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Influence of FCGR3A-V212F and TNFRSF1B-M196R genotypes in patients with rheumatoid arthritis treated with infliximab therapy

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ABSTRACT

Objective. *Anti-TNF-α therapies are widely used in rheumatoid arthritis (RA) patients. Despite their clearly proven efficacy, some discrepancies were observed in the treatment response with 40% of non-responder patients. The aim of this study is to determine whether two functional single-nucleotide polymorphisms, V212F in the FCGR3A, and M196R in the TNFRSF1B genes correlate with rheumatoid arthritis susceptibility and response to anti-TNF-α therapy.*

Methods. *The population study was composed of a French cohort of 78 RA patients and 70 healthy controls. Allele and genotype frequencies were compared between patients and controls, according to their response to infliximab therapy, using the American College of Rheumatology (ACR) response criteria.*

Results. *No association was found between these two SNPs and RA susceptibility. A significant correlation was found between 196R allele carriers and low response to infliximab therapy.*

Conclusion. *This is the first report of a statistically significant association between the TNFRSF1B-M196R SNP and response to infliximab in a French cohort. Larger studies are needed to confirm the relevance of this association.*

Introduction

Rheumatoid arthritis (RA) is the most frequent chronic inflammatory rheumatic disease in the world. Therapeutic agents blocking Tumor Necrosis Factor α (TNF-α) are used for the treatment of active RA after the inefficacy of a full trial of traditional effective disease modifying antirheumatic drugs (DMARDs) (1). The overexpression of TNF-α in RA synovium, along with the data obtained from *in vitro* synovial cell cultures and the results from TNF-α blockade in animal models of arthritis, confirmed the central role of this cytokine in the pathogenesis of RA. There are currently three anti-TNF-α drugs available in the treatment of RA patients: infliximab (a chimeric mouse/human monoclonal antibody), etanercept (a fusion protein combining two p75 TNF receptors with a Fc fragment of human IgG_1) and adalimumab (a fully human monoclonal antibody). Nevertheless, only about 60% of RA patients exhibit a positive response to anti-TNF-α therapy, and no significant results were shown about clinical or biological predictor factors of the outcome of the treatment (2). Due to the high cost and potential side effects of these therapies, the identification of genetic or other predictors of treatment response would provide valuable information for therapeutic decisions.

The biological effects of TNF-α are mediated through two specific transmembrane receptors TNFRSFIA (CD120a, TNF-R55) and TNFRSFIB (CD120b, TNF-R75).

A SNP has been reported in exon 6 of the *TNFRSF1B* gene, which leads to an amino acid substitution at codon 196 (M196R). Molecular and functional analysis of this *TNFRSF1B* gene polymorphism suggests that the 196R allele is functionally distinct from the wild-type 196M allele (3). Furthermore, an association of this allele with RA severity has also been highlighted in two different cohorts (4, 5).

In a way similar to soluble receptors, anti-TNF monoclonal antibodies block soluble TNF. However, another mechanism of action has been suggested, as these molecules bind to transmembrane TNF-α on activated immune cells, thereby inducing lysis of these cells by complement activation or antibody-dependent cellular cytotoxicity via Fcγ receptors. One SNP within exon 5 of the *FCGR3A* gene resulting in an amino acid substitution at codon 212 (V212F) has been reported to have an influence on RA susceptibility and disease severity (6, 7), whereas contradictory results have also been published (8, 9). More recently, Tutuncu *et al.* highlighted an association between V212F SNP and response to TNF inhibitors (the *FCGR3A*-212FF genotype was associated with a better response) in a cohort of psoriatic and RA patients (10), which was not confirmed by a larger study on Swedish RA patients treated with infliximab and etanercept (11).

The present study was performed with two goals. The first goal was to evaluate the association between both

FCGR3A - V212F and *TNFRSF1B* - M196R SNPs and the susceptibility to RA. The second goal was to seek for a correlation between these two SNPs and response to infliximab.

Patients and methods

Patients and controls

All RA patients included were aged ≥18 years and met ACR (American College of Rheumatology) criteria for RA diagnosis. At each follow-up visit, the Disease Activity Score 28 (DAS28), and the French version of Health Assessment Questionnaire (F-HAQ) were evaluated. Each individual signed an informed consent form after receiving information regarding the study. The protocol was approved by the local committee for protection of persons participating in biomedical research (French law 88-1138; December 20, 1988). Infliximab was administered at week 0, then at weeks 2, 6 and 14, by an infusion of 3 mg/kg. The efficacy of therapy after 14 weeks was assessed according to variations of DAS28 score and ACR response criteria.

A population of unrelated individuals, from the same region, constituted the control group. These individuals had been informed that their biological samples would be used within the framework of research projects, according to the French legal requirements.

FCGR3A and TNFRSF1B genotyping

DNA was extracted from peripheral blood leukocytes using the Wizard Genomic DNA Purification Kit (Promega). Primers for PCR amplification were designed using Primer 3.0 (http://frodo.wi.mit.edu/). *FCGR3A* primers around the V212F SNP (rs396991) were: FCGR3Af 5'-TGGCAAAGGCAGGAAGTATT-3'; FCGR3Ar 5'-CAACTCAACTTCCCAGTGTCAT-3'. The reverse primer was designed to specifically amplify *FCGR3A,* and not *FCGR3B* (these two genes share 99 % of nucleotide homology in this region). *TNFRSF1B* primers around the M196R SNP (rs1061622) were: TNFRSF1Bf 5'-CTCTCCTATCCTGCCTGCTG-3' and TNFRSF1Br 5'-AGTGCTGGGTTCTGGAGTTG-3'. The two purified PCR fragments were used as templates for primer extension using the ABI PRISM®SNaPshotTM Multiplex System (Applied Biosystems). The SNaPshot primers were: FCGR3A-SS 5'-AAATGAAGACACATTTTTACTCCCAA-3', TNFRSF1B-SS 5'-CCGACGTGCAGACTGCATCC-3'. Thermal cycling was conducted in *PCRExpress* Thermal Cycler (Hybaid), as follows: 10 seconds at 96°C, 5 seconds at 50°C, 30 seconds at 60°C, for 25 cycles. All samples were run on an ABI-PRISM®3130xl, and analyzed using the Genescan®3.7 software (Applied Biosystems).

Statistical analysis

FCGR3A and *TNFRSF1B* genotype frequencies were compared between RA patients and controls. Response rates were compared across *FCGR3A* and *TNFRSF1B* genotype frequencies after 14 weeks of therapy, using chi-square test or Fisher exact test when needed. Treatment response was assessed using ACR response criteria, thus defining four groups of patients: non-responders, ACR20, ACR50 and ACR70 responders. Variations of DAS28 score were also compared according to genotype frequencies.

Test thresholds were determined using a 0.05 alpha risk. Statistical analyses were performed with the STATA 7.0 SE Software (Stata, College Station, TX).

Results

Population study

A population of 104 patients was included in this study: 24 patients were excluded due to premature treatment interruption, and 2 patients were lost during follow-up. A final population of 78 patients was considered and genotyped for the two selected SNPs.

Demographic characteristics of included patients and therapy outcome are presented in Table I.

Definition of ACR groups of patients

After 14 weeks of anti-TNF treatment, 34 patients were classified as non-responders (43.6%), as they did not meet any ACR response criteria, while 44 were ACR20 responders (56.4%), 27 were ACR50 responders (34.6%), and 17 were ACR70 responders (21.8%).

Genotyping results

Genotyping results for the two SNPs in RA cases and controls are summarized in Table II.

1. FCGR3A-V212F polymorphism

There was no association between any of the genotypes (VV, FF or VF) and susceptibility to RA (p=0.51). No significant difference was observed in DAS28 score variation between the different genotypes (p=0.69). Concerning the responsiveness to infliximab, there was no statistical difference between

Table I. Demographic characteristics of included patients and therapy outcome.

Characteristic	No response (n=34)	ACR20 (n=44)	ACR50 (n=27)	ACR70 (n=17)
Female sex, %	65	68	63	59
Age, mean ± SD years	57.8 ± 11.4	56.2 ± 12.8	53.7 ± 12.5	53.3 ± 13.6
Disease duration, mean ± SD years	16.8 ± 9.3	18 ± 8.7	18.4 ± 8.2	17.5 ± 7.5

Table II. Genotyping results for RA patients and controls according to treatment response.

Group of patients	FCGR3A-V212F			TNFRSF1B-M196R		
	FF (n=59)	VF (n=77)	VV (n=12)	MM (n=91)	MR (n=50)	RR (n=7)
RA patients						
- No response	13	18	3	16	16	2
- ACR20	17	22	5	34	9	1
- ACR50	9	16	2	22	4	1
- ACR70	5	11	1	12	4	1
Controls	29	37	4	41	25	4

the non-responder group and ACR20 (p=1), ACR50 (p=0.924) and ACR70 (p=0.813) groups, according to the genotype distribution.

2. TNFRSF1B-M196R polymorphism

There was no association between the different genotypes (RR, MM or MR) in patients and RA susceptibility (p=0.99). A tendency to higher values of DAS28 score was observed in R allele carriers than in R allele non-carriers, but without statistical significance (p=0,10).

R allele carriers were found to be associated with a significantly lower response to infliximab, compared with R allele non-carriers, according to ACR criteria (OR=3.825, $IC_{95\%}$= [1.30-11.44], p=0.006). This was confirmed by genotype analyses when comparing non-responders with ACR20 responders (p=0.013) and ACR50 responders (p=0.011). No statistical association was found with ACR70 responders (p=0.174).

Discussion

No significant association was found between the two SNPs and susceptibility to RA in our cohort of 78 RA patients. Regarding the responsiveness to infliximab, the frequency of non responders was about 40% in our cohort, which is comparable with previous results in the literature (2).

We found no association between the *FCGR3A* genotypes and response to infliximab. These results are concordant with a recent study (11). Interestingly, this SNP, within the FCGR2A-FCGR3A haplotype, was found to be associated with susceptibility to giant cell arteritis, which is also a polygenic disease associated with HLA-DRB1*04 (12).

The *TNFRSF1B*-196R allele carriers are less susceptible to respond to treatment. This is the first report of a statistically significant association between this SNP and response to infliximab in a cohort of RA patients. This result confirms a trend already found by Fabris *et al*. in 2002 (5). The functional influence of the M196R SNP has been extensively studied. Codon 196 is located in the fourth cysteine-rich domain of the extracellular region of TNFRII, close to the putative cleavage site and to the "stalk" that is an important domain for its dimer or trimer formation (13). Experiments performed by expressing the full-length cDNA for TNFRII carrying either 196M or 196R in HeLa cells showed a functional difference between alleles: IL-6 production and cytotoxic activity were increased in cells carrying the R allele, without modifying receptor affinity to TNF-α (3). These results suggest that the R allele triggers a stronger inflammatory response via the TNF-α pathway and this might be related to a weaker response to anti-TNF-α treatment. In other studies, the TNFRSF1B-M196R genotype correlated with levels of the soluble TNFRII in healthy controls and RA patients (14, 15).

The association between the R allele and poor response to treatment should be confirmed with a larger cohort of patients in multicentric studies, as it would provide a useful test for infliximab indication in patients with RA.

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