The 158V polymorphism of Fc gamma receptor type IIIA in early rheumatoid arthritis: increased susceptibility and severity in male patients (the Swedish TIRA* project)

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Objectives. To evaluate the influence of Fcγ receptor IIIA (FcγRIIIA) 158V/F polymorphism on susceptibility and disease severity in early rheumatoid arthritis (RA).

Methods. In 181 Swedish patients (128 women, 53 men) with RA of recent onset, disease and disability variables such as erythrocyte sedimentation rate, 28-joint disease activity score (DAS28) and health assessment questionnaire (HAQ) scores were monitored regularly during 3 yr. Three hundred and sixty-two controls were recruited from the same geographical area as the patients. FcγRIIIA genotyping was performed using denaturing high-performance liquid chromatography.

Results. In all RA patients, FcγRIIIA-158V/F was significantly over-represented compared with controls [odds ratio (OR) 1.9, 95% confidence interval (CI) 1.01–3.5, P < 0.05]. After stratifying for sex, the difference remained in the male population (OR 3.2, 95%CI 1.03–11, P < 0.05) but disappeared among women (OR 1.4, 95%CI 0.7–3.1, P = 0.4). In addition, 158VV patients were more likely to exhibit early joint erosions (OR 6.1, 95%CI 1.4–28, P < 0.01). At baseline, patients with different FcγRIIIA genotypes did not differ with respect to measures of disease activity or functional ability. Thereafter, in male patients with at least one V allele the mean DAS28 and HAQ scores were higher compared with 158FF. In contrast, female patients with at least one 158V allele displayed lower mean DAS28 and HAQ scores compared with those with 158FF. In a male population, the FcγRIIIA-158VV genotype is associated with an increased risk of developing RA, and the 158V allele with more severe disease in early RA.

Conclusions. In a male population, the FcγRIIIA-158VV genotype is associated with an increased risk of developing RA, and the 158V allele with more severe disease in early RA.

KEY WORDS: Disease course, Early rheumatoid arthritis, Fc receptor, Single-nucleotide polymorphism.

As a link between humoral and cellular immune responses, receptors recognizing the Fc region of immunoglobulin (Ig) G (FcγR) have attracted much interest concerning the aetiology and pathogenesis of autoimmune diseases [1, 2]. In addition to the placental FcγR (FcγRn), which transfers maternal monomeric IgG to the fetus, three distinct classes of FcγR have been characterized: FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16). Human FcγRII is divided into subclasses A, B and C, and for FcγRIII subclasses A and B have been described [3]. Except for FcγRIIB, all FcγRs are activating, e.g. they initiate antibody-dependent cellular cytotoxicity, proinflammatory cytokine secretion and phagocytosis, ultimately resulting in immune complex clearance and antigen presentation by antigen-presenting cells [4, 5]. In contrast to FcγRIIIA and FcγRIII, FcγRII is a high-affinity receptor with the ability to bind IgG monomers [3].

Rheumatoid arthritis (RA) is an autoimmune disease characterized by a high prevalence of autoantibodies directed against IgG-Fc, i.e. rheumatoid factors (RFs). RFs interact with IgG immune complexes and may interfere with their physiological elimination via FcγR-bearing cells [6]. The old hypothesis of the contribution of RF to the pathogenesis of arthritis has attracted new interest after the introduction of B-cell targeted therapy in RA [7]. Although the pathogenetic importance of RF in RA remains speculative, there are clear indications from animal models that immune complexes and FcγRs are of critical importance for the development of arthritis. Knockout mice lacking the gene encoding the γ-chain common to murine FcγRI and FcγRIII are protected against induction of collagen-induced arthritis (CIA) [8], an effect also seen in FcγRII –/– mice, indicating a specific function of FcγRIII in CIA [9]. Furthermore, in K/BxN murine RA-like polyarthritis, FcγRIIIs appear to be crucial for disease development and progression [10].

The FcγR gene cluster is located at human chromosome 1q21–23, a region not associated with RA in linkage analyses [11]. However, a large number of single-nucleotide polymorphisms (SNPs) have been identified and several have been associated with different inflammatory and autoimmune diseases, including systemic lupus erythematosus (SLE), periodontitis, idiopathic thrombocytopenic purpura and RA [12–14].

In 1997, Wu et al. [15] described a functional polymorphism in the extracellular domain 2 of FcγRIIIA, where a T to G substitution at nucleotide 559 changes phenylalanine to valine at codon 158 (158V/F). This polymorphism is sometimes called 176V/F when counted from the N-terminus of the mature protein.
after cleavage of the signal peptide. The presence of the V allele on NK cells was shown to result in more avid binding of IgG1 and IgG3 compared with the F allele and to enhance Ca$^{2+}$ influx and cell activation. Since then, the 158V/F polymorphism has been studied extensively, with remarkably contradictory results, in RA case-control studies, possibly reflecting methodological difficulties due to the extreme homology to FcyRIIB [16]. Both 158FF and 158VV have been reported to be associated with RA, with or without the presence of the shared epitope (SE) [17–23].

RA is a heterogeneous disease in which reliable predictors of outcome would be of great importance for individually tailored anti-rheumatic interventions, where potentially toxic side-effects and costs must be balanced against expected benefits. Previous reports on the 158V/F polymorphism of FcyRIIIA have been based upon patients with RA of medium or long duration, comparing clinical phenotypes in a cross-sectional manner. In this study, we investigated the influence of FcyRIIIA-158V/F on susceptibility and disease progression in 181 Swedish patients with RA of recent onset.

Patients and methods

Patients and referents

One hundred and eighty-one patients (71% women, median age 57.5 yr) from southeastern Sweden recruited to the Swedish TIRA multicentre cohort in 1996–1998 [24] were included in this study. To be included, the patients should fulfill at least four of the seven ACR classification criteria for RA, or the following: symmetrical arthritis, small-joint arthritis (metacarpo-/metatarso-phalangeal/proximal interphalangeal joints/wrists) and morning stiffness ≥60 min. Furthermore, symptom onset (joint swelling) should have occurred at least 6 weeks previously, but not more than 12 months ago. Ninety-six per cent of the patients fulfilled the ACR classification criteria for RA. Disease-modifying anti-rheumatic drugs (DMARDs), corticosteroids and analgesics were prescribed as found appropriate by the physicians. Follow-up was conducted regularly for 3 yr, monitoring clinical and laboratory variables such as erythrocyte sedimentation rate (ESR), plasma C-reactive protein (CRP) level, and 28-joint disease activity score (DAS28) [25]. The Swedish version of the Health Assessment Questionnaire (HAQ) was used to assess functional ability [26]. The control group of 362 individuals (63% women, median age 57 yr), without history of any rheumatic disease, was recruited from the same geographical area as the patients.

Laboratory analyses

ESR, CRP and agglutinating RF were measured at the laboratories affiliated to the patients’ local hospitals. Anti-cyclic citrullinated peptide (CCP) antibody was analysed using a commercial second-generation enzyme-linked immunosorbent assay kit (Immunoscan RA CCP2; Euro-Diagnostica, Arnhem, the Netherlands).

FcyRIIIA genotyping

The genotypes of FcyRIIIA-158 were assessed by denaturing high-performance liquid chromatography. DNA was extracted and purified from whole blood as previously described [27]. A 231 base-pair (bp) product was amplified by PCR using the forward primer 5′-CAC CAG GAG GGA ACC ACA ATA-3′ previously used by Morgan et al. [19, 20], together with a reverse primer corresponding to nucleotides 266–247 in the flanking intron, 5′-TCA CAT ATT TAC AGA ATG GCA ATG G-3′ and purified from whole blood as previously described [27]. A 231 bp PCR product was amplified by PCR using the forward primer 5′-CAC CAG GAG GGA ACC ACA ATA-3′ (GenBank accession numbers X52645 and AF162790). Fifty nanograms of genomic DNA were used in a 20 μl PCR with 2 mM MgCl$_2$, 200 μM of dNTPs, 0.2 μM of each primer and 1 unit of Taq polymerase (Invitrogen, Stockholm, Sweden) in buffer provided by the manufacturer. Samples were amplified in a thermal cycler (PTC 100; MJ Research, Watertown, MA, USA) at 94°C for 3 min followed by 35 cycles of 94°C for 45 s, 54°C for 30 s and 72°C for 20 s. Finally, samples were incubated at 72°C for 3 min. PCR products (5 μl) were injected into an automated liquid chromatography system (Transgenomic, Dallas, TX, USA), which used a linear gradient of acetonitrile as recommended by the manufacturer in 0.1 M triethylamine acetate buffer at 61°C to distinguish the retention time of heteroduplexed DNA from that of homoduplexed DNA. As shown in Fig. 1, the chromatograms of samples forming heteroduplexes (158VF) were clearly identified, while samples forming homoduplexes (158FF and 158VV) appeared nearly identical. After annealing 5 μl of this sample with 5 μl PCR product of a known wild-type sample at 95°C for 3 min followed by 40 cycles of 1 min lowering the temperature with 1.5°C each cycle, 158V samples formed heteroduplexes while 158FF remained unchanged (Fig. 1b).

Twenty-five samples, all genotypes represented, were sequenced by automated fluorescence-based automated cycling (MegaBace 500; Amersham Biosciences, Piscataway, NJ, USA) showing equal results in every case. FcyRIIIA specificity compared with FcyRIIB was established by examining nucleotide 473, where guanine is present in FcyRIIIA while FcyRIIB displays an adenine.

HLA-DRB1 typing

DRB1 alleles were typed by PCR amplification with sequence-specific primers (GenoVision, Oslo, Norway). The shared epitope

Statistical analyses

Genotype distributions were compared by \( \chi^2 \) analysis with continuity correction or Fisher’s exact test when expected cell count was less than 5. Mean values of disease activity measures from the scheduled follow-up visits (3–36 months) were calculated for each patient and Student’s \( t \)-test was used for comparisons. Statistical calculations were carried out using StatCalc (Epi Info v.3.2.2; Centers for Disease Control and Prevention, Atlanta, GA, USA) and SPSS statistical software (v.11.5; SPSS, Chicago, IL, USA). \( P \) values less than 0.05 were considered significant.

Ethical considerations

The ethical committees at the participating hospitals approved the study protocol. All patients gave written informed consent to participation.

Results

There were no significant differences in median age or proportion of women between patients and controls. Neither did pharmacological therapy or the presence of the SE in RA patients differ significantly across FcγRIIIA genotypes or between sexes.

Genotype and allele distribution in patients and controls

Genotype and allele frequencies of FcγRIIIA-158V/F in this population were in agreement with previous reports [17, 19, 20], and the control population was in Hardy–Weinberg equilibrium. With 158FF as a reference group, both 158VF and 158VV were more prevalent in RA patients than in the controls, reaching significance for 158VV (\( P < 0.05 \)) (Table 1). When patients and controls were stratified for sex, the observed risk for RA among 158VV was carried by males (OR 3.2, 1.03–10.2), whereas no risk was evident for females (Table 1). As shown in Table 2, the 158V allele frequency again showed increased risk for all RA patients and male patients, but not females. Anti-CCP, RF or HLA-DRB1 status did not influence the ORs in any of the strata (data not shown).

Genotypes and disease severity in RA patients

Disease activity measures were investigated in men and women separately, comparing 158FF patients with those possessing at least one V allele. No differences were found at inclusion, but during follow-up disparate trends were found in women and men. As shown in Fig. 2, 158V male patients showed higher mean DAS28 and HAQ scores compared with patients with 158FF (\( P < 0.05 \) for both). The opposite was seen for 158V female patients, who displayed lower mean values of DAS28 and HAQ score (\( P < 0.01 \) and \( P < 0.05 \)) during the 3-yr follow-up. There were no significant differences regarding mean ESR or CRP (data not shown).

Data on radiographic signs of joint damage were available from inclusion. Of the 20 patients (11%) who displayed radiological findings typical of RA (erosions and/or periarticular osteopenia), patients homozygous for the 158V allele were significantly at risk compared with 158FF (OR 6.1, 1.4–28, \( P < 0.01 \)) (Table 3).
Table 3. FcγRIIIA genotype versus baseline X-ray findings

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Damage (n = 20)</th>
<th>No damage (n = 161)</th>
<th>Odds ratio (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>FcγRIIIA-158FF</td>
<td>4</td>
<td>66</td>
<td>1.0</td>
<td>0.008</td>
</tr>
<tr>
<td>FcγRIIIA-158VF</td>
<td>9</td>
<td>76</td>
<td>1.95 (0.5–8)</td>
<td>0.4</td>
</tr>
<tr>
<td>FcγRIIIA-158VV</td>
<td>7</td>
<td>19</td>
<td>6.1 (1.4–28)</td>
<td>0.008</td>
</tr>
</tbody>
</table>

Damage is defined as the presence of erosions or periarticular osteopenia.

Discussion

After many years in the cold, the roles of antibodies, immune complexes, complement, stimulating and inhibiting Fc-receptors, as well as complement receptors, attract increasing interest in relation to the aetiology and pathogenesis of RA. FcR-mediated signals, both activating and inhibiting, are central in immune complex-stimulated inflammation. Apart from FcγRIIIA, we have investigated the functional 232-I/T polymorphism of the inhibiting FcγRIIB, without finding any association with RA or disease severity. The present study confirms that the 158VV genotype of FcγRIIIA is associated with RA [20], and is the first report to describe that the risk for RA susceptibility and disease severity is attributable to men.

The surface expression of FcγRIII on monocytes/macrophages in the circulation and in the synovium as well as the number of CD16⁺ monocytes has been shown to be increased in RA patients compared with healthy controls [28, 29]. Also, in RA patients, the expression level of FcγRIIIA on monocytes is higher in synovial fluid than in peripheral blood [30]. FcγRIIIA ligation is known to induce production of tumour necrosis factor α (TNF-α) by monocytes [31], and elevated FcγRIII expression on macrophages yields increased production of both TNF-α and matrix metalloproteinase 1 in RA synovium [29]. Moreover, the distribution of FcγRIII expression in human tissues other than synovium corresponds well to the pattern of extra-articular involvement seen in RA [32]. Taking these results together, it is conceivable that functional polymorphisms of FcγRIIIA have important implications in RA.

Since FcγRIIIA-158VV appears to be a susceptibility factor and since the V allele binds immune complexes with higher affinity compared with 158F, it may be assumed that the presence of 158V results in a more pronounced proinflammatory response upon ligation with IgG compared with 158F. Consistently with this, although baseline disease activity measures did not differ significantly, we found that 158VV was associated with the presence of early joint damage in RA (i.e. 6–52 weeks after onset of symptoms). Although this finding suggests 158VV to be a severity marker, it should be pointed out that there were few prevalent cases since radiographs were taken at baseline, and after stratifying for sex the results were inconclusive. Male RA patients with at least one V allele showed higher disease activity and significantly lower functional ability (HAQ) compared with 158FF. In women with RA, however, the presence of 158V was associated with a less severe disease course, both regarding disease activity and functional ability. The explanation of this discrepancy is not immediately apparent, but is likely to be multifactorial. For instance, oestrogen is known to influence FcγRIII expression and cytokine release in macrophages [33]. Also, the differences in DAS28 and HAQ were seen not at inclusion but at subsequent follow-ups, i.e. when the majority of the patients had been prescribed DMARD therapy, possibly implying effects of FcγRIIIA genotypes and gender on responses to therapy. A decreased number of synovial macrophages is a key element in RA remission [34, 35], and the expression of activating FcγRs on monocytes is decreased following initiation of therapy [36].

However, the effect of traditional DMARDs on FcγR-mediated functions and vice versa remains largely unknown.

Very interestingly, the therapeutic efficacy of monoclonal antibodies (mAbs) against CD20 (rituximab) and TNF-α (infliximab) in lymphoma, SLE and Crohn’s disease has been shown to be directly affected by the FcγRIIIA-158VF polymorphism [37–39]. Also, immunosuppressive therapies other than mAbs have shown different response rates across FcγRIIIA-158VF genotypes in thrombocytopenic purpura [14]. Therapeutic response to TNF-α inhibitors in relation to FcγRIIIA genotype cannot be evaluated in the present study, in which such therapy was instituted in only a small number of patients.

We conclude that, in men, FcγRIIIA-158VV is associated with an increased risk of developing RA and more aggressive disease in its early phase. In females, we are not able to identify 158VV as a risk factor; but instead, carrying at least one 158V allele seems to be associated with milder disease, as measured by the HAQ and DAS28. These findings need to be replicated in larger data sets, and investigations on FcγR biology in relation to steroid hormones are also warranted. Future studies on FcγRs in autoimmune disease should be performed with a gender perspective.

Rheumatology

Key messages

- FcγRIIIA-158V is associated with RA and disease severity in a Swedish male population. In females, 158V is not associated with RA but predicts milder disease.

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The authors have declared no conflicts of interest.

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