Fc receptor gamma subunit polymorphisms and systemic lupus erythematosus

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ABSTRACT

Objective: To investigate the possible association between Fc receptor (FcR) gamma polymorphisms and systemic lupus erythematosus (SLE).

Methods: We have investigated the full FcR gamma gene for polymorphisms using polymerase chain reaction (PCR)-single strand conformational polymorphism and DNA sequencing. The polymorphisms identified were genotype using PCR-restriction fragment length polymorphism. Systemic lupus erythematosus cases and controls were available from 3 ethnic groups: Turkish, Spanish and Caucasian. The study was conducted in the year 2001 at the Arthritis Research Campaign, Epidemiology Unit, Manchester University Medical School, Manchester, United Kingdom.

Results: Five single nucleotide polymorphisms were identified, 2 in the promoter, one in intron 4 and, 2 in the 3'UTR. Four of the 5 single nucleotide polymorphisms (SNPs) were relatively common and investigated in the 3 populations. Allele and genotype frequencies of all 4 investigated SNPs were not statistically different between cases and controls.

Conclusion: Fc receptor gamma gene does not appear to contribute to SLE susceptibility. The identified polymorphisms may be useful in investigating other diseases where receptors containing the FcR gamma subunit contribute to the pathology.

Saudi Med J 2004; Vol. 25 (10): 1445-1448

The Fc receptor (FcR) gamma subunit is a type 1 membrane protein, which is essential for assembly and signal transduction of the high-affinity receptor for IgE (FcεRI) and the low-affinity receptor for IgG (FcγRIII), associated with the high-affinity receptor for IgG (FcγRI) and the T cell receptor-CD3 complex.[1,2] In addition, it is important in transmembrane signal transduction by the FcαR.[3] Deletion of the FcRγ subunit affects phagocytosis of antibody-coated particles by macrophages, NK cell-mediated antibody-dependent cytotoxicity and mast cell-mediated allergic responses.[2] Systemic lupus erythematosus (SLE) is an autoimmune disease where immune complex-triggered inflammatory cascade plays a major role. T cells (CD4 and CD8) from SLE patients express increased levels of the FcRγ subunit.[4] In addition, Clynes et al,[5] have shown that immune complexes formation can be prevented from eliciting subsequent inflammatory responses by disrupting the FcRγ subunit gene in the New Zealand black or New Zealand white (NZB/NZW) mouse, a spontaneous model of lupus nephritis. In humans, genetic linkage analysis mapped a susceptibility locus to an interval on chromosome 1q23-1q25. The FcRγ gene is located to the same region, and is thus a potential candidate SLE susceptibility gene. The FcRγ subunit is located on chromosome 1q23[1] consists of 5 exons and spans 4 kilobases.[6] The first exon contains the

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Received 23rd November 2003. Accepted for publication in final form 11th May 2004.

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entire 5'-untranslated region and most of the leader peptide. Intron one is 2.6 kb, representing 67% of the total gene and it contains Alu sequences (**Figure 1**). The second exon encodes the last part of the leader peptide, the extracellular domain, the membrane region and the first part of the intracellular region. Exons 3, 4 and 5 encode the remainder of the intracellular tail. The entire 3'-untranslated sequence is in the fifth exon and the polyadenylation signal (AATAAA) is found 25 bp downstream the fifth exon. In this study, we have investigated 5 FcRγ gene single nucleotide polymorphisms (SNPs) in 3 different populations of SLE cases and healthy controls.

Methods. Three groups of SLE patients were recruited for this study: Caucasian SLE cases from the Northwest region of the United Kingdom, Spanish cases from Barcelona, Spain and Turkish SLE patients from Istanbul, Turkey. All SLE patients satisfied the 1982 revised ACR criteria for the classification of SLE.[7] Ethnically matched control groups were also recruited. Genomic DNA was extracted from ethylenediaminetetraacetic acid anticoagulated whole blood using the DNAce™ MaxiBlood Purification System (Bioline kit, London, UK). This work was conducted at the Arthritis Research Campaign, Epidemiology Unit, Manchester University Medical School, Manchester, United Kingdom.

Polymerase chain reaction-single strand conformational polymorphism (PCR-SSCP). The published DNA sequence of human FcRγ subuit (GenBank accession number M33196) was used to design the primers used. As shown in **Figure 1**, 3 pairs of primers were used to amplify 3 regions of the gamma chain (fragments A, B, and C), which covered most of the gene.

The PCR amplified products were digested with restriction enzymes to produce suitable fragments (200-300bp) for SNP detection by SSCP. Equal volumes of the digested product and denaturing solution (95% formamide, 0.1% bromophenol blue, 0.1% Xylene cyanol) were mixed and heated (to denature double stranded DNA) at 95^{0}C for 5 minutes. This was chilled on ice before being subjected to electrophoresis on a 6% polyacrylamide gel (acrylamide: bisacrylamide 29:1). Non-denaturing electrophoresis using Protean II Xi Cell (BIO-RAD) apparatus was performed at constant power (5W) for 3.5 hours. A circulating water jacket was used to maintain the gel system at a constant temperature of 4^{0}C. Following electrophoresis the gel was fixed in 50% methanol for 2 hours at room temperature with gentle agitation. The gel was washed quickly with

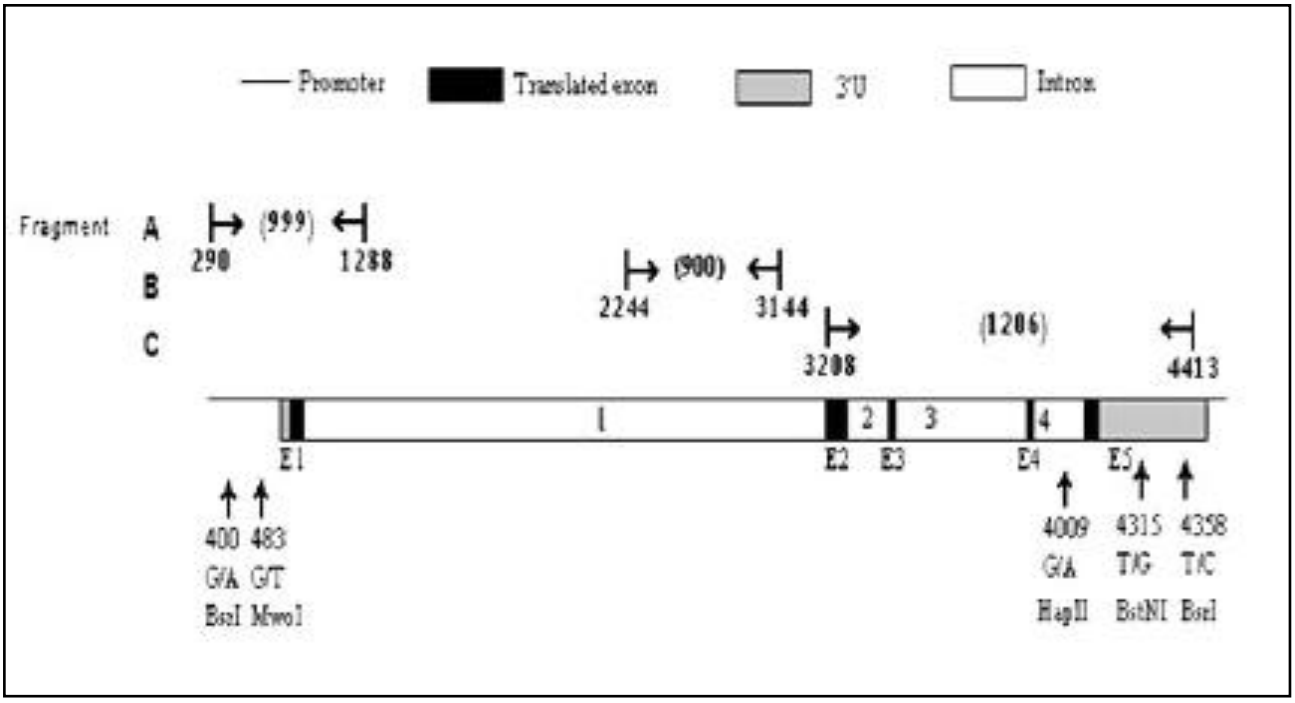

Figure 1 - The FcRγ subunit genomic organization with the primer design for polymerase chain reaction-single strand conformational polymorphism (PCR/SSCP) analysis and the identified polymorphisms sites. The gene regions are shown based on Kuster et al.[6] Horizontal arrows show the directions of primers with the position in nucleotide numbers. The nucleotide number is shown based on the sequence under accession number M33196. The size (base pairs) of the products (fragments A, B, and C) is shown in parentheses.

Table 1 - Single nucleotide polymorphisms (SNP) identified in the FcRγ subunit. Polymerase chain reaction-restriction fragment length polymorphism conditions and position (according to GenBank accession # M33196).

SNP	Region and nt Positions	Restriction enzyme	Primer : 5'-3'sequence	Size	Annealing temperature
G/A	Promoter, nt 400	BsrI	F2: 5'-CCT GAG CGT GAG AGT CTC CT-3'	999bp	56°C
G/T	Promoter, nt 483	MowI	R2: 5'-CCA GCT CTT CAT GCC ATA CA-3'		
T/G	3'U, nt 4315	BstNI	F4: 5'-CTG CAT GCC ATT AAC ACC AG-3'	229bp	56°C
T/C	3'U, nt 4358	BsrI	R4: 5'-GTC CAG TCC ATG GCA GTT TT-3'		
C/T	Intron 4, nt 4009	HpaII	F5: 5'-TCT GCA GAA ATC AGA TGG TGT T-3' R5: 5'-TCG TAA GTC TCC TGG TTC CTG-3'	217bp	56°C

Table 2 - Allele and genotype frequencies of the FcRγ subunit single nucleotide polymorphisms in 3 systemic lupus erythematosus (SLE) groups. Polymorphisms are numbered according to GenBank accession number M33196.

Allele and genotype frequencies	Spanish SLE n (%)	Spanish controls n (%)	Caucasian SLE n (%)	Caucasian controls n (%)	Turkish SLE n (%)	Turkish controls n (%)
Promoter position 483						
Allele frequency						
G	81 (79)	139 (72)	121 (80)	140 (77)	145 (77)	129 (71)
T	21 (21)	54 (28)	31 (20)	42 (23)	41 (23)	53 (29)
Genotype frequency						
GG	32 (63)	55 (62)	48 (63)	52 (57)	60 (57)	49 (54)
TT	2 (4)	5 (5)	3 (4)	3 (3)	8 (3)	10 (11)
GT	17 (33)	29 (33)	25 (33)	36 (40)	25 (40)	31 (34)
Intron 4 position 4009						
Allele frequency						
C	215 (96)	168 (93)	250 (96)	320 (95)	177 (95)	194 (93)
T	9 (4)	12 (7)	10 (4)	18 (5)	9 (5)	14 (7)
Genotype frequency						
CC	103 (92)	80 (89)	121 (93)	153 (91)	86 (93)	90 (87)
TT	0 (0)	2 (2)	1 (1)	2 (1)	2 (2)	0 (0)
TC	9 (8)	8 (9)	8 (6)	14 (8)	5 (5)	14 (13)
3'UTR position 4315						
Allele frequency						
T	243 (91)	251 (90)	257 (95)	145 (92)	178 (95)	165 (92)
G	25 (9)	29 (10)	14 (14)	13 (8)	10 (5)	15 (8)
Genotype frequency						
TT	109 (81)	112 (80)	120 (87)	67 (85)	86 (92)	75 (83)
GG	0 (0)	1 (1)	1 (1)	1 (1)	2 (2)	0 (0)
TG	25 (19)	27 (19)	17 (12)	11 (14)	6 (6)	15 (17)
3'UTR position 4358						
Allele frequency						
T	215 (83)	219 (79)	209 (84)	140 (87)	148 (79)	146 (81)
C	43 (17)	57 (21)	39 (16)	18 (11)	40 (21)	34 (19)
Genotype frequency						
T/T	91 (70)	89 (64)	89 (71)	61 (77)	57 (61)	58 (64)
C/C	5 (4)	8 (6)	4 (3)	0 (0)	3 (3)	2 (2)
T/C	33 (26)	41 (30)	31 (25)	18 (23)	34 (36)	30 (33)

distilled water, and was silver-stained for 15 minutes. Following staining the gel was washed twice for 5 minutes with 200 ml of deionized water. Silver staining of the single DNA strands was visualized using a developer that containing 1% Citric acid and formaldehyde. After adequate exposure, the gel was washed with distilled water and finally stop solution was added to halt the reaction.[8]

Identification of single nucleotide polymorphisms by direct sequencing. Samples showing variation in the SSCP banding patterns were DNA sequenced using the Big-dye sequencing kit (Applied Biosystems).

Polymerase chain reaction conditions for single nucleotide polymorphisms typing. Five SNPs were identified. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) methods were developed to detect these polymorphisms. Polymerase chain reaction was performed under the following conditions: 95^{0}C for 5 minutes followed by 35 cycles denaturation at 95^{0}C for 1 minute, annealing at 56^{0}C for 1 minute and extension at 72^{0}C for 1 minute. A final extension step was carried out at 72^{0}C for 5 minutes. The primer sequences details are listed in **Table 1**.

Restriction fragment length polymorphism analysis. Seven µl of each PCR product were digested overnight with 4U of the appropriate enzyme (New England Biolabs, UK) followed by electrophoresis on agarose gel for separating the restriction fragments. The specific enzymes for SNPs genotyping are given in **Table 1**.

Data analysis. Hardy-Weinberg equilibrium was examined to determine if the observed number of alleles did not differ significantly from those expected (p>0.05). Calculations were performed using Stat for Windows 95 (5.0). Allele and genotype frequencies were compared between cases and controls using x^2 analysis with the appropriate degrees of freedom.

Results. ***Identification of polymorphisms.*** Five polymorphisms have been identified (**Figure 1**). Two polymorphisms in the promoter, one in intron 4 and 2 in exon 5, located within the 3'-untranslated domain. All 5 polymorphisms are listed in **Table 1** and the positions are given on the bases of their location in the GenBank M33196 sequence. The genotype, and allele frequencies for each polymorphism in 3 case control groups are presented in **Table 2**. The frequencies of all 4 biallelic genotype polymorphisms satisfied the Hardy-Weinberg equilibrium. None of the polymorphisms showed significant association with SLE. The FcRγ subunit BsrI- promoter SNP was very rare, at position 400, therefore it was not genotype in 3 population.

Discussion. Fc receptor γ subunit is important for the assembly and signal transduction of the FcRs, FcεR and FcγR.[1] Its coding gene is located within the SLE linkage area on chromosome 1, thus making it a strong potential candidate susceptibility gene in SLE. We have investigated SNPs in the 5' and 3' end, all the exons, intron 2-4 and part of intron 1. We have identified 5 SNPs within the gene. None of 5 polymorphisms identified found in the coding region or known conserved sequences. However, the first promoter polymorphism is found immediately following the GC box and it is uncommon (1%). Thus, it is rather referred to as a mutation. The second polymorphism in the promoter is the highest in heterozygosity (54-64%) compared with the other 3 typed polymorphisms, which makes it the most informative.

In order to examine the role of the FcR γ subunit gene in SLE etiology, we performed a case-control association study in 3 populations, Turkish, Spanish and Caucasian. The 4 genotype SNPs were present in all 3 populations investigated, and were found at a very similar frequency. Allele frequencies were not significantly different between cases and controls. No significant association were detected with SLE in any of the 3 populations, suggesting that this gene is unlikely to be SLE susceptibility gene.

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