Pediatric rheumatology review

Non-HLA gene polymorphisms in juvenile rheumatoid arthritis

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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 pacuciarticular);
HLA   (human leukocyte antigen);
HPA   (hypothalamic-pituitary-adrenal);
HPG   (hypothalamic-pituitary-gonadal);
CRH   (coricotrophin-releasing hormone);
ESR1   (estrogen receptor 1);
PRL   (prolactin);
IFNA1 (interferon- 1);
IFNG   (interferon-);
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 auto-immunity 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.

Marker	Study Design	Subtype of JRA / JIA	Odds Ratio	Ethnicity	Reference	Comments
Amyloid P	Case Control	JA with amyloidosis	2.4	Northern European (UK)	[6][2]	No replication
IgA Deficiency	Case Control	Pauciarticular onset, polyarticular course JRA	2.5	Northern European (UK)	[8][4]	
Immunoglobulin Genes (IgG allotypes)	Case control with family derived haplotypes – historical controls (Dutch)	Pauciarticular JCA	Small sample size	Northern European (UK)	[11][7]	
-1-antitrypsin	Case Control	Polyarticular JCP	7.2	Northern European (UK)	[12][8]	No replication
IL1 SNP (promoter polymorphisms)	Case Control	Oligo (early pauci) JCA	2.1 (6.2 for chronic iridocyclitis)	Northern European (Norwegian)	[15][9]	
IL1 (a) SNP (b) intronic microsatellite	Case Control	Oligo JCA	0.7	Northern European (UK)	[16][10]	
IL1 (SNP promoter)	Case Control	Oligo JIA	0.8	Northern European (UK)	[17][11]	
IL-10 (SNP haplotype, 5 prime flanking region)	Case Control	Extended Oligo	1.7 (extended Oligo) 0.9 (Oligo)	Northern European (UK)	[18][12]	
IL-10 (SNP promoter)	Case Control	All JIA	1.3	Northern European (UK)	[17][11]	
NRAMP1 (promoter polymorphisms and D2S1471 oligonucleotide repeat)	Case Control	Pauci and Poly	Allele2: 0.4 Allele3: 2.3	Northern European (Latvia)	[20][14]	Other autoimmune disease, Crohn's disease and diabetes. No replication in JRA
TNF (SNP -1031C, -863A, -857T)	Case Control	Systemic JRA	-1031C 1.8 -863A 1.8 857T 1.8	(Far East)		
TNF (SNPs 238A, -308A promoter region)	Case Control	JIA	Turkish: -238A 0.7 -308A 0.8 Czech: -238A 1.5 -308A 2.0	Middle East Central Europe (Turkey/Czech)	[41] [17]	No significant association with disease overall but association of 308A with poor outcome in Turkish group
TNF (microsatellites) 13 alleles	Case Control	JIA	100bp 4.4 114bp 0.1	Northern European (Latvia)	[26][18]	
TNF (SNPs promoter introns and exons)	Case Control (Family based haplotypes) TDT	JIA (oligo- arthritis)	All oligo: 2.0-2.5 Persistent oligo only: 2.4-4.0	Northern European (UK)	[33][19]	
IRF1 (microsatellite 3 prime UTR)	Case Control	All JIA	3.3	Northern Europe (U.K.)	[17][11]	
MICA Allele4 (179bp)	Case Control	JIA	2.3	Northern European (Latvia)	[26][18]	An MHC gene, nonclassical HLA
TCR V 6.1 (Bg1 II RFLP)	Case Control	EOPA-JRA	6.0	North America (Caucasian)	[29][20]	Among HLA-DQA1*0101 patients
TCRV 6.1 (Intronic oligonucleotide repeat) (null gene)	Case Control	Polyarticular disease course	1.8	North America (Caucasian)	[31][22]	A replication study
TCRV 6.1 null gene	Case Control	Pauciarticular-onset JRA	1.4	North America (Caucasian)	[32][23]	A replication study
TCRV 6S1*2		Pauciarticular	1.1	Northern European (Norwegian)	[30][24]	
MIF (SNP 5' prime flanking)	Case Control	Systemic Onset JCA	2.3	Northern European (UK)	[17][25]	Systemic subtype only
MIF (5' prime SNP -173 M*C allele SNP haplotypes)	Case Control	JIA Systemic JIA	1.9	Northern European (UK)	[33][26]	
IL-6 (5'-flanking region SNP)	Case Control	Systemic JCA	1.4	Northern European (UK)	[38][31]	
IL-6 (Promoter SNP)	Case Control	All JIA Systemic	1.3 0.6	Northern European (UK)	[17][11]	
Neurogenic candidate genes (SNPs and introgenic micro-satellites, CRH, ESR1, PRL)	Case Control	JIA	CRH 1.0 ESR1 1.0 PRL 1.1	Northern European (UK)	[42]	Corticotrophin Release Hormone (CRH) Estrogen Receptor 1 (ESR1) Prolactin (PRL)
IL-4 (Promoter SNP)	Case Control	JIA	1.0	Northern European (UK)	[17] [11]	
IL-4 (Promoter SNP)	Family Based Associated (TDT)	Early Poly JRA	2.0 IgM RF Poly)	North American (Caucasian)	[43][36]	

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 (-1-antitrypsin) inhibits human granulocyte collagenase and elastase. Deficient -1-antitrypsin activity may predispose an individual to joint damage. Inheritance of the Z allele has been associated with de-creased -1-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 1 alpha

IL-1 is a potent pro-inflammatory cytokine that has catabolic effects on cartilage (13) and bone (14). It increases the release of inflammatory mediators from synovial cells (15). IL-1 is also thought to contribute to the pathogenesis of inflammatory joint disease. In a study of Norwegian JRA patients, McDowell *et al.* reported an association between the promoter region polymorphism at -889 IL-1 C → T mutation among early-onset pauciarticular (EOPA) patients (OR = 2.1) (15) (9).

When early- and late-onset pauciarticular patients were considered together, the odds ratio was 1.7. In this Norwegian cohort, the -899 C → T polymorphism was more strongly associated with the EOPA-JRA patients with chronic iridocyclitis compared to those without chronic iridocyclitis (OR =6.2) (15).

Studies from Britain gave different results. Analysis of the same promoter region polymorphism in 159 UK oligoarticular JCA patients and 151 controls failed to show any association between the T allele and oligoarticular JCA patients (16) (OR = 0.7*). In another British cohort, 330 JIA patients and 236 controls were studied by Donn *et al.* (17). Examination of the polymorphism in the oligoarticular (persistent and extended) patients did not confer susceptibility for juvenile arthritis (OR = 0.8*).

Interleukin-10

IL-10 is an anti-inflammatory cytokine that inhibits the synthesis of the pro-inflammatory cytokines IL-1, IL-1, IL-6, IL-8, IL-12, and TNF. IL-10 also blocks the action of IL-1 and IL-1 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 5' flanking region haplotype 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 5' 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 oligoarticular 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 chemoattractant KC genes, TNF, IL-1, 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, TNF 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 TNF 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 TNF promoter polymorphisms (24). The fre-

quencies of the TNF -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 TNF -857T alleles were present with the inheritance of DRB1*0405 (24).

Another study examined the TNF 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 TNF microsatellite polymorphisms in 112 Latvian JIA patients and 108 controls. Thirteen alleles were identified. The authors reported an association of allele TNF 2 with JIA patients (OR = 4.4). Allele TNF 9 conferred a protective effect for the development of JIA (OR = 0.1) (26). A negative finding for association of the TNF for JRA patients was also reported as part of this study.

Zeggini et al. analyzed 14 single-nucleotide polymorphisms (SNPs) in the TNF gene in 144 simplex families with an affected child and 88 controls (27). There were eight SNPs in the TNF promoter region, four SNPs in intron 1, one SNP in intron 3, and one SNP in exon 3. Significant associations were found between juvenile oligo-arthritis patients and four of the TNF SNPs. The G → A nucleotide substitu-tion at position -308 in the promoter region also showed a positive associa-tion (OR=2.1). The -238 G → A substi-tution in the promoter region also show-ed 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 sub-stitution (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
IRF-1 is a transcription factor involved in the regulation and expression of IFN -, IFN -, and IFN -inducible genes (17). IRF-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 IRF-1 3'-untranslated region (3'UTR). An association was found when genotype frequencies were com-pared 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 con-text 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- V6.1 based on restriction fragment sizes resulting from *Bgl* II digestion.

The TCR- V6.1 allele was examined in 126 Caucasian EOPA-JRA patients and 207 Caucasian controls (29). Initial analysis revealed that the TCR- V6.1 allelic frequencies did not differ signif-icantly between the patient and control populations. When comparisons were made between subjects who were posi-tive for the HLA class II allele, *HLA-*

*DQA1*0101*, a significant association was found. *HLA-DQA1*0101* patients with the TCR- V6.1 12.5 kb *Bgl* II fragment had a higher susceptibility than *HLA-DQA1*0101* controls who were lacking the TCR- V6.1 12.5 kb *Bgl* II fragment (OR = 6.0*) (29). A Norwegian population was used to ana-lyze the role of TCRBV6S1*2 allele in conferring susceptibility to pauciarticu-lar JCA among *DQA1* 0101*-positive patients (30). No association was found.

There are also polymorphisms of TCR- V6.1 that result in a non-functional gene (31). These null alleles are due to a tyrosine substitution of a highly con-served cysteine residue at position 92. Loss of cysteine prevents the formation of disulfide bridges. Thus, the gene product cannot participate in the for-mation of a functional TCR.

The TCR- V6.1 null allele was ana-lyzed in 42 American pauciarticular-onset JRA patients and 56 Caucasian controls (32). The null allele showed a mild association with disease suscepti-bility (OR=1.4*).

In a larger study, Epplen et al. (see also the list in the Discussion section) examined TCRBV6S1 alleles in a Ger-man population of 120 EOPA-JCA patients and over 500 controls (21). They re-ported 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 investi-gators 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 dis-ease.

Macrophage migration inhibitory factor
MIF up-regulates macrophage intracel-

lular killing and phagocytosis. TNF 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 TNF). 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 genotype frequencies 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*C 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 hexacetonide 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*).

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 hypothalamic-pituitary-adrenal (HPA) axis and the hypothalamic-pituitary-gonadal (HPG) axis can affect the development of autoimmune disease (47). Coricotrophin-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- 1 (IFNA1) and interferon- (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-

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

Disease*	Locus	Frequency	Relative risk
Alzheimer's disease	APOE-APOE4	0.09-0.22	4.0-15.0
	APOE-APOE2	0.04-0.08	0.5
Thrombosis	Factor V Leiden	0.00-0.08 (Eur)	5.0-10.0
Hemochromatosis	Hfe	0.02-0.22	4.0
NIDDM	PPARg	0.85 (Eur)	1.5-2.5
HIV	CCR5	0.01-0.14 (Eur)	High (resistance), moderate (non-progression)
Crohn's disease	NOD2ICARD15	0.02 (Eur)	6.0
Breast cancer	CRCA2	0.25 (Eur)	1.3
Colon cancer	APC	0.03 (AJ)	2.0
Neural tube defects	MTHFR	0.30 (Eur)	2.0
FMF	MEFV	0.02 (AJ)	7.0
Graves disease	CTLA4	0.35 (Eur)	1.5-2.0
Creutzfeld-Jakob	PRNP	0.65 (Eur)	3.0
Autoimmune diseases	HLA B, DR, DQ	Polymorphic	Low to moderate

AJ: Ashkenazi Jews; Eur: European; FMF: familial Mediterranean fever; IDDM: insulin-dependent diabetes mellitus; NIDDM: non-insulin-dependent diabetes mellitus.
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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
- Ethnic variability
  - As seen with HLA and JRA
- Cohort size
- Individual variability
- Dataset interpretation variability
- Classification heterogeneity
  - JRA, JCA, JIA
- Patient pooling
  - Subtype specific findings may be masked
- Statistical power
  - Differs 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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