Review
Genetic epidemiology
Juvenile idiopathic arthritis genetics – What's new? What's next?

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Abstract

Studies have established the magnitude of the genetic basis of juvenile idiopathic arthritis (JIA). JIA is a complex genetic condition and the genes that influence susceptibility are actively being sought. A candidate gene approach is being used by several groups. MHC-, cytokine- and T-cell-related genes have all been positively associated with JIA. Here we review some of the latest genetic data, and discuss ways in which JIA genetic research might proceed.

Keywords: candidate genes, cytokines, genetics, juvenile idiopathic arthritis

Introduction

It is necessary to define the genetic component of any disease in order to enhance the understanding of its pathogenesis, imply its aetiology and refine its treatment. The most rapid progress towards such aims is best achieved when there is homology of the expressed form (phenotype). Unfortunately, progress in defining the genetic components of childhood arthritis has long proven difficult, for two reasons. Firstly, childhood inflammatory arthritis is not a single disease but a group of clinical syndromes. Secondly, since its first description in the late 1890s, classification of the chronic arthritides of childhood has been problematic. Ansell and Bywaters [1] first proposed that classification should be based on the characteristics of disease at onset and this basic premise still remains. This premise has led to the development of two related but importantly different classifications, juvenile chronic arthritis as defined by EULAR and juvenile rheumatoid arthritis (JRA) as defined by the American Rheumatism Association (ARA). The major differences are the disease duration (minimum of 3 months for EULAR, 6 weeks for ARA) and the fact that the EULAR criteria are inclusive of other forms of juvenile arthritis, such as juvenile ankylosing spondylitis, inflammatory bowel disease and juvenile psoriatic arthritis, whereas the ARA criteria are exclusive.

These problems have hindered genetic studies. In addition, the relatively small patient numbers in some of the disease subgroups significantly reduces the power of any study to detect a 'real' effect. Also, the determination of true genetic effects relies heavily on the replication of results in identical patient populations but transatlantic differences in classification have made this virtually impossible. In an attempt to solve these issues, a new classification system, the ILAR classification, has been developed. It aims both to unify the previous classification systems so as to minimise international differences in disease definition and to identify clinically homogenous disease subgroups within the umbrella term JIA and thus facilitate research [2].

Juvenile idiopathic arthritis (JIA) indicates a disease of childhood (i.e. less than 16 years of age) of no known aetiology, characterised by arthritis persistent for at least 6 weeks. Classification is made at 6 months after diagnosis into one of eight disease categories, each of which has its own specific characteristics, exclusions and descrip-

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ARA = American Rheumatism Association; EULAR = European League Against Rheumatism; HLA = human leukocyte antigen; IL = interleukin; ILAR = International League of Associations for Rheumatology; JIA = juvenile idiopathic arthritis; JRA = juvenile rheumatoid arthritis; λs = sibling recurrence risk; MIF = macrophage migration inhibitory factor; SNP = single nucleotide polymorphism; TCR = T-cell receptor; TNF = tumour necrosis factor.

tors. Validation of the ILAR classification is still in progress. Despite these challenges, progress within the area of JIA genetics is being achieved.

Evidence for a genetic component to juvenile idiopathic arthritis

Evidence for a genetic component to a disease can come from a variety of sources such as twin studies, family studies or association studies. As JIA is a relatively rare disease, accounts of twin and family studies are quite uncommon and often based on small numbers. Recent data from the USA and Finland, however, suggest that the genetic contribution to JIA may be quite considerable.

Within the USA, the National Institute of Arthritis and Musculoskeletal and Skin Diseases has sponsored a research registry for JRA-affected sibling pairs. Initial analysis of 71 affected sibling pairs showed that 63% were concordant for gender and 76% for onset type [3]. This study also provided the first estimate of the sibling recurrence risk (λs) for JRA; this was 15 (a value similar to those for insulin dependent diabetes mellitus and multiple sclerosis), although this is likely to vary between subgroups. Such a high λs is indicative of a factor shared between siblings – genetic or environmental. In a more recent analysis of 118 affected sibling pairs, 14 pairs of twins were identified in which both twins have arthritis. One pair comprises a girl with polyarthritis and a boy with persistent oligoarthritis. The other 13 pairs (11 monozygotic, 2 dizygotic and 2 of unknown zygosity) were concordant for gender (nine female, four male), disease onset (10 pauciarticular, 3 polyarticular) and disease course (eight pauciarticular, five polyarticular) [4].

Within Finland, 41 JIA multicase families with 88 affected siblings have been collected over a period of 15 years. This study estimates the λs of JIA to be nearer 20 [5]. Within this set of families there were eight sets of monozygotic twins, two of which were concordant for JIA. Both sets of twins were concordant for disease course but were unexpectedly different for disease onset [6]. A concordance rate of 25% for a disease with a population prevalence of 1 per 1000 implies a relative risk of 250 for a monozygotic twin. All these data (American and Finnish) taken together provide convincing evidence that there is a substantial genetic component to JIA.

Juvenile idiopathic arthritis and the MHC
Much of the genetic work undertaken in the past three decades centred round HLA genes. These earlier studies of HLA and JIA included children classified according to either the EULAR or the ACR criteria. Numerous studies of associations of JIA with both HLA class I and class II genes have been described, with the class I associations being consistently more limited than those for class II. These studies have been reviewed elsewhere [7]. More

recently, linkage to HLA has now been confirmed in two populations [8,9].

Studies of non-HLA genes within the MHC have been limited. Positive associations have recently been described, however, between *LMP7* and early-onset pauciarticular JRA, and between the gene encoding Tapasin with systemic-onset JRA [10,11].

Candidate gene selection in juvenile idiopathic arthritis

Several aspects can be considered when selecting genes for investigation in JIA. The nature of the histopathology of the inflamed synovium is one starting point. Evidence for the underlying driving force for the chronic synovitis of JIA being antigen-driven and T-cell mediated has been well documented in a recent review by Grom & Hirsch [12].

Arguably, another important starting point for genetic investigation is raised levels of protein expression in affected children. Keys to the pathogenesis of JIA may be provided by changes in the secretion patterns of particular proteins, as measured by bioassays; alternatively, these proteins could simply be present at altered levels as a result of 'bystander effects', having little to do with the pathogenic mechanism. Studying the genetic variation of such candidates should help to elucidate this 'cause' or 'effect' conundrum. To be effective the functional polymorphism(s) need to be studied. These are generally not predetermined. Hence to fully investigate a gene it may be necessary to study all the single nucleotide polymorphisms (SNPs) within it.

Cytokine gene polymorphisms and juvenile idiopathic arthritis
Tumour necrosis factor
The involvement of tumour necrosis factor (TNF) protein and its receptors in the pathology of JIA has been suggested by multiple studies. The genetic evidence in support of these observations, however, is much scarcer. Date *et al.* [13] showed the frequencies of polymorphisms at the −1031, −863 and −857 positions of the TNF promoter to be significantly higher in a group of Japanese systemic-onset JIA patients compared with those observed in controls. Also, particular alleles of a microsatellite marker in the TNF-α gene were found to be strongly associated with early-onset pauciarticular juvenile arthritis in German patients [14]. Ozen *et al.* [15] studied the TNF −308 and −238 polymorphisms in Czech and Turkish JIA patients, but found no association with either polymorphism. In contrast, Zeggini *et al.* [16] have reported positive association with TNF polymorphisms in a large panel of UK Caucasian oligoarticular JIA patients. The TNF locus is highly polymorphic and several of the SNPs that have so far been described have potential functional significance. Clearly, more studies of the polymorphisms of TNF in JIA patients are required.

Interleukin 6

Many of the clinical features of systemic-onset JIA are typical of excessive IL-6 production, for example fever, hypergammaglobulinaemia, thrombocytosis, anaemia and stunted growth. A functional polymorphism that determines the transcriptional response of the IL-6 gene to IL-1 and lipopolysaccharide was identified recently (as −174 in the regulatory region of the IL-6 gene). There was a significant lack of the protective genotype (CC: low producer of IL-6 on stimulation by IL-1/lipopolysaccharide) in children that develop systemic JIA at age 5 and under [17]. In a recent study by Pignatti et al. [18], however, this was not replicated. Similarly, the −174 polymorphism was not shown to be associated with UK systemic-onset JIA (or any other JIA subgroup) in a study by Donn et al. [19]. Further SNPs have been found and analyses of haplotypes suggest a more complex genetic regulation of IL-6 [20]. Identification of functional SNP haplotypes and re-examination of these disease cohorts will be necessary.

Interleukin 10

The hypothesis that the expression of the anti-inflammatory cytokine IL-10 is genetically lower in the more severe JIA subtype was tested by case–control and transmission disequilibrium test association studies. The production of IL-10 was lower in the parents of children with persistent oligoarticular-onset JIA, and these parents have a significantly increased frequency of nucleotide changes at positions −1082, −819 and −592 that combine to give the 'ATA' IL-10 haplotype [21]. The children with the more severe disease (extended JIA) have a significantly increased frequency of the IL-10 ATA haplotype. Transmission disequilibrium test confirmed the disease association of the IL-10 ATA allele with this group of children. In contrast, Donn et al. [19] did not find evidence of IL-10 as a susceptibility gene for JIA when the frequency of nucleotide changes at positions −1082, −819 and −592 was compared in JIA patients and controls in a large association study.

Interferon regulatory factor 1

A positive association with a novel polymorphism in the 3′ untranslated region of the interferon regulatory factor (IRF)-1 gene, which maps to a 'cytokine gene cluster' on the long arm of chromosome 5 (5q31), has been described [19]. In a study of synovial tissue cytokine mRNA expression, Scola et al. [22] found a predominantly Th1 bias, with a significant role of IL-12 in contributing to this effect. A particular allele of a variable number tandem repeat within the IL-1 receptor antagonist gene (IL1RN*2) has been studied in JIA patients and a positive association observed. Vencovsky et al. [23] also suggested that the IL1RN*2 allele could be a useful prognostic marker for extended oligoarticular JIA. The numbers included in this study were relatively small, however, suggesting that further investigation should now be considered, using a large panel of well characterised oligoarticular-JIA patients with a defined study outcome.

Macrophage migration inhibitory factor

Meazza et al. [24] have described raised levels of macrophage migration inhibitory factor (MIF) protein in Italian JIA patients. A novel polymorphism in the 5′ flanking region of the MIF gene was reported to be associated, initially with UK systemic-onset JIA [25], and subsequently with susceptibility to all types of JIA, irrelevant of subgroup [26]. MIF is a unique molecule that has pro-inflammatory, hormonal and enzymatic properties (reviewed in [27]). The unique induction of MIF that takes place at low glucocorticoid concentrations, together with its ability to counter-regulate glucocorticoid immunosuppressive actions, implies a potentially important role of MIF in the control of the immune response. The functional significance of the −173 polymorphism of MIF has now also been determined and supports the genetic association observed for JIA [28].

T cell studies and JIA

JIA is thought to be an autoimmune condition (or possibly group of conditions) in which the immune response to self-antigen, present within the inflamed joint, plays a central role. But what is the nature of this antigen? Since several well-described HLA associations are known for juvenile arthritis it has been tempting to try to extrapolate from these to suggest the initiating pathogenic organism(s). Albani [29] has shown that the Escherichia coli heat shock protein DNAJ specifically binds within the groove of HLA class II alleles known to be associated with pauciarticular juvenile chronic arthritis. Subsequently, work with synthetic peptides from E. coli DNAJ suggested that T-lymphocyte reactivity may be critical to T-cell regulatory mechanisms that affect the course of joint inflammation in oligoarticular-JIA patients [30]. In an allied approach Kamphuis et al. [31] generated putative self-epitopes in silico from the rat model of adjuvant arthritis. A selection of human analogues of the recognized peptides in adjuvant arthritis were made and tested for T-cell recognition in JIA patients, by measuring proliferative activity of peripheral blood mononuclear cells. Four of the selected peptides were recognised by 20–40% of JIA patients. Amongst these were peptides from matrix metalloproteinases, and also from proteoglycan/aggregan molecules. Such an approach has implications for the better identification of autoreactive T cells involved in JIA and the initiating micro-organisms that may be involved.

Wedderburn et al. [32], using high-resolution heteroduplex TCR analysis, have recently shown multiple clonal T-cell expansions that are present and persistent within the joints of patients with enthesitis-related (HLA-B27-positive) arthritis and oligoarthritis. The dominant T-cell subset containing these expansions showed disease-specific divergence, however, such that for the class I associated

subgroup the dominant clones were in the CD8$^+$ synovial T cell population whereas for class II associated oligoarthritis the dominant clones were within the CD4$^+$ synovial T cell population. This is supportive evidence that the recognition of MHC/peptide complexes by T cells plays a critical role in the pathogenesis of JIA [32].

It is also noteworthy that analysis of the third hypervariable region of HLA-associated alleles (HLA-DRB1*08,*11,*13) reveals that they share a common motif (FLED) in their protein sequence. Studies aimed at identifying relevant homologies to potential organisms that could be cross-reactive to FLED could offer a useful approach to further understanding JIA pathology.

Conclusion: What should be the future direction of JIA genetics?

Unified nomenclature, universally accepted and applied, would be beneficial. In addition, we need to develop ways to avoid the 'pre-emptive strike' of selecting candidate genes. The idea of identifying 'novel' transcripts of relevance to disease is obviously attractive. Expression (microarray-based) technology could be useful if the correct sample material were available, such as paired blood and synovial fluids. This would allow us to look at alterations in mRNA levels as an indication of gene activation or regulation. This type of work, however, requires serial samples (say, before and after treatment) that may not always be ethically acceptable when studying disease in children.

The majority of the genetic research conducted for JIA so far has been retrospective in nature. This may identify genes involved in disease susceptibility. Perhaps the more useful and clinically relevant genetic approach is to define genetic predictors of outcome or disease severity. This can be attempted by prospectively following a cohort of clinically well-defined patients. Again the issue of multiple samples becomes important and possibly limiting. Identifying, at initial presentation, patients who are most likely to have the more aggressive course of disease would have substantial implications for treatment interventions.

For our understanding of JIA genetics to progress, international collaborations that maximise the resource potential would appear to be the way forward. This should allow larger-scale association and linkage studies to be carried out. Coupled with the rapid advances in genomic and proteomic technologies, these studies should pave the way forward to a better understanding of this complex genetic disease.

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