Non-HLA gene polymorphisms in juvenile rheumatoid arthritis

P. Rosen, S. Thompson, D. Glass

Cincinnati Children’s Hospital Medical Center, Cincinnati, Ohio, USA.
Paul Rosen, MD, Susan Thompson, PhD, David Glass, MD.
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Please address correspondence and reprint requests to: David Glass, MD, Division of Pediatric Rheumatology, Children’s Hospital Medical Center Pavilion 1-129, Elland and Bethesda Avenues, 45229 Cincinnati, Ohio, USA. E-mail: David.Glass@cchmc.org

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Key words: JRA/JIA, susceptibility, non-HLA, genetics.

Abbreviations:
OR (odds ratio);
RR (relative risk);
SOJRA (systemic onset juvenile rheumatoid arthritis);
SAA (serum amyloid A protein);
Ig (Immunoglobulin);
JCA (juvenile chronic arthritis);
JCP (juvenile chronic polyarthritis);
JIA (juvenile idiopathic arthritis);
JRA (juvenile rheumatoid arthritis);
EOPA (early-onset pauciarticular);
HLA (human leukocyte antigen);
HPA (hypothalamic-pituitary-adrenal);
HPG (hypothalamic-pituitary-gonadal);
CRH (coricotomy-releasing hormone);
ESR1 (estrogen receptor 1);
PRL (prolactin);
IFNA1 (interferon-α1);
IFNG (interferon-γ);
SNP (single nucleotide polymorphism).

*Odds ratios calculated by Rosen, Thompson and Glass.

ABSTRACT
A substantial amount of work has gone into elucidating the non-HLA genetic associations in JRA. In this paper, we attempt to provide an overview of this body of knowledge. Direct comparison of the different studies is difficult. Different ethnic populations, different JRA/JIA subgroups, and different systems of nomenclature and classification all impose various limitations. Adding to the complexity is the polygenic nature of chronic childhood arthritis. Family based studies will be necessary to overcome ethnicity related issues. A candidate gene approach complemented by genome wide screen data will hopefully advance our knowledge of the genetics of JRA.

Introduction
For over three decades, researchers have been working to elucidate gene based mechanisms underlying JRA. Since a family history of JRA is rare, a genetic component is not readily apparent. However, a family history of autoimmunity is common. It is now evident that juvenile rheumatoid arthritis is a complex genetic trait or a series of such traits (1). The genetic components of the pathogenesis can be considered as two elements: HLA and non-HLA. The HLA effect is recognized with JRA subtype specific Class I and Class II associations for both susceptibility and protective effects. Additional MHC coded non-traditional HLA polymorphisms may also be relevant. In addition, genetic linkage has been demonstrated (2-4). The non-HLA associations in JRA have been less reproducible.

In this paper, we review the work that has been done on non-HLA associations in JRA (Table I). We report odds ratios (OR) to quantify the effects of these genetic variations. The significance of these different genetic polymorphisms is commented on.

Data interpretation is complicated by the use of alternative patient classifications. These include juvenile rheumatoid arthritis (JRA), juvenile chronic arthritis (JCA) and juvenile idiopathic arthritis (JIA). Although similar, these classifications differ with respect to inclusion and exclusion criteria (5).

Amyloid P
Amyloidosis develops in a small proportion of children with systemic onset JRA (SOJRA) some years after onset of disease. SOJRA patients with high inflammatory activity have high levels of serum amyloid A (SAA) protein. This protein is the precursor of amyloid fibers. However, the high SAA levels in SOJRA patients do not distinguish patients with amyloidosis from those without amyloidosis. A possible genetic susceptibility to develop amyloidosis has been identified (6). A DNA polymorphic site, distinguishable by digestion with the restriction endonuclease MspI, and 5’ to the serum amyloid P component gene, was found to be associated with the development of amyloidosis in SOJRA patients. Examination of allele frequencies showed that SOJRA patients lacking the MspI site were more likely to develop amyloidosis compared to SOJRA patients without the polymorphism (OR = 2.4*).

Immunoglobulin A
IgA deficiency is associated with JRA (7-9). The incidence of IgA deficiency in juvenile polyarthritis patients has been reported as 8% (9). The prevalence of IgA deficiency in the general population has been calculated as 0.1% (10). When the sera of 582 children with polyarticular course JRA were examined for immunoglobulin deficiency, the children in the pauciarticular onset group were found to be at highest risk for IgA deficiency (8)(4).
## Table I.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Study Design</th>
<th>Subtype of JRA / JIA</th>
<th>Odds Ratio</th>
<th>Ethnicity</th>
<th>Reference</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amyloid P</td>
<td>Case Control</td>
<td>JA with amyloidosis</td>
<td>2.4</td>
<td>Northern European (UK)</td>
<td>[6][2]</td>
<td>No replication</td>
</tr>
<tr>
<td>IgA Deficiency</td>
<td>Case Control</td>
<td>Panniculitis, polyarticular course JRA</td>
<td>2.5</td>
<td>European (UK)</td>
<td>Northenm</td>
<td>[8][4]</td>
</tr>
<tr>
<td>Immunoglobulin Genes (IgG allotypes)</td>
<td>Case control with family derived haplotypes – historical controls (Dutch)</td>
<td>Panniculitis JCA</td>
<td>Small sample size</td>
<td>Northern European (UK)</td>
<td>[11][7]</td>
<td></td>
</tr>
<tr>
<td>tα-1-antitrypsin</td>
<td>Case Control</td>
<td>Polyarticular JCP</td>
<td>7.2</td>
<td>Northern European (UK)</td>
<td>[12][8]</td>
<td>No replication</td>
</tr>
<tr>
<td>IL1α SNP (promoter polymorphisms)</td>
<td>Case Control</td>
<td>Oligo (early panu) JCA</td>
<td>2.1</td>
<td>Northern European (Norwegian)</td>
<td>[15][9]</td>
<td></td>
</tr>
<tr>
<td>IL1α (a) SNP (b) intronic microsatellite</td>
<td>Case Control</td>
<td>Oligo JCA</td>
<td>0.7</td>
<td>Northern European (UK)</td>
<td>[16][10]</td>
<td></td>
</tr>
<tr>
<td>IL1α (SNP promoter)</td>
<td>Case Control</td>
<td>Oligo JIA</td>
<td>0.8</td>
<td>Northern European (UK)</td>
<td>[17][11]</td>
<td></td>
</tr>
<tr>
<td>IL-10 (SNP haplotype, 5 prime flanking region)</td>
<td>Case Control</td>
<td>Extended Oligo</td>
<td>1.7 (extended Oligo)</td>
<td>Northern European (UK)</td>
<td>[18][12]</td>
<td></td>
</tr>
<tr>
<td>IL-10 (SNP promoter)</td>
<td>Case Control</td>
<td>All JIA</td>
<td>1.3</td>
<td>Northern European (UK)</td>
<td>[17][11]</td>
<td></td>
</tr>
<tr>
<td>NRAMP1 (promoter polymorphisms and D251471 oligonucleotide repeat)</td>
<td>Case Control</td>
<td>Panniculitis Poly JRA</td>
<td>Allele2: 0.4 Allele3: 2.3</td>
<td>Northern European (Latvia)</td>
<td>[20][14]</td>
<td></td>
</tr>
<tr>
<td>TNFα (SNP -1031C,-863A,-857T)</td>
<td>Case Control</td>
<td>Systemic JRA</td>
<td>-1031C 1.8 -863A 1.8 -857T 1.8</td>
<td>(Far East)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNFα (SNPs promoter introns and exons)</td>
<td>Case Control</td>
<td>JIA</td>
<td>Turkish: -238A 0.7 -308A 0.8</td>
<td>Middle East Central Europe (Turkey/Czech)</td>
<td>[41][17]</td>
<td></td>
</tr>
<tr>
<td>IRF1 (microsatellite 3 prime UTR)</td>
<td>Case Control</td>
<td>JIA</td>
<td>All oligo: 2.0-2.5 Persistent oligo only: 2.4-4.0</td>
<td>Northern European (UK)</td>
<td>[17][11]</td>
<td></td>
</tr>
<tr>
<td>MICA Allele4 (179bp haplotype)</td>
<td>Case Control</td>
<td>JIA</td>
<td>100bp 4.4 11bp 0.1</td>
<td>Northern European (Latvia)</td>
<td>[26][18]</td>
<td></td>
</tr>
<tr>
<td>TCRVβ6.1 (Bg1 II RFLP)</td>
<td>Case Control</td>
<td>EOPA-JRA</td>
<td>6.0</td>
<td>North America (Caucasian)</td>
<td>[29][20]</td>
<td></td>
</tr>
<tr>
<td>TCRVβ6.1 (Intronic oligonucleotide repeat (null gene)</td>
<td>Case Control</td>
<td>Polyarticular disease course</td>
<td>1.8</td>
<td>North America (Caucasian)</td>
<td>[31][22]</td>
<td></td>
</tr>
<tr>
<td>TCRVβ6.1 null gene</td>
<td>Case Control</td>
<td>Panniculitis-onset JRA</td>
<td>1.4</td>
<td>North America (Caucasian)</td>
<td>[32][23]</td>
<td></td>
</tr>
<tr>
<td>MIF (SNP 5’ prime flanking)</td>
<td>Case Control</td>
<td>Systemic Onset JCA</td>
<td>2.3</td>
<td>Northern European (UK)</td>
<td>[17][25]</td>
<td></td>
</tr>
<tr>
<td>MIF (5’ prime SNP -173 M4C allele SNP haplotypes)</td>
<td>Case Control</td>
<td>JIA</td>
<td>1.9</td>
<td>Northern European (UK)</td>
<td>[33][26]</td>
<td></td>
</tr>
<tr>
<td>IL-6 (5’-flanking region SNP)</td>
<td>Case Control</td>
<td>Systemic JCA</td>
<td>1.4</td>
<td>Northern European (UK)</td>
<td>[38][31]</td>
<td></td>
</tr>
<tr>
<td>IL-6 (Promoter SNP)</td>
<td>Case Control</td>
<td>All JIA</td>
<td>1.3</td>
<td>Northern European (UK)</td>
<td>[17][11]</td>
<td></td>
</tr>
<tr>
<td>Neurogenic candidate genes (SNP and intronse microsatellites, CRH, ESR1, PRL)</td>
<td>Case Control</td>
<td>JIA</td>
<td>CRH 1.0 ESR1 1.0 PRL 1.1</td>
<td>Northern European (UK)</td>
<td>[42]</td>
<td></td>
</tr>
<tr>
<td>IL-4 (Promoter SNP)</td>
<td>Case Control</td>
<td>JIA</td>
<td>1.0</td>
<td>Northern European (UK)</td>
<td>[17][11]</td>
<td></td>
</tr>
<tr>
<td>IL-4 (Promoter SNP)</td>
<td>Case Control</td>
<td>Early Poly JRA</td>
<td>2.0 IgM RF Poly</td>
<td>North American (Caucasian)</td>
<td>[43][36]</td>
<td></td>
</tr>
</tbody>
</table>
Pauciarticular onset JRA patients were more likely to have no or low serum IgA levels when compared to the combined group of systemic onset and polyarticular onset JRA patients (OR = 2.5*).

Immunoglobulin allotypes
Ig genetic markers have been associated with immune responsiveness and disease susceptibility. Hall et al. typed 20 British Caucasian pauciarticular JCA patients and their families for multiple Ig allotypes including Gm and Km. The Gm and Km phenotypes of the patients and families (75 individuals) were compared to those of 798 controls. The authors concluded that the study provided no evidence of an association between pauciarticular JCA patients and immunoglobulin subtypes (11).

Alpha-1-antitrypsin
Alpha-1-protease inhibitor (α-1-antitrypsin) inhibits human granulocyte collagenase and elastase. Deficient α-1-antitrypsin activity may predispose an individual to joint damage. Inheritance of the Z allele has been associated with decreased α1-antitrypsin activity. To investigate if deficient alleles were more common in juvenile polyarthritis patients, 96 English Caucasian patients were studied (12). An increased frequency of the Z allele was found in the juvenile polyarthritis patients compared to 4,565 controls. The study found that 10.4% of patients carried the Z allele compared to 1.6% of the controls (OR=7.2*). This finding to our knowledge has not been confirmed.

Interleukin-10
IL-10 is an anti-inflammatory cytokine that inhibits the synthesis of the proinflammatory cytokines IL-1α, IL-1β, IL-6, IL-8, IL-12, and TNFα. IL-10 also blocks the action of IL-1α and IL-1β by increasing the release of soluble IL-1 receptor antagonist. Because of these properties, IL-10 may play an anti-inflammatory role in arthritis. The ATA haplotype region was shown to be associated with low IL-10 production in transient transfection studies and whole-blood culture (18). In the same report, the ATA haplotype of the IL-10 polymorphic Z′ flanking region was studied to determine if a genetically determined low level of IL-10 production may play a role in JRA. Eighty-six British Caucasian oligoarthritis patients and 78 British Caucasian extended oligoarthritis patients were compared with 274 controls. While there was no association of the ATA haplotype with oligoarthritis (OR=0.9*), a significant association was found with extended oligoarticular JRA (OR=1.7*) (18).

In a separate study, the frequency of the ATA haplotype was determined in the parents of children with JIA. The parents were studied so that any observable difference in IL-10 production would not be attributed to medication received by the children. The results showed an association between the ATA haplotype in the parents and polyarticular JIA in the children (OR = 1.7) (19). Examination of the ATA genotype by Donn et al. comparing all JIA patients (n = 348) to controls (n = 239) revealed a significant association (17) (OR=1.3*).

Natural resistance-associated macrophage protein (NRAMP)
NRAMP has multiple effects on macrophage function. NRAMP modulates chemokine/ cytokine neutrophil chemotactant KC genes, TNFα, IL-1β, inducible nitric oxide synthetase, and MHC class II expression. These features make NRAMP a likely candidate for a susceptibility trait in autoimmune diseases. A functional repeat polymorphism in the promoter of NRAMP1 was studied using 190 subjects of Latvian descent (102 JRA patients, 88 controls) and 40 subjects of Russian descent (17 JRA patients, 23 controls). Significant associations for allele 2 and allele 3 were found. Allele 2 conferred a protective effect against the development of JRA (OR = 0.4). Allele 3 conferred an increased risk for development of JRA (OR = 2.3) (20).

The NRAMP1 allele conferring susceptibility to JRA drives high levels of NRAMP1 expression, while the allele associated with protection drives low levels. These two alleles are inversely associated with susceptibility to infectious disease. This finding is consistent with their maintenance in populations through balancing selection. To our knowledge no additional cohorts have been studied.

Tumor necrosis factor α
Located in the Class III region of the MHC, TNFα is one of the central cytokines involved in the pathogenesis of JRA (21-23). Epplen et al. reported an increased relative risk (2.2) for a TNFα microsatellite polymorphism in female EOPA-JCA patients (21). The relative risk (RR) rose to 12.8 in patients with HLA-DRB1*11. A study from Japan analyzed TNFα promoter polymorphisms (24). The fre-
quantities of the TNFα -1031C, -863A and -857T alleles were compared for 111 JRA patients and 575 controls (24). When only the 50 systemic JRA patients were considered, there were significant associations with the allele frequencies: -1031C allele (OR=1.9*), -863A allele (OR=1.7*) and the -857T allele (OR=2.1*). An increased odds ratio (3.8) was found when both TNFα -857T alleles were present with the inheritance of DRB1*0405 (25).

Another study examined the TNFα G A -238 and G A -308 polymorphisms in Turkish and Czech JIA patients (25). The polymorphisms were associated with different effects in the two different populations. In the Turkish population the -238A polymorphism was slightly protective (OR = 0.7*), whereas in the Czech population this polymorphism conferred susceptibility (OR = 1.5*). Analysis of -308A polymorphism conferred slight protection in the Turkish population (OR = 0.8*), compared to a risk for susceptibility in the Czech population (OR = 2.0*).

Zake et al. studied TNFα microsatellite polymorphisms in 112 Latvian JIA patients and 108 controls. Thirteen alleles were identified. The authors reported an association of allele TNFα2 with JIA patients (OR = 4.4). Allele TNFα9 conferred a protective effect for the development of JIA (OR = 0.1) (26). A negative finding for association of the TNFα for JRA patients was also reported as part of this study.

Zeggini et al. analyzed 14 single-nucleotide polymorphisms (SNPs) in the TNFα gene in 144 simplex families with an affected child and 88 controls (27). There were eight SNPs in the TNFα promoter region, four SNPs in intron 1, one SNP in intron 3, and one SNP in exon 3. Significant associations were found between juvenile oligoarthritis patients and four of the TNFα SNPs. The G A nucleotide substitution at position -308 in the promoter region also showed a positive association (OR = 2.1). The -238 G A substitution in the promoter region also showed a positive association (OR = 2.5). At position 489, the G A substitution was associated with susceptibility (OR = 2.3), as in the Czech population studied by Ozen et al. (25). An association was found with a position 851 A G substitution (OR = 2.0). Considering only the persistent oligoarthritis patients, stronger associations were found (-308 A, OR = 3.1 - 238A, OR = 4.0; +489A, OR = 2.4; +851G, OR = 3.9) (27).

Interferon regulatory factor 1

IFR-1 is a transcription factor involved in the regulation and expression of IFNα-, IFNβ-, and IFNγ-inducible genes (17). IFR-1 deficiency results in an elevation of Th2 cytokines (17). Donn et al. analyzed the association between a single base A G transition in the IFR-1 3'-untranslated region (3'UTR). An association was found when genotype frequencies were compared between the total JIA patient group and controls (17) (OR=3.3*).

Major histocompatibility complex class I chain related (MIC) A gene

MICA gene is a polymorphic sequence localized to the HLA region. It is believed to play a role in autoimmunity. MICA was analyzed in Latvian JIA patients by Zake et al. (26). Five MICA alleles were examined in patients and controls. MICA allele A4 (179bp) was significantly increased in the JIA group (OR = 2.3) (26).

T-cell receptor variable genes

The antigen-specific T-cell receptor (TCR) recognizes antigen in the context of HLA molecules. HLA-DQA1*0101 is positively associated with the progression to polyarticular disease in EOPA-JRA patients (28). TCR variable (V) region genes directly influence antigen-MHC recognition. Two alleles (12.5 kb and 5.7 kb) have been defined for TCR-βV6.1 based on restriction fragment sizes resulting from Bgl II digestion. The TCR-βV6.1 allele was examined in 126 Caucasian EOPA-JRA patients and 207 Caucasian controls (29). Initial analysis revealed that the TCR-βV6.1 allelic frequencies did not differ significantly between the patient and control populations. When comparisons were made between subjects who were positive for the HLA class II allele, HLA-DQA1*0101, a significant association was found. HLA-DQA1*0101 patients with the TCR-βV6.1 12.5 kb Bgl II fragment had a higher susceptibility than HLA-DQA1*0101 controls who were lacking the TCR-βV6.1 12.5 kb Bgl II fragment (OR = 6.0*) (29). A Norwegian population was used to analyze the role of TCRBV6S1*2 allele in conferring susceptibility to pauciarticular JCA among DQA1*0101-positive patients (30). No association was found.

There are also polymorphisms of TCR-βV6.1 that result in a non-functional gene (31). These null alleles are due to a tyrosine substitution of a highly conserved cysteine residue at position 92. Loss of cysteine prevents the formation of disulfide bridges. Thus, the gene product cannot participate in the formation of a functional TCR. The TCR-βV6.1 null allele was analyzed in 42 American pauciarticular-onset JRA patients and 56 Caucasian controls (32). The null allele showed a mild association with disease susceptibility (OR=1.4*).

In a larger study, Epplen et al. (see also the list in the Discussion section) examined TCRBV6S1 alleles in a German population of 120 EOPA-JCA patients and over 500 controls (21). They reported no association with TCRBV 6S1 in HLA-DQA*0101 JCA female patients (21). From Cincinnati (USA) an association was reported between HLA-DQA1*0101 EOPA individuals and the BV6S1 null allele (29). To determine whether the association was present in other clinical groups, the Cincinnati investigators analyzed BV6S1 genotypes for TCRBV6S1 null alleles in 316 JRA patients (166 pauciarticular onset, 118 polyarticular onset, and 32 systemic onset) and 190 controls (30). Among the 205 patients with a polyarticular course, there was an association with the TCRBV6S1 null allele (OR = 1.8*). This association was most prominent among patients with early onset disease.

Macrophage migration inhibitory factor

MIF up-regulates macrophage intracel-
ular killing and phagocytosis. TNFα secretion by macrophages increases in the presence of MIF. Macrophage derived MIF promotes secretion of pro-inflammatory cytokines (IL-1, IL-6, IL-8, and TNFα). MIF has a role in autoimmune conditions and is involved in inflammatory arthritis (17). A SNP in the 5'-flanking region of the MIF gene was identified as a G → C transition at position -173. Comparison of MIF-173 genotypes between 117 British systemic-onset JIA patients and 172 controls showed a significant association with the C allele (OR = 2.3) (17). Study of MIF polymorphisms was extended to include all JIA subgroups (33). A tetranucleotide repeat and three SNPs (including -173 G → C) were identified. Genotyping was performed in 526 UK Caucasian JIA patients and in 259 controls. In agreement with the earlier finding, the -173-MIF*G allele conferred increased risk of susceptibility to JIA (OR = 1.9) (33). A similar association was seen among the systemic JIA patients (OR = 2.2*).

De Benedetti et al. extended these findings by providing evidence that carriage of a MIF-173*C allele is associated with worse clinical outcomes (34). These include an increased number of joints with active arthritis, a shorter clinical response to triamcinolone hexacetone intraarticular injection, increased systemic glucocorticoid requirement, and poorer functional outcome.

**Chromosomal deletions**

Chromosome 22q11.2 deletion syndrome (velocardiofacial syndrome) has a characteristic phenotype that includes hypocalcemia, immunodeficiency, and conotruncal cardiac anomalies (35). In addition, there is an association of chromosome 22q11.2 deletion syndrome with polyarthritis (35). The prevalence of polyarthritis in this population is 50 times greater than that seen in the general population (35). There are also case reports of arthritis in patients with deletion 18q syndrome (36, 37).

**Interleukin-6**

IL-6 is elevated in the serum and synovial fluid of inflamed joints (38-40). In systemic JCA patients, the IL-6 levels rise and fall in the serum parallels the quotidian fever seen clinically (38). Analysis of a polymorphism in the 5′ flanking region in the IL-6 gene was studied in 92 systemic-JCA patients and 383 controls (38). There was an association of a -174 G → C polymorphism with systemic-JCA (OR = 1.4*).

**Table II. Examples of polymorphic, moderate to low risk variants.**

<table>
<thead>
<tr>
<th>Disease*</th>
<th>Locus</th>
<th>Frequency</th>
<th>Relative risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alzheimer's disease</td>
<td>APOE-APOE4</td>
<td>0.09-0.22</td>
<td>4.0-15.0</td>
</tr>
<tr>
<td></td>
<td>APOE-APOE2</td>
<td>0.04-0.08</td>
<td>0.5</td>
</tr>
<tr>
<td>Thrombosis</td>
<td>Factor V Leiden</td>
<td>0.09-0.08 (Eur)</td>
<td>5.0-10.0</td>
</tr>
<tr>
<td>Hemochromatosis</td>
<td>Hfe</td>
<td>0.02-0.22</td>
<td>4.0</td>
</tr>
<tr>
<td>NIDDM</td>
<td>PPARγ</td>
<td>0.85 (Eur)</td>
<td>1.5-2.5</td>
</tr>
<tr>
<td>HIV</td>
<td>CCR5</td>
<td>0.01-0.14 (Eur)</td>
<td>High (resistance), moderate (non-progression)</td>
</tr>
<tr>
<td>Crohn's disease</td>
<td>NOD2/CARD15</td>
<td>0.02 (Eur)</td>
<td>6.0</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>CRCA2</td>
<td>0.25 (Eur)</td>
<td>1.3</td>
</tr>
<tr>
<td>Colon cancer</td>
<td>APC</td>
<td>0.03 (AJ)</td>
<td>2.0</td>
</tr>
<tr>
<td>Neural tube defects</td>
<td>MTHFR</td>
<td>0.30 (Eur)</td>
<td>2.0</td>
</tr>
<tr>
<td>FMF</td>
<td>MEFV</td>
<td>0.02 (AJ)</td>
<td>7.0</td>
</tr>
<tr>
<td>Graves disease</td>
<td>CTLA4</td>
<td>0.35 (Eur)</td>
<td>1.5-2.0</td>
</tr>
<tr>
<td>Creutzfeld-Jakob</td>
<td>PRNP</td>
<td>0.65 (Eur)</td>
<td>3.0</td>
</tr>
<tr>
<td>Autoimmune diseases</td>
<td>HLA B, DR, DQ</td>
<td>Polymorphic</td>
<td>Low to moderate</td>
</tr>
</tbody>
</table>


In contrast, the analysis of the -174 G → C transition by Donn et al. (17) bore out a protective effect for the polymorphism in systemic JIA patients (OR = 0.6*). When JIA patients of all subtypes were considered, the protective effect was lost (OR = 1.3*).

A more recent multicenter study demonstrated an IL-6 gene effect using the same gene polymorphisms. The design was family based and utilized TDT (41).

**Neuroendocrine genes**

Gene polymorphisms of the hypothalamo-pituitary-adrenal (HPA) axis and the hypothalamic-pituitary-gonadal (HPG) axis can affect the development of autoimmune disease (47). Corticotrophin-releasing hormone (CRH) is the main mediator of cortisol secretion and subsequent anti-inflammatory effects. Estrogen receptor 1 (ESR1) is both the main receptor for estrogen and a member of a superfamily of nuclear receptors that transduce extracellular signals into transcriptional responses. Prolactin (PRL) is a hormone with immunoregulatory capacity (47). Donn et al. analyzed genetic markers within the CRH, ESR1, and PRL genes to discover a potential role in JIA (47). Polymorphisms in all three neuroendocrine genes were not associated with JIA susceptibility (47) (CRH, OR = 1.0*; ESR1, OR = 1.0*; PRL, OR = 1.1*).

**Interferons/ Interleukin 2/ Interleukin 4**

Interferon-α1 (IFNA1) and interferon-γ (IFNG) are part of the immune response against viral infection. Donn et al. investigated microsatellites within both genes and found no significant association with JIA (17).

No significant association between IL-2 polymorphisms or IL-4 polymorphisms and JIA were reported by Donn et al. (17). An association was, however, reported between early polyarticular JRA patients and an IL-4 SNP in North American Caucasian patients (OR = 2.0) (43).

**Discussion and conclusions**

Consensus on the role of non-HLA genes has been difficult. Few observa-
tions would meet the firm criteria for significant association with odds ratios consistently above 2. Polymorphisms that meet these criteria are limited. They would likely include IL-6 and MIF.

The Vβ6.1 observation illustrates some of the challenges. Replication studies that were positive in the USA were equivocal or negative in Europe. Do these TCR data represent false or true positives? It is difficult to be certain at this stage despite the considerable effort that has gone into generating these observations. Polymorphisms in null genes have potential biological importance. IL-10 gene polymorphisms have been associated with many autoimmune diseases. However, the low odds ratios do not substantiate a significant genetic effect. Replication in TDT may be helpful in this situation. These data illustrate the problems in elucidating the extent of complex traits beyond the major effects of HLA in JRA. The problem is demonstrated by the low odds ratios reported in JRA studies. This compares with a recent analysis of the relatively few non-MHC gene associations reported in other complex traits (44).

The higher number relative risks in these studies provides greater confidence in the relevance of the observations (Table II). The levels of odds ratios in this dataset from this review are noteworthy. The JRA findings reviewed in this report may or may not have similar significance.

Confounding issues include:

- Failure to replicate
- Population stratification
  - Use of TDT instead of case control studies may be a solution

Individual variability

- As seen with HLA and JRA

Cohort size

Dataset interpretation variability

Classification heterogeneity

JRA, JCA, JIA

Patient pooling

Subtype specific findings may be masked

Statistical power

Diffs for sib pairs studies, case control studies, and multiplex family studies

Age-related effects.

- Seen in HLA and non-HLA polymorphisms
- These are not always incorporated into analyses.

Linkage disequilibrium.

- Population variability.
- Chromosome location variability

(45, 46)

Candidate gene studies, complemented by genome wide screens, will be more robust if these variables are factored into the design especially for the detection of small genetic effects. The use of population sensitive genetic markers would be important in case control studies (46). Transmission disequilibrium testing has advantages and working with a pediatric population given the availability of parents and grandparents.

For genome wide screens, a focus on SNPs would help to create extended datasets and lead to more powerful analytic tools, particularly promoter and exonic polymorphisms. Many such SNPs have significant functional effects.

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