CONCISE REPORT

The F158V polymorphism in FcγRIIIA shows disparate associations with rheumatoid arthritis in two genetically distinct populations

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Objectives: To investigate the association of the FcγRIIIA gene with rheumatoid arthritis (RA) in two genetically distinct groups: a white group from the United Kingdom and a northern Indian group.
Methods: The distributions of the two alleles of the FcγRIIIA F158V polymorphism were determined in 398 white patients from the United Kingdom and 63 Indian patients with RA and compared with those from 289 United Kingdom and 93 Indian healthy controls, respectively.
Results: Among the Indian patients, the frequency of the rare 158V allele and the proportion of 158VV homozygotes were reduced (relative risk (RR)=0.3, 95% confidence interval (95% CI) 0.1 to 1.1, p<0.06), reaching statistical significance for carrying the 158VV phenotype relative to 158FV or FF (RR=0.2, 95% CI 0.05–0.9, p<0.02). Conversely, no significant deviation in allelic frequencies was noted between the patients and controls from the United Kingdom.
Conclusions: The 158VV phenotype showed a weak protective effect against developing RA in the Indian group. However, this sample was small (resulting in a low power for statistical analysis) and no independent confirmation was found in the larger white United Kingdom group. Thus the FcγRIIIA locus is unlikely to be of major importance in causing RA.

Rheumatoid arthritis (RA) is thought to have an important genetic component, with heritability estimated at around 60%.[1] An oligogenic contribution is suspected,[2] but to date only the HLA-DRB1 locus, contributing up to 40% of the genetic component of the disease, has been identified with certainty.

Rheumatoid arthritis is characterised by inflammation in the synovial joints and the presence of rheumatoid factor (RF)—autoantibodies directed against the (Fc) region of IgG—in the peripheral blood and the synovial fluid.[3] IgG rheumatoid factors in particular have been associated with severe disease. These autoantibodies can self associate into immune complexes which, through the interaction with their receptors, trigger inflammatory events and have been implicated in the pathogenesis of RA.[3]

The receptors for IgG recognise the Fc region of the immunoglobulin and divide into three main classes: FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16), all of which are encoded at loci on chromosome 1q21–24. CD16 has two forms, IIIA and IIIB, encoded by the highly homologous FcγRIIIA and FcγRIIIb genes. The Fcγ receptor IIIA (FcγRIIIA) is a transmembrane molecule of moderate affinity, involved in signal transduction on binding to the Fc region of IgG.[4] It is expressed on the surface of natural killer (NK) cells, macrophages, differentiating monocytes, and γ/δ T cells and is the key mediator in some immune defence functions including degranulation, phagocytosis, antibody dependent cytotoxicity (ADCC), transcription of cytokine genes, and release of inflammatory mediators.[4] Studies of FcγRIIIA deficient mice have shown an important role for this receptor in inflammatory responses and immune complex mediated disease.[5]

A single nucleotide polymorphism exists at position 559 (T/G) of the FcγRIIIA molecule,[6] which results in a phenylalanine (F) to valine (V) substitution at residue 158 (or 176 in some publications). It has been reported that IgG stimulation of NK cells from FcγRIIIA-158Val homozygous people (158VV) results in higher Ca^{2+} influx, higher concentrations of interleukin-2 (IL2) receptor (CD25) expression, and reduced survival of NK cells after activation induced cell death when compared with 158FV heterozygotes or 158FF homozygotes.[7] IgG binding studies have also shown that NK cells from 158VV homozygotes have a higher affinity for binding IgG than NK cells from 158FV or 158FF donors, so the 158F and 158V variants have respectively been designated the low and high binding affinity alleles. A gene-dosage effect was also found, with the NK cells from 158FV heterozygotes showing intermediate levels of IgG binding.[8]

These results indicate a functional significance of the FcγRIIIA F158V transition which may have implications for the aetiology of autoimmune diseases. Association has been reported between the homozygosity for the low binding variant of FcγRIIIA (158FF) and susceptibility to systemic lupus erythematosus (SLE) in white people[9] and Hispanic subjects,[10] although this was not confirmed in Korean[11] and Japanese people.[12]

A recent study of this polymorphism in white people with RA found a weak positive association with the 158V allele, and an overrepresentation of 158VV homozygotes (odds ratio (OR)=1.6, p<0.05).[13] Conversely, a Spanish study reported an overrepresentation of the 158FF phenotype in patients with RA.[14] No association was found in Japanese patients with RA.[12] To further investigate any association between this FcγRIIIA polymorphism and RA, we have analysed its distribution in two ethnically diverse populations: a large group of white United Kingdom people and a northern Indian sample.

Abbreviations: ADCC, antibody dependent cytotoxicity; CI, confidence interval; F, phenylalanine; FcγRIIIA, Fcγ receptor IIIA; ICA, immune complex mediated arthritis; IL2, interleukin-2; NK, natural killer; OR, odds ratio; PCR–RFLP, polymerase chain reaction restriction fragment length polymorphism; RA, rheumatoid arthritis; RF, rheumatoid factor; RR, relative risk; SLE, systemic lupus erythematosus; V, valine

Table 1 Distribution of the genotype frequencies of the FcγRIIIA 158F/V polymorphism among the patients with RA and controls in the United Kingdom and Indian groups

	RA (n=401)	Controls (n=420)	χ^2	RR	95% CI	p Value
United Kingdom genotype:						
158FF	165 (41%)	172 (41%)	0.0	1.0	0.7 to 1.4	NS
158FV	189 (47%)	213 (51%)	0.7	1.1	0.9 to 1.4	NS
158VV	47 (12%)	35 (8%)	0.1	1.1	0.7 to 1.7	NS
Northern Indian genotype:						
158FF	36 (57%)	44 (47%)	0.0	1.0	0.5 to 2.0	NS
158FV	25 (40%)	35 (38%)	1.3	0.7	0.4 to 1.3	NS
158VV	2 (3%)	14 (15%)	3.5	0.3	0.1 to 1.1	<0.06

Table 2 Assessing the relative risk for carrying two V alleles compared with one or no V alleles

	158FF/158FV	158VV	χ^2	RR	95% CI	p Value
United Kingdom:						
Patients with RA	354 (88%)	47 (12%)	2.6	1.5	0.9 to 2.4	0.1
Controls	385 (92%)	35 (8%)				
Indian:						
Patients with RA	61 (97%)	2 (3%)	5.8	0.2	0.04 to 0.9	<0.02
Controls	79 (85%)	14 (15%)				

PATIENTS AND METHODS

Genomic DNA was obtained from samples of peripheral venous blood from 398 white patients from the United Kingdom with RA (Nuffield Orthopaedic Centre, Oxford, UK) and 289 ethnically matched healthy controls (Oxford Regional Transfusion Centre), and from 63 patients with RA and 93 ethnically matched controls from Uttar Pradesh, northern India. All patients with RA satisfied the 1987 revised American Rheumatism Association criteria.[15]

An experimental review of the published methods for typing the 559T/G polymorphism disclosed a high error rate in genotyping (over 10%), mainly due to the existence of the highly homologous FcγRIIIb gene which at position 559 has an invariant G (158V) allele. To ensure unequivocal results, typing was done using two different methods: amplification by polymerase chain reaction (PCR) followed by restriction digestion (PCR–RFLP)[14] and allele specific PCR amplification.[16]

Statistical analysis

Allele and genotype frequencies were determined by direct counting. The level of significance for the phenotypic frequencies was determined from 2x2 contingency tables using the χ^2 statistic, with odds ratios (ORs) calculated from the cross product ratio. Two sided p values were set at the 5% significance level. Genotypic relative risk was determined by the method of Lathrop.[17] In the comparison of the 158GG phenotype versus phenotypes with no or one 158G allele, the χ^2 statistic was used. This study had 90% statistical power to detect a genotypic relative risk (RR) of 1.6 and a significant allelic association with an OR=1.4 in the white group. The power to detect a significant allelic and genotypic association in the northern Indian group was significantly lower (70% power to detect a significant genotypic association with OR=2.5, and 80% power to detect a significant allelic association with OR=2.0).

RESULTS

The distributions of the FcγRIIIA 158F and 158V alleles between the patients with RA and the controls were similar in both the United Kingdom and Indian groups. The frequencies of the V allele in patients compared with controls were 35% versus 34% in the United Kingdom group and 28% versus 33% in the Indian group. In the Indian group, the frequency of the 158V allele was non-significantly reduced among the patients and the proportion of the 158VV homozygotes was also correspondingly non-significantly reduced (table 1). However, as there have been reports of a gene-dosage effect in this polymorphism, we analysed the RR for 158VV compared with that of 158FV or 158FF. A moderate protective effect from 158VV was found among the northern Indian patients with RA (RR=0.2; p<0.02, 95% confidence interval (95% CI) (0.04 to 0.9); table 2). The genotypic frequencies did not differ significantly in the United Kingdom group, although the proportion of 158VV homozygotes was slightly higher among the patients.

DISCUSSION

Testing polymorphisms in candidate genes across different ethnic groups is potentially a rigorous method for identifying relevant genetic influences. Studies on FcγRIIIA have now been undertaken in RA in white patients from the United Kingdom, and Indian, Spanish, and Japanese patients.[12–14] If similar associations had been found in these different racial groups there would be strong evidence of a causal relationship with the FcγRIIIA gene. However, results from this study do not support an association between the 158VV FcγRIIIA phenotype and RA.[13] They are in agreement with the negative finding from a smaller study of Japanese patients with RA.[12] A comparison of the published frequencies of this polymorphism among healthy controls in various populations has disclosed significant discrepancies among the published studies, most of which have analysed samples of between 100 and 200 people.[7 8 14] The allelic and genotypic frequencies obtained in the United Kingdom control group in our study differed from the United Kingdom control frequencies published previously by Morgan *et al* on a smaller sample.[13] Combining our data with those from Morgan *et al* (patients with RA 542, controls 544) showed no significant allelic or genotypic association. Thus it seems likely that the earlier positive association in the

white United Kingdom group is misleading. The protective effect of the 158VV phenotype found in the Indians with RA in our study reached statistical significance but it is also likely to be spurious as it was not found in the other groups and the sample studied was small.

One possible explanation for the discrepancies in the results reported so far is that one of the other neighbouring FcγR genes (FcγRII or FcγRI) is the true disease susceptibility locus. Incomplete linkage disequilibrium between the 158FV polymorphism and the actual RA predisposing allele could account for different associations in different ethnic groups.

Another significant factor influencing the activity of FcγRIIIA is its density on the cell surface, as aggregation of Fcγ receptor triggers cell activation.[18] A study of a mouse model of immune complex mediated arthritis (ICA) has shown an interdependence between the degree of joint inflammation and cartilage destruction and the levels of FcγRIII expression on synovial macrophages.[5] If FcγRIIIA is a true RA susceptibility factor, then the regions regulating gene expression, rather than polymorphisms of the coding sequence, may harbour the genetic elements for its involvement in the cause of disease. Furthermore, the signalling function of FcγRIIIA is mediated by two closely related intracytoplasmic subunits: the ζ chain of the T cell receptor and the γ chain of the IgE receptor. Comparison of the abilities of these two subunits in mediating activation signals showed that the cross linking of FcγRIIIA associated with a γ chain was significantly more efficient in signal transduction and phagocytosis than signalling through a ζ subunit.[19 20] Genes for the γ and ζ subunits map to the same region of chromosome 1q as the Fcγ receptor cluster. It is conceivable that a potential polymorphism predisposing to RA in one of these two subunits may be reflected by the differential findings of genetic associations between RA and the FcγRIIIA. Further studies of the genes in linkage disequilibrium with this polymorphism in large samples of genetically diverse populations could clarify the involvement of the IgG receptors in the cause of RA.

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