

# Transforming Growth Factor- $\beta$ 2 Polymorphism and Systemic Lupus Erythematosus

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**ABSTRACT. Objective.** To determine whether transforming growth factor- $\beta$ 2 (TGF- $\beta$ 2) gene polymorphism is associated with systemic lupus erythematosus (SLE) susceptibility. TGF- $\beta$  is a multifunctional family of cytokines important in tissue repair, inflammation and immunoregulation. SLE is thought to be a T cell dependent autoimmune disorder with T cell dysfunction. Due to its known suppressive effects on interleukin 2 dependent T cell growth, TGF- $\beta$ 2 is considered to be a candidate SLE susceptibility gene. Furthermore, SLE has been linked with a region to which the TGF- $\beta$ 2 gene has been mapped.

**Methods.** Association studies were performed in 3 case-control populations, from Spain, Turkey, and UK, using a TGF- $\beta$ 2 5'-untranslated region (5'-UTR) 4 base pair (bp) insertion polymorphism. Genotyping was performed using fluorescent labeled polymerase chain reaction product sizing.

**Results.** No significant differences were detected in TGF- $\beta$ 2 5'-UTR polymorphism allele frequencies between SLE patients and matched controls in the 3 populations studied.

**Conclusion.** The 4 bp insertion polymorphism within the TGF- $\beta$ 2 gene does not appear to be associated with SLE. However, this does not rule out the possible involvement of TGF- $\beta$ 2 in the disease pathogenesis. (J Rheumatol 2002;29:1189-91)

## Key Indexing Terms:

SYSTEMIC LUPUS ERYTHEMATOSUS

TRANSFORMING GROWTH FACTOR  $\beta$ 2

Systemic lupus erythematosus (SLE) is a heterogeneous autoimmune disease of unknown etiology that presents with a broad variety of symptoms involving multiple organ systems<sup>1</sup>. The etiopathogenesis is likely to be multifactorial, consisting of both genetic and environmental components. SLE is associated with a number of immune response abnormalities such as the appearance of autoantibodies, altered T cell function and defects of the complement system and phagocytosis.

A number of genetic linkage studies have been performed to identify SLE susceptibility regions<sup>2-4</sup>. Positive linkage of SLE to the 1q41-1q42 chromosomal region was shown in more than one study<sup>5</sup> and, potentially, several genes mapped to this region may play a role in SLE risk.

One of the genes in this region is transforming growth

factor- $\beta$ 2 (TGF- $\beta$ 2), a member of the TGF- $\beta$  family. TGF- $\beta$  is a family of multifunctional signalling molecules with immunoregulatory properties that regulate cellular proliferation and differentiation<sup>6</sup>. The TGF- $\beta$  isoforms 1, 2, and 3 signal through the same surface receptors and have similar cellular targets. Each isoform is expressed in a distinct pattern, which has been thought to be important in controlling their effects<sup>6</sup>.

TGF- $\beta$  are recognized as regulators of the inflammatory response that occurs in various types of animal models of autoimmune diseases, especially those mediated by excessive Th1 responses<sup>7,8</sup>. In a murine model of SLE, high levels of TGF- $\beta$  expressed by T cells, as a consequence of *Plasmodium* infection, have been found to delay the disease onset<sup>9</sup>. Mechanisms leading to protection in this model have suggested an involvement of T cell regulation. In addition, TGF- $\beta$  somatic gene therapy has been shown to decrease autoantibody production in the MRL-*lpr/lpr* murine SLE model<sup>10</sup>.

In patients with SLE, low levels of both total and active TGF- $\beta$  have been reported, and it has been suggested that this is more likely to be due to its decreased production, which did not seem to be secondary to other cytokine defects<sup>11</sup>. Whether the decreased levels reported are due to mRNA syntheses or translation rates has not been investigated. In the same study, it has been shown that the combination of TGF- $\beta$  and interleukin 2 abolish spontaneous IgG production in SLE patients with B cell hyperactivity.

The circumstantial evidence implicating TGF- $\beta$  in SLE

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and the colocalization of the TGF- $\beta$ 2 gene within an area of SLE linkage, 1q41<sup>12</sup>, suggests that it represents a good candidate for this condition.

We therefore tested the possible role of TGF- $\beta$ 2 in SLE susceptibility, using an insertion polymorphism in the 5'-UTR of the TGF- $\beta$ 2 gene<sup>13</sup> in a case-control association study of 3 populations. This is a common polymorphism that has been found to create a potential recognition sequence for hepatocyte nuclear factor 3 $\beta$  (HNF-3 $\beta$ ) and genesis, members of the winged helix family of transcription factors. However, no analysis was carried out to confirm its functional significance.

## MATERIALS AND METHODS

**Cases and controls.** Three cohorts of SLE patients were recruited for this study: UK Caucasoid SLE cases from the North West region (n = 90), Spanish cases from Barcelona (n = 111), and Turkish SLE patients from Istanbul (n = 92). All patients satisfied the 1982 revised American College of Rheumatology criteria for the classification of SLE<sup>14</sup>. Ethnically and geographically matched control groups were available from the UK (n = 187), Spain (n = 84), and Turkey (n = 90). Controls were recruited from either general practice registers or from healthy blood donor volunteers. Consent and samples were obtained from all cases and controls following the guidelines of the local ethics committees, and the study was performed in compliance with the principles of the Declaration of Helsinki.

**Genotyping.** Genomic DNA was extracted from EDTA anticoagulated whole blood using the DNAcetM MaxiBlood Purification System (Bioline kit, London, UK). The 1249\_1250insACAA polymorphism tested resides within the 5'-UTR of the gene, 109 base pairs (bp) away from the initiation codon (GenBank: M87843). The polymerase chain reaction (PCR) mixture contained 100 ng genomic DNA, 67 mM Tris-HCl (pH 8.8), 1.5 mM MgCl<sub>2</sub>, 16 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.1% Tween-20, 0.5 U Taq DNA polymerase (Bioline, London, UK), 200 mM dNTP and 0.5 mM of each primer (forward primer; 5'-TTTGGAACTACTGGCCTTC-3'; reverse primer; 5'-AGCGCTCAGCACACAGTAGT-3') in a 25  $\mu$ l reaction. The forward PCR primer was fluorescently labeled at the 5' end with 6-FAM. PCR was performed under the following conditions: 95°C for 5 minutes followed by 35 cycles denaturation at 95°C for 1 minute, annealing at 56°C for 1 minute and extension at 72°C for 1 minute. A final extension step was carried out at 72°C for 5 minutes.

Semi-automated analysis for genotyping was performed using an Applied Biosystems 377 DNA sequencer with Genescan analysis (Version 2.1) and Genotyper software (Version 1.1.1) (Applied Biosystems). Aliquots of the labeled PCR product were denatured and electrophoresed on 0.2 mm 4% polyacrylamide gels run for 2 hours at 3000 V and a running temperature of 51°C. ROX 400 size standard (Applied Biosystems) was included in every lane. Allele sizes were expressed as mobility units and

measured by Genescan 2.1 analysis software. The PCR product with the insertion was 240 bp and without was 236 bp.

**Data analysis.** Hardy-Weinberg equilibrium was examined to determine if the observed number of alleles did not differ significantly from that expected ( $p > 0.05$ ). Calculations were performed using Stata for Windows 95 (5.0). Allele and genotype frequencies were compared between cases and controls using chi-square analysis with the appropriate degrees of freedom.

## RESULTS

Genotype and allele frequencies in the 3 case control groups are presented in Table 1. The genotype frequencies satisfied Hardy-Weinberg equilibrium in all 3 groups. No significant differences were observed among the 3 case-control groups examined.

## DISCUSSION

No significant association was detected between TGF- $\beta$ 2 and SLE in any of the 3 populations tested, using a 5'-UTR polymorphism within the gene. Furthermore, no significant difference in genotypes was detected for renal versus non-renal patient subgroups (data not shown).

Given that TGF- $\beta$ 2 represents a potential candidate in a replicated region of SLE linkage on chromosome 1q41, it is important to consider how much confidence can be placed in this observed lack of association. The TGF- $\beta$ 2 gene spans approximately 100 kb and the polymorphism tested mapped to the 5' end of the gene. It has been reported that a complex disease polymorphism can be detected for an average distance of 40 kb on either side in case-control based studies<sup>15</sup>. Thus, if the polymorphism is not itself contributing to disease risk, it is likely that it could detect disease association with other functional polymorphisms within its physical proximity. Therefore, it is likely to detect association with other potential functional polymorphisms within the first part of the gene. In addition, the TGF- $\beta$ 2 less frequent (insert) allele frequency in the 3 populations varied between 22.6 and 30.6%, which is greater than an allele frequency previously shown to detect disease association (14%)<sup>15</sup>.

It would seem unlikely that our result represents a false negative finding for the first part of the gene for several reasons. First, the study performed had adequate statistical

Table 1. TGF- $\beta$ 2 genotypes and allele frequencies in 3 SLE populations.

	Spanish		UK		Turkish	
	SLE (n = 11)	Controls (n = 84)	SLE (n = 90)	Controls (n = 187)	SLE (n = 92)	Controls (n = 90)
Allele frequency						
1 (del) (%)	160 (72.1)	130 (77.4)	127 (70.6)	273 (73.0)	141 (76.6)	125 (69.4)
2 (ins) (%)	62 (27.9)	38 (22.6)	53 (29.4)	101 (27.0)	43 (23.4)	55 (30.6)
Genotype frequency						
1 (del) (%)	55 (49.5)	51 (60.7)	49 (54.4)	102 (54.5)	54 (58.7)	46 (51.1)
2 (ins/del) (%)	50 (45.1)	28 (33.3)	29 (32.2)	69 (36.9)	33 (35.9)	33 (36.7)
3 (ins) (%)	6 (5.4)	5 (6.0)	12 (13.3)	16 (8.6)	5 (5.4)	11 (12.2)

power to detect an odds ratio of at least 2.5 in each of the 3 populations tested [ $\alpha = 0.05$  (2 sided), power = 93% in UK, 83% in Spanish and 84% in Turkish]. Second, it is possible that a weaker association may have been missed with the numbers, but more confidence can be based in this lack of association given that 3 different populations have been studied.

In support of our finding, no association was previously found between SLE and a dinucleotide repeat within the TGF- $\beta$ 2 gene using 81 Caucasian nuclear families<sup>16</sup>. In that study another candidate gene (PARP) was mapped to the same interval and was proposed as being responsible for the significant linkage results, using a polymorphic CA dinucleotide repeat located within the promoter region of the gene. However, 3 independent studies have failed to replicate this result<sup>17-19</sup>, leaving the potential candidate or candidates accountable for linkage to 1q41-q42 interval yet to be determined.

In summary, the 4 bp insertion polymorphism within the TGF- $\beta$ 2 gene does not appear to be associated with SLE. However, this does not exclude the potential role that TGF- $\beta$ 2 may have in SLE pathology.

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