

Association between Tumor Necrosis Factor- α -308 G/A Polymorphism and Multiple Sclerosis: A Systematic Review and Meta-Analysis

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Abstract

Multiple sclerosis (MS) is a complex polygenic disease in which gene-environment interactions are important. A number of studies have investigated the association between tumor necrosis factor- α (TNF- α) -308 G/A polymorphism (substitution G \rightarrow A, designated as TNF1 and TNF2) and MS susceptibility in different populations, but the results of individual studies have been inconsistent. Therefore, performing a systematic review and meta-analysis of the published studies is desirable. We sought to quantitatively summarize the association between TNF- α -308 G/A polymorphism and MS. The Medline and Scopus databases were searched to identify potentially relevant case-control studies published in English journals up to January 2010. A meta-analysis of these studies was performed. Summary odds ratios (ORs) and 95% confidence intervals (CIs) were calculated under fixed and random effects models. Twenty-one eligible studies, comprising 2880 patients with MS and 3579 controls, were included in the meta-analysis. The overall pooled ORs (95%CI) for TNF2 versus TNF1 and TNF2 carriers (2/2+2/1) versus non-carriers (1/1) were 1.02 (0.86-1.21) and 0.99 (0.8-1.24), respectively. In the European populations, the pooled ORs (95%CI) for TNF 2/1 versus 1/1 were 0.85 (0.73-0.98), which was statistically significant. However, the other results did not support this finding. The pooled ORs (95%CI) for TNF 2/1 versus 1/1 and TNF 2/2 versus 2/1 were not statistically significant in the overall population. In addition, the pooled ORs for TNF2/2 versus TNF2/1+1/1 and TNF2/2 versus TNF1/1 were not statistically significant. Our meta-analysis does not support the role of TNF- α -308 G/A polymorphism in developing MS.

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Introduction

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system. The etiology of MS is complicated, and both environmental and genetic factors play important roles in the pathogenesis of the disease.^{1,2} Several genes have been investigated, but studies on association with human leukocyte antigen (HLA) have been the most consistent.²⁻⁴ Tumor necrosis factor- α (TNF- α) is a

pro-inflammatory cytokine, secreted by activated macrophages, with a wide range of biological activities, including induction of tumor regression, fever, cachexia, shock, and cellular immune responses.⁵ High levels of TNF- α have been found in the blood and cerebrospinal fluid (CSF) of MS patients.⁶ TNF- α gene is located on chromosome 6, within the class III region of HLA.⁷ A single-nucleotide polymorphism (SNP) at position -308 in the TNF- α gene promoter, defined as TNF1 (-308G) and TNF2 (-308A), has been identified,^{8,9} in which the less common TNF2 allele is associated with a high production of TNF- α .^{10,11}

A large number of case-control studies have been conducted to investigate the association between TNF- α -308 G/A polymorphism and MS in different populations. However, the results of the individual studies are conflicting, inconsistent, and inconclusive.¹²⁻³² Because of small sample sizes in most of these studies, they lacked enough power to detect the probable relationship between this SNP and MS. Since no quantitative summarization of evidence has been performed to date and in order to do a well-powered study in this regard, we conducted a systematic review to find all relevant published studies and performed a meta-analysis to quantitatively summarize the evidence for such a relationship.

Methods

Search Strategy

The Medline (using PubMed) and Scopus databases (last updated search being 1 January 2010) were searched to identify potentially relevant case-control studies. The following keywords were used: polymorphism; multiple sclerosis; and tumor necrosis factor. To find any additional published studies not found by computer search, the reference lists of review articles and all retrieved articles were searched manually at the same time. If more than one article was published by the same author(s) using the same participants, the study that comprised the most individuals or/and had more complementary information was selected. When the written information was insufficient, efforts were made to contact the investigators so as to obtain the needed information. If a reply was not forthcoming or when the contact was impossible, the study was excluded from the meta-analysis.

The title and abstract of all potentially relevant articles were reviewed to determine their relevance. Additionally, full articles were reviewed if the title and abstract were ambiguous. All the searches were conducted independently by three reviewers and disagreements about the inclusion

of a study were resolved by consensus.

Inclusion and Exclusion Criteria

The following criteria were used to include studies in the meta-analysis: the study design had to be case-control; the outcome had to be MS; there had to be at least two comparison groups (MS vs. control groups); the number of MS cases and controls and also the frequency of genotypes in both groups had to be identified; and participants could be of any age. English articles and also articles of other languages which had English abstracts with sufficient information (one article) were included in the meta-analysis. Major reasons for the exclusion of studies were: 1) lack of a control group; 2) basing the design on family or sibling pairs; 3) duplication; and 4) not reporting usable data (despite correspondence with authors).

Assessment of Study Quality

Quality assessment was conducted by two investigators using the Little criteria³³ for genetic studies and the Lichtenstein criteria³⁴ for case-control studies. A number of those criteria were: 1) Do the controls and cases come from the same population; 2) Is the same sample used in both groups (e.g. blood); 3) Is there any ethnic matching between the groups?; and 4) Are the methods of genotyping in both groups the same? Subjective assessment was avoided by refraining from the generation of an overall quality score; instead, these criteria were utilized to rank the studies and they are illustrated in tables and forest plots according to their quality ranks. The quality assessors were blinded to the authors, journals, and results of the studies.

Data Extraction

Data were extracted from each study independently by two reviewers using a predefined form. To increase reliability and decrease probable biases in data extraction, the following actions were performed:

Before starting, the reviewers had an orientation meeting about how to enter the data or transform some indices. When there was a difference between the reports in the abstracts and full texts, the latter was chosen. Before the confirmation of the final form, a pilot extraction was performed on a number of articles and defects of forms were modified by consensus.

Statistical Analysis and Heterogeneity Assessment

Summary odds ratios (ORs) and 95% confidence intervals were calculated from the raw data of the selected studies. For summarizing

ORs, the Mantel-Haenzel method based on the fixed effects model was used when there was no heterogeneity between the studies. Otherwise, the DerSimonian and Laird method based on the random effects model was employed. A P value smaller than 0.05 was considered statistically significant. Heterogeneity among the studies was assessed via the χ^2 -based Q test, and a P value smaller than 0.1 was considered statistically significant in the Q test because of its low power. Visual assessment of heterogeneity was illustrated by the Galbraith plot. Subgroup analysis was also conducted only in the European studies, because the number of studies in the other regions was not sufficient.

The Begg rank correlation³⁵ and the Egger weighted regression methods³⁶ were used to statistically assess publication bias. A P value smaller than 0.05 was considered statistically significant for publication bias tests. The funnel plot was also drawn upon for the visual assessment of publication bias. (Asymmetry shows the probable publication bias.) Statistical analysis was performed using STATA 9.0 (Stata

Corp., College Station, TX, USA).

Results

Characteristics of Included Studies

In the first step, 72 papers were identified. Our manual search of the references identified 4 more articles. However, after the screening stage of the titles and abstracts, forty were excluded because they were either review articles, animal studies, or irrelevant to our study. Full texts of 35 potentially relevant studies and an English abstract of a Chinese article were retrieved and reviewed (totally, 36 studies). From them, 15 were excluded for the following reasons: three articles were duplicates;³⁷⁻³⁹ two did not report usable data;^{40,41} and 10 investigated other polymorphisms of TNF- α rather than TNF- α -308.^{5,42-50} Finally, twenty-one case-control studies, which comprised 2880 MS cases and 3579 controls, were included in the study.¹²⁻³² The selected characteristics of the 21 case-control studies included in this meta-analysis are summarized in table 1 (figure 1).

Table 1: Characteristics of the 21 studies included in the meta-analysis of tumor necrosis factor- α (TNF- α) -308 polymorphism and multiple sclerosis (MS)*

Study (year)	Source of cases (Number)	Source of controls (Number)
Drulovic (2003)	Belgrade, Serbia (143)	Belgrade, Ethnically matched blood donors (123)
De Jong (2002)	Netherlands, Amsterdam (109) Leiden (50)	Ethnically-matched Dutch organ donors (273)
Ristic (2007)	Croatia (175) Slovenia (163)	Healthy blood donors matched for age, sex, and ethnicity (460)
Fernandes-Filho (2002)	Norway (133)	Healthy controls, same region (148)
Kamali-Sarvestani (2007)	Iran, Shiraz (270)	Healthy volunteers matched for ethnicity and sex (439)
Favorova (2006)	Russia, Moscow (223)	Healthy controls, ethnically matched, free of internal and neurological diseases (222)
Sarial (2008)	Iran, Tehran MS Society (99)	Tehran, random blood samples from Iranian Blood Transfusion Organization (137)
Bing He (1995)	Sweden (93)	Healthy blood donors, ethnically matched (95)
Mihailova (2005)	Bulgaria (55)	Healthy blood donors, ethnically matched (86)
Fernandez Arquero (1999)	Spain (238)	Spain, Madrid (324)
Kirk (1997)	Northern Ireland (189)	Healthy blood donors (106), normal spouses of individuals with single-gene disorder (100), ethnically matched
Forte (2006)	Italy, West Sicily (91)	Healthy controls matched for age, sex, and region(220)
Lucotte (2000)	France (74)	Healthy controls matched for age, sex, and ethnicity (75)
Dong (2006)	South China (68)	South China, ethnically matched (106)
Huizinga (1997)	Nursing home, Belgium (57) and outpatient clinic, Netherlands (98)	Healthy Dutch controls (186)
Mycko (1998)	Poland (53)	Poland, ethnically matched (81)
Braun (1996)	Germany (50)	Healthy controls, ethnically matched (22)
Anlar (2001)	Turkey (24)	DNA bank, Turkey (93)
Wirz (2004)	Italy, Sardinia (32)	Sardinia, healthy controls (35)
Wingerchuk (1997)	US, prevalence cohort of MS in Olmsted county, Mayo Clinic (110)	Patients of other diseases in Mayo Clinic, matched for age, sex, and ethnicity (110)
Maurer (1999)	Germany (283)	Germany, patients of amyotrophic lateral sclerosis (72) and stroke (66)

*Studies were ranked based on their quality.

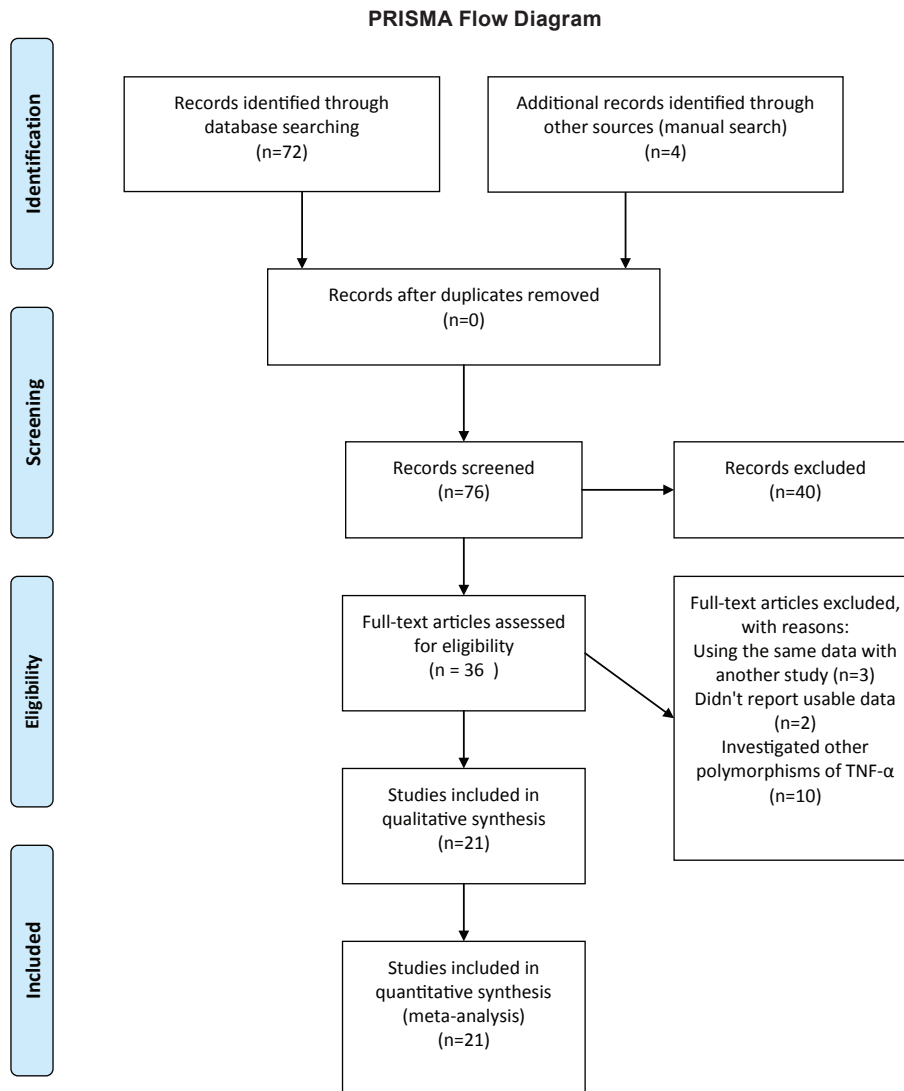


Figure 1: Flow diagram of study results reviewed in our systematic review (n=76).

Association between Alleles and Genotypes of TNF- α -308 and MS

The pooled ORs and 95% CIs in both overall and subgroup populations are depicted in table 2. From the meta-analysis, an association between MS and TNF2 allele was not found in the overall (figure 2) and European populations. The Galbraith plot of heterogeneity shows that the Sarial and De Jong studies were the leading causes of heterogeneity between the studies (figure 3). What is more, the Begg and Egger tests and also the funnel plot revealed that there was no significant publication bias in this meta-analysis (table 2, figure 4). The pooled ORs for TNF2/2+2/1 (TNF2+) versus TNF 1/1 (TNF2-) were not significant in the overall and European publications. The pooled ORs for TNF2/1 versus 1/1 were not statistically significant in the overall population, but this association was significant in European studies (OR=0.84, 95% CI: 0.73-0.98).

The pooled ORs for TNF2/2 versus TNF 2/1+1/1 and also for TNF2/2 versus TNF 1/1 were not significant in all the comparisons. In addition, the pooled ORs for TNF 2/2 versus TNF 2/1 were not significant either in the overall or in the European publications.

Discussion

MS is a complex polygenic disease, and the dissection of its genetic background is very complicated because of the combinatorial possibilities of gene-gene interactions. Our meta-analysis reveals that the inheritance of TNF2 allele does not change the risk of MS. In the meta-analysis of genotypes, although we witnessed that 2/1 heterozygote decreased the risk of MS in comparison with 1/1 homozygote in the European publications, other comparisons did not support this result. For instance, considering the dose-response

Table 2: Meta-analysis of tumor necrosis factor- α (TNF- α) -308 gene polymorphism and multiple sclerosis association

Comparisons	Population	N	Test of association		Test of homogeneity		Publication bias	
			OR(95%CI)	P	Q	P†	Begg (P)	Egger (p)
TNF2 versus TNF1	Total	21	1.02(0.86-1.21)	0.76	48.4	<0.001	0.27	0.51
	European	17	0.97(0.83-1.14)		29.3	0.02		
TNF2+versus TNF2-	Total	18	0.99(0.8-1.24)	0.98	42.4	0.001	0.075	0.15
	European	14	0.86(0.75-1)	0.056	17.6	0.17		
TNF2.1 versus TNF1.1	Total	18	0.97(0.78-1.2)	0.78	39.5	0.001	0.12	0.23
	European	14	0.84 (0.73-0.98)	0.03*	15.46	0.27		
TNF2.2 versus TNF2.1+1.1	Total	15	1.11(0.73-1.71)	0.6	14.3	0.42	1	0.38
	European	13	1.12(0.72-1.75)	0.59	10.7	0.46		
TNF2.2 versus TNF2.1	Total	15	1.36(0.9-2.05)	0.14	12.3	0.58	0.76	0.69
	European	13	1.36(0.88-2.1)	0.16	9.3	0.59		
TNF2.2 versus TNF1.1	Total	15	1.13(0.76-1.68)	0.52	15.2	0.36	0.8	0.3
	European	13	1.07(0.68-1.67)	0.73	11.4	0.41		

OR, odds ratio; CI, confidence interval; *Statistically significant; †P<0.1 is considered statistically significant for Q statistics

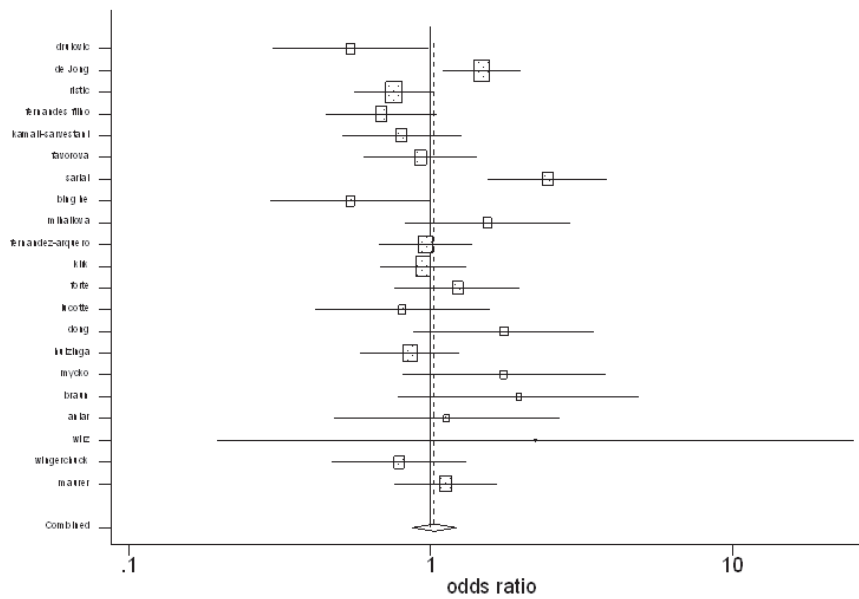


Figure 2: The figure demonstrates the pooled odds ratios and 95% confidence intervals for multiple sclerosis when comparing TNF2 allele with TNF1 allele. The studies are listed based on quality ranking.

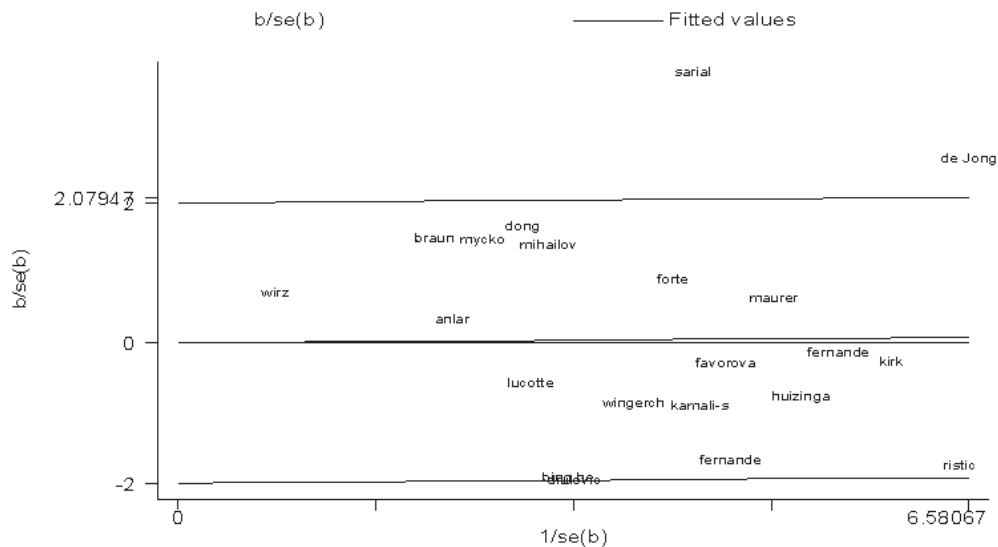


Figure 3: This figures illustrates the Galbraith plot of heterogeneity among the studies in our meta-analysis of tumor necrosis factor- α (TNF- α) -308 gene polymorphism and multiple sclerosis association (TNF2 vs. TNF1 alleles).

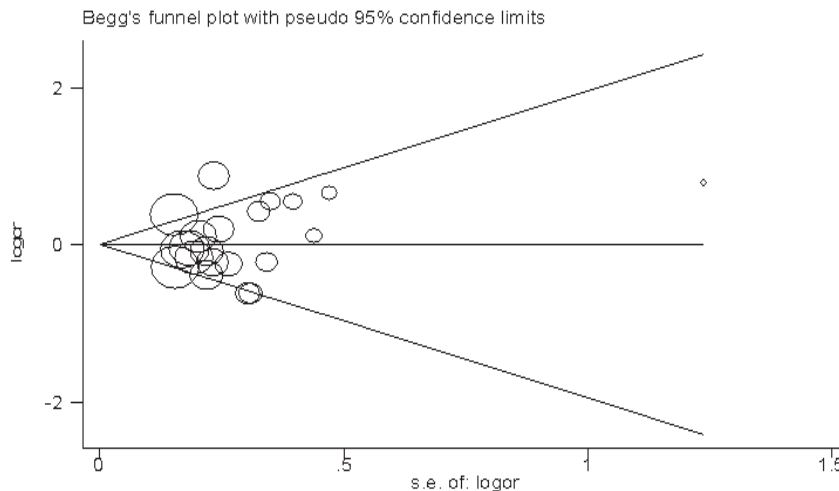


Figure 4: This figure depicted the Begg funnel plot of publication bias in our meta-analysis of tumor necrosis factor- α (TNF- α)-308 gene polymorphism and multiple sclerosis association (TNF2 vs. TNF1 alleles).

correlation, it was expected that 2/2 homozygote would exhibit a stronger negative association than 2/1 heterozygote with MS, but we did not find these results in our different comparisons.

On the other hand, some studies have suggested that TNF2 allele is associated with a high production of TNF- α ^{10,11} and that the level of TNF-alpha in the CSF correlates with the severity and progression of MS.⁶ It is, therefore, expected that the carriers of TNF2 alleles have more chance of developing MS than TNF1 carriers. Be that as it may, our meta-analysis did not support this interpretation.

It seems that other polymorphisms in different positions of TNF- α , other cytokines, and also their interaction should be taken into account in the study of MS susceptibility. Recently, some genome-wide association studies (GWAS) were performed by analyzing a large number of SNPs, simultaneously, based on chip technology and demonstrated no significant relationship between TNF- α -308 gene polymorphism and MS,⁵¹⁻⁵⁴ which is consistent with our findings. It is crystal clear that these kinds of studies that consider different gene variations at the same time and also studies that analyze gene/gene and gene/environment interactions would be more reliable to reach the concise results about the exact contribution of genes in this complex disease.

There were some limitations in this meta-analysis. Firstly, in some comparisons, the pooled ORs were obtained from heterogeneous studies. Secondly, only published studies were included in this meta-analysis; consequently, publication bias may have occurred, although the funnel plots and statistical tests did not show it in our meta-analysis. Thirdly, assessment and quality ranking of the studies was according to their reports and

also was very subjective, precluding us from considering this ranking as a definite criterion. Finally, meta-analysis is a retrospective research that is subject to methodological deficiencies and potential biases in the studies included.

Conclusion

Our meta-analysis does not support the role of TNF- α -308 G/A polymorphism in developing MS.

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Conflict of Interests: None declared.

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