FCGR polymorphisms in the treatment of Rheumatoid Arthritis with Fc-containing TNF inhibitors

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Short title: *FCGR* gene polymorphisms in the response of RA to TNFi

Abstract

We have explored if three FcγR functional polymorphisms could be biomarkers of the response of rheumatoid arthritis (RA) to the Fc-containing TNF inhibitors (TNFi) in samples from 429 patients. We also performed a meta-analysis of studies addressing FCGR3A F158V polymorphism, which is the most studied of the three. None of the three functional polymorphisms was associated with the response to TNFi. Meta-analysis of the seven FCGR3A F158V studies with available data, which included 899 patients with RA, showed no significant association (OR=1.11, 95% CI =0.8-1.5; P = 0.5). These results are notable given the large size of this study relative to others addressing these polymorphisms and the appearance given by previous studies of association of the FCGR3A F158V SNP.

~~**Aims:** We aimed to explore if the response of patients with rheumatoid arthritis (RA)~~

Keywords: Rheumatoid arthritis, TNF inhibitors, biologics, genetics, biomarkers, Fc receptor, FCGRT, infliximab, etanercept, adalimumab

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Introduction

Treatment of RA frequently involves the use of biologic drugs [1, 2]. The first biologics introduced in RA treatment were the TNF inhibitors (TNFi) and they still are the first choice for many patients. The most frequently used are infliximab (INX), etanercept (ETC) and adalimumab (ADM). The three include the Fc of human IgG1. INX and ADM include it as part of their monoclonal antibody molecule, whereas ETC includes the Fc as part of the fusion between sTNFR and IgG1. This situation is common among biologics because the Fc of IgG1 provides favorable pharmacokinetics, including a long half-life [3]. As a consequence, these three TNFi are influenced by the Fc gamma receptors (FcγR), which are pharmacogenetic modifiers with known functional polymorphisms [4-11]. These polymorphisms could explain part of the variable response of RA patients to TNFi treatments, with some patients very effectively responding whereas others, about a third, do not respond to the treatment. Variability that poses a major problem for the control of RA because about a third of the patients do not respond to the treatment [1, 2, 12, 13]. This lack of efficacy is largely partly drug-specific and the same non-responder patient improves when switched to a different drug, other TNFi or a biologic directed against a different target. The causes of the variable response are still unknown, but there is a lot of interest in finding biomarkers to avoid the trial and error approach that has been followed up to now. These trials are very inefficient because knowing whether a treatment is effective or not for a given patient can take up to 6 months [14].

The FcγRs are a family of glycoprotein surface receptors, including the high affinity receptor FcγRI, and the low affinity receptors FcγRIIA, FcγRIIIA, FcγRIIC, FcγRIIIB and FcγRIIB [15]. All of them are expressed in immune cells. They have appeared by duplication and diversification and they still conserve a high degree of homology.

Reflecting their common origin, all the genes encoding the low affinity receptors are tightly placed on chromosome 1q23 (in the above-listed order). These receptors mediate, upon recognition of immune-complexes, the activation of innate and acquired immunity. The exception is FcγRIIB, which is inhibitory. In addition to the FcγRs, there are other proteins binding the Fc. They are heterogeneous, expressed outside the immune system and known as Fc-receptor related proteins. The most notable is FcRn, which is encoded by the *FCGRT* gene [16-18]. It was originally described in the placenta, where it mediates the transfer of IgG from the mother to the fetus. Now, we know that FcRn expression is widespread and that its principal function is to maintain IgG prolonged half-life. It recovers the IgG that has been secreted in the urine and the gut. In addition, it also rescues IgG from intracellular lysosomal degradation, bringing it back to the circulation.

The FcγRs have a less noticiable effect on IgG half-life than FcRn, but they can modify the efficacy or bioavailability of Fc-containing biologics also through their involvement in multiple immune pathways, including phagocytosis, antibody-dependent cellular cytotoxicity and cellular signaling [15-18]. Therefore, the functional polymorphisms affecting the FcγRs and FcRn are potential biomarkers associated with ~~the~~ patient variability in the response to TNFi. These possible biomarkers include non-synonymous SNPs affecting the protein sequence of FcγRs and a variable number tandem repeat, VNTR, of 37 bp in the *FCGRT* promoter that has been associated with differences in FcRn expression and binding to IgG [19]. Recently, we have found an association between one of these polymorphisms, the *FCGR2A* H131R SNP, and the response to INX [20]. Other FcγR polymorphisms have also been reported as associated with the response to TNFi, most notably the *FCGR3A* F158V SNP [4, 7, 9, 10]. However, not all studies are concordant [5, 6, 8,

11], and no definitive validation of these biomarkers has yet been obtained. Here, we have examined two FcγR functional polymorphisms and the VNTR of *FCGRT* in the same 429 patients with RA in which we found association of the *FCGR2A* SNP [20], and we have also summarized the available evidence for the most studied of them, the *FCGR3A* F158V SNP, through meta-analysis of all available studies.

Material and methods

Patients. Biologic-naive patients with RA according to the 1987 revised American College of Rheumatology classification criteria were included [21]. They were recruited from six Spanish and two Greek Rheumatology Units during the course of a previous study [20]. They were treated with INX, ETC, or ADM between 2000 and 2010. The indication of treatment, the choice of drugs, and the control of disease evolution were performed with independence of this study, during standard care of the patients. Evaluations included the DAS28 and the EULAR criteria at the start of treatment and at 3, 6, and 12 months. The DAS28 is a composite index of RA activity including the number of tender joints and swollen joints (28 joints maximum), laboratory results and patient status assessment. The EULAR criteria divide patients into three classes based on change in DAS28 from baseline (ΔDAS28 = current DAS28 – baseline DAS28) and current DAS28 ~~both measured at the time of evaluation~~: good responders are those with ΔDAS28 ≥ 1.2 and current DAS28 ≤ 3.2; non-responders are all patients with ΔDAS28 ≤ 0.6 and those with ΔDAS28 > 0.6 but ≤ 1.2 and with current DAS28 > 5.1; all the remaining patients are moderate responders [22]. There was a total of 429 patients, 245 of them completed follow-up at 3, 6, and 12 months. Six of the 429 patients initially included in the study were

excluded because they showed a baseline DAS28 <3.2. Clinical characteristics are detailed in Table 1. All the patients provided blood samples for DNA extraction and their informed written consent to participate in the study. The study was approved by the local ethics committees and by the Comite Etico de Investigacion Clinica de Galicia (Santiago de Compostela, Spain).

Genotyping. The nsSNPs, rs1050501 in *FCGR2B* and rs396991 in *FCGR3A* were genotyped by PCR amplification followed by single-base extension with the SNaPshot Multiplex Kit (Applied Biosystems, Foster City, CA, USA). These SNPs are commonly referred to by their protein alleles with the following correspondences: rs1050501 T>C as Ile>Thre or I232T, and rs396991 T>G as Phe>Val or F158V. The VNTR of FcRn is difficult to amplify because of the high-GC content of the sequence. Its protocol is described in more detail: we used PCR amplification with enhancer PCR buffer [23] and a Touchdown PCR protocol [24]. Number of copies of the VNTR was determined by size in agarose gel electrophoresis. We obtained valid VNTR results from 97.0 % of our original discovery collection (295 patients) but no additional samples were genotyped given the problems and lack of difference observed. ~~In addition,~~The VNTR genotypes of 44 samples were verified with a second PCR method in which 2'deoxyguanosine was substituted by 7-deaza-2`deoxyguanosine (7-deaza-dGTP) (Roche Diagnostics, Mannheim, Germany) following the protocol previously described [25]. Finally, samples with different genotypes of the three polymorphisms were sequenced to assess the accuracy of results. All primer sequences and details of the PCR protocols are provided (Supplementary Note 1 and Supplementary Table 1).

Statistical analysis. The Statistica 7.0 (Statsoft, Tulsa OK) software was used thoroughly. Allele frequencies, odds ratios (O.R) and their 95% confidence intervals

(95% CI) were calculated from 2x2 contingency tables. The HWE of the SNPs was analyzed with the Guo and Thompson exact method [26]. Pairwise D´and r^2 measures of linkage disequilibrium (LD) were obtained with Haploview [27]. Treatment outcomes were considered as ΔDAS28, main outcome, or according to the EULAR criteria, secondary outcome [28]. A general~~ized~~ linear model in the first case, and a logistic regression model in the second were fitted. In ~~both cases~~ all analyses, genotypes were considered according with an additive genetic model, which is the model that best accounts for complex traits. EULAR categories were compared either as good + moderate responders *versus* non-responders, or as good responders *versus* non-responders (leaving moderate responders out of the analysis). The specific type of analysis is indicated in the text. We also tested for interaction involving *FCGR2A* and *FCGR3A* nsSNPs because it has been previously reported [29, 30]. Two approaches were followed. First, we grouped the diplotypes (two-gene genotypes) according to the presence of high affinity alleles (H131 in FCGR2A and 158V in FCGR3A). This classification resulted in a variable with four levels ranging from 0 for patients that were homozygotes for 131R and F158, to 4 high affinity alleles for patients that were homozygotes for H131 and 158V. This new variable was regressed against the treatment outcomes. The second approach was to analyze the interaction term in regression models including the two SNPs and the multiplicative interaction between them. All these analyses included three possible confounding factors as covariates: baseline DAS28, patient gender and the specific TNFi. In addition, some analyses were done with the center of recruitment and the year of treatment as additional covariates to account for heterogeneity in the treatment and evaluation of the patients. Baseline DAS28, the specific TNFi and the year of treatment were significantly associated with response to treatment. In addition, there were differences in response

to treatment between some centers of recruitment. Gender was included because it is a common covariate in most RA studies and some reports indicate that it is an important factor in the response to treatment, but no significant difference was observed in our study. Bonferroni correction by the number of FcγR polymorphisms we have studied (4 including ref. [20]) was applied for interpretation of the results. Power of our study for the main outcome was estimated with the GPower software [31].

We searched PubMed, the ISI Web of Science using the following terms "Arthritis" and "FCGR3A" and "Polymorphism" or "TNF" or "treatment", and the references of all the articles we have found for studies analyzing the role of *FCGR3A* F158V SNP in the response of RA patients to TNFi. All the studies describing original studies addressing genetic association of response to treatment with any of the three TNFi mentioned above (INX, ADM, ETC) in patients with rheumatoid arthritis were selected. Information relative to the sample size, the outcome, the genetic model, and the percentages of responders and non-responders was manually extracted. One type of analysis was the most common ~~to most~~in previous studies. It involved comparison of responders and non-responders following a FF genotype recessive model (FF *vs.* FV-VV). This model~~It~~ was selected due to the wide availability of the corresponding effect size for fixed-effects meta-analysis with the inverse variance weighting method as available in the *meta* library of the R project [32]. Fixed-effects meta-analysis was used because this model is generally more powerful than the random effect model. Heterogeneity of effect sizes was evaluated with the inconsistency parameter I^2 derived from the Cochran Q statistic [33]. A high, moderate and low level of inconsistency was attributed to levels of I^2 over 75%, 50% and 25%, respectively. Power of the meta-analysis was evaluated with the approach described by Hedges and Pigot [34].

Results

Patient characteristics and their response to TNFi. ~~Six of the 429 patients initially included in the study showed a baseline DAS28 <3.2. They were excluded from analysis because this low disease activity makes evaluation unreliable.~~ The ~~remaining~~ 423 selected ~~RA~~ patients showed characteristics of severe RA (Table 1) with a high percentage of erosive arthritis (84%). In addition, high mean baseline DAS28 (5.9) and high mean baseline Health Assessment Questionnaire (HAQ) score (1.5) showed their active disease with moderate to severe disability before starting treatment with TNFi. This status was observed in spite of the previous treatment with a mean of 2.5 different Disease Modifying Anti-Rheumatic Drugs (DMARDs). These patients were followed during treatment with their first biologic drug, which specifically was one of the three most common TNFi, INX (60.8 %), ETC (20.8 %) or ADM (18.4 %). Most patients (94.6 %) received combined therapy with a DMARD accompanying the TNFi. This treatment resulted in improvement of DAS28 at all times of follow-up, but about 20 % of the patients were non-responders.

Analysis of *FCGR3A* and *FCGR2B* nsSNPs and of the VNTR in *FCGRT*. The two nsSNPs were successfully genotyped in 98.8 % of the 423 samples. The nsSNP in *FCGR2B* is located in a sequence that is identical to the sequence of *FCGR2C,* which is a pseudogene bearing the ancestral allele at the site homologous with the I232T polymorphism. This circumstance makes it impossible to distinguish IT heterozygotes from TT rare allele homozygotes. Therefore, a carrier analysis of the 232T allele was performed. In turn, the VNTR in *FCGRT* was difficult to amplify, but it was genotyped with a 97.0 % success rate in the first 295 samples (no additional samples

were analyzed). Only the 2 (11.9 %) and 3 (87.2 %) repeats of the VNTR were common. The alleles of 4 and 5 repeats were observed, but in less than 1% of the patients and were excluded from further analysis. All the quality control filters were passed: genotypes were in HWE, replicated samples showed concordant genotypes, and the frequencies observed in our patients were similar to the previously reported in Europeans.

Association with the response to TNFi was analyzed considering ΔDAS28 ~~as the main outcome~~ and classification by the EULAR criteria ~~as a secondary outcome~~. None of these outcomes showed significant association with the nsSNP or with the VNTR genotypes at any of the three times analyzed (Table 2). The *FCGR2B* polymorphism was nominally associated with the response assessed by the EULAR criteria at 3 months of follow-up ($P = 0.03$). However, this association is not considered as significant because it did not pass Bonferroni correction by the number of tests, and it was only present when comparing responders (good + moderate responders) with non-responders. It was not present when comparing good responders with non-responders ($P = 0.08$), or in analysis of the primary outcome, ΔDAS28, which is ~~more sensitive~~ a continuous quantitative variable supporting more sensitive regression analysis than the EULAR criteria, a categorical variable [35]. The results were not modified by the inclusion of the center of recruitment and the year in which treatment with the TNFi was started as covariates (Supplementary Table 2). These analyses were done to account for possible heterogeneity in the treatment and evaluation of the patients.

Some previous studies have reported interactions between the *FCGR3A* F158V and the *FCGR2A* H131R nsSNPs [29, 30]. We explored this possibility taking advantage of our previous analysis of the *FCGR2A* nsSNP in the same RA patients considered

here [20]. The combined analysis showed that the two *FCGR2A/FCGR3A* nsSNPs were in weak LD (pairwise D' = 0.38, 95% CI = 0.26-0.48, and $r^2 = 0.074$) in our samples. However, there was not significant interaction between them (Supplementary Table 3) and the total number of the high affinity alleles (H131 at FCGR2A and 158V at FCGR3A) at the two nsSNPs was not associated with the response to TNFi (not shown).

Meta-analysis of studies addressing *FCGR3A* F158V association. We completed our analyses by summarizing the studies that have addressed the role of the *FCGR3A* F158V nsSNP in the response or RA to TNFi. We searched for articles and abstracts in the ISI Web of Science, in PubMed and in the bibliography of the manuscripts we were finding. Eight reports were found according to our inclusion criteria (Table 3). They were considered together with the current study. The nine studies are heterogeneous in sample size, in the TNFi used for treatment, in the outcomes and in the results. Four studies (including the current study) were of more than 280 patients each, whereas the other five studies were of less than 80 patients each. Together the five small studies add to fewer patients (254) than any one of the three large studies. Four of the five small studies showed a significant association with the response to TNFi, in contrast with none of the four large studies. This overrepresentation of significant association in the small studies was indicative of publication bias as shown by analyzing a funnel plot of the studies (Supplementary Figure 1) [36]. The four studies reporting a significant association showed an improved response in the FF homozygous patients. They included either the three TNFi or only INX, but no differences between the TNFi have been reported.

Combination of the results by meta-analysis could not include two of the large studies because of incomplete information [5] or a different pattern of genotype frequencies

[11]. The other seven studies contained information allowing for the comparison of treatment responders and non-responders between patients with the FF genotype and patients with the FV-VV genotypes. Specifically, responders (good + moderate responders) and non-responders according to the EULAR criteria were available for 5 studies, whereas in two other studies responders and non-responders according to ACR20 were available. The two classifications were considered as equivalent for meta-analysis. This decision was supported by the fact that two associated studies had used the EULAR criteria [22], and the other two the ACR20 criteria [37]. There was also some heterogeneity in the times of assessment. However, the three months assessment was available in five studies and we choose this time point for evaluation. In the two studies lacking this time point, the nearest evaluation was considered. Two of the studies did not include patients that were both FF homozygotes and non-responders [9, 10], making impossible to obtain an OR for them. These two studies were pooled with the most similar of the other studies to allow their inclusion in the meta-analysis. In this way, we analyzed 899 patients from seven studies (Figure 1). They did not show association with the response to TNFi (OR = 1.11, 95% CI = 0.8-1.5; $P = 0.5$). This result was observed with moderate heterogeneity between the studies ($I^2 = 62$ %). A priori power of a meta-analysis as the performed was sufficient (with $1-\beta = 0.8$, for $\alpha = 0.05$) to exclude an effect size equivalent to OR = 1.31. We also did not find association in a second meta-analysis. This meta-analysis combined results at different times, choosing the time of follow-up showing the strongest association in each study (Supplementary Figure 2). It was designed to offset possible losses of sensitivity due to the heterogeneity in the assessment.

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Discussion

We did not find significant association of any of the three functional polymorphisms analyzed, *FCGR3A* F158V, *FCGR2B* I232T, and the VNTR of *FCGRT,* with the response of RA patients to TNFi. These disappointing results were obtained in spite of the sample size of the study, the largest analyzing any of these polymorphisms in relation with the response to TNFi. In addition, the three polymorphisms were plausible biomarkers of the efficacy of Fc-containing TNFi. Two of them, *FCGR3A* F158V and *FCGR2B* I232T, have been previously associated with several disease phenotypes in patients with RA including response to TNFi [4-10, 38, 39]. The third, VNTR in *FCGRT*, has not been studied in this context, but its involvement was likely given the strong influence of FcRn on the IgG half-life [16-18]. Finally, the study was feasible as shown by the identification in the same RA patient samples of a reproducible association of response to INX and the *FCGR2A* H131R polymorphism [20] and by the *a priori* power of a study of this size to detect (with 1-β = 0.8 power for α = 0.05) effects of the *FCGR3A* polymorphism corresponding to a slope of 0.3 or larger, a slope of ≥ 0.46 for the *FCGR2B* SNP, and a slope ≥ 0.52 for the VNTR in *FCGRT* at 6 months of follow-up. Our results are more conclusive regarding the *FCGR3A* F158V nsSNP, because the availability of previous studies allowed combining them by meta-analysis. This meta-analysis rules out its value as a biomarker of the RA response to TNFi and suggests that previously reported *FCGR3A* F158V associations are likely ascribable to false positive results.

Previous support for *FCGR3A* F158V as a biomarker of the response to TNFi in RA patients was apparently strong. However, our meta-analysis has shown its weakness. The distribution of the results of all the studies in function of their sample sizes (Supplementary Figure 1) is very indicative of publication bias, with the significant

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associations overrepresented among the small studies [36]. Therefore, it is likely that studies of small sample size not finding association have also been done, but remain unpublished. This bias gives the appearance of a notable fraction of studies with significant association, but this is misleading as shown by the summary statistic of our meta-analysis. However, a note of caution on this meta-analysis is required because it had not enough power to detect mild effects, and because previous studies were heterogeneous in the follow-up times and in the assessment of response to treatment.

The *FCGR3A* F158V SNP has also been proposed as a biomarker of the response to other biologics. The most consistent results involve increased Antibody Dependent Cell Citotoxycity (ADCC) or killing by NK cells afforded by the V allele of *FCGR3A* F158V [40-42]. This mechanism seems to explain the improved response of some lymphomas and other hematological cancers to rituximab, an anti-CD20 chimeric monoclonal antibody, and other cancers to biologics that act by killing cancer cells [43]. It could also explain the better response to rituximab in patients with RA and other autoimmune diseases who carried the V allele of *FCGR3A* F158V [44-47]. In these diseases, rituximab efficacy is associated with the killing of autoimmune B cells. Therefore, the *FCGR3A* F158V SNP is a possible biomarker of the response to rituximab in RA, but we have ruled it out as a biomarker of the response to TNFi. This is in agreement with the lack of a role for ADCC and NK killing in the response to TNFi.

An additional complexity in the relationship of the *FCGR3A* F158V with Fc-containing drugs becomes evident when considering the studies analyzing Crohn's disease. In this disease, the VV genotype of *FCGR3A* F158V has been associated with the response to one of the TNFi, INX, but the improvement was observed in a biological response, not in the clinical response. In effect, several studies report a

larger decrease of CRP in the VV homozygotes than in the other genotypes that implies some type of biological effect, but this was not translated in the clinical response [42, 48, 49]. We checked our patients for this effect, but no association between *FCGR3A* F158V and the decrease in CRP levels after treatment with INX was observed (Supplementary Table 4). All these results suggest that the *FCGR3A* F158V polymorphism influences the responses to treatment with some Fc-containing biologics with the clinical effect depending on the mode of action of the specific drug and on the specific disease.

The *FCGR2B* I232T SNP was not associated with the response to TNFi except for an isolated result observed at 3 months of follow-up with a particular comparison according to the EULAR criteria. This isolated observation obtained with our secondary outcome and not passing Bonferroni correction would most likely be due to chance assortment of the patients in the different response classes. However, lack of association with the response to TNFi should not be interpreted as a sign of the irrelevance of the polymorphism for other clinical phenotypes. In effect, there are sound studies showing association of the T allele with SLE susceptibility, especially in Asians [50].

The current study was conducted when four GWAS have already explored the response to TNFi in patients with RA [54-57]. These GWAS are small in relation with the studies analyzing disease susceptibility or other complex traits. The largest study, combining patients from the other 3 GWAS, included 2706 patients [57]. These numbers show the current difficulties in assembling large sets of patients with sufficient data for this type of genetic studies. Until now, the GWAS have identified some promising associations, as the association of CD84 with the response to ETC,

but none at the level of GWAS significance. None of these studies reported association with the FcγR genes, which is in concordance with our results. However, this lack of reporting is hard to interpret because of the strict significance thresholds that GWAS apply.

In summary, none of the three functional polymorphisms in FcγR genes explored here, the *FCGR3A* F158V and *FCGR2B* I232T nsSNPs and the VNTR in *FCGRT*, showed an association with the response to TNFi in patients with RA. These results were obtained in spite of the larger sample size relative to all the previous studies addressing these polymorphisms in the response to TNFi. In addition, meta-analysis of studies on the *FCGR3A* F158V SNP also showed lack of association. Therefore, the search of biomarkers to guide treatment of RA patients with TNFi should follow other routes.

Conflict of interest and sources of funding

There is not conflict of interest.

The present work was supported by Fondo de Investigacion Sanitaria of the Instituto de Salud Carlos III (Spain), Grants PI11/01048, PI12/01909, RD08/0075/0019, and RD12/0009/0008 that are partially financed by the European Regional Development Fund of the European Union.

Acknowledgements

We thank Cristina Rodriguez-Fontenla for help in genotyping design, Carmen Pena-Pena for technical assistance and Manuel Calaza for help with data management.

Future prespectives

One of the main concerns in the management of RA patients is how to choose the most efficacious drug for a given patient given the individual variability in responses. This need is aggravated by the high cost of the biologics and the need to initiate an effective treatment early in the disease process to obtain the largest benefit for the patient. Already, there are several biomarkers for prediction of response to specific drugs that have been identified in sound studies, but none has yet been established as sufficiently reproducible or as clinically useful. Some of these biomarkers are pharmacogenetic biomarkers. They require further validation in additional sample collections as the evidence supporting them is not incontrovertible. This validation will be obtained by replication in new studies with large sample collections. In addition, they require an assessment of their predictive potential because studies until now have been centered in demonstrating a significant association not predictive power. This will require a different type of studies, most likely including clinical trials that incorporate the biomarkers in the assignement of patients to different treatment branches.

In addition, it is very likely that most genetic biomarkers are still undiscovered given the relative small size of the studies done until now. It is clear that this is the largest barrier for future progress: the lack of large collections of samples with response to treatment information. The recent availability of a variety of new drugs for treating RA does not make the tasks of collection these samples any easier, but the conscience of the problem and of the technical possibilities we have should convince more researchers to participate in these efforts.

It is likely that after further study and validation the integration of different biomarkers from the genetics field and from other biological or clinical fields will lead to a clinical prediction model to help choose the most appropriate drug for each patient.

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Table 1. Clinical characteristics of the patients with RA included in this study

Patients, number	423
Women, %	83.9
Age at diagnosis, median (IQR)	45 (36-55)
Diagnosis to anti-TNF, median years (IQR)	7 (3-12)
Rheumatoid factor, %	76.7
Anti-CCP antibodies, % [a]	70.2
Erosive arthritis, %	84.0
Smoking, % [a]	16.4
DMARD before anti-TNF, mean ± SD	2.5 ± 1.3
Concomitant DMARD, % [a]	94.6
Baseline CRP (mg/L), median (IQR) [a]	8 (4-20)
Baseline ESR (mm/h), median (IQR) [a]	35 (19-56)
Baseline HAQ, median (IQR) [a]	1.5 (1-2.1)
TNF inhibitor, % (number)	
Infliximab (INX)	60.8 (257)
Adalimumab (ADM)	18.4 (78)
Etanercept (ETC)	20.8 (88)
DAS28, mean ± SD	
baseline	5.9 ± 1.2
3 months	3.9 ± 1.4
6 months	3.8 ± 1.4
12 months	3.6 ± 1.4
EULAR response, %	
3 months	
responder	29.9
moderate	50.5
non-responder	19.6
6 months	
responder	39.9
moderate	39.7
non-responder	20.4
12 months	
responder	43.6
moderate	38.3
non-responder	18.1

Years from diagnosis to TNFi, median (IQR)		7 (3-12)
Rheumatoid factor, %		76.7
Anti-CCP antibodies, % [a]		70.2
Erosive arthritis, %		84.0
Smoking, % [a]		16.4
DMARD before anti-TNF, mean ± SD		2.5 ± 1.3
Concomitant DMARD, % [a]		94.6
Baseline CRP (mg/L), median (IQR) [a]		8 (4-20)
Baseline ESR (mm/h), median (IQR) [a]		35 (19-56)
Baseline HAQ, median (IQR)[a]		1.5 (1-2.1)
TNF inhibitor, %		INX / ADM / ETC[b]
		60.8 / 18.4 / 20.8
DAS28, mean ± SD		
	baseline	5.9 ± 1.2
	3 months	3.9 ± 1.4
	6 months	3.8 ± 1.4
	12 months	3.6 ± 1.4
EULAR response, %		R / M / NR[c]
3 months		29.9 / 50.5 / 19.6
6 months		39.9 / 39.7 / 20.4
12 months		43.6 / 38.3 / 18.1

[a] Data from < 85 % of the patients were available: 349 for anti-CCP antibodies, 311 for smoking, 261 for CRP, 304 for ESR, and 329 for baseline HAQ.

[b] INX = infliximab, ADM = adalimumab, ETC = etanercept

[c] R = responder, M = moderate responder, NR = non-responder

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Table 2. Distribution of the *FCGR* genotypes in the patients with RA.

	***FCGR3A* F158V**					***FCGR2B* I232T[a]**				***FCRGT*[b]**			
	FF	FV	VV			II	IT+TT			3/2	3/3		
Total	35.6	51.4	12.9			74.6	25.4			19.3	80.7		
ΔDAS28 (mean ± SD)				β	*P*			β	*P*			β	*P*
3 months, n = 364	1.9 ±1.5	2.0 ±1.4	2.0 ±1.3	0.05	0.2	1.9 ±1.5	2.1 ±1.3	0.05	0.3	2.2 ±1.5	2.1 ±1.4	-0.07	0.3
6 months, n = 377	2.1 ±1.6	2.0 ±1.6	2.4 ±1.6	0.07	0.1	2.1 ±1.6	2.2 ±1.6	0.03	0.5	1.9 ±1.6	2.2 ±1.5	0.03	0.5
12 months, n = 284	2.4 ±1.6	2.2 ±1.8	3.0 ±1.7	0.06	0.2	2.3 ±1.7	2.4 ±1.7	0.001	1.0	2.2 ±1.8	2.5 ±1.7	0.02	0.7
EULAR criteria ~~(n)~~				OR (95% CI)	*P*			OR (95% CI)	*P*			OR (95% CI)	*P*
3 months, n = 364 ~~(364)~~													
Responder	34.5	51.9	16.7	1.2	0.4	74.3	25.7	2.3	0.03	20.1	79.9	0.75	0.5
Non-responder	39.4	49.3	11.3	(0.8-1.8)		87.3	12.7	(1.1-5.0)		17.4	82.6	(0.3-1.8)	
6 months, n = 377 ~~(377)~~													
Responder	35.6	50.7	13.8	1.0	0.9	72.6	27.4	1.6	0.2	18.0	82.0	1.8	0.6
Non-responder	32.0	54.7	13.3	(0.7-1.5)		79.0	21.0	(0.8-3.0)		29.8	70.2	(0.8-4.0)	
12 months, n = 284 ~~(284)~~													
Responder	34.5	52.6	12.9	1.4	0.2	72.5	27.5	1.03	0.9	16.8	83.2	1.4	0.5
Non-responder	32.7	63.5	3.8	(0.8-2.3)		72.6	27.4	(0.5-2.1)		22.2	77.8	(0.5-3.6)	

Top row shows the genotype % for all patients. Upper rows present mean ΔDAS28 (± SD) for each genotype at the different evaluations. Lower rows show the genotype frequencies as percentage of the responder (good + moderate responders) and non-responder subgroups according to EULAR criteria. All analyses were adjusted by gender, TNFi and baseline DAS28

[a] Only carrier analysis was possible for *FCGR2B*

[b] The *FCGRT* VNTR was genotyped in all samples of our original discovery collection (295 patients) and exclude low frequency genotypes (2/2, 2/4, 3/4 and 3/5 that were observed only in 6, 2, 2 and 1 patients, respectively). Remaining patient numbers were: 245, 252 and 215 at 3, 6 and 12 months, respectively

Table 3. Summary of the studies addressing association of the *FCGR3A* F158V nSNP with the response to TNFi in patients with RA.

Study	Time[a]	Number	Criteria	TNFi	% responders[b]	Association with response
Sarsour *et al.*	6	390	CDAI[c]	-	-	No
Criswell *et al.*	12	301	ACR50	ETC	-	No
Kastbom *et al.*	3	282	ACR20	INX ETC	66.3	No
Rooryck *et al.*	3	78	ACR20	INX	56.4	No
Tutuncu *et al.*	3	35	EULAR	INX ADM ETC	65.7	FF genotype
Tsukahara *et al.*	5	29	EULAR	INX	93.1	FF genotype
Morales-Lara *et al.*	3	38	ACR20 EULAR	INX	76.3	No
	6	37			62.2	No
	12	32			68.8	FF genotype (ACR20)
Cañete *et al.*	1.5	73	ACR20 EULAR	INX ETC ADM	67.5	FF genotype (ACR20)
	7	74			61.0	No
Current study	3	364	EULAR	INX ETC ADM	80.5	No
	6	377			79.9	No
	12	286			81.7	No

[a] Time of follow-up in months
[b] Frequency of responders considering EULAR criteria (good + moderate responder) when available, ACR20 otherwise
[c] CDAI = Clinical Disease Activity Index

Figure Legends

Figure 1. Fixed-effects meta-analysis of the studies comparing *FCGR3A* F158V between TNFi responder and non-responder patients with RA at 3 months of treatment. The Tutuncutu *et al.,* Morales-Lara *et al.* and Tsukahara *et al*. studies were joined to avoid zero counts in the OR denominator. EULAR response (good + moderate) was used when available; the ACR20 response otherwise. FF genotypes were compared with FV+VV genotypes.

Supplementary Material

It includes Supplementary Note 1 describing details of the protocols and Supplementary Table 1 with the primers and probes used for genotyping; Supplementary Table 2 with the association analysis after adjusting by center of recruitment and year of start of treatment as additional covariates; Supplementary Table 3 with the analysis of interaction between *FCGR2A* and *FCGR3A* nsSNPs; Supplementary Table 4 with analysis of the association between *FCGR3A* nsSNP and change in CRP at 6 months in patients treated with INX; and Supplementary Figure 1 with the funnel plot of all the studies addressing *FCGR3A* association with response to TNFi in RA patients; and Supplementary Figure 2 with the forest plot of a second meta-analysis including each study at the time of follow-up showing maximum association.

Executive summary

Need of biomarkers to select rheumatoid arthritis biologic drugs and opportunity among the *FCGR* genes

- A third of the patients with rheumatoid arthritis fail to respond to specific biologic drugs, but can respond after changing to another drug. The trial and error approach currently followed is inefficient and very costly.
- The pharmacokinetics and action of some of these biologic drugs (such as the TNF inhibitors: infliximab, etanercept and adalimumab) can be modulated by polymorphisms in the genes of FcγR because their molecules contain the Fc region of IgG1.

Study design and protocol

- Three functional *FCGR* polymorphisms were selected for study: *FCGR3A* F158V, which FF genotype has previously been associated with better response to TNF inhibitors in some studies, VNRT of *FCGRT*, which modulates the level of expression of the major determinant of IgG half-life, and *FCGR2B* I232T, which is associated with specific phenotypes in rheumatoid arthritis and other diseases
- Response to treatment was evaluated in 429 patients with RA treated with TNF inhibitors as the first biologic drug either as change in DAS28 or according to the EULAR response criteria.
- A meta-analysis of all published studies addressing the association of *FCGR3A* F158V with response to TNF inhibitors in rheumatoid arthritis was also performed.

Association of polymorphisms and clinical response to TNF inhibitors

- None of the three functional polymorphisms showed significant association with the response to TNF inhibitors.
- The meta-analysis of seven *FCGR3A* F158V with sufficient information showed also lack of significant association. This result contrast markedly with the previous appearance of association due to the small size of the four studies reporting association.

Interpretation

- None of the three *FCGR* polymorphisms studied influence response to TNF inhibitors in rheumatoid arthritis. However, other *FCGR* polymorphisms do modify this response as we have shown in a reproducible way for *FCGR2A* H131R.
- The apparent reproducibly association of *FCGR3A* FF genotype with a better response to TNF inhibitors can be ascribed to false positive results and publication bias as none of the larger studies, nor the meta-analysis supported this result.
- However, the *FCGR3A* F158V polymorphism can modify the clinical response to drugs with a different mechanism of action, as rituximab that cause cell cytotoxicity, or other types of responses as the modulation of CRP levels observed in Crohn's disease.

Conclusions

- None of the three functional FcγR polymorphisms explored here was associated with the response to TNFi in patients with RA.

Meta-analysis of all the *FCGR3A* F158V showed lack of association.