HLA class II haplotype and autoantibody associations in children with juvenile dermatomyositis and juvenile dermatomyositis–scleroderma overlap


Introduction

Juvenile dermatomyositis (JDM) is the most common of the childhood idiopathic inflammatory myopathies (IM). Despite improvements in management, JDM is still associated with significant morbidity and mortality [1–3]. Certain clinical features in JDM, such as skin ulceration, calcinosis, major organ vasculopathy, dysphagia and respiratory involvement, have been proposed as predictors of a severe disease course in JDM [4]. There are, however, no serological or genetic tools currently available to predict who will suffer these extramuscular complications.

A further source of considerable morbidity in JDM is the occurrence of overlap features with other connective tissue disorders, including systemic sclerosis (SSc), systemic lupus erythematosus (SLE) and mixed connective tissue disease (MCTD) [5–7]. In children with JDM, scleroderma overlap commonly presents with sclerodactyly, facial skin changes and Raynaud’s phenomenon, and may progress to include many SSc features [3, 8, 9]. Some authors suggest that myositis with features of scleroderma should be considered a separate entity known as ‘scleromyositis’ [10]; however, in JDM many ‘overlap’ features may develop after an initial typical JDM presentation [4].

A greater understanding of the genetic and pathological mechanisms of overlap between the paediatric CTDs could provide knowledge with which to design predictive tests, select subpopulations of children who require specific monitoring or therapies, and generate targets for novel therapies.

The strongest genetic associations for autoimmune conditions, including JDM, are with genes in the major histocompatibility complex (MHC) region, in which are clustered multiple genes coding for proteins central to the immune system, many of them highly polymorphic. Reported associations between JDM and the MHC genes include HLA-B*08, DRB1*0301 and DQA1*0501, as well as that between the tumour necrosis factor (TNF) allele, 308A, and disease course or severity [11–14]. These alleles are known to be linked as a haplotype, sometimes referred to as the 8.1 ancestral haplotype. HLA-A1-B8-DRB1*0301-DQA1*0501 (8.1 AH) [15–17], which is common in Caucasians and confers risk for many autoimmune diseases. Strong associations of these alleles with adult DM have also been demonstrated [16, 18, 19].

Autoantibodies, including myositis-specific antibodies (MSAs) and myositis-associated antibodies (MAAs), are common in the IIMs [18, 20–22]. Association studies show that HLA alleles and haplotypes are more strongly linked to the serological profile than to the clinical subtypes of adult IIM [19, 23–25], to the extent that a serological classification of myositis has been proposed [18, 26–28]. However, autoantibodies have been less fully characterized in JDM and JDM–scleroderma overlap (JDM–SSc) [8, 29]. The PM-Scl antibody has been shown to be associated with juvenile scleroderma and JDM–scleroderma overlap [5], but other specific associations are less clear in childhood disease.

There are significant differences in the clinical manifestations of myositis between the adult and paediatric IIM, such as a low incidence of malignancy in JDM, but a higher prevalence of
calcinosi

Haplotype-autoantibody association in JDM

Haplotype–autoantibody association in JDM

Serological testing

Methods

Patients, clinical phenotypes and control subjects

All patients had probable or definite JDM according to the Bohan and Peter criteria [34, 35] and were recruited to the National JDM Registry and Repository (UK and Ireland) [3], through the UK Juvenile Dermatomyositis Research Group (JDRG). The current study had multi-centre ethical approval and all subjects had full written parental consent, according to the Declaration of Helsinki. DNA for genotyping was available from 114 Caucasian children with JDM, of which 99 had serum available for antibody analysis. Clinical data were collected on all children using standardized baseline and follow-up proforma sheets, as described [3, 33].

There are no internationally agreed definitions of ‘overlap’ syndromes in children, although JDM–SSc overlap is well-recognized and typically defined by the presence of Raynaud’s phenomenon, sclerodactyly and sclerodermatous changes on other areas such as around the mouth [5, 9, 29]. In a recent large study of childhood scleroderma, Raynaud’s phenomenon, sclerodactyly and skin induration were among the most common clinical features [36]. For the current study, children were defined as having JDM–SSc overlap if they had two or more of the following features: Raynaud’s phenomenon, sclerodactyly and sclerodermatous skin changes. Nail-fold capillary data were not collected on all cases. Caucasian normal control subjects (537) were recruited from a number of UK sources as described [19].

DNA was extracted from peripheral blood samples obtained from both cases and controls using the standard phenol-chloroform method. Cases were broad-typed for the HLA-DRB1 and DQB1 loci, using a commercially available polymerase chain reaction sequence-specific oligonucleotide probe typing system (Dynal Biotech GmbH, Hamburg, Germany). All 537 controls were HLA-DRB1 typed, while 153 were HLA-DQB1 typed. As strong linkage disequilibrium (LD) exists across the MHC class II region, the DQA1 alleles for patients and 142 controls were derived from the DRB1 and DQB1 results, using well-documented Caucasian haplotype tables [38].

Statistical analysis

Patient-specific HLA and autoantibody associations were derived from 2 × 2 contingency tables. Probabilities were calculated using Fisher’s exact test, and corrected for multiple comparisons using the Bonferroni correction (12 for DRB1, 6 for DQA1, 5 for DQB1) to give a corrected P-value (Pcorr). In the free text, unless otherwise stated, stated P-values are uncorrected. Data were also expressed as odds ratios (ORs) with 95% confidence intervals (CIs). ORs were calculated according to Woolf’s method with Haldane’s correction when critical entries were zero. Analyses were repeated after stratification for myositis serology and clinical features. Multivariate logistic regression analysis was undertaken to determine significant associations after adjustment for other features. Assumed DRB1-DQA1-DQB1 haplotypes were assigned to individuals where data for all three loci were available. Haplotypes were estimated for selected loci using the Expectation/Maximization algorithm, as implemented in HelixTree (version 3.1.2, Golden Helix, Inc., Bozeman, MT, USA). Unless otherwise stated, the statistical package Stata (release 8, Stata Corp., College Station, TX, USA) was used to perform the statistical analysis.

Results

Demographics

One hundred and fourteen patients with definite or probable JDM were recruited. Of these, 87 had ‘classical’ JDM (hereafter simply referred to as JDM) and 27 had JDM overlapping with sclerodermatous features (hereafter referred to as JDM–SSc) (Table 1). A female predominance was found, more marked in the JDM–SSc group compared with the JDM group (JDM–SSc vs JDM, P = 0.08). The average age at diagnosis was 7.2 ± 3.6 yrs in the group as a whole, and was significantly higher in the JDM–SSc group compared with the JDM group (P = 0.03), even after adjustment for gender. The average time from onset of symptoms to diagnosis was higher in the JDM–SSc group (JDM–SSc 1 yr vs JDM 0.7 yrs, P = 0.2).
Serology

The overall frequency of ANA positivity (titre >1:40) by immunofluorescence was 71.7% (Table 1). Seven of the 99 sera tested had an MSA, while 20 sera had an MAA (Table 1). Five patients had more than one MSAs or MAAs. All three patients with anti-Sm antibody also possessed an anti-U1-RNP antibody, and both JDM–SSc patients with anti-Jo-1 antibodies also had anti-Ro antibodies. The anti-Jo-1, anti-topo-1, and most anti-PM-Scl and anti-U1-RNP antibody-positive patients were found in the JDM–SSc group, while all five patients with an anti-Mi-2 antibody were in the JDM group. Since several of the MSAs and MAAs were present in small numbers of children (as expected for a cohort of this size), our results must be interpreted with caution. The presence of any MAA, including anti-PM-Scl and anti-U1-RNP antibodies, represented a significant risk factor for JDM–SSc compared with JDM (54% JDM–SSc vs 4% JDM, Pcorr < 0.0001, OR 28.4, 95% CI 6.1–170.1). Significantly more unidentified specificities were detected in the JDM group. No patients with anti-SRP antibody and apart from the one patient with anti-topo-1 antibody, there were no other patients with scleroderma-associated antibodies, (e.g. anti-centromere or RNA polymerases I, II or III).

**HLA data**

There were significant increases in the frequencies of HLA-DRB1*03 and DQA1*05 for all juvenile myositis cases (as a whole) vs controls (Table 2). These associations of HLA-DRB1*03 and DQA1*05 were strongest for the JDM–SSc subset of patients. The DQB1*02 association in all cases compared with controls (Supplementary Table A). Furthermore, three of the seven anti-PM-Scl antibody-positive patients were DQB1*02 homozygotes (43% anti-PM-Scl vs 9% controls, Pcorr = 0.01). All six (100%) anti-U1-RNP antibody-positive patients had one copy of HLA-DRB1*04, DQA1*05 and DQB1*02, although no homozygosity risk factors were identified. Both patients with anti-Jo-1 antibodies possessed one copy each of the 8.1 AH alleles. Furthermore, four of five anti-Mi-2 positive patients possessed one copy of HLA-DRB1*04, DQA1*03 and
DQA1/*03, and two of five possessed one copy of HLA-DRB1/*07, DQA1/*02 and DQB1/*02.

We also determined whether associations existed independent of the association between anti-PM-Scl and anti-Jo-1 antibodies, and members of the 8.1 AH. After allowing for the presence of these antibodies, the strong associations of the 8.1 AH alleles previously seen in the JDM–SSc group vs controls lost statistical significance, and the 8.1 AH differences between the JDM–SSc and JDM group were also lost. This indicates that the 8.1 AH associations observed are due to the presence of anti-PM-Scl and anti-Jo-1 antibodies.

Haplotype frequencies

Assumed HLA class II haplotypes existed at a frequency of >1% in the control population and captured 89% of the variation (Supplementary Table B is available at Rheumatology Online). The frequency of the HLA-DRB1/*03-DQA1/*05-DQB1/*02 haplotype was significantly increased in both JDM–SSc and MAA-positive groups vs controls (Supplementary Table B). Examining specific antibodies, the HLA-DRB1/*04-DQA1/*03-DQB1/*03 haplotype was a significant risk factor in anti-U1-RNP-positive patients vs controls (50% U1-RNP vs 20% controls, P = 0.02, OR 3.9, 95% CI 0.9–15.1). As expected, the HLA-DRB1/*03-DQA1/*05-DQB1/*02 haplotype was a significant risk factor in the anti-PM-Scl-positive patients vs controls (57.1% PM-Scl vs 16.5% controls, P = 0.001, OR 6.7, 95% CI 1.9–24.4).

Correlation of clinical, serological and HLA associations

Table 3 summarizes the clinical features divided into serological subsets. An absence of calcinosis was noted in MSA-positive patients, but was frequent in ANA-negative patients. Those with unidentified specificities had a significantly lower frequency of sclerodermatous skin changes and Raynaud’s phenomenon compared with MSA/MAA-positive patients. MAA-positive patients had a significantly increased frequency of Raynaud’s phenomenon and sclerodermatous skin changes compared with patients without MAAs. The frequency of patients with mouth ulcers and ulcerative skin disease in the MAA-positive group was low.

Examining specific MAAs, sclerodermatous skin changes were noted in 6/7 anti-PM-Scl-positive patients (PM-Scl-positive vs PM-Scl-negative patients, P corr < 0.00001), and the presence of arthritis was noted in 5/6 anti-U1-RNP-positive cases (P = NS). No specific significant associations were observed in the MSA group (n = 7), although absence of calcinosis and increased frequency of arthritis (present in both anti-Jo-1-positive cases), mouth ulcers and alopecia were noted. In contrast, the MAA group were more likely to have calcinosis but less likely to have ulcerative skin disease, mouth ulcers or alopecia. ANA-negative patients were more likely to develop calcinosis than those with ANA positivity. Finally, ANA-positive patients with unidentified or absent RIP status were associated with less sclerodermatous changes, Raynaud’s phenomenon and calcinosis.

HLA class II data were also stratified by the clinical features in Table 3, to see if further positive associations existed. The 8.1 AH alleles were significant risk factors for the presence of DM-specific rash, skin ulceration, arthritis, sclerodermatous changes and Raynaud’s phenomenon, but not for calcinosis, dysphagia, mouth ulcers or alopecia. Using a stepwise regression model containing all of the clinical features, it was found that the 8.1 AH alleles best predicted the presence of sclerodermatous skin changes. However, after repeating the analysis of the significant clinical associations in Table 3, and adjusting for the presence of 8.1 AH alleles and serology, in each case the serological, rather than the genetic, factor was the stronger predictor of clinical features.

Discussion

This large study brings together HLA haplotype data, detailed autoantibody analysis and clinical data in children with JDM and JSM–SSc overlap. In stratifying our JDM patients by the presence of sclerodermatous features, we demonstrate clear genetic and serological differences between the JDM and JDM–SSc subgroups, and highlight a distinct clinical subtype within JDM, which has been described in the context of childhood-onset SSc [9]. Over 70% of the overall cases are ANA-positive, and MAAs are more frequently detected in JDM–SSc than in the JDM group. The known association of JDM with HLA class II members of the 8.1 AH (HLA-DRB1/*03-DQA1/*05-DQB1/*02) is confirmed. Furthermore, the serological associations appear to define certain clinical features.

The high frequency of ANA positivity in juvenile myositis patients is consistent with both paediatric and adult IIM studies, where the ANA test by indirect immunofluorescence is positive in 30–70% [18, 29, 39–41]. Our finding of a higher ANA frequency in JDM–SSc patients than those with classical JDM is also consistent with adult data, where ANA frequency is higher in myositis-overlap syndromes than in classical DM [27]. While we found some antibodies typical of SSc in the JDM–SSc group (in particular, U1-RNP and PM-Scl), other specificities associated with scleroderma in adults, such as anti-centromere or anti-RNA polymerases I, II or III, were absent, emphasizing the differences between paediatric and adult IIM populations [9, 27]. As expected, the MSA profile of our JDM patients is different to
adult DM. Frequencies of anti-Jo-1 and anti-Mi-2 antibodies in adult DM studies range 16–23% [19, 25, 27, 42]. The frequencies of anti-Jo-1 and anti-Mi-2 antibodies in our JDM cohort are considerably lower than this, consistent with other studies, [29]. The frequency of MAAs detected here in JDM is similar to that in adult DM, and in the JDM–SSc group, it approximates to that seen in adult CTD/myositis overlap [25].

In addition to the ANA specificities tested here, we and others have recently reported a novel specificity which can lead to a positive ANA and detects a protein of 155kDa, frequently associated with a weaker band at 140kDa [43–46]. In our previous report of a series of JDM patients, 23% of JDM sera were positive for anti-p155/140 [45] and this antibody was no more common in those with sclerodermatous features, than those without. There may also be other novel autoantibodies present that account for unidentifiable bands on radioimmunoprecipitation gels, and it is possible that RNA immunoprecipitation that was not performed in the current study may detect yet further specificities.

Considering HLA profiles associated with the MSAs in the current study, both anti-Jo-1-positive JDM–SSc patients had a copy of the 8.1 AH, as described in adult DM. In the five anti-Mi-2-positive patients, the HLA-DRB1*04-DQA1*04-DQB1*03 haplotype was more frequent in JDM, in contrast to the HLA-DRB1*07-DQA1*02-DQB1*02 haplotype more commonly associated with anti-Mi-2 positive adult DM [19, 25, 47]. The strong DRB1*04-DQA1*03-DQB1*03 association in anti-U1-RNP cases parallels that in adult myositis and adult SLE [19, 48, 49]. The HLA class II associations found in our JDM study are also consistent with previous evidence that HLA-DQA1*0501 and DRB1*0301 are risk factors in JDM [12, 50]. However, we have also demonstrated that the HLA class II associations lose statistical significance after accounting for anti-Jo-1 and anti-PM-Sci. It is possible that the presence of the 8.1 AH is more critical for generation of a particular autoantibody repertoire than it is for the development of myositis. One limitation of our study is that even with a large initial cohort (n = 114), once analysis of specific autoantibodies or clinical features is undertaken, some groups are very small, and therefore may be underpowered to reveal all associations.

There is evidence that the TNF-308A allele, a single nucleotide polymorphism which is in strong linkage disequilibrium (LD) with the 8.1 AH, is a risk factor for calcinosis and prolonged disease course [13]. In our study, we have not tested for TNF polymorphisms or analysed clinical data using measures of disease severity. ANA-negative patients showed a higher frequency of calcinosis, and it would therefore be interesting to analyse the ANA status of known TNF-308A carriers. We have not demonstrated an HLA class II association with the presence of calcinosis in this study. Changes in JDM treatment in recent years may explain the falling incidence of calcinosis in JDM, making comparisons with the results of previous studies difficult [2, 3, 51].

Recent high-resolution typing IIM studies of HLA subtypes enabled the derivation of putative peptide-binding motif sites [16, 25]. Since our HLA typing produced broad-type data, we are currently unable to derive such information. Furthermore, our DQA1 results were derived from DRB1 and DQB1 data, on the basis of very strong LD between these loci in UK Caucasians. An exercise in comparing genotyped to derived DQA1 data (unpublished observations in control subjects), produced uncommon typed HLA class II haplotypes not observed in the derived data, which accounted for less than 1% of the variation. Therefore, typing at DQA1 would not have affected our overall results. In keeping with this observation, the control subjects’ HLA profiles, including the derived DQA1 frequencies, were highly comparable with published allelic frequencies for UK Caucasians [30]. The current study was cross-sectional in nature, so it is difficult to assess the prognostic value of the genetic and serological risk factors identified. However, despite these limitations, the well-characterized clinical data have enabled their detailed correlation with serotype and genotype. Stratification of data by disease subtype and serology will inevitably lead to problems with sample size and statistical power. Despite this, the results show that HLA class II status associates with JDM serological status and that serological rather than HLA class II status is the stronger predictor of clinical features. The genetic data, therefore, appear to define certain serological subtypes which in turn identify distinct clinical phenotypes, akin to a suggested system in adult IIM [18].

In conclusion, this study presents clinical and HLA class II haplotype data, stratified by serological subtype. We demonstrate differences in the JDM–SSc overlap subgroup which clearly distinguish these patients from those with classical JDM. We suggest that JDM myositis serology may be a powerful tool for predicting clinical phenotypes. Longitudinal studies of disease course, morbidity and mortality are now required to test this hypothesis. Overall, our data support the concept that JDM is a complex disease, with multiple genetic and environmental risk factors influencing disease susceptibility. In the future, more specific autoantibody typing in JDM, perhaps combined with genetic testing, may provide better prognostic or predictive tests to assist in the management of these complex patients.

**Rheumatology key messages**

- This study analyses a large cohort of carefully characterized patients with JDM and those with overlap with scleroderma.
- Serological analysis and HLA typing of these two groups shows clear genetic and serological differences between the two groups.
- In the future genetic typing combined with high-resolution specificity analysis of autoantibodies may provide new prognostic markers for children with JDM and JDM–scleroderma overlap.

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