Association of *FCGR2A* with response to Infliximab treatment of patients with Rheumatoid Arthritis

Running head: *FCGR2A* polymorphism in response to Infliximab

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COMPETING INTERESTS: The authors have not conflict of interest to declare

FUNDING: 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.

ABSTRACT

Objectives: We aimed to assess a functional polymorphism in *FCGR2A*, H131R, for association with treatment response to Fc-containing inhibitors of TNF.

Methods: A total of 429 biologic-naïve patients with rheumatoid arthritis (RA) collected in two sets (299 and 130) were treated during standard care with Infliximab, Etanercept or Adalimumab. Response to treatment was evaluated at 3, 6 and 12 months of follow-up as change in Disease Activity Score (DAS) 28 from baseline and as response by the European League Against Rheumatism (EULAR) criteria. These variables were analyzed for association with linear and logistic regression models that included gender, inhibitor of TNF and baseline DAS28 as covariates.

Results: Significant association was found between the *FCGR2A* H131R polymorphism and response to treatment with Infliximab, but not with the other two TNF inhibitors. The 131R allele was associated with a lower change in DAS28 (P = 0.04 to 0.008 at different times) in the first set of patients and confirmed in the second group of patients (P = 0.026 at 3 months of follow-up). Association was also found in the comparison between non-responders and responders to Infliximab by the EULAR criteria.

Conclusions: We have found association of the *FCGR2A* 131R allele with poor response to Infliximab. This finding could be of utility to understand the mechanisms behind treatment failure and contribute to biomarker panels for Infliximab response prediction.

Keywords: Rheumatoid arthritis, anti-TNF, genetics, biomarkers, Fc receptor, *FCGR2A*, Infliximab

INTRODUCTION

Treatment of RA improved dramatically with the introduction of the TNF inhibitors [1, 2]. However, a significant fraction of patients do not respond adequately, even if they respond after switching to a different TNF inhibitor or to other biologic [3, 4]. Therefore, to make efficient use of the available options we need to know the best drug for each patient [5-7]. Multiple attempts to find predictors of response to TNF inhibitors have already been done [6]. Many parameters have been studied. Some of them are associated with response, but none has shown yet its utility for clinical practice. The most reproducible predictor is baseline disease activity. Other predictors are less consistent, like gender, RF, anti-CCP, disability and concurrent use of DMARDs. In the genetics field, there have already been four GWAS [8-11] that have showed some associated loci: CD84 associated with response to Etanercept (ETC) near the GWAS level; and others that need to be further validated [10, 12, 13]. Other studies have followed the candidate gene approach. A prominent candidate has been the TNF gene itself, and very particularly the -308 SNP in its promoter. The results of several studies supported its association with anti-TNF response [14], but the most recent large analyses did not found significant association [10, 15]. Other candidate genes have been the receptors for the Fc of IgG or *FCGR*s [16-22]. They are considered candidates because most biologics incorporate the Fc either because they are monoclonal antibodies or to improve their pharmacokinetics and function [23, 24].

The most commonly used inhibitors of TNF, Infliximab (INX), Etanercept (ETC) and Adalimumab (ADM), include the Fc of IgG1. INX and ADM because they are monoclonal antibodies; ETC because it is a fusion molecule between sTNFR and the Fc of IgG1. Therefore, their function and availability can be affected by functional polymorphisms in the FcγRs. Some of these polymorphisms have effects in the

transport and half-life of IgG, and also in how IgG binding regulates cell activation status, phagocytosis and cytotoxicity. The *FCGR2R* H131A SNP was associated with response to anti-TNF in one study [18], but not in a second one [22].

We have analyzed the *FCGR2R* H131A functional polymorphism for association with response to the three most common TNF inhibitors in patients with RA that were biologic-naïve. The *FCGR2A* SNP showed association with response to treatment at all times analyzed in patients treated with INX and this association was replicated in a second independent set of patients.

METHODS

Patients. Biologic-naïve patients with RA according to the 1987 revised American College of Rheumatology classification criteria were included [25]. They were treated with Infliximab (INX), Etanercept (ETC) or Adalimumab (ADM). The indication of treatment, choice of drug and control of evolution were done during standard care and with independence of this study. Evaluations included DAS28 at the start of treatment and at 3, 6 and 12 months [26].

There were two sets of patients, 299 collected in six Rheumatology Units from Spanish Hospitals at the start of the study, of which 217 have complete follow-up at 3, 6 and 12 months and were considered the discovery group. The second set, of 130 patients, was recruited from four hospitals (2 Spanish and 2 Greek) once the results from the discovery group were known.

All the patients provided blood samples for DNA extraction and their written informed consent to participate in the study. The study was approved by the Comite Etico de Investigacion Clinica de Galicia.

Genotyping. The nsSNP rs1801274 in *FCGR2A* was genotyped by PCR amplification followed by single-base extension with the SNaPshot Multiplex Kit (Applied Biosystems, Foster City, CA, USA). This SNP is commonly referred to by its protein alleles with the following correspondence rs1801274 A>G as His131Arg or H131R. Samples with different genotypes of the H131R polymorphism were sequenced to assess the accuracy of results. Oligonucleotide sequences were (5→3´sense): forward primer GGAATCTATCCTTACAACTTTTT, reverse primer AACAGCGTGTAGCCTATG, and minisequencing probe CTTGTGGGATGGAGAAGGTGGGATCCAAA

Statistical analysis. The Statistica 7.0 (Statsoft, Tulsa OK) software was used thoroughly. 2x3 and 3x3 contingency tables, ANOVA and Kruskal-Wallis tests were performed to compare the three strata defined by the TNF antagonist. The type of test used was determined by the variable type. Allele frequencies, odds ratios (O.R) and their 95% confidence intervals (95% CI) were calculated from 2x2 contingency tables. Response to treatment was considered as change from baseline in DAS28 (ΔDAS28) or according to the EULAR criteria [27]. A generalized linear model in the first case and a logistic regression model in the second (confronting responders + moderate responders with non-responders) were fitted. An additive genetic model was considered. Covariates were baseline DAS28, gender and treatment.

RESULTS

Patient characteristics and response to TNF inhibitors. Samples and data from 429 patients with RA were obtained in two phases. Nineteen were excluded from study because of poor genotyping quality (13 samples, 9 from the first set and 4 from the second) or low DAS28 at baseline (6 patients with DAS28 < 3.2, 4 from the first set and

2 from the second). These patients were not included in any of the analyses. The remaining 410 patients (286 from the first set and 124 from the second set) showed characteristics of severe RA (Table 1). Severity was reflected in the high percentage showing erosive arthritis and lack of disease control with previous treatments including a mean of 2.4 disease-modifying antirheumatic drugs (DMARDs). Mean baseline DAS28 was 5.9 and median baseline Health Assessment Questionnaire (HAQ) was 1.6 indicating an active disease with moderate to severe disability. More than 75 % of the patients showed high activity (DAS28 > 5.1). Other signs associated with severity and activity of RA, as high prevalence of rheumatoid factor (RF) or anti-citrullinated protein antibodies (ACPA), and elevated C reactive protein (CRP) and erythrocyte sedimentation rate (ESR), were also present. A characteristic that is different from other European series is the low prevalence of smokers among our patients.

Some baseline characteristics were different between the three treatment groups as prevalence of RF and ACPA, which were largest in patients treated with ETC, and CRP levels that were higher in the patients treated with ADM and lowest in the treated with INX (Table 1). Most patients received combined therapy with a DMARD accompanying the TNF inhibitor with any treatment, this was almost uniform in the INX group, very common in the ADM group and less so in the ETC group. Treatment with any of the TNF inhibitors resulted in an improvement in all the parameters of disease activity and disability: CRP, ESR, HAQ (not shown) and DAS28 (Table 1). However, there were differences in response between the three TNF inhibitors at all times of follow-up (Table 1). DAS28 showed less improvement in the patients treated with INX than in those treated with the other two drugs. At 12 months of follow-up the decrease was maximum in the patients treated with ETC. Response evaluated with the

EULAR criteria was also different between the three drugs at all times indicating the need of considering the TNF inhibitor as an important variable in analysis.

Association of *FCGR2A* H131R with response to Infliximab. Analysis of the association of the *FCGR2A* H131R polymorphism with response to treatment was done considering either ΔDAS28 at each of the three time points evaluated (main outcome) or comparing responders (good + moderate responders) versus non responders according to the EULAR criteria (secondary outcome). Data from patients with complete follow-up was analyzed first and incomplete data were incorporated later. Baseline DAS28, gender and TNF inhibitor were included as covariates. Congruency of association at different follow-up times was considered relevant, not an isolated p value below 0.05.

The nsSNP in *FCGR2A* showed association with response to treatment to the three TNF inhibitors in some analysis (not shown), but it was clear that this association was only due to the patients that were treated with INX (Figure 1). Therefore, detailed results are only presented for INX that showed association in multiple analyses (Tables 2 and 4).

In the first group of patients with complete follow-up (n = 105), the 131R allele was associated with less improvement in ΔDAS28 at the 6 and 12 months' time points in the patient treated with INX (P = 0.044 and 0.008, respectively) and showed a trend to association at the 3 months' evaluation (P = 0.099; Table 2). The effect was more marked with time as reflected by the beta coefficients that increased from -0.15 at 3 months to -0.23 at 12 months. Incorporation of data from patients with incomplete follow-up did not alter association at 12 months, make it significant at 3 months (P = 0.034) and canceled it at 6 months.

The second set of samples replicated association at 3 months of follow-up in the patients treated with INX (P = 0.026), but not at the 6 or 12 months' time points (Table

2). However, it is important to consider that this second set of samples was smaller (n = 95 treated with INX) than the first one and the global analysis with all data showed significant associations at 3 and 12 months (P = 0.0067 and 0.044, respectively).

We considered interesting to search the possible causes of the lost association at 6 months when patients with incomplete follow-up were included (Table 2). We found that patients with incomplete data showed less improvement at 6 months (0.8 units lower) and lower age (4 years lower) than patients with complete data (Table 3). None of the other clinical variables analyzed, including the shown in table 1, were different (not shown). The lower improvement in DAS28 could suggest that incomplete follow-up of these patients was due to interruption of treatment. However, this was not the case because a large fraction of these patients were from centers with response data at less than the three time points irrespective of patient evolution. In addition, patients with information only at 6 and 12 months also showed less improvement at 6 months than patients with complete follow-up (n = 14; ΔDAS28 = 0.99, P = 0.009). In addition, we found that the two trends, to younger age and to less improvement in DAS28 at 6 months, were also present in patients with incomplete follow-up treated with ETC or ADM (not shown).

The secondary outcome in our analyses, the EULAR response criteria, also showed significant association with the H131R *FCGR2A* polymorphism at the three evaluated times in at least one of the patient sets (Table 4). However, results were less striking as should be expected from a dichotomous variable as the EULAR response/non-response criteria with respect to the quantitative variable, DAS28, from which it is derived. The R allele was associated with non-responders at the 6 and 12 months of follow-up in the first set of patients with complete data (Table 4). This association was only significant at 3 months when all data from the first set of samples was considered (at 12 months

was almost significant). Association at 3 months was replicated in the second set of samples, and was the only that remained when all data from the first and the second sets were considered together.

Response of patients treated with ETC or with ADM either considered separately or together did not show association with the H131R *FCGR2A* polymorphism at any time point (Figure 1B and not shown).

DISCUSSION

Our results have shown a poorer response to INX in the patients with RA bearing the 131R allele of the *FCGR2A* nsSNP. This effect was modest but the differences were significant at the three times of evaluation in at least one of the analyses. Replication of association at 3 months was obtained in a second set of independent samples. No differences were detected with the other two TNF inhibitors analyzed.

The relative resistance to INX associated with the 131R allele of *FCGR2A* was replicated, but will need further confirmation given the confusing previously reported evidence. In one side, four GWAS of response to treatment with inhibitors of TNF have already been published and none has communicated association with this SNP [8-11], which is included in the genotyping panels of three of these studies [8, 10, 11]. However, this lack of association in GWAS is not very compelling because some of these studies were small, three of the four did not separate the different TNF inhibitors, and GWAS do not report association results with a p value that does not withstand correction by a high number of tests. In the other side, there are several candidate gene studies focused in this SNP in relation with response to treatment with TNF inhibitors and other Fc-bearing biologic drugs. Two of them relate with the treatment of RA. One showed association of the *FCGR2A* nsSNP with response to INX in 91 Spanish patients

with RA at a single time point and with an analysis based in a recessive model of inheritance [18]. The direction of change was contrary to the found by us: the ACR20 response was better in the RR patients than in the HR+HH patients at week 30 of follow-up. There are some differences between the studies that could justify the discrepancy, including a higher prevalence of non-responders, 38.8 % at 30 weeks *vs.* 27.5 % at 6 months in our study (although the previous study used DAS28-CRP, which overestimates EULAR response [28]), and less patients with high basal disease activity (mean DAS28-CRP = 4.5 that is lower than the threshold of 4.9 for this index [29] *vs.* our DAS28-ESR = 6.0, which is over the 5.1 threshold for high activity in our patients). A second study in RA included 457 RA patients treated with ETC [22]. No association was found in agreement with our results with this same anti-TNF. A positive association in the same direction as the found by us has been communicated in patients with psoriatic arthritis [30]. However, this study concerns a different disease and the association was predominantly observed in patients treated with ETC and therefore adds little as a confirmation of our results. Others studies have shown association of the H131R polymorphism with response to monoclonal antibodies in cancer treatment [31]. Two antibodies have shown association in several studies, Trastuzumab targeting HER2 in breast cancer and Cetuximab directed to EGFR that is overexpressed in epithelial tumors. All studies showing association found a better response associated with the H allele. These results are congruent with our study, but none of these associations can yet be considered as established.

Influence of the H131R polymorphism in the response to INX could be through three types of mechanisms. The most direct would be by differential binding of INX through its Fc. However, this mechanism cannot be assumed without experimental support because INX bears the IgG1 Fc and there are not marked differences between the two

alleles of FcγRIIA in their binding to polyclonal human IgG1 [32, 33]. However, they affect binding of some monoclonal IgG1 antibodies as shown by the higher affinity of the H131 allele for two of the three IgG1 monoclonal antibodies assayed [33]. The two monoclonal antibodies showing differential binding with the FcγRIIA alleles were a chimeric anti-CD20 and a fully human anti-RhD, whereas a chimeric anti-HLA-DR antibody was not affected. It is tempting to postulate that INX also binds differentially with the FcγRIIA alleles, whereas ETC and ADM are not affected. This would explain the discordant association with response to treatment we have found. Binding with FcγRIIA could induce phagocytosis, cytolysis, degranulation, and the transcriptional activation of cytokine genes, uptake and degradation of immunocomplexes that contributes to the shortening of the drug half-life and to presentation of its peptides as antigens. Some of these functions could impinge in the efficacy of INX treatment. The second type of mechanisms would be through differential effect of H131R polymorphism on the RA immune responses. This possibility is suggested by association of this polymorphism with inflammatory and autoimmune diseases. The most convincing associations have been found with ulcerative colitis [34, 35], Kawasaki vasculitis [36] and systemic lupus erythematosus [37, 38]. Finally, association with *FCGR2A* H131R could be due to linkage disequilibrium with polymorphisms in other *FCGR* and related genes mapping together with *FCGR2A* in a cluster of chromosome *1q23*.

A possible cause of the different results obtained with INX and with ETC or ADM has already been signaled in the preceding paragraph. But the discrepancy could be also explained by known differences between the three TNF inhibitors [23, 24]. Differences that could explain the efficacy of switching between them [39, 40] and that include affinity, molecular structure (antibodies *vs.* chimeric molecule, human *vs.* mouse/human

chimera), pharmacokinetics, immunogenicity, complement activation and glycosylation at Asn297 [23, 24]. A relevant example is that ETC-TNF complexes bind poorly to FcγRIIA in comparison with INX-TNF or ADM-TNF complexes even when the three anti-TNF share the same IgG1 Fc [24]. Unfortunately, no similar study comparing the two alleles of FcγRIIA is available.

Our results showed a less clear association of *FCGR2A* 131R with poor response at 6 months of follow-up that was the time including more patients with incomplete follow-up in our study. This abundance of incomplete information at 6 months was due to some centers that only evaluated patients at this specific time point or at 3 and 6 months. The patients in this group were younger and showed less improvement in DAS28. The causes of these differences are unknown but could indicate that the association we have found requires circumstances allowing a significant effect of INX in RA disease activity.

CONCLUSIONS

Our results show association between poor response to INX and the 131R allele at *FCGR2A*. This association has been found with consistency in several analyses with the same patients and replicated at 3 months in a second set of patients. This result could contribute to biomarker panels of clinical utility if further confirmed. In addition, it will help us understand factors affecting the response to treatment of RA patients.

ACKNOWLEDGMENTS

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

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

	All [a]	Infliximab	Etanercept	Adalimumab	*P*-value [b]
Patients, number (%)	410	246 (60.0)	87 (21.2)	77 (18.8)	
Female, %	83.4	86.6	81.8	75.9	0.06
Age at diagnosis, median (IQR[c])	45 (36- 55)	45 (36 - 54)	47 (35-54)	47 (39-59)	ns
Diagnosis to anti-TNF, median (IQR)	6 (3-12)	7 (3-12)	6 (3-13)	7 (2-12)	ns
RF, %	76.4	72.4	88.4	75.3	0.01
ACPA, % [a]	78.3	77.8	92.0	68.9	0.01
Erosive arthritis, %	84.0	83.1	84.7	87.2	ns
Smoking, % [a]	14.7	15.5	15.2	10.9	ns
DMARD before anti-TNF, mean ± SD [a]	2.4 ± 1.3	2.4 ± 1.3	2.4 ± 1.3	2.4 ± 1.1	ns
Concomitant DMARD, % [a]	88.4	91.2	77.6	88.0	0.03
Baseline CRP (mg/L), median (IQR) [a]	8.0 (3.9-18.4)	6.6 (3.0-18.8)	10.4 (4.1 -16.3)	15.7 (6.0-23.0)	0.03
Baseline ESR, median (IQR) [a]	35 (19-56)	36 (20-56)	28 (15-56)	41 (19-57)	ns
Baseline HAQ, median (IQR) [a]	1.6 (1.0-2.1)	1.6 (1.1-2.1)	1.4 (1.0-2.0)	1.5 (0.9-2.0)	ns
DAS28, mean ± SD					
baseline	5.9 ± 1.1	6.0 ± 1.2	5.7 ± 1.1	5.8 ± 1.0	ns
3 month	3.9 ± 1.4	4.2 ± 1.4	3.6 ± 1.4	3.6 ± 1.1	0.001
6 months	3.8 ± 1.4	4.1 ± 1.5	3.2 ± 1.2	3.3 ± 1.2	2×10^{-5}
12 months	3.6 ± 1.4	3.8 ± 1.4	3.0 ± 1.2	3.5 ± 1.6	0.03
EULAR response, %					
3 months					
responder	29.6	23.1	38.8	37.3	0.04
moderate	50.5	55.2	38.8	50.7	
non responder	19.8	21.7	22.5	12.0	
6 months					
responder	39.3	30.2	56.6	49.3	0.0006
moderate	40.7	40.7	35.5	40.8	
non responder	20.1	27.5	7.9	9.9	
12 months					
responder	43.8	36.0	56.2	53.8	0.04
moderate	38.8	44.5	34.2	23.1	
non responder	17.4	19.5	9.6	23.1	

a. Data from <85 % of the patients: 309 for ACPA, 299 for smoking, 340 for DMARDs before anti-TNF, 268 for concomitant DMARD, 245 for baseline CRP, 294 for baseline ESR, and 317 patients for baseline HAQ.

b. 2x3, 3x3 contingency tables, ANOVA or Kruskal-Wallis tests were performed to compare the three treatment groups depending on the variable.

c. IQR = interquartile range; RF = rheumatoid factor; ACPA = anti-citrullinated protein antibodies; DMARD = disease-modifying antirheumatic drug; SD = standard deviation; CRP = C-reactive protein; ESR = erythrocyte sedimentation rate; HAQ = Health Assessment Questionnaire; DAS28 = Disease Activity Score 28 joints; EULAR = The European League Against Rheumatism.

Table 2. **Association of the 131R allele with less improvement in DAS28 at multiple times and in the two sets of patients treated with Infliximab**.

	3 months			6 months			12 months		
1st set of patients	n	Beta	*P-value*	n	Beta	*P-value*	n	Beta	*P-value*
Complete follow-up	105	-0.15	0.099	105	-0.18	0.044	105	-0.23	0.008
Complete + incomplete	127	-0.18	0.034	143	-0.10	0.2	109	-0.22	0.008
2nd set of patients	75	-0.22	0.026	79	-0.04	0.6	55	-0.02	0.88
Combined (1st + 2nd)	202	-0.17	0.0067	222	-0.06	0.3	164	-0.13	0.047

Effects of the H131R genotypes according to an additive model on ΔDAS28 in generalized linear models are shown separately for patients of the first set with complete follow-up; all patients in the first set (complete + incomplete follow-up); all patients in the second set; and jointly, all patients in the first and the second sets together.

Table 3. **Differential characteristics between patients with complete and incomplete follow-up treated with Infliximab (first and second sets of patients together)**.

Characteristic	Complete	Incomplete	*P-value*
number	141	81	-
Age	61 ± 12 [a]	57 ± 13	0.02
Baseline DAS28[b]	6.1 ± 1.5	5.8 ± 1.4	0.20
DAS28 at 6 months	3.8 ± 1.5	4.5 ± 1.3	0.005
ΔDAS28 at 6 months	2.2 ± 1.6	1.4 ± 1.6	0.0006

[a] mean ± SD

[b] DAS28 = Disease Activity Score on 28 joints.

Table 4. **Association of the *FCGR2A* H131R polymorphism with response to treatment with Infliximb as evaluated with the EULAR criteria.**

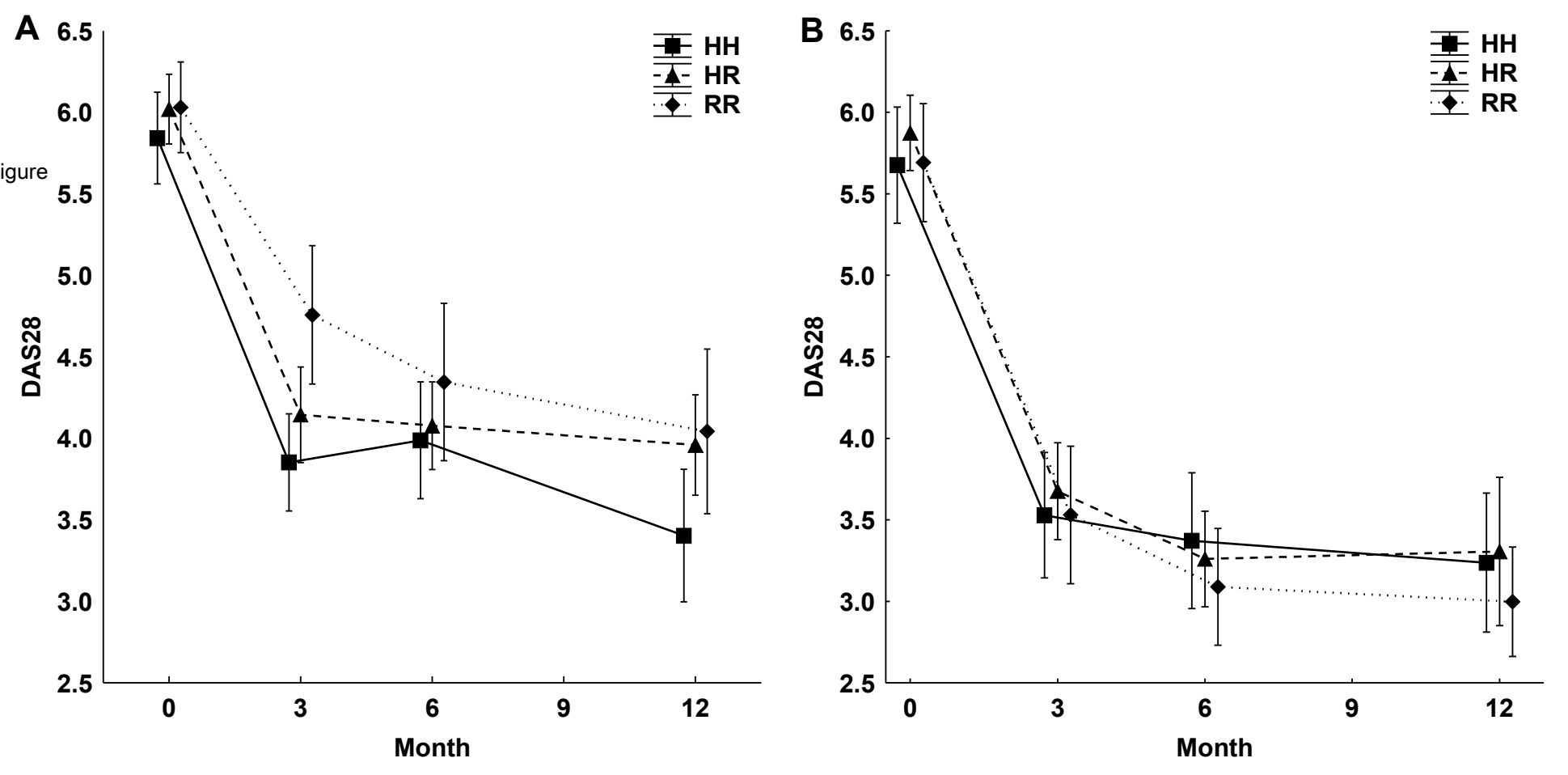
	3 months				6 months				12 months			
	n	R [a]	NR	*P-value*[b]	n	R	NR	*P-value*	n	R	NR	*P-value*
1st set of patients												
Complete follow-up	105	26.5/54.2/19.3	18.2/45.5/36.4	0.1	105	27.7/54.2/18.1	13.6/45.5/40.9	0.028	105	29.4/49.4/21.1	5.0/65.0/30.0	0.046
Complete + incomplete	127	28.7/53.5/17.8	15.4/50.0/34.6	0.049	143	27.6/53.3/19.0	21.1/50.0/28.9	0.2	109	28.4/50.0/21.6	4.8/66.7/28.6	0.052
2nd set of patients	75	35.1/47.4/17.5	5.6/72.2/22.2	0.027	79	30.4/55.4/14.3	30.4/60.9/8.7	0.5	55	29.5/52.3/18.2	36.4/63.6/0.0	0.4
Combined (1st + 2nd)	202	31.0/51.3/17.7	11.4/59.1/29.5	0.0055	222	28.6/54.0/17.4	24.6/54.1/21.3	0.2	164	28.8/50.8/20.5	15.6/65.6/18.8	0.3

[a] R = responder + moderate response; NR = non-responder; values are % of patients with the HH/HR/RR genotypes at the *FCGR2A* H131R polymorphism

[b] Results of the logistic regression analysis according to an additive genetic model including baseline DAS28 and gender as covariates for the same patients subgroups and evaluation times as in Table 2 are shown.

FIGURE CAPTIONS

Figure 1: Evolution of DAS28 in the patients with RA. Data from all patients (1^{st} +2^{nd} sets) are shown in function of the *FCGR2A* H131R genotypes and of the TNF antagonist. A) Patients treated with Infliximab; B) patients treated with Etanercept or Adalimumab. Means ± SD are represented.


A
B
HH
HR
RR
DAS28
Month
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
0
3
6
9
12