31.3
Wilson *et al*[13]	European	168 (17)	81 (24)	1.6	1.0–2.5	NA	24.5	61.2
Goldstein *et al*[28]	European	91 (32)	91 (41)	1.4	0.9–2.2	0.83	21.1	53.5
Fugger *et al*[27]	European	131 (29)	20 (48)	2.2	1.1–4.4	0.20	10.7	24.4
Azizah *et al*[23]	Asian	59 (20)	70 (37)	1.6	0.9–2.9	NA	16.1	40.4
Wang *et al*[33]	Asian	70 (33)	89 (56)	2.6	1.6–4.1	0.17	18.8	47.6
Wang *et al*[12]	Asian	187 (8)	51 (7)	0.9	0.4–2.1	0.25	19.1	48.5
Chen *et al*[24]	Asian	107 (14)	100 (10)	0.6	0.4–1.2	0.93	23.4	58.8
Fong *et al*[26]	Asian	89 (13)	67 (19)	1.5	0.8–2.9	NA	18.4	46.7
Tomita *et al*[10]	Asian	23 (22)	20 (45)	2.9	1.2–7.5	NA	8.1	16.7
Atsumi *et al*[22]	Asian	20 (43)	74 (31)	0.6	0.3–1.3	0.59	10.0	22.5
Parks *et al*[30]	African	73 (15)	144 (14)	0.9	0.5–1.6	0.55	22.2	56.1
Sullivan *et al*[32]	African	88 (8)	64 (19)	2.7	1.3–5.8	NA	18.0	45.6
Rudwaleit *et al*[31]	African	81 (18)	49 (14)	0.8	0.4–1.5	NA	15.6	38.9
Zuniga *et al*[35]	Mexican	55 (3)	51 (8)	3.0	0.8–11.8	0.84	14.1	34.5

A = Disease allele; NS = not significant; NA = not available; OR = odds ratio; CI = confidence interval; HWE = Hardy–Weinberg equilibrium of genotypes of controls.

Table 2 Meta-analysis of the TNF-α promoter −308 A/G polymorphism and SLE association

		Sample Size			Test of association			Test of heterogeneity			
Polymorphism	*Population*	*SLE*	*Control*	*No. of Studies*	*OR*	*95% CI*	*P-value*	*Model*	*Q*	*P-value*	I^2
Promoter −318 A allele	Overall	3060	4479	21	1.7	1.4–2.2	<0.001	R	69.9	<0.001	71.4
	European	1454	2814	10	2.1	1.6–2.7	<0.001	R	27.2	0.001	66.9
	Asian	942	1110	7	1.4	0.8–2.3	0.20	R	24.2	<0.001	75.2
	African	562	436	3	1.2	0.6–2.5	0.59	R	71.4	0.030	71.4
Promoter −318 A/A* (recessive)	Overall	932	1294	13	3.2	2.0–5.3	<0.001	R	21.9	0.025	45.2
	European	474	969	7	4.0	2.5–6.4	<0.001	R	9.8	0.136	38.8
	Asian	263	197	4	1.3	0.3–6.3	0.76	R	7.5	0.023	73.3
Promoter −318 A/A + A/G* (dominant)	Overall	983	1481	13	2.0	1.3–3.1	<0.001	R	53.4	<0.001	77.5
	European	474	969	7	2.9	2.0–4.2	<0.001	R	14.3	0.027	58.0
	Asian	314	384	4	1.3	0.5–3.1	0.44	R	16.1	<0.001	81.4
Promoter −318 A/A *vs* G/G	Overall	623	1081	13	4.3	2.3–7.9	<0.001	R	30.3	0.001	63.7
	European	251	620	7	5.4	2.6–11.4	<0.001	R	13.1	0.02	61.8
	Asian	201	285	4	1.6	0.17–14.3	0.70	R	11.3	0.01	73.5
Promoter −318 A/G *vs* G/G	Overall	889	1437	13	1.8	1.2–2.7	0.003	R	43.6	<0.001	72.5
	European	465	991	7	2.5	1.7–3.6	<0.001	R	14.1	0.05	50.4
	Asian	285	374	4	1.2	0.6–2.7	0.59	R	10.9	0.01	72.5

R = Random model.
*Genotype-based meta-analysis was not performed on the Africans, as the genotypes data was available for only one study.

shown in Table 1. The summary of meta-analysis for the TNF-α promoter −308 polymorphism with SLE is shown (Table 2). The *Q*-test of heterogeneity was almost always significant and we conducted analyses using random effect models except in one case which was in a subgroup analysis and the *Q*-test was likely to have been not statistically significant owing to lack of power. So we also performed the subgroup analysis using random effect model.

An association between SLE and A/A risk genotype (assuming A allele as recessive allele) was found in the overall population (OR = 3.2, 95% CI = 2.0–5.3, $P<0.001$) (Figure 1). However, stratification by ethnicity indicates that the A/A genotype is significantly associated with SLE in Europeans (OR = 4.0, 95% CI = 2.5–6.4, $P<0.001$). Conversely, there was no association detected for the A/A genotype with SLE patients from the Asian samples (OR = 1.3, 95% CI = 0.3–6.3, $P=0.76$). Genotype data on TNF-α −308 A/G polymorphism was available for one of three studies in African population,[30] therefore, genotype-specific meta-analysis was not performed in African samples. Assuming A allele as dominant allele, the overall OR for the combined A/A + A/G genotypes was 2.0 (95% CI = 1.3–3.1, $P<0.001$). Similarly, using ethnic-specific analysis, OR was increased significantly in the European samples (OR = 2.9, 95% CI = 2.0–4.2, $P<0.001$), but not in Asians (OR = 1.3, 95% CI = 0.5–3.1, $P=0.44$). The overall OR for the A allele of the TNF-α promoter −308 was 1.7 (95% CI = 1.4–2.2, $P<0.001$) (Table 2 and Figure 1). Stratification by ethnicity indicates that the A allele is a risk factor for SLE in European (OR = 2.1, 95% CI = 1.6–2.7, $P<0.001$) but not in Asian (OR = 1.4, 95% CI = 0.8–2.3, $P=0.20$) or African (OR = 1.2, 95% CI = 0.6–2.5, $P=0.59$) (Figure 2). We also performed a 'model-free' analysis by considering the G/G genotype as the reference and estimated the OR for the A/A *versus* G/G and A/G *versus* G/G genotype. We found gene dosage effect of the A allele (Table 2).

Overall, the meta-analysis of the TNF-α −308 A/A genotype (recessive effect), A/A + A/G genotype (dominant effect), and the risk allele A was associated with susceptibility of SLE in Europeans.

Discussion

TNF-α is an inducible cytokine with a broad range of pro-inflammatory and immuno-stimulatory actions.[5] The importance of TNF-α in the pathogenesis of SLE is unclear but even minor differences in TNF-α production can influence upon the resulting autoimmune disease. In mice, lupus-prone strains have been shown to have different phenotypes. The (NZB X NZW) F1 strain of genetic murine lupus produces relatively low level of TNF-α. Therapy with recombinant TNF-α caused a significant delay in the onset of nephritis and an improved survival rate.[36] In contrast, the MRL-lpr/lpr and BXSB strains of murine lupus produce relatively high TNF-α levels.[37] The increase of TNF-α in SLE and the development of antinuclear antibodies and anti-DNA antibodies, and an SLE-like illness in some patients treated with TNF antagonists lead some to suspect that the TNF-α axis may be important in susceptibility.[38,39]

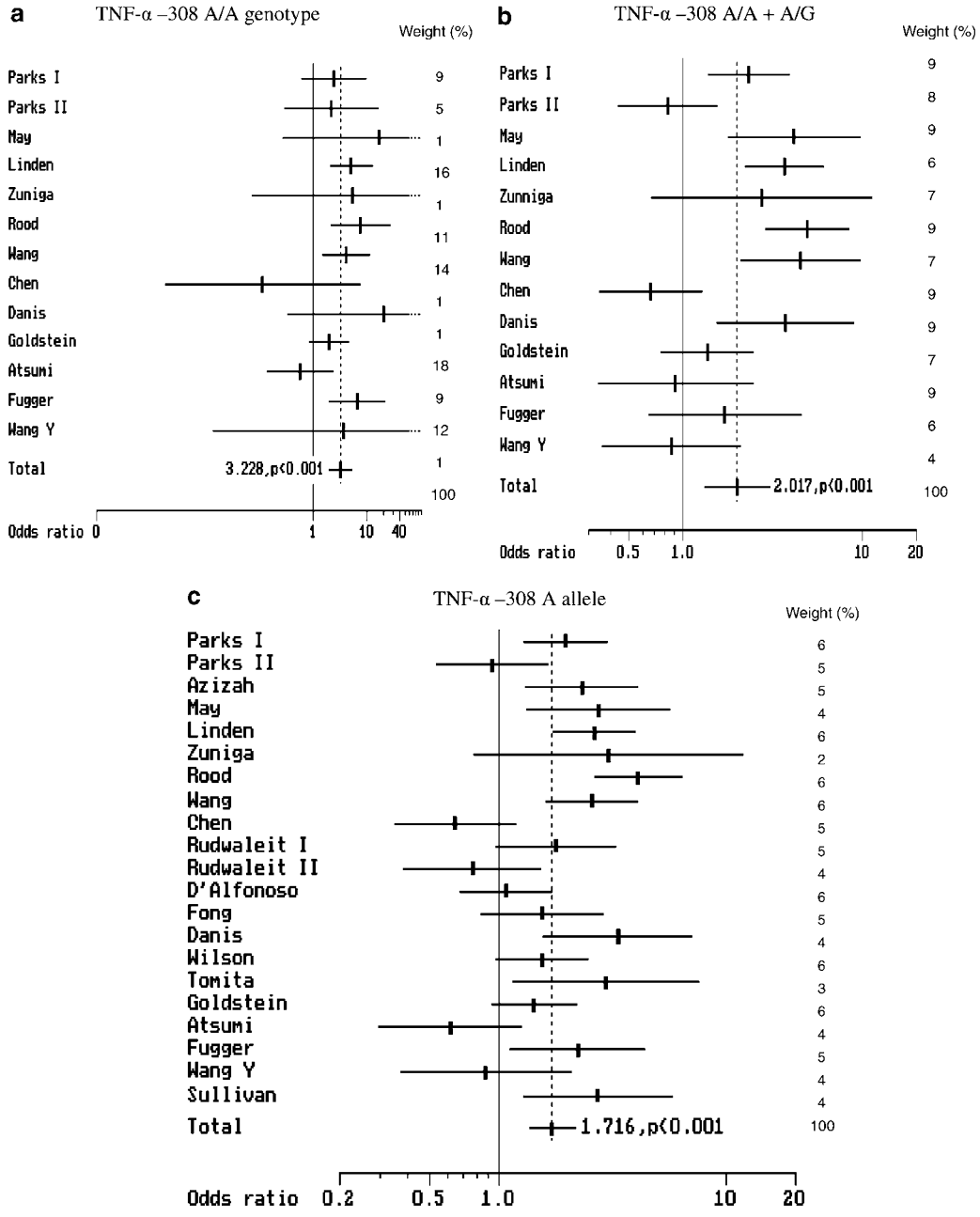

Figure 1 ORs and 95% CI of individual studies and pooled data for the association of the TNF-α −308 A/G polymorphism and SLE with (**a**) risk genotype A/A, (**b**) genotype A/A + A/G, and (**c**) A allele.

TNF-α is located within the MHC class III region in chromosome 6p21.3. The TNF-α A/G polymorphism has been reported to be associated with several autoimmune disorders including SLE.[7,27,40] However, studies of an association of TNF-α A/G polymorphism with SLE have reported conflicting results (Table 1). The most obvious explanation is that individual studies have not had sufficient statistical power to detect the small differences between cases and controls, and that is reflected from our power analysis. None of the 21 individual studies has shown a statistical power greater than 80% to detect the association between TNF-α −308 A/G polymorphism and SLE.

Meta-analysis is a method to integrate previous research, providing increased statistical power and resolution by combining the results of independent analyses. Meta-analysis is a promising method to overcome the problem of small sample size and inadequate statistical power of

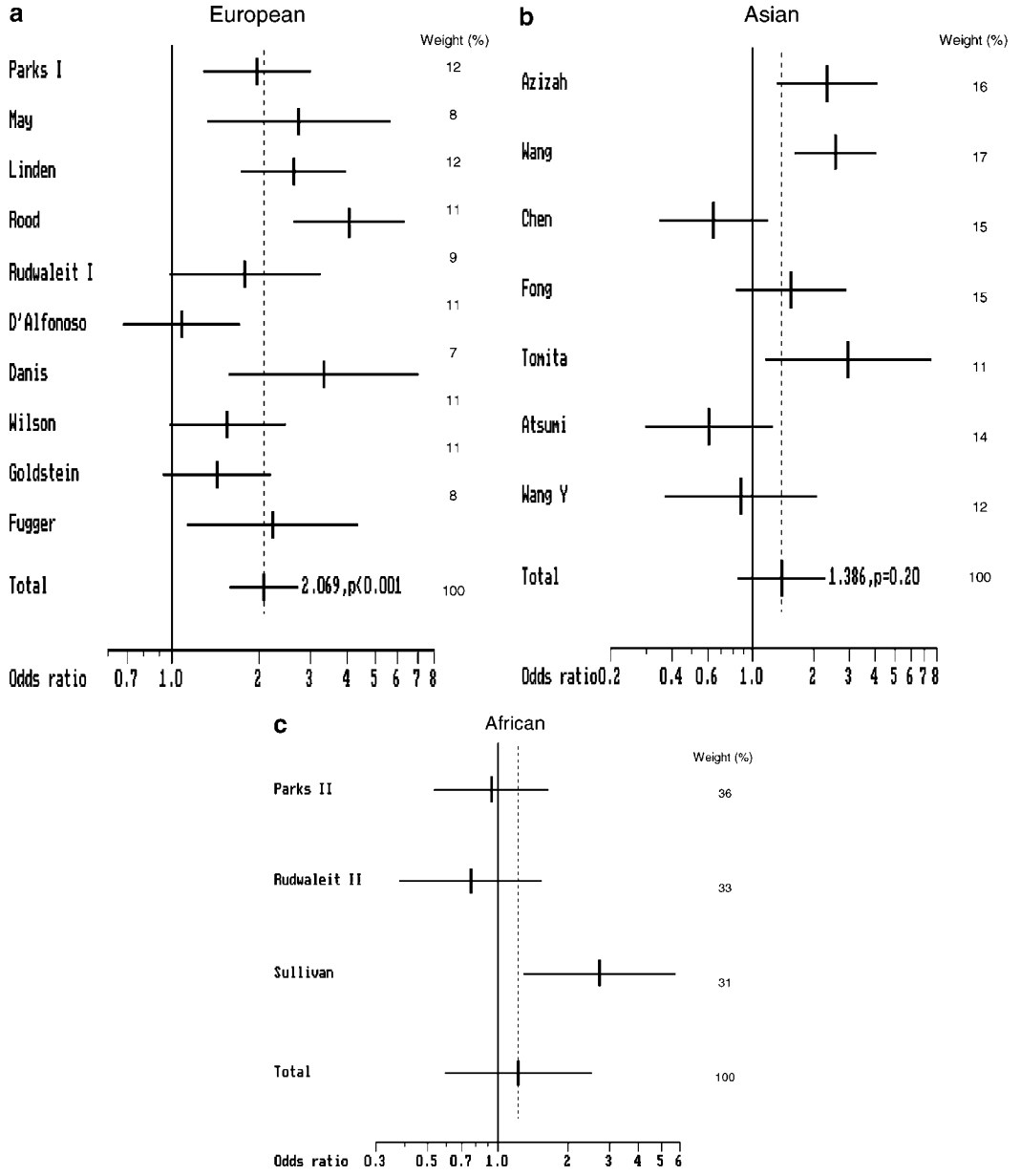

Figure 2 OR and 95% CI of individual studies and pooled data for the association of the TNF-α −308 risk allele A with SLE in (**a**) European, (**b**) Asian and (**c**) African populations. The A allele is a risk factor for SLE in European (OR = 2.1, 95% CI = 1.6–2.7, $P < 0.001$) but not in Asian (OR = 1.4, 95% CI = 0.8–2.3, $P = 0.20$) or African (OR = 1.2, 95% CI = 0.6–2.5, $P = 0.59$).

genetic studies of complex traits. Our meta-analysis showed significant susceptibility from this TNF-α −308 promoter G/A polymorphism in the overall population samples. Subsequently, a meta-analysis after stratification by ethnicity detected a significant association with the TNF-α polymorphism in the European-derived samples, but not in Asian-derived or African-derived samples according to the allele-based comparisons of the 21 studies. The available data support TNF-α −308 A/G polymorphism being an ethnic population-specific risk factor for SLE, although the small number of Asian-derived and African-derived studies available to date reduces our confidence in this conclusion. The finding of the different association according to ethnicity is somewhat surprising. It needs to be explained why the TNF-α promoter −308 A allele is not associated with SLE in non-European populations, especially Asian population. Ioannidis *et al* revealed that the frequencies of the genetic marker of interest in the control

population (58%) often showed large heterogeneity between races. Conversely, they saw large heterogeneity in the genetic effects between races in only 14% of cases.[41] Our finding might be partially explained by ethnic difference. The allele-based comparisons show results that have absolutely no racial heterogeneity and the 95% CIs amply overlap. I^2 for the between-racial descent heterogeneity were quite small and nowhere near statistical difference (data not shown).

The present study may have three caveats. First, cases and controls in the studies contributing to this meta-analysis were not age- and sex-matched, with the exception of two studies.[10,34] Provided that the ethnic background is similar among patients and controls and the frequency of the polymorphism does not change with age among controls, the lack of matching by age should not introduce bias. Second, our study identified a stronger association between the disease causing allele or carrier genotypes and SLE, especially in the patients with the European-derived population. However, the lack of association in the Asian-derived and African-derived populations from this study might not be very conclusive owing to the relatively small number of Asian-derived and African-derived populations used in the analysis. Third, 10 European studies found a formally statistically significant effect on their own, although none of the studies had enough power to detect an effect that would be plausible. This may be indirect evidence that some selective reporting bias is operating here, and it may be quite substantial.

There has been controversy whether the TNF-α promoter −308 A/G polymorphism has a functional significance. The construct with TNF-α promoter containing the −308 A allele produced up to six times more mRNA than that with −308 G allele,[40] though this has not been a consistent findings.[42,43] Five of six previous studies observed higher levels of production of TNF-α by cells of −308 A/G individuals than cells of −308 G/G individuals. The degree of increase was generally approximately 20–40% and whether or not the result was considered to be significant appeared to be related to the number of individuals tested,[14] suggesting that this polymorphism may be associated with a small but significant increase in gene transcription. Our findings are likely to favor the functional studies that the A allele of the TNF-α −308 is susceptible to autoimmune diseases including SLE. Our results seem to support the idea that TNF-α may be detrimental in SLE, by both inducing inflammation and apoptosis, which could fuel the autoimmune response.

The region spanning the TNF cluster has been implicated in susceptibility to numerous immunopathological diseases, including SLE. Recently, using the ancestral recombinants information, Graham *et al*[44] demonstrated that the disease-associated haplotypes in HLA class II region containing DRB1 and DQB1 alleles were strong risk factors for human SLE. However, possible linkage disequilibrium across the HLA region has hampered the identification of the precise genes involved. 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. The TNF-α promoter −308 A allele and HLA_DR alleles contributed independently to susceptibility to SLE in South African patients,[33] whereas the TNF-α A allele was in linkage disequilibrium with the HLA-DR3 allele in Caucasian SLE patients.[13] Therefore, whether TNF-α alone and/or in combination with the other genes in this region is conferring the susceptibility to lupus is unknown, and this is beyond the scope of the present study.

Previous two meta-analyses of FcγRIIa and FcRγIIa in SLE have shown a modest relative risk for SLE.[45,46] A meta-analysis of 17 studies has concluded that the low-binding R131 allele confers a 1.3-fold risk for developing SLE[45] and a meta-analysis of more than 1000 subjects has concluded that the F158 allele confers a 1.2-fold risk for developing lupus nephritis.[46] Taken together, it is suggested that multiple susceptibility genes with small or modest effect contribute to the development of SLE.

In conclusion, combined results of independent association studies by meta-analysis showed significant association between disease susceptible allele 'A', A/A genotype and A/A + A/G genotype of the TNF-α −308 A/G polymorphism and SLE. The race-specific meta-analysis demonstrates a significant statistical association with European-derived samples but not with Asian or African-derived samples. Therefore, our meta-analysis demonstrates that the TNF-α −308 A/G polymorphism may be a risk factor for SLE, especially in those with European ancestry.

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