# Association of Single Nucleotide Polymorphisms within Cytokine Genes with Juvenile Idiopathic Arthritis in the Czech Population

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*ABSTRACT.* ***Objective.*** To examine the possible association of juvenile idiopathic arthritis (JIA) with polymorphisms within cytokine genes in the Czech population.

***Methods.*** In a case-control study, genotypes of 130 patients with JIA (63 male, 67 female; age at onset 7.6 ± 4.4 yrs; 43 oligoarticular, 72 polyarticular, 15 systemic form) were compared to 102 healthy unrelated blood donors. Using the polymerase chain reaction technique with sequence-specific primers from the 13th IHWG workshop, we analyzed 19 single nucleotide polymorphisms within 12 different cytokine genes [interleukin (IL)-1α, IL-1ß, IL-2, IL-4, IL-6, IL-10, IL-12, transforming growth factor (TGF)-ß, interferon (IFN)-γ], and related molecules (IL-1R, IL-1RA, IL-4Rα). Genotype frequencies were compared using chi-square analysis, and the significance level was corrected for the number of independent tests.

***Results.*** Significant positive association was found for the G allele of the IL-4 –1098 T/G polymorphism, which was carried by 10% of cases and 25% of controls [odds ratio (OR) 0.32, 95% confidence interval (CI) 0.16–0.67, corrected p = 0.038). Also, a nonsignificant increase in the frequency of the IL-1ß +3962 C allele was detected in cases (96%) versus controls (84%) (OR 4.65, 95% CI 1.64–13.2, corrected p = 0.091). We did not replicate previously found associations with the IL-1α, IL-6, IL-10, and IL-1RA polymorphisms.

***Conclusion.*** Our study showed association with JIA for the IL-4 –1098 T/G polymorphism. It also underlines the genetic contribution of IL-1 polymorphisms to the pathogenesis of JIA, as another polymorphism within the IL-1ß may influence the risk of the disease. (J Rheumatol 2004;31:1206–10)

*Key Indexing Terms:*
JUVENILE IDIOPATHIC ARTHRITIS CYTOKINE GENETIC ASSOCIATION
SINGLE NUCLEOTIDE POLYMORPHISM

Juvenile idiopathic arthritis (JIA) is a multifactorial autoimmune disease. Genetic susceptibility and resistance have been attributed not only to the genes of the major histocompatibility complex (MHC), but also to non-MHC genes participating in the immune response. Polymorphisms within the cytokine genes are promising candidates since some of them are known to influence the quantity of cytokine expression. Association with JIA, with its subtype, or with its course has been already shown for polymorphisms of several cytokine genes: e.g., interleukin (IL)-6[1], IL-10[2], tumor necrosis factor (TNF)[3-5], or IL-1α[6]. Nevertheless, the nature of the JIA association with these genes is very complex and heterogeneous: the positive results mentioned above were not confirmed in other populations or patient groups: Donn, *et al*[7] did not replicate association of IL-6 and IL-10; another study by Donn, *et al*[8] did not confirm association of IL-1α; and a recent study by Ozen, *et al*[9] did not find association of TNF-α with risk of JIA. Our study investigated associations of single nucleotide polymorphisms (SNP) within cytokine genes with juvenile idiopathic arthritis (JIA) in Czech Caucasians.

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## MATERIALS AND METHODS

*Study design.* In a case-control study, genotypes of SNP within cytokine genes were compared between patients with JIA and healthy unrelated blood donors. All cases and controls were Czechs of Caucasian origin. One hundred thirty patients with JIA (63 male, 67 female) were recruited at the outpatient rheumatology clinic of the Motol University Hospital, Prague. Patients were classified according to the International League of Associations for Rheumatology (ILAR) criteria: 43 presented with oligoarticular, 72 with polyarticular, and 15 with the systemic form of JIA. The age (mean ± SD) at disease onset was 7.6 ± 4.4 years, and the followup length was 1–46 years, median 6.0 years. Comprehensive medical history and

longitudinal followup data were available for each individual. The characteristics of patients are summarized in Table 1.

The controls were 103 unrelated healthy blood donors from the Prague region, collected for the Anthropology study and the Cytokine Gene Polymorphism component of the 13th International Histocompatibility Workshop and Conference (IHWC). In 4 of the controls, several polymorphisms were not tested due to lack of DNA.

*Polymerase chain reaction (PCR)*. Cytokine genotypes were investigated using PCR with sequence-specific primers designed for the 13th IHWC. The accuracy of our laboratory controlled by testing the IHWC proficiency panels achieved 98%. Twenty-one single nucleotide polymorphisms were tested within 12 different cytokine genes [IL-1α –889, IL-1ß –511 and +3962, IL-2 –330 and +160, IL-4 –1098 and –590, IL-6 –174 and +565, IL-10 –1082, –819 and –590, IL-12 –1188, TNF-α –308 and –238, transforming growth factor (TGF)-ß codon 10 and 25, interferon (IFN)-γ UTR 5644] and related molecules (IL-1R pst 1970, IL-1RA mspa 11100, IL-4Rα +1902). The design of the sequence-specific primers allowed us also to directly test the phase of single nucleotide polymorphisms in haplotypes of the TGF-ß1, TNF-α, IL-2, IL-4, IL-6, and IL-10. As complete data on HLA-DR genotypes in cases were not available, we did not analyze the risk conferred by polymorphisms of TNF-α.

*Statistical analysis*. The distribution of genotypes or haplotypes was tested for significant departure from Hardy-Weinberg equilibrium. The cases and controls were tested for linkage disequilibria between alleles of individual SNP. Association with JIA was tested using heterogeneity tests in allelic distribution between the cases and controls, and the risk was expressed using odds ratios (OR) calculated together with its confidence intervals (CI) using Woolf's formula. The p values were corrected for 13 tests (i.e., number of independent SNP or haplotypes). If there was a significant difference in genotype distribution between cases and controls, tests were also performed separately for each of the 3 subsets of patients.

## RESULTS

Distribution of cytokine genotypes in cases and controls is shown in Table 2. It followed the Hardy-Weinberg distribution with the exception of the IL-4 –590 and IL-10 –1082 polymorphisms. In both cases and controls, significant linkage disequilibria were observed between alleles of the IL-1ß –511 and IL-1ß +3962; IL-2 –330 and IL-2 +160; IL-6 –174 and IL-6 +565; and IL-10 –1082, –819, and –590. Further, in cases, a borderline-significant linkage disequilibrium was observed between the alleles of IL-1α +511 and IL-1ß +3962, and between the alleles of TGF-ß codon 10 and 25.

Apart from testing linkage disequilibria between alleles of individual SNP, the TGF-ß1, IL-2, IL-4, IL-6, and IL-10 haplotypes were directly assigned using PCR-SSP, which tests whether the alleles in the haplotype are in the cis- or trans- phase. The haplotype frequencies and respective OR are shown in Table 3.

*Table 1*. Patient characteristics.

| | Total | Oligoarticular | Polyarticular | Systemic |
|---|---|---|---|---|
| Patients, n | 130 | 43 | 72 | 15 |
| Male | 63 | 26 | 27 | 10 |
| Female | 67 | 17 | 45 | 5 |
| Age at investigation, yrs, mean ± SD | 15.7 ± 8.1 | 14.7 ± 5.8 | 16.6 ± 8.8 | 14.5 ± 9.5 |
| Age at disease onset, yrs, mean ± SD | 7.6 ± 4.4 | 6.9 ± 3.9 | 8.6 ± 4.7 | 4.7 ± 2.4 |

Association with JIA was observed for the IL-4 –1098 G allele. The G allele of the IL-4 –1098 polymorphism (carried by 10% of cases and 25% of controls) was negatively associated with JIA in the total group of cases (OR 0.32, 95% CI 0.16–0.67, $p_{uncorrected}$ = 0.0029, $p_{corrected}$ = 0.038), and tendency towards negative association was consistently present in all 3 subsets. The C allele of the IL-1ß +3962 polymorphism was increased in cases (96%) compared to controls (84%), but this difference lost its statistical significance after correction for the number of tests (OR 4.65, 95% CI 1.64–13.2, $p_{uncorrected}$ = 0.0070, $p_{corrected}$ = 0.091). A tendency towards increased representation of the allele was also present in oligo- and polyarticular, but not in the systemic form of the disease.

## DISCUSSION

We observed a significant negative association with JIA of the G allele of the IL-4 –1098 T/G polymorphism. IL-4 is an antiinflammatory cytokine mainly produced by activated $T_H2$ cells, mastocytes, and basophils. The IL-4 shifts the immune response towards $T_H2$, diminishing the inflammatory function of monocytes and macrophages, thus possibly accounting for protection from $T_H1$ mediated diseases such as JIA. As no published data are available about the effect of the IL-4 –1098 T/G polymorphism on the expression level, it is difficult to judge whether our finding may reflect causality due to differences between alleles in expression of this antiinflammatory cytokine in the affected joint.

The C allele of the IL-1ß +3962 T/C polymorphism exhibits a positive association with JIA. This association lost statistical significance after correction for the number of independent tests, but it may still be relevant with respect to previous findings on the association of another polymorphism within the IL-1 gene cluster, the IL-1α –889[6]. IL-1ß is a potent proinflammatory cytokine produced mainly by monocytes and macrophages and it plays an important role in destruction of the joint. The T allele of the IL-1ß +3962 T/C polymorphism has been previously associated with the high-secretor phenotype[10]; however, it is the C and not the T allele that is positively associated with JIA in our study. The genes encoding IL-1α, IL-1ß, and IL-1RA lie in a cluster on chromosome 2. Since the results on association of these genes with JIA are generally heterogeneous[6,8,11], it may be speculated that our observed association might be secondary due to linkage disequilibrium with other polymorphisms within the IL-1 gene cluster. In this respect, investigation of polymorphisms IL-1ß –35 T/C and nt5810 A/T, as well as IL-1α +4345 T/G may help in disclosing the etiological mutation.

We did not detect the previously described association with polymorphisms within IL-6[1], IL-10[2], IL-1RA[11], and IL-1α[6]. The discrepancies between studies on cytokine gene polymorphisms may be ascribed either to true differences among populations in genetic susceptibility to JIA, or to the

*Table 2*. Distribution of genotypes of cytokine gene polymorphisms in patients with JIA and controls. Data are presented as OR conferred by phenotypic positivity of the variants, and p values of heterogeneity testing of genotype distribution. The OR for IL-4-590 and IL-10-1082 were not calculated, as these 2 SNP did not follow Hardy-Weinberg equilibrium (H-W).

| | Individuals with the Genotype, n (% of total) | | Risk Conferred by Phenotypic Allele | Positivity of the Allele |
|---|---|---|---|---|
| | Patients | Controls | | OR (95% CI) p |
| IL-1α -889 | | | | |
| C/C | 72 (55) | 66 (65) | C | 1.31 (0.58–2.98) |
| C/T | 45 (35) | 23 (23) | T | 1.48 (0.87–2.52) |
| T/T | 13 (10) | 13 (13) | | p = 0.16 |
| IL-1ß -511 | | | | |
| C/C | 63 (48) | 57 (55) | C | 0.61 (0.22–1.68) |
| C/T | 55 (42) | 40 (39) | T | 1.32 (0.78–2.21) |
| T/T | 12 (9) | 6 (6) | | p = 0.46 |
| IL-1ß +3962 | | | | |
| C/C | 75 (58) | 49 (48) | C | 4.65 (1.64–13.2) |
| C/T | 50 (38) | 37 (36) | T | 0.68 (0.40–1.14) |
| T/T | 5 (4) | 16 (16) | | p = 0.0070 |
| IL-1R pst 1970 | | | | |
| C/C | 54 (42) | 41 (40) | C | 0.98 (0.46–2.08) |
| C/T | 58 (45) | 48 (47) | T | 0.93 (0.55–1.58) |
| T/T | 18 (14) | 14 (14) | | p = 0.95 |
| IL-1RA mspa 11100 | | | | |
| C/C | 20 (15) | 10 (10) | C | 1.04 (0.62–1.76) |
| C/T | 57 (44) | 50 (49) | T | 0.59 (0.26–1.33) |
| T/T | 53 (41) | 43 (42) | | p = 0.42 |
| IL-4RA +1902 | | | | |
| A/A | 80 (62) | 55 (53) | A | 0.78 (0.25-2.45) |
| A/G | 42 (32) | 43 (42) | G | 0.72 (0.42–1.21) |
| G/G | 8 (6) | 5 (5) | | p = 0.33 |
| IL-12 -1188 | | | | |
| A/A | 77 (59) | 58 (59) | A | 0.42 (0.11–1.59) |
| A/C | 44 (34) | 38 (38) | C | 0.97 (0.57–1.66) |
| C/C | 9 (7) | 3 (3) | | p = 0.37 |
| IFN-γ UTR 5644 | | | | |
| A/A | 35 (27) | 25 (27) | A | 1.32 (0.71–2.45) |
| A/T | 67 (52) | 44 (47) | T | 0.98 (0.54–1.79) |
| T/T | 28 (22) | 25 (27) | | p = 0.66 |
| TGF-ß1 codon 10 | | | | |
| C/C | 21 (16) | 15 (15) | C | 1.39 (0.81–2.38) |
| C/T | 67 (52) | 47 (46) | T | 0.88 (0.43–1.82) |
| T/T | 42 (32) | 41 (40) | | p = 0.49 |
| TGF-ß1 codon 25 | | | | |
| C/C | 1 (1) | 2 (2) | C | 1.29 (0.63–2.68) |
| C/G | 21 (16) | 12 (12) | G | 2.55 (0.23–28.6) |
| G/G | 108 (83) | 89 (86) | | p = 0.47 |
| IL-2 -330 | | | | |
| G/G | 23 (18) | 11 (11) | G | 1.17 (0.69–1.97) |
| G/T | 54 (42) | 45 (45) | T | 0.57 (0.26–1.23) |
| T/T | 53 (41) | 45 (45) | | p = 0.35 |
| IL-2 +160 | | | | |
| G/G | 71 (55) | 48 (48) | G | 1.20 (0.51–2.85) |
| G/T | 47 (36) | 42 (42) | T | 0.75 (0.45–1.27) |
| T/T | 12 (9) | 11 (11) | | p = 0.56 |
| IL-4 -1098 | | | | |
| G/G | 1 (1) | 0 (0) | G | 0.32 (0.16–0.67) |
| G/T | 12 (9) | 26 (25) | T | 0.63 (0.06–7.06) |
| T/T | 117 (90) | 76 (75) | | p = 0.0029 |
| IL-4 -590 | | | | |
| C/C | 91 (70) | 77 (75) | | Departure from H-W, |
| C/T | 31 (24) | 20 (20) | | not analyzed |
| T/T | 8 (6) | 5 (5) | | |

*Table 2*. Continued.

| | Individuals with the Genotype, n (% of total) Patients | Controls | Risk Conferred by Phenotypic Allele | Positivity of the Allele OR (95% CI) p |
|---|---|---|---|---|
| IL-6 -174 | | | | |
| C/C | 31 (24) | 20 (19) | C | 1.12 (0.63–1.98) |
| C/G | 64 (49) | 53 (51) | G | 0.77 (0.41–1.45) |
| G/G | 35 (27) | 30 (29) | | p = 0.71 |
| IL-6 +565 | | | | |
| A/A | 30 (23) | 16 (16) | A | 1.14 (0.65–2.00) |
| A/G | 62 (48) | 54 (52) | G | 0.61 (0.31–1.20) |
| G/G | 38 (29) | 33 (32) | | p = 0.35 |
| IL-10 -1082 | | | | |
| A/A | 28 (22) | 21 (20) | | Departure from H-W, |
| A/G | 77 (59) | 71 (69) | | not analyzed. |
| G/G | 25 (19) | 11 (11) | | |
| IL-10 -819 | | | | |
| C/C | 75 (58) | 48 (47) | C | 1.61 (0.42–6.14) |
| C/T | 51 (39) | 50 (49) | T | 0.64 (0.38–1.08) |
| T/T | 4 (3) | 5 (5) | | p = 0.23 |
| IL-10 -590 | | | | |
| A/A | 4 (3) | 6 (6) | A | 0.67 (0.40–1.12) |
| A/C | 51 (39) | 48 (47) | C | 1.95 (0.53–7.10) |
| C/C | 75 (58) | 49 (48) | | p = 0.24 |

*Table 3*. Haplotypes directly assigned using PCR-SSP, their carrier rates (phenotypic positivity) in patients and controls, and the respective OR for JIA.

| | Cases, n (%) | Controls, n (%) | OR (95% CI) |
|---|---|---|---|
| TGF-ß1 codon 10 C/T, codon 25 C/G | | | |
| CC | 22 (17) | 108 (14) | 1.3 (0.6–2.7) |
| CG | 74 (57) | 56 (50) | 1.3 (0.8–2.3) |
| TG | 109 (84) | 21 (85) | 0.9 (0.4–1.8) |
| IL-2 -330 G/T, +160 G/T | | | |
| GG | 77 (59) | 53 (55) | 1.2 (0.7–2.0) |
| GT | 0 (0) | 130 (1) | 0.4 (0.0–4.3) |
| TG | 68 (52) | 62 (54) | 0.9 (0.5–1.5) |
| TT | 59 (45) | 71 (50) | 0.8 (0.5–1.4) |
| IL-4 -1098 G/T, -590 C/T | | | |
| GC | 13 (10) | 117 (22) | 0.4 (0.2–0.8) |
| TC | 119 (92) | 11 (92) | 1.0 (0.4–2.5) |
| TT | 39 (30) | 91 (22) | 1.5 (0.8–2.7) |
| IL-6 -174 C/G, +565 A/G | | | |
| CA | 92 (71) | 38 (67) | 1.2 (0.7–2.1) |
| CG | 4 (3) | 126 (8) | 0.4 (0.1–1.3) |
| GA | 0 (0) | 130 (1) | 0.4 (0.0–4.4) |
| GG | 99 (76) | 31 (81) | 0.8 (0.4–1.4) |
| IL-10 -1082 A/G, -819 C/T, -590 A/C | | | |
| ACC | 63 (48) | 67 (59) | 0.6 (0.4–1.1) |
| ATA | 55 (42) | 75 (48) | 0.8 (0.5–1.4) |
| GCC | 102 (78) | 28 (75) | 1.2 (0.7–2.3) |

variations in patient group characteristics and study methodology. The latter 2 reasons may be of particular importance in JIA, whose diagnosis and classification rely mainly on subjective judgment of clinical patterns.

We report an association of JIA with 2 polymorphisms of cytokine genes, the IL-4 –1098 T/G and the IL-1ß +3962 T/C. Using larger groups of cases of a single JIA subtype, typing a more dense set of markers within the most promising regions, and investigating other populations will give more clues as to whether these associations reflect causality.

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