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

A. Kastbom1,2, A. Ahmadi2, P. Söderkvist2 and T. Skogh1

Objectives. To evaluate the influence of Fcy 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-158VV 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.

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.
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 Ca2+ 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 FcRIIIA [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 FcRIIIA 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 FcRIIIA-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 (metacarpophalangeal/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 analyses 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).

**FcγRIIIA genotyping**

The genotypes of FcγRIIIA-158 were assessed by denaturing high-performance liquid chromatography. DNA was extracted and purified from whole blood as previously described [27]. A 231 bp PCR product was amplified by PCR using the forward primer 5'-TCA CAT ATT TAC AGA ATG GCA ATG G-3' previously used by Morgan et al. [19, 20], together with a reverse primer corresponding to nucleotides 266–247 in the flanking intron, 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 MgCl2, 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 (Transgenic, 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. FcγRIIIA specificity compared with FcγRIIIB was established by examining nucleotide 473, where guanine is present in FcγRIIIA while FcγRIIIB 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 χ² 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 1. Genotype frequencies in patients and controls

<table>
<thead>
<tr>
<th>FcγRIIIA genotype</th>
<th>RA patients (%)</th>
<th>Controls (%)</th>
<th>Odds ratio (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>All subjects</td>
<td>n = 181</td>
<td>n = 362</td>
<td></td>
<td></td>
</tr>
<tr>
<td>158FF</td>
<td>70 (39)</td>
<td>168 (46)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>158VF</td>
<td>85 (47)</td>
<td>161 (45)</td>
<td>1.3 (0.9–1.9)</td>
<td>0.27</td>
</tr>
<tr>
<td>158VV</td>
<td>26 (14)</td>
<td>33 (9)</td>
<td>1.9 (1.01–3.5)</td>
<td>0.046</td>
</tr>
<tr>
<td>Women</td>
<td>n = 128</td>
<td>n = 228</td>
<td></td>
<td></td>
</tr>
<tr>
<td>158FF</td>
<td>50 (39)</td>
<td>96 (42)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>158VF</td>
<td>61 (48)</td>
<td>109 (48)</td>
<td>1.1 (0.7–1.8)</td>
<td>0.9</td>
</tr>
<tr>
<td>158VV</td>
<td>17 (13)</td>
<td>23 (10)</td>
<td>1.4 (0.7–3.1)</td>
<td>0.4</td>
</tr>
<tr>
<td>Men</td>
<td>n = 53</td>
<td>n = 134</td>
<td></td>
<td></td>
</tr>
<tr>
<td>158FF</td>
<td>20 (38)</td>
<td>72 (54)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>158VF</td>
<td>24 (45)</td>
<td>52 (39)</td>
<td>1.7 (0.8–3.5)</td>
<td>0.2</td>
</tr>
<tr>
<td>158VV</td>
<td>9 (17)</td>
<td>10 (8)</td>
<td>3.2 (1.03–10.2)</td>
<td>0.041</td>
</tr>
</tbody>
</table>

### Table 2. Allele frequencies in patients and controls

<table>
<thead>
<tr>
<th>RA patients (%)</th>
<th>Controls (%)</th>
<th>Odds ratio (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>All subjects</td>
<td>n = 362</td>
<td>n = 724</td>
<td></td>
</tr>
<tr>
<td>158F</td>
<td>225 (62)</td>
<td>497 (69)</td>
<td>1.0</td>
</tr>
<tr>
<td>158V</td>
<td>137 (38)</td>
<td>227 (31)</td>
<td>1.3 (1.02–1.8)</td>
</tr>
<tr>
<td>Women</td>
<td>n = 256</td>
<td>n = 456</td>
<td></td>
</tr>
<tr>
<td>158F</td>
<td>161 (63)</td>
<td>301 (66)</td>
<td>1.0</td>
</tr>
<tr>
<td>158V</td>
<td>95 (37)</td>
<td>155 (34)</td>
<td>1.2 (0.8–1.6)</td>
</tr>
<tr>
<td>Men</td>
<td>n = 106</td>
<td>n = 268</td>
<td></td>
</tr>
<tr>
<td>158F</td>
<td>64 (60)</td>
<td>196 (73)</td>
<td>1.0</td>
</tr>
<tr>
<td>158V</td>
<td>42 (40)</td>
<td>72 (27)</td>
<td>1.8 (1.08–3.0)</td>
</tr>
</tbody>
</table>

**Fig. 2.** Mean DAS28 and HAQ scores during 3-yr follow up in women (n = 128) and men (n = 53), comparing FcγRIIIA-158FF and FcγRIIIA-158V. Mean scores and s.e.m. are shown. *P < 0.05; **P < 0.01.
on monocytes is decreased following initiation of therapy [36].

in RA remission [34, 35], and the expression of activating Fc

of Fc

been prescribed DMARD therapy, possibly implying effects

quent follow-ups, i.e. when the majority of the patients had

in DAS28 and HAQ were seen not at inclusion but at subse-

osteopenia.

For instance, oestrogen is known to influence Fc

is not immediately apparent, but is likely to be multifactorial.

the explanation of this discrepancy

associated with a less severe disease course, both regarding disease

severity marker, it should be pointed out that there were few

of symptoms). Although this finding suggests 158VV to be a

significantly, we found that 158VV was associated with the

this, although baseline disease activity measures did not differ

response to TNF-α inhibitors in relation to FcγRIIA genotype
cannot be evaluated in the present study, in which such therapy

We conclude that, in men, FcγRIIA-158VV is associated with an

in vivo

Downloaded from https://academic.oup.com/rheumatology/article-abstract/44/10/1294/1788369 by guest on 07 February 2019

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 activation, both activating and inhibiting, are central in immune complex-stimulated inflammation. Apart from FcγRIIA, 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 FcγRIIA is associated with RA [20], and is the first report to describe that the risk for RA susceptibility and disease severity is attributed to men only.

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]. FcγRIIA 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 FcγRIIA have important implications in RA.

Since FcγRIIA-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 FcγRIIA genotypes and gender on responses to therapy. An increased 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 FcγRIIA-158VV/F polymorphism [37–39]. Also, immunosuppressive therapies other than mAbs have shown different response rates across FcγRIIA-158V/F genotypes in thrombocytopenic purpura [14]. Therapeutic response to TNF-α inhibitors in relation to FcγRIIA 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, FcγRIIA-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.

<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>FcγRIIA-158FF</td>
<td>4</td>
<td>66</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>FcγRIIA-158VF</td>
<td>9</td>
<td>76</td>
<td>1.95 (0.5–8)</td>
<td>0.4</td>
</tr>
<tr>
<td>FcγRIIA-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 osteoporosis.

### Table 3. FcγRIIIA genotype versus baseline X-ray findings

![Table 3](https://example.com/table3.png)

### Key messages

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

Acknowledgements

We thank Mrs Annette Molbæk for expert technical assistance, Mrs Ylva Billing and all the TIRA participants in Eskilstuna, Jönköping, Kalmar, Lindesberg, Linköping, Motala, Norrköping, Orebro, Oskarshamn and Västervik for excellent cooperation. The study was financed by grants from the Swedish Rheumatism Association, the Swedish Research Council (project K2003–74VX–14594–01A), the County Council of Östergötland, the Linköping Society of Medicine, King Gustaf V 80-year Foundation and the National Board of Health and Welfare.

The authors have declared no conflicts of interest.

### References

6. Devys ME, Hogben DN. The effect of rheumatoid factor on the clearance of endogenous immune complexes formed in low-affinity