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# Association of the Tumor Necrosis Factor Alpha Gene Polymorphism with Susceptibility and Clinical-Immunological Findings of Systemic Lupus Erythematosus

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SUMMARY The etiology of systemic lupus erythematosus (SLE) is unknown but genetic factors seem to play a role in the disease pathogenesis. The tumor necrosis factor alpha (TNFα) gene, encoded at the TNF locus in the MHC class III region, is now known to be an important candidate gene in SLE, due to the proinflammatory activities of the TNFα. The objectives of this study were to examine the role of the TNFα polymorphism for the susceptibility of Malaysian Chinese lupus patients to SLE and to determine its association with organ involvement. The allelic frequencies of the TNFα polymorphic variant (TNF2) of seventy lupus patients were determined during follow-up at the Medical Clinic of the National University Hospital Malaysia by PCR-RFLP technique. Sixty-four females and 6 males with a mean age of 33 ± 12 years were included. Clinical data were obtained from case records. Autoantibody levels were measured by ELISA. Fifty-nine ethnically-matched blood donors were used as controls. The allelic frequency of the TNF2 variant was found to be significantly increased in the patients compared to the controls (52.8% vs 33.8%). SLE patients with the polymorphic TNF2 variant were found to be at increased risk of central nervous system involvement ($p$ = 0.004, RR = 2.59) and to have an increased frequency of anti-La antibodies ($p$ = 0.03). In view of these findings we suggest that TNF2 variant is playing a role in conferring susceptibility to SLE and in the disease pathogenesis.

Systemic lupus erythematosus (SLE) is a chronic inflammatory disease of autoimmune nature and appears to be influenced by the genetic make-up. Family aggregation among patients[1] and increased concordance of SLE among monozygotic versus dizygotic twins[2] highlight the role of genes in its etiology. Cytokine imbalances are responsible for the pathogenesis of certain autoimmune diseases and among the cytokines, the TNFα plays a central role. The TNFα gene is a central mediator of the inflammatory response and has important immunological activities. The first bi-allelic TNFα polymorphism detected in humans involved a single base change from G to A at the position –308 in the promoter region of the gene.[3] The less common TNFα allele, TNF2 (-308A) was shown to be strongly associated with the DR3 allele.[4] Due to its localization within the MHC region (between the HLA class II and HLA B locus) and its biological activities, it is not surprising that TNF2 was later reported to be associated with SLE.[5,6] The purpose of the present study was to determine the distribution of the polymorphism in the TNFα alleles in Chinese patients with SLE and whether the rare allele (TNF2) has a role in disease susceptibility and pathogenesis.

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## MATERIALS AND METHODS

Seventy patients were recruited during their follow-up at the Medical Clinic of the National University Hospital Malaysia. All patients met the American College of Rheumatology (ACR) classification criteria for SLE.[7] There were 64 females and 6 males with a mean age of 33 ± 12 years (mean ± SD) and a mean disease duration of 8 ± 5 years. Present clinical characteristics were noted by the physician while past features were reviewed from medical records. Informed consent was obtained from all patients. Five milliliters of blood were taken from the patients as well as the fifty-nine healthy ethnically-matched controls. Auto-antibody (anti-dsDNA, anti-SM, anti-RNP, anti-Ro and anti-La) levels were determined at the time of serum sampling by a commercial ELISA kits (IMMCO, USA). Extraction of genomic DNA from peripheral blood mononuclear cells was carried out using the QIAamp blood extraction kit (Qiagen, Hilden, Germany). The extracted DNA was stored at -20°C before the test was carried out.

PCR amplification of the TNFα promoter region was carried out using the following primers: 5'-AGGCAATAGGTTTTGAGGGC-CAT-3'-, antisense: 5'-TCCTCCT-GCTCCGATTCCG-3'[3] and amplification was performed using a GeneAmp PCR System 9600 (PE Applied Biosystems) programmed at an initial denaturing step of 94°C for 3 minutes followed by 35 cycles of the following: 94°C for 10 seconds, 60°C for 50 seconds and 72° C for 50 seconds and a final extension at 72°C for 4 minutes. The resulting amplified products were digested with *Nco* 1 (Research Biolabs) at 37°C for 3 hours and finally analyzed by electrophoresis on a 2% ethidium bromide stained agarose gel. A 100 bp molecular ladder (Fermentas, Lithuania) was used to determine the size of the PCR fragments. Homozygous TNF 1 showed one fragment of 87 bp, homozygous TNF2 showed a single 107 bp fragment while heterozygous TNF1/2 showed both fragments (Fig. 1).

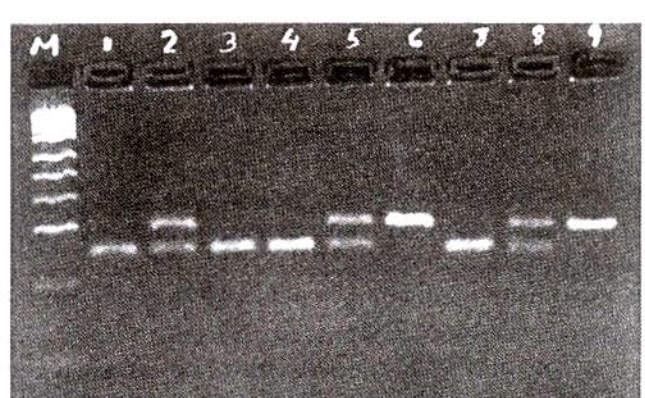
### Statistical analysis

Results are presented as allele frequencies. The results from the control and test groups were compared using the $\chi^2$ test or the Fischer's exact test (2 x 2 contingency tables) for statistical significance. For the analysis of an association between clinical involvement and autoantibody positivity, a comparison was made with SLE patients without the given symptom. Odds ratios equal to approximate relative risks have been calculated for the disease in carriers of specific alleles. *P* values less than 0.05 were considered to be significant.

## RESULTS

Seventy Chinese patients with SLE were recruited into the study. The cumulative clinical and immunological features are shown in Table 1. The majority of patients (90%) suffered mucocutaneous involvement followed by musculoskeletal (69%) and renal (61%) involvements. Thirty patients (43%) had anti-La antibodies while anti-dsDNA antibodies were seen only in 22 (31%) of patients. Antinuclear antibodies were present in 95% of patients. The distribution of the TNFα alleles (TNF1 and TNF2) in patients and controls is shown in Table 2. In the patient population, the allele frequencies of the TNF1 and 2 were 63% and 37% while among the controls it was 80% and 20% respectively. There was a statistically significant difference in the frequency of the

**Fig. 1** Patterns of *Nco1* RFLP on PCR amplified TNFα gene products.




Lane M, 100 bp ladder, Lanes 1,3,4,7 homozygous TNF1 (87 bp), lanes 2,5,8 heterozygous TNF1/2 (87 and 107 bp), Lanes 6,9 homozygous TNF2 (107 bp). Electrophoresis was carried out in 2% ethidium bromide stained agarose gel.

TNF2 allele in patients compared to controls ($p$ = 0.003, RR = 1.42) demonstrating the role of this variant in conferring susceptibility to lupus. TNF2 was significantly increased in patients with central nervous system (CNS) involvement compared to those without (40% *vs* 15%, $p$ value < 0.05, RR = 2.59). As for immunological abnormalities, TNF2 was also increased significantly in patients with anti La antibodies (28% *vs* 13%, $p$ < 0.05, RR = 1.42). However, there was no difference in anti-dsDNA antibody positivity between the TNF2 positive and TNF2 negative groups (Table 3).

**Table 1** Clinical and immunological features of patients with systemic lupus erythematosus

| Characteristics | Number of patients (%) (n = 70) |
|---|---|
| **Females** | 64 (91) |
| **Males** | 6 (9%) |
| **Mean age ± SD (yrs)** | 33 ± 12 |
| **Organ involvements** | |
| **- Musculoskeletal** | 48 (69) |
| **- Mucocutaneous** | 63 (90) |
| **- Cardiorespiratory** | 13 (19) |
| **- Renal** | 43 (61) |
| **- Central nervous system** | 14 (20) |
| **- Haematological** | 22 (31) |
| **Autoantibodies** | |
| **- Anti-ds DNA** | 22 (31) |
| **- Anti-Sm** | 10 (14) |
| **- Anti-RNP** | 28 (40) |
| **- Anti-Ro** | 28 (40) |
| **- Anti-La** | 30 (43) |

**Table 2** Allele frequency of TNF1 and TNF2 in patients (expressed as % of total alleles)

| Alleles | Patients (n = 70) | Controls (n = 59) |
|---|---|---|
| **TNF1** | 63 | 80 |
| **[a]TNF2** | 37 | 20 |

[a] $p$ value < 0.05

## DISCUSSION

The role of the TNFα gene is quite well known in autoimmune disorders.[8,9,10] The first studies in transgenic mice demonstrated systemic or organ specific inflammation.[11] However, in humans, studies on the genetic diversity of this locus point to the fact that differences in the TNF or its production play a role in various autoimmune diseases. Studies have shown that polymorphism of the TNFα is associated with increased expression of TNFα by monocytes in SLE.[10,12]

This present study showed a significant association of the TNF2 allele with lupus. This finding was supported by some authors[5,13] but negated by others.[14,15,16] The fact that this polymorphic variation lies within the promoter region of the TNFα locus makes it a likely candidate to play a regulatory role in the production of TNFα.

It is interesting to speculate that the TNF2 allele may play a role in disease pathogenesis. We analyzed whether the TNF2 variant is associated with any of the clinical and immunological characteristics of SLE. There was a significant increase of the TNF2 variant in patients with central nervous system (CNS) involvement. This finding indicates that this gene polymorphism may contribute to the increased manifestation of CNS involvement among patients. There was no association of the TNF-2 allele with neuropsychiatric lupus patients.[17] A significant increase was also seen in those patients with anti-La antibodies. However, in another study, no such association was found with clinical or immunological characteristics.[13]

The hallmark of SLE is the production of autoantibodies. In this study we found that the TNF2 variant was significantly associated with anti-La antibodies among our patients. However, both anti-Ro and anti-La autoantibodies were found to be associated with the TNF2 variant.[5] Differences in the

**Table 3** Comparison of the frequency of clinical and immunological features of patients with and without TNF2 variant (%)

| Characteristics | Patients' allele positivity | | | | |
|---|---|---|---|---|---|
| | TNF2+ | TNF2- | *p*-value | OR | RR |
| **Mucocutaneous** | 21 | 79 | NS | - | - |
| **Musculoskeletal** | 22 | 78 | NS | - | - |
| **Cardiorespiratory** | 15 | 85 | NS | - | - |
| **Renal** | 20 | 80 | NS | - | - |
| **Central nervous system** | 40 | 60 | 0.004 | 3.62 | 2.59 |
| **Hematological** | 20 | 80 | NS | - | - |
| **Autoantibodies** | | | | | |
| **Anti-Sm** | 35 | 65 | NS | - | - |
| **Anti-RNP** | 23 | 77 | NS | - | - |
| **Anti-Ro** | 18 | 72 | NS | - | - |
| **Anti-La** | 28 | 72 | 0.03 | 2.48 | 1.58 |

NS, not significant difference

results between this study and previous ones could be due to a genetic heterogeneity between the patient groups. Individual genes involved in SLE susceptibility in one population may not be the same in another population. In view of these findings, it seems likely that the TNF2 polymorphism predisposes to SLE in the Malaysian Chinese lupus population and can be considered as a marker for clinical and immunological outcome of the disease.

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