

RESEARCH ARTICLE

Variation at *FCGR2A* and Functionally Related Genes Is Associated with the Response to Anti-TNF Therapy in Rheumatoid Arthritis

Gabriela Avila-Pedretti¹, Jesús Tornero², Antonio Fernández-Nebro³, Francisco Blanco⁴, Isidoro González-Alvaro⁵, Juan D. Cañete⁶, Joan Maymó⁷, Mercedes Alperiz⁸, Benjamín Fernández-Gutiérrez⁹, Alex Olivé¹⁰, Héctor Corominas¹¹, Alba Erra¹², Adrià Aterido¹, María López Lasanta¹, Raül Tortosa¹, Antonio Julià¹, Sara Marsal^{1*}



OPEN ACCESS

Citation: Avila-Pedretti G, Tornero J, Fernández-Nebro A, Blanco F, González-Alvaro I, Cañete JD, et al. (2015) Variation at *FCGR2A* and Functionally Related Genes Is Associated with the Response to Anti-TNF Therapy in Rheumatoid Arthritis. PLOS ONE 10(4): e0122088. doi:10.1371/journal.pone.0122088

Academic Editor: Cordula M. Stover, University of Leicester, United Kingdom

Received: December 29, 2014

Accepted: February 18, 2015

Published: April 7, 2015

Copyright: © 2015 Avila-Pedretti et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: This work was supported by the Spanish Ministry of Economy and Competitiveness grants (PSE-010000-2006-6 and IPT-010000-2010-36). The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

1 Vall d'Hebron Hospital Research Institute, Rheumatology Research Group, Barcelona, Spain, **2** Hospital Universitario De Guadalajara, Rheumatology Department, Guadalajara, Spain, **3** UGC Reumatología, Instituto de Investigación Biomédica en Málaga, Hospital Regional Universitario de Málaga, Universidad de Málaga, Málaga, Spain, **4** INIBIC-Hospital Universitario A Coruña, Rheumatology Department, A Coruña, Spain, **5** Hospital Universitario de La Princesa, IIS La Princesa, Rheumatology Department, Madrid, Spain, **6** Hospital Clínic de Barcelona, Rheumatology Department, Barcelona, Spain, **7** Hospital del Mar, Barcelona, Rheumatology Department, Barcelona, Spain, **8** Hospital Universitario Central de Asturias, Rheumatology Department, Oviedo, Spain, **9** Hospital Clínico San Carlos, Madrid, Rheumatology Department, Madrid, Spain, **10** Hospital Universitari Germans Trias i Pujol, Rheumatology Department, Barcelona, Spain, **11** Hospital Moisès Broggi, Rheumatology Department, Barcelona, Spain, **12** Hospital Sant Rafael, Rheumatology Department, Barcelona, Spain

* sara.marsal@vhir.org

Abstract

Objective

Anti-TNF therapies have been highly efficacious in the management of rheumatoid arthritis (RA), but 25–30% of patients do not show a significant clinical response. There is increasing evidence that genetic variation at the Fc receptor *FCGR2A* is associated with the response to anti-TNF therapy. We aimed to validate this genetic association in a patient cohort from the Spanish population, and also to identify new genes functionally related to *FCGR2A* that are also associated with anti-TNF response.

Methods

A total of 348 RA patients treated with an anti-TNF therapy were included and genotyped for *FCGR2A* polymorphism rs1081274. Response to therapy was determined at 12 weeks, and was tested for association globally and independently for each anti-TNF drug (infliximab, etanercept and adalimumab). Using gene expression profiles from macrophages obtained from synovial fluid of RA patients, we searched for genes highly correlated with *FCGR2A* expression. Tag SNPs were selected from each candidate gene and tested for association with the response to therapy.

Results

We found a significant association between *FCGR2A* and the response to adalimumab ($P=0.022$). Analyzing the subset of anti-CCP positive RA patients (78%), we also found a significant association between *FCGR2A* and the response to infliximab ($P=0.035$). *DHX32* and *RGS12* were the most consistently correlated genes with *FCGR2A* expression in RA synovial fluid macrophages ($P<0.001$). We found a significant association between the genetic variation at *DHX32* (rs12356233, corrected $P=0.019$) and a nominally significant association between *RGS12* and the response to adalimumab (rs4690093, uncorrected $P=0.040$). In the anti-CCP positive group of patients, we also found a nominally significant association between *RGS12* and the response to infliximab (rs2857859, uncorrected $P=0.042$).

Conclusions

In the present study we have validated the *FCGR2A* association in an independent population, and we have identified new genes associated with the response to anti-TNF therapy in RA.

Introduction

The introduction of Tumor Necrosis Factor (TNF) inhibitors has revolutionized the treatment of rheumatoid arthritis (RA). In the clinical practice, anti-TNF alpha agents have made it possible to achieve a minimal inflammatory activity or even disease remission [1,2]. Despite their clear efficacy in RA management, there is a substantial group of patients who will fail to respond to this therapeutic approach [3]. The high costs of these therapies as well as the availability of alternative biologic therapies in RA, clearly increase the need to identify markers of response to anti-TNF agents [4].

Genetic variation has shown to influence many aspects of RA heterogeneity, including the response to anti-TNF therapy [5,6]. Genome-wide association studies (GWAS) are a powerful genetic analysis approach and have allowed the identification of new genomic regions associated with treatment response in RA [7,8]. Candidate-gene studies, although limited to the knowledge of the biological pathways associated to a particular disease or trait, have also been successful in identifying new candidate loci for the response to anti-TNF therapy [9]. One such candidate gene is *FCGR2A*, encoding an Fc receptor mainly expressed in macrophages and dendritic cells [10], and for which there is increasing evidence supporting its association to anti-TNF therapy in RA [11].

Fc receptors for IgG immunoglobulins (FCGRs) are expressed in different immune cells but predominantly in phagocytic cells like macrophages [12,13]. In these cells, FCGRs bind to extracellular IgG immunoglobulins, and this binding can lead to either cell activation or repression [13,14,15]. Consequently, the genetic variants that affect the activity of FCGRs [16,17,18,19], could also influence the efficacy of immunoglobulin-based therapies like anti-TNF agents. Infliximab, adalimumab and etanercept are the most commonly used anti-TNF agents in RA, and are characterized by having an IgG1 Fc portion that can bind to FCGRs. Therefore, variations in the Fc binding affinity between the different anti-TNF agents could also influence the response to these biological therapies [20,21,22].

FCGR2A (CD32A) SNP rs1801274 is a nonsynonymous polymorphism that leads to an amino acid change at position 131 of the Fc receptor (i.e. R131H). This change in the protein

sequence has shown to have important implications in the binding of the receptor to different IgG subclasses [23,24]. Consequently, rs1801274 is a strong candidate for influencing the response to IgG-based treatments, like anti-TNF agents. There is increasing evidence that variation at this SNP is associated with a differential response to anti-TNF therapy in RA [11,25]. Importantly, there is recent evidence that the association between *FCGR2A* and the clinical response in RA could be dependent on the type of anti-TNF agent, with a significant association in patients treated with infliximab [25,26] and a lack of association on etanercept-treated patients [26,27]. Despite the increasing evidence of a strong and differential genetic background associated with patients positive for anti-cyclic citrullinated protein antibodies (anti-CCP, ~70–80% of patients) [28,29,30], very few pharmacogenetic studies in RA have evaluated testing for association in this subgroup of patients. If confirmed, this drug specific associations would be of major relevance for RA. First, it would allow the identification of biological pathways that are specifically targeted by each anti-TNF agent, and secondly, it could lead to the development of new and more specific therapies and finally improve treatment personalization in RA.

The first objective of this study was to validate the association between *FCGR2A* and the clinical response to the main anti-TNF agents infliximab, adalimumab and etanercept. Next, we hypothesized that patients positive for anti-CCP antibodies could show stronger genetic associations to drug response. Also, we hypothesized that analyzing the gene expression correlation of *FCGR2A* in a crucial cell type in RA, synovial fluid macrophage, we could identify new candidate genes associated with anti-TNF response. Using a cohort of well-characterized RA patients we have been able to validate and further characterize *FCGR2A* association, as well as identify new candidate genes for anti-TNF response in RA.

Materials and Methods

Study population

A total of 348 RA patients that had received an anti-TNF therapy (infliximab, etanercept or adalimumab) as their first biological treatment, were included in the present study. This patient cohort was collected as part of the Immune-Mediated Inflammatory Disease Consortium (IMIDC) [9], which includes a network of rheumatology departments from 12 university hospitals from Spain. All patients fulfilled the 1987 American College of Rheumatology classification criteria for RA [31] and had >2 years of follow-up since diagnosis. All recruited individuals had an erosive disease defined as 1 \geq erosions in, at least, 2 joint groups in hands and/or feet. Only RA patients naïve to biologic therapies were included in this study. Patients were Caucasian European born in Spain and with all four grandparents also born in Spain.

Informed consent was obtained from all participants and protocols were reviewed and approved by local institutional review boards. The present study was conducted according to the Declaration of Helsinki principles.

The response to anti-TNF treatment was measured at week 12 following the EULAR treatment response criteria [32]. For all patients, the DAS28 activity score [33] was measured at baseline and after 12 weeks of anti-TNF treatment. According to the change in the DAS28 score and the endpoint DAS28, patients were categorized into good, moderate and none responders. As described previously, EULAR good and moderate responders were combined into a single anti-TNF responder group [9].

FCGR2A correlated genes in synovial RA macrophages

The NCBI Gene Expression Omnibus database of microarray data [34] was used to identify previous studies analyzing the gene expression profiles of macrophages obtained from synovial fluid from rheumatoid arthritis patients. Using the terms "rheumatoid arthritis + macrophage

+ synovial fluid" we found three different datasets (GSE49604, GSE11575 and GSE10500). From these, we selected the gene expression profiles from RA CD14+ synovial macrophages obtained by positive selection (i.e. GSE49604 and GSE10500). The gene expression profiles from RA macrophages generated by *in vitro* differentiation of blood monocytes (GSE11575 dataset) was not considered in this study. The selected gene expression data was processed and analyzed using the R statistical software [35] and the Bioconductor repository packages (www.bioconductor.org). The details on dataset selection, data preprocessing and correlation analyses are given in the Supporting Information (S1 Protocol). The correlation between *FCGR2A* gene expression and the genes expressed in synovial fluid macrophages was analyzed using the statistical test based on Pearson's product moment correlation. A significance threshold of $\alpha = 0.001$ was considered to select the genes most strongly correlated with *FCGR2A*.

DNA collection, SNP selection and genotyping

Whole blood samples were obtained from 348 RA patients and genomic DNA was extracted using the Chemagic Magnetic Separation Module I (Perkin Elmer, US). The *FCGR2A* polymorphism previously associated with the response to anti-TNF therapy, SNP rs1801274 (*FCGR2A-R131H*), was analyzed in the cohort of RA patients using the TaqMan Real-Time PCR platform (Life Technologies, US) with the C__9077561_20 predesigned assay.

Tag SNPs for *DHX32* and *RGS12* genes were selected using the genetic information from the Caucasian European cohort (CEU) sequenced in the 1,000 Genomes Project (1KGP) [36]. Briefly, the dense SNP genotype generated from the 1KGP data from the sequences of the two genes +/- 5 kb was downloaded. Using the Haploview genetic analysis tool (v4.2) [37] we identified the most relevant haplotype blocks in each gene and we selected the corresponding tagSNPs. Additional details on the haplotype analysis and tagSNP selection are given in the Supplementary Material (S1 Protocol). For *DHX32* association analysis we selected tagSNP rs12356233 (chromosome 10, pb 127,534,930), and for *RGS12* locus we selected tagSNP rs2857859 (pb 3,322,140) and rs4690093 (pb 3,412,196). Genotyping was performed with the TaqMan RT-PCR technology (Life Technologies, US) using predesigned assays C__31490226_30, C__26934339_10 and C__11283507_10 for rs12356233, rs2857859 and rs4690093 genotyping, respectively.

For all Taqman analyses RT-PCR thermal conditions were as follows: 50°C for 2 min and 95°C for 10 min, followed by 40 cycles of 92°C for 15 s and 60°C for 1 min. The PCR assay and point fluorescent readings were performed using an ABI PRISM 7900HT sequence detection system (Life Technologies, USA). Genotyping error was determined by re-genotyping 20% of the patients (<1% genotyping error).

Statistical analysis

The association of the *FCGR2A* SNP rs1801274 with treatment response was performed using Fisher's exact test. Given that the association between *DHX32* and *RGS12* SNPs and response to anti-TNF therapy was novel, we used the allelic chi-square test [38]. All statistical tests were performed using the R statistical software version 3.0.1 (www.R-project.org).

Results

Study population

A total of 348 RA patients treated with anti-TNF agents were included. The patients had a mean (\pm SD) age at diagnosis of 43.5 years (\pm 12.6), and an average disease duration of 10.2 years (\pm 8.3). From these, 126 patients had been treated with infliximab, 95 with adalimumab

Table 1. Epidemiological and Clinical Features of the Patient Study Cohort.

Variable	All (n = 348)	Infliximab (n = 126)	Adalimumab (n = 95)	Etanercept (n = 127)
Female, n (%)	287 (82.4)	107 (84.9)	76 (80.0)	104 (81.8)
Age at diagnosis (years)	43.5 ± 12.6	43.1 ± 11.6	45.9 ± 12.6	42.2 ± 13.3
Disease duration (years)	10.2 ± 8.3	10.1 ± 6.7	9.7 ± 9.1	10.8 ± 9.1
RF (+), n (%)	270 (77.8)	96 (76.1)	72 (76.6)	102 (80.3)
Anti-CCP (+), n (%)	261 (77.9)	87 (72.5)	79 (85.8)	95 (77.2)
Erosions, n (%)	314 (90.2)	115 (91.3)	85 (89.5)	114 (89.7)
Smokers, n (%)	148 (42.7)	52 (41.6)	43 (45.2)	43 (45.7)
Previous DMARDs	2.7 ± 1.7	3.1 ± 1.7	2.0 ± 1.4	2.7 ± 1.6
Concomitant corticoids, n (%)	182 (52.3)	64 (50.8)	49(51.6)	69 (54.3)
DAS28 (mean+/-SD), baseline	5.6 ± 1.1	5.6 ± 1.1	5.3 ± 1.0	5.7 ± 1.2
DAS28 (mean+/-SD), 12 weeks	3.9 ± 1.4	4.3 ± 1.4	3.5 ± 1.2	3.9 ± 1.5
ΔDAS28 (mean+/-SD)	1.6 ± 1.3	1.4 ± 1.3	1.7 ± 1.2	1.7 ± 1.4
EULAR Responders, n(%)	261(75%)	88 (70%)	76 (80%)	97 (76%)
EULAR Non-Responders, n(%)	87 (25%)	38 (30%)	19 (20%)	30 (24%)

Except where indicated otherwise, values are the mean ±SD. RF: rheumatoid factor; Anti-CCP: anti-citrullinated protein antibodies; DMARDs: disease-modifying antirheumatic drugs; ΔDAS28; delta DAS28 (DAS28 baseline—DAS28 endpoint); EULAR: European League Against Rheumatism response, where Good and Moderate EULAR responders were merged into a single Responder category.

doi:10.1371/journal.pone.0122088.t001

and 127 with etanercept. The complete clinical features of the patient cohort are shown in [Table 1](#).

Association of FCGR2A with anti-TNF response

Comparing *FCGR2A* genotype frequencies between the global cohort of anti-TNF therapy responders and non-responders, we found no significant association ($P = 0.15$, [Table 2](#)). When analyzing the association within each different anti-TNF agent, we found a statistically significant association between *FCGR2A* and the clinical response to adalimumab ($P = 0.022$, [Table 2](#)). In infliximab-treated patients, we did not observe a statistically significant association ($P = 0.11$), while in etanercept-treated patients we found no evidence of association with *FCGR2A* SNP ($P = 0.96$).

RA patients that are positive for anti-cyclic citrullinated protein antibodies (anti-CCP), have shown to have a differential and much stronger genetic background compared to anti-CCP negative patients [[28,29,30](#)]. Based on this observation, we explored the *FCGR2A* association with the response to anti-TNF therapy in the anti-CCP positive group of patients only (78% in our cohort) ([Table 3](#)). Analyzing each treatment separately, we found that *FCGR2A* SNP was significantly associated to the response of both adalimumab and infliximab ($P = 0.047$ and $P = 0.035$, respectively). By contrast, we found no association between *FCGR2A* and the clinical response to etanercept ($P = 1$).

Identification and analysis of FCGR2A functionally-related genes

We identified two microarray-based studies in RA analyzing the transcriptome of synovial macrophages (*GSE49604* and *GSE1050*, $n = 8$ samples each). In both studies, synovial macrophages were isolated from the synovial fluid by positive CD14+ selection. In each study we found one gene showing a consistent correlation with *FCGR2A* ($P < 0.001$). In *GSE49604* study we found *DEAH (Asp-Glu-Ala-His) box polypeptide 32* gene (*DHX32*) levels to be strongly associated to *FCGR2A* gene expression, while in *GSE1050* RA study we found *regulator of G-*

Table 2. Genotype frequencies of the FCGR2A polymorphism rs1801274 according to the clinical response at week 12.

Anti-TNF agent	Genotype	Responders n(%)	Non-responders n(%)	P-value ^a	OR(95%CI)
All (n = 348)					
	AA	67 (25.7)	25 (28.7)	-	-
	AG	143 (54.8)	38 (43.7)	-	-
	GG	51 (19.5)	24 (27.6)	0.15	1.1(0.78–1.56)
Infliximab (n = 126)					
	AA	23 (26.1)	16 (42.1)	-	-
	AG	49 (55.7)	14 (36.8)	-	-
	GG	16 (18.2)	8 (21.1)	0.11	0.76(0.44–1.32)
Adalimumab (n = 95)					
	AA	21 (27.6)	3 (15.8)	-	-
	AG	40 (52.6)	6 (31.6)	-	-
	GG	15 (19.7)	10 (52.6)	0.022	2.54(1.19–5.4)
Etanercept (n = 127)					
	AA	23 (23.7)	6 (20)	-	-
	AG	54 (55.7)	18 (60)	-	-
	GG	20 (20.6)	6 (20)	0.96	1.06(0.6–1.9)

^aFisher's exact test; OR: Odds ratio using allele G as reference.

doi:10.1371/journal.pone.0122088.t002

protein signaling 12 gene (*RGS12*) to correlate significantly with *FCGR2A* levels (S1 Fig). *DH32* was found to positively correlate with *FCGR2A* (average $r^2 = 0.93$) and *RGS12* to correlate negatively with *FCGR2A* expression (average $r^2 = -0.96$).

For each functionally-related gene we selected tagSNPs and performed an association test with the clinical response to anti-TNF therapy in the RA patient cohort (Table 4). Analyzing

Table 3. Genotype frequencies of the FCGR2A polymorphism rs1801274 in anti-CCP positive RA patients according to the clinical response at week 12.

Anti-TNF agent	Genotype	Responders n(%)	Non-responders n(%)	P-value ^a	OR (95%CI)
All (n = 261)					
	AA	52 (26.8)	21 (31.3)	-	-
	AG	104 (53.6)	26 (38.8)	-	-
	GG	38 (19.6)	20 (29.9)	0.079	1.12(0.76–1.66)
Infliximab (n = 87)					
	AA	15 (24.6)	13 (50)	-	-
	AG	36 (59)	8(30.8)	-	-
	GG	10 (16.4)	5 (19.2)	0.035	0.62(0.32–1.22)
Adalimumab (n = 79)					
	AA	18 (30)	3(15.8)	-	-
	AG	29 (48.3)	6 (31.6)	-	-
	GG	13 (21.7)	10 (52.6)	0.047	2.56(1.18–5.54)
Etanercept (n = 95)					
	AA	19 (26)	5(22.7)	-	-
	AG	39 (53.4)	12 (54.5)	-	-
	GG	15 (20.5)	5 (22.7)	1	1.12(0.57–2.19)

^aFisher's exact test; ANTI-CCP: anti-citrullinated protein antibodies; OR: Odds ratio using allele G as reference.

doi:10.1371/journal.pone.0122088.t003

Table 4. *DHX32* and *RGS12* association with clinical response in RA patients according to anti-TNF therapy.

SNP (gene)	Minor Allele	Major Allele	MAF	All anti-TNF OR(95%CI)	P	Infliximab OR(95%CI)	P	Adalimumab OR(95%CI)	P	Etanercept OR(95%CI)	P
rs12356233 (<i>DHX32</i>)	G	A	0.39	1.01(0.71–1.43)	0.96	0.65(0.37–1.15)	0.14	2.7(1.3–5.61)	0.0064*	0.85(0.47–1.56)	0.61
rs2857859 (<i>RGS12</i>)	T	C	0.3	0.87(0.59–1.28)	0.48	0.8(0.43–1.48)	0.48	0.98(0.45–2.15)	0.96	0.89(0.47–1.71)	0.73
rs4690093 (<i>RGS12</i>)	G	A	0.29	0.75(0.51–1.12)	0.16	0.95(0.52–1.71)	0.85	0.4(0.17–0.98)	0.04	0.88(0.44–1.76)	0.71

MAF: minor allele frequency in all RA patients; OR: odds ratio; CI: odds ratio confidence interval; P: nominal significance in association test with clinical response at week 12.

*significant (P<0.05) after multiple test correction.

doi:10.1371/journal.pone.0122088.t004

Table 5. *DHX32* and *RGS12* association with clinical response in anti-CCP positive RA patients according to anti-TNF therapy.

SNP (gene)	Minor Allele	Major Allele	MAF	All anti-TNF OR(95%CI)	P	Infliximab OR(95%CI)	P	Adalimumab OR(95%CI)	P	Etanercept OR(95%CI)	P
rs12356233 (<i>DHX32</i>)	G	A	0.39	1.09(0.73–1.64)	0.66	0.85(0.42–1.7)	0.64	2.65(1.25–5.6)	0.0095*	0.7(0.35–1.42)	0.32
rs2857859 (<i>RGS12</i>)	T	C	0.29	0.64(0.4–1.03)	0.062	0.4(0.17–0.99)	0.042	1.01(0.45–2.25)	0.99	0.68(0.31–1.48)	0.33
rs4690093 (<i>RGS12</i>)	G	A	0.3	0.85(0.55–1.32)	0.46	1.13(0.57–2.24)	0.73	0.4(0.16–0.99)	0.049	1.06(0.48–2.31)	0.89

MAF: minor allele frequency in all anti-CCP RA patients; OR: odds ratio; CI: odds ratio confidence interval; P: nominal significance in association test with clinical response at week 12.

*significant (P<0.05) after multiple test correction.

doi:10.1371/journal.pone.0122088.t005

all anti-TNF agents together, we found no association between *DHX32* or *RGS12* SNPs with the clinical response at week 12. However, when analyzing each treatment separately, we found a significant association between *DHX32* SNP rs12356233 with the response to adalimumab (corrected P = 0.019). We also found a nominally significant association between *RGS12* SNP rs4690093 and the response to adalimumab (uncorrected P = 0.040), but this was no longer significant after multiple test correction. When analyzing the anti-CCP positive group of RA patients (Table 5), rs12356233 was still significantly associated with adalimumab response (corrected P = 0.028), and *RGS12* SNP rs4690093 was also associated at the nominal level only (P = 0.044). Importantly, in this group of RA patients we also found a nominally significant association between *RGS12* SNP rs2857859 and the response to infliximab (uncorrected P = 0.042). This association was not significant after multiple test correction.

Discussion

Anti-TNF agents have been a major success in RA treatment, significantly improving the prognosis of many patients. There is, however, a group of RA patients that does not respond significantly to this therapeutic approach. Consequently, there is a major need to identify biomarkers that can help predict anti-TNF response and therefore guide anti-TNF therapy. In the present study we have used a cohort of RA patients from the Spanish population to validate the association between *FCGR2A* and anti-TNF agents infliximab, adalimumab and etanercept. Also, we have identified the genes most strongly correlated with *FCGR2A* expression in synovial fluid macrophages from RA studies, and we have found an association between these new, functionally-related genes with the response to anti-TNF therapy.

In our patient cohort we have found, for the first time, that *FCGR2A* is significantly associated with the response to infliximab in anti-CCP positive RA patients. Anti-CCP antibodies are

known to bind to citrullinated antigens expressed in the synovial joint like filaggrin, and lead to the formation of immune complexes which are powerful activators of the immune response [39]. FCGR2A receptor has been shown to be key in the internalization of immune complexes by phagocytic cells and consequently, could influence citrullinated peptide presentation by HLA proteins and the subsequent activation of autorreactive T cells [40]. In this context, differences in the affinity of FCGR2A for citrullinated immune complexes could be counteracting the beneficial effect of ADCC by inducing a major response to citrullinated autoantigens. The results of this study, therefore, support the importance of anti-CCP antibodies in the FCGR2A-mediated response to anti-TNF agents.

The genetic analysis of genes functionally related to *FCGR2A* in synovial macrophages from RA patients, has lead to the identification of two new genes, *DHX32* and *RGS12* with the response to anti-TNF therapy. *DHX32* gene encodes for a putative RNA helicase, and has been associated with lymphocyte differentiation and activation [41,42,43,44]. Importantly, RNA helicases have also shown to be important in innate immunity inactivation of viral RNA [45], and there is an increasing evidence that deregulation in this pathogen-sensing pathways could contribute to the development of autoimmunity, including RA [46,47,48,49]. Together, these results support the contribution of innate immunity in the differential response to anti-TNF therapies.

RGS12 encodes for a member of the 'regulator of G protein signaling' gene family although its biological still role remains to be characterized. Transcriptomic analysis on mouse monocyte progenitor cells activated with Receptor Activator of NF-kappa-B (RANKL) have shown a high induction of *RGS12* gene [50]. *RGS12* was shown to be highly and specifically expressed in human osteoclasts, and the inhibition of *RGS12* in mouse monocyte cultures impaired RANKL-mediated differentiation to osteoclasts. These results suggest that *RGS12* could participate in the macrophage to osteoclast transition, and therefore contribute to bone erosion and disease activity in RA.

Like most genetic studies for drug response, the present study has limitations. Issues like the uncertainty on the true genetic model underlying *FCGR2A* association, the definition of clinical response or the presence of environmental or genetic interactions could have reduced the statistical power of the study. For example, analyzing only those patients with a more extreme clinical response (i.e. EULAR good vs. none responders), only the *FCGR2A* association with adalimumab in all patients is still significant (S1 and S2 Tables). In this case, discarding the EULAR moderate group of patients, which is also the most frequent type of response (i.e. >40% of anti-TNF treated patients), clearly reduces the statistical power to identify the genetic associations. Also, from the observed genotype *FCGR2A* frequencies, there is no obvious gene dosage effect. However, in a complex trait like anti-TNF response there is likely to be a polygenic component, with multiple risk genes of moderate to low effect size where genetic models are more difficult to characterize. The presence of interaction effects can also limit our ability to fully characterize the present pharmacogenetic association. In anti-CCP positive RA patients, smoking has shown to strongly interact with *HLA-DRB1* genotypes in the risk to develop the disease [51]. In our cohort of anti-CCP positive RA patients we did not find a significant interaction between *FCGR2A* and smoking status (data not shown). Finally, the identification of new anti-TNF response genes using gene expression profiles is clearly subject to isolating the relevant cell type where the gene is expressed as well as the methodological approach used to characterize the gene expression profiles. However, the association of genetic variants in *DHX32* and *RGS12* loci associated with anti-TNF response, strongly supports the role of these two new genes in RA pathophysiology.

In the present study, we confirm the association between *FCGR2A* and the response to anti-TNF therapy. Also, using transcriptomic data from synovial fluid macrophages from RA

studies, we have identified two genes, *DHX32* and *RGS12*, strongly correlated with *FCGR2A* expression. Analyzing variants in these two new candidate genes, we have found new genetic associations with treatment response. The results of this study demonstrate a complex genetic basis for anti-TNF response and are an important advance in the understanding of the molecular mechanisms associated with the response to these therapies.

Supporting Information

S1 Fig. Significantly correlated genes with *FCGR2A* in synovial macrophages. Plots of the two probes measuring *FCGR2A* expression in SMPA and SMPB microarray studies with respect to the most significantly correlated genes ($P < 0.001$, *DHX32* in SMPA and *RGS12* in SMPB). The red line depicts the linear regression model of each gene against

FCGR2A expression.
(TIFF)

S1 Protocol. Includes the detailed description of the synovial macrophage microarray dataset selection, data preprocessing and association analysis. It also includes details on the methodology used to select tagSNPs for each gene, *DHX32* and *RGS12*, functionally associated with *FCGR2A*.

(DOCX)

S1 Table. *FCGR2A* polymorphism frequencies according to the EULAR extreme clinical response.

(DOCX)

S2 Table. *FCGR2A* polymorphism frequencies in anti-CCP positive RA patients according to the EULAR clinical extreme response.

(DOCX)

Acknowledgments

We thank the patients and clinical specialists collaborating in the IMID Consortium for participation.

Author Contributions

Conceived and designed the experiments: GAP JT AFN AJ SM. Performed the experiments: GAP AA MLL RT. Analyzed the data: GAP AA AJ SM. Contributed reagents/materials/analysis tools: JT AFN FB IGA JDC JM MA BFG AO HC AE RT. Wrote the paper: GAP JT AFN FB IGA JDC JM MA BFG AO HC AE AJ SM.

References

1. Felson DT, Smolen JS, Wells G, Zhang B, van Tuyl LHD, Funovits J, et al. (2011) American College of Rheumatology/European League Against Rheumatism provisional definition of remission in rheumatoid arthritis for clinical trials. *Arthritis Rheum* 63: 573–586. doi: [10.1002/art.30129](https://doi.org/10.1002/art.30129) PMID: [21294106](https://pubmed.ncbi.nlm.nih.gov/21294106/)
2. Smolen JS, Aletaha D (2006) What should be our treatment goal in rheumatoid arthritis today? *Clin Exp Rheumatol* 24 Suppl 43: S-7–13. PMID: [17083756](https://pubmed.ncbi.nlm.nih.gov/17083756/)
3. Salliot C, Finckh A, Katchamart W, Lu Y, Sun Y, Bombardier C, et al. (2011) Indirect comparisons of the efficacy of biological antirheumatic agents in rheumatoid arthritis in patients with an inadequate response to conventional disease-modifying antirheumatic drugs or to an anti-tumour necrosis factor agent: a meta-analysis. *Ann Rheum Dis* 70: 266–271. doi: [10.1136/ard.2010.132134](https://doi.org/10.1136/ard.2010.132134) PMID: [21097801](https://pubmed.ncbi.nlm.nih.gov/21097801/)
4. van den Broek M, Visser K, Allaart CF, Huizinga TWJ (2013) Personalized medicine: predicting responses to therapy in patients with RA. *Curr Opin Pharmacol* 13: 463–469. doi: [10.1016/j.coph.2013.03.006](https://doi.org/10.1016/j.coph.2013.03.006) PMID: [23578763](https://pubmed.ncbi.nlm.nih.gov/23578763/)

5. Emery P, Dorner T (2011) Optimising treatment in rheumatoid arthritis: a review of potential biological markers of response. *Ann Rheum Dis* 70: 2063–2070. doi: [10.1136/ard.2010.148015](https://doi.org/10.1136/ard.2010.148015) PMID: [22039166](https://pubmed.ncbi.nlm.nih.gov/22039166/)
6. Burska AN, Roget K, Blits M, Soto Gomez L, van de Loo F, Hazelwood LD, et al. (2014) Gene expression analysis in RA: towards personalized medicine. *Pharmacogenomics J* 14: 93–106. doi: [10.1038/tpj.2013.48](https://doi.org/10.1038/tpj.2013.48) PMID: [24589910](https://pubmed.ncbi.nlm.nih.gov/24589910/)
7. Cui J, Stahl EA, Saevarsdottir S, Miceli C, Diogo D, Trynka G, et al. (2013) Genome-wide association study and gene expression analysis identifies CD84 as a predictor of response to etanercept therapy in rheumatoid arthritis. *PLoS Genet* 9: e1003394. doi: [10.1371/journal.pgen.1003394](https://doi.org/10.1371/journal.pgen.1003394) PMID: [23555300](https://pubmed.ncbi.nlm.nih.gov/23555300/)
8. Krintel SB, Palermo G, Johansen JS, Germer S, Essioux L, Benayed R, et al. (2012) Investigation of single nucleotide polymorphisms and biological pathways associated with response to TNFalpha inhibitors in patients with rheumatoid arthritis. *Pharmacogenet Genomics* 22: 577–589. doi: [10.1097/FPC.0b013e3283544043](https://doi.org/10.1097/FPC.0b013e3283544043) PMID: [22569225](https://pubmed.ncbi.nlm.nih.gov/22569225/)
9. Acosta-Colman I, Palau N, Tornero J, Fernandez-Nebro A, Blanco F, Gonzalez-Alvaro I, et al. (2013) GWAS replication study confirms the association of PDE3A-SLCO1C1 with anti-TNF therapy response in rheumatoid arthritis. *Pharmacogenomics* 14: 727–734. doi: [10.2217/pgs.13.60](https://doi.org/10.2217/pgs.13.60) PMID: [23651021](https://pubmed.ncbi.nlm.nih.gov/23651021/)
10. Guillems M, Bruhns P, Saeys Y, Hammad H, Lambrecht BN (2014) The function of Fc gamma receptors in dendritic cells and macrophages. *Nat Rev Immunol* 14: 94–108. doi: [10.1038/nri3582](https://doi.org/10.1038/nri3582). Epub 2014 Jan 1021. PMID: [24445665](https://pubmed.ncbi.nlm.nih.gov/24445665/)
11. Montes A, Perez-Pampin E, Narvaez J, Canete JD, Navarro-Sarabia F, Moreira V, et al. (2014) Association of FCGR2A with the response to infliximab treatment of patients with rheumatoid arthritis. *Pharmacogenet Genomics* 24: 238–245. doi: [10.1097/FPC.0000000000000042](https://doi.org/10.1097/FPC.0000000000000042) PMID: [24667440](https://pubmed.ncbi.nlm.nih.gov/24667440/)
12. Ivan E, Colovai AI (2006) Human Fc receptors: critical targets in the treatment of autoimmune diseases and transplant rejections. *Hum Immunol* 67: 479–491. PMID: [16829303](https://pubmed.ncbi.nlm.nih.gov/16829303/)
13. Siberil S, Dutertre CA, Boix C, Bonnin E, Menez R, Stura E, et al. (2006) Molecular aspects of human Fc gamma R interactions with IgG: functional and therapeutic consequences. *Immunol Lett* 106: 111–118. PMID: [16797726](https://pubmed.ncbi.nlm.nih.gov/16797726/)
14. Ravetch JV, Lanier LL (2000) Immune inhibitory receptors. *Science* 290: 84–89. PMID: [11021804](https://pubmed.ncbi.nlm.nih.gov/11021804/)
15. Nimmerjahn F, Ravetch JV (2008) Fc gamma receptors as regulators of immune responses. *Nat Rev Immunol* 8: 34–47. PMID: [18064051](https://pubmed.ncbi.nlm.nih.gov/18064051/)
16. Zhang W, Gordon M, Schultheis AM, Yang DY, Nagashima F, Azuma M, et al. (2007) FCGR2A and FCGR3A polymorphisms associated with clinical outcome of epidermal growth factor receptor expressing metastatic colorectal cancer patients treated with single-agent cetuximab. *J Clin Oncol* 25: 3712–3718. PMID: [17704420](https://pubmed.ncbi.nlm.nih.gov/17704420/)
17. Musolino A, Naldi N, Bortesi B, Pezzuolo D, Capelletti M, Missale G, et al. (2008) Immunoglobulin G fragment C receptor polymorphisms and clinical efficacy of trastuzumab-based therapy in patients with HER-2/neu-positive metastatic breast cancer. *J Clin Oncol* 26: 1789–1796. doi: [10.1200/JCO.2007.14.8957](https://doi.org/10.1200/JCO.2007.14.8957) PMID: [18347005](https://pubmed.ncbi.nlm.nih.gov/18347005/)
18. Weng WK, Levy R (2003) Two immunoglobulin G fragment C receptor polymorphisms independently predict response to rituximab in patients with follicular lymphoma. *J Clin Oncol* 21: 3940–3947. PMID: [12975461](https://pubmed.ncbi.nlm.nih.gov/12975461/)
19. Paiva M, Marques H, Martins A, Ferreira P, Catarino R, Medeiros R (2008) Fc gamma RIIa polymorphism and clinical response to rituximab in non-Hodgkin lymphoma patients. *Cancer Genet Cytogenet* 183: 35–40. doi: [10.1016/j.cancergencyto.2008.02.001](https://doi.org/10.1016/j.cancergencyto.2008.02.001) PMID: [18474295](https://pubmed.ncbi.nlm.nih.gov/18474295/)
20. Arora T, Padaki R, Liu L, Hamburger AE, Ellison AR, Stevens SR, et al. (2009) Differences in binding and effector functions between classes of TNF antagonists. *Cytokine* 45: 124–131. doi: [10.1016/j.cyto.2008.11.008](https://doi.org/10.1016/j.cyto.2008.11.008) PMID: [19128982](https://pubmed.ncbi.nlm.nih.gov/19128982/)
21. Guillems M, Bruhns P, Saeys Y, Hammad H, Lambrecht BN (2014) The function of Fc gamma receptors in dendritic cells and macrophages. *Nat Rev Immunol* 14: 94–108. doi: [10.1038/nri3582](https://doi.org/10.1038/nri3582) PMID: [24445665](https://pubmed.ncbi.nlm.nih.gov/24445665/)
22. Mimoto F, Kadono S, Katada H, Igawa T, Kamikawa T, Hattori K (2014) Crystal structure of a novel asymmetrically engineered Fc variant with improved affinity for Fc gamma Rs. *Mol Immunol* 58: 132–138. doi: [10.1016/j.molimm.2013.11.017](https://doi.org/10.1016/j.molimm.2013.11.017) PMID: [24334029](https://pubmed.ncbi.nlm.nih.gov/24334029/)
23. Dijkstra HM, van de Winkel JG, Kallenberg CG (2001) Inflammation in autoimmunity: receptors for IgG revisited. *Trends Immunol* 22: 510–516. PMID: [11525942](https://pubmed.ncbi.nlm.nih.gov/11525942/)
24. Bruhns P (2012) Properties of mouse and human IgG receptors and their contribution to disease models. *Blood* 119: 5640–5649. doi: [10.1182/blood-2012-5601-380121](https://doi.org/10.1182/blood-2012-5601-380121). Epub 382012 Apr 380125. PMID: [22535666](https://pubmed.ncbi.nlm.nih.gov/22535666/)
25. Canete JD, Suarez B, Hernandez MV, Sanmarti R, Rego I, Celis R, et al. (2009) Influence of variants of Fc gamma receptors IIA and IIIA on the American College of Rheumatology and European League

- Against Rheumatism responses to anti-tumour necrosis factor alpha therapy in rheumatoid arthritis. *Ann Rheum Dis* 68: 1547–1552. doi: [10.1136/ard.2008.096982](https://doi.org/10.1136/ard.2008.096982). Epub 092008 Oct 096917. PMID: [18930989](https://pubmed.ncbi.nlm.nih.gov/18930989/)
26. Montes A, Perez-Pampin E, Narvaez J, Canete JD, Navarro-Sarabia F, Moreira V, et al. (2014) Association of FCGR2A with the response to infliximab treatment of patients with rheumatoid arthritis. *Pharmacogenet Genomics* 24: 238–245. doi: [10.1097/FPC.0000000000000042](https://doi.org/10.1097/FPC.0000000000000042) PMID: [24667440](https://pubmed.ncbi.nlm.nih.gov/24667440/)
 27. Criswell LA, Lum RF, Turner KN, Woehl B, Zhu Y, Wang J, et al. (2004) The influence of genetic variation in the HLA-DRB1 and LTA-TNF regions on the response to treatment of early rheumatoid arthritis with methotrexate or etanercept. *Arthritis Rheum* 50: 2750–2756. PMID: [15457442](https://pubmed.ncbi.nlm.nih.gov/15457442/)
 28. Padyukov L, Seielstad M, Ong RT, Ding B, Ronnelid J, Seddighzadeh M, et al. (2011) A genome-wide association study suggests contrasting associations in ACPA-positive versus ACPA-negative rheumatoid arthritis. *Ann Rheum Dis* 70: 259–265. doi: [10.1136/ard.2009.126821](https://doi.org/10.1136/ard.2009.126821). Epub 122010 Dec 126814. PMID: [21156761](https://pubmed.ncbi.nlm.nih.gov/21156761/)
 29. Kurreeman F, Liao K, Chibnik L, Hickey B, Stahl E, Gainer V, et al. (2011) Genetic basis of autoantibody positive and negative rheumatoid arthritis risk in a multi-ethnic cohort derived from electronic health records. *Am J Hum Genet* 88: 57–69. doi: [10.1016/j.ajhg.2010.1012.1007](https://doi.org/10.1016/j.ajhg.2010.1012.1007) PMID: [21211616](https://pubmed.ncbi.nlm.nih.gov/21211616/)
 30. Bossini-Castillo L, de Kovel C, Kallberg H, van 't Slot R, Italiaander A, Coenen M, et al. (2014) A genome-wide association study of rheumatoid arthritis without antibodies against citrullinated peptides. *Ann Rheum Dis* 14: 2013–204591.
 31. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. (1988) The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 31: 315–324. PMID: [3358796](https://pubmed.ncbi.nlm.nih.gov/3358796/)
 32. van Gestel AM, Haagsma CJ, van Riel PL (1998) Validation of rheumatoid arthritis improvement criteria that include simplified joint counts. *Arthritis Rheum* 41: 1845–1850. PMID: [9778226](https://pubmed.ncbi.nlm.nih.gov/9778226/)
 33. Prevoo ML, van 't Hof MA, Kuper HH, van Leeuwen MA, van de Putte LB, van Riel PL, et al. (1995) Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. *Arthritis Rheum* 38: 44–48. PMID: [7818570](https://pubmed.ncbi.nlm.nih.gov/7818570/)
 34. Barrett T, Edgar R (2006) Gene expression omnibus: microarray data storage, submission, retrieval, and analysis. *Methods Enzymol* 411: 352–369. PMID: [16939800](https://pubmed.ncbi.nlm.nih.gov/16939800/)
 35. Team RC (2013) R: A Language and Environment for Statistical Computing. (R Foundation for Statistical Computing, Vienna, 2009). doi: [10.3758/s13428-013-0330-5](https://doi.org/10.3758/s13428-013-0330-5) PMID: [23519455](https://pubmed.ncbi.nlm.nih.gov/23519455/)
 36. Abecasis GR, Auton A, Brooks LD, DePristo MA, Durbin RM, Handsaker RE, et al. (2012) An integrated map of genetic variation from 1,092 human genomes. *Nature* 491: 56–65. doi: [10.1038/nature11632](https://doi.org/10.1038/nature11632) PMID: [23128226](https://pubmed.ncbi.nlm.nih.gov/23128226/)
 37. Barrett JC, Fry B, Maller J, Daly MJ (2005) Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 21: 263–265. Epub 2004 Aug 2005. PMID: [15297300](https://pubmed.ncbi.nlm.nih.gov/15297300/)
 38. Zondervan KT, Cardon LR (2004) The complex interplay among factors that influence allelic association. *Nat Rev Genet* 5: 89–100. PMID: [14735120](https://pubmed.ncbi.nlm.nih.gov/14735120/)
 39. Kuhn KA, Kulik L, Tomooka B, Braschler KJ, Arend WP, Robinson WH, et al. (2006) Antibodies against citrullinated proteins enhance tissue injury in experimental autoimmune arthritis. *J Clin Invest* 116: 961–973. PMID: [16585962](https://pubmed.ncbi.nlm.nih.gov/16585962/)
 40. Takai T (2002) Roles of Fc receptors in autoimmunity. *Nat Rev Immunol* 2: 580–592. PMID: [12154377](https://pubmed.ncbi.nlm.nih.gov/12154377/)
 41. Abdelhaleem M (2002) The novel helicase homologue DDX32 is down-regulated in acute lymphoblastic leukemia. *Leuk Res* 26: 945–954. PMID: [12163057](https://pubmed.ncbi.nlm.nih.gov/12163057/)
 42. Alli Z, Chen Y, Abdul Wajid S, Al-Saud B, Abdelhaleem M (2007) A role for DHX32 in regulating T-cell apoptosis. *Anticancer Res* 27: 373–377. PMID: [17352256](https://pubmed.ncbi.nlm.nih.gov/17352256/)
 43. Alli Z, Nam EH, Beimnet K, Abdelhaleem M (2005) The activation-induced expression of DHX32 in Jurkat T cells is specific and involves calcium and nuclear factor of activated T cells. *Cell Immunol* 237: 141–146. PMID: [16414036](https://pubmed.ncbi.nlm.nih.gov/16414036/)
 44. Abdelhaleem M, Sun TH, Ho M (2005) DHX32 expression suggests a role in lymphocyte differentiation. *Anticancer Res* 25: 2645–2648. PMID: [16080506](https://pubmed.ncbi.nlm.nih.gov/16080506/)
 45. Meylan E, Tschopp J (2006) Toll-like receptors and RNA helicases: two parallel ways to trigger antiviral responses. *Mol Cell* 22: 561–569. PMID: [16762830](https://pubmed.ncbi.nlm.nih.gov/16762830/)
 46. Baccala R, Gonzalez-Quintal R, Lawson BR, Stern ME, Kono DH, Beutler B, et al. (2009) Sensors of the innate immune system: their mode of action. *Nat Rev Rheumatol* 5: 448–456. doi: [10.1038/nrrheum.2009.1136](https://doi.org/10.1038/nrrheum.2009.1136) PMID: [19597511](https://pubmed.ncbi.nlm.nih.gov/19597511/)

47. Narendra SC, Chalise JP, Hook N, Magnusson M (2014) Dendritic cells activated by double-stranded RNA induce arthritis via autocrine type I IFN signaling. *J Leukoc Biol* 95: 661–666. doi: [10.1189/jlb.0613320](https://doi.org/10.1189/jlb.0613320) PMID: [24304616](https://pubmed.ncbi.nlm.nih.gov/24304616/)
48. Mitoma H, Hanabuchi S, Kim T, Bao M, Zhang Z, Sugimoto N, et al. (2013) The DHX33 RNA helicase senses cytosolic RNA and activates the NLRP3 inflammasome. *Immunity* 39: 123–135. doi: [10.1016/j.immuni.2013.07.001](https://doi.org/10.1016/j.immuni.2013.07.001) PMID: [23871209](https://pubmed.ncbi.nlm.nih.gov/23871209/)
49. Mathews RJ, Robinson JI, Battellino M, Wong C, Taylor JC, Eyre S, et al. (2014) Evidence of NLRP3-inflammasome activation in rheumatoid arthritis (RA); genetic variants within the NLRP3-inflammasome complex in relation to susceptibility to RA and response to anti-TNF treatment. *Ann Rheum Dis* 73: 1202–1210. doi: [10.1136/annrheumdis-2013-203276](https://doi.org/10.1136/annrheumdis-2013-203276) PMID: [23687262](https://pubmed.ncbi.nlm.nih.gov/23687262/)
50. Yang S, Li YP (2007) RGS12 is essential for RANKL-evoked signaling for terminal differentiation of osteoclasts in vitro. *J Bone Miner Res* 22: 45–54. PMID: [17042716](https://pubmed.ncbi.nlm.nih.gov/17042716/)
51. Lundstrom E, Kallberg H, Alfredsson L, Klareskog L, Padyukov L (2009) Gene-environment interaction between the DRB1 shared epitope and smoking in the risk of anti-citrullinated protein antibody-positive rheumatoid arthritis: all alleles are important. *Arthritis Rheum* 60: 1597–1603. doi: [10.1002/art.24572](https://doi.org/10.1002/art.24572) PMID: [19479873](https://pubmed.ncbi.nlm.nih.gov/19479873/)