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# Fc γ R IIB gene polymorphisms in Indian systemic lupus erythematosus (SLE) patients

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***Background & objectives*: Receptors for the Fc fragment of immunoglobulin G (Fc γ Rs) represent the link between humoral and cellular immune responses. Polymorphisms in Fc γ Rs have been identified as genetic factors influencing susceptibility to various autoimmune diseases. This study was aimed to identify *Fc γ R IIB* genotypes in Indian systemic lupus erythematosus (SLE) patients and to correlate these with clinical presentation and autoantibody profile.**

***Methods*: Eighty consecutive clinically diagnosed SLE patients were included. SLE patients were classified according to the American College of Rheumatology (ACR) criteria. Disease activity was assessed by Systemic Lupus Erythematosus Disease Activity Index (SLEDAI). PCR-RFLP method was used to detect Fc γ R IIB polymorphism.**

***Results*: Of the 80 SLE patients, 53 were LN and 27 were SLE without nephritis. The mean SLEDAI score at evaluation was 6.5 ± 5.8. Among SLE patients *Fc γ R IIB* genotype frequency was 61.2 per cent for Ile/Thr, 20.0 per cent for Thr/Thr and 18.8 per cent for Ile/Ile as compared to 65, 12.5 and 22.5 per cent respectively among normal population. There was no significant difference for *Fc γ R IIB* genotypes between SLE and normals. The allele frequency for Thr allele in SLE patients was slightly higher (0.51) than in normals (0.45). Thr allele frequency in LN patients was slightly higher (0.53) than in SLE patients without nephritis (0.49). Though a higher percentages of symptoms like renal manifestations (81.3%), arthritis (62.5%) and oral ulcer (56.3%) were noted in patients with Thr/Thr genotypes, no significant difference was noted when these patients were compared with Ile/Ile and Ile/Thr genotypes.**

***Interpretation & conclusions*: The findings of this study indicate towards an involvement of Thr allele with SLE disease severity and clinical presentation in Indian SLE patients. Future study on a large sample is needed to support this finding to understand the association of *Fc γ R IIB 232Thr/Thr* genotype as a susceptibility factor in SLE.**

**Key words** Autoantibodies - Fc γ RIIB genotypes - lupus nephritis - SLE without nephritis - systemic lupus erythematosus (SLE)

Systemic lupus erythematosus (SLE) is a prototype autoimmune disease of multifactorial origin. The age of onset is between 16 and 55 yr and there is a higher frequency of SLE in women typically during their child bearing years[1]. Aetiopathogenesis of SLE involves one or more immune mechanisms like autoantibody production, complement activation, multiple inflammation, immune complex deposition and organ tissue damage. The pathogenesis of SLE has a strong and complex genetic base, the strongest

evidence points to HLA, Fcγ receptors, complement and tumour necrosis factor (TNF) polymorphisms as factors operative in SLE pathogenesis. Numerous specific candidate genes have been identified and most relate to the handling of immune complexes or antigen presentation[2].

Human type II low affinity receptor of immunoglobulin G (Fc γ R II, CD32) is encoded by three highly homologous genes (*Fc γ R IIA*, *Fc γ R IIB* and *Fc γ R IIC*) clustered on chromosome 1q23. *Fc γ R II B* is expressed on B cells and on myeloid lineage effector cells such as monocytes, macrophages, myeloid dendritic cells, neutrophils, eosinophils and mast cells. *Fc γ R II B* is not expressed on T cells and natural killer (NK cells)[3]. The basic structure of *Fc γ RII* consists of two extracellular Ig like domains, a transmembrane TM region and a cytoplasmic tail. *Fc γ R IIA* and *Fc γ R IIC* contain an activating signal motif (immunoreceptor tyrosine-based activation motif, ITAM) on their cytoplasmic tails, whereas *Fc γ R IIB* contains a unique immunoreceptor tyrosine based inhibitory motif (ITIM)[4]. *Fc γ R IIB* encodes for receptor expressed on B cells and monocytes which is an inhibitory receptor for B cell receptor (BCR) signaling and is considered to be highly relevant to the pathogenesis of SLE. *Fc γ R IIB* deficient mice have been shown to become susceptible to lupus like disease and some lupus prone mice have shown to have polymorphism in the *Fc γ R IIB* gene[5,6]. Polymorphisms of *Fc γ R IIB* in mice have been reported to be associated with SLE and target disruption of *Fc γ R IIB* renders mice susceptible to induced or susceptible autoimmunity, depending on the genetic background. In mice the inhibitory signaling cascade via Fc γ R IIB is crucial for the suppression of autoimmunity[7].

The present study was designed to identify *Fc γ R IIB* genotypes in Indian SLE patients and to find their association with clinical presentation of the disease and autoantibody profile in lupus nephritis (LN) and SLE without nephritis patients.

## Material & Methods

This cross-sectional study was conducted in 80 SLE patients (74 females, 6 males) selected consecutively from the Rheumatology, Dermatology and Nephrology departments of KEM hospital, Mumbai, India, for a period of two years (2006-2008). All these patients were diagnosed according to the American College of Rheumatology (ACR) criteria[8]. The study protocol was approved by the Institute's Ethics Committee approval and a written consent was obtained from all the patients. The disease activity was assessed using the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI)[9]. Thirty seven patients (46.3%) met 5 to 8 ACR criteria and remaining 43 patients (53.7%) met more than 8 ACR criteria at the time of evaluation. The mean age was 27.5 ± 9.52 yr and mean duration of SLE disease was 6.5 ± 3.0 months before evaluation. The mean SLEDAI score at clinical evaluation was 6.5 ± 5.8. Based on the renal histopathology, 53 patients (66.3%) were LN and remaining 27 patients (33.7%) were SLE without nephritis. Renal histopathology revealed that among LN patients, 16 patients (30.2%) were focal proliferative glomerulonephritis (FPGN) (Type III), 34 patients (64.2%) were diffuse proliferative (DP) GN (Type IV) and remaining 3 patients (5.7%) were memberanoplioiferative glomerulonephritis (MPGN) (Type V).

Normal control group consisted of 80 age and sex matched healthy blood bank donors (70 females and 10 males) with mean age 25.4 ± 8.5 yr. After blood collection, serum was stored in aliquots at -80$^0$C until tested. Renal biopsies of LN cases were examined by light microscopy using hematoxylin, eosin, periodic acid Schiff (PAS) staining. Immunofluorescence microscopy was done using anti-IgG, anti-IgM, anti-IgA, anti-C3, anti-C4 and anti-fibrinogen fluorescein isothiocyanate conjugate (FITC). In LN patients the renal histology was classified according to WHO criteria[10]. Anti-nuclear antibodies (ANA) were tested using Bio-Rad Laboratories, USA kit. Confirmation of unusual ANA patterns was done using a Confocal Laser Scanning Microscope (LSM -510, Karl Zeiss, Germany). Anti-dsDNA and anti-neutrophil cyto plasmic antibody (ANCA) were detected by Euroimmune kits (Germany) using Nikon Optiphot II fluorescence microscope (Germany). Anti-C1q antibodies and anti-histone antibodies were detected by ELISA kits (Euroimmune, Lubeck, Germany).

The genomic DNA was extracted from the peripheral blood mononuclear cells of SLE patients and healthy normal individuals using the standard protocol[11]. DNA samples were stored at -20$^0$C until the genotype analysis was performed.

*Genotyping for Fc γ R IIB*: FcγRIIB-Ile232Thr polymorphism was genotyped by PCR-restriction fragment length polymorphism (RFLP) method[12]. A 100 bp fragment was amplified using the specific sense and anti-sense primer pair 5'- TGC CTG TCC TGA TGT CTG TC 3' and 3' CAG CAA CAA TGG

**Table I.** Distribution of *Fc γ R* IIB genotypes in SLE patients and control group

| Fc γ R IIB | Total SLE patients (n= 80) | Lupus nephritis (LN) (n=53) | SLE without LN (n=27) | Normal (n= 80) |
|---|---|---|---|---|
| *Genotype*: | | | | |
| Ile/Ile | 15 (18.8) | 10 (18.8) | 5 (18.5) | 18 (22.5) |
| Ile/Thr | 49 (61.2) | 30 (56.7) | 18 (66.6) | 52 (65) |
| Thr/Thr | 16 (20.0) | 13 (24.5) | 4 (14.8) | 10 (12.5) |
| *Genotype frequencies*: | | | | |
| Ile/Ile | 0.19 | 0.19 | 0.18 | 0.23 |
| Ile/Thr | 0.61 | 0.57 | 0.67 | 0.65 |
| Thr/Thr | 0.20 | 0.24 | 0.15 | 0.12 |
| *Allele positivity*: | | | | |
| IIB Ile | 64 | 40 | 23 | 70 |
| IIB Thr | 65 | 43 | 22 | 62 |
| *Allele frequencies*: | | | | |
| IIB Ile | 0.49 | 0.47 | 0.51 | 0.55 |
| IIB Thr | 0.51 | 0.53 | 0.49 | 0.45 |

Values in parentheses are percentages

**Table II.** Distribution of Fc γIIB genotypes and SLEDAI in SLE patients (n= 80)

| Fc γIIB genotype | Mild (<8) | Moderate (8-18) | Severe (> 18) |
|---|---|---|---|
| Ile/Ile (n= 15) | 3 (20) | 7 (46.7) | 5 (33.3) |
| Ile/Thr (n=49) | 12 (24.5) | 30 (61.2) | 7 (14.3) |
| Thr/Thr (n= 16) | 3 (18.7) | 7 (43.8) | 6 (37.5) |
| Total (n=80) | 18 (22.5) | 44 (55.0) | 18 (22.5) |

Values in parentheses are percentage

**Table III.** Association of the distribution of Fc γ IIB genotypes with the clinical presentation and autoantibodies in SLE (n=80)

| Organ involvement (%) | Total SLE (n=80) | Ile/Ile (n=15) | Ile/Thr (n=49) | Thr/Thr (n=16) |
|---|---|---|---|---|
| Rash (62.5) | 50 | 7 | 35 | 8 |
| Photosensitivity (52.5) | 42 | 6 | 32 | 9 |
| Oral ulcers (22.5) | 18 | 5 | 4 | 9 |
| Arthritis (56.3) | 45 | 8 | 27 | 10 |
| Serositis (15) | 12 | 2 | 6 | 4 |
| Renal (66.3) | 53 | 10 | 30 | 13 |
| Haematological (12.5) | 10 | 3 | 3 | 4 |
| Neurological (6.3) | 5 | 1 | 2 | 2 |
| *Autoantibodies* (%): | | | | |
| ANA (100) | 80 | 15 | 49 | 16 |
| Anti-dsDNA (90) | 72 | 13 | 48 | 12 |
| ANCA (28.8) | 23 | 5 | 10 | 8 |
| Anti-histone (35) | 28 | 8 | 12 | 8 |
| Anti-C1q (80) | 64 | 12 | 40 | 12 |

ANA, anti-nuclear antibodies; ANCA, anti-neutrophil cytoplasmic antibodies

CCG CGA CAG CA 5' after a 4 min incubation at $96^0$C; 35 cycles of PCR were performed ($96^0$C for 30 sec, $60^0$C for 30 sec and $72^0$C for 90 sec), followed by a 7 min extension at $72^0$C until genotype analysis was performed following a standard protocol. PCR was performed in 25 µl volumes. Ten µl of the PCR product was incubated with *Pshal* restriction endonuclease (New England Biolabs, England) at $37^0$C overnight. PCR fragments containing 232 Thr/Thr are digested into two fragments (100, 22 bp), whereas PCR fragments containing 232 Ile/Thr are digested into three fragments (100, 78 and 22 bp), the 22 bp fragments were not visible on 3 per cent agarose gel, therefore, these were visualized on 10 per cent PAGE.

*Statistical analysis*: Continuous variables were expressed as mean ± SD. Pairs of groups were compared using student 't' test for normally distributed continuous variables. Chi square test was used for the categorical variables if needed.

## Results

*Distribution of Fc γ R IIB genotypes*: Among SLE patients' 15 (18.8%) were Ile/Ile homozygous, 16 (20%) showed Thr/Thr homozygous and 49 (61.2%) patients were Ile/Thr heterozygous. Normal individuals showed 22.5, 12.5 and 65 per cent distribution, respectively. The difference between *Fc γ R IIB* genotype distribution in SLE and normals was not significant. The distribution of *Fc γ RIIB* genotypes in LN and SLE without nephritis is shown in Table I. The allele frequency for Thr allele in SLE

**Table IV.** *Fc γ R IIB* genotypes (%) in normal population and SLE patients in various population of the world

| Population | Normals | | | SLE | | |
|---|---|---|---|---|---|---|
| | Ile/Ile | Ile/Thr | Thr/Thr | Ile/Ile | Ile/Thr | Thr/Thr |
| Japanese[14] | 60.4 | 34.3 | 5.3 | 54.9 | 34.2 | 10.9 |
| African American[15] | 53 | 36 | 11 | 61.0 | 31.0 | 9.0 |
| Thai[16] | 58.8 | 33.9 | 7.3 | 48.1 | 36.7 | 15.2 |
| Caucasian[17] | 77 | 20 | 3 | 76.0 | 20 | 4.0 |
| Chinese[18] | 60 | 35.3 | 4.7 | 45.4 | 44.4 | 10.2 |
| Taiwanese[19] | 55 | 39 | 6.0 | 54.0 | 35.0 | 11.0 |
| Present study | 22.5 | 65 | 12.5 | 18.8 | 61.2 | 20.0 |

was slightly higher (0.51) than in normals (0.45). LN patients showed Thr allele frequency (0.53) which was slightly higher than in SLE patients without nephritis (0.49). Based on the SLEDAI scores it was observed that 22.5 per cent had severe disease (SLEDAI > 18), 55 per cent had moderate disease (SLEDAI 8-18) and the remaining 22.5 per cent had mild disease (SLEDAI <8) (Table II). It was observed that among Ile/Thr heterozygous patients, 61.2 per cent had moderate disease (SLEDAI 8-18) and among Thr/Thr homozygous patients, 37.5 per cent had severe disease activity (SLEDAI > 18), 43.8 per cent had moderate disease and remaining 18.7 per cent had mild disease (SLEDAI <8) indicating the involvement of Thr allele in severity of disease activity.

*Fc γ R IIB genotypes with clinical manifestations and autoantibodies in SLE*: The association between *Fc γ R IIB* genotypes and clinical features according to ACR criteria were analyzed. Though a higher percentages of symptoms like renal manifestations (81.3%), arthritis (62.5%) and oral ulcer (56.3%) were noted in patients with Thr/Thr genotypes, there was no statistically significant difference noted when these patients were compared with Ile/Ile and Ile/Thr genotypes. Profiling of autoantibodies showed positivity for ANA in all patients (100%), 90 per cent had anti-dsDNA, 28.8 per cent had ANCA, 35 per cent had anti-histone antibodies and 80 per cent had anti-C1q antibodies (Table III).

## Discussion

The low affinity receptor Fc gamma receptor IIB (Fc γ R IIB) is an immunoreceptor tyrosine-based inhibitory motif (ITIM) in the cytoplasmic domain that downregulates humoral immune responses and modulates the risk of autoimmunity in humans and in experimental animal models[13]. Fc γ R IIB has B cell inhibitory receptor function in the disease onset, progress and severity of the disease and it plays crucial role in the maintenance of immune tolerance. Variations in *Fc γ R IIB* expression and function could have profound effects on modulation of B cell activity and immune phenotypes in SLE. Our study showed that the allele frequency for Thr allele in Indian SLE patients was slightly higher than in normals and Thr allele frequency in LN patients was slightly higher than in SLE patients without nephritis. Table IV gives various reports available on *Fc γ R IIB* genotypes in normal population and SLE patients worldwide, and in Indian SLE patients. Also, a higher percentages of symptoms like renal manifestations, arthritis and oral ulcer were noted in patients with Thr/Thr genotypes, there was no statistically significant difference noted when compared with Ile/Ile and Ile/Thr genotypes. Though our preliminary study indicates an involvement of Thr allele as a susceptible factor in SLE, a larger study is needed to support this finding for the possible association of Fc γ R IIB 232Thr/Thr genotype and clinical presentation of the disease.

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