Genetic variants contribute to differences in response and toxicity to drugs used in autoimmune diseases: Rheumatoid arthritis and systemic lupus erythematosus

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Chapter 7

Association of the *FCGR3A-158F/V* gene polymorphism with the response to rituximab treatment in Spanish systemic autoimmune disease patients

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Abstract

Rituximab is being used as treatment for systemic autoimmune diseases. The objective of this study was to determine whether the genetic variant in the *Fc gamma-receptor III a* (*FCGR3A*) gene, 158F/V, contributes to the observed variation in response to rituximab in patients with systemic autoimmune diseases. DNA samples from 132 Spanish patients with different systemic autoimmune diseases receiving rituximab were genotyped for *FCGR3A-158F/V* (rs396991) gene polymorphism using the TaqMan allelic discrimination technology. Six months after infusion with rituximab we evaluated the response to the drug: 61% of the patients showed a complete response, partial 27% and 12% did not respond to the treatment. A statistically significant difference was observed in V allele frequency between responder (38%) and non-responder (16%) patients (p = 0.01; odds ratio [OR] = 3.24, 95% confidence interval [CI] 1.17–11.1). Rituximab was also more effective in V allele carriers (94%) than in homozygous FF patients (81%): p = 0.02; OR = 3.96, 95% CI 1.10–17.68. These results suggest that *FCGR3A*-158F/V (rs396991) gene polymorphism play a role in the response to rituximab in autoimmune diseases. Validation of these findings in independent cohorts is warranted.

INTRODUCTION

Systemic autoimmune diseases are a heterogeneous group of diseases with different pathogenesis and clinical manifestations but with common pathogenic mechanisms, including an aberrant B-cell regulation. The crucial role of B cells in autoimmune disorders has evidenced the importance of biological treatments that blockade these cells in refractory patients (1). Rituximab is a chimeric mouse-human monoclonal immunoglobulin G1 (IgG1) antibody that specifically targets the human B-lymphocyte CD20 surface protein (2), resulting in peripheral B-cell depletion (3). Rituximab use has been approved by the FDA for B-cell non-Hodgkin's lymphomas (4), for non-responders to patients with first-line antitumor necrosis factor-a rheumatoid arthritis (RA) (5), and recently, for vasculitis (6, 7) or lupus nephritis (8, 9), but there is growing evidence from observational studies and registries of patients that their usefulness can be extended to other autoimmune diseases (10-12). Studies have shown several different mechanisms by which rituximab can selectively deplete B cells, such as antibody-dependent cell-mediated cytotoxicity (ADCC), complement-mediated cytotoxicity, and direct induction of B-cell apoptosis (13).

Several factors have been associated with a better response to rituximab, for example, in patients with RA, the presence of positive rheumatoid factor (14), positive Epstein-Barr virus (15), low levels of B lymphocyte stimulator (16), and low levels of type I interferons (17).

The Fc portion of rituximab binds specifically to cell surface Fc-g receptors (FcGR), and this may affect certain immune responses such as removal of antigen–antibody complexes from the circulation, ADCC, or phagocytosis. There are three major classes of human FCGR that are encoded by 8 genes (*FCGR1A*, *B*, and *C*; *FcGR2A*, *B*, and *C*; *FcGR3A* and *B*), all located on chromosome 1. Three polymorphisms, two in positions 48 and 158 of *FcR3A* and one at codon 131 of *FcGR2A*, have been reported to affect receptor affinity for IgG (18-20). Functional studies have described a correlation between the *FcGR3A*-158 genotype and rituximab efficacy, but no correlation has been found with *FcGR2A*-131 (21). Moreover, genetic linkage of FcGR-48 and *FcGR2A*-131 with *FcGR3A*-158 has been demonstrated and points to the primacy of *FcGR3A*-158 in predicting rituximab response (22-25).

FcGR3A, also known as CD16, is expressed on macrophages, monocytes, and natural killer (NK) cells, all of which are involved in B-cell depletion. The nonsynonymous *FcGR3A*-158 polymorphism results in either a phenylalanine (F158) or a valine (V158) at this position in the membrane proximal domain of the molecule. The *FcGR3A*-158V single nucleotide polymorphism (SNP) exhibits a higher affinity for IgG subtypes than the *FcGR3A*-158F SNP (22, 26). This SNP has been associated to different autoimmune diseases such as type 1 diabetes, celiac disease, RA (27), and systemic lupus erythematosus (SLE) (28). In patients

with giant cell arteritis, an association was observed with the *FcGR2A FcGR3A* 131R-158F haplotype (29). However, no association between *FcGR3A*-158 and systemic sclerosis was described (30). Homozygosity for the higher-affinity V allele has also been shown to be associated with susceptibility to antibody-positive RA (31, 32).

An important pharmacogenetic association with biological response to rituximab has been shown in this polymorphism. Patients carrying the V/V isoform with non-Hodgkin lymphoma (NHL) and SLE showed a better biological response to rituximab (21, 33). Later, two studies conducted in healthy donors determined that this improved response observed in individuals expressing at least one valine at *FcGR3A*-158 seems to be due to an increased CD16 expression, rituximab binding, and ADCC activity mediated by NK cells (26, 34). In other diseases such as Sjögren's Syndrome (35) or chronic lymphocytic leukemia (36), this association was not observed, which may indicate that mechanisms of action of rituximab other than ADCC may be more important in these pathologies.

Only one study has examined the influence of *FcGR3A*-158F/V in the clinical response to rituximab in autoimmunity. This study conducted in patients with RA found that the V allele carriage was significantly associated with a higher response rate (37). It is possible however that the relative importance of ADCC as a mechanism for the activity of rituximab may differ between autoimmune disorders. The aim of our work was to investigate the possible involvement of the *FcGR3A*-158F/V polymorphism in the clinical response to rituximab in Spanish patients with different systemic autoimmune diseases.

MATERIALS AND METHODS

Patients and treatment

In total, 132 unselected patients with systemic autoimmune diseases treated with rituximab were recruited from three university medical centers (Hospital Universitario San Cecilio, Granada; Hospital Carlos Haya, Málaga; Hospital Virgen del Rocío, Sevilla). The characteristics of the patients enrolled in this study are shown in Table 7.1. Of the 132 patients, 81 (61.4%) were patients with SLE; 16 (12.1%) presented different inflammatory myopathies such as polymyositis and dermatomyositis; 13 (9.8%) were patients with ANCA mediated vasculitis, including Wegener's granulomatosis, Churg-Strauss Syndrome, and microscopic polyangiitis; and the remaining 22 patients presented other systemic autoimmune diseases such as Sjögren syndrome, systemic sclerosis, or autoimmune hemolytic anemia. The majority of patients received rituximab when conventional treatment had failed caused side effects or was contraindicated. Four 375 mg/m^2 doses

Table 7.1: Main characteristics of patients with systemic autoimmune diseases treated with rituximab included in this study

Characteristics	n (%)
Female	108 (82%)
Male	24 (18%)
Systemic autoimmune diseases	
Systemic lupus erythematosus	81 (61%)
Inflammatory myopathies	16 (12%)
ANCA-mediated vasculitis	13 (10%)
Sjögren's syndrome	4 (3%)
Systemic sclerosis	4 (3%)
Hemolytic autoimmune anemia	3 (2%)
Pemphigus vulgaris	3 (2%)
Mixed connective tissue disease	2 (1.5%)
Idiopathic thrombocytopenic purpura	2 (1.5%)
Rheumatoid arthritis	1 (1%)
Axonal polyneuropathy associated with HCV	1 (1%)
Autoimmune thrombocytopenia	1 (1%)
Sarcoidosis + RA	1 (1%)
Previous therapies	
Corticosteroids	127 (96%)
Cyclophosphamide	85 (64%)
Methotrexate	48 (36%)
Mycophenolate	47 (36%)
Intravenous immunoglobulins	46 (35%)
Antimalarials	38 (29%)
Azathioprine	35 (26%)
Cyclosporine A	11 (8%)
Leflunomide	5 (4%)
Other biologic therapies	5 (4%)
Plasma exchange	3 (2%)
Thalinomide	2 (1.5%)
Other therapies	9 (7%)
Concomitant therapies	
Corticosteroids	127 (96%)
Antimalarials	13 (10%)
Methotrexate	8 (6%)
Azathioprine	8 (6%)
Mycophenolate	7 (5%)
Cyclophosphamide	5 (4%)
Cyclosporine A	2 (1.5%)
Intravenous immunoglobulins	1 (0.5%)
Other therapies	2 (1.5%)
Response (n = 132)	
Complete	80 (61%)
Partial	36 (27%)
No response	16 (12%)

of rituximab (the recommended for treatment of lymphoma) were administered by intravenous infusion on days 1, 8, 15, and 22, in most cases, although some patients received 1000 mg twice at an interval of 15 days. Clinical response was evaluated according to the American College of Rheumatology (ACR) and European League Against Rheumatism (EULAR) recommendations at 6th month after the first infusion (12, 38-40). Complete response was defined as disappearance of all symptoms and signs that led to the use of rituximab, while concomitant immunosuppressive therapy remained stable and in acceptable levels in clinical practice; partial response was defined as a significant improvement (at least 50%) of initial disease activity, based on clinical judgment, but not reaching complete remission; no response was defined as no significant improvement or a worsening of the disease. Previous and concomitant treatments are shown in Table 7.1. The study protocol was approved by an ethics committee, and all patients gave written, informed consent before participation.

FcGR3A genotyping

For genotyping, cellular DNA was isolated from peripheral blood, using QIAamp DNA blood midi/maxi extraction kit (Qiagen GmbH, Germany). *FcGR3A*-158F/V SNP (rs396991) was genotyped using a TaqMan allelic discrimination Assay-By-Design method (Applied Biosystems, Foster City, CA). The genotype call rate was 100% for the tested *FcGR3A* genetic variants. The probes were labeled with the fluorescent dyes 2-chloro-7-phenyl-1,4-dichloro-6-carboxyfluorescein (VIC) and 6-carboxyfluorescein (FAM), and a polymerase chain reaction was carried. Endpoint fluorescent readings were performed on an ABI PRISM 7500 Sequence Detection Systems using SDS 2.3 software for allelic discrimination (Applied Biosystems).

Statistical analysis

Statistical analysis for allelic and genotypic distributions was calculated by chi-squared test or Fisher's exact test, when necessary, using the Statcalc software packages (EpiInfo 2002; Centers for Disease Control and Prevention, Atlanta, GA); p-values, odds ratio (OR), and 95% confidence intervals (95% CIs) were calculated. The results were considered to be statistically significant when $p < 0.05$. The presence of heterogeneity between SLE and the remaining patients with autoimmune disease was tested on the basis of the Breslow-Day test using a significance level of 0.05 (StatsDirect, v. 2,6,6).

RESULTS

The response to rituximab was evaluated at month 6 after first infusion, according to the EULAR and ACR criteria (12, 38-40). Eighty of the patients (61%) were considered to be good responders (complete remission of the symptoms and clinical characteristics that recommended the use of the drug); 36 patients (27%) were partial responders (reduction in at least 50% of the disease activity); and sixteen (12%) were classified as non-responders (reduction in < 50% of the disease activity). Of the 132 patients analyzed, 62 (47%) were homozygous for F allele, 48 (36%) were heterozygous FV, and 22 (17%) were homozygous for V allele, similarly to previous studies in SLE in Caucasian populations (41).

When all patients were pooled, after checking for homogeneity of odds ratios between patients with SLE and the remaining patients with autoimmune diseases by Breslow-Day test ($p > 0.05$), and stratified into two groups according to the response to rituximab, genotypic frequencies in patients presenting total or partial response to rituximab were as follows: 50 patients (43%) were FF; 45 patients (39%) were FV; and 21 patients (18%) were VV, whereas in the subgroup of patients presenting non-response to rituximab, 12 (75%) were FF; 3 (19%) were FV; and 1 patient (6%) was VV. In responders, the frequency of V allele carriers (FV +VV) was significantly increased with respect to non-responders (66 patients = 57% vs. 4 patients = 25%; $p = 0.02$; OR = 3.96, 95% CI 1.10–17.68). A significant association was also found when comparing the FCGR3A-158V allele frequency between responders (87 patients = 38%) and non-responders (5 patients = 16%); $p = 0.01$; OR = 3.24, 95% CI 1.17–11.13 (Table 7.2).

Table 7.2: Distribution of *FCGR3A-158F/V* (rs396991) single-nucleotide polymorphism and efficiency in patients with systemic autoimmune diseases treated with rituximab

	Responders n = 116 n (%)	Non-responders n = 16 n (%)	Efficiency rituximab %	p-value	OR (95% CI)
Genotype					
FF	50 (43)	12 (75)	81	0.02	0.25 (0.06–0.91)
FV	45 (39)	3 (19)	94	0.12	2.75 (0.70–15.7)
VV	21 (18)	1 (6)	95	0.21	3.32 (0.46–146.1)
Carriers					
FV + VV	66 (57)	4 (25)	94	0.02	3.96 (1.10–17.68)
FF	50 (43)	12 (75)	81	-	-
Allele					
F	145 (62)	27 (84)	84	0.01	0.31 (0.09–0.86)
V	87 (38)	5 (16)	95	0.01	3.24 (1.17–11.13)

OR, odds ratio; 95% CI, 95% confidence interval; p-value compares the frequency and efficiency in responders versus non-responders.

In correlation with these results, rituximab was effective in 94% of the patients carrying V allele (66 responders carrying the V allele/70 patients carrying the V allele) and 81% of the homozygous FF patients (50 responders FF homozygotes/62 patients FF homozygotes) (p = 0.02) (Table 7.2).

Finally, we analyzed separately patients with SLE (81/132, 61.4% of the patients) and patients with other autoimmune diseases (51/132, 38.6% of the patients). In both groups, we found a similar trend to those observed in the global analysis. In patients with SLE, 49% of the

Table 7.3: Distribution of *FCGR3A-158F/V* (rs396991) single-nucleotide polymorphism and efficiency in patients with systemic lupus erythematosus treated with rituximab

	Responders n = 71 n (%)	Non-responders n = 10 n (%)	Efficiency rituximab %	p-value	OR (95% CI)
Genotype					
FF	36 (51)	8 (80)	82	0.08	0.26 (0.02–1.44)
FV	23 (32)	1 (10)	96	0.14	4.31 (0.53–197.10)
VV	12 (17)	1 (10)	92	0.5	1.83 (0.21–86.83)
Carriers					
FV + VV	35 (49)	2 (20)	95	0.08	3.89 (0.70–39.51)
FF	36 (51)	8 (80)	82	-	-
Allele					
F	95 (68)	17 (85)	85	0.10	0.36 (0.06–1.33)
V	47 (32)	3 (15)	94	0.10	2.80 (0.75–15.58)

P-value compares the frequency and efficiency in responders versus non-responders.

Table 7.4: Distribution of *FCGR3A-158F/V* (rs396991) single-nucleotide polymorphism and efficiency in patients with no systemic lupus erythematosus treated with rituximab

	Responders n = 45 n (%)	Non-responders n = 6 n (%)	Efficiency rituximab %	p-value	OR (95% CI)
Genotype					
FF	15 (33)	4 (67)	79	0.13	0.25 (0.02–2.03)
FV	22 (49)	2 (33)	92	0.39	1.91 (0.24–22.88)
VV	8 (18)	0 (0)	100	0.34	Undefined
Carriers					
FV + VV	30 (67)	2 (33)	94	0.13	4.00 (0.49–47.55)
FF	15 (33)	4 (67)	79	-	-
Allele					
F	52 (58)	10 (83)	85	0.08	0.27 (0.03–1.41)
V	38 (42)	2 (17)	94	0.08	3.65 (0.71–35.81)

P-value compares the frequency and efficiency in responders versus non-responders.

responders carried the V allele while it was present in 20% of non-responders (p = 0.08, OR = 3.89, 95% CI 0.70–39.51). Rituximab was effective in 95% of the V carriers and 82% of the homozygousFF (p = 0.08) (Table 7.3). Likewise, in patients with no SLE, the frequency of the V allele was increased in responder versus non-responder patients (42% vs. 17%; p = 0.08, OR = 3.65, 95% CI 0.71–35.81). Rituximab was effective in 94% of the V carriers and 79% of the patients with FF homozygotes (p = 0.13) (Table 7.4).

DISCUSSION

The establishment of pharmacogenetic markers to predict the response to rituximab therapy becomes a pivotal requirement, given the expanding clinical use of this drug in the treatment of several autoimmune diseases.

Rituximab is recognized and bound to the surface of NK cells and macrophages through the FCGR, triggering ADCC immune system mechanism, essential for the activity of rituximab to deplete B cells. FCGR3A is expressed by immune effector cells and shows specific affinity for IgG monoclonal antibodies, such as rituximab. The importance of FCGR3A in the response to rituximab has been shown in studies where mice lacking FCGR3 presented a decrease in the response to this drug (42).

In the present study, we have analyzed the association of the *FCGR3A-158F/V* polymorphism with the response to rituximab in patients with autoimmune diseases. Genotypic frequencies for this SNP were similar to those described previously for several patients with autoimmune diseases in Caucasian populations (43, 44, 27). It is remarkable that frequencies were elevated for V carriers in responders, which correlates with the fact that patients carrying the V allele at this position presented a better response to the treatment with the drug than those with homozygous FF genotype.

Functional studies have demonstrated that the 158V allele is correlated with a better biological response to rituximab in autoimmunity. Anolik et al. (21) showed that in patients with SLE carrying the high-affinity V allele (FV or VV), rituximab was more effective in depleting peripheral B cells than in those homozygous for the low-affinity FF. Recently, the *FCGR3A-158F/V* SNP has been associated with the clinical response to rituximab in RA. This study conducted in 111 patients found that the V allele carriage was significantly associated with a higher response rate (91% of responder vs. 70%; p = 0.006, OR = 4.6, 95% CI 1.5–13.6) (37).

The findings in SLE and RA are in line with our results that showed a better response to rituximab in patients with autoimmune diseases that carried the V allele (FV or VV) than in

patients with homozygous FF. Additionally, based on the previous association observed in patients with SLE and on the fact that this was the largest group, we analyzed separately patients with SLE. We found a similar pattern, and patients carrying the V allele showed a better response to rituximab treatment, although it did not reach statistical significance ($p = 0.08$). Finally, we examined the group of patients with no SLE to establish whether this association is shared by different autoimmune disorders. As in the case of patients with SLE, we observed a similar effect, but this association did not reach statistical significance either ($p = 0.08$). This suggests that the influence of the 158F/V polymorphism in the therapeutic response to rituximab is common to various autoimmune diseases; however, the reduced numbers involved in these stratified analysis leads to poor statistical power, and therefore the conclusions are provisional.

It should be noted that copy number variation (CNV) has been shown to be present in the *FCGR3A* gene (45-47). The presence of common CNVs can cause false SNP genotyping results that can lead to fail the Hardy–Weinberg equilibrium (HWE) and may blur the association of the studied SNPs with disease susceptibility. In our study, the genotypic frequencies were significantly different from those predicted by HWE, but only in the group of patients with SLE. This may be due to existence of an association between the *158F/V* polymorphism and this disease (48). In fact, in our cohort of healthy controls (previously published genotypic data), genotype frequencies for this SNP were in the HWE (49). Moreover, the frequency of CNV has been reported to vary significantly in different ethnic populations, which can result in contradictory findings, but in this case, frequencies observed in patients were similar to those previously described, and the results reported to date are fairly consistent.

Previous findings showed that patients carrying the V allele in *FCGR3A-158F/V* increased expression of CD16 in NK cells (34). A correlation between the number of cell surface CD16 receptors and the enhancing of the ADCC activity mediated by NK cells was found. These observations would explain the better response to rituximab observed in patients with systemic autoimmune diseases carrying the V allele and would highlight the importance of the ADCC mechanism for clearance of B cells by rituximab in autoimmune diseases.

In summary, our results together with previous findings (21, 50, 37) suggest that FCGR3A plays an important role in response to rituximab in patients with systemic autoimmune diseases and support the hypothesis that the 158F/V variant could be used as a potential predictor of those patients who will respond better to treatment with rituximab.

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