

# Lack of Association Between Juvenile Idiopathic Arthritis and Fas Gene Polymorphism

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**ABSTRACT.** *Objective.* Juvenile idiopathic arthritis (JIA) is a complex genetic disease of autoimmune etiology. Fas is a molecule with a pivotal role in apoptosis and hence in immune regulation. Elevated transcriptional levels of Fas in the synovial fluid of patients with JIA suggest that it might be implicated in disease etiopathogenesis. We investigated whether a polymorphism in the *Fas* promoter region (–670) confers susceptibility to JIA.

*Methods.* In this association study, 342 UK patients with JIA and 255 healthy individuals were genotyped for the polymorphism using polymerase chain reaction restriction fragment length polymorphism. Comparisons of the genotypic frequencies were made using chi-square analysis.

*Results.* No statistically significant differences were found when the genotype frequencies of the –670 *Fas* polymorphism were compared between the JIA cases and the control panel. Similarly, no differences were seen between the JIA subgroups, or when the patients were divided on the basis of rheumatoid factor or antinuclear antibody positivity.

*Conclusion.* The –670 polymorphism of *Fas* does not appear to be associated with susceptibility to JIA. (J Rheumatol 2002;29:166-8)

*Key Indexing Terms:*

JUVENILE IDIOPATHIC ARTHRITIS

FAS GENE

Juvenile idiopathic arthritis (JIA), previously called juvenile chronic arthritis within Europe, and juvenile rheumatoid arthritis in the USA, is the commonest arthritic condition of childhood. In accord with recent nomenclature, JIA encompasses a heterogeneous group of conditions, in which the onset of disease occurs before the age of 16 years with a minimum duration of 6 weeks<sup>1</sup>. JIA appears to be the result of both genetic and environmental influences, the genetic predisposition involving several contributory loci<sup>2</sup>. We examined whether *Fas* constitutes one of these susceptibility genes.

Fas (or CD95 or Apo1) is a transmembrane molecule, belonging to the tumor necrosis factor (TNF) receptor family, that plays an important role in apoptosis and, therefore, immune regulation<sup>3</sup>. It possesses domains of specific sequence that can activate downstream components of the apoptotic pathway. When bound to homotrimers of its ligand (FasL), Fas can initiate intracellular cascades that culminate in programmed cell death. Cell death suppressor molecules, such as Bcl-2 and Bcl-x<sub>L</sub>, are integral to this process and act to inhibit the transduction of pro-apoptotic Fas signals<sup>4</sup>.

The Fas gene (*Fas*) has been mapped to chromosome 10q23 in humans<sup>5</sup>. It has been found to consist of 9 exons and to encompass a 2 kb promoter sequence. Screening of the latter region has led to the identification of various single nucleotide polymorphisms (SNP), for instance those at positions –1377, –690, –670, and –397 relative to the transcription start site<sup>6</sup>. The –670 SNP resides in the enhancer region and creates a *Mva*I restriction site, while abolishing the binding site for the nuclear transcription element gamma activation site<sup>7</sup>.

*Fas* can be alternatively spliced to produce a soluble form of the receptor (sFas), which is incapable of triggering the apoptotic machinery and thought to compete with Fas for FasL. Aberrant expression of sFas has been correlated with various autoimmune diseases<sup>3,8</sup>. Similarly, upregulated Fas expression has been demonstrated in the synovial fluid (SF) of patients with JIA<sup>9,10</sup>. Promoter polymorphisms are possible phenotype causative factors, especially if they disturb recognition sequences. Investigation of the involvement of the –670 SNP in multiple sclerosis, rheumatoid arthritis, and systemic lupus erythematosus pathogenesis indicated a possible effect in disease susceptibility<sup>11,12</sup>. The contribution of this SNP to JIA etiology was the subject of investigation in this case-control association study.

## MATERIALS AND METHODS

The patient group consisted of 342 UK Caucasian JIA cases from the British Paediatric Rheumatology Group (BPRG) National Repository for JIA. They all fulfilled the International League of Associations for Rheumatology (ILAR) classification criteria<sup>1</sup>, which subdivide JIA into 8 subgroups. Patients fulfilling the “unclassifiable” ILAR category were not used in this study. Two

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hundred fifty-five healthy, unrelated UK Caucasian individuals served as the control group.

As the -670 A to G substitution creates a *Mva*I restriction site, both the cases and the controls were genotyped using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Genomic DNA (50 ng) was amplified in a 25 µl PCR reaction as described<sup>7</sup>. Five microliters of the PCR product were subsequently digested with 10 U of *Mva*I enzyme at 37°C overnight. The digestion product was then analyzed on a 4% agarose gel, and stained with ethidium bromide. The data were statistically analyzed using Stata v6. A value of  $p \leq 0.05$  was regarded as nominally significant.

RESULTS

The control samples were tested and found to comply with the Hardy-Weinberg equilibrium law. Genotype frequencies were generated for the controls, the total number of JIA cases, as well as each JIA subgroup separately (Table 1), and analyzed using the Pearson chi-square test. No differences were observed when the -670 SNP genotypic frequencies of all patients with JIA were compared with the controls ( $p = 0.45$ ). To reduce the amount of multiple testing, and the increased possibility in type I error that this can generate, identification of any JIA subgroup-specific associations with the -670 SNP was carried out by examining the difference in genotype frequency between the 7 ILAR subgroups studied. Only if evidence of a difference (at the 5% significance level) had been shown would tests be performed to separately compare genotype frequencies for each subgroup with the controls. No significant variations were found when comparing the genotypic frequencies between the 7 JIA ILAR subgroups ( $p = 0.12$ ).

To define any associations of the polymorphism with distinct clinical manifestations of the disease, patients with the relevant clinical data available were categorized into rheumatoid factor (RF) negative and RF positive individuals, as well as antinuclear antibody (ANA) negative and positive cases (Table 1). Comparisons of the genotypic frequencies between the subsets of each of these categories led to nonsignificant results ( $p_{RF} = 0.34$  and  $p_{ANA} = 0.45$ ).

Table 1. Frequencies (%) of -670 Fas genotype in JIA cases and controls.

Sample group	Genotype Frequency (%)		
	AA	AG	GG
Systemic JIA (n = 57)	35.1	47.4	17.5
Persistent oligo-JIA (n = 95)	28.4	52.6	19.0
Extended oligo-JIA (n = 51)	21.6	49.0	29.4
RF- poly-JIA (n = 49)	24.5	42.9	32.6
RF+ poly-JIA (n = 43)	14.0	58.1	27.9
Enthesitis (n = 18)	38.9	50.0	11.1
Psoriatic arthritis (n = 29)	10.3	69.0	20.7
RF- JIA (n = 268)	26.5	51.1	22.4
RF+ JIA (n = 43)	16.3	55.8	27.9
ANA- JIA (n = 209)	27.3	51.7	21.0
ANA+ JIA (n = 95)	23.1	49.5	27.4
All JIA (n = 342)	25.1	51.8	23.1
Controls (n = 255)	26.7	54.5	18.8

Oligo-JIA: oligoarthritis; poly-JIA: polyarthritis; enthesitis: enthesitis-related arthritis.

DISCUSSION

Both animal models of disease and serological studies in autoimmune conditions point toward the involvement of Fas in disease etiopathogenesis<sup>8</sup>. Elevated levels of Fas in the SF of patients with JIA suggest a role for the molecule, although its exact contribution has not yet been elucidated. This study used a case-control association analysis to investigate the involvement of the -670 SNP in Fas upregulation. This polymorphism does not appear to have a role in susceptibility to JIA within the UK. Moreover, it does not seem to be associated with RF nor ANA positivity within JIA. The observed increase in Fas transcription, however, may be explained by the contribution of other polymorphisms within the same gene.

Fas functions on the top of a complex process, a pathway governed by the presence of feedback loops and regulatory mechanisms<sup>4</sup>. Variations in the genetic, transcriptional, or translational control levels of modulatory molecules can affect their function and result in the increased induction of Fas production. We have additionally considered Bcl-2 to be a likely contributory molecule, as its expression was shown to be decreased in SF T cells in RA and polyarticular JIA<sup>10</sup>. However, in an association study (unpublished data) we have found no statistically significant differences between JIA cases and controls for a Bcl-2 intragenic microsatellite marker ( $p = 0.35$ ).

TNF receptor II (TNF-RII), another pro-apoptotic receptor belonging to the same family as Fas, has also been found in augmented levels in JIA SF<sup>13</sup>. The pathways that these 2 molecules mediate are very similar and share many regulatory molecules, therefore implying that their overproduction might have a common underlying cause.

This study has shown that the -670 Fas SNP is not associated with JIA susceptibility within a UK Caucasian population. This finding will require replication in patients with JIA from different populations.

**APPENDIX:** The British Paediatric Rheumatology Study Group: Drs. M. Abinun, M. Becker, A. Bell, Professor A. Craft, Drs. E. Crawley, J. David, H. Foster, J. Gardener-Medwin, J. Griffin, A. Hall, M. Hall, A. Herrick, P. Hollingworth, L. Holt, S. Jones, G. Pountain, C. Ryder, Prof. T. Southwood, Drs. I. Stewart, H. Venning, Prof. P. Woo, Dr. S. Wyatt.

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