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Polymorphism in IgG Fc receptor gene *FCGR3A* and response to infliximab in Crohn's disease: a subanalysis of the ACCENT I study

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Abstract

Recently, it has been shown that *FCGR3A*-158 gene polymorphism is associated with biological and possibly clinical response to infliximab in Crohn's disease. We further assessed this association in a subset of 344 patients from the large and well-defined cohort of 573 patients with Crohn's disease from the ACCENT I study. No association could be observed between *FCGR3A*-158 gene polymorphism and the clinical response to infliximab, which was primarily defined as a decrease of ≥ 70 points in the Crohn's disease activity index or clinical remission (Crohn's disease activity index <150). We did, however, confirm a trend towards a greater decrease in C-reactive protein after infliximab in V/V homozygotes as compared with V/F heterozygotes and F/F homozygotes (-79.4, -76.5, and -64.3%, respectively, at week 6; *P*=0.085; one-tailed *P*= 0.043). This finding has no immediate clinical impact but may enhance the understanding of the complex mechanisms of action of anti-tumor necrosis factor agents in Crohn's disease.

Keywords: C-reactive protein, Crohn's disease, IgG Fc receptor, infliximab, polymorphism, tumor necrosis factor

Introduction

While the mechanism of action of infliximab in Crohn's disease (CD) is not completely understood, lysis of mononuclear cells expressing membrane tumor necrosis factor-alpha (TNF-α) through apoptosis, complement activation, and/or antibody-dependent cell-mediated cytotoxicity (ADCC) are suspected [1-4]. A functionally significant polymorphism in *FCGR3A,* the gene coding for FcγRIIIa, a receptor for the Fc portion of IgG expressed on macrophages and natural killer cells, and involved in ADCC, has been found to be associated with a positive response to another recombinant IgG1 antibody, ritux-imab, in non-Hodgkin's lymphoma [5].

Recently, it has been reported that CD patients with *FCGR3A*-158V/V genotype had a better biological, and possibly better clinical response to infliximab [6]. Specifically, the median relative decrease in C-reactive protein (CRP) following infliximab therapy was significantly greater in V/V homozygotes, and all patients with this genotype had a CRP decrease of at least 25% 4 weeks after a single infliximab infusion [6]. Confirmation of this finding in a large cohort was a key step before considering this genetic marker as potentially relevant for infliximab treatment of CD patients. We therefore tested the hypothesis of an association between *FCGR3A* gene polymorphism and response to infliximab in the prospective, randomized, controlled ACCENT I study (A Crohn's disease Clinical Trial Evaluating infliximab in a New long-term Treatment regimen) [7].

Patients and methods

Study population

In ACCENT I [7], 573 patients with active intestinal CD and a Crohn's disease activity index (CDAI) between 220 and 450 received a single infusion of infliximab 5 mg/kg at baseline. At week 2, patients were randomized to one of three treatment regimens administered through week 46: (1) single-dose group: placebo at weeks 2 and 6 and then every 8 weeks, (2) 5-mg/kg maintenance group: infliximab 5 mg/kg at weeks 2 and 6 and then every 8 weeks, and (3) 10-mg/kg maintenance group: infliximab 5mg/kg at weeks 2 and 6 and then infliximab l0mg/kg every 8 weeks. *FCGR3A-158* polymorphism was determined in 344 patients who consented to participate in this

substudy.

Classification of response to infliximab

For this analysis, clinical response was assessed at week 2, at which point all patients had received one infusion of infliximab 5 mg/kg. Clinical response was defined as a CDAI decrease of at least 70 points, and clinical remission was defined as a CDAI score lower than 150. The decrease in CDAI was also compared among patient genotypes. With regard to biomarkers of disease activity, serum CRP concentration data were available at weeks 2 and 6. A positive response, assessed at both weeks 2 and 6, was defined by a CRP decrease of at least 25% from baseline. The actual decrease in CRP levels was also compared between patient genotypes.

DNA analysis

FCGR3A polymorphism was assessed using allelic discrimination by TaqMan technology (ABI 7700, Applied Biosystems, Foster City, California, USA) [8] after validation by an allele-specific polymerase chain reaction [6]. The following primers and probes were used: (1) primer sequences: FCRG3A_TG-F1 ATTCCAAAAGC-CACACTCAAAGA and FCRG3A_TG-R1 ATGGT-GATGTTCACAGTCTCTGAAG; (2) probes: FCRG3A_TG-V1 CTCCCAACAAGCC VIC and FCRG3A_TG-M1 TACTCCCAAAAAGC FAM. Primers and probes were designed with Primer Express (Aplera, Foster City, California, USA) and synthesized through Applied Biosystems. The amplification conditions involved two pre-PCR steps of 2 min at 50°C and 10 min at 95°C, followed by 35 cycles including a denaturation step at 95°C for 15 s and an annealing step of 1 min at 62°C in a final volume of 5 µl.

Statistical analysis

The proportions of patients with clinical and CRP responses to infliximab were compared between genotypes using a χ^2 or Fisher's exact test, as appropriate. Decreases in CDAI and CRP were compared across genotypes using an analysis of variance on the van der Waerden normal scores. For relative decreases in CRP, the analysis was first performed on the entire cohort, and then on the subgroup of patients having CRP concentration data at baseline within the two highest quartiles. Furthermore, as this analysis was intended as a confirmatory study of a previous one [6], a one-tailed test was also applied.

Results

FCGR3A genotypes and baseline characteristics

Baseline characteristics of the ACCENT I population have been described previously [7]. In this substudy, 95.3% of patients were Caucasian, 57.3% were women, and the median (range) age was 35 (18-76) years. Patients had a median (range) disease duration of 8 (0.2-38.9) years. Regarding disease location, 25.5% of patients had ileum involvement, 20.8% had the colon involved, and 53.7% had ileum and colon involvement. The median (range) baseline CDAI score was 293 (200-450), and the median (range) baseline CRP level was 0.8 (0.4-16.2) mg/d. The proportions of patients with *FCGR3A* V/V, V/F, and F/F genotypes were 14.2, 43.6, and 42.2%, respectively. No deviation was found from Hardy-Weinberg equilibrium. At baseline, there was no significant difference between genotypes as far as CDAI (306.2 ± 49.1, 300.8 ± 53.6, and 296.9 ± 56.1 for V/V V/F, and F/F patients, respectively) and CRP serum concentrations (2.1 ± 2.5, 1.9 ± 2.2, and 1.6 ± 2.1 mg/dl, for V/V, V/F, and F/F patients, respectively).

FCGR3A genotypes according to clinical and biological response to infliximab

At week 2, in the overall cohort, there was no significant difference in clinical or CRP response to infliximab when assessed by *FCGR3A* genotype (Table 1).

The analysis of the subgroup both at baseline and at week 6 CRP values within the two highest quartiles (n = 156) showed a trend toward a greater relative change in CRP at week 6 in V/V homozygotes (-79.4, -76.5, and -64.3%, in V/V, V/F, and F/F genotypes, respectively; P = 0.085; one-tailed P = 0.043). Numerical differences were already present at week 2 but were not statistically significant (Fig. 1). In this subgroup, all (95.8%) but one patient with V/V genotype had a decrease of at least 25% in CRP at week 6, while this was the case for only 87.1% of V/F patients and 79.0% of F/F patients (P = 0.12; one-tailed P =0.06). This trend was also evident at week 2 (96, 88.6, and 87.7%, for V/V, V/F, and F/F patients, respectively; P = 0.497).

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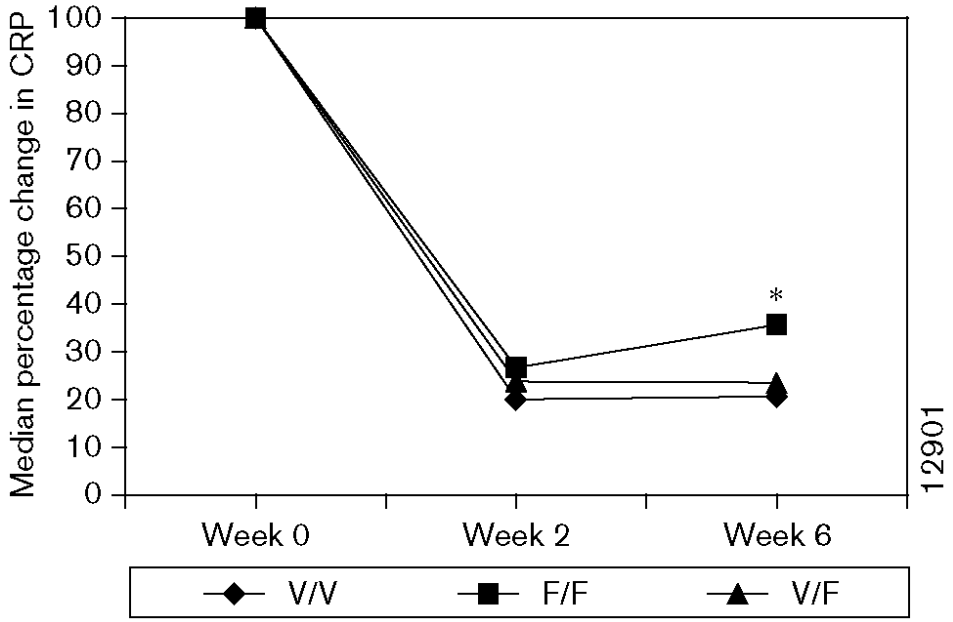
Table 1 *Clinical and CRP response 2 weeks after a single infusion of infliximab 5 mg/kg by FCGR3A genotype among the 344 ACCENT I substudy patients*

Genotype	*FCGR3A* V/V	*FCGR3A* V/F	*FCGR3A* F/F	*P*-value
Patients evaluated	49 (14.2%)	150 (43.6%)	145 (42.2%)	
Clinical response (CDAI $\geq$ 70)	61.2%	56.7%	66.9%	0.195
Clinical remission (CDAI <150)	20.4%	26.0%	31.0%	0.314
Median CDAI decrease	-97.0	-84.5	-101.0	0.487
Median CRP decrease	-0.4	-0.2	-0.1	0.255
Median % CRP change	-43.7%	-33.3%	- 20.0%	0.195

CRP, C-reactive protein; CDAI, Crohn's disease activity index.

Fig. 1 : *Median relative decrease in C-reactive protein (CRP) following the administration of infliximab 5 mg/kg at week 2 in patients with Crohn's disease in the ACCENT I study.* Only patients with a CRP concentration within the two highest quartiles at both baseline and week 6 CRP were considered for this analysis (n= 156; CRP range: 0.9-16.2 mg/dl). Patients are separated into three groups according to FCGR3/4-158 genotype. At week 2, the largest decrease was observed in V/V patients and the smallest decrease in F/F patients, but this difference was not statistically significant. This difference reached a borderline level of significance by week 6 (P= 0.086; one-tailed P= 0.043).



Discussion

Our findings indicate an absence of association between *FCGR34-158* polymorphism and clinical response to infliximab in CD. Furthermore, in this large cohort of patients from the ACCENT I study, there was also no strong influence on biological (CRP) response, although there was a numerical difference in the magnitude of the CRP decrease following infliximab therapy. In a previous study, there was a significant association between the same *FCGR3A* polymorphism and the proportion of patients having a decrease in CRP after infliximab, as well as with the magnitude of CRP decrease [6]. In that series of patients from the Belgian Expanded Access Program for infliximab in CD, there was also no strong association between polymorphism and infliximab clinical response, although in multivariate analysis of patients with elevated baseline CRP, immunosuppressant use and *FCGR3A* polymorphism were independently associated with a positive clinical response to infliximab. These data were therefore suggestive of an influence of this variant on the control of the inflammation induced by infliximab in CD. This hypothesis was reinforced by previous data showing a similar effect with another IgG1 therapeutic monoclonal antibody, rituximab, in non-Hodgkin's lymphoma [5], and by functional data showing a better affinity of the FcγRIIIa-158V allotype for IgGl and an ADCC EC_{50} at lower rituximab concentrations in V/V patients [9]. It was assumed that the analysis of a larger cohort would demonstrate a significant influence of this polymorphism on the clinical response to infliximab in CD patients. This was not, however, the case in the present large and very well-defined ACCENT I cohort.

FCGR3A polymorphism is not a practical marker to assist clinicians in defining the best candidates for infliximab treatment and has yet to find its place in the management of patients receiving infliximab. In this ACCENT I cohort, as in the Belgian cohort, the relative decrease in CRP was greatest in V/V homozygotes and lowest in F/F homozygotes. In these studies, discrepancies in the number of infliximab infusions and timing of response evaluations do not allow strict comparison or metaanalysis, the difference between genotypes was of the same magnitude.

The mechanism of action of infliximab in CD appears to be multifactorial. Along with neutralizing soluble TNF-α, inducing apoptosis of cells expressing membrane-bound TNF is the most well-characterized mechanism of action [2-4]. Other mechanisms however, including lysis of TNF-expressing cells by complement activation and ADCC, have also been suggested [1]. Although in-vivo data are lacking to demonstrate ADCC involvement in infliximab effect in CD, the ability of infliximab to bind transmembrane TNF-α has been clearly demonstrated [2,4,10]. Owing to the complexity of infliximab action in CD, it is unlikely that a single biological or clinical parameter would have a strong influence on the response to the agent. This is particularly true for clinical response, owing to the influence of subjective variables in the scores used in clinical evaluations and to the influence of factors not directly related to inflammation in the clinical evolution of the patients, for example, fibrotic stricture-related pain or functional diarrhea. Therefore, the finding of the same phenomenon (influence of *FCGR3A* genotype on CRP decrease) in two large independent cohorts is certainly of value and is not a trivial finding. Importantly, this finding may help further the understanding of the mechanism of action of infliximab in CD and even possibly assist in the design of new anti-TNF molecules. The precise mechanism by which this variant may influence response to infliximab in CD remains to be elucidated. Further study of the *CRP* gene located close to the *FCGR3A* gene, however, confirmed that the association seen in the Belgian cohort was indeed with the *FCGR3A* variants and not with the *CRP* gene itself [10]. With regard to the mechanism of this association, the most attractive hypothesis is more efficient destruction or a destruction at a lower concentration of membrane TNF-expressing cells by ADCC in W homozygous patients. In this hypothesis, this variant would not create a dichotomy of response-non-response but rather enhance the response to infliximab in a subgroup of patients. The evolution of CRP decrease at weeks 2 and 6 (Fig. 1), suggests the possibility of a shorter duration of inflammation control in F/F homozygous patients. This may fit with the lower affinity of the FcγRIIIa-158F allotype for IgG1. As for rituximab, a higher concentration of infliximab would be needed for ADCC in these patients. In this hypothesis, the week-2 time point may be too early to discern a significant difference between genotypes. On the other hand, week-6 data are not as easy to interpret because patients in the ACCENT I study had received either one or two infusions of 5 mg/kg by this time point. In patients who received higher doses of infliximab, the influence of *FCGR3A* polymorphism may be attenuated.

In conclusion, no association between the *FCGR3A-158* polymorphism and the clinical response to infliximab in CD could be observed. Hence, this polymorphism has no direct clinical impact on the management of the patients. As previously suggested however, this polymorphism seems to influence the magnitude of CRP decrease in these patients. This finding may shed new light on the complex mechanism of action of infliximab in CD.

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