EXTENDED REPORT

IL1 and TNF gene polymorphisms in patients with juvenile idiopathic arthritis treated with TNF inhibitors

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Objective: To investigate the genetic contribution of cytokine gene polymorphisms (interleukin 1 (IL1) and tumour necrosis factor α (TNFα)) on disease phenotype and on response to TNF-blocking agents in a population of patients with juvenile idiopathic arthritis (JIA).

Methods: A cohort of 107 consecutive patients with JIA who were receiving treatment with anti-TNF agents was enrolled in this study. Analysis of genetic polymorphisms for IL1B +3954, IL1RA +2018, TNFα −238 and TNFα −308 was performed by enzyme-linked oligo sorbent assay, and compared with those obtained from 630 healthy Caucasians and 263 adult patients with rheumatoid arthritis. Relevant demographic, clinical and laboratory data were collected from clinical charts and entered into a customised database, and χ^2 analysis was performed to compare cytokine polymorphisms with disease type according to the International League of Associations for Rheumatology criteria, presence of uveitis, rheumatoid factor and anti-nuclear antibody positivity, erosive disease, frequency of adverse effects to anti-TNF and clinical response after 3 months.

Results: The T/T genotype of the IL1B +3954 polymorphism was absent in patients with JIA and present in 5% of controls (p = 0.015). No significant correlation was found between the studied polymorphisms and clinical or laboratory variables considered. Clinical response to TNF inhibitors at 3 months was not associated with the genetic polymorphisms considered.

Conclusion: In our cohort, the absence of the rare IL1B +3954 gene polymorphism was associated with JIA, but without specificity to particular disease phenotypes. The TNF and IL1 gene polymorphism studied did not seem to be associated with response to anti-TNF treatment.

Juvenile idiopathic arthritis (JIA) is characterised by chronic arthritis of unknown aetiology with onset before 16 years of age. Although a large percentage of patients can achieve durable remissions, a substantial proportion will have progressive joint destruction. JIA pathogenesis is complex and includes genetic and environmental factors. Numerous major histocompatibility complex and non-major histocompatibility complex associations have been described; however, different ethnic populations, patient groupings, and systems of nomenclature and classification all impose limitations to direct comparisons of existing studies.[1]

Single nucleotide polymorphisms (SNPs) are single base changes that can occur at any site in the DNA, and some of these SNPs may influence variations in in vitro transcription or protein synthesis, thereby differentially affecting inflammatory processes and disease outcome. Cytokines such as IL1 and TNFα are central mediators of joint inflammation in patients with JIA, and different polymorphisms of these and other cytokines have already been studied in patients with JIA.[2–4]

The availability of biological agents such as TNF inhibitors for children has substantially improved the prognosis and quality of life of paediatric patients with JIA, even after failure with conventional second-line treatments, but the response to treatment is not constant. The aim of the present study was to investigate possible genetic contributions of selected cytokine polymorphisms (IL1 and TNFα) on clinical response to TNF-blocking agents in a population of patients with JIA. Moreover, possible associations with particular JIA phenotypes were investigated.

MATERIALS AND METHODS

Patients

A total of 107 patients with JIA who were receiving treatment with anti-TNF agents in three tertiary care paediatric rheumatology centres (Edouard Herriot, Lyon, France; Gaetano Pini Hospital, Milan, Italy; and Department of Pediatrics, University of Padova, Padova, Italy) during 2005 were enrolled. All were classified according to the revised International League of Associations for Rheumatology (ILAR) criteria for JIA.[5] Parameters recorded included age, sex, disease category, disease duration, presence of extra-articular manifestations (namely, chronic uveitis) and treatment received. Biological data included erythrocyte sedimentation rate, rheumatoid factor, antinuclear antibodies (indirect immunofluorescence on Hep2 cells, positivity >1:80), and IL1 and TNFα polymorphisms. Joint damage was evaluated by plain *x* rays, and radiographic erosions were classified in a dichotomous manner as either present or absent. Type and duration of anti-TNF treatment, as well as possible side effects, was also recorded.

Infliximab therapy was given at the dosage of 3 mg/kg of body weight according to a standardised protocol,[6] etanercept was given at the dosage of 0.4 mg/kg subcutaneously twice a week and adalimumab was given at the dosage of 24 mg/m^2 subcutaneously on alternate weeks. Clinical response to anti-TNF was assessed after 3 months of therapy according to the ACR Pedi 30 (American College of Rheumatology Pediatric 30%).[7]

A cohort of 263 adult patients with rheumatoid arthritis (RA) followed up in Lyon was also studied, and the results were compared with those obtained from patients with JIA. The same genotyping was performed in 630 adult blood donors of French origin, who acted as a control group.

Abbreviations: IL, interleukin; ILAR, International League of Associations for Rheumatology; JIA, juvenile idiopathic arthritis; RA, rheumatoid arthritis; SNP, single nucleotide polymorphism; TNFα, tumour necrosis factor α

Informed consent was obtained from parent or guardian, or by the patients if they are aged >18 years. The protocol was approved by the committee for protection of persons participating in biomedical research and by the ethical committees of the participating hospitals.

Polymorphism and gene typing

Uncoagulated blood was taken from patients during routine venipuncture, and stored frozen at −20°C until DNA extraction. DNA was extracted using the QIAamp DNA Blood Mini Kit (Qiagen GmbH, Hilden, Germany), as recommended by the manufacturer. A multiplex PCR amplification was performed using three sets of primers to amplify a 245 bp sequence of the IL1β gene, a 230 bp sequence of the IL1Ra gene and a 374 bp sequence of the TNFα gene. PCR primers were as follows. IL1β gene: 5′ TTCAGTTCATATGGACCAGA 3′; 5′ GTTGTCATCAGA CTTTGACC 3′. IL1Ra gene: 5′ GGGCACATGGTGGCTGTGCA 3′; 5′ ACCTAGGGTTTGTGCAGGCA 3′. TNFα gene: 5′ TCAACGG ACTCA GCTTTCTGAA 3′; 5′ CGGAAAACTTCCTTGGTGGAG 3′. PCRs were performed in 100 µl reaction volume containing 100 ng of genomic DNA, 0.24 µM of deoxyribonucleotide triphosphates (dNTPs), IL1β and IL1Ra primers at 0.36 µM each, TNFα primers at 0.54 µM each and 2.5 units of Taq DNA polymerase (Perkin Elmer, Waltham, Massachusetts, USA) in a Bio-Rad thermal cycler (Bio-Rad Laboratories, Hercules, California, USA). PCR cycles were as follows: (95°C, 2 min) ×1; (94°C, 30 s; 55°C, 30 s; 68°C, 1 min) ×40; and (68°C, 10 min) ×1. The amplicon products of multiplex PCR were genotyped by enzyme-linked oligo sorbent assay as previously described.[8]

Statistical analysis

Relevant demographic, clinical and laboratory data were collected from clinical charts and entered into a customised Excel database. We performed χ^2 analysis to compare cytokine polymorphisms with disease type (ILAR criteria), presence of uveitis, rheumatoid factor and anti-nuclear antibody positivity, radiographic erosive changes, frequency of adverse effects to anti-TNF and clinical response after 3 months (ACR Pedi 30). p Values <0.05 were considered significant. Both genotypic frequency (the frequency of a genotype in that population) and allelic frequency (the frequency with which a particular allele appears among the possible alleles in that population) were considered in the statistical comparisons.

RESULTS

Characteristics of the patient population

A total of 107 patients with JIA (83 females, 24 males) were included in the study, almost all (n = 103) were of Caucasian origin. Mean (SD) age at disease onset was 6.5 (4.9) years (range, 1 month–15 years), while mean age at onset of anti-TNF treatment was 16.3 (8.1) years (range, 3–43 years). The Gaetano Pini Hospital is a referral centre that includes an adult rheumatology and a paediatric rheumatology unit, therefore some patients with JIA who were diagnosed in childhood but received anti-TNF treatment during adulthood in the same centre were also included in this study. According to the ILAR classification criteria, diagnoses were: systemic arthritis (n = 29); persistent oligoarthritis (n = 4); extended oligoarthritis (n = 27); rheumatoid factor-negative polyarthritis (n = 24); rheumatoid factor-positive polyarthritis (n = 5); psoriatic arthritis (n = 6); and enthesitis-related arthritis (n = 12). All patients had active disease despite being treated with disease-modifying antirheumatic drugs (notably, by definition, they all had to have failed methotrexate treatment) before initiation of anti-TNF therapy. In 16 cases, the disease course had been complicated by the presence of chronic uveitis. Across patients, 71 had received etanercept, 34 infliximab and 2 adalimumab. Table 1 summarises the general demographic and clinical features of the study patients, in total, and subdivided by ILAR classification.

Response to anti-TNF treatment after 3 months was positive in 84/106 (79%) evaluable patients. It was not possible to evaluate the response according to standardised criteria for only one patient (lost to follow-up). There was no statistically significant difference in response rates to the different anti-TNF used (data not shown), nor any significant difference in response among the different disease diagnoses.

IL1 and TNF gene polymorphisms and disease phenotype

The results of cytokine polymorphisms on our patients were as follows: for IL1b +3954: C/C 62 (58%), C/T 45 (42%), T/T 0 (0%); for IL1RA +2018: C/C 8 (7%), C/T 50 (47%), T/T 49 (46%); for TNFα –238: G/G 95 (90%), G/A 10 (9%), A/A 1 (1%); and for TNFα –308: G/G 86 (81%), G/A 19 (18%), A/A 1 (1%).

When the SNPs considered were compared among patients with JIA and healthy controls, we found that the T/T genotype of the IL1B +3954 polymorphism was absent in our study population and present in 5% of controls; this difference was significant (p = 0.015; fig 1). The other SNPs considered did not differ in patients and controls.

In addition, we compared these results with data obtained from adult patients with RA followed up in Lyon, and found that the prevalence of the A allele of the –238 TNF polymorphism was 6% in those with JIA and 3% in those with RA (p = 0.046; fig 1). All other allelic frequencies, and all genotypic frequencies for the different polymorphisms were not statistically different between JIA and RA (data not shown).

With regard to our patients, no link was observed between the TNF and IL1 SNPs and the selected clinical variables (data not shown).

IL1 and TNF gene polymorphisms and response to TNF inhibitors

When we compared the SNPs with clinical response after 3 months, we were not able to find significant differences both for allelic and genotypic frequencies between responders and non-responders (fig 2).

DISCUSSION

Genetic contribution in the susceptibility for JIA is well established, and cytokine gene polymorphisms have been studied by several groups. We focused on polymorphisms in the IL1B gene (at +3954), in the IL1-RN gene (at +2018) and in the TNFα promoter (at –238 and –308), as they had been already studied in patients with RA, and previously described as severity markers.[9] We were able to find a statistical difference between our patient group and healthy blood donors with regard to the IL1B +3954 genotype, in that the T/T genotype was not present in any of our cases.

Most of the studies on cytokine gene polymorphisms in JIA were limited to the study of one or two cytokines, with only few comprehensive studies being performed.[2–4] In our study, we did not find any association between the SNPs that we analysed and disease phenotype or severity; however, our study population was, by definition, already selected, in that all patients were receiving anti-TNF treatment, and therefore already had a severe and progressive disease. A direct comparison of our result with a previous series is therefore not possible. Moreover, it is widely acknowledged that JIA is not a single disease but a term that encompasses several categories, now often represented within the ILAR classification. This is of interest as it is known that pathogenesis and response to treatment might

Table 1 General demographic and clinical features of the study patients, in the whole group and subdivided by International League of Associations for Rheumatology classification

	JIA	SoJIA	PO	ExO	RFneg	RFpos	PsA	ERA
N° patients	107	29	4	27	24	5	6	12
Male	24	10	0	2	3	1	1	7
Female	83	19	4	25	21	4	5	5
Disease duration at onset anti-TNF therapy (Mean (SD), years	9.8 (8.7)	9.6 (8.1)	5 (2.8)	11.3 (8)	8.7 (7.2)	6.1 (4)	20 (9.7)	7.2 (5)
Anti-TNF type								
Infliximab	34	8	1	8	6	3	5	3
Etanercept	71	19	3	19	18	2	1	9
Adalimumab	2	2	0	0	0	0	0	0
Uveitis								
Yes	16	1	3	7	2	0	2	1
No	91	28	1	20	22	5	4	11
Erosions								
Yes	49	17	1	12	9	2	5	3
No	30	5	2	13	8	1	1	0
Complications of anti-TNF treatment								
Yes	28	11	1	6	3	2	4	1
No	77	18	2	20	21	3	2	11
Response to anti-TNF at 3 months								
Yes	84	19	3	25	19	3	6	9
No	22	10	1	2	4	2	0	3
Not applicable	1	0	0	0	1	0	0	0

ERA, enthesitis-related arthritis; ExO, extended oligoarthritis; JIA, juvenile idiopathic arthritis (total); PO, persistent oligoarthritis; PsA, psoriatic arthritis; RFneg, polyarthritis RF-negative; RFpos, polyarthritis RF-positive; SoJIA, systemic arthritis.
There was no patient with undifferentiated arthritis. Erosions have been evaluated in 79 patients, complications in 105 and clinical response in 106.

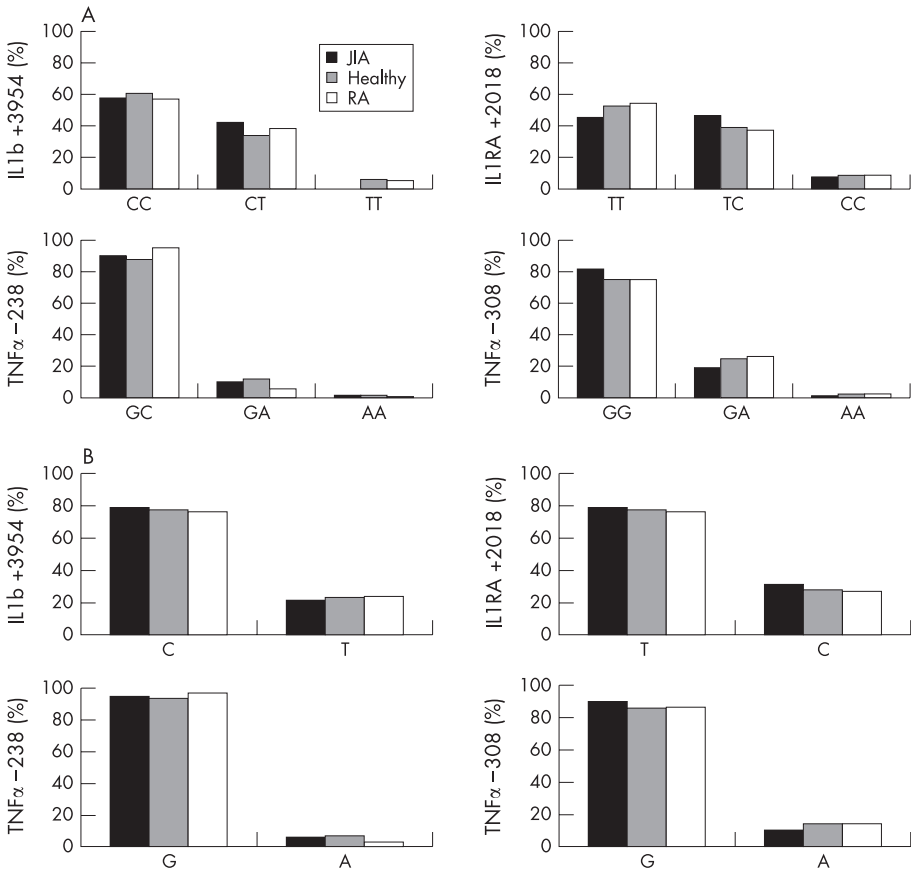

Figure 1 Distribution of frequencies of selected interleukin (IL) 1 and tumour necrosis factor α (TNFα) gene polymorphisms in patients with juvenile idiopathic arthritis (JIA), rheumatoid arthritis (RA) and healthy controls. (A) Genotypic frequencies: difference for IL1b +3954 polymorphism between patients with JIA and controls ($p=0.015$). (B) Allelic frequencies: difference for TNFα −238 polymorphism between patients with JIA and those with RA ($p=0.046$).

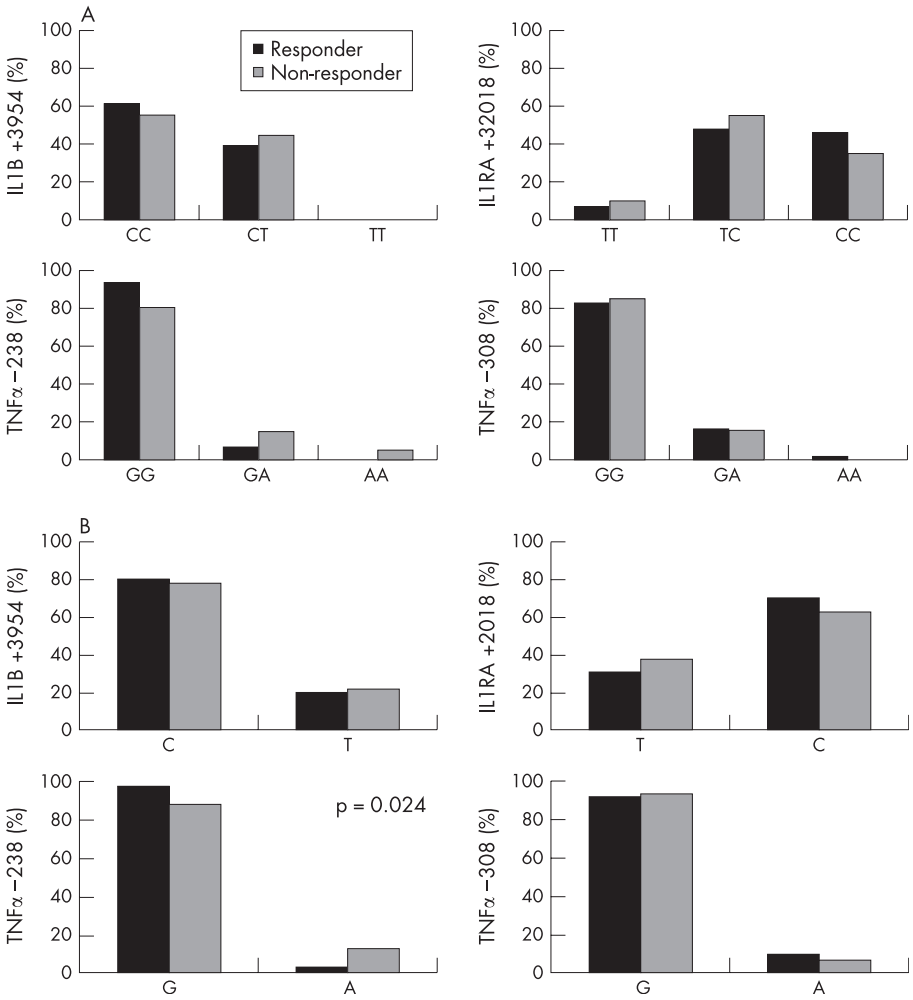

Figure 2 (A) Comparison of genotypic frequencies of selected IL1 and TNFα gene polymorphisms with clinical response to anti-TNF agents. (B) Comparison of allelic frequencies of selected interleukin (IL) 1 and tumour necrosis factor α (TNFα) gene polymorphisms with clinical response to anti-TNF agents.

differ among these groups; in particular, it has been recently shown that systemic-onset disease might be mediated by IL1 and might respond to IL1 blockage.[10] However, in our series, even if there was a trend towards a difference in clinical response to anti-TNF between patients with systemic-onset JIA and those with other JIAs, this did not reach significance ($p = 0.06$), maybe because of an insufficient sample size.

The use of therapies targeting TNF has remarkably improved the prognosis of JIA; however, clinical responses to TNF inhibitors are variable among patients.[11] There is no evidence that simple determination of plasma TNFα levels by ELISA allows such prediction for treatment with TNFα inhibitors.[12] However, recent results from our laboratory have indicated that, using a functional cell-based assay, it is possible to link the response to TNF inhibitor infliximab to the functional circulating level of TNF activity in plasma, with patients with high circulating levels of TNF activity being more susceptible to respond to TNF inhibition.[12]

Therefore, the contribution of cytokine polymorphisms to the response to anti-TNF is of interest, but to date no disease-related features, patient characteristics, genetic associations or other factors that reliably correlate with treatment outcome have been identified.[13] We were not able to find a link between any of the selected cytokine SNPs (in particular, a polymorphism at the TNF locus) and clinical response to anti-TNF. All of our patients, despite different onset types, had a severe and polyarticular disease, and were therefore grouped together for this particular analysis. In a previous study from our hospital on patients with RA,[8] no association between the same SNPs and response to anti-TNF was found. In a study from another French group[14] performed on 59 patients with RA, those carrying the rare allele A were two times more likely to have no response to infliximab than those with the common G/G genotype of the –308 TNFα polymorphism. In another cohort of 110 patients with RA treated with infliximab, a relationship was detected between ACR criteria of improvement and increased circulating TNFα levels, but not between TNFα promoter SNPs and clinical response.[15] Also, Criswell *et al*[16] showed that an extended haplotype, which included HLA-DRB1 alleles and SNPs in the lymphotoxin TNFα region (including those at position –238 and –308), was associated with the response to treatment with methotrexate or etanercept, even if the precise genetic markers on this extended haplotype and their mechanism of action related to RA treatment response remained to be defined.

Perhaps not single SNPs but rather a combination of them might be more useful in predicting a clinical response, such as those seen in the study by Padyukov *et al*[17] who found that TNF and IL10 SNPs did predict clinical response to etanercept only when the combined genotype was considered. Finally, there are several polymorphic sites within the TNF locus, and preliminary results suggest that other TNF SNPs may predict clinical response to etanercept in RA.[18]

TNFα gene polymorphisms have already been studied in JIA,[19] but to our knowledge there is only one other published study focusing on cytokine SNPs and response to anti-TNF in

JIA.[20] We acknowledge some limits of our study, such as heterogeneity of study population in terms of disease onset type. However, we have selected only patients who had a polyarticular course and who had symptoms severe enough to receive anti-TNF treatment. Moreover, all our healthy controls were of French and not Italian origin; however, the statistical difference between patients and controls was not influenced by the ethnic origin of the patients (data not shown).

In summary, our study showed that the absence of the rare IL1B +3954 gene polymorphism was associated with JIA, but without specificity to particular disease phenotypes. The studied TNF and IL1 gene polymorphisms did not seem to be associated with the response to anti-TNF treatment. We are currently studying other genetic and biological factors that might predict the clinical response to anti-TNF, in order to be able to tailor specific treatments based on individual patient profile.

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