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Original article

Association between transforming growth factor-β1 T869C polymorphism and rheumatoid arthritis: a meta-analysis

Wei-Wei Chang[1], Hong Su[1], Li He[1], Ke-Fu Zhao[1], Jia-Ling Wu[1] and Zhi-Wei Xu[1]

Abstract

Objective. The results of studies on association between TGF-β1 T869C polymorphism and susceptibility to RA are controversial. The absence of a replication of linkage might be due to different ethnicities. The aim of this study was to perform a preliminary investigation on the effect size of TGF-β1 T869C polymorphism on RA susceptibility through a meta-analysis.

Methods. Case–control studies on the association of TGF-β1 T869C with RA were searched up to March 2009, and the genotype frequencies in the control group were found to be consistent with the Hardy–Weinberg equilibrium. The effect summary odds ratio (OR) and 95% CIs were obtained. Publication bias was tested by funnel plot with Egger's regression test, and heterogeneity was assessed.

Results. Seven studies comprising 1122 cases and 1132 controls were included. Heterogeneity was observed ($\chi^2 = 17.16$; $P = 0.009$). Under the random effects model, the common OR was 1.38 (95% CI 0.95, 2.01; $P = 0.09$). In the subgroup meta-analysis, there was an association between TGF-β1 T869C polymorphism and RA in the people of Asian descent (OR = 1.93; 95% CI 1.42, 2.62; $P < 0.0001$), but not in the people of non-Asian descent (OR = 0.88; 95% CI 0.65, 1.19; $P = 0.41$). There was no evidence of publication bias according to Funnel plot and Egger's regression test ($a = 4.778$; $P = 0.14$).

Conclusions. There was heterogeneity between studies, and no clear evidence of an association on a worldwide population was observed. Subgroup analysis results suggest that TGF-β1 T869C might play a role in RA susceptibility for Asians but not for non-Asians. Further studies are required for definite conclusions.

Key words: Rheumatoid arthritis, Gene polymorphism, transforming growth factor-β1, Meta-analysis.

BASIC SCIENCE

Introduction

RA is an inflammatory, systemic and autoimmune disease, characterized by chronic joint inflammation and destruction [1]. It is a complex and heterogeneous genetic disease in which multiple genetic and non-genetic factors interact over time. The role of genetic factors in the pathogenesis of RA has been established by data from twin and family studies, which estimate that the heritability of RA liability may be as high as 60% [2]. The HLA class II molecules are the most powerful recognized genetic factors for RA. However, results of family studies suggest that this association accounts for only one-third of the genetic susceptibility, and that non-HLA factors are also involved in disease susceptibility [3]. Jawaheer *et al*. [4, 5] reported that non-HLA genes, particularly on chromosomes 1p, 1q and 18q, are involved in RA susceptibility. And currently, PTPN22 (rs2476601), 6q23 (rs6920220), STAT4 (rs7574865), TRAF1/C5 (rs3761847), CTLA4 (rs3087243), PADI4 (rs2240340) and IL2/IL21 (rs6822844) have been consistently reported in RA susceptibility [6–9].

In order to better understand the aetiology of RA, genes other than HLA should be explored. TGF-β1 is a multifunctional cytokine, involved in the regulation and proliferation of cells. The cytokine promotes differentiation of leucocytes, but has inhibitory effects on proliferation of

[1]Epidemiology and Biostatistics School of Public Health, Anhui Medical University, Hefei, China.

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Correspondence to: Hong Su, Epidemiology and Biostatistics School of Public Health, Anhui Medical University, Hefei 230032, China.
E-mail: suhong5151@sina.com.

T lymphocytes and activation of macrophages, suggesting a regulatory role in inflammatory states [13]. TGF-β1 has been detected in the synovial tissue of RA patients [14, 15]. The TGF-β1 869T/C (rs1982073) polymorphism is located in the signal peptide sequence of TGF-β, which is thought to target the newly synthesized protein to the endoplasmic reticulum [13]. Yamada *et al*. [14, 15] had previously shown that the serum concentration of TGF-β1 increased according to the rank order of 869T/C TT < TC < CC. The association suggested that the 869T/C substitution may affect the function of the signalling peptide, most likely influencing the intracellular trafficking or export efficiency of the protein.

As the function and location of the TGF-β1 T869C gene makes it a good candidate for association with RA, a candidate gene analysis of TGF-β1 T869C was recently performed. It had been reported that functional variants of the gene encoding TGF-β1 T869C were associated with RA in Japanese individuals [16]. In addition, Zhu *et al*. [17, 18] reported that there was an association between TGF-β1 T869C polymorphism and RA. Thus, the TGF-β1 T869C susceptibility gene has an important role in the pathogenesis of RA.

Despite the association between TGF-β1 T869C and RA observed in Japanese and Chinese populations, the results in later studies [19–22] were inconsistent. Considering the possibly small effect size of this genetic polymorphism to RA and the relatively small sample size in each study, the discrepancy will become apparent because a single study may have been underpowered to detect a small but real association.

Given the amount of accumulated data now available, it is important to perform a quantitative synthesis of the evidence using rigorous methods. The aim of this study was to assess the association of TGF-β1 T869C polymorphism with the risk of RA by conducting a meta-analysis of individual datasets from all eligible case–control studies published to date.

Materials and methods

Identification of eligible studies and data extraction

Electronic databases, PubMed, Chinese biomedical database (CBM), Chinese National Knowledge Infrastructure (CNKI) and Wan fang (Chinese) database, were searched up to March 2009 for all genetic association studies evaluating the TGF-β1 gene polymorphism and RA in humans. Computer search of CNKI, PubMed, CBM and Wan fang was done using the following keywords and subjects terms: 'transforming growth factor β1', 'TGF-β1', 'TGF beta', 'TGF β', 'polymorphism', 'rheumatoid arthritis', 'RA', 'DPD1', 'camurati–engelmann syndrome', 'CED' and 'camurati–engelmann disease'. No restrictions were placed on language, race, ethnicity or geographical area.

The following criteria were used to identify the relevant published studies: (i) the diagnosis of RA was established using the ACR classification criteria for RA [23], and ethical approval and informed patient consent was obtained; (ii) the study followed a case–control design and all controls were healthy people; (iii) enough information had to be provided to calculate the odds ratio (OR); (iv) the manuscript was published in peer-reviewed journals as a full paper, and not as an abstract or review; and (v) the distribution of genotypes in the control groups was in the Hardy–Weinberg equilibrium. We excluded the following: (i) studies in which the number of wild genotypes could not be ascertained and (ii) studies without an appropriate case–control design. The following information was extracted from this study: first author, year of publication, study population (country), the number of patients and controls for the study and genotyping information and frequencies of alleles.

Statistical analysis

Because case–control studies were involved, ORs were used to assess the strength of association between the TGF-β1 T869C polymorphism and RA. We calculated the OR and respective 95% CIs by comparing the carriers of rare alleles with the wild homozygote, as CT + TT *vs* CC.

Statistical heterogeneity among studies was assessed with the *Q*- and I^2-statistics [24]. I^2-values of 25, 50 and 75% were assigned as low, moderate and high estimates, respectively. Heterogeneity was considered significant for $P < 0.10$.

A fixed effects model using the Mantel–Haenszel method and a random effects model using the DerSimonian and Laird method were used to pool the results [25]. Random effects are more appropriate when heterogeneity is present. The significance of the pooled OR was determined by the *Z*-test. Chi-square test was used for the Hardy–Weinberg equilibrium of genotypes in the control group of each study.

Publication bias was investigated using a funnel plot, in which the standard error of log (OR) of each study was plotted against its OR. An asymmetric plot suggested possible publication bias. Funnel plot asymmetry was assessed by the method of Egger's linear regression test, which used a linear regression approach to measure the funnel plot asymmetry on the natural logarithm scale of the OR [26].

Analyses were performed using the software Stata version 10 (StataCorp LP, College Station, TX, USA), Review Manager 4.2 (Cochrane Collaboration, http://www.cc-ims.net/RevMan/relnotes.htm). The significance of each study was computed as the probability of detecting an association between the TGF-β1 T869C SNP and RA at $P = 0.05$, assuming medium effect size (0.3). The power analysis was performed using the statistical program G*Power (http://www.psycho.uni-duesseldorf.de/aap/projects/gpower). All the *P*-values were two sided. $P < 0.05$ was considered statistically significant.

Results

Study selection and subject characteristics

Seven association studies relating to TGF-β1 gene polymorphism and susceptibility to RA accomplished the inclusion requirements for the meta-analysis [16–22].

TABLE 1 Characteristics of published studies about the association between TGF-β1 T869C gene polymorphism and RA included in the meta-analysis

No.	References	Country (origin)	T869C								
			Cases				Controls				
			CC	CT	TT	Total	CC	CT	TT	Total	Power[a], %
1	Sugiura *et al*. [16]	Japan (Asian)	29	92	34	155	33	53	24	110	99.83
2	Pokorny *et al*. [19]	New Zealand (non-Asian)	27	58	32	117	26	73	41	140	99.78
3	Kim *et al*. [21]	Korean (Asian)	32	76	35	143	40	65	43	148	99.79
4	Zhu *et al*. [17]	China (Asian)	11	41	24	76	33	47	20	100	97.83
5	Wang *et al*. [18]	China (Asian)	17	52	36	105	34	46	20	100	99.02
6	Alayli *et al*. [22]	Turkey (non-Asian)	21	54	56	131	21	64	48	133	99.82
7	Panoulas *et al*. [20]	UK (non-Asian)	58	176	161	395	54	186	161	401	100

[a]Power calculations assume $a = 0.05$ and medium effect size (0.3). PCR-RFLP: PCR restriction fragment length polymorphism.

One study was excluded because it was not a case–control study and the controls were not healthy people [27]. A total of 1122 cases and 1132 controls were investigated. Two studies were from China [17, 18], one from New Zealand [19], one from Japan [16], one from Korea [21], one from Turkey [22] and one from the UK [20] (Table 1). All the cases of RA were diagnosed by the ACR classification criteria for RA [23]. Selected characteristics of the seven case–control studies for the relationship between TGF-β1 T869C polymorphisms and the risk of RA are summarized in Table 1. The genotype frequencies in the control group were consistent with the Hardy–Weinberg equilibrium (supplementary table 1, available as supplementary data at *Rheumatology* Online). The distribution of ORs from individual studies with respect to their respective S.D. was not very symmetrical in the funnel plot. However, an Egger's test was performed to provide statistical evidence for funnel plot symmetry ($a = 4.778$; $P = 0.14$) (supplementary figures 1 and 2, available as supplementary data at *Rheumatology* Online). These data provided no significant evidence for publication bias.

Association between TGF-β1 T869C and RA

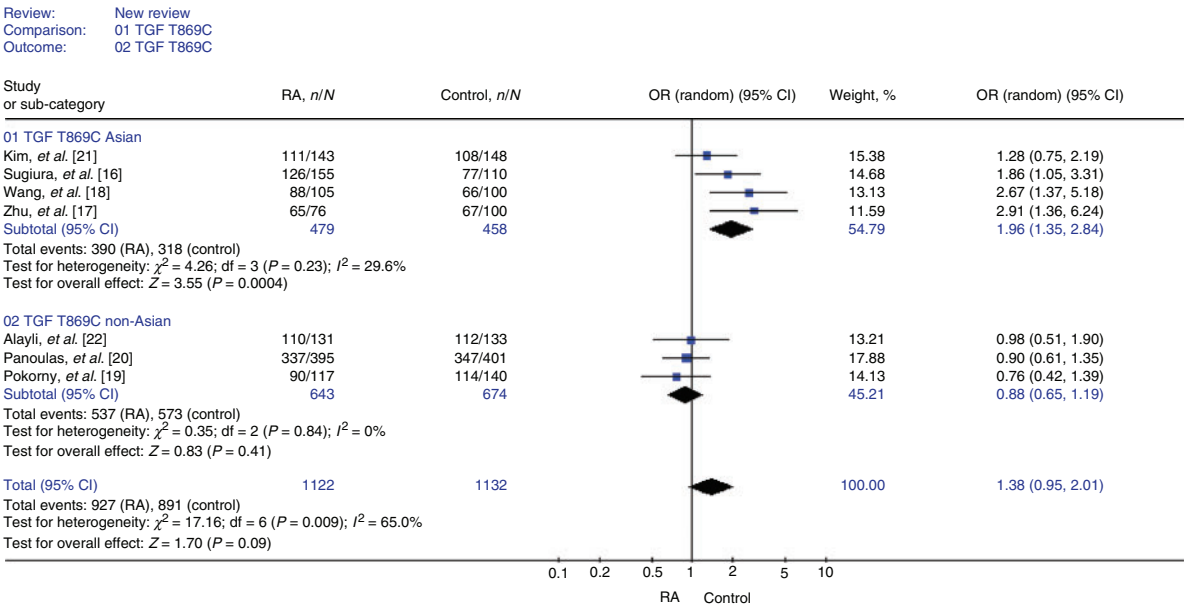
Heterogeneity was observed among individual estimates of the ORs ($\chi^2 = 17.16$; $P = 0.009$) and the original data were combined by means of the random effects model. First, statistics calculated for each study were shown in the forest plot (Fig. 1). The summary OR was 1.38 (95% CI 0.95, −2.01; $P = 0.09$) by the random effects model and 1.29 (95% CI 1.05, 1.60; $P = 0.02$) by the fixed effects model. There was no evidence that TGF-β1 T869C polymorphism resulted in an increased susceptibility to RA on a worldwide population. Secondly, the reported discrepancies on the association between TGF-β1 T869C and RA may be due to the different ethnicities [16, 17, 19]. And there was heterogeneity between studies. In order to look for an ethnic effect, we performed the subgroup meta-analysis in populations of Asian and non-Asian decent, respectively. No effect of T allele on susceptibility was observed in subgroups of non-Asian descent (three comparisons, OR = 0.88; 95% CI 0.65, 1.19; no significant between-study heterogeneity). However, an association was observed in Asian descent subgroups under the fixed effect models (OR = 1.93; 95% CI 1.42, 2.62; $P = 0.00$; low between-study heterogeneity) [Fig. 1; and supplementary table 2 (available as supplementary data at *Rheumatology* Online)].

Discussion

Since the first positive association between TGF-β1 T869C and RA was reported in a Japanese population [16], six studies have been undertaken to replicate the association [17–22]. However, subsequent studies in populations were inconsistent [17–22]. Therefore, we did a meta-analysis to estimate the relationship between TGF-β1 polymorphisms and susceptibility to RA. Our study is the first meta-analysis to examine the T869C polymorphism of the TGF-β1 gene and its relationship with susceptibility to RA. In the meta-analysis, the overall data showed that the TGF-β1 T869C may not be an RA susceptibility gene across populations. The common OR for the risk allele is 1.38 (95% CI 0.95, 2.01; $P = 0.09$). There was an association between TGF-β1 T869C polymorphism and RA in Asian populations in the subgroup meta-analysis, but not in non-Asian populations.

The reasons that the same polymorphism plays a different role in different ethnic populations or across different studies may arise from many aspects. First, RA is a complex disease and genetic heterogeneity exists in different populations. Whole genome linkage studies on RA have confirmed this genetic heterogeneity [4, 5, 28–30]. Secondly, clinical heterogeneity may also explain the discrepancy. The potential contribution of differences in patient populations (e.g. age and years from onset, female proportion, disease severity and so on) might lead to different results. Sometimes association can only be found in stratification analysis according to the clinical character. In this article, this information

Fig. 1 The overall and pooled ORs with 95% CI for overall analysis and subgroup analysis testing association of TGF-β1 T869C polymorphism with RA. The aggregate OR and 95% CI of the risk allele are also given. The weighting factors (weight %) used to calculate the aggregate OR, calculated from the inverse of the variance, are given for each study.



cannot be gotten completely. Moreover, there were only seven studies, which are inconvenient for subgroup study on 'years from onset *et al.*'. They may be the main reason for the reported heterogeneity in this article.

The characteristic of meta-analysis is to combine comparable studies, to increase the sample size and statistical power and draw a more compelling result. However, meta-analysis has confounding factors such as publication bias, method of sampling, different genetic backgrounds of subjects, different protocols and quality of analysis. We followed the inclusion and exclusion criteria strictly to reduce the selection bias. A funnel plot and Egger's linear regression test were used to assess the publication bias. And the test of the Hardy–Weinberg equilibrium for distribution of the genotypes in control groups suggested that there was no significantly different genetic background among the participants. The seven studies would seem to be comparable, relevant to the meta-analysis.

Nevertheless, there are several limitations in this meta-analysis. First, the number of total samples in the meta-analysis is small, and the amount of ethnic stratification in subgroup analysis is smaller; more studies are required for definite conclusions. Secondly, there is heterogeneity between studies, maybe owing to covariates—age and years from onset, fraction of patients with erosions or RF and so on. Thirdly, the selection of control participants in case–control studies may influence the results because hospital-based controls may not be as representative as population-based *N* controls [31], although no evidence of the effect of control population was detected in this meta-analysis.

There was heterogeneity between studies, and no clear evidence of an association between TGF-β1 T869C and RA in a worldwide population was observed. Subgroup analysis results suggest that TGF-β1 T869C would not be a risk factor for RA in non-Asians but might play a role in RA susceptibility for Asians. As the number of studies is relatively small, more studies are required for definite conclusions.

Rheumatology key messages

- There was heterogeneity between studies and no clear evidence of an association in a worldwide population.
- There seemed to be a difference between Asian and non-Asians studies, but further studies are required for definite conclusions.

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Supplementary data

Supplementary data are available at *Rheumatology* Online.

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