Rheumatology 2005;44:1115–1121 doi:10.1093/rheumatology/keh689
Advance Access publication 18 May 2005

Cytokine genotypes correlate with pain and radiologically defined joint damage in patients with juvenile rheumatoid arthritis

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Objectives. **Single nucleotide polymorphisms (SNPs) in cytokine genes have been associated with risk of a number of autoimmune diseases. Moreover, some SNPs are associated with variations in rates of *in vitro* gene expression, and it is therefore possible that these functional polymorphisms may differentially affect inflammatory processes and disease outcome. This project's objective was to determine whether cytokine genotypes correlate with disease outcomes in patients with juvenile rheumatoid arthritis (JRA).**

Methods. **Genotypes of SNPs of pro-inflammatory cytokines, tumour necrosis factor-α −308G→A, interleukin-6 (IL-6) −174G→C and interferon-gamma +874G→A, and anti-inflammatory, immunosuppressive cytokines, interleukin-10 −1082G→A, −819C→T and −592A→C and transforming growth factor-β1 (TGF-β1) codon 10T→C and codon 25G→C, were determined for patients with JRA who previously participated in a long-term outcome study. Cytokine genotypes and clinical variables showing significant correlations with clinical outcomes at the $\alpha = 0.100$ level in univariate analyses were entered in multivariate tests.**

Results. **In multivariate tests, the IL-6 genotype −174G/G was positively correlated with pain [regression coefficient $B = 0.899$, 95% confidence intervals (CI) 0.185, 1.612, $P = 0.014$]. The homozygous TGF-β1 codon 25G/G genotype showed a protective effect against joint space narrowing on radiographs taken within 2 yr of disease onset, but confidence intervals were wide [odds ratio (OR) 0.176, 95% CI 0.037, 0.837 $P = 0.029$].**

Conclusions. **The correlation of IL-6 genotype with pain and the possible association of the TGF-β1 codon 25 genotype with short-term radiographic damage (G/C with greater risk and G/G with decreased risk) suggests that both these polymorphisms may be useful early prognostic indicators. Further studies of the relation between cytokine genotypes and outcomes in patients with all forms of juvenile idiopathic arthritis (JIA) are warranted.**

KEY WORDS: Juvenile idiopathic arthritis, Juvenile rheumatoid arthritis, Cytokine genes, Long-term outcomes.

Many patients with juvenile idiopathic arthritis (JIA) may have persistent disease and long-term disability [1–3]. Predictors of poor outcomes that are present at, or soon after, diagnosis would be valuable in determining prognosis and guiding treatment. As genetic polymorphisms are constant, they might be ideal prognosticators of long-term outcomes. To date immunogenetic analyses in JIA have concentrated on the major histocompatibility locus, while fewer studies have examined polymorphisms at other loci.

In JIA an excess of pro-inflammatory cytokines in synovial fluid or peripheral blood might be important in perpetuating chronic inflammatory disease [4–6]. Levels of such mediators are partly genetically controlled. For example, certain functional cytokine gene polymorphisms are associated with differential rates of gene transcription or protein synthesis *in vitro*, although some associations have been controversial [7–10]. Arguably differential transcription rates may heighten inflammatory responses and increase disease risk, or conversely protect against disease occurrence. Similarly, some cytokine genotypes may affect disease severity. Associations of interleukin-6 (IL-6) single nucleotide polymorphisms (SNPs) and systemic JIA have already been reported [9, 11], and IL-10 SNPs may differentiate patients with extended and persistent oligoarthritis [8].

To facilitate early identification of patients with potentially poor outcomes we sought to determine whether cytokine gene polymorphisms correlate with JIA outcomes. In the present study we assayed pro- and anti-inflammatory cytokine SNPs in a subset of patients from our earlier study and analysed associations with previously determined outcomes [1]. Clinical outcomes and correlations between clinical variables and functional outcome, pain and radiological outcome have been previously published [1, 12–14]. Since patients were diagnosed by the 1977 American College of Rheumatology (ACR) criteria [15], we have used the JRA nomenclature when referring to this cohort. Corresponding diagnoses for these patients in the International League Against Rheumatism (ILAR) classification of JIA are systemic, oligoarticular, polyarthritis rheumatoid factor negative and polyarthritis rheumatoid factor positive [16].

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Submitted 24 January 2005; revised version accepted 26 April 2005.

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Methods

Patients

Participants in a previous long-term outcome study were re-contacted to obtain consent for analysis of stored blood samples collected during the preceding study [1]. Criteria for inclusion in the original study were a diagnosis of JRA by 1977 ACR criteria [15], an interval of ≥5 yr since onset, and an age of ≥8 yr at the time of study. Patients were diagnosed and followed at British Columbia's Children's Hospital in Vancouver, Royal University Hospital in Saskatoon or the Children's Hospital in Winnipeg. Blood samples had been collected from 282 of the 392 participants in the original study. Of these 180 consented to the present studies and the remainder could not be contacted or did not reply. Seventeen additional patients were recruited using the same selection criteria and study protocol. Sixteen blood samples could not be found or were in an unsuitable condition leaving a total of 181 samples which were analysed.

Written informed consent was obtained from all participants and/or their parents or guardians according to the Declaration of Helsinki. Studies were approved by the Clinical Review Ethics Board at the University of British Columbia, the Biomedical Research Ethics Board at the University of Saskatchewan and the Health Research Ethics Board at the University of Manitoba.

The following outcome measures were obtained during the original study: the Childhood Health Assessment Questionnaire (CHAQ) completed by participants, pain measured by a 10 cm visual analogue scale (VAS) completed by participants, active joint count determined by participating paediatric rheumatologists, examining physician's global assessment of disease activity (PGA), remission, and active disease duration [1]. CHAQ scores were calculated as the mean score of eight categories of activities; the minimum score was 0 and the maximum 3.0 [17]. Moderate to severe disability was defined as a score of ≥0.75 [12, 18]. PGA was scored as 0 for inactive, 1 for mild, 2 for moderate and 3 for severe disease activity [1]. Active arthritis was defined as joint swelling or limitation of movement with tenderness or pain on movement [1]. Uveitis was not considered in the definition of active disease. Remission was defined as inactive arthritis while off treatment for a minimum of 2 yr. Active disease duration was defined as the interval from symptom onset to either the date arthritis was last active by examination or to date of study if arthritis was still active. For patients with an intermittent disease course, the active articular disease duration was the sum of periods of active arthritis.

A patient was considered North American Native if both parents were Native. Patients with only one Native parent were listed as 'part Native' (in Table 1) but were included as non-Native in all other analyses.

Joint radiographs taken within the first 2 yr after onset (early radiographs) and the latest films prior to clinical outcome studies (late radiographs) were reviewed. Late films selected for this study were taken at least 5 yr after onset. All radiographs were read blindly by a single paediatric radiologist. Except for films of the additional patients, the results of these radiographic findings have been published previously [13].

Cytokine gene alleles

Cytokine gene allele typing was performed by polymerase chain reaction (PCR) with sequence-specific primers for the following SNPs: tumour necrosis factor-α (TNF-α) promoter $-308G \rightarrow A$, transforming growth factor-β1 (TGF-β1) codon $10T \rightarrow C$, TGF-β1 codon $25G \rightarrow C$, interleukin-10 (IL-10) promoter $-1082G \rightarrow A$, IL-10 promoter $-819C \rightarrow T$ and IL-10 promoter $-592A \rightarrow C$, IL-6 promoter $-174G \rightarrow C$ and interferon-gamma (IFN-γ) intron $+874T \rightarrow A$ (One Lambda Inc, Canoga Park, CA). These SNPs were chosen as they have been associated with differential rates of gene transcription or protein synthesis *in vitro* [7–9, 19–22]. Furthermore elevations of these cytokines (IL-6 and TNF-α) or their expression in synovial fluid cells or synovial tissues (IL-6, TNF-α, IFN-γ, IL-10 and TGF-β) in patients with JIA have been reported [4, 23–30]. Alleles or haplotypes with reported high rates of *in vitro* gene transcription and/or production of these cytokines are: TNF-α $-308A$, TGF-β1 codon 10T, TGF-β1 codon 25G, IL-10 $-1082G$, $-819C$, $-592C$ haplotype, IL-6 $-174G$ and IFN-γ $+874T$ [7–9, 19–22]. GCC, ACC and ATA are the three haplotypes of IL-10 -1082, -819 and -592 SNPs in most populations studied [21, 31–33]. However, additional haplotypes have been reported in southern Chinese (ATG) and African-Americans (ATC) [32, 34]. Haplotypes in North American Native races included in our studies have not been confirmed. Since our patient population consisted of a number of ethnic groups, IL-10 genotypes are listed separately for each polymorphism. For Caucasian groups only, IL-10 haplotypes were inferred from IL-10 -1082, -819 and -592 genotypes.

The distribution of cytokine genotypes was determined in previous studies of healthy Caucasian blood bank donors [35].

Calculations

Only Caucasian subjects were entered for comparisons of frequencies of cytokine genotypes and IL-10 haplotypic genotypes among JRA onset subgroups and controls. χ^2 tests or Fisher's exact tests were used for these comparisons and the level of significance was set at $\alpha = 0.006$ using the Bonferroni adjustment for multiple comparisons (eight comparisons, one for each cytokine polymorphism, were made).

Correlations of cytokine SNP genotypes with the following outcomes were sought: active disease duration, remission, joint count, PGA, CHAQ score ≥0.750, pain and joint space narrowing

Table 1. Patient characteristics

	Disease onset category			
	Systemic	Oligoarticular	Polyarticular RF negative	Polyarticular RF positive
Number	21	99	46	15
Female:male	11:10	80:19	38:8	13:2
Median age at onset (yr) (range)	5.0 (0.3–15.8)	2.9 (0.8–13.8)	4.2 (0.8–15.7)	8.7 (0.7–15.4)
Median time from onset to first visit (months) (range)	1.0 (0–12)	2.1 (0–79)	5.1 (0–125)	6.1 (0.3–84)
Median age at study (yr) (range)	15.2 (8.1–32.1)	15.4 (7.8–28.7)	15.3 (8.2–29.2)	19.1 (9.6–30.4)
Median time from onset to study (yr) (range)	9.2 (4.8–17.7)	10.6 (5.0–21.8)	9.6 (4.7–21.7)	10.4 (5.4–21.4)
Race (number) [Caucasian:Native (part Native) North American:other]	19:1:1	92:1(3):3	39:4:3	11:4:0
ANA positive (no. positive/no. done) (%)	3/20 (15.0)	70/97 (72.2)	25/43 (58.1)	10/15 (66.7)

Range = minimum – maximum; ANA, antinuclear antibody; RF, rheumatoid factor; no., number.

or erosions on early and late radiographs. Joint space narrowing included joint space loss, ankylosis and carpal collapse [13]. Radiographic outcomes were defined as the presence of these abnormalities at one or more sites, or their absence at all sites radiographed at each time point. As all outcomes were non-normally distributed, non-parametric tests were used. Univariate correlations of the three possible genotypes for each cytokine gene SNP with outcome measures were made by Kruskal–Wallis, χ^2 or Fisher's exact tests. For active disease duration and remission Cox regressions were performed separately for each SNP and patients with active disease, or those not in remission, were censored to the time of study. Univariate tests for clinical variables were performed to identify which needed to be controlled for to determine independent effects of cytokine genotype. Spearman rank correlation coefficients were calculated for continuous measures. Other correlations were tested as above.

Cytokine SNP genotypes and clinical variables which correlated with outcomes at a level of significance of $\alpha = 0.100$ in univariate tests were then entered into multivariate tests to determine independent effects. Multivariate tests were performed only for those outcomes for which univariate correlations with cytokine genotypes were found. Stepwise linear regressions were performed for continuous measures and both forward and backward conditional multiple logistic regressions for dichotomous outcomes. The level of significance for multivariate tests was set at $\alpha = 0.05$ and a sample size requirement of at least 10 times the number of variables was assumed [36]; however, binary outcome analyses are reliable for fewer variables. For PGA, the outcome variable was a score of 2 or 3. Calculations were performed with SPSS Version 11.5.

The effect of subtype of JRA was controlled for in the multivariate tests. A stratified multivariate analysis for each subtype was not done due to small numbers of patients in some of the groups. To eliminate possible ethnic differences in cytokine genotype distribution, additional separate multivariate tests were performed for Caucasian patients only.

Results

Patient characteristics

Patients studied were comparable to the 228 others in the original cohort who were not studied with respect to onset subtypes, sex ratio, interval from onset to time of outcome assessments, and outcome measures—active joint count, CHAQ scores, PGA, pain scores, remission and active disease duration. However, those in the present study were younger both at onset [median age 3.7 yr (range 0.3 to 15.8 yr) *vs* 6.2 yr (0.4 to 16.3 yr), $P = 0.001$] and at time of outcome assessment [median 15.5 yr (7.8 to 32.1 yr) *vs* 16.1 yr (8.1 to 32.1 yr) $P = 0.007$], and there were proportionately fewer patients of North American Native origin (88.9% Caucasian, 7.2% Native and 3.9% other races, versus 78.4% Caucasian, 17.2% Native and 4.4% other races, $P = 0.010$). The characteristics of patients studied are shown in Table 1.

Radiographs were available for 113 patients in this study, 52 had both early and late, 28 only early and 33 only late films. Among all patients with late films, the median follow-up time from onset to time of late radiographs was 9.7 yr (range 5.1 to 24.1 yr).

Correlations of cytokine genotypes with onset subtype

Frequencies of cytokine genotypes among the onset subtypes are shown in Table 2. No differences could be detected among Caucasian patient groups and healthy controls (Table 2) or between patients with persistent (72 patients) and extended (16 patients) oligoarticular JRA (data not shown). There were also no differences in the frequencies of haplotypic genotypes for the IL-10 promoter −1082, −819 and −592 polymorphisms, ATA/ATA, ATA/ACC, ACC/ACC, GCC/ATA, GCC/ATA, GCC/GCC and GCC/GCC, among Caucasian patient groups and controls (data not shown).

Correlations of cytokine genotypes with outcomes

Univariate tests. Significant correlations with $P < 0.100$ were found between pain and IL-6 −174 genotypes ($P = 0.020$); between PGA and IL-10 −819 ($P = 0.015$) and −592 ($P = 0.015$) genotypes; and between joint space narrowing on early radiographs and TGF-β1 codon 25 ($P = 0.095$) and IL-10 −1082 ($P = 0.083$) genotypes, respectively. There were no correlations between cytokine SNP genotypes and active disease duration, remission, joint count, CHAQ score ≥ 0.750, erosions on early or late radiographs, or joint space narrowing on late radiographs.

Among other clinical variables indicated in Tables 3 and 4, onset subtype correlated with pain ($P = 0.023$), early joint space narrowing ($P = 0.016$) and PGA ($P = 0.001$).

No correlations could be detected between the IL-10 haplotypic genotypes and any of the outcomes among Caucasian patients.

Multivariate tests. To determine whether the correlations between cytokine genes and outcome measures noted above were independent associations, multivariate analyses were performed. The homozygous IL-6 −174G/G genotype was significantly correlated with pain scores when clinical variables with independent effects (active disease duration, PGA and age at study) were controlled for (Table 3). In this analysis, JRA onset subtype did not have an independent effect on pain. However, the total variation in pain scores explained by the model was only 27.2%, and the contribution of the IL-6 −174G/G genotype only 2.7%. The analysis was based on 170 patients, and 11 variables were entered.

In a separate multivariate analysis of 151 Caucasian patients only, similar results were obtained and independent variables showing significant effects on pain were: IL-6 −174G/G genotype (regression coefficient $B = 0.780$, 95% CI 0.038, 1.522, $P = 0.039$), active disease duration ($B = 0.163$, 95% CI 0.091, 0.234, $P < 0.0001$), PGA 2 or 3 ($B = 1.853$, 95% CI 0.917, 2.790, $P < 0.0001$) and age at study ($B = 0.090$, 95% CI, 0.018, 0.162, $P = 0.015$). (The regression model could explain 28.4% of the variance in pain.)

The odds of early joint space narrowing were reduced by 83% by the TGF-β1 codon 25G/G genotype compared with the heterozygous C/G genotype, and increased nearly 13-fold by North American Native race compared with other races, however confidence intervals were wide (Table 4). There were no subjects with the C/C genotype in this analysis. It was not possible to assess the effects of onset subtype or IL-10 −1082 genotypes due to insufficient power (Table 4). The analysis was based on 74 patients and five variables were entered. Similarly it was not possible to assess correlations in separate multivariate analyses for Caucasian patients only as the number of patients was reduced to 63 (not shown).

For a PGA score of 2 or 3, there were no independent correlations with IL-10 SNP genotypes and the only remaining significant associations in multivariate analyses were systemic onset [odds ratio (OR) 5.28, 95% confidence limits (CI) 1.67, 16.72, $P = 0.005$], and polyarticular RF positive onset (OR = 11.07, 95% CI 3.19, 38.45, $P < 0.0001$) (not shown). In this analysis, patients with the homozygous IL-10 819T/T or IL-10 −592A/A were removed as none had the outcome of interest (data not shown).

Discussion

Correlations between cytokine SNPs and outcome measures in a subset of patients with JRA are reported. A correlation between

Table 2. Cytokine genotype frequencies

Genotype	Disease onset category				
	Systemic	Oligoarticular	Polyarticular RF negative	Polyarticular RF positive	Caucasian controls
TNF-α −308G→A					
Number tested (Caucasian only)	21 (19)	97 (90)	44 (37)	15 (11)	(92)
% with genotype (Caucasian only):					
G/G	81.0 (78.9)	74.2 (74.4)	72.7 (67.6)	73.3 (63.6)	(68.5)
G/A	19.0 (21.1)	23.7 (23.3)	27.3 (32.4)	20.0 (27.3)	(29.3)
A/A	0 (0)	2.1 (2.2)	0 (0)	6.7 (9.1)	(2.2)
TGF-β codon 10T→C					
Number tested (Caucasian only)	17 (16)	95 (88)	42 (35)	14 (10)	(92)
% with genotype (Caucasian only):					
C/C	23.5 (25.0)	17.9 (15.9)	2.4 (2.9)	14.3 (10.0)	(10.9)
C/T	47.1 (43.8)	49.5 (51.1)	45.2 (42.9)	71.4 (80.0)	(53.3)
T/T	29.4 (31.3)	32.6 (33.0)	52.4 (54.3)	14.3 (10.0)	(35.9)
TGF-β codon 25G→C					
Number tested (Caucasian only)	16 (15)	95 (88)	42 (35)	14 (10)	(92)
% with genotype (Caucasian only):					
C/C	6.3 (6.7)	2.1 (2.3)	0 (0)	0 (0)	(0)
C/G	6.3 (6.7)	10.5 (9.1)	14.3 (14.3)	35.7 (50.0)	(15.2)
G/G	87.5 (86.7)	87.4 (88.6)	85.7 (85.7)	64.3 (50.0)	(84.8)*
IL-10 −1082G→A					
Number tested (Caucasian only)	21 (19)	99 (92)	46 (39)	15 (11)	(91)
% with genotype (Caucasian only):					
A/A	38.1 (36.8)	26.3 (27.2)	34.8 (30.8)	40.0 (18.2)	(25.3)
A/G	42.9 (47.4)	52.5 (52.2)	43.5 (46.2)	33.3 (45.5)	(53.8)
G/G	19.0 (15.8)	21.2 (20.7)	21.7 (23.1)	26.7 (36.4)	(20.9)
IL-10 −819C→T					
Number tested (Caucasian only)	21 (19)	99 (92)	46 (39)	15 (11)	(92)
% with genotype (Caucasian only):					
T/T	4.8 (5.3)	3.0 (3.3)	10.9 (7.7)	6.7 (0)	(5.5)
T/C	14.3 (10.5)	32.3 (33.7)	23.9 (20.5)	53.3 (45.5)	(31.9)
C/C	81.0 (84.2)	64.6 (63.0)	65.2 (71.8)	40.0 (54.5)	(62.6)
IL-10 −592C→A					
Number tested (Caucasian only)	21 (19)	99 (92)	46 (39)	15 (11)	(91)
% with genotype (Caucasian only):					
A/A	4.8 (5.3)	3.0 (3.3)	10.9 (7.7)	6.7 (0)	(5.5)
A/C	14.3 (10.5)	32.3 (33.7)	23.9 (20.5)	53.3 (45.5)	(31.9)
C/C	81.0 (84.2)	64.6 (63.0)	65.2 (71.8)	40.0 (54.5)	(62.6)
IL-6 −174G→C					
Number tested (Caucasian only)	20 (19)	99 (92)	46 (39)	15 (11)	(91)
% with genotype (Caucasian only):					
C/C	15.0 (15.8)	16.2 (15.2)	21.7 (25.6)	13.3 (18.2)	(14.1)
C/G	45.0 (42.1)	48.5 (48.9)	37.0 (38.5)	40.0 (45.5)	(44.6)
G/G	40.0 (42.1)	35.4 (35.9)	41.3 (35.9)	46.7 (36.4)	(41.3)
IFN-γ+874T→A					
Number tested (Caucasian only)	20 (18)	94 (87)	43 (36)	15 (11)	(91)
% with genotype (Caucasian only):					
A/A	30.0 (27.8)	37.0 (36.8)	25.6 (19.4)	53.3 (36.4)	(29.7)
T/A	45.0 (50.0)	50.0 (50.6)	65.1 (69.4)	26.7 (36.4)	(45.1)
T/T	25.0 (22.2)	12.8 (12.6)	9.3 (11.1)	20.0 (27.3)	(25.3)

The homozygous genotype corresponding to reported low rates of gene transcription are listed first, followed by the heterozygous genotype, and the homozygous genotype corresponding to reported high *in vitro* transcription rates. Comparisons among Caucasian patients in each subgroup and Caucasian controls were made by χ^2 or Fisher's exact tests. For each test the level of significance was set at $\alpha = 0.006$ after applying the Bonferroni adjustment. There were no comparisons with significant results.

*$P = 0.022$.

IL-6 −174G/G and pain, and between TGF-β1 codon 25G/G and a lower risk, or conversely between TGF-β1 codon 25C/G and a higher risk, of early joint damage were documented. Correlations with other outcomes, including remission and disability, were not detected. The correlation of IL-6 genotype with pain is of potential relevance to disability as we and others have previously found that pain is one of the largest contributors to the variation in physical function [13, 37, 38]. The correlation of TGF-β1 codon 25 genotypes with early radiological damage needs to be interpreted with caution as it was based on a small number of patients. Similarly, the absence of an effect of the TGF-β1 codon 25 polymorphism on long-term joint damage may be due to inadequate power. Alternatively, other variables may have a greater effect as disease duration increases.

To eliminate possible effects of ethnic differences in cytokine SNP gene or genotype frequencies [9, 32, 39] and IL-10 haplotypes [32–34], separate analyses were performed for Caucasian patients only. A significant correlation of IL-6 genotype with pain was confirmed. However, the association of TGF-β1 codon 25 genotypes with early joint space narrowing could not be confirmed,

Table 3. Multivariate tests for pain (R square = 0.272)

Variable entered	Coefficient B	95% CI	P	Change in R square
Active disease duration	0.156	0.085, 0.227	<0.0001	0.144
PGA 1	E			
PGA 2 or 3	1.955	1.060, 2.850	<0.0001	0.070
Age at study	0.092	0.022, 0.163	0.011	0.031
IL-6 −174C/G	E			
IL-6 −174G/G	0.899	0.185, 1.612	0.014	0.027
Male	E			
Joint count	E			
Systemic	E			
Polyarticular RF negative	E			
Polyarticular RF positive	E			
Constant	−1.408	−2.617, −0.198	0.023	

Variables with significant correlations with pain at $\alpha = 0.100$, in univariate analyses, were entered. Reference values were: PGA 0, IL-6 −174C/C (the homozygous low expressing genotype), female, and oligoarticular, respectively. In the final model, the reference conditions were 0 and 1 for PGA, and C/C and C/G genotypes for IL6 −174. Active disease duration, PGA 2 or 3, age at study, and IL-6 −174G/G had significant effects on pain. PGA = physician's global estimate of disease activity; 95% CI = 95% confidence intervals; E = eliminated in the regression. The analysis was based on 170 patients.

Table 4. Multivariate analysis for joint space narrowing within 2 yr (Cox and Snell R square = 0.117)

Variable entered	Odds ratio	95% CI	P
Non-North American Native race	1.0		
North American Native race	12.750	1.914, 84.946	0.009
TGF-β1 codon 25G/C	1.0		
TGF-β1 codon 25G/G	0.176	0.037, 0.837	0.029
IL-10 −1082G→A genotypes	E		
Onset age	E		
Oligoarticular	1.0		
Systemic	E		
Polyarticular RF negative	E		
Polyarticular RF positive	E		
Constant	0.444		0.177

Variables with significant correlations with joint space narrowing on radiographs taken within 2 yr of onset, at $\alpha = 0.100$ in univariate analyses were entered. Reference values were: non-North American Native race (Caucasian and other), the heterozygous expressing genotypes TGF-β1 codon 25C/G, and homozygous IL-10 −1082A/A, and oligoarticular onset, respectively. There were no patients with the homozygous TGF-β1 codon 25 homozygous C/C genotype. North American Native race increased, and TGF-β1 codon 25 genotype G/G decreased the odds of early joint space narrowing. Forward and backward conditional entry of variables gave identical results. See Table 3 for abbreviations. The analysis was based on 74 patients.

and no correlations with IL-10 haplotypes were detected, again perhaps due to inadequate power.

Previous studies have shown that cytokine gene polymorphisms may affect the risk of autoimmune conditions, including JIA [9, 11, 40, 41]. For example, associations between the IL-6 −174G allele and systemic JIA, and between the TNF-α +851A allele and oligoarthritis have been reported [9, 11, 41]. Other studies have shown a higher frequency of the IL-10 −1082A, −819T, −592A (ATA) haplotype in patients with extended oligoarthritis compared with those with persistent oligoarthritis [8], but reported associations between IL-1α −889 polymorphisms and oligoarthritis are inconsistent [42, 43]. It was not a primary aim to determine associations between cytokine genotypes and the risk of developing specific subtypes of JRA in this study, and the failure to detect previously reported associations or significant new associations was probably due to insufficient power of small sample sizes in the small subgroups. For example *post hoc* power calculations suggested the best power for any of the comparisons was only 72% and half were <10%.

In vitro, an IL-6 promoter −174G construct has a higher transcription rate than a C construct, and genotypes containing the G allele are associated with higher serum levels of IL-6 in healthy controls, suggesting that IL-6 levels are affected by genetic influences [9]. If so, the present correlation between IL-6 G/G and pain may be due to higher IL-6 responses. However, regulation of IL-6 transcription may be affected by other functional polymorphisms in the promoter region and haplotypes may reflect correlations with transcription rates more accurately [44, 45]. The correlation between the IL-6 −174G→C polymorphism and IL-6 responses *in vivo* has also been controversial [9, 46–48]. Therefore, an explanation for our findings requires further study.

The importance of IL-6 in the pathogenesis of systemic JIA has been underscored by the association of IL-6 −174G/G genotype with this onset subtype, the correlation of elevated serum IL-6 levels with febrile episodes and disease activity, increased levels of IL-6 receptor (an agonist of IL-6) and a recent clinical trial suggesting a beneficial effect of anti-IL6 receptor antibody in systemic JIA [4, 25–27, 49]. However, IL-6 levels are also elevated in other JIA subtypes [4, 23, 26], and the present correlation between IL-6 genotype and pain is also not subtype-specific. Moreover, the observed association is consistent with previous clinical and laboratory-based reports of a relation between IL-6 and pain [50–52].

TGF-β inhibits inflammatory cells, promotes extracellular matrix and inhibits collagenase and metalloproteinases [53, 54]. These actions of TGF-β might have beneficial effects in patients with arthritis. Indeed, TGF-β1 has protective effects in animal models of arthritis [55, 56]. The TGF-β1 codon 25G/G genotype is associated with higher rates of *in vitro* TGF-β1 synthesis, although the absolute differences are small [7]. The present observation of a possible protective effect of this genotype on joint damage is consistent with the enhancing effect of this cytokine on extracellular matrix production [53, 54].

TGF-β1 also has potentially adverse effects on synovial inflammation as it induces vascular endothelial growth factor (VGEF) in synovial fibroblasts and inhibits apoptosis of synovial cells [57, 58]. These considerations complicate the interpretation of our observation. However, reported correlations of cytokine genotype with gene expression are controversial and *in vivo* gene expression may be affected by disease states [10]. Further study of this cytokine in JIA may clarify a biological basis for the possible associations suggested in the present study.

The limitations of this study include a lack of measurements of cytokines in serum and lack of inclusion of other relevant cytokine genes. The failure to detect correlations with other outcome measures may have been due to lack of power.

In summary, the present report describes an association between an IL-6 gene polymorphism and pain and a possible correlation between a TGF-β1 polymorphism and joint damage in patients with JRA. Although it is important to detect subtype-specific associations in a larger patient cohort, the present study suggests correlations that are common to patients with JRA as a whole. Thus in some instances, predispositions may be shared. The reported findings require confirmation in all seven subsets of JIA. Nevertheless the present results do suggest that further study of cytokine genotypes in relation to patient outcome is warranted as they may prove to be useful prognostic markers.

Rheumatology	Key messages
	• An IL-6 single nucleotide polymorphism correlates with pain in patients with JRA. • A correlation between TGF-β1 genotypes and radiological damage needs further confirmation. • Cytokine genotypes may prove to be useful early prognostic indicators in JIA.

Acknowledgements

Supported by grants from the Health Sciences Centre Foundation, Winnipeg, the Winnipeg Foundation, the Children's Hospital Foundation, Winnipeg, and the Arthritis Society. The authors are grateful to the participants of this study, and thank Ms M. Cheang, M Math (Stat), Biostatistical Consultant, Department of Community Health Sciences, University of Manitoba, for assistance with the statistical analyses, and Ms I. Dembinski for performing the cytokine SNP assays.

The authors have declared no conflicts of interest.

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