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An overview of genetics of paediatric rheumatic diseases

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Abstract

The evidence so far suggests that the paediatric inflammatory diseases encountered in rheumatology practice may be largely genetic in origin, where common single nucleotide polymorphisms (SNPs) in multiple genes contribute to risk, with real but variable environmental components. As far as genetic susceptibility to common paediatric rheumatic diseases is concerned, only juvenile idiopathic arthritis (JIA) has been investigated in any substantial way so far. This article discusses susceptibility for different types of JIA, the different methods used and their advantages and disadvantages. The genetic code is also modifiable by epigenetic mechanisms and examples of these in immunity and rheumatoid arthritis are given to indicate another area of research in the elucidation of the genetics of paediatric rheumatic diseases.

Keywords

juvenile idiopathic arthritis; candidate gene association study; genome-wide association study; microarray; epigenetics

The most common group of paediatric rheumatic diseases are the juvenile idiopathic arthritides followed by juvenile-onset systemic lupus erythematosis (SLE), and rarer diseases such as juvenile dermatomyositis, primary vasculitides and scleroderma. Evidence suggests that these diseases may be largely genetic in origin, where common single nucleotide polymorphisms (SNPs) in multiple genes contribute to risk, with real but variable environmental components. There is also a group of rare autoinflammatory diseases described elsewhere, many of which result from mutations of genes that affect the innate immune response. As far as genetic susceptibility to common paediatric rheumatic diseases is concerned, only JIA has been investigated in any substantialway so far. This article discusses the state-of-the-art and future research directions.

Juvenile idiopathic arthritis

Juvenile idiopathic arthritis (JIA), as defined by the International League of Associations of Rheumatology (ILAR), is a group of persistent (> 6 weeks duration) arthritides of unknown aetiology, occurring in children from 0 to 16 years of age [1]. Classification is made 6 months after disease onset into one of seven subtypes, one of which is in fact a mixed group where the clinical picture either does not fit that of any of the categories or fits criteria for

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more than one. The purpose of this classification was to provide a scheme that would define more clearly distinctive clinical phenotypes, thus facilitating research into the underlying genetic background, disease processes, as well as prognosis and response to therapy in this group of conditions.

In the UK, the incidence of JIA as a whole is 10 per 100 000 children-years [2], whilst prevalence in Europe and North America, as reviewed by Thomson and Silman in 2001, ranges from 40 to 160 per 100 000 children-years [3]. However, incidence/prevalence rates vary depending on the subtype and within different ethnic groups. In Caucasian populations with European ancestries, the most common ILAR subtype is oligoarticular JIA (oligoJIA), which accounts for approximately 50% of all JIA [2]. These children have four or less joints affected at onset (within the first 6 months). A proportion further develops polyarthritis from 6 months to 1 year after onset of arthritis: extended oligoartiular JIA (EOJIA). About 10–15% of JIA cases are rheumatoid factor negative polyarticular JIA (polyJIA) in which more than four joints are involved at the onset of disease. Rheumatoid factor positive polyJIA is clinically indistinguishable from adult-onset rheumatoid arthritis and is very rare. In cases of both EOJIA and polyJIA, the course of disease tends to be more prolonged compared with persistent oligoJIA and disease-modifying drugs are usually required. The third most common subtype is systemic JIA (sJIA), comprising 5–15% of all JIA in Caucasians with European ancestry. These children have clinical features distinct from the other types of JIA, such as a typical evanescent rash, high quotidian fevers and arthritis. Lymphadenopathy, hepatosplenomegaly and serositis are also found in the more severe cases. The most severe ones exhibit clinical features of macrophage activation syndrome. Prevalence figures for the fourth type, enthesitis-related arthritis (ERA), appear to vary according to ethnic groups and may be more prevalent in Mexico and some Oriental countries. A proportion develops ankylosing spondylitis (AS) meeting the modified New York criteria as they enter teenage years (juvenile AS) or later (juvenile-onset AS). Psoriatic arthritis is rare and genetic association studies in children have involved small numbers of cases.

Genetics of JIA

In keeping with other autoimmune and inflammatory diseases, JIA is considered a complex genetic disease, caused by a combination of multiple genetic and environmental factors. Given that JIA represents a clinically heterogeneous group of conditions, but having similar inflammatory changes in the joints, it has been postulated that some of the genetic factors will be shared between the subgroups and also with other autoimmune diseases, whilst other genes will differentiate between them. Evidence for a genetic component to disease comes from both twin and family studies. The monozygotic twin concordance rates for JIA range between 25% and 40%. Due to the low prevalence of JIA, family studies are sparse. However, the sibling recurrence risk ratio (λs) has been calculated to be between 15 and 30, similar to that calculated for insulin-dependent diabetes. In addition, it was shown that sibling pairs tend to show concordance both in terms of disease-onset type, disease course and age at onset [4]. The lack of large multi-case families for JIA has limited the use of linkage studies in this disease and therefore, to date, those involved in trying to elucidate the underlying genetic component of the disease have taken a largely candidate gene-based association study approach, using the inherited variations within the genome, commonly known as single nucleotide polymorphisms (SNPs). In this approach, the cases and control populations must be well matched genetically. Moreover, confirmation in a different cohort (also known as replication) is needed usually before one has some confidence that the result does not represent a false positive.

As with other autoimmune diseases, initial studies focussed on the human leucocyte antigen (HLA) genes within the major histocompatibility complex (MHC). There are a number of

well-documented, replicated HLA associations in JIA, which vary between the subgroups. For HLA class I alleles, HLA-A2 has been consistently shown to be associated with JIA, particularly in children with an early disease onset. HLA-B27 has long been recognised as a contributing factor to the development of spondyloarthritis, and, in particular, axial inflammation with hip involvement with subsequent AS [5]. The strongest HLA class II associations are seen in the oligoJIA subgroup, in particular, with increased frequency of HLA-DRB1*08, 11 and 13 and DPB1*02, whereas DRB1*04 and 07 are consistently reduced. HLA-DRB1*08 is also associated with rheumatoid factor negative polyJIA [6]. The effect of the HLA locus has been calculated to account for approximately 17% of the sibling recurrence risk [7]. Interestingly, there is an apparent lack of association between sJIA and HLA, with the possible exception of HLADRB1* 04, which has been weakly associated with sJIA in some studies.

The ERA subgroup of JIA includes children with undifferentiated spondyloarthritis, some of whom have axial disease and will eventually develop AS either later during childhood or after reaching adulthood. In addition to HLA-B27, which comprises up to 40% of the overall genetic susceptibility to AS, non-MHC genes *IL23R* and *ERAP1* (also known as *ARTS1*) have recently been identified as susceptibility factors [8]. *IL23R* encodes a protein that pairs with IL-12Rβ1 to form the IL-23 receptor, which is critical for the development of Th17 T cells and their production of IL-17 [9]. *IL23R* polymorphisms have previously been identified in susceptibility to inflammatory bowel disease (IBD) [10] and psoriasis [11], conditions that can overlap with spondyloarthritis. However, it should be noted that the effect of *IL23R* polymorphisms in AS is independent of both psoriasis and IBD. *ERAP1* encodes an endoplasmic reticulum (ER) aminopeptidase that has at least two functions. It is involved in the processing of peptide antigens in the ER [12], and therefore may be important in determining the quality and quantity of HLA class I complexes assembled in the cell. Interestingly, Levine and colleagues independently discovered this gene product and named it aminopeptidase regulator of tumour necrosis factor (TNF) receptor shedding (ARTS1) to reflect its role in promoting proteolytic removal of the receptor from the cell surface [13]. It has also been shown to influence shedding of the IL-1 and IL-6 receptors [14,15]. Its role in receptor removal does not appear to be through its aminopeptidase activity, and this function would require cellular localisation to a non-ER compartment. It will be important to determine which function of ERAP1 is involved in determining susceptibility and/or severity of AS.

It should be emphasised that genetic susceptibility to the phenotype subsumed in the ERA subgroup of JIA has not been studied. Since ERA encompasses most forms of undifferentiated spondyloarthritis, including patients previously classified as having SEA syndrome (seronegative enthesitis and arthritis), we know it will be associated with HLA-B27. However, the inclusion of HLA-B27 as a minor criterion for classifying someone with JIA as ERA precludes any valid determination of the risk conferred by this gene [1]. It is nevertheless tempting to speculate that once we know the majority of additional genes conferring susceptibility to AS, it will be possible (and cost-effective) to predict which individuals with ERA will develop AS. Predicting long-term outcome may be more feasible in the context of clinical features at presentation. Tools for establishing these features are being developed for adults and need to be considered for children because of differences in paediatric disease at presentation. Nevertheless, the primary rationale for using genotypes to predict outcome is to more effectively treat the underlying disease in hopes of reducing morbidity and preventing ankylosis. This still requires a better understanding of pathogenesis, and, in particular, the mediators of excess bone formation in this disease, which occurs during and after inflammation along the spine.

Candidate gene association versus whole genome association studies

Although many different non-HLA candidate loci have been investigated for associations with all JIA and with subtypes of JIA, very few have been confirmed. In a recent review of the literature, Prahalad and Glass [7] reported that whilst over 100 different candidate genes have been studied in over 150 association-based studies of JIA, only a handful show replication (*MIF*, *NRAMP1*, *PTPN22*, *TNFA* and *WISP3*); some of these are microsatellite- and not SNP-based and for some, the replication is a family-based study within the same population. There are only two loci in which a genetic variant has been shown to be associated with JIA in the same direction in more than one case-control study in independent populations: *PTPN22* (rs2467701) and *IL2RA* (rs2104286) [16,17], although neither of these loci reach genome-wide significance thresholds for association.

It is reasonable to suggest that the small sample sizes in most of these studies, as well as positive association publication bias, have led to a likely scenario of over-representation of false positives reported in the literature. It is also true that in many cases, the lack of replication may merely reflect the lack of availability of sufficiently large cohorts in which to seek replication. It is also likely that in some instances, combining clinically distinct entities, such as sJIA and oligoJIA, might confound the results. Indeed, there is a growing body of evidence to suggest that sJIA should be considered separate to other JIA subgroups [18]. Recent recognition of a group of systemic inflammatory illnesses that are largely genetically determined and characterised by unprovoked episodes of inflammation (the autoinflammatory syndromes) [19] has caused many clinicians and researchers in JIA to reconsider whether sJIA fits better within this group rather than JIA on clinical and genetic grounds. As already mentioned, HLA associations with sJIA are, at best, weak. There has been only one reproducible association with sJIA (rs1800795 in the promoter of *IL*-6), although this does not reach genome-wide significance levels [20]. Other cytokine genes have also been described to be associated with sJIA, but not yet replicated in other populations. These findings, in addition to the clinical features, suggest that the genetic background of sJIA may differ substantially from oligoJIA and polyJIA. Gene expression studies comparing differentially expressed genes in active sJIA with genes from polyJIA, an autoinflammatory disease (chronic infantile neurologic, cutaneous, arthropathy syndrome, or CINCA; also known as neonatal onset multisystem inflammatory disease or NOMID) and other inflammatory diseases of childhood, showed more genes in common with CINCA than with Kawasaki disease, polyJIA or SLE [21]. However, the *NLRP3* gene that is mutated in CINCA/NOMID, a syndrome that used to be confused with sJIA, has not as yet been found to be associated with sJIA. More recently, gene expression profiling in active JIA showed striking differences between subtypes, with sJIA being the most distinct [22].

For many complex diseases, the candidate gene approach has proved to be largely unsuccessful; this may, in part, be explained by poor study design, but it may also be that a lack of understanding of underlying biological mechanisms of disease has led to poor selection of candidates. Furthermore, there has tended to be a focus on the downstream products of a pathway (e.g., a cytokine) rather than upstream genes that influence pathways leading to production of the cytokine (e.g., transcription factors) or differential cellular responsiveness to the cytokine (e.g., receptors and their signal transducers). In recent years, the candidate-gene approach has been largely superceded by the genome-wide approach (genome-wide association study or GWAS), in which hundreds of thousands of SNPs across the entire genome are screened in a single assay. This has led to a plethora of novel susceptibility loci being identified for a variety of complex diseases, many of which have since been confirmed in replication studies [23]. Although expensive, the per genotype cost of genome-wide studies has come down considerably, making it cost-effective. In addition, the genome-wide approach has the advantage of being largely hypothesis-free, in that it

makes no assumptions about which genes may be important in disease. Many of the associations identified to date in other complex diseases are not in genes that would have been selected as candidates; indeed, many are not in genes at all but lie in intergenic regions, presumed to be regulatory.

Given the success of the GWAS approach in other autoimmune conditions, notably rheumatoid arthritis, type 1 diabetes and Crohn's disease, it is highly likely that further key pathways affecting immune regulation and inflammation will be revealed by a GWAS approach in JIA. Limited work has already been performed in small cohorts leading to the identification of novel loci [24]. Large-scale genome-wide association studies on oligoJIA and polyJIA are ongoing in Cincinnati, following up on previous genome-wide analyses showing evidence of genetic linkage [25]. More recent developments have led to technologies that allow simultaneous testing of common, rare and copy number variants and this now represents the most comprehensive way to study complex genetic diseases and has to be the approach of choice for JIA. Data from the study of other complex genetic diseases demonstrate that more often than not, the genetic effects are small (odds ratios <2.0). To have the power to detect these effects, and to rule out the involvement of other genes, large sample sizes and replication are necessary for GWAS. Therefore, in order to use this approach for JIA, international collaboration is essential.

With the use of international consortia, there are potential problems with heterogeneity since the frequency of many alleles varies with race and ethnicity. For a case-control GWAS, ideally only clinically homogeneous cases should be used, and these cases should be compared with a closely matched population of healthy controls. With international consortia, it is essential that this rule is adhered to. Different geographical populations of similar ethnicity/ancestry will still need to be matched with their own geographic controls. Meta-analysis of the frequencies of the genetic variants of each group with appropriate statistical methods will then provide the power needed for detection of genetic variants associated with a disease. As mentioned earlier, it is also very important to repeat the process with a separate cohort to confirm any positive findings. Although the number of cases and controls required to detect disease-causing alleles depends on minor allele frequencies and the odds ratio for the gene, most GWAS now include at least around 1000 cases and 1000 controls for discovery and replication phases [26].

Gene expression profiling in JIA

Complementary to the search for genes that confer susceptibility to disease, gene expression profiling on microarray gene chips represents a powerful comprehensive approach that allows one to probe inside cells to look for differences that characterise normal and disease states, and even disease subtypes, by making comparisons with healthy individuals. In paediatric rheumatic diseases, micro-array-based expression profiling has shown what genes/functional groups of genes are involved in the clinical state. Most studies use purified peripheral blood mononuclear cells (PBMC) or whole blood, and a variety of signatures have been seen. One consistent finding has been increased IL-10 mRNA or changes in IL-10-regulated genes in sJIA, oligoJIA and polyJIA [21,22]. In addition, innate immunity and complement and coagulation genes are differentially expressed in sJIA [21,22,27,28]. In sJIA with subclinical macrophage activation, there is an additional group of haematopoietic genes being differentially expressed, and include MUNC13-4 [28]. Mutations in MUNC13-4 are seen in familial forms of macrophage activation, also known as familial haemophagocytic lymphohistiocytosis. This observation led the authors to follow up with a candidate gene association study and found a MUNC13 4 genetic variant associated with MAS in sJIA [29]. These publications illustrate that profiling of cellular mRNA in disease states can lead to novel susceptibility genes being identified.

Differentially expressed genes can be used to distinguish the major subtypes of JIA at disease onset [22], which is quite remarkable given that there may only be subtle clinical differences that distinguish certain subtypes. By identifying the cellular origin of differentially expressed genes, results can inform us about the pathological pathways involved [21]. Gene expression profiles may also be useful in the prediction of disease outcome [30] or in response to treatment [27]. Together with genetic and clinical data, it is expected that these approaches can be used to re-classify JIA [31] and to establish more rational and specific treatment protocols for each subgroup.

Genotype to clinical manifestations (i.e., phenotype)–How do we use genetics to unravel pathogenic mechanisms?

The rapid pace of discovery of genes that predispose to autoimmune or immune-mediated inflammatory diseases promises to provide important clues to identify relevant dysregulated pathways, understand pathogenic mechanisms and ultimately finding new therapeutic targets for more specific treatments and cures. However, finding the correct and most direct route from genotype to phenotype is difficult, as exemplified by lack of understanding of how MHC-encoded HLA genes contribute to disease. HLA-B27 is probably the most humbling example. It is associated with several forms of spondyloarthritis, most notably AS, where it is present in >90% of affected individuals, compared with 7–8% of healthy controls. There is a paucity of evidence to support the existence of the long-sought autoreactive CD8 + T cells, and no arthritogenic or spondylogenic peptides have been found [32]. Furthermore, there is conclusive evidence from HLA-B27 transgenic rats that CD8 + T cells do not mediate inflammation [33,34]. Parallel studies have highlighted unusual properties of the HLA-B27 heavy chain, including its tendency to misfold and generate ER stress and to form cell surface homodimers [32], both of which could be responsible for its role in disease. With the recent discovery of additional susceptibility alleles for AS, *IL23R* and *ERAP1*, it is tempting to look for ways in which the products of these genes may interact with HLA-B27 to better understand its role in disease. For example, in cells from transgenic rats when HLA-B27 is up-regulated, misfolding is exacerbated, leading to ER stress and activation of the unfolded protein response (UPR) [35]. Recent results have linked the UPR with increased production of IL-23 in macrophages, and expansion and activation of Th17 T cells in the inflamed colon of HLA-B27 transgenic rats [36]. There is increasing evidence for Th17 activation in humans with spondyloarthritis including AS, supporting the relevance of this animal model. This raises the possibility that one unanticipated link between HLA-B27 and disease might be through upstream increased IL-23 production, with *IL23R* polymorphisms then determining responsiveness to the cytokine. Although this remains to be proven, it is an attractive paradigm that can be tested.

Does ERAP1 influence the effect of HLA-B27 on disease? This is possible given its role in trimming peptides that are presented by MHC class I molecules. It could be hypothesised that ERAP1 polymorphisms might alter the abundance of putative spondylogenic peptides, yet results from HLA-B27 transgenic rats would argue that this is not the mechanism. One alternative possibility is that ERAP1 influences other properties of HLA-B27 such as its folding efficiency, which in turn may affect misfolding or its tendency to lose peptides and/or β_2m and then dimerise after reaching the cell surface. One must also consider the reported effect of ERAP1 (ARTS1) on cytokine receptor shedding. Understanding the immunobiology of ERAP1/ARTS1 promises to be interesting and hopefully will shed more light on the pathogenesis of spondyloarthritis, and in particular the role of HLA-B27.

Many genes being identified in genome-wide association studies of immune-mediated or autoimmune inflammatory diseases carry a low risk in the population, and may only contribute to disease in the context of several other susceptibility alleles. Risk alleles are

often present at only a slightly higher frequency in cases compared with controls, and, in fact, are absent in many individuals with the phenotype. In many instances, the functional significance of the risk allele may be difficult to establish. These studies may require identifying and studying individuals with the risk allele, but no disease, thus eliminating the possibility of artefacts induced by the disease state. Approaches using animal models expressing the allele of interest, either in the absence or presence of the non-risk allele may also be helpful, but are time consuming and do not always provide answers that are relevant to human disease.

Epigenetics and lessons for the future in paediatric rheumatic disease

Heritable differences in DNA sequence are responsible for much of the phenotypic (i.e., clinical) variation seen across the population, and for susceptibility to many diseases. By contrast, epigenetic alterations are defined as heritable differences in gene expression that do not involve a change in the DNA sequence [37]. Epigenetic differences are caused by modifications in chromatin structure related to nucleosome arrangement. DNA that is tightly packaged in nucleosomes, which are comprised largely of proteins known as histones, is less accessible to transcription factors and thus not readily transcribed. Covalent modification of DNA or histones can dramatically alter nucleosome structure. Histones can be covalently modified (e.g., methylated, acetylated and phosphorylated), altering their chemical and physical properties, which, in turn, results in changes in nucleosome structure and, ultimately, gene expression. DNA methylation (i.e., methylation of deoxycytosine residues next to deoxyguanosine, or CpG sequences where p is a phosphate group) is another epigenetic mechanism that contributes to stable changes in gene expression. For example, the X chromosome has heavily methylated genes that are normally expressed in Y chromosome. In oncology, certain genes are demethylated in tumour cells, for example, the RASSF tumour suppressor genes are inactivated in childhood leukaemias [38]. Interestingly, the degree of methylation decreases with age, and is affected by UV light exposure.

Epigenetic changes are established during development and form the basis for developmentally regulated gene expression, and are thus responsible for tissue-specific gene expression patterns. Epigenetic changes to DNA structure are largely reversible, yet they are replicated during mitosis and therefore are heritable from parent to daughter cells. Environmental agents that affect histone and DNA modification during mitosis can prevent proper replication of epigenetic patterns, and altered gene expression patterns that may be further replicated in the next generation of cell division.

There are well-recognised environmental triggers of human disease including auto-immunity, particularly systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) [39]. One of the ways that the environment contributes is through epigenetic changes in gene expression. For example, in SLE, there is strong evidence, albeit circumstantial, for impaired T cell DNA methylation, and that CD4 + T cells treated with DNA methylation inhibitors can be made autoreactive. Furthermore, transferring these cells into genetically identical hosts can cause a lupus-like disease [40,41]. This led to the idea that drugs known to induce anti-nuclear antibodies (ANA) and to cause lupus-like disease in certain individuals might be inhibiting DNA methylation. This was demonstrated for procainamide and hydralazine [42], including the ability of these drugs to induce autoreactivity in mouse T cells and lupus-like disease in transfer experiments. It remains possible that additional environmental agents including medications contribute to lupus-like disease in susceptible individuals. While many of these effects have been ascribed to the inhibition of Dnmt1 (DNA methyltransferase-1), effects on other genes such as the one encoding CD11a have been shown to cause up-regulation of LFA-1, which is a heterodimer of CD11a and CD18 [43]. This is postulated to stabilise the interaction between T cells and antigen-presenting cells,

and contribute to autoimmunity by increasing interactions with self-MHC class I molecules. Additional examples of how DNA hypomethylation may contribute to autoreactivity provide a compelling argument linking epigenetic changes in gene expression to human SLE [39].

Less is known about epigenetic influences on the pathogenesis of RA. Hypomethylated T cell DNA has been reported [44] and could lead to autoreactive T cells in this disease similar to SLE. There are also reports of CD21 promoter demethylation in PBMC and synovial fluid from RA patients [45]. There are strong environmental links to RA, particularly with cigarette smoking, but whether this causes epigenetic changes is unknown. A possible role for epigenetic changes linking environmental exposures and disease pathogenesis exists in other autoimmune diseases, including multiple sclerosis and type 1 diabetes. However, cause-and-effect relationships have not been proven at this point. We are not aware of any published data implicating epigenetic changes in JIA pathogenesis, yet it seems likely that they exist. Much more needs to be learned in order to understand how the environment contributes to autoimmune and autoinflammatory disease pathogenesis, and the area of epigenetics promises to be interesting. For example, there are major epigenetic influences on T-helper (Th) cell differentiation and function. Access by lineage-specific Th cell transcription factors to their target genes is strongly influenced by epigenetic changes, with downstream effects on Th1, Th2 and Th17 development and activity [46]. The relatively recent discovery of the CD4 + T cell Th17 lineage and the role of Th17 cytokines such as IL-17 and IL-22, along with Th1 cytokines (e.g., IFN-γ, TNF-α), in immune-mediated inflammatory diseases underscores the importance of understanding how epigenetic changes regulate these processes.

An additional area of interest in genomics involves a group of small RNA molecules (microRNA or miRNA) that act as regulators of gene expression. They are transcribed from parts of the genome that do not code for proteins. The RNAs miRNAs regulate gene expression by binding the 3′ untranslated region of specific mRNAs and targeting them for degradation or suppressing their translation. The miRNA-mediated gene regulation is critical for normal cellular functions such as cell division, differentiation and apoptosis. As many as one-third of human mRNAs may be miRNA targets. Many oncological translocations and mutations have been found in miRNA loci [47]. The miRNA (146a) has been found to be over-expressed in PBMC of RA compared with healthy controls, and this may regulate TNF-α production from cells in culture [48]. Cell culture experiments using synovial tissue and fibroblasts for example has revealed that there is altered miRNA expression in RA compared with osteoarthritis patients, in particular miRNA 155 [49], which targets matrix metalloproteinase-3 (MMP3), implicated in synovial pathology.

In summary, the complex genetic basis of many paediatric rheumatic diseases is clear. Our ability to catalogue the genes involved, and understand the contribution of gene products to pathogenesis, will depend on well-designed large-scale studies that require co-operation from a number of investigators, as well as patients and families. Emerging data suggest that common polymorphisms may be responsible for a great deal of genetic susceptibility, even though, in many cases, the effects of individual genes may be small. In addition, there are common susceptibility genes that underlie several diseases, as well as unique genes that are likely to determine phenotypic differences, thus accounting for the diversity of clinical manifestations. Understanding ways in which the environment may trigger and/or perpetuate disease is a major challenge. While environmental triggers are frequently conceptualized as microbial in origin, and thought to contribute as antigens initiating cross-reactivity, their role in stimulating the innate immune system may be equally as important. In addition, the role of environmental factors leading to epigenetic changes in gene expression must be considered.

References

1. Petty RE, Southwood TR, Manners P, et al. International League of Associations for Rheumatology classification of juvenile idiopathic arthritis: second revision, Edmonton, 2001. J Rheumatol 2004 Feb 31;31(2):390–392. [PubMed: 14760812]
2. Symmons DP, Jones M, Osborne J, et al. Pediatric rheumatology in the United Kingdom: data from the British Pediatric Rheumatology Group National Diagnostic Register. J Rheumatol 1996 Nov; 23(11):1975–1980. [PubMed: 8923378]
3. Adib N, Silman A, Thomson W. Outcome following onset of juvenile idiopathic inflammatory arthritis: I. frequency of different outcomes. Rheumatology (Oxford) 2005 Aug;44(8):995–1001. [PubMed: 15827045]
4. Glass DN, Giannini EH. Juvenile rheumatoid arthritis as a complex genetic trait. Arthritis Rheum 1999 Nov;42(11):2261–2268. [PubMed: 10555018]
5. O'Shea FD, Boyle E, Riarh R, et al. Comparison of clinical and radiographic severity of juvenile-onset versus adult-onset ankylosing spondylitis. Ann Rheum Dis 2008 Sep;9
6. Thomson W, Barrett JH, Donn R, et al. Juvenile idiopathic arthritis classified by the ILAR criteria: HLA associations in UK patients. Rheumatology (Oxford) 2002 Oct;41(10):1183–1189. [PubMed: 12364641]
7. Prahalad S, Glass DN. A comprehensive review of the genetics of juvenile idiopathic arthritis. Pediatr Rheumatol Online J 2008;6:11. [PubMed: 18644131]
8. Burton PR, Clayton DG, Cardon LR, et al. Association scan of 14,500 nonsynonymous SNPs in four diseases identifies autoimmunity variants. Nat Genet 2007 Nov;39(11):1329–1337. [PubMed: 17952073]
9. Annunziato F, Cosmi L, Liotta F, et al. Type 17T helper cells-origins, features and possible roles in rheumatic disease. Nat Rev Rheumatol 2009 Jun;5(6):325–331. [PubMed: 19434074]
10. Duerr RH, Taylor KD, Brant SR, et al. A genome-wide association study identifies IL23R as an inflammatory bowel disease gene. Science 2006 Dec 1;314(5804):1461–1463. [PubMed: 17068223]
11. Cargill M, Schrodi SJ, Chang M, et al. A large-scale genetic association study confirms IL12B and leads to the identification of IL23R as psoriasis-risk genes. Am J Hum Genet 2007 Feb;80(2):273–290. [PubMed: 17236132]
12. Hammer GE, Gonzalez F, James E, et al. In the absence of aminopeptidase ERAAP, MHC class I molecules present many unstable and highly immunogenic peptides. Nat Immunol 2007 Jan;8(1): 101–108. [PubMed: 17128277]
13. Cui X, Hawari F, Alsaaty S, et al. Identification of ARTS-1 as a novel TNFR1-binding protein that promotes TNFR1 ectodomain shedding. J Clin Invest 2002 Aug;110(4):515–526. [PubMed: 12189246]
14. Cui X, Rouhani FN, Hawari F, Levine SJ. Shedding of the type II IL-1 decoy receptor requires a multifunctional aminopeptidase, aminopeptidase regulator of TNF receptor type 1 shedding. J Immunol 2003 Dec 15;171(12):6814–6819. [PubMed: 14662887]
15. Cui X, Rouhani FN, Hawari F, Levine SJ. An aminopeptidase, ARTS-1, is required for interleukin-6 receptor shedding. J Biol Chem 2003 Aug 1;278(31):28677–28685. [PubMed: 12748171]
16. Hinks A, Worthington J, Thomson W. The association of PTPN22 with rheumatoid arthritis and juvenile idiopathic arthritis. Rheumatology (Oxford) 2006 Apr;45(4):365–368. [PubMed: 16418195]
17. Hinks A, Ke X, Barton A, et al. Association of the IL2RA/CD25 gene with juvenile idiopathic arthritis. Arthritis Rheum 2009 Jan;60(1):251–257. [PubMed: 19116909]
18. Ramanan AV, Grom AA. Does systemic-onset juvenile idiopathic arthritis belong under juvenile idiopathic arthritis? Rheumatology (Oxford) 2005 Nov;44(11):1350–1353. [PubMed: 15956091]
19. Masters SL, Simon A, Aksentijevich I, Kastner DL. Horror autoinflammaticus: the molecular pathophysiology of autoinflammatory disease (*). Annu Rev Immunol 2009;27:621–668. [PubMed: 19302049]

20. Ogilvie EM, Fife MS, Thompson SD, et al. The -174G allele of the interleukin-6 gene confers susceptibility to systemic arthritis in children: a multicenter study using simplex and multiplex juvenile idiopathic arthritis families. Arthritis Rheum 2003 Nov;48(11):3202–3206. [PubMed: 14613283]
21. Ogilvie EM, Khan A, Hubank M, et al. Specific gene expression profiles in systemic juvenile idiopathic arthritis. Arthritis Rheum 2007 Jun;56(6):1954–1965. [PubMed: 17530721]
22. Barnes MG, Grom AA, Thompson SD, et al. Subtype-specific peripheral blood gene expression profiles in recent-onset juvenile idiopathic arthritis. Arthritis Rheum 2009 Jul;60(7):2102–2112. [PubMed: 19565513]
23. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 2007 Jun 7;447(7145)(7145):661–678. [PubMed: 17554300]
24. Hinks A, Barton A, Shephard N, et al. Identification of a novel susceptibility locus for juvenile idiopathic arthritis by genome-wide association analysis. Arthritis Rheum 2009 Jan;60(1):258–263. [PubMed: 19116933]
25. Thompson SD, Moroldo MB, Guyer L, et al. A genome-wide scan for juvenile rheumatoid arthritis in affected sibpair families provides evidence of linkage. Arthritis Rheum 2004 Sep;50(9):2920–2930. [PubMed: 15457461]
26. Gregersen PK. Pathways to gene identification in rheumatoid arthritis: PTPN22 and beyond. Immunol Rev 2005 Apr;204:74–86. [PubMed: 15790351]
27. Allantaz F, Chaussabel D, Stichweh D, et al. Blood leukocyte microarrays to diagnose systemic onset juvenile idiopathic arthritis and follow the response to IL-1 blockade. J Exp Med 2007 Sep 3;204(9):2131–2144. [PubMed: 17724127]
28. Fall N, Barnes M, Thornton S, et al. Gene expression profiling of peripheral blood from patients with untreated new-onset systemic juvenile idiopathic arthritis reveals molecular heterogeneity that may predict macrophage activation syndrome. Arthritis Rheum 2007 Nov;56(11):3793–3804. [PubMed: 17968951]
29. Zhang K, Biroschak J, Glass DN, et al. Macrophage activation syndrome in patients with systemic juvenile idiopathic arthritis is associated with MUNC13-4 polymorphisms. Arthritis Rheum 2008 Sep;58(9):2892–2896. [PubMed: 18759271]
30. Hunter P, Nistala K, Jina N, et al. Biological predictors of extension of oligoarticular juvenile idiopathic arthritis from synovial fluid cellular composition and gene expression. Arthritis Rheum. (in press).
31. Griffin TA, Barnes MG, Ilowite NT, et al. Gene expression signatures in polyarticular juvenile idiopathic arthritis demonstrate disease heterogeneity and offer a molecular classification of disease subsets. Arthritis Rheum 2009 Jul;60(7):2113–2123. [PubMed: 19565504]
32. Smith JA, Marker-Hermann E, Colbert RA. Pathogenesis of ankylosing spondylitis: current concepts. Best Pract Res Clin Rheumatol 2006 Jun;20(3):571–591. [PubMed: 16777583]
33. May E, Dorris ML, Satumtira N, et al. CD8 alpha beta T cells are not essential to the pathogenesis of arthritis or colitis in HLA-B27 transgenic rats. J Immunol 2003 Jan 15;170(2):1099–1105. [PubMed: 12517979]
34. Taurog JD, Dorris ML, Satumtira N, et al. Spondylarthritis in HLA-B27/human beta(2)-microglobulin-transgenic rats is not prevented by lack of CD8. Arthritis Rheum 2009 Jul;60(7):1977–1984. [PubMed: 19565478]
35. Turner MJ, DeLay ML, Bai S, et al. HLA-B27 up-regulation causes accumulation of misfolded heavy chains and correlates with the magnitude of the unfolded protein response in transgenic rats: implications for the pathogenesis of spondylarthritislike disease. Arthritis Rheum 2007 Jan;56(1):215–223. [PubMed: 17195225]
36. DeLay ML, Turner MJ, Klenk EI. HLA-B27 misfolding and the unfolded protein response augment IL-23 production and are associated with Th17 activation in transgenic rats. Arthritis Rheum 2009;60:2633–2643. [PubMed: 19714651]
37. Jaenisch R, Bird A. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nat Genet 2003 Mar;33 (Suppl.):245–254. [PubMed: 12610534]

38. Hesson LB, Dunwell TL, Cooper WN, et al. The novel RASSF6 and RASSF10 candidate tumour suppressor genes are frequently epigenetically inactivated in childhood leukaemias. Mol Cancer 2009;8:42. [PubMed: 19570220]
39. Hewagama A, Richardson B. The genetics and epigenetics of autoimmune diseases. J Autoimmun 2009 Aug;33(1):3–11. [PubMed: 19349147]
40. Quddus J, Johnson KJ, Gavalchin J, et al. Treating activated CD4 + T cells with either of two distinct DNA methyltransferase inhibitors, 5-azacytidine or procainamide, is sufficient to cause a lupus-like disease in syngeneic mice. J Clin Invest 1993 Jul;92(1):38–53. [PubMed: 7686923]
41. Yung RL, Quddus J, Chrisp CE, et al. Mechanism of drug-induced lupus. I. Cloned Th2 cells modified with DNA methylation inhibitors in vitro cause autoimmunity *in vivo*. J Immunol 1995 Mar 15;154(6):3025–3035. [PubMed: 7533191]
42. Cornacchia E, Golbus J, Maybaum J, et al. Hydralazine and procainamide inhibit T cell DNA methylation and induce autoreactivity. J Immunol 1988 Apr 1;140(7):2197–2200. [PubMed: 3258330]
43. Alcolado JC, Laji K, Gill-Randall R. Maternal transmission of diabetes. Diabet Med 2002 Feb; 19(2):89–98. [PubMed: 11874423]
44. Richardson B, Scheinbart L, Strahler J, et al. Evidence for impaired T cell DNA methylation in systemic lupus erythematosus and rheumatoid arthritis. Arthritis Rheum 1990 Nov;33(11):1665–1673. [PubMed: 2242063]
45. Schwab J, Illges H. Silencing of CD21 expression in synovial lymphocytes is independent of methylation of the CD21 promoter CpG island. Rheumatol Int 2001 May;20(4):133–137. [PubMed: 11411956]
46. Wilson CB, Rowell E, Sekimata M. Epigenetic control of T-helper-cell differentiation. Nat Rev Immunol 2009 Feb;9(2):91–105. [PubMed: 19151746]
47. Calin GA, Croce CM. MicroRNA signatures in human cancers. Nat Rev Cancer 2006 Nov;6(11): 857–866. [PubMed: 17060945]
48. Pauley KM, Satoh M, Chan AL, et al. Upregulated miR-146a expression in peripheral blood mononuclear cells from rheumatoid arthritis patients. Arthritis Res Ther 2008;10(4):R101. [PubMed: 18759964]
49. Stanczyk J, Pedrioli DM, Brentano F, et al. Altered expression of MicroRNA in synovial fibroblasts and synovial tissue in rheumatoid arthritis. Arthritis Rheum 2008 Apr;58(4):1001–1009. [PubMed: 18383392]