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Major histocompatibility complex (MHC) class I and II alleles which confer susceptibility or protection in the Morphea in Adults and Children (MAC) cohort

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Abstract

Objective—To determine human leukocyte antigen class I (HLA-class I) and II (HLA-class II) alleles associated with morphea (localized scleroderma) in the Morphea in Adults and Children (MAC) cohort by a nested case–control association study.

Methods—Morphea patients were included from MAC cohort and matched controls from the NIH/NIAMS Scleroderma Family Registry and DNA Repository and Division of Rheumatology at the University of Texas Health Science Center at Houston. HLA- Class II genotyping and SSCP typing was performed of HLA-A, -B, -C alleles. Associations between HLA-Class I and II alleles and morphea as well as its subphenotypes were determined.

Results—There were 211 cases available for HLA-class I typing with 726 matched controls and 158 cases available for HLA Class-II typing with 1108 matched controls. The strongest associations were found with *DRB1*04:04* (OR 2.3, 95% CI 1.4–4.0 P=0.002) and *HLA-B*37* conferred the highest OR among Class I alleles (3.3, 95% CI 1.6–6.9, P= 0.0016). Comparison with risk alleles in systemic sclerosis determined using the same methods and control population revealed one common allele (*DRB*04:04*).

Conclusion—Results of the present study demonstrate specific HLA Class I and II alleles are associated with morphea and likely generalized and linear subtypes. The associated morphea alleles are different than in scleroderma, implicating morphea is also immunogenetically distinct. Risk alleles in morphea are also associated with conditions such as rheumatoid arthritis (RA) and other autoimmune conditions. Population based studies indicate patients with RA have increased risk of morphea, implicating a common susceptibility allele.

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Conflicts of interest: None

Introduction

Morphea (localized scleroderma) is a disorder characterized by inflammation and sclerosis of the dermis and sometimes subcutaneous fat or deeper structures. It affects both adults and children, often producing impairment of function, cosmesis, and quality of life (1–3).

Studies to date implicate that morphea is an immunologically mediated disease due to its association with other autoimmune disorders (2, 3) and the presence of autoantibodies including ANA (4–6) with greater frequency than expected in comparison to a healthy population. Further, studies examining the histopathological changes in morphea have identified an inflammatory cell infiltrate composed of T and B lymphocytes, plasma cells, and plasmacytoid dendritic cells (7). Limited studies have also identified elevation of cytokines associated with T helper cells in the sera and tissue of morphea patients (8–10). HLA-class II (HLA-DR) expression is also seen on keratinocytes in biopsy specimens from morphea patients (11). However, this ectopic expression of HLA- class II may be secondary to the T-cell infiltrate and indicative of inflammation or cell injury. Despite these studies, the molecular pathogenesis of morphea is poorly understood.

Many autoimmune disorders, including systemic sclerosis, have significant genetic associations with particular alleles of the class I and II human major histocompatibility complex (MHC), and study of these associations has led to significant insight into the molecular underpinnings of these disorders (12). The MHC region on chromosome 6 encodes the highly polymorphic DR, DQ, and DP heterodimers which are present on antigen presenting cells of the immune system and dictate the T-cell repertoire exerting control over immune responsiveness (13, 14). In addition, HLA-A, B, C proteins (Class I) present small polymers from inside the cell (intracellular pathogens or self antigens) to induce cytotoxic T cell responses and are increasingly associated with autoimmunity (13, 14). HLA association studies in morphea are limited. Putative associations made via small case control studies include: HLA-DR1 and –DR5 (*DRB1*01* and *DRB1*11* and *12* respectively with current nomenclature) in a group of 23 German patients (15), HLA DR2 and DRW8 (*DRB1*16:06* and *DRB1*08* respectively with current nomenclature) in 42 German patients (16), and one paper reported that the number of morphea patients was too small for statistical analysis so no associations were made (16). The validity of these associations is uncertain due to limited sample size as well as possible misidentification of cases. Class- I HLA associations have never been reported in morphea.

Morphea and systemic sclerosis are thought to exist in the same spectrum of disorders, as indicated by the designation “localized scleroderma,” largely because they are indistinguishable on histopathology. Consequently, many studies examining pathogenesis group morphea and systemic sclerosis together as a single entity (17). Despite their histological similarity, morphea and systemic sclerosis are distinct in terms of demographics, clinical course, and autoantibody profile, implicating they might be unique entities. However, the assumption that morphea and systemic sclerosis exist within the same spectrum of pathogenesis has never been tested on a molecular level.

The present study, referred to as the MAC cohort (Morphea in Adults and Children), was designed to examine demographic, clinical, antibody, and immunogenetic features in a carefully phenotyped cohort of adults and children with morphea. By studying patients in a nested case-control fashion, we aimed to determine specific HLA-class I and II alleles influencing susceptibility to or protection from morphea, its major subtypes, and antinuclear antibody subsets in the largest cohort to date.

Methods

A case control association study was performed (total morphea cases 211 for Class-I HLA and 158 for Class-II HLA; total controls 726 for Class-I HLA and 1008 for Class-II HLA) with subanalyses by race (Caucasian), morphea subtype (linear and generalized), and presence or absence of antinuclear antibodies. The number of cases is different for Class I and II typing due to limitations in the amount or quality of DNA samples.

Selection of Morphea Cases and Normal Controls

Morphea Cases—Morphea cases were included from the MAC cohort which contains 264 adults (18 years old at enrollment) and children (17 years old at enrollment). All patients or guardians consented by written agreement to inclusion in this study, which was approved by the University of Texas (UT) Southwestern Medical Center Institutional Review Board. The study protocol and informed consent were in compliance with Declaration of Helsinki Principles. Criteria for inclusion in the study reported herein included: eligibility for enrollment in MAC cohort (the details of eligibility have been reported previously) (5, 18); age 4 years or older; and availability of adequate clinical information, sera, and DNA for analysis (in the case of DNA both quantity and quality were ascertained).

The MAC cohort was designed to capture prevalent and incident cases of morphea. Patients were recruited from within the UT Southwestern Medical Center system, encompassing two dedicated pediatric care facilities, a county hospital, and a faculty-based practice. In addition, patients were routinely enrolled through regional and national referrals from dermatologists and rheumatologists, both pediatric and adult. This represents a conscious effort to enroll patients of varied disease severity, subtypes, and socioeconomic backgrounds. After patients (or guardians) signed consent, all data were abstracted using a comprehensive clinical report form (CRF) designed prior to the study, including demographic, clinical, medical history, and family history data. Medical records were obtained and reviewed for confirmation of patient-reported findings. At the time of enrollment, all patients were examined by one examiner with expertise in morphea (H.J.), who assigned each patient one of five clinical subtypes as defined by the preliminary criteria of Zulian and Laxer. (Table 1) (19). Blood samples for immunologic and immunogenetic studies were obtained from patients at the time of enrollment. Patients were excluded from the present study for the following reasons: if the morphea subtype was indeterminate or if insufficient clinical information, sera, or DNA was present for analysis (n=53).

Control Subjects—Controls were included from the NIH/NIAMS Scleroderma Family Registry and DNA Repository (20) and the Division of Rheumatology at the University of

Texas Health Science Center at Houston-(UTHSC-H) (12). Registry controls were primarily spouses or friends of systemic sclerosis subjects, and UTHSC-H controls were healthy medical center personnel or blood bank donors from Texas. All controls were screened for a personal or family history of any autoimmune diseases and excluded if positive. Controls were matched by race only. The same control subjects were selected as those previously published by Arnett, et al (12).

Autoantibody Identification

Antinuclear antibodies (including anti-centromere and anti-mitochondrial) were determined in all patients with morphea by indirect immunofluorescence on HEp-2 cells (Antibodies, Davis, Inc., California, USA) in a single laboratory by a single investigator (F.A.). A titer of 1:80 was considered positive (5).

HLA Class I and II Genotyping

HLA Class I Genotyping—Single Stranded Conformational Polymorphism (SSCP)-typing of HLA-A, -B and -C alleles was carried out using commercially available kits (Dynal Biotech LLC, Biotech Ltd., Oslo, Norway) according to manufacturers' instructions.

HLA Class II Genotyping—HLA-DRB1, DQA1, and DQB1 typing was carried out by standard oligotyping techniques using primers and probes recommended by the 13th International Histocompatibility Testing Workshop (held in Victoria, Canada, May 2002) (21, 22) with high resolution DRB1 typing further achieved by nucleotide sequence analysis of PCR-amplified DRB1 exon 2 as previously published (12, 21, 22).

Statistical analyses

We used statistical analysis software SAS 9.1.3. Descriptive statistics such as frequency counts, median, and interquartile range were used for demographic and clinical variables of interest. Chi-square tests or Fisher's exact tests were used to compare HLA-class I and II allele frequencies between the controls and morphea cases. Subgroups analyzed included: morphea subtypes (only linear and generalized were analyzed due to insufficient numbers in the other morphea subgroups for meaningful analysis), Caucasian subjects, and morphea cases with (out) ANA. We report the odds ratio (OR) and the corresponding 95% confidence interval (CI). P values were adjusted by Bonferroni correction and only those <0.017 were considered significant.

RESULTS

Demographic and Clinical Features

Of the 264 patients in the MAC cohort 211 were included in the present study for HLA-class I and 158 for class II typing. Fifty-three patients were excluded from Class I and 106 from Class II typing due to insufficient or degraded DNA that was not analyzable for HLA determination, indeterminate subtype, or refusal of blood draw. The demographic and clinical features of the cases are available in Table 2. Because the morphea patients were predominantly Caucasian and female, comparisons between race and sex were not performed.

HLA-class II Alleles in Morphea

HLA-class II (DQA1, DQB1, DRB1) genotyping was completed in 158 morphea cases and 1002 normal controls (Table 3). For all cases versus controls an association was found with *HLA-DRB1*04* but specifically with *DRB1*04:04*. *HLA-DQB1*02*, specifically *DQB1*02:01* and *DRB1*03*, specifically *DRB1*03:01* were also increased. The highest odds ratio was conferred by *HLA-DRB1*04:04* (OR 2.3, 95% CI 1.4–4.0, P=0.002). *HLA-DQA1*03:00* was present exclusively in patients with morphea. The following allele was less frequent in morphea versus controls: *HLA-DQA1*0401*.

For the 113 Caucasian patients available for analysis of Class II alleles, the OR of *HLA-DRB1*04* was increased (OR 2.9, 95% CI 1.4–5.8, P=0.0023) as compared with the OR present in the analysis of the group overall (Table 3). In addition, the proportion of Caucasians with *HLA-DRB1*04* was higher than in the overall group, implying the association in the overall group was driven by the Caucasian patients. The risk association of *HLA-DQB1*02:01* and *DQA1*03:00* was maintained with similar OR and proportion of cases with the allele as in the overall group analysis, implying that the association was with the group overall. The remaining Class II associations present in the cases overall became statistically insignificant when Caucasian cases and controls were analyzed. No other Class II alleles were associated with Caucasian cases.

HLA-class I Alleles in Morphea

HLA-class I genotyping was completed in 211 morphea cases and 675 controls (Table 4). *HLA-B*37* conferred the highest odds ratio (OR 3.2, 95% CI 1.5–6.5, P= 0.001). *HLA-C*08* (OR 2.6, 95% CI 1.5–4.6, P=0.0006) and *HLA-C*15* (OR 2.7, 95% CI 1.2–6.0, P=0.014) were also associated with morphea cases. In comparing Caucasian cases with controls the risk association with *HLA-B*37* maintained statistical significance and the allele was present in similar proportion to the cases overall thus producing a similar OR, implying the association was with the overall group of cases. The other Class I associations reported in the overall morphea cases became statistically insignificant when comparing Caucasian cases to controls. No other alleles attained statistical significance in this comparison.

HLA Allele Frequencies in Morphea Subtypes

The results of HLA I and II association with morphea subtypes are available in Tables 5 and 6. In comparing patients with generalized morphea and controls (Table 5) the same HLA Class I and II alleles were present as when comparing overall cases to controls including *DQA1*0300*, *DQB1*0201*, *C*08* and *C*15*. To determine whether alleles associated with generalized morphea were present in greater proportion in the generalized subtype than the group overall Fisher's exact test was performed. All the alleles were present generalized cases in similar proportion to the morphea group overall, implying they are associated with the overall group of cases, not the generalized subtype. The exception was *HLA-DRB1*15* and *DRB1*15:01* which conferred the highest OR in the generalized subgroup, and was present in greater proportion in generalized morphea than in the group overall (p<0.001). This allele missed statistical significance for association in the group overall, implying it is associated with the generalized subtype. Additional analysis revealed that the majority of

cases with *HLA-DRB1*15* had generalized morphea implying an association between generalized morphea and this allele.

Alleles associated with linear morphea were the same as in the group overall, with the exception of *DQA1*01:02*, which was protective in linear morphea and not present in the analysis of the group in its entirety (Table 6). The proportion of these alleles present in linear morphea was compared to the proportion present in the morphea group overall and there were no statistically significant differences, suggesting the association of these alleles was with the group overall, rather than the linear subtype.

HLA Allele Frequencies in Antinuclear Antibody Subsets of Morphea

ANA were present in 49 (23.2%) of the morphea cases. Morphea patients enrolled in the MAC cohort were tested for numerous autoantibodies (extractable nuclear antigens, anti-histone antibodies, anti-ssDNA antibodies, and the like), but they were present so infrequently that analysis was not feasible (anti-ssDNA n=13, anti-histone n=24). The details of these studies were previously published (6). The results of HLA class I and II association analyses in ANA negative and positive patients did not reveal any associations with the presence or absence of ANA.

DISCUSSION

We undertook this case control association study of 211 patients with morphea and 675 controls (HLA Class I) and 158 cases and 1002 controls (HLA Class II) in order to determine which HLA class I and II alleles were associated with morphea, its subtypes, and presence of ANA. Our results demonstrate that specific alleles are associated with morphea overall and certain alleles may be specific to the generalized subtype.

The present study is the first to provide strong evidence for the association of HLA class II alleles with morphea and its subtypes. The strongest association was with *HLA-DRB1*04* specifically with *DRB1*04:04*, which conferred an OR of 2.3 (95% CI 1.4–4.0, adjusted p-value 0.002). This association was strengthened (the proportion with this allele was significantly higher in Caucasians with morphea than non-Caucasians) implying the association of this allele might be due to racial differences. *DQB1*02:01* as well as *DRB1*03:01* also conferred risk in morphea. New to this study, HLA class I alleles were also found in association with morphea. *HLA-B*37* conferred the highest odds ratio (3.2, 95% CI 1.5–6.5, P= 0.001) as well as *HLA-C*08* and *HLA-C*15*. The association of morphea with specific class -I HLA alleles supports the role of CD8 or natural killer (NK) cell-associated immune responses in the pathogenesis of morphea and implicates loss of tolerance to an unknown self -antigen. Older literature implicated *HLA-B*37* in susceptibility to psoriasis (23), though this was subsequently found to be better explained by the linkage of *HLA-B*37* to *C*06*, which is well established as an important psoriasis susceptibility factor. However in the present study, no association was seen with *HLA-C*06*, suggesting a primary association with *B*37*.

Further examination of the frequency of HLA alleles within subtypes of morphea revealed an association with the generalized subtype (*HLA-DRB1*15:01*). This association was

significant when comparing patients with generalized morphea with controls and retained significance when comparing generalized morphea with the group overall. Although there were class-I and II alleles associated with linear morphea when comparing linear morphea cases and controls, they did not retain statistical significance when comparing the proportion of linear morphea cases with these alleles to the proportions in morphea group overall. This implies that the association was with the group overall and not specific to the linear subtype. However, these data should be interpreted with caution as the sample size might not have been large enough to detect a difference. Taken together, this data implies that generalized morphea is not only phenotypically unique from other morphea subtypes in terms of clinical manifestations and demographic features, but also immunogenetically distinct.

HLA Class II alleles associated with morphea in the present study do not overlap with those previously reported (15, 16). Given that these studies were in very small groups of patients and controls, it is possible that the previously reported associations occurred by chance alone or reflect associations in a different group of cases.

Current paradigms suggest that morphea and systemic sclerosis exist in a continuous spectrum of sclerosing disorders, with morphea occupying the skin dominant end of the spectrum and systemic sclerosis the systemic. This is reflected in calls to retain the designation “localized scleroderma” in the literature and the inclusion of patients with morphea and systemic sclerosis as one group in studies of pathogenesis (19). We compared the susceptibility alleles previously reported by Arnett et al (Ref 12) with the morphea susceptibility alleles identified in the present study. This study was chosen because HLA alleles were determined in the same laboratory and analysis was performed using the same controls as in the present study. However, we found morphea and systemic sclerosis have few susceptibility alleles in common. In fact, alleles that conferred susceptibility in morphea are reported to be protective in systemic sclerosis and vice versa. These include: HLA-*DRB1*15:01* (increased risk in generalized morphea, decreased risk in systemic sclerosis). The only allele that is associated with both disorders is HLA-*DRB1*04:04*, which confers risk in both. Rather, the alleles associated with morphea are strongly associated with rheumatoid arthritis (RA) (24) (*DRB1*04:04*), multiple sclerosis (MS) (*DRB1*15:01*) autoimmune thyroid disease (AITD) (*DQB1*02:01*, *DRB1*03:01*, and *DRB1*04*) (26); and diabetes mellitus type I (2) (*DRB1*03:01*). Interestingly, population based studies examining the autoimmune profile of RA, MS, and AITD have identified increased risk (SIR) of morphea in patients with these disorders, implicating a common genetic susceptibility (26–28). Additionally, studies of morphea patients report increased frequency of RA, further supporting common susceptibility (2). This indicates morphea and systemic sclerosis may be immunogenetically distinct.

This study is subject to several limitations. First, despite the fact that this is the largest series to date, the sample size is relatively small, especially in reference to subgroups of morphea patients. This limited comparison across sex, race, and morphea subtypes. Consequently associations or (or lack of an association) in subgroups should be interpreted with caution. It is possible additional HLA Class II associations exist, but were not detected because the present study included only HLA DRB1, DQA1, and DQB1 alleles. Further, validation in a second independent cohort is needed to confirm HLA associations.

In summary, the presence of specific HLA class I and II alleles is associated with both susceptibility and protection in morphea; but few of these alleles are shared with systemic sclerosis. This implies that the paradigm which places morphea and systemic sclerosis as a continuum of the same process is inaccurate from an immunogenetic perspective. Alleles which confer risk in morphea are also reported in conditions that have increased risk for morphea clinically, implicating common susceptibility.

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References

1. Kroft EB, de Jong EM, Evers AW. Physical burden of symptoms in patients with localized scleroderma and eosinophilic fasciitis. *Arch Dermatol*. 2008; 144:1394–5. [PubMed: 18936410]
2. Leitenberger JJ, Cayce RL, Haley RW, Adams-Huet B, Bergstresser PR, Jacobe HT. Distinct autoimmune syndromes in morphea: a review of 245 adult and pediatric cases. *Arch Dermatol*. 2009; 145:545–50. [PubMed: 19451498]
3. Zulian F, Vallongo C, Woo P, Russo R, Ruperto N, Harper J, et al. Localized scleroderma in childhood is not just a skin disease. *Arthritis Rheum*. 2005; 52:2873–81. [PubMed: 16142730]
4. Falanga V, Medsger TA Jr, Reichlin M. High titers of antibodies to single-stranded DNA in linear scleroderma. *Arch Dermatol*. 1985; 121:345–7. [PubMed: 3883904]
5. Arkachaisri T, Fertig N, Pino S, Medsger TA Jr. Serum autoantibodies and their clinical associations in patients with childhood- and adult-onset linear scleroderma. A single-center study. *J Rheumatol*. 2008; 35:2439–44. [PubMed: 19004036]
6. Dharamsi JW, Victor S, Aguwa N, Ahn C, Arnett F, Mayes MD, et al. Morphea in adults and children cohort III: Nested case-control study: the clinical significance of autoantibodies in morphea. *JAMA Dermatol*. 2013; 149:1159–65. [PubMed: 23925398]
7. Torres JE, Sanchez JL. Histopathologic differentiation between localized and systemic scleroderma. *Am J Dermatopathol*. 1998; 20:242–5. [PubMed: 9650695]
8. Ghoreishi M, Vera Kellet C, Dutz JP. Type 1 IFN-induced protein MxA and plasmacytoid dendritic cells in lesions of morphea. *Exp Dermatol*. 2012; 21:417–9. [PubMed: 22507598]
9. Ihn H, Sato S, Fujimoto M, Kikuchi K, Takehara K. Demonstration of interleukin-2, interleukin-4 and interleukin-6 in sera from patients with localized scleroderma. *Arch Dermatol Res*. 1995; 287:193–7. [PubMed: 7763091]
10. Alecu M, Geleriu L, Coman G, Galatescu L. The interleukin-1, interleukin-2, interleukin-6 and tumour necrosis factor alpha serological levels in localised and systemic sclerosis. *Rom J Intern Med*. 1998; 36:251–9. [PubMed: 10822522]
11. Aubock J, Romani N, Grubauer G, Fritsch P. HLA-DR expression on keratinocytes is a common feature of diseased skin. *Br J Dermatol*. 1986; 114:465–72. [PubMed: 2421756]
12. Arnett FC, Gourh P, Shete S, Ahn CW, Honey RE, Agarwal SK, et al. Major histocompatibility complex (MHC) class II alleles, haplotypes and epitopes which confer susceptibility or protection in systemic sclerosis: analyses in 1300 Caucasian, African-American and Hispanic cases and 1000 controls. *Ann Rheum Dis*. 2010; 69:822–7. [PubMed: 19596691]
13. Klein J, Sato A. The HLA system. First of two parts. *N Engl J Med*. 2000; 343:702–9. [PubMed: 10974135]

14. Klein J, Sato A. The HLA system. Second of two parts. *N Engl J Med.* 2000; 343:782–6. [PubMed: 10984567]
15. Luderschmidt C, Scholz S, Mehlhaff E, König G, Albert E. Association of progressive systemic sclerosis to several HLA-B and HLA-DR alleles. *Arch Dermatol.* 1987; 123:1188–91. [PubMed: 3498440]
16. Kuhn P, Sibrowski W, Kalmar G, Holzmann H, Sollberg S, Böhm BO. HLA-antigen frequencies in patients with progressive systemic sclerosis and morphea. *Beitr Infusionsther.* 1990; 26:287–9. [PubMed: 1703854]
17. Milano A, Pendergrass SA, Sargent JL, George LK, McCalmont TH, Connolly MK, et al. Molecular subsets in the gene expression signatures of scleroderma skin. *PLoS One.* 2008; 3:e2696. [PubMed: 18648520]
18. Freudenberg J, Holzmann H, Schneider S, Korting GW. HLA antigen frequencies in patients with progressive systemic sclerosis and morphea (author's transl). *Arch Dermatol Res.* 1978; 263:197–205. [PubMed: 736604]
19. Laxer RM, Zulian F. Localized scleroderma. *Curr Opin Rheumatol.* 2006; 18:606–13. [PubMed: 17053506]
20. Mayes MD. The establishment and utility of a population-based registry to understand the epidemiology of systemic sclerosis. *Curr Rheumatol Rep.* 2000; 2:512–6. [PubMed: 11123106]
21. Hansen, JA.; Dupont, B. HLA 2004: Immunobiology of the Human MHC. The Proceedings of the 13th IHWC; Seattle: IHWG Press; 2004.
22. Olsen ML, Arnett FC, Reveille JD. Contrasting molecular patterns of MHC class II alleles associated with the anti-Sm and anti-RNP precipitin autoantibodies in systemic lupus erythematosus. *Arthritis Rheum.* 1993; 36:94–104. [PubMed: 7678744]
23. Gazit E, Brenner S, Efer T, Orgad S, Mizrahi Y, Krakowski A. HLA antigens in patients with psoriasis. *Tissue Antigens.* 1978; 12:195–9. [PubMed: 83016]
24. Weyand CM, Hicok KC, Conn DL, Goronzy JJ. The influence of HLA-DRB1 genes on disease severity in rheumatoid arthritis. *Ann Intern Med.* 1992; 117:801–6. [PubMed: 1416553]
25. Oksenberg JR, Baranzini SE, Barcellos LF, Hauser SL. Multiple sclerosis: genomic rewards. *J Neuroimmunol.* 2001; 113:171–84. [PubMed: 11164900]
26. Simmonds MJ, Gough SC. Unravelling the genetic complexity of autoimmune thyroid disease: HLA, CTLA-4 and beyond. *Clin Exp Immunol.* 2004; 136:1–10. [PubMed: 15030506]
27. Hemminki K, Li X, Sundquist J, Hillert J, Sundquist K. Risk for multiple sclerosis in relatives and spouses of patients diagnosed with autoimmune and related conditions. *Neurogenetics.* 2009; 10:5–11. [PubMed: 18843511]
28. Hemminki K, Li X, Sundquist J, Sundquist K. Familial associations of rheumatoid arthritis with autoimmune diseases and related conditions. *Arthritis Rheum.* 2009; 60:661–8. [PubMed: 19248111]

Table 1

Preliminary proposed classification of juvenile localized scleroderma

Main group	Subtype	Description
Circumscribed morphea	(a) Superficial (b) Deep	Oval or round circumscribed areas of induration limited to epidermis and dermis. Oval or round circumscribed deep induration of the skin involving subcutaneous tissue extending to fascia and may involve underlying muscle.
Linear morphea *	(a) Trunk/limbs (b) Head	Linear induration involving dermis, subcutaneous tissue and, sometimes, muscle and underlying bone. <i>En coup de sabre</i> (ECDS). Linear induration that affects the face and the scalp and sometimes involves muscle and underlying bone. Includes Parry Romberg (progressive hemifacial atrophy).
Generalized morphea		Induration of the skin starting as individual plaques (four or more and larger than 3 cm) that become confluent and involve at least two out of seven anatomic sites.
Pansclerotic morphea *		Circumferential involvement of limb(s) affecting the skin, subcutaneous tissue, muscle and bone.
Mixed morphea		Combination of two or more of the previous subtypes.

Adapted from R Laxer, F Zulian. Localized scleroderma. *Current Opinions in Rheumatology*. 2006.18:606–613.

* This is a modification from the classification as originally published which reads ‘linear scleroderma.’ The authors avoid the term “linear scleroderma” due to confusion with “limited scleroderma” among patients and providers and use linear morphea in this manuscript.

Table 2

Demographic features of cases

Total no. of patients	211*	
Age at Onset, Yrs, median(IQR)	27	(12–37)
Gender, N (%)		
Male	37	(17.5%)
Female	174	(82.5%)
Race, N (%)		
Caucasian	161	(76.3%)
Latino	29	(13.7%)
African American	8	(3.8%)
Asian	7	(3.3%)
Other	6	(2.8%)
Subtype, N (%)		
Linear	95	(45.0%)
Generalized	75	(35.5%)
Plaque	25	(11.8%)
Other	21	(10.0%)
Positive ANA, N (%)	49	(23.2%)

Table 3

HLA-class II alleles which confer susceptibility or protection in patients with morphea

Allele	Controls (n=1002) [‡]	Cases (n=158) [‡]	Odds Ratio (95% CI)	p-value *
<u>DRB1</u> *03:01	186/1001(18%)	43/158 (27%)	1.6 (1.1 – 2.4)	0.011
<u>DRB1</u> *04:04	59/1001 (6%)	20/158 (13%)	2.3 (1.4 – 4.0)	0.002
<u>DQA1</u> *03:00	0/1002 (0%)	26/101 (26%)	N/A	< 0.0001
<u>DQA1</u> *04:01	120/1002 (12%)	3/101 [‡] (3%)	0.2 (0.1 – 0.7)	0.006
<u>DQB1</u> *02:01	205/1002 (20%)	50/154 (32%)	1.89 (1.3 – 2.7)	0.0008

* P value <0.017 was considered statistically significant. All P values are chi square tests unless indicated by [†] where Fisher's exact test was performed. Alleles conferring risk are underlined.

[‡] Numbers of cases and controls vary by each allele due to incomplete HLA typing.

Table 4

HLA-class I alleles which confer susceptibility or protection in patients with morphea

Allele	Controls (n=675) [†]	Cases (n=211) [†]	Odds Ratio (95% CI)	p-value *
A *33	13/595 (2%)	11/211 (5%)	2.5 (1.1 – 5.6)	0.026
B *37	16/675 (2%)	15/211 (7%)	3.2 (1.5 – 6.5)	0.001
C *08	31/579 (5%)	23/178 (13%)	2.6 (1.5 – 4.6)	0.0006
C *15	14/579 (2%)	11/178 (6%)	2.7 (1.2 – 6.0)	0.014

* P value less than 0.017 was considered statistically significant. All p-values are from chi-square tests

[†] Numbers of cases and controls vary by each allele due to incomplete HLA typing.

Table 5

HLA-class I and II allelic frequencies associated with generalized morphea

Allele	Controls [†]	Generalized	Odds Ratio (95% CI)	p-value *
Class II				
<u>DRA1</u> * <u>15</u>	246/1001 (25%)	23/58 8(40%)	2.0 (1.2 – 3.5)	0.010
<u>DRA1</u> * <u>15:01</u>	185/1001 (18%)	21/58 (36%)	2.5 (1.4 – 4.4)	0.0009
<u>DQA1</u> * <u>03:00</u>	0/1002 (0%)	11/38 (29%)	N/A	< 0.0001
<u>DQA1</u> * <u>02</u>	353/1002 (35%)	30/58 (52%)	2.0 (1.2 – 3.4)	0.011
<u>DQA1</u> * <u>02:01</u>	205/1002 (20%)	22/58 (38%)	2.4 (1.4 – 4.1)	0.002
Class I				
<u>C</u> * <u>08</u>	31/579 (5%)	11/62 (18%)	3.8 (1.8 – 8.0)	0.0002
<u>C</u> * <u>15</u>	14/579 (2%)	7/83 (8%)	3.7 (1.5 – 9.5)	0.010

* P value less than 0.017 was considered statistically significant. All p-values are from chi-square tests

[†] Numbers of cases and controls vary by each allele due to incomplete HLA typing.

Table 6

HLA-class I and II allelic frequencies associated with linear morphea

HLA Class II	Controls [†]	Linear	Odds Ratio (95% CI)	p-value [*]
Class II				
<u>DRB1</u> * <u>04:04</u>	59/1001 (6%)	9/68 (13%)	2.4 (1.2 – 5.2)	0.016
<u>DQA1</u> * <u>01:02</u>	352/1002 (35%)	8/46 (17%)	0.4 (0.2 – 0.8)	0.013
<u>DQA1</u> * <u>03:00</u>	0/1002 (0%)	12/46 (26%)	N/A	< 0.0001 [*]
Class I				
<u>A</u> * <u>33</u>	13/595 (2%)	7/93 (8%)	3.6 (1.4 – 9.4)	0.012 [*]
<u>C</u> * <u>15</u>	14/579 (2%)	7/83 (8%)	3.7 (1.5 – 9.5)	0.010 [*]

^{*} P value less than 0.017 was considered statistically significant. All p-values are from chi-square tests

[†] Numbers of cases and controls vary by each allele due to incomplete HLA typing.