

Tumor necrosis factor alpha promoter polymorphisms in patients with juvenile idiopathic arthritis

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Abstract

Objective

To investigate the potential association of tumor necrosis factor- α (TNF) promoter alleles within subtypes of juvenile idiopathic arthritis (JIA) compared to healthy controls in a Caucasian population.

Methods

TNF- α promoter polymorphisms at positions -163, -238, -244, -308, -376 were determined in 228 patients with JIA and 196 healthy individuals. Genomic DNA was isolated and a PCR fragment of about 500 base pairs of the TNF gene promoter were amplified by PCR. Detection of polymorphisms was achieved by a single sequencing procedure.

Results

The TNF -238A allele was more frequent in the psoriatic arthritis JIA subgroup compared to healthy controls as well as to non-psoriatic JIA patients ($p < 0.001$, chi-square-test) and was associated with the more frequent occurrence of joint erosion ($p < 0.05$, chi-square-test). The frequency of the TNF -308A allele was significantly lower in patients with rheumatoid factor negative polyarthritis JIA patients compared to healthy controls, respectively ($p < 0.05$, chi-square-test). Joint erosions were detectable more often in rheumatoid factor negative polyarthritis JIA patients with the G/A genotype (80%) than in those with the G/G genotype (45%) ($p = 0.20$). The rare alleles at position -376 or at positions -163 and -244 were found very infrequently.

Conclusion

TNF promoter polymorphisms may play a role in the pathogenesis of JIA. The TNF-238A allele seems to be associated with juvenile psoriatic arthritis. The TNF-308A allele is less frequently found in rheumatoid factor negative but not in rheumatoid factor positive polyarthritis and may therefore be associated with a more severe disease, while the more common TNF-308G allele may be protective.

Key words

Juvenile idiopathic arthritis, TNF alpha promoter polymorphisms.

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Introduction

Juvenile idiopathic arthritis (JIA) is the most common systemic and chronic autoimmune disease occurring in childhood with an incidence of 10-20 per 100,000 children below the age of 16 years (1, 2). According to the International League Against Rheumatism (ILAR) classification, patients can be classified in seven subgroups depending on the number of affected joints during onset and course of the disease and the presence of extraarticular manifestations (3).

JIA is a complex disease of unknown aetiology. Certain genetic factors acting in a concert are believed to predispose the host to the development of JIA (4, 5). Linkage studies and association studies have been carried out to delineate the factors involved in various rheumatic diseases. The attempt to dissect the genetic basis of JIA has primarily revealed disease associations with the major histocompatibility complex (HLA) loci (6-8). Further molecules, however, have also been implicated in the aetiopathogenesis of JIA both through genetic and serological studies (9-15). TNF- α , a polypeptide cytokine, is a potent molecule that stimulates the production of many other cytokines, including IL1 β , IL6, GM-CSF, IL8, and secretion of degradatory molecules including several metalloproteinases. TNF- α mediates the cytokine cascade that causes inflammation possibly leading to joint destruction in JIA. The level of TNF- α in the serum and synovial fluid of JIA patients has been shown to fluctuate with disease activity (16, 17). Several single nucleotide polymorphisms (SNPs) have been noted in the TNF promoter (18). Some reports have shown that production of TNF- α is influenced by these TNF promoter polymorphisms; for example cells of patients with TNF- α G/A heterozygosity at position -308 showed an increased *in vitro* production of TNF- α (19-21). Polymorphisms in the promoter region of the TNF- α gene has been associated with a number of autoimmune disease, including systemic lupus erythematosus (22-24). Rood *et al.* have shown that both the TNF -308 A/A and the -308 G/A genotype occur-

red at a higher frequency in SLE patients than in controls. Their study also showed that the TNF -308A allele was a susceptibility factor for SLE, and this effect was independent of HLA-DR3 (25).

The aim of our study was to investigate the potential association of tumor necrosis factor- α (TNF) promoter alleles within subtypes of juvenile idiopathic arthritis in a Caucasian population.

Methods

Patients

TNF- α promoter polymorphisms at positions -163, -238, -244, -308, -376 were determined in 228 German Caucasian JIA patients (systemic arthritis 23, rheumatoid factor negative polyarthritis 62, rheumatoid factor positive polyarthritis 14, persistent oligoarthritis 38 and extended oligoarthritis 28, enthesitis related arthritis 37, psoriatic arthritis 13, undifferentiated arthritis 13). Patients were selected from 11 pediatric rheumatology departments in Germany. Diagnosis of JIA was performed according to the ILAR classification criteria for JIA (3) by a pediatric rheumatologist, certified by the German Society of Pediatric and Adolescent Rheumatology. Written informed consent was obtained from all patients and parents from whom data were collected.

Clinical characteristics such as gender (139 females, 89 males), age of onset, disease duration, presence of ANA, rheumatoid factor, HLA-DR4 or HLA-B27 were determined. The clinical course of disease was evaluated, laboratory parameters C-reactive protein (CRP) and the erythrocyte sedimentation rate (ESR), the use of disease-modifying anti-rheumatic drugs (DMARDs) and the number of DMARDs used, the presence of joint erosion and the history of uveitis. The means of all determined ESR and CRP values over the period of the disease were calculated. Remission of disease was defined by the absence of morning stiffness, tender and swollen joints in presence of normal ESR (below 20 mm/h) and normal CRP (below 6 mg/l) as defined in reference 26. 196 healthy

German Caucasian blood donors of the same geographic and ethnic origin without history of rheumatic diseases were used as controls.

The study has been approved by the ethics committee of the Martin-Luther University Halle-Wittenberg.

DNA analysis

Genomic DNA was extracted from peripheral blood samples using the QIAamp DNA Blood Mini Kit (QIAGEN) following the manufacturer's protocol. A PCR fragment of about 500 base pairs of the TNF gene promoter was amplified with the forward primer: 5' CAAACACAGGCCTCAGGACTC 3' and the reverse primer: 5' AGGGAG CGTCTGCTGGCTG 3'. PCR was performed with 300 ng DNA in 50 µl reactions containing 1.5 U Taq polymerase, 50 pmol of each primer, 0.5 mM of each dNTP, 2 mM MgCl₂. The PCR conditions were follows: denaturation at 94°C for 10 min, then 30 cycles for denaturation at 94°C for 1 min, annealing at 64°C for 1 min and extension at 72°C for 1 min, followed by final extension at 72°C for 10 min. PCR products (single bands) were purified after gel separation on 2.0% agarose gel using the MinElute Gel Extraction Kit (QIAGEN). PCR fragments (100 mg) were sequenced directly following the cycle sequencing procedure (BigDye Terminator Cycle Sequencing Ready Reaction Kit, Applied Biosystems) in a volume of 10 µl by using the same primer (0.5 µM). Cycling parameters were follows: 25 cycles of denaturation at 94°C for 10 sec, annealing at 60°C for 4 min. The cycle sequencing products were purified by ethanol precipitation and then separated and analysed on an automatic sequencing analyser (ABI-PRISM-310, Perkin Elmer).

Statistical analysis

The allele frequencies of each SNP were compared between cases and controls using the chi-square test (http://schnoodles.com/cgi-bin/web_chi_form.cgi).

Results

The genotype –238G/G was present in 213 JIA patients while the genotype

–238A/A was not found in any patient. 15 patients were heterozygous. Thus, the A allele frequency in the total patient group was 3.3%, which was not different from the A allele frequency of healthy blood donors (Table I).

However, the TNF–238A allele was found more frequently in the psoriatic arthritis JIA subgroup compared to healthy controls as well as to non-psoriatic arthritis JIA patients ($p < 0.001$). Moreover, joint erosions occurred more often in psoriatic arthritis patients with a TNF–238A allele than without ($p < 0.05$).

None of the patients within the enthesitis related arthritis subtype group ($n = 37$) presented the –238G/A genotype, all were –238 G/G ($p = 0.10$ compared to controls). In the group of JIA patients with the –238G/G genotype HLA-B27 was insignificantly more frequent than in –238G/A patients (35% vs. 14%, $p > 0.05$). Therefore, it could be speculated that this particular combination of alleles plays a role in the susceptibility for the enthesitis related arthritis subtype.

Since a number of JIA patients reached a remission of their disease, either spontaneously or induced by intensive combination pharmacotherapy, it was interesting to investigate the TNF-pro-

motor polymorphisms in those patients. Upon anti-TNF therapy suppression of signs and symptoms of the disease is seen in the vast majority of polyarticular JIA patients. Patients treated with TNF-antagonists were therefore analysed separately. In patients without anti-TNF therapy who demonstrated the –238G/G genotype a remission was insignificantly more frequent than in patients with the –238G/A genotype (31% vs. 13%, $p = 0.20$). Therefore more patients with the –238G/A genotype showed ongoing active disease and did receive treatment with the TNF antagonist etanercept (73% versus 51%, $p = 0.10$). After institution of etanercept the rate of remission reached by therapy was equal in patients regardless of the genotype.

The mean serum level of CRP over time was insignificantly higher in patients with the –238G/G genotype (10.1 mg/l vs. 6.9 mg/l) than in patients with the –238G/A genotype. No influence was found regarding the ESR.

Uveitis occurred in 21 patients (9.9%) with the –238G/G genotype and in none of the patients with the –238G/A genotype. However, this difference was not statistically significant.

The genotype –308G/G was present in 169 patients and the –308A/A genotype

Table I. TNF- α promoter –238 and –308 polymorphism allele frequency in JIA subgroups and controls.

Patients and controls	Allele frequency (%) –238		Allele frequency (%) –308	
	G	A	G	A
Systemic arthritis ($n = 23$)	95.7	4.3	82.6	17.4
Rheumatoid factor negative polyarthritis ($n = 62$)	96.8	3.2	91.1	8.9 ^s
Rheumatoid factor positive polyarthritis ($n = 14$)	96.4	3.6	82.1	17.9
Persistent oligoarthritis ($n = 38$)	97.4	2.6	84.2	15.8
Extended oligoarthritis ($n = 28$)	100	0	87.5	12.5
Enthesitis related arthritis ($n = 37$)	100	0	83.8	16.2
Psoriatic arthritis ($n = 13$)	84.6	15.4* [#]	89.5	11.5
Undifferentiated arthritis ($n = 13$)	92.3	7.7	80.8	19.2
Total JIA patients ($n = 228$)	96.7	3.3 [#]	86.2	13.8
Controls ($n = 196$)	97.4	2.6*	82.9	17.1 ^s

The frequency of the A –238 allele was significantly higher in the psoriatic arthritis subgroup compared to controls* and to the entire JIA patient group[#] ($p < 0.001$, chi-square-test). The frequency of the A –308 allele was significantly lower in the rheumatoid factor negative polyarthritis subgroup compared to controls^s ($p < 0.05$, chi-square-test).

was present in 4 patients. 55 patients were heterozygous. The A allele frequency therefore was 13.8%. In the healthy control group the genotype -308G/G was present in 132 persons and the -308A/A in 3 persons. 61 persons were heterozygous. The A allele frequency was 17.1%. Therefore, the presence of the A allele in JIA patients is insignificantly lower ($p=0.20$) (Table I). Heterozygosity of both, the -238G/A and the -308G/A genotype was found in two control persons and in none of the JIA patients.

In patients with rheumatoid factor negative polyarthritis the frequency of TNF-308A was significantly lower than in healthy controls (8.9% vs. 17.1%, $p < 0.05$) or in rheumatoid factor positive polyarthritis (17.9%) (Table I). Joint erosions seem to occur more frequently in rheumatoid factor negative polyarthritis patients with the genotype G/A (80%) than in those with the genotype G/G (45%) ($p = 0.20$).

Uveitis occurred in 18 patients (11%) demonstrating the -308G/G genotype and in 3 patients (5%) with the -308G/A genotype ($p > 0.05$).

None of the JIA patients and only two controls had the rare allele at position -376 and no patient or control had the rare allele at position -163 and -244.

There was no relationship between the several promoter polymorphisms and gender, age of onset, disease duration or presence of ANA.

Discussion

According to the present study TNF polymorphisms may play a role in the pathogenesis of at least distinct JIA subgroups, particularly in the juvenile psoriatic arthritis.

The TNF-238A allele seems to be associated with juvenile psoriatic arthritis since it was found more frequently in these patients compared to healthy controls as well as to non-psoriatic arthritis JIA patients. In addition, joint erosion occurred more often in patients with the presence of the TNF-238A allele.

Until now there were no studies of TNF polymorphisms in JIA-patients with psoriatic arthritis. But in agreement with our study in adult patients with psoriasis the -238G/A genotype was

significantly more frequent than in controls (27). Hohler *et al.* found the -238A allele in 20 of 62 patients (32%, $p < 0.03$) with psoriatic arthritis and in 23 of 60 patients with juvenile onset psoriasis (38%, $p < 0.008$), compared with seven of 99 (7%) Caucasian controls. These data demonstrate a significant association of the -238A allele to psoriasis as well as to psoriatic arthritis (28). This findings suggest that the -238A allele predispose to the development of psoriasis in Caucasian patients, whereas in a Japanese patient group with psoriasis no association to the -238 and -308 polymorphisms was found (29).

In our patients the rare TNF-308A allele was less frequently found in rheumatoid factor negative but not in rheumatoid factor positive polyarthritis and may therefore be associated with disease severity, while the common TNF-308G allele may be protective. Moreover, joint erosions occurred more often in rheumatoid factor negative polyarthritis JIA patients with the genotype -308 G/A (80%) than in those with the genotype -308 G/G (45%).

The association to the destructive behaviour may be due to higher levels of TNF- α production associated with this particular allele. Indeed, high TNF levels have been associated with more severe disease in JIA (16, 17).

In a study of Turkish ($n = 51$) and Czech patients ($n = 159$) no aberrant distribution of genotypes of the -308 and -238 polymorphisms was found in the several subtypes of JIA. However, there were no patients with psoriatic arthritis included. In concordance to our observation, the TNF-308A allele was significantly associated with a poor outcome in the Turkish group ($p = 0.005$) but there was no association in the Czech patients (30). Currently, there is no explanation for this. In a Japanese population (systemic type $n = 50$, oligoarticular type $n = 29$, polyarticular type $n = 32$) an association of TNF promoter SNPs -1031, -863, and -857 with systemic JIA, but not with other subtypes of JIA was shown. No association was found to the -308 and -238 polymorphisms. A positive association was found between DRB1*0405 and systemic JIA. This is uncom-

mon in Caucasian populations (31). Interestingly, in Japan more than 50% of the JIA patients have a systemic type whereas in Caucasian population the frequency of this subtype is only 10%. These observations indicate the influence of geographic and ethnic origin. In agreement with our study, Eppelen *et al.* observed no difference in the frequencies of the -308 and -238 TNF SNP alleles between German patients with juvenile oligoarthritis and controls (32). It is interesting to note that Zeggini examined TNF SNPs in juvenile oligoarthritis and showed that the promoter SNP alleles -238G ($p = 0.032$), -308A ($p = 0.007$) and the intronic SNP alleles +489A ($p = 0.021$) and +851A ($p = 0.024$) were all associated with the juvenile oligoarthritis (33). Further JIA subtypes were not investigated.

In summary, some of the published data are in agreement to our observations, others are not. Etanercept, a soluble tumor necrosis factor (TNF) receptor fusion protein, has proven efficacious for treatment of joint inflammation in children with polyarticular JIA (34). No relationship was found between the several TNF promoter gene SNPs and the occurrence of clinical efficacy of etanercept therapy. Some studies on adult patients with rheumatoid arthritis demonstrate an influence of TNF promoter polymorphisms and clinical or radiological outcome, other did not (35-43).

In conclusion, TNF-polymorphisms seem to be associated with several JIA subgroups and to the course of the disease. The complex genetic mechanisms that predispose to autoimmune diseases have not yet been elucidated, it appears that some of these genetic variants in combination with other genes confer increased susceptibility to autoimmunity. That these allelic variants act in combination explains both the difficulty in mapping them and the observation that autoimmune diseases are not inherited in a simple mendelian way.

The incidence and prevalence of JIA varies substantially among different ethnic and geographically distinct populations throughout the world. Genetically determined differences in disease

susceptibility may readily account for such interethnic variation.

However, the results are preliminary and will need confirmation by further observation since the total number of patients in several JIA subgroups was low.

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