

Influence of *FCGR3A* genotype on the therapeutic response to rituximab in rheumatoid arthritis: an observational cohort study

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ABSTRACT

Objectives: To determine whether a polymorphism in the Fc γ receptor type IIIA (*FCGR3A*-F158V), influencing immunoglobulin G binding affinity, relates to the therapeutic efficacy of rituximab in rheumatoid arthritis (RA) patients.

Design: Observational cohort study.

Setting: Three university hospital rheumatology units in Sweden.

Participants: Patients with established RA (n=177; 145 females and 32 males) who started rituximab (Mabthera) as part of routine care.

Primary outcome measures: Response to rituximab therapy in relation to *FCGR3A* genotype, including stratification for sex.

Results: The frequency of responders differed significantly across *FCGR3A* genotypes ($p=0.017$ in a 3 $\times$ 2 contingency table). Heterozygous patients showed the highest response rate at 83%, as compared with patients carrying 158FF (68%) or 158VV (56%) ($p=0.028$ and 0.016 , respectively). Among 158VV patients, response rates differed between male and female patients ($p=0.036$), but not among 158FF or 158VF patients ($p=0.72$ and 0.46 , respectively).

Conclusions: Therapeutic efficacy of rituximab in RA patients is influenced by *FCGR3A* genotype, with the highest response rates found among heterozygous patients. This may suggest that different rituximab mechanisms of action in RA are optimally balanced in *FCGR3A*-158VF patients. Similar to the previously described associations with RA susceptibility and disease course, the impact of 158VV on rituximab response may be influenced by sex.

INTRODUCTION

The growing arsenal of biological agents available in rheumatoid arthritis (RA) pharmacotherapy increases the demand for predictors of therapeutic responses and/or side effects. Human Fc γ receptors mediate effector functions

ARTICLE SUMMARY

Article focus

- A functional polymorphism in the gene encoding Fc γ receptor type IIIA (*FCGR3A*) influences the outcome of B cell-depleting therapy with rituximab in malignancies.
- Although rituximab is frequently used for the therapy of severe RA, studies on RA patients have been lacking.
- We wished to determine if *FCGR3A*-F158V genotype associates with rituximab efficacy in RA.

Key messages

- *FCGR3A* heterozygous patients experienced significantly higher response rates than 158FF and 158VV patients.
- The results are discordant to a similar recently published study based on 111 RA patients.
- There are indications of a sex-specific effect of the 158VV genotype, as have been previously described regarding RA susceptibility and disease course.

Strengths and limitations of this study

- Although limited by the small numbers of males and 158VV patients, this is until now the largest published study on *FCGR3A* and rituximab in RA patients.
- Differences could have been attenuated by the 'real-life' approach, that is, that therapy was not administered by a standardised scheme, and the time to evaluation varied between patients.

of immunoglobulin G (IgG) antibodies and may modulate the therapeutic efficacy of all biological substances containing IgG-Fc parts. Hence, a functional single-nucleotide polymorphism in *FCGR3A* (*FCGR3A*-F158V, rs396991), influencing IgG binding affinity to the activating Fc γ receptor type IIIA (Fc γ RIIIA),¹ has been associated with the therapeutic efficacy of rituximab (RTX), that is, an anti-CD20 B cell-depleting IgG1 monoclonal antibody, in B cell

malignancies.^{2–3} RTX-coated B cells may be eliminated by several mechanisms; complement-dependent cytotoxicity, antibody-dependent cytotoxicity (ADCC) by natural killer (NK) cells, and/or phagocytosis by FcγR-bearing cells. Although the relevance in humans has not been established, mice lacking proper complement or NK-cell functions are equally well B cell depleted as native animals, thus pointing towards a major role of FcγR-bearing macrophages.⁴ It has been convincingly shown that RTX binds with higher affinity to 158V in an allele-dose-dependent manner and that ADCC by NK cells are affected accordingly.⁵ Data regarding *FCGR3A* genotype and RTX efficacy in RA has been lacking until recently, when Ruysen-Witrand *et al*⁶ reported an association between carriage of the high-affinity binding valine (V) allele of *FCGR3A* and higher response rates to RTX.

Numerous case-control studies have been performed with regard to *FCGR3A*-F158V and RA susceptibility. Although initial studies showed remarkably discordant results, subsequent investigations have shown an association between homozygosity of the high-affinity allele (158VV) and an increased risk of RA in Europeans.^{7–10} In studies presenting data stratified for sex, the increased risk conferred by the 158VV genotype only attributes to the male population.^{7–10} In line with this, male patients with early RA carrying 158V have a more severe disease course whereas, intriguingly, in female patients the opposite is seen.⁷ The biological basis for this sex difference is yet to be elucidated, but it has been shown that oestrogen influences both FcγRIIIA expression and FcγRIIIA-mediated release of tumour necrosis factor and IL-1β from monocytic cells.¹¹

The current study was conducted to explore the influence of *FCGR3A* genotype on RTX efficacy in RA patients.

PATIENTS AND METHODS

Patients with established RA (n=177; 145 females and 32 males) who started RTX (Mabthera) as part of routine care at three rheumatology clinics in Sweden (Karolinska University Hospital, Solna; Linköping University Hospital, Linköping; and Umeå University Hospital, Umeå, Sweden) were included in the study. Baseline characteristics and concurrent medication are shown in table 1. The therapeutic response was assessed by the European League Against Rheumatism (EULAR)

response criteria after 3–6 months.¹² DNA was extracted from whole blood by standard techniques and *FCGR3A*-F158V was genotyped by a commercially available TaqMan assay (Applied Biosystems, Foster City, CA, USA, ID C_25815666_10). Validation of the TaqMan results was performed in 30 samples (10 of each genotype), yielding 100% concordance with a previously described direct sequencing assay specific for *FCGR3A*.⁷

Statistical analysis

Response rates were compared by χ^2 test across *FCGR3A* genotypes, and by Fisher's exact test between sexes. Baseline characteristics were tested by χ^2 test for categorical variables and by one-way analysis of variance for continuous variables. Two-sided p values <0.05 were considered significant. Analyses were performed using SPSS V.19.

Ethical considerations

Informed consent was obtained from all patients according to the Declaration of Helsinki. The study protocol was approved by the regional ethics committees in Linköping, Stockholm and Umeå.

RESULTS

Overall, 130 patients (73%) achieved a moderate or good EULAR response, whereas 47 (27%) were non-responders. The distribution of *FCGR3A* genotypes was 81 (46%) 158FF, 78 (44%) 158VF and 18 (10%) 158VV, which is in agreement with previous findings in Swedish RA populations,^{7–13} and in Hardy-Weinberg equilibrium ($p>0.95$). The proportion of EULAR good responders did not differ significantly across *FCGR3A* genotypes (21%, 26% and 28% for 158FF, 158VF and 158VV, respectively). However, the proportion of good and moderate responders together, compared to non-responders, was significantly different across *FCGR3A* genotypes ($p=0.017$ in a 3×2 contingency table), where heterozygous patients showed the highest response rate (65 of 78 patients, 83%), as compared to 55/81 (68%) patients carrying 158FF (OR 2.36, 95% CI 1.04 to 5.41, $p=0.028$), and 10/18 (56%) 158VV patients (OR 4.0, 95% CI 1.16 to 13.9, $p=0.016$; figure 1). The frequency of responders was not significantly different between 158FF and 158VV

Table 1 Baseline characteristics of the 177 RA patients according to *FCGR3A* genotype

	158VV (n=18)	158VF (n=78)	158FF (n=81)	Total (n=177)
Mean (SD) disease duration, years	11 (6)	15 (10)	14 (12)	14 (11)
Females, n (%)	13 (72)	61 (78)	71 (88)	145 (82)
Rheumatoid factor positive, n (%)	12 (67)	62 (81)	63 (78)	137 (78)
Baseline DAS28, mean (SD)	5.2 (1.0)	5.5 (1.1)	5.5 (1.2)	5.5 (1.1)
Concurrent DMARD therapy, n (%)	15 (83)	48 (62)	59 (73)	122 (69)
Oral glucocorticoid therapy, n (%)*	13 (72)	55 (72)#	48 (64)##	116 (69)###
Number of previous TNF inhibitors, mean (SD)	1.4 (1.2)	1.3 (1.1)	1.2 (1.1)	1.3 (1.1)

*Data available on: #76/78 patients, ##75/81 patients, ###169/177 patients.
DMARD, disease modifying antirheumatic drugs; TNF, tumour necrosis factor.

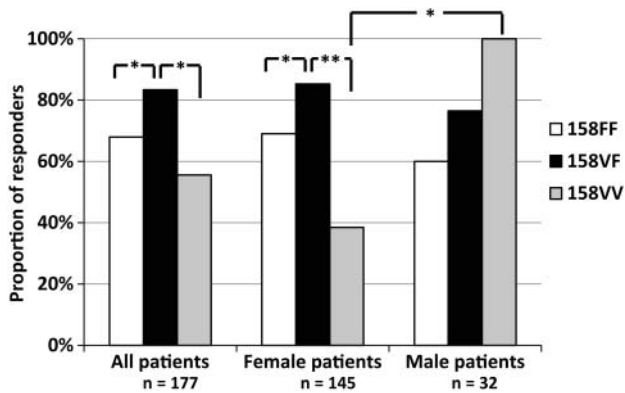


Figure 1 Proportion of rituximab responders in relation to *FCGR3A* genotype in RA patients. Significant differences are indicated as * $p < 0.05$, ** $p < 0.01$.

(OR 1.69, 95% CI 0.53 to 5.37, $p = 0.4$). Baseline characteristics (table 1) revealed no significant differences across *FCGR3A* genotypes. Experiencing a therapeutic response was more common among rheumatoid factor (RF)-positive cases as compared with RF-negative cases (OR 2.38, 1.05 to 5.39, $p = 0.038$), and in the RF-positive group, response rates remained significantly different across *FCGR3A* genotype ($p = 0.004$), but did not reach statistical significance among RF-negative cases ($p = 0.056$). After stratifying for sex, response rates among female patients remained different in 158VF as compared with 158FF ($p = 0.047$) and 158VV ($p = 0.001$), but this was not seen in the smaller group of males ($p > 0.4$; figure 1). Furthermore, among 158VV patients, men had significantly higher response rates than women (100% vs 39%, respectively, $p = 0.036$), while therapeutic response among 158FF or 158VF patients did not differ between men and women ($p = 0.72$ and 0.46 , respectively). Absolute changes in DAS28 in relation to *FCGR3A* yielded similar results as for categorical EULAR responses (data not shown).

DISCUSSION

Although an influence of *FCGR3A*-F158V on RTX efficacy was in line with our hypothesis, the lack of a clear allele-dose effect points towards a more complex role of *FCGR3A* in RTX therapy of RA than anticipated. On the basis of previous reports of increased RTX-induced ADCC,⁵ more pronounced peripheral B cell depletion,¹⁴ better clinical outcomes in B cell malignancies,^{2 3} we expected any difference to appear in favour of the 158VV genotype. Also, a recent study on 111 RA patients described an association between the 158V allele and response to RTX.⁶ Our study is, to our knowledge, the largest on this topic to date, and we surprisingly found a significantly larger proportion of responders among *FCGR3A*-F158V heterozygous patients, a finding to which there is no immediate explanation from previous experimental work. Regarding the *in vivo* situation little is known, however, as the mechanisms whereby RTX reduces signs and symptoms of RA remain incompletely

understood. The initial view that the disease-modifying action of RTX depends on depletion of B cells and the eventual disappearance of pathogenic autoantibodies is contradicted by several observations. For instance, although circulating autoantibodies may indeed decline, they seldom disappear,¹⁵ and both non-circulating B cells and autoantibody production in the synovium are clearly less affected by RTX than their circulating counterparts.¹⁶ Other proposals for RTX mode of action in RA include the immune complex decoy hypothesis, suggesting that RTX-opsonised B cells keep disease-promoting FcγR-expressing phagocytic cells busy eliminating B cells instead of perpetuating synovial inflammation.¹⁷ In this context, one would expect that the more pronounced activation of *FCGR3A*-158VV macrophages would render them less prone to divert from the immune complex-driven rheumatoid inflammation in the synovium, and hence corresponding to a worse therapeutic outcome in 158VV patients as seen in the current study. Alternatively, *FCGR3A*-158VV individuals could, as compared to *FCGR3A*-158FF, handle RTX-opsonised B cells more efficiently and may thereby, to a greater extent and more rapidly, return from this drug-induced diversion. Our finding of a significantly higher response rate among heterozygous patients could possibly point towards several mechanisms being involved in RTX action and that, in RA, these are most optimally balanced in individuals with the intermediate binding variant of FcγRIIIA, that is, *FCGR3A*-158VF.

A previous study on RA patients reported that the proportion of responders among 158VF patients was significantly higher compared to 158FF cases, but that the proportion of responders among 158VV were similar to 158VF, albeit failing to reach statistical significance when compared with 158VF or 158FF.⁶ Merging the response data from the two studies yields a significantly better response rate among 158VF as compared to 158FF (OR 2.82, 95% CI 1.44 to 5.56, $p = 0.001$), whereas the 158VV group does not differ significantly from neither 158VF (OR 0.41, 0.15 to 1.15, $p = 0.067$) nor 158FF (OR 1.17, 95% CI 0.46 to 2.99, $p = 0.83$).

Shortcomings of the current study include the fact that therapeutic response was assessed with up to 3 months variation between patients. However, a separate analysis of patients with response assessed at 6 months ($n = 108$) yielded similar results, making a major impact of follow-up time-point unlikely. Our finding that 158VV patients less frequently experience response to RTX is discordant with the findings of Ruysen-Witrand *et al.* and the limited number of 158VV patients (in both studies) adds uncertainty to firm conclusions regarding this particular genotype. Further, the relatively limited number of male patients calls for cautious interpretation of the findings regarding sex differences. Still, we believe that the 158VV sex difference is of interest, as no tendencies towards sex differences were found among 158FF and 158VF patients, despite being substantially larger groups. Also, there are previously described sex-dependent associations of 158VV

in RA.^{7 10} The *FCGR* locus is subject to copy number variation (CNV), and in the current study CNV of *FCGR3A* was not investigated. However, the functional consequences of *FCGR3A* CNV remains unknown, and a previous study showed that only 5% of Swedish individuals carry $\neq 2$ copies of *FCGR3A*.¹⁸

On the basis of currently available data, we conclude that *FCGR3A-F158V* heterozygosity is associated with a better response to RTX in RA patients as compared with homozygosity of the low-affinity F allele. Data regarding patients carrying two high-affinity alleles, 158VV, remains inconclusive and needs to be resolved before *FCGR3A-F158V* may become clinically relevant as a predictor of RTX response in RA.

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Contributors AK was involved in conception and design of the study, acquisition of patient data, genotyping and drafted the paper. LÅ and LP were involved in design of the study, genotyping and interpretation of results. LC, AC, RFV, SR-D and SS were involved in design of the study, acquisition of patient data and interpretation of result. All authors revised the draft paper.

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