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 4

Confirmation of *-174G/C interleukin-6* gene promoter polymorphism as a genetic marker predicting anti-TNF treatment outcome

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Pharmacogenetics and Genomics. 2014;24:1-5

Abstract

Background

The *IL-6* -174G/C genetic variant has been recently associated with the clinical response to etanercept therapy in rheumatoid arthritis (RA) patients. Considering previous results, the aim of our study was to validate the role of this polymorphism as a predictor of the anti-TNF treatment outcome in RA.

Methods

Our study population was composed of 199 Spanish patients with RA receiving anti-TNF therapy. The *IL6* -174G/C (rs1800795) genetic variant was genotyped using the TaqMan® allelic discrimination technology. Patients were classified, according to the European League Against Rheumatism (EULAR) criteria, as responders (good and moderate response) and non-responders at 6, 12, 18 y 24 months after the first infusion.

Results

The -174*G allele was significantly associated with a good or moderated EULAR response at 12 (p = 0.015, OR = 2.93, 95% CI 1.29–6.70), 18 (p = 4.54E-03, OR = 5.17, 95% CI 1.80–14.85) and 24 months (p = 4.54E-03, OR = 14.86, 95% CI 2.91–75.91). A meta-analysis combining these data with the results from a previous study confirmed this association (p = 1.89^{E-02}, OR = 1.80, 95% CI 1.13–2.87, at 12 months).

Conclusion

Our results support the role of the-174G/C *IL-6* polymorphism as a genetic marker of responsiveness to anti-TNF therapy.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, systemic inflammatory disease characterized by polyarthritis, joint damage and functional disability (1). The cutaneous and systemic over-expression of several proinflammatory cytokines such as IL-2, IL-6, IL-8 and tumor necrosis factor-alpha (TNF-α), has been suggested to be responsible for the initiation, maintenance and recurrence of skin lesions and joint inflammation and destruction in RA (2, 3).

Research on the complex biology of TNF has uncovered many mechanisms and pathways by which TNF may be involved in the pathogenesis of RA (3, 4). The introduction of TNF-blocking agents, such as infliximab, etanercept and adalimumab revolutionized the treatment of RA, most notably because of the excellent clinical efficacy and ability of these agents to prevent further structural damage in patients who failed to respond to treatment with conventional disease-modifying antirheumatic drugs (DMARDs) (3, 5). However, the response to treatment with anti-TNF agents is variable and a substantial proportion of patients (20–50%) do not display any significant clinical improvement or lose an initially favorable response over time (5-8). The identification of pharmacogenetic markers of treatment response may be useful in predicting clinical response to anti-TNF therapies and would facilitate the development of individualized treatment (6, 9).

IL-6, produced by a variety of cell types, including monocytes, macrophages, fibroblasts, T-helper 2 cells and vascular endothelial cells, is a multifunctional cytokine that plays important roles in host defense, acute-phase reactions, immune responses and haematopoiesis (10-14). The *-174G/C* polymorphism (rs1800795), which is located in the negative regulative domain of the *IL-6* gene promoter, has been found to affect transcriptional regulation (15, 16). The *IL-6* -174*C allele has been associated in vivo with increased levels of IL-6 (17, 18), and C-reactive protein (CRP) (19) in the general population and in RA patients (20).

A recent study (21) has described a significant association of -174G/C with the clinical response to etanercept therapy at 12 months in Serbian patients with RA. This article showed that a higher number of responders were present among patients with the -174*GG genotype compared with patients carrying the -174*C allele, suggesting that this polymorphism may be a genetic marker of responsiveness to etanercept in RA. Replication of these results in independent and larger data sets is required in order to confirm the role of this genetic variant as predictor of anti-TNF outcome.

The aim of this study was to validate the reported association of the *IL-6* -174G/C polymorphism with the anti-TNF response in an independent cohort of Spanish RA patients.

MATERIAL AND METHODS

Patients and treatment

A total of 199 anti-TNF treated RA patients were recruited from five Spanish university medical centres (Hospital San Cecilio, Granada; Hospital Virgen de la Arrixaca, Murcia; Hospital La Paz, Madrid, Hospital Doctor Peset, Valencia; Hospital Virgen de la Victoria, Málaga). All patients were diagnosed with RA according to the 1987 American College of Rheumatology (ACR) criteria. Informed written consent from all participants and approval from the local ethical committees were obtained in accordance with the tenets of the Declaration of Helsinki. The characteristics of the patients enrolled in this study are shown in Table 4.1.

Table 4.1: Baseline characteristics of the rheumatoid arthritis

Parameters	N = 199	GG (N = 98)	GC (N = 83)	CC (N = 18)	p-value
Age (years) (mean ± SD)	53.08 ± 14.28	53.25 ± 13.84	51.64 ± 14.76	59 ± 13.52	0.608
Sex (female) [N (%)]	163 (81.9%)	83 (50.92%)	65 (39.88%)	15 (9.20%)	0.608
Swollen joints (mean ± SD)	6.10 ± 4.08	6.04 ± 4.18	5.79 ± 3.53	7.94 ± 5.45	0.138
Tenders joints (mean ± SD)	4.5 ± 3.14	4.11 ± 2.91	4.81 ± 3.20	5.64 ± 3.79	0.100
DAS28 (mean ± SD)	5.21 ± 1.12	5.10 ± 1.14	5.25 ± 1.11	5.62 ± 0.93	0.185
NSAID [N (%)]	132 (73.74%)	63 (47.73%)	59(44.7%)	10(7.58%)	0.179
Corticosteroids [N (%)]	130 (65.33%)	66 (50.77%)	54(41.54%)	10(7.69%)	0.833
MTX/DMARDS [N (%)]	157 (78.89%)	76 (48.41%)	68(43.31%)	13(8.28%)	0.471
ESR (> 8) [N (%)]	139 (69.85%)	69 (49.64%)	58(41.73%)	12(8.63%)	0.972
CRP ≥ 5 [N (%)]	25 (12.56%)	12 (12.44%)	10(12.04%)	3 (16%)	0.210
Positive RF ≥ 20 [N (%)]	70 (35.17%)	31 (31.63%)	32 (38.5%)	7 (38%)	0.110
Infliximab [N (%)]	61 (30.65%)	31(50.82%)	25(40.98%)	5(8.20%)	0.940
Etanercept [N (%)]	21(10.5%)	12(57.14%)	7(33.33%)	2(9.52%)	0.941
Adalimumab [N (%)]	117(58.8)	57(48.72%)	50(42.72%)	10(8.55%)	0.942

SD, standard deviation; DAS28, disease activity score 28; NSAID, non-steroidal anti-inflammatory drugs; MTX, methotrexate; ESR, erythrocyte sedimentation rate; CRP, c-reactive protein, RF, rheumatoid factor.

Infliximab was given intravenously and continuously at a dose of 5 mg/kg at weeks 0, 2 and 6 and every 8 weeks thereafter. Etanercept was given at a dose of 50 mg once per week subcutaneously. Adalimumab was subcutaneously administered at dose of 40 mg every two weeks. The choice between infliximab, etanercept and adalimumab was made according to the typology of patients and disease features, the onset of action in terms of clinical response.

Disease severity was evaluated using the disease activity score 28 (DAS28). DAS28 was measured at baseline and at four time points after the first infusion: 6, 12, 18 and 24 months. According to the European League Against Rheumatism (EULAR) response criteria (22, 23) patients were classified as good responders (good and moderate) or non-responders, using the individual amount of change in DAS28 (ΔDAS28) and DAS28 values at 6, 12, 18 and 24 months. Briefly, a good responder was classified if ΔDAS28 > 1.2, moderate responders were patients with ΔDAS28 ≤ 1.2 and > 0.6. Patients were classified as non-responders if they do not fall into any of these categories.

Following this criteria, most patients were responders to anti-TNF therapy at 6 (84.8%), 12 (87.6%), 18 (83.5%) and 24 months (88.5%).

-174G/C *IL-6* genotyping

For genotyping, cellular DNA was isolated from saliva using standard procedures. The *IL-6* -174G/C (rs1800795) gene promoter single-nucleotide polymorphism (SNP) was genotyped using the TaqMan® allelic discrimination assay technology from Applied Biosystems (Foster City, California, USA) on a LightCycler® 480 Real-time PCR system (Roche Applied Science).

Statistical analysis

Plink (v1.07) (http://pngu.mgh.harvard.edu/purcell/plink/) and StatsDirect v.2.6.6 (StatsDirect Ltd, Cheshire, UK) were used to perform 2x2 contingency tables and χ^2 test and/or Fisher's exact test. Odds ratios (OR) and 95% confidence intervals (CI) were obtained according to Woolf's method (24). The Benjamini & Hochberg (25) step-up false discovery rate (FDR) control correction for multiple testing was applied to the P-values. After correction, P-values lower than 0.05 were considered statistically significant. The analysis of the combined data from our study and the previous report was performed using Plink and StatsDirect. Breslow–Day (BD) test method (26) was used to estimate the homogeneity among populations. Pooled analyses were performed by Mantel-Haenszel test under fixed effects.

Clinical variables previously identified as being independent predictors of efficacy of anti-TNF agents, including age, gender, baseline DAS28, smoking status, rheumatoid factor status, previous and concomitant treatments, and, anticyclic citrullinated protein antibodies (anti-CCP) status, were assessed for association with treatment response. In the multivariate analysis using Plink, only baseline DAS28 was strongly associated with the efficacy of the therapy. Accordingly, analyses were adjusted for DAS28 at baseline.

RESULTS

Demographic and clinical features

A total of 199 RA patients receiving anti-TNF therapy were included. RA patients were aged (mean ± SD) 53.08 ± 14.28 years, 81.9% were female, 48.24% were rheumatoid factor (RF) positive and 78.89% had taken methotrexate/DMARDS. 30.65% were treated with infliximab, 10.5% with etanercept and 58.8% adalimumab. Demographic and clinical features at baseline according to genotype distribution are showed in Table 4.1. There were not significant differences in baseline features.

EULAR response in RA patients

The response to anti-TNF therapy was evaluated at months 6, 12, 18 and 24 after first infusion, according to the EULAR criteria. We consider good and moderate as responders. The EULAR responses were: 84.8% responders (162 out of 191 patients) and 15.18% non-responders (29 out of 191 patients) at 6 months, 87.6% responders (113 out of 129 patients) and 12.4% non-responders (16 out of 129 patients) at 12 months, 83.5% responders (66 out of 79 patients) and 16.45% non-responders (13 out of 79 patients) at 18 months and 88.4% responders (69 out of 78 patients) and 11.53% non-responders (9 out of 78 patients) at 24 months.

Association of *IL-6* polymorphism with response to anti-TNF-therapy

As shown in Table 4.2, when allelic frequencies were compared between responder and non-responder patients, the presence of the *IL-6* -174*G allele was associated with a good or moderated EULAR response at 12 (p_{FDR} = 0.015, OR = 2.93, 95% CI 1.29–6.70), 18 (p_{FDR} = 4.54^{E-03}, OR = 5.17, 95% CI 1.80–14.85) and 24 months (p_{FDR} = 4.54E-03, OR = 14.86, 95% CI 2.91–75.91). At 6 months, the number of patients carrying the -174*G allele was slightly increased in the group of responder patients compared with non-responders, however this association did not reach statistically significance (p = 0.456).

The administered anti-TNF agent did not affect the responder/non-responder status since none of them was associated independently with the response in any of the time points evaluated (data not shown).

Table 4.2: Genotype and allele distribution of the *IL-6 –174G/C* genetic variant in responder and non-responder rheumatoid arthritis patients treated with antitumor necrosis factor therapy

		Genotype, N (%)				Allele test		
	Subgroup (N)	CC	GC	GG	G allele frequency (%)	P-value*†	P_{FDR}**	OR (95% CI)***
6 months	Non-responders (n = 29)	3 (10.34)	13 (44.83)	13 (44.83)	67.24			
	Responders (n = 162)	15 (9.26)	65 (40.12)	82 (50.62)	70.68	0.456	0.456	1.27 (0.68–2.35)
12 months	Non-responders (n = 16)	6 (37.50)	4 (25.00)	6 (37.50)	50			
	Responders (n = 113)	7 (6.19)	48 (42.48)	58 (51.33)	72.57	**0.011**	**0.015**	2.93 (1.29–6.70)
18 months	Non-responders (n = 13)	7 (53.85)	2 (15.38)	4 (30.77)	38.46			
	Responders (n = 66)	4 (6.06)	31 (46.97)	31 (46.97)	70.45	**2.27^{E-03}**	**4.54^{E-03}**	5.17 (1.80–14.85)
24 months	Non-responders (n = 9)	6 (66.67)	2 (22.22)	1 (11.11)	22.22			
	Responders (n = 69)	5 (7.25)	32 (46.38)	32 (46.38)	69.57	**1.18^{E-03}**	**4.54^{E-03}**	14.86 (2.91–75.91)

* All p-values have been calculated for the allelic model.

† Adjusted for DAS28 at baseline; ** Benjamini and Hochberg step-up false discovery rate control; *** Odds ratio for the comparison of the G allele frequency between responder and non-responder patients.

Table 4.3: Meta-analysis of the *IL-6* -174 G/C genetic variant in non-responder and responder RA patients from Spain and Serbia at 6 and 12 months considering responder as patients with DAS28 improvement > 1.2

			Genotype, N (%)				Allele test	
6 months	Population	Subgroup (N)	CC	GC	GG	G allele frequency (%)	P-value*	OR (95% CI)**
	Spain	Non-responders (n = 61)	8 (13.11)	25 (40.98)	28 (45.90)	66,39		
		Responders (n = 130)	10 (7.69)	53 (40.77)	67 (51.54)	71,92	0.127	1.48 (0.89–2.45)
	Serbia (21)	Non-responders (n = 13)	2 (15.38)	8 (61.54)	3 (23.08)	53,85		
		Responders (n = 64)	7 (10.94)	37 (57.81)	20 (31.25)	60,16	0.551	1.30 (0.56–2.99)
	Overall meta-analysis	Non-responders (n = 74)	10 (13.51)	33 (44.59)	31 (41.89)	64,19		
		Responders (n = 194)	17 (8.76)	90 (46.39)	87 (44.85)	68,04	0.252	1.30 (0.86–1.95)
12 months	Population	Subgroup (N)	CC	GC	GG	G allele frequency (%)	P-value*	OR (95% CI)**
	Spain	Non-responders (n = 33)	6 (18.18)	11 (33.33)	16 (48.48)	65,15		
		Responders (n = 96)	7 (7.29)	41 (42.71)	48 (50.00)	71,35	0.069	1.83 (0.95–3.52)
	Serbia (21)	Non-responders (n = 17)	5 (29.41)	11 (64.71)	1 (5.88)	38,24		
		Responders (n = 60)	4 (6.67)	34 (56.67)	22 (36.66)	65,00	**5.10E-03**	3.00 (1.28–7.18)
	Overall meta-analysis	Non-responders (n = 50)	11 (22.00)	22 (44.00)	17 (34.00)	56,00		
		Responders (n = 156)	11 (7.05)	75 (48.08)	70 (44.87)	68,91	**1.89E-02**	1.80 (1.13–2.87)

* All p-values have been calculated for the allelic model.

** Odds ratio for the comparison of the G allele frequency between responder and non-responder patients.

Meta-analysis

Subsequently, as no heterogeneity between the ORs from our study and the previously published report (21) was evident by BD test ($p > 0.05$), a pooled analysis was performed (Table 4.3). Since in Jancic et al. (21) only the patients who had DAS28 improvement > 1.2 were defined as responders, the meta-analysis was performed following this criteria. Overall meta-analysis showed a consistent association between the *IL-6* -174*G allele and anti-TNF treatment response at 12 months ($p_{MH} = 1.89^{E-02}$, OR = 1.80, 95% CI 1.13–2.87) (Table 4.3). Again, no significant differences were found when responder and non-responder patients at 6 months were compared ($p_{MH} = 0.252$).

DISCUSSION

Treatment with anti-TNF agents results in a reduction of disease activity in most RA patients; however, a percentage of patients do not respond to this therapy for unknown reasons. Due to the extremely high costs of anti-TNF therapy and the risk of event adverse, it would be beneficial to predict whether an individual patient will respond to treatment in advance.

Our results confirm the role of the *IL-6* -174G/C polymorphism as a genetic predictor of the response to anti-TNF therapy in RA patients. A similar study (21) was achieved to address the potential influence of the -174G/C *IL-6* gene promoter polymorphism on disease activity and clinical response to etanercept therapy in patients with RA following 6 and 12 months after the initial treatment. According with our results, the authors of this article showed that a higher number of responders were present among patients with the -174GG genotype compared with the patients with the -174GC or CC genotypes (C alleles carriers), suggesting that the -174G/C *IL-6* gene polymorphism may be a genetic marker of responsiveness to etanercept in RA.

The combined analysis of our data and those previously published showed an association between this genetic variant and the clinical response to TNF-α blockers (Table 4.3).

IL-6 has the ability to induce an acute inflammatory reaction and, in the chronic phase, to support the activation of lymphocytes and myeloid cells, which may elevate the serum levels of *IL-6*, leading to increased inflammation. It may, therefore, be responsible for many of the systemic manifestations of RA (27). It has been shown that the neutralisation of the TNF-α results in the suppression of various proinflammatory cytokines, including *IL-6* (28, 29). Functional studies have reported that the -174*C allele is associated with higher serum levels of *IL-6* (15, 16) thus suggesting that increased expression of this cytokine in patients carrying the -174*C allele would result in a poorer response to anti-TNF treatment. In fact,

it has been shown that although both TNF-α and *IL-6* are major targets of therapeutic intervention in RA, baseline serum *IL-6* but not baseline TNF-α level is a potential biomarker reflecting disease activity (30).

According to our data, *-174G/C* was significantly associated with a good or moderated EULAR response at 12, 18 and 24 months, but not at 6 months. Moreover, the larger the treatment period the stronger the observed association signal. This highlights the importance of assessing the response to long-term anti-TNF treatment. This may be the reason that an association between this polymorphism and the clinical efficacy of anti-TNF therapy has not been reported in previous pharmacogenomic studies, most of which did not evaluate the clinical response beyond 6 months of treatment (8, 31-33).

CONCLUSION

The original effect on anti-TNF treatment response caused by the change *IL-6* -174G/C was successfully replicated in an independent population, supporting the role of this polymorphism as a genetic marker predicting anti-TNF treatment outcome.

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