REVIEW ARTICLE

Influence of the Human Leukocyte Antigen Complex on the Development of Cutaneous Fibrosis: An Immunogenetic Perspective

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A number of aesthetically and physically distressing disorders of the skin come under the general term “cutaneous fibrosis”, all sharing a common abnormal wound healing process. These disorders are often incurable and effective treatments remain to be established and, as such, they present a significant burden for patients and a therapeutic challenge for clinicians. The aim of this review is to investigate the evidence of either positive or negative associations of the human leukocyte antigen (HLA) system with various types of cutaneous fibrosis, focussing in particular on keloid scars, hypertrophic scars and scleroderma. A standard systematic literature search was performed. The strengths and limitations of studies were evaluated in terms of significance, methodology and reproducibility. There is a clear association between specific HLA alleles and predilection or protection to cutaneous fibrosis. Of these candidate HLA alleles, the class II loci seem to be the most promising in terms of a genetic biomarker, with the DQ and DR alleles having significant associations with abnormal wound healing and cutaneous fibrosis. There is strong evidence of a significant immune component in the pathogenesis of each type of fibrotic disorder explored in this review. However, the exact mechanisms remain to be elucidated, since the pathogenesis of cutaneous fibrosis and abnormal wound healing are not fully understood. Key words: cutaneous fibrosis; human leukocyte antigen; major histocompatibility complex; keloid; hypertrophic scars; scleroderma; systemic sclerosis; abnormal wound healing.

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Cutaneous fibrosis covers a variety of human disorders with differing aetiology, but with a common dysregulation of connective tissue metabolism, particularly of dermal fibroblasts (9). Specific examples of cutaneous fibrosis include keloid disease, hypertrophic scars (HS) and scleroderma. Since the discovery of the human leukocyte antigen (HLA) system (Fig. 1), there has been considerable research interest into the mechanisms of disease and genetic association with the HLA gene loci (10–22), covering a wide range of both fibrotic and non-fibrotic disorders. Some allelic associations have already been identified as predictors of prognosis, demonstrated by functional effects of mutations resulting in an altered immune response.

Positive associations have been made between HLA antigens and diseases with a suspected immunological and/or malignant aetiology. The HLA complex system is one of the most polymorphic systems in the human genome (Figs 1–3). It is, therefore, an ideal target for identification of potential genetic biomarkers of disease. The source of HLA allele diversity differs from that of antibodies and T-cell receptors (23). Diversification of antibodies and T-cell receptors is a continual process involving random rearrangements and somatic mutation (23). In contrast, major histocompatibility complex (MHC) diversity does not change over time in an individual, but alleles may differ significantly between individuals (23). This makes the MHC complex an even more promising biomarker target.

However, it remains difficult to construct case-control studies to analyse specific HLA alleles and haplotypes.
with disease association, given the extent of the polymorphism and linkage disequilibrium (13, 23, 24). Nevertheless, there have been a number of mechanisms elucidated for the role of certain HLA alleles in the pathogenesis of cutaneous fibrosis. The aim of this review is to explore the current literature surrounding the area of immunogenetic associations with cutaneous fibrosis, focussing in particular on HLA allelic associations, contributing perspectives on the direction of future research and the possible outcomes and clinical relevance such research may initiate.

METHODS

Relevant research articles were identified via a systematic search of scientific databases, specifically PubMed, Scopus, Science Direct and Scirus. A number of key search terms were used, including: cutaneous fibrosis, keloid disease, fibrosis, human leukocyte antigen (HLA), hypertrophic scars, scleroderma, systemic sclerosis, genetic association and linkage. These search terms yielded a considerable amount of literature surrounding the area of research explored in this review, which was then analysed in terms of results, methodology and study limitations.

RESULTS OF THE LITERATURE SEARCH

Overview – the role of immune cells and human leukocyte antigen molecules in cutaneous fibrosis

A number of functional immune cells, particularly T lymphocytes and macrophages, have been shown to be augmented in skin lesions undergoing fibrosis, particularly within the dermis (3, 16, 25). These cells, along with immune incompetent cells (i.e. keratinocytes in the epidermis, fibroblasts in the dermis and endothelial cells in the dermis), are often associated with HLA class II molecules (3, 6, 26). These HLA molecules may increase the binding and presenting capacity of antigen-associated lymphocytes, which mediate the actions of other immune components (27). Such molecules also act as co-stimulatory molecules that augment inflammation and mediate cytokine production (28–32). In turn, cytokine production has been linked to the increase in collagen deposition in fibrotic lesions (4, 33). A number of initiating events of cutaneous fibrosis have been suggested, including a response to cutaneous antigens or a possible reaction to environmental super-antigens including viral infection (31, 32, 34, 35). This could suggest either an autoimmune response mechanism or delayed-type hypersensitivity following dermal or epidermal injury (Fig. 4 and Table I).
**Human leukocyte antigen and keloids**

Keloids are benign, fibroproliferative dermal lesions resulting from an aberrant or exaggerated wound healing process (2, 4–6, 8, 36, 37), more often than not following minor trauma to the skin (2, 5). The regulated sequence of events during wound healing is disrupted, resulting in a wound healing process that remains in the proliferative phase for an extended time period, leading to elevated deposition of extracellular matrix (ECM) (7, 38). This stage is preceded by the inflammatory stage (7).

There appears to be a genetic predisposition to keloid development, although the exact incidence and genesis

<table>
<thead>
<tr>
<th>HLA allele</th>
<th>Associated disease</th>
<th>Positive or negative association</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-DRB1*15</td>
<td>Keloid disease</td>
<td>Positive</td>
<td>Brown et al. (6)</td>
</tr>
<tr>
<td>HLA-DRB1*08</td>
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<td>Lu et al. (21)</td>
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<tr>
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<td>Lu et al. (21)</td>
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<tr>
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<td>Negative</td>
<td></td>
</tr>
<tr>
<td>HLA-DQB1*0201</td>
<td>Keloid disease</td>
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</tr>
<tr>
<td>HLA-DQB1*0402</td>
<td>Keloid disease</td>
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<td></td>
</tr>
<tr>
<td>HLA-B14</td>
<td>Keloid disease/hypertrophic scar</td>
<td>Positive</td>
<td>Laurentaci &amp; Dioguardi (70)</td>
</tr>
<tr>
<td>HLA-Bw16</td>
<td>Hypertrophic scar (following thermal injury)</td>
<td>Positive</td>
<td>Castagnoli et al. (30)</td>
</tr>
<tr>
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<td>Scleroderma</td>
<td>Positive (for PSS-DS)</td>
<td>Lynch et al. (104)</td>
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<td>Positive (for non-diffuse scleroderma)</td>
<td>Black et al. (99)</td>
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<tr>
<td>HLA-DR1/DR5</td>
<td>Scleroderma</td>
<td>Positive (in Caucasian population)</td>
<td>Livingston et al. (103)</td>
</tr>
<tr>
<td>HLA-DR6.1</td>
<td>Scleroderma</td>
<td>Positive (in black population)</td>
<td>Livingston et al. (103)</td>
</tr>
<tr>
<td>HLA-DR5</td>
<td>Scleroderma</td>
<td>Positive</td>
<td>Gladman et al. (102)</td>
</tr>
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</table>

PSS-DS: Progressive Systemic Sclerosis-Diffuse Scleroderma

*Fig. 4. Role of immune cells and human leukocyte antigen (HLA) molecules in fibrotic skin. Evidence has shown that an immune response mounted against cutaneous antigens or delayed-type hypersensitivity may lead to the elevated levels of immune mediators and thus augmented collagen levels which contribute to cutaneous fibrosis. Certain HLA alleles may be more efficient at antigen binding following trauma in susceptible individuals leading to an overactive immune cascade. HLA molecules are bound to antigen-presenting cells, which in turn activate the T-cell response. An increase in inflammation, cytokines and autoantibodies has been shown in a number of studies of cutaneous fibrotic lesions. As a result, fibroblasts proliferate at a higher rate than those in normal skin and cause collagen levels to increase leading to the characteristic changes seen in fibrotic skin. SC: stratum corneum; EP: epidermis; PD: papillary dermis; RD: reticular dermis; HD: hypodermis; SG: sebaceous gland; ECM extracellular matrix; APC: antigen presenting cell.*
of the disease remains uncertain (4, 6, 39–43). Evidence suggests a predilection to keloid formation with autosomal dominant, autosomal recessive or X-linked recessive inheritance patterns (7, 39, 42, 43). Keloids only affect humans (7, 44), and this is a major barrier to research into the aetiology of the disease due to the lack of a beneficial animal model (2, 44, 45). As such, keloids continue to be a burden to patients, who often present with both physical and psychological symptoms (1–6). There is no single consistently effective treatment available (2, 4, 6). Furthermore, surgery to an elevated recurrence rate (2, 7, 8) (50–70%) (8), since the new wound is prone to the same mechanical, immunological and biochemical mechanisms as the initial wound (7).

There is a clear ethnic variation in the prevalence of keloid formation, advocating a strong genetic predisposition: keloids are more common among darker-skinned races, with high incidence rates among people of African and Asian descent compared with Caucasians (46, 47). Furthermore, keloids rarely affect individuals with albinism (4). There is also concordance of keloid formation in identical twins (6). There is an equal risk of keloid development between both sexes (7, 48), although females may be more likely to report the disorder given the aesthetic implications.

There have been a number of studies that have focused on the immune aspect of keloid formation (4, 26, 27, 34, 49–53). As noted by Olabanji et al. (52), the increased incidence of keloid disease in younger individuals, particularly of the second to fourth decades of life, could be due to either the increased predisposition to trauma at a young age or the genetic differences in tissue repair (52). Furthermore, the immune system is more efficient at this stage in life. In addition, it has been noted that keloid scars of older patients are generally formed at a younger age (52). Yet, the age at onset and the anatomical site of keloid disease is often reflective of the initial causative trauma relative to age, sex and ethnic origin (2, 7, 36, 53). For example, keloids of the earlobes are often a consequence of ear piercing in younger individuals (7), whereas in older patients keloid formation is often due to an increase in mid-chest operative procedures and coronary artery bypass surgery at this stage in life, thus increasing the risk of sternal keloids (2). Additionally, the skin of younger individuals has greater tension (7, 36), which is a major influential factor in keloid formation, along with an abundance of ECM components (54). Concurrently, high incidence rates in Sudan have been partly accounted for by the practice of tribal markings in specific anatomical sites (53).

With respect to the immune response theory, a number of mechanisms have been hypothesized (Fig. 4). These include an immune response mounted against cutaneous antigens (51), sebum (4, 53) and melanin (52). Delayed-type hypersensitivity reaction to cutaneous antigens (51, 52) and antinuclear antibodies to fibroblasts as mediators in immune activity has also been proposed (34). While Cohen & McCoy (27) state that levels of α-globulin, serum complement, T lymphocytes and B lymphocytes remain normal in serum of keloid patients, others have shown an increase in these immune response mediators within keloid lesions. Additionally, levels of IgG within keloid scar tissue have been shown to be augmented in comparison with normal tissue and that of HS (1, 37, 55). Similarly, it has been shown that keloid scars arise from multiple cells of origin (26), including interactions between fibroblasts, myofibroblasts, macrophages, T cells, and endothelial cells (8). This was demonstrated by Knapp et al. (49), who found that multiple phenotypic differences occurred in cells derived from keloid lesions. However, the interrelation between such immune mechanisms and the initial injury remains uncertain. In addition, the degree of keloid scar tissue bears little or no resemblance to the initial underlying injury, with trivial trauma resulting in severe, abnormal scar tissue (7). Although keloids have been previously reported to occur spontaneously (1, 7, 56, 57), it is likely that these lesions had been caused by trivial or over-looked injury, such as insect bites or razor cuts (7, 52). As such, the early classification of keloids into two groups; namely “true” keloids (spontaneous lesions devoid of previous trauma) and “false” keloids (arising from an initial trauma) has been entirely rejected (7). Nevertheless, Muir (50) states that keloids in Caucasians may occur without any obvious causative injury, given that the keloid-susceptible areas in white-skinned individuals are only rarely exposed to accidental injury. Additionally, Muir (50) recorded no evidence of keloid scars in children that showed invasion of normal tissue or failed to regress after 6 months. Furthermore, two patients were seen with keloid scars on the pinna of only one ear following bilateral operations (50). Moreover, the time lapse between initial cutaneous injury and keloid formation is often several weeks to years (7), perhaps bringing into question the immune response hypothesis. Yet, although keloids were initially thought to be limited to the abnormal healing process of deep skin wounds of the dermis (36, 57, 58), several recent studies have reported epidermis-dermis interactions (59, 60), which could account for keloid formation following minor injury breaching the epidermal-dermal junction.

An early study by Yagi et al. (53) noted that keloids very rarely develop in areas lacking sebaceous glands, such as the genitalia, the palms and the soles of the feet (53). In addition, there is a greater abundance of sebaceous glands on the face, which often leads to acne formation, particularly during puberty (61). Acne is a common initial cause of keloid formation (36). Furthermore, since body piercings are another common cause of keloid scarring (62), sebum build-up around piercings may provide a triggering event for keloid formation. Additionally, reports of acne as the causative cutaneous...
injury in elderly patients (36) may be explained by the higher incidence of sebaceous hyperplasia in old age (63), which shares characteristics with acne (63). This could be one explanation for the difference in incidence of keloids between different ethnic populations and age groups. It could also explain why only humans develop keloids, since humans are the only mammals with true sebaceous glands (64).

It may be more logical to study the association of the immune aetiology of keloid formation in conjunction with melanin distribution, given the higher incidence of keloid formation among darker-skinned individuals and the increased risk in anatomical areas with a high concentration of melanin (65). This increased incidence in certain races may be secondary to alterations in the normal metabolism of melanotropin (66). Incidentally, this could coincide with the suggestions of an aberrant immune response to melanin and/or other cutaneous antigens. However, there is not sufficient evidence to support such speculation. Yet, it is known that an increase in melanin-synthesizing hormone (MSH) occurs during puberty and pregnancy (67), which are periods of increased keloid susceptibility (65).

In light of such strong evidence suggesting both genetic and immune response aetiology for keloid formation, it seems plausible to study the possible association with specific HLA alleles. Although the scale of genetic studies is limited in terms of HLA associations, there are some promising data to suggest a link between keloid disease and the HLA-DR loci. In a recent study, Brown et al. (6) compared the HLA-DRB1 phenotype frequencies of Northern European Caucasians with keloid scars with a control population. The resulting data showed a higher frequency of the HLA-DRB1*15 phenotype in keloid patients (38.8%) compared with the control group (20.9%), (p = 0.017). In addition, HLA-DRB1*08 occurred at a frequency of 6.9% in the control group, but was absent from the keloid patients.

A similar study by Lu et al. (21) recognized an association between keloid-positive patients and HLA-DQA1 and DQB1 alleles in Chinese Han subjects, using the polymerase chain reaction-sequence specific primers (PCR-SSP) typing method in 192 keloid patients. In this study HLA-DQA1*0104, DQB1*0501 and DQB1*0503 were all found to be significantly more frequent in diseased cases (p = 0.0063, < 10^-7 and < 10^-7, respectively), while HLA-DQA1*0501, DQB1*0201 and DQB1*0402 were all found to be significantly less frequent in the same patients (p = 0.0099, < 10^-4 and 0.0054, respectively). In addition to these allelic associations, specific alleles were also linked to presentation of single or multiple site lesions, keloid disease severity and family history. However, the mechanisms for such associations are yet to be confirmed. One possible mechanism, however, is that certain alleles associated with the disease could be more efficient at binding and presenting antigen to immune cells. Furthermore, Lu et al. (68) found that the frequencies of the class I HLA alleles HLA-A*03, A*25, B*07 and Cw*0802 were all significantly increased among a keloid-affected Chinese Han population compared with an ethnically matched control population. On the other hand, the HLA-A*01 allele was decreased in frequency among the keloid patient group compared with the control group. The relationship between these specific alleles and keloid susceptibility may be explained by the local and transient release of cytokines following activation of lesional lymphocytes associated since HLA class I antigens interact with killer immunoglobulin receptors (KIRs) that are present on certain immune cells (68, 69).

The density of HLA-DR+ dendritic cells and keratinocytes in the epidermis, and fibroblasts and endothelial cells in the dermis of keloid tissue has also been shown to be augmented in comparison with normal skin (3, 6, 26). While Cohen et al. (37) found no correlation between the class I HLA-B or HLA-A alleles with keloid susceptibility, Laurentaci & Dioguardi (70) did find a link between keloids and HS with HLA-B14 and HLA-Bw16. However, these early studies may have limited reliability, since serological typing of the HLA system cannot identify many HLA alleles that can be detected at the genomic level (21). It is therefore apparent that further multi-cohort studies are needed to confirm the HLA associations with keloid scar formation using highly sensitive PCR-SSP methods in combination with allele sequencing.

Human leukocyte antigen and hypertrophic scars

Like keloids, HS have a definite immune response trait with characteristic immune cell infiltration comprising primarily of T cells along with macrophages and Langerhans’ cells (30, 71, 72) in conjunction with ectopic expression of HLA class II molecules (30, 56) and pro-inflammatory cytokines (30, 73) (Fig. 4). Such cellular infiltration can be associated with excessive fibroblast stimulation (56, 74). Although HS can prove difficult to differentiate from keloids, there are distinctive clinical differences between the two: HS remain within the boundary of the original injury but increase in size by pushing out the margins of the scar without invading the surrounding normal skin, while keloid scars extend beyond the initial causative injury (75–77). Despite their benign characteristics, both keloid and HS can show aggressive clinical phenotypes (78). However, it is difficult to differentiate the two scar types in terms of biochemical characteristics (71, 79). Consequently, it becomes complicated to establish discrete biomarkers of disease for the individual scar types. Evidence strongly emphasizes the importance of treating keloids and HS as separate disorders (71). For example, in contrast to the fibroblasts of HS and
normal scars, those of keloid scars are resistant to specific types of apoptosis, namely fatty acid synthase (FAS)-mediated and staurosporine-induced apoptosis (80). As such, treatment of keloid-derived fibroblasts with transforming growth factor-beta 2 (TGF-β2) can overcome this apoptotic resistance (80), proving to be an example of the differing outcomes of treatment strategies between the two scar types.

HS seem to share much of the same epidemiology of keloids in terms of age at onset, male: female spread and likely causative injuries (81). Yet, HS have a higher incidence in the general population than keloids, which are more abundant in the black and Hispanic population (71, 82). One notably significant difference between the two scar types is that the size of HS is reflective of initial injury, in contrast to keloid scars, which are not (78).

In terms of the cellular pathology and immunological characteristics of HS, a number of studies have confirmed the increased presence of lymphocytes in affected tissues, which links to the increase in HLA class II molecules expressed on keratinocytes and fibroblasts (57, 58) which are necessary for potentiating inflammation (30).

Castagnoli et al. (30) conducted a study to verify the difference in immune cell infiltrates between active and remission phase HS and found that in active lesions the number of CD1+, CD11+, CD22+, CD3+, CD56+, CD57+, CD25+ and HLA-DR+ cells were all greater than in the remission-phase lesions, with CD3+ cells being the most profuse (30). Macrophages were also found to be in lower abundance in the remission phase lesions (30). In contrast, neither B cells nor natural killer (NK) cells were found to be associated with any type of HS (30). Santucci et al. (3) also showed that levels of immune cell infiltrates reflect the age and type of HS (3).

The co-existence of T lymphocytes and HLA class II molecules on keratinocytes and fibroblasts supports the hypothesis that the formation of HS may be potentiated by infection, given that the presence of HLA class II molecules and leukocyte function-associated antigen-1 (LFA-1)/intracellular adhesion molecule-1 (ICAM-1) (both present in the tissue of HS) act as co-stimulatory signals (30), which may generate T-cell activation, possibly subsequent to environmental-derived antigens (31, 32).

Of the HLA class II molecules it is the HLA-DR allo-genotype that seems to be the most strongly linked in terms of a genetic predisposition to the formation of HS (15, 16). Castagnoli et al. (15) found the HLA-DRB*16 allele to be significantly associated with the formation of HS following thermal injury, with a relative risk of 12.25 (15). However, the HLA typing method was based on restriction fragment length polymorphism (RFLP) analysis, but with no confirmatory sequencing data. Furthermore, the results were based on only 19 patients (15). A further study by Castagnoli et al. (16) showed increased expression of the HLA-DR molecules on the non-immunocompetent cells of the affected skin, i.e. keratinocytes and fibroblasts (16), yet the exact role of such HLA+ cells in scar formation/progression is unclear. However, the use of HLA-detection antibodies in immunohistochemical techniques fails to distinguish between HLA-DP and HLA-DR molecules (16). In comparison, HLA-DQ alleles were rarely detected in these cells (16).

One explanation for the increased prevalence of HLA-DR2+ patients with HS is the association of this HLA group with production of tumour necrosis factor-alpha (TNF-α) (28). Bendtzen et al. (28) demonstrated that HLA-DR2+ cells secrete significantly lower amounts of TNF-α than DR2- cells. However, the overall level of TNF-α production was minimal in all cases (28). The lower abundance of this cytokine in the tissue of HS may result in an increase in collagen production, given that TNF-α can increase collagenase activity via the stimulation of matrix metalloproteinase 2 (MMP-2) (a type IV collagenase) secretion (83). On the other hand, this cytokine has also been shown to increase collagen production (84).

**Human leukocyte antigen and scleroderma**

Sclerodermy (systemic sclerosis) has an immunological pathogenesis characterized by immune cell infiltrate in early sclerotic lesions with evidence of serological abnormalities and autoantibody production (85–88). Scleroderma is a multisystem disease (86) of variable course (89, 90) and increased mortality (91) with typical cutaneous fibrosis and excessive collagen production (92). Although the disease is of unknown aetiology, there is affirmation of both genetic and immunological pathogenesis, with some evidence that environmental and ethnic risk factors may play a part in its pathology (90). As with keloid and hypertrophic scars, there is also evidence that the excessive collagen deposition in sclerodermal lesions is in association with abnormal fibroblast proliferation and the resulting uncontrolled connective tissue accumulation (93). These are thought to be important factors in the pathogenesis of scleroderma (93). However, like the other fibrotic diseases described, the initial event for fibroblast activation is largely speculative (94). Early evidence has shown that the most characteristic feature of progressive systemic sclerosis is cutaneous sclerosis resulting from elevated synthesis of collagen, glycosaminoglycans (GAG) and other connective tissue products of dermal fibroblasts that may be important in connective tissue thickening (95). Furthermore, the increased transcription of type I and III collagen genes, which leads to the accumulation of collagen, is believed to be secondary to the control of various hormones and cytokines (94, 96), which may also be secondary to increased fibroblast proliferation.
There have been consistent reports of immune cell infiltrates in diseased tissues that correlate with the stage of disease progression and extent of fibrosis in scleroderma (87). Evidence strongly suggests an increase in T-cell, macrophage, monocyte and mast cell activation, with an ensuing increase in cytokine levels along with the production of autoantibodies in sclerodermal lesions (85–87, 97, 98). Roumm et al. (87) found that 50% of the untreated progressive systemic sclerosis patients studied had significant dermal mononuclear cell infiltrate, which paralleled the increased thickening of the skin. The distinctive increase in collagen may be linked to this immune response. Treatments used to deplete T cells, for example, of patients with sclerodermal skin thickening, demonstrated a positive reduction in skin tautness caused by a reduction in type III collagen (92). Additionally, immune cell infiltrates has been shown to precede any fibrosis in skin biopsies from patients with sclerosis (86).

Of the HLA regions associated with the onset and clinical progression of systemic sclerosis, it is the HLA-DR class II loci that have been most commonly associated with the disease, in particular with progressive systemic sclerosis (PSS) and diffuse sclerosis (DS) (99–105). Some significant associations have been made between scleroderma and the class II DR1, DR3, DR5 and DR8 loci, with discrepancies in results being attributable to differences in ethnic, racial and geographical orientation (100, 101). In addition, different HLA allele subgroups have been associated with different forms of scleroderma and with clinical phenotype. While the study by Lynch et al. (104) found no significant differences in HLA class I allele frequencies among cases and controls, they did find a significant association between PSS-DS and the Bw35 and DR1 alleles. Likewise, Whiteside et al. (105) found a weak association between DR1 and PSS-DS. Similarly, the HLA-DR5 loci has been identified as a potential biomarker of different types of systemic sclerosis, with Black et al. (99) showing that patients with non-diffuse scleroderma had a higher frequency of the DR1 and DR5 antigens compared with a control population. On the other hand, no association was found between HLA status and specific organ involvement. Similarly, as with keloid susceptibility, diffuse cutaneous systemic sclerosis has been associated with the HLA-DRB1*15 allele among the Japanese population (106). Tikly et al. (107) also found specific class II HLA alleles to be associated with either limited cutaneous systemic sclerosis (lcSSc) or diffuse cutaneous systemic sclerosis (dcSSc), with DRB1*0301 having an elevated frequency among lcSSc patients (OR = 9.0) and DQB1*0301/4 having an increased frequency among dcSSc patients (OR = 9.0). In comparison, the HLA-DR2 allele was found to be more common among the overall SSC population compared with the controls (OR = 2.4). Livingston et al. (103) observed differences in HLA allele frequencies among different ethnic populations, with both the DR1 and DR5 loci proving to be increased in frequency among the Caucasian population and an overall increase in the DR6.1 allele among the black population. Furthermore, Gladman et al. (102) found that DR5* status potentiated a relative risk of 5.01 for scleroderma. However, other DR antigen frequencies were not significantly different between patients and controls. One very notable pathological change in sclerodermal skin is the production of autoantibodies, in particular anti-topo I antibodies (97). Whether these autoantibodies have a direct influence on the development of sclerotic lesions and their interaction with HLA antibodies remains to be clarified. However, Briggs & Welsh (108) noted that the 5’ flanking DNA of dermal collagen genes is particularly susceptible to the action of Sel-70 (topoisomerase I). This may provide the pathological link between HLA and increased collagen gene expression with the presence of autoantibodies in sclerodermal lesions (108).

**DISCUSSION**

There is a strong association between the HLA system and cutaneous fibrosis. However, its aetiopathogenesis remains to be fully elucidated given the lack of sufficient research to identify the immunological parameters among individuals affected by cutaneous fibrosis (55). There is limited knowledge of factors that inhibit the activity of normal wound restoration (50). One of the most limiting factors in research progression has been the lack of an appropriate animal model for cutaneous fibrosis in certain conditions e.g. keloid disease. As such, *in vitro* cell culture studies incur a number of problems in generating data that can be related directly to the *in vivo* biological and genetic events occurring in the wound, given the variation in *in vivo* and *in vitro* environments (109). Difficulties in studying genetic associations with cutaneous fibrosis are a result of the multifaceted and intricate pathological alterations in many different disorders with cutaneous fibrosis (4). It would be of major diagnostic benefit to be able to accurately predict the biological and immunological behaviour of abnormal wound healing associated with cutaneous fibrosis (55).

In particular, the association between immune mechanisms and excessive collagen production and aberrant fibroblast activity remains to be clarified. As described by O’Sullivan et al. (7) mesenchymal wound healing is comprised of four well-defined phases; namely, the haemostatic, inflammatory, proliferative and remodelling stages. Defects in wound healing resulting in abnormal scar formation may occur at any or all of these phases (7). However, much evidence has mounted with respect to the involvement of the immune system along with various genetic links, with specific interest
in candidate genes of the highly polymorphic HLA loci, in particular the HLA class II loci given the immuno-regulatory role and antigen presentation capacity of these molecules. For example, there is some evidence to suggest the potential of the HLA-DRB1 loci to provide allelic biomarkers of keloid formation (6). Likewise, the HLA-DR loci have also been linked to the predilection for the formation of HS (15, 16, 28). Such genetic associations may provide potential biomarkers of disease, which may lead to early diagnosis and prevention strategies and thus better disease management and prophylaxis. It has already been suggested that HLA typing is important in the diagnosis of other diseases, such as coeliac disease, and that HLA typing in patients with diseases with a strong HLA linkage may be useful in differential diagnosis when not all of the characteristic symptoms are presented (110). Furthermore, the cost saving of HLA typing in such patients in comparison with alternative diagnostic strategies may prove to be economically beneficial and may reduce the need for invasive diagnostic procedures that can cause distress to the patient (110). Thus, if a common HLA biomarker of disease is identified then such diagnostic tools may be useful in the future for the diseases discussed.

The evidence of varying candidate HLA alleles in different types of cutaneous fibrosis indicates the need to differentially identify fibrotic lesions and highlights the need for specific treatments for various forms of cutaneous fibrosis. Although no consistent gene mutation has been reported in connection with cutaneous fibrosis, these reports, amongst other genetic associations found in linkage with abnormal scarring (4), seem to reach the conclusion of a significant genetic link (Fig. 5). This is further supported by the positive association found in twin studies (6). However, given the complex biochemical and physiological complications of such disorders, it seems likely that there is an interaction of several disease-associated genes with environmental, geographical and other demographic risk factors. Yet, the biological mechanism of HLA and other gene associations remains ambiguous.

There remains a number of limitations that need to be taken into consideration in the design and conduct of research projects and in the interpretation of results, in order to avoid the risk of missing subtle but significant clues to disease pathogenesis and to enable the appreciation of the significance of observations, thus avoiding the production of spurious associations (111). In terms of tissue-based experimental protocols, there are a number of confounding variables that need to be taken into account: anatomical site of scar, family history, medical history including presence or past history.
of autoimmune and/or other immune disorders, initial causative insult, environmental factors (e.g. microbial infection, drug exposure) (111), age, sex and race (it may prove difficult to identify individuals of complete single race). Such variables may affect the characteristic morphological features and pathological behaviour that can lead to differences in treatment responsiveness (36, 112). As noted by Muir (50), such risk factors may also relate to the degree of scar hypertrophy. Some of these variables are often overlooked when recruiting patient and control cohorts, resulting in potential discrepancies in results, thus limiting the reliability of such data. In addition, when designing family-based studies, familial clustering of disease might not be obvious even where there is a known genetic linkage, which is especially true of diseases with multifactorial aetiology (111). Despite the long-established guidelines, the differentiation of scars in the clinical setting still seems to be inconsistent specifically in the differentiation of keloids and HS. Such discrepancies could lead to varied treatment outcomes and possible iatrogenic complications. Studies based on such inconsistent diagnostic criteria result in data that are difficult to reproduce. Stringent diagnostic criteria are needed in the recruitment process to avoid confounding effects due to misdiagnosis and disease heterogeneity (75). Another noticeable problem among the majority of HLA typing studies is the lack of allele sequencing as a clarification step following PCR-SSP or serological-based typing methods. Furthermore, the inherent limitations of early serology-based studies need to be considered when making comparisons between data from these and more sophisticated studies. Epidemiological studies are also difficult to analyse, since evidence is based primarily on sufferers who attain medical advice. For example, it is probable that more young females will request therapy given the cosmetic implications. Another area of consideration when designing case-control studies is in the analysis of possible HLA associations of diseases that have early mortality as a feature of their natural progression (111). Finally, it is important to recognize the impact of linkage disequilibrium, which can limit the authenticity of allelic associations. Also, multiple comparisons need to be accounted for during statistical analysis, since such comparisons will mitigate the significance of some associations (111). The complexities of linkage disequilibrium within the HLA loci have been explored extensively, yet this remains a major problem in many gene linkage studies. There is also evidence that linkage disequilibrium varies between races, as demonstrated by Kischer (79) who identified linkage disequilibrium between the HLA-DQB1 and DRB1 loci only in white populations. It also seems to prove more difficult to define negative allelic associations with protective effects.

In terms of future research, it seems logical to pursue research into the genetic basis of cutaneous fibrosis to enable the development of diagnostic strategies and more appropriate intervention methods, since the majority of studies have focussed on clinical trials and effectiveness of current treatments. A major limitation observed in a number of studies was case/control numbers. It may prove beneficial, therefore, to utilize multi-centre collaborations as most studies are limited to one clinical setting (113). As noted by Rockwell et al. (114) it would also be useful to explore the triggers for regression of fibroblasts and blood vessels in normal scars so as to re-introduce these triggers into developing keloids as a means of intervention. Continued advances in immunology and genetic analyses would probably generate a uniform hypothesis to explain the imbalance of enhancing and inhibitory factors that must be present during abnormal wound healing (50). Early recommendations for research that will reveal the similarities between pathogenic mechanisms producing a wide range of disorders and whether these similarities can be attributable to a single causative mechanism (111) still need to be achieved. One main area of research needing further focus is the affirmation of the association between immunological mechanisms and fibroblast-collagen metabolism (55). The observations of scarless surgery in the foetus and the near flawless healing of neonates may also help to provide insight into the pathogenesis of abnormal scarring and deserves further attention (52).

Justification for such future studies stems from the number of case studies showing evidence that cutaneous fibrosis and abnormal scarring often result in significant psychological, psychosocial and physical morbidity. There is a clear lack of set therapeutic options and evidence-based strategies on individual treatments. The capacity for early diagnosis through clearly defined HLA biomarkers could lead to the development of immunomodulatory therapies and would enable clinicians to generate individual treatment plans including genetic screening, lifestyle counselling and the avoidance of unnecessary surgical procedures and trauma in susceptible individuals, especially those with a positive family history of cutaneous fibrotic disorders. More research is needed to identify the link between HLA status and the primary biochemical disease characteristic of each fibrotic disorder described, which is over-production of collagen. In turn, there is a potential predictive value of biomarkers within the HLA region that could be important in the diagnosis, theragnosis and prognosis of these diseases.

CONCLUSION

The pathogenesis of cutaneous fibrosis and abnormal wound healing is not fully understood, though clear evidence has suggested some of the key molecules involved in this complex process. The most significant of HLA associations with the fibrotic disorders explored are of
the class II loci, in particular the HLA-DQ and DR allele regions. Significant limitations in study design seems to have hampered progress in this area of research and, given the physical and social burden of cutaneous fibrosis and abnormal scarring, further research into early diagnosis and prevention is justified. Of importance when designing case-control studies is strict differentiation between scar types, a flaw inherent in many research studies of this kind, which can lead to varied treatment outcomes. The ability to accurately assess an individual's potential immunogenetic susceptibility to the fibrotic conditions discussed may aid in the establishment of more personalized diagnostic, therapeutic and prognostic management in the future.

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