Review article

Using proteomic and genomic methods to understand JDM

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summary

Juvenile dermatomyositis is a complex illness characterized by vascular/perivascular inflammation, primarily in the skin and muscles. In this review, we discuss how proteomic and genomic technologies have expanded our understanding of the immune pathogenesis of this disease. We will also discuss further directions that the field may take to use existing and developing technologies to further our understanding of this often-perplexing disease.

Key words—juvenile dermatomyositis; proteomics; anti-endothelial cell antibodies (AECA); genomics; gene expression profiling

Introduction

Juvenile dermatomyositis (JDM) is the most common clinical phenotype of myositis in children and accounts for approximately 85% of the juvenile idiopathic inflammatory myopathies (JIIM)^{1,2}. While classified as a “myopathy,” JDM, is also considered a systemic autoimmune vasculopathy that includes muscle tissue but can also involve other organ systems. We are increasingly recognizing that the spectrum of clinicopathologic groups and serologic phenotypes in pediatric myopathies, including JDM, is quite heterogeneous. Among other things, we are finding the these different groups can be defined by the presence of myositis-specific or myositis-associated autoantibodies, each with differing demographics, clinical manifestations, laboratory findings, and prognoses. However, the etiology of JDM is unknown, and we lack biomarkers in patients with JDM for diagnosis.

This paper, we will discuss how new approaches and technologies are broadening our understanding of the heterogeneity of autoantibody expression in JDM and discuss how unbiased proteomics approaches may further advance understanding. In addition, we discuss the new insights that genomics approaches, and gene expression profiling in particular, have added new insights into disease pathogenesis.

1. The clinical spectrum of JDM

As noted above, JDM is a systemic, inflammatory,

idiopathic disease of affecting the skin, the muscles and other organs. In the USA, the incidence rate of JDM is estimated to be 3.2 per 1 million children per year, with the median age at onset between 5.7 and 7.5 years. There is a slight female preponderance in new cases, with male:female ratios varying between 1:2.2 to 1:2.7^{2–6}. In Japan, the prevalence of JDM is estimated to be 17.4 per 1 million children, with male:female ratios 1:2.3⁷. While it is difficult to compare incidence statistics in one country with prevalence data from another, it is reasonable to conclude that JDM is one of the rarer childhood-onset rheumatic diseases in both Japan and North America. At the same time, older data suggests that, if anything, the disease is more common in Japanese children than in North American^{7,8}.

The clinical feature at presentation include Gottron's papules, heliotrope rash, proximal muscle weakness, myalgia, arthralgia and/or arthritis and described as “dermatomyositis” exist in both children and adults, there are differences between in children and in adults that suggest that the entity we designate “JDM” may be pathologically distinct. Vascular and perivascular inflammation in the skin, gastrointestinal tract, lungs, kidneys, eyes and heart are prominent pathological features of JDM not encountered to the same degree in the adult form of the disease. Furthermore subcutaneous and intramuscular calcinosis, which is often refractory to treatment, remains a vexing clinical problem for anywhere from 15–50% of JDM patients^{2,9,10}. A retrospective review of sub-

Saharan African children with JDM found a cumulative frequency of calcinosis in 71% of the affected children¹¹. In addition, there are cases in children where rash is prominent but muscle involvement either absent or minimal. These entities are sometimes referred to as clinically amyopathic dermatomyositis (CADM) or hypomyopathic dermatomyositis. Despite the absence of prominent muscle symptoms, these children are at risk for rapidly progressive interstitial lung disease (RP-ILD), which can be fatal. It is reported that CADM occurs with a frequency of 1%¹.

2. Autoantibodies in JDM

Previous studies showed there are significant correlations between clinical phenotype and the presence and/or the type of autoantibodies in patients with JDM. For example, antinuclear antibodies (ANA) are detected in 27% to 72% of children with JDM (vs. 10–30% of healthy children)^{3, 4, 12}, and anti-TIF1 (anti-p155/140) and anti-NXP2 (anti-p140/anti-MJ), myositis-specific autoantibodies (MSAs) that are detected frequently in 23% to 38% and 18% to 28% of JDM patients, respectively^{12–18}. Shah et al.³ reported that anti-Ro (5% of patients), anti-UI-RNP (2% of patients) and anti-PM-Scl (3% of patients) can rarely be detected in JDM patients; these antibodies are sometimes referred to as myositis-associated autoantibodies (MAAs). It is useful to note that these antibodies are far from a universal phenomenon in JDM. Shah et al.³ reported that 28% of the patients they studied with JDM had no myositis autoantibody. Anti-Jo-1 were negative in almost all of patients with JDM (< 5%)⁸.

Expression of specific autoantibodies often correlates with specific clinical features. For example, in patients with anti-TIF1, clinical features include cutaneous ulceration and edema, severe photosensitive rashes, lower creatine kinase levels, and a chronic course¹. In patients expressing anti-NXP2, dysphonia, muscle cramps, and increased calcinosis are distinguishing clinical features, although these patients typically pursue a monocyclic course¹. Patients expressing MAAs as well as MSAs, have extra-muscular involvement, for example, Raynaud's phenomenon, sclerodactyly and arthritis seen in patients with anti-UI-RNP, and Raynaud's phenomenon, arthritis, dysphagia and interstitial lung disease in patients with anti-PM-Scl. anti-Ro antibodies, are common in patients

with overlap myositis (i.e., patients expressing complex phenotypes that may include clinical features of scleroderma, systemic lupus, or rheumatoid arthritis). However, little has been reported on the relationship between these autoantibodies and specific clinical features². Although the number of JDM patients expressing anti-Jo-1 antibody is very small, these patients frequently have ILD, arthritis, Raynaud's phenomenon, fevers, and mechanic's hands¹⁹.

Furthermore there are significant differences between JDM and juvenile polymyositis (JPM) in presence of autoantibodies such as anti-p155/140, anti-Jo-1, anti-Mi-2, anti-SRP and anti-UI-RNP³. As well as JPM, there are significant differences between JDM and JDM overlapping with another autoimmune or connective tissue disease in median ANA titer and in presence of autoantibodies such as anti-p155/140, anti-aminoacyl-tRNA synthetase, anti-Jo-1, anti-Ro, anti-UI-RNP, anti-PM-Scl, anti-Sm and anti-La³.

As noted above, ILD, including RP-ILD, is a well-described feature of CADM. This complication is associated with the presence of anti-melanoma differentiation-associated gene 5 (MDA5) antibodies in patients with PM, DM and JDM. Kobayashi et al.²⁰ showed that anti-MDA5 antibodies were detected in all eight patients with RP-ILD and in 71% of the patients with chronic ILD, but in no JDM patients without ILD. In addition serum levels of anti-MDA5 antibody were also significantly higher in the JDM patients with RP-ILD than in those with chronic ILD or non-ILD. Tansley et al.²¹ investigated relationship between the presence of anti-MDA5 autoantibodies and clinical phenotype of the patients with JDM. They showed that anti-MDA5 antibodies were closely related to clinical phenotypes, including skin and oral ulcerations and milder muscle disease. In addition, 21% of JDM patients with anti-MDA5 had ILD, but none of those had RP-ILD²¹. It should be noted that ILD is still rare in JDM, affecting only 3–5% of patients compared with DM^{3, 5}. At the same time, it must be noted that RP-ILD is one of the fatal complication of JDM.

3. Anti-endothelial cell antibodies (AECA)

Anti-endothelial cell antibodies (AECA) are autoantibodies against antigens on endothelial cell (EC) surfaces.

AECA have been detected in patients with connective tissue diseases, inflammatory diseases, and primary and secondary vasculitis^{22, 23}. There are many reports that AECA may be involved in EC activation and vascular disorders via increased expression of adhesion molecules, complement activation, increased levels of pro-inflammatory cytokines and chemokines and induction of apoptosis and thrombosis^{22, 24–26}. Furthermore it is reported the titers of AECA are associated with disease activity and the presence of AECA may be reliable biomarkers for vasculitis^{27–30}.

JDM is an autoimmune vasculopathy that may involve multiple organ systems in addition to skin and muscle. Intestinal ischemia due to vessel inflammation and thrombotic occlusion, for example, is a rare but serious, sometimes lethal event. Thus AECA are attractive candidates for biomarkers in patients with JDM. Yu et al.¹² reported that IgG AECA were detected in 76.0% of 25 JDM patients with active disease and in 29.4% of 17 control patients by ELISA, when using human umbilical vein endothelial cell lysates as a means of detection. On the other hand, anti-p155/140 antibodies and anti-p140 antibodies were detected in 36% and 28% of the patients with JDM and in none of the control patients¹².

To understand mechanistic roles of AECA in the pathophysiology of diseases, it is useful to identify the specific antigens targeted by these antibodies. This is best done with an unbiased and comprehensive proteomic analysis. Karasawa et al.²³ identified target antigens for AECA in patients with primary systemic vasculitis such as Takayasu's arteritis and with secondary systemic vasculitis such as systemic lupus erythematosus using proteomic approaches. Protein extracted from human umbilical vein endothelial cells (HUVEC) were used as an antigen source for this research. To select autoantigens expressed specifically in ECs, protein extracted from HeLa cells were used as a control. Extracted proteins were separated by two-dimensional electrophoresis, and transferred them onto membranes. Western blotting using sera samples from patients with systemic vasculitis was used to select the autoantigens-candidates by choosing only positive blots in HUVEC samples but not in HeLa samples. By peptide mass finger printing, the sequence of them was identified. One of identified proteins was peroxiredoxin2 (Prx2), an antioxidant enzyme. IgG

antibodies to recombinant Prx2 were detected in 60% of the patients with systemic vasculitis and in 7% of the patients with collagen diseases without vasculitis but not in the healthy donors. The titers of anti-Prx2 antibodies were correlated with disease activity and the presence of anti-Prx2 antibodies was closely related to increased level of both D-dimer and thrombin-antithrombin complex. Furthermore anti-Prx2 antibodies induced increased inflammatory cytokines and chemokines secretion from HUVEC including IL-6, growth regulated oncogene (GRO)- α and IL-1 β ²³.

Fujieda et al.³¹ reported that IgG antibodies to recombinant Prx2 were detected in 43.3% of the patients with Kawasaki disease (KD) and in 10% of the patients with sepsis but not in the healthy donors. Furthermore, the anti-Prx2-positive group showed a longer period of fever, decreased responses to high-dose γ -globulin therapy and increased excretion of urinary 8-isoprostaglandin as compared with anti-Prx2-negative group.

Although there are several reports about pathogenic roles of AECA in vascular disorders, the pathogenic roles of AECA remain incompletely understood. We believe, however, that such a comprehensive, unbiased approach to the identification of target antigens on/within AECA in patients with JDM will significantly expand the repertoire of known JDM-associated antibodies in the same way that this approach has expanded our knowledge of the autoantibody repertoire in other forms of vasculitis.

4. Detection of biomarkers in patients with JDM using proteomic techniques

Proteomic techniques make it possible to identify specific proteins, modification of proteins, and protein expression levels^{32, 33}. Proteomic techniques may also be helpful for analyzing the differences in time-course, such as between pre- and post-treatment patients. These same approaches may also characterize subtly different disease phenotypes. An additional advantage to this approach is its suitability for many kinds of samples, including sera, plasma, urine, saliva, cerebrospinal fluid and synovial fluid³⁴. An example of the utility of an unbiased proteomic approach can be found in the recent work of O'Hanlon et al.³⁵. These authors reported results from proteomic analysis of plasma samples from monozygotic (MZ) twin pairs discordant for systemic autoimmune dis-

eases (SAID; their sample included 2 patients with JDM as well as juvenile arthritis and systemic lupus) as well as matched healthy controls. Proteomic approaches consisted of examination of patterns of protein expression using differential quantitative proteomic analysis (one-dimensional reverse-phase liquid chromatography-mass spectrometry). Affected twins expressed higher levels of retinoblastoma-binding protein 1 and leucine-rich $\alpha 2$ -glycoprotein 1 compared to either unaffected twins or unrelated controls. In addition, decreased plasma levels of paraoxonase 1 were detected in affected twins compared to same groups. Random Forest multivariate analysis identified seven proteins that crisply segregated affected and unaffected twins by their expression levels. Four of these were already identified on the univariate analysis. Thus, using a combination of both wet lab and computational approaches, as is done in genomics, is likely to yield the best means for identifying useful biomarkers for JDM.

5. Genetic basis of JDM

In many ways, the field of genomics as applied to JDM is still in its infancy. For example, while we have some understanding of the genetic contribution to JDM³²⁾, these studies have been largely undertaken using “candidate gene” approaches. For example, increased risk for JDM has been reported in individuals carrying an HLA B8-DR3 haplotype³⁶⁾. However, the best-documented genetic associations for JDM are with the class II HLA antigen, DQA1*0501, associations that appear to be common for multiple ethnic groups³⁷⁾. In addition to being a risk factor for disease *per se*³⁸⁾, affected children expressing the DQA1*0501 allele may be at higher risk for pursuing a chronic or persistent disease course than are children with JDM who do not express this allele.

Alleles within the TNFA gene have also shown associations with JDM. At position -308 within the TNFA promoter, a G → A substitution occurs in approximately 20% of healthy Caucasians. In *in vitro* models, the “A” allele is associated with more robust TNF α production to specific stimuli than is the more common -308 “G” allele. It was therefore of great interest when Pachman and colleagues reported a strong association between the -308 “A” allele and JDM in Caucasian children. Furthermore, this allele was strongly associated with a

more severe clinical course (including calcifications) in children with this disease^{39, 40)}. Because TNF α is a critical initiator and regulator of both innate and adaptive immune responses, these findings support the hypothesis that JDM, like other childhood rheumatic diseases⁴¹⁾, involves complex interactions between innate and adaptive immunity.

While these observations provide some useful insight, we are largely in the dark as to whether there are other genetic loci that contribute to disease susceptibility. Conspicuously absent from the literature is a genome-wide association study (GWAS), such as the one recently completed for juvenile idiopathic arthritis (JIA)⁴²⁾. Even that study was small by GWAS standards (1,800 subjects), and, given the rarity of JDM relative to JIA, gathering a sufficient number of subjects to perform a meaningful GWAS would be extraordinarily difficult. However, continuing with candidate gene approaches alone is unlikely to significantly advance the field. Among other things, most genetic risk for complex traits doesn’t reside within the canonical coding regions of genes. In the Hinks study of JIA, for example, 22 of the 24 risk-associated single nucleotide polymorphisms (SNPs) were located within intronic or intergenic regions of the genome. This finding is consistent with almost every other GWAS for complex traits⁴³⁾ and has naturally led to the question, “What’s in those regions?” For JIA, we have some answers, as we have recently demonstrated that all of the JIA-associated SNPs that are not within the coding genome are located within linkage disequilibrium (LD) blocks that contain H3K4me1/H3K27ac-marked enhancers in either T cells or neutrophils⁴⁴⁾.

Given that a GWAS approach to understanding genetic risk in JIA is impractical, what alternatives are there for us to derive a better understanding of this disease? There are two approaches that might be taken. One approach would be exome sequencing, which queries only the coding parts of the genome. While this approach has been typically applied to Mendelian traits, there is emerging interest in using this approach to identify rare variants that may be associated with complex human diseases⁴⁵⁾. An advantage to this approach is that meaningful information could be acquired with hundreds of patients, rather than the thousands or tens of thousands required to obtain meaningful information from GWAS. The disadvantage,

of course, is that this approach provides no information about non-coding parts of the genome, where disease risk for most complex traits is typically located, as noted previously³⁹⁾. Alternatively, deep sequencing of whole genomes is becoming increasingly attractive as costs of next-generation sequencing continue to fall and new platforms continue to evolve.

6. Transcription profiling

Transcription profiling using hybridization-based microarrays is an attractive approach for both understanding disease pathogenesis and to develop clinically relevant biomarkers⁴⁶⁾. Despite their limitations⁴⁷⁾, this technology has provided some useful insights into JDM pathogenesis. For example, in 2002, Tezak et al reported a distinct type 1 interferon “signature” in muscle biopsies of a select group of patients with JDM⁴⁸⁾. Specifically, muscle biopsies in children with JDM were distinguished from children with non-inflammatory myopathies as well as healthy controls by the expression of genes known to be induced by type 1 interferons. Disease duration was subsequently shown to have a significant impact on muscle expression profiles in JDM⁴⁹⁾. The type 1 interferon signature has also been demonstrated on whole blood expression profiling in treated patients with JDM (this study included both adult and pediatric JDM subjects)⁵⁰⁾. In this study, the signature was not as strong as in the Tezak study but was clearly associated with active disease. Each of these studies is limited by the number of samples or patients studied (for example, in the Chen study, only 3 samples were studied in the short disease duration group; in the Baechler study, only 2 of the subjects were children, and all subjects were already on medication). These small numbers are therefore prone to high false discovery rates that can’t be completely eliminated with any bioinformatics approach. A larger patient population of untreated patients (9 girls and 8 boys) was described by Frank et al in a study aimed at discovering gene expression network structures in both JIA and JDM⁵¹⁾. These authors demonstrated that, like physiologic systems, gene co-expression networks in childhood rheumatic diseases like JDM display the same hub-and-node structures as physiologic systems^{52, 53)}. Furthermore, such networks could be identified in both peripheral blood mononuclear cells and neutrophils. These findings

are consistent with the increasingly-accepted theory that complex human diseases are associated with cellular “rewiring” that must be corrected in order to achieve therapeutic response^{54, 55)}. At the same time, achieving this rewiring may be challenging, as network structures that display hub-and-node configurations are particularly resistant to external impingement⁵⁶⁾.

In summary, preliminary studies using genomic approaches have added some valuable insights into JDM that have simultaneously added more questions. Future work will likely be aimed at: (1) a better understanding of the genetic contribution to JDM; (2) understanding the origins of the type 1 interferon signature; and (3) understanding the biology of therapeutic response, as it currently under way for JIA⁵⁷⁾.

Conclusion

In conclusion, both proteomic and genomic approaches have provided both useful insights into disease pathogenesis and provided direction for biomarker development. A focused effort to compare Japanese and North American patients with JDM would likely provide new and valuable information regarding the genetics and epigenetics of this disease.

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