FcγRIIa polymorphism in systemic lupus erythematosus

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Abstract

***Objectives*—Polymorphism of the phagocyte IgG receptor FcγRIIa may modulate immune complex mediated inflammation, particularly when immune complexes contain IgG2. Previous studies suggest that this polymorphism may be an important risk factor for lupus nephritis. FcγRIIa is biallelic, the alleles R and H each having a gene frequency of about 50%. Nephritis has been associated with an increased frequency of the R allele. The frequency of common FcγRIIa alleles was examined in white subjects from the United Kingdom and Greek subjects with systemic lupus erythematosus (SLE) and healthy controls.**

***Methods*—FcγRIIa genotyping was performed using a single step polymerase chain reaction technique, which differentiates the two major alleles, R and H. Two study populations were examined: (a) white subjects from the United Kingdom : 66 controls and 81 with SLE (19 of whom had renal disease) and (b) Greek: 52 controls and 42 with SLE (19 with renal disease).**

***Results*—No significant relation was observed between FcγRIIa genotype and susceptibility to SLE or SLE nephritis.**

***Conclusions*—The FcγRIIa R allele does not seem to be associated with SLE (with or without renal disease) in our United Kingdom white or Greek populations.**

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Immunoglobulins can interact with the effector mechanisms of the immune system by activation of the complement cascade or by direct binding to effector cells via receptors for the Fc region of the immunoglobulin heavy chain. Families of Fc receptors exist for each of the immunoglobulin isotypes. Fc receptors with specificity for IgG (FcγR) fall into three sub-types: FcγRI (CD64), a high affinity receptor capable of binding monomeric IgG, and the FcγRII (CD32) and FcγRIII (CD16) receptor families, both of lower affinity capable of binding multimeric IgG in immune complexes or IgG opsonised particles.[1] The A isoform of human CD32 (FcγRIIa) is the only receptor on human phagocytic cells capable of significant interaction with IgG2.[2] As IgG2 is also a poor activator of the classic complement pathway, FcγRIIa represents the only means for interaction of IgG2 with the inflammatory response, other than activation of the alternative complement pathway by large insoluble immune complexes. Two common allotypic variants of FcγRIIa have been described, differing in their affinity for human IgG2 and, to a lesser extent IgG3. The molecular basis for this allotypic variation has now been defined. Table 1 summarises the phenotypic and genotypic differences between the two alleles. The amino acid at position 131 is thought to be critical for interaction with IgG.[3–5]

This polymorphism may play a particular part in the expression of antibody responses mediated by IgG2. Previous studies have suggested that this CD32 polymorphism may influence susceptibility to infection by capsulate bacteria. IgG2 antibodies, directed against capsular polysaccharides, seem to be critical for defence against organisms such as pneumococci. People with low values of anti-polysaccharide antibodies may fail to opsonise these organisms if they possess the R form of FcγRIIa, but opsonise adequately if they have the H form.[6 7]

CD32 polymorphism may also potentially affect the expression of disorders in which immune complex deposition plays a pathogenic part, such as systemic lupus erythematosus (SLE), with the clearance of IgG2 immune complexes (and to a lesser extent IgG3 complexes) being impaired in RR homozygous people. Two recent studies have suggested that FcγRIIa RR homozygosity may constitute a risk factor for lupus nephritis in both African Americans[8] and European white populations,[9] although a further study has found no association in white, Afro-Caribbean, and Chinese subjects.[10] We have examined CD32 polymorphism in a Greek population and a further white population with SLE.

We have examined the role of CD32 polymorphism using a novel single step polymerase chain reaction method.

Methods

STUDY POPULATION

We determined FcγRIIa genotypes in two study populations: (a) UK white population (from the north west of the United Kingdom): 66 controls and 81 with SLE (19 of whom had renal disease) and (b) Greek: 52 controls and 42 with SLE (19 with renal disease). Renal disease was defined by ACR criteria (>500 mg/24 h proteinuria or cellular casts on urine microscopy). The age/sex distribution was similar in both populations. Further demographic and clinical details are available from the authors.

FCγRIIA GENOTYPING

FcγRIIa genotyping was performed using a single step allele specific polymerase chain

Table 1 Comparison of the two major alleles of FcγRIIa. The affinity for IgG3 is slightly higher for the H-131 allele

Allele	Affinity for human IgG subclasses	Amino acid differences	Corresponding nucleotide differences
R-131	IgG3=IgG1>>IgG2>IgG4	27 Glu 131 Arg	236/7 CA 519 G
H-131	IgG3>IgG1=IgG2>>IgG4	27 Tyr 131 His	236/7 TG 519 A

Table 2 FcγRIIa R and H gene frequencies and genotypes in patient groups and controls. Genotype frequencies expressed as numbers of patients in each group (percentage in brackets)

	Number	H	R	HH	RH	RR
Greek controls	52	0.62	0.38	20 (39)	24 (46)	8 (15)
Greek SLE (all)	42	0.52	0.48	14 (33)	16 (38)	12 (29)
Greek SLE with nephritis	19	0.5	0.5	5 (26)	9 (48)	5 (26)
UK controls	66	0.47	0.53	12 (18)	38 (58)	16 (24)
UK SLE (all)	81	0.43	0.57	10 (12)	49 (60)	22 (27)
UK SLE with nephritis	19	0.52	0.48	5 (26)	10 (52)	4 (21)

reaction method, which allows rapid detection of the single base polymorphism at base 519. Two separate polymerase chain reactions were performed for each sample: in each reaction primers were used to amplify a 398 base pair segment of the fourth exon on the FcγRIIa gene. This spanned the site of the point mutation at base 519. These primers were used together with one of two mutation specific nested primers, which differed only in the final base at the 3' end of the primer, with specificity for the position +519 point mutation. These nested primers, generated a 224 base pair allele specific product. The presence of the 398 base pair fragment generated by the flanking primers acts as a positive control for the reaction.

The polymerase chain reaction products were run on a 2% agarose gel stained with ethidium bromide and visualised by UVA illumination.

Primer sequences were as follows: flanking primer forward: GAAAAACCCTTGGAATC, flanking primer reverse: TCTCAGACCTCCATGTAG, R alleles pecific: AATCCCAGAAATTCTCCCG, H allele specificAATCCCAGAAATTCTCCCA.

Polymerase chain reaction conditions were as follows: 95°C for five minutes (one cycle), and then 35 cycles of: 95°C for 45 seconds, 50°C for 45 seconds, 72°C for 45 seconds, and finally 72°C for five minutes (one cycle).

To ensure the allele specific primers 32-H and 32-R were promoting amplification of the correct alleles, sequencing analysis was carried out on four subjects (two homozygotes HH, one heterozygote RH, and one homozygote RR). The two flanking primers were used to make sufficient quantity of the 398 base pair product on which the sequencing reaction was carried out using the Taq Dye Deoxy Terminator Cycle Sequencing Kit. A fifth primer (CTGGTCAAGGTCACATTC, located 40 base pairs 5' upstream from the allele specific primers) was used in the sequencing reaction, which enabled the detection of the nucleotide present at the polymorphic site (base 519).

STATISTICS

The χ^2 test was used to examine skewing of genotype frquencies between patient and control groups (using 2 × 3 contingency tables). Individual genotype frequencies were compared using odds ratios and 95% confidence intervals calculated using Epi-info v.6 software.

Results

The polymerase chain reaction method was found to give repeatable results and the results of the method were validated by sequencing in four subjects (two HH homozygotes, one HR heterozygote, and one RR homozygote).

Table 2 shows the genotype frequencies in the patient groups and controls. R/H genotype frequencies have not been reported previously in a Greek population. The observed frequencies are comparable to those found in other European populations.[6–10] The genotype frequencies were not significantly different from those predicted from the gene frequnecy by the Hardy-Weinberg equation.

No significant difference in distribution of RR, RH, and HH genotypes was seen between controls and patients with SLE (UK SLE versus controls: χ^2=0.9998, df=2, p=0.61. Greek SLE versus controls: χ^2=2.42, df=2, p=0.30). Sub-group analysis also showed no relation between the R allele and lupus nephritis in either ethnic group (UK SLE nephritis: χ^2=0.616, df=2, p=0.74. Greek SLE nephritis versus controls: χ^2=1.496, df=2, p=0.47). A small excess of RR homozygotes was seen in the Greek SLE patients but this was not significant (total SLE versus controls: OR 1.48, 95%CI 0.94, 2.33). In the UK white population, the proportion of HH homozygotes was increased in the SLE nephritis group. This difference also failed to reach significance (SLE nephritis versus controls: OR 1.2, 95% CI 0.35, 7.5)

Discussion

The FcγRIIa polymorphism at base +519/amino acid 131 does not seem to influence susceptibility to SLE in UK white or Greek subjects. In contrast with previous studies,[8–10] our results do not offer any support to the hypothesis that RR homozygosity is an important risk factor for renal disease in SLE. This is in contrast with the findings of Duits and colleagues[9] who found a significant excess of RR homozygotes among European white patients with SLE complicated by renal disease, but in agreement with two further studies of North American and UK patients with lupus nephritis.[8 10] Although Salmon and colleagues[8] found no association in a US white population they did show a strong association between possession of FcγRIIa R alleles and lupus nephritis in African-Americans. However, Botto and colleagues[10] found no association with renal disease in Afro-Caribbean subjects resident in the UK. How can these differences be resolved? Type I and type II errors cannot be excluded. However, our sample size should be sufficient to exclude a genetic relative risk of 3.5 for the RR homozygous genotype with regard to SLE with a power of 80% at the 95% significance (a relative risk less than that found by Salmon *et al* in African-American SLE patients). Alternatively, the

differences may reflect ethnic variation in genetic susceptibility in SLE, which in turn may contribute to the heterogeneity in disease phenotype seen between different ethnic groups; African-Americans tend to have more severe lupus than white people with a higher frequency of nephritis. Ethnic variation in genetic association with disease may reflect population differences in allele frequency rather than any difference in mechanism of disease; for example, the association of rheumatoid arthritis with class II alleles bearing the QKRAA/QRRAA shared epitope varies depending upon which class II allele commonly carries the shared epitope in the study population.[11] However, the FcγRIIa R and H allele frequencies are comparable in healthy white people, African-Americans, and Afro-Caribbeans. The differences in association of R and H alleles with SLE may therefore reflect heterogeneity in disease pathophysiology between these ethnic groups. The functional differences between R and H FcγRIIa alleles are most pronounced in terms of interaction with IgG2. This is of particular biological significance as no other Fc receptor interacts significantly with IgG2, and this IgG subclass is a poor activator of the classic complement pathway. This suggests that differential handling of immune complexes containing predominantly IgG2 autoantibodies may underlie the reported association of FcγRIIa polymorphism with SLE and nephritis in African-Americans. Most autoantibodies found in lupus are, however, considered to be of IgG1 or IgG3 subclass,[12] with the exception of anti-C1q antibodies.[13] It is of particular interest that anti-C1q antibodies have been implicated in the pathogenesis of lupus nephritis. Most of the data on autoantibody IgG subclass are derived from white populations and no systematic comparison has been made between different ethnic groups. It is also not clear whether anti-C1q antibodies occur more frequently in African-Americans. Further studies are required of CD32 polymorphism, autoantibody production, autoantibody IgG subclass, and disease expression in lupus patients of differing ethnic background.

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