The molecular pathogenesis of morphoea: from genetics to future treatment targets

Running title: The molecular pathogenesis of morphoea

Authors:
A.M. Saracino$^{1,2}$, C.P Denton$^{2,3}$, C.H. Orteu$^1$

Institutions:
1. The Royal Free London NHS Foundation Trust
   Department of Dermatology
2. University College London
   Department of Rheumatology and Connective Tissue Diseases
   Division of Medicine
3. The Royal Free London NHS Foundation Trust
   Department of Rheumatology

Corresponding author:
Name - Dr Amanda Saracino
Address - Department of Dermatology
          The Royal Free Hospital
          Pond Street
          London
          NW3 2QG
Email - a.saracino@nhs.net
Phone - +44 (0)20 7830 2376

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WHAT IS ALREADY KNOWN ABOUT THIS TOPIC?
- Morphoea has a number of distinct clinical subsets with variable anatomical distribution, morphology and depth of tissue involvement

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• Susceptibility mechanisms (including genetics and underlying autoimmunity) and environmental triggers are at play in morphoea pathogenesis
• A variety of innate and adaptive inflammatory and profibrotic immunopathogenic mechanisms and signalling pathways have demonstrated roles in the pathogenesis of sclerotic skin disease

WHAT DOES THIS STUDY ADD?
• This review draws together what is currently known in the pathogenesis of systemic sclerosis and other fibrotic processes, to expand on and provide links with our current limited understanding of morphoea pathogenesis.
• We propose the key-inciting role of epidermal keratinocytes in morphoea pathogenesis based on the Blaschkoid nature of linear morphoea, and recognised role of keratinocyte derived factors and epidermal-dermal signalling pathways in the pathogenesis of sclerotic skin disease.
• We review underlying genetic and immunopathological mechanisms in morphoea, and suggest how these may link to recognised reproducible clinical subsets.
• Finally, we look ahead at possible future targeted and individualised therapeutic approaches in sclerotic skin disease and morphoea.

ABSTRACT
A number of immunoinflammatory and profibrotic mechanisms are recognised in the pathogenesis of broad sclerotic skin processes, and more specifically, morphoea. However, precise aetiopathogenesis is complex and remains unclear. Morphoea is clinically heterogeneous, with variable anatomical patterning, depth of tissue involvement, and sclerotic, inflammatory, atrophic and dyspigmented morphology. Underlying mechanisms determining these reproducible clinical subsets are poorly understood, but of great clinical and therapeutic relevance. Regional susceptibility mechanisms (such as environmental triggers, mosaicism and positional identity) together with distinct pathogenic determinants (including innate, adaptive and imbalanced pro and anti-fibrotic signaling pathways) are likely implicated. In the age of genetic profiling and personalised medicine, improved characterisation of the environmental, systemic, local, genetic and immunopathologic factors underpinning morphoea pathogenesis, may open the door to novel targeted therapeutic approaches.

INTRODUCTION
A review of morphoea pathogenesis is timely with improved understanding of underlying molecular mechanisms likely to bring the advent of more targeted therapeutic approaches. However the clinical heterogeneity of morphoea, with variable anatomical patterning, morphology and depth of tissue involvement observed both within and between individuals, poses many clinical and pathogenic challenges (see Figure 1). Whilst key aetiologic, immunoinflammatory and profibrotic molecular mechanisms are likely shared across these subsets of morphoea, there must be underlying determinants of clinical heterogeneity.
Unmasking the genetic and molecular basis of this reproducibly observed clinical variability in morphoea has great therapeutic potential.

**SUSCEPTIBILITY MECHANISMS**

Systemic and regional genetic and epigenetic susceptibility mechanisms are all potentially at play in morphoea (see Figure 1 and 2). Certain HLA subtypes, including HLA-DRB1*04:04 and HLA-B*37, infer increased susceptibility, especially in generalized and linear subtypes\(^1\), and morphoea is reported in first or second-degree relatives in 2% of cases\(^2\)\(^3\)\(^4\). A personal or family history of autoimmune or rheumatologic diseases is seen in up to 46% of those with morphoea\(^2\)\(^5\)\(^6\)\(^7\)\(^8\)-\(^9\), sometimes more frequently in generalized or linear subtypes\(^2\)\(^7\). Hence, one may speculate that a shared susceptibility locus exists for these disorders. As such, the systemic autoimmune nature of morphoea is further recognised by a variety of autoantibodies\(^2\)\(^7\)\(^10\)\(^11\); antinuclear antibodies (ANA) can occur in 18 to 68%\(^2\)\(^9\)\(^12\)\(^20\) and notably, more of those with ANA positivity appear to have extracutaneous manifestations\(^11\).

Distinct genetic ‘intrinsic subsets’ in systemic sclerosis (SSc) have been described, with reproducible and temporally stable gene expression profiles correlating to inflammatory and fibroproliferative signatures, and related clinical heterogeneity\(^21\)-\(^25\). Significantly, differences in gene expression can be mapped not only to fibroblasts, but also to epithelial, endothelial, smooth muscle, T and B cells\(^24\)\(^26\). Genome wide expression profiling of skin biopsies from three morphoea patients linked them to the inflammatory signature\(^23\), hence implicating mediators such as interferon (IFN), interleukin (IL)-13, chemokine (C-C motif) ligand (CCL)-2, early growth response (EGR)-1 gene and others in pathogenesis\(^21\)-\(^25\). Indeed, IFN-γ-inducible protein 10 (IP10, CXCL10) is elevated in children with morphoea\(^27\) and acts via CXC-chemokine-receptor (CXCR)-3 to attract inflammatory cells to the skin.

Of great potential relevance to regional genetic susceptibility is evidence that linear morphoea follows Blaschko’s lines of epidermal development; suggesting likely mosaicism for a mutation that causes increased susceptibility to morphoea at certain sites\(^28\)-\(^39\) (see Figure 1 and 2). However, that a fibroblast driven dermal process can be Blaschkoid raises many unanswered questions, but its feasibility is perhaps substantiated by the existence of other similar conditions, including focal dermal hypoplasia (FDH; Goltz syndrome)\(^40\) and a recent report of Blaschkoid granuloma annulare\(^41\). This may highlight the potential role of epidermally-derived signalling and immunoinflammatory pathways in morphoea pathogenesis.

Finally, epigenetic mechanisms may provide a potential link between genetic susceptibility, environmental aetiology and site specificity (see Figure 1). Altered patterns of histone acetylation and DNA-methylation at multiple genetic loci have been demonstrated in SSc. This has included transcriptional silencing of repressive genes in SSc fibroblasts, resulting in increased profibrotic gene expression and transforming growth factor (TGF)-β induced responses\(^42\)\(^43\). The importance of microRNAs (miRNAs) in the pathogenesis of skin fibrosis is also emerging. Twenty-six miRNAs are shown to be deregulated in SSc skin and isolated fibroblasts, some forming part of a positive feedback profibrotic mechanism activated by TGF-β\(^44\). miR-21 and miR-155 are profibrotic and show elevated expression in SSc skin\(^45\). These miRNAs are in turn regulated by TGF-β and hence have, as their predicted targets, genes involved in matrix repair and remodelling; such as collagens, matrix
metallopeptidases (MMPs) and integrins. Conversely, miR-30b levels are inversely correlated to modified Rodnan skin scores (mRSS) and appear to be related to increased platelet-derived growth factor (PDGFR) expression on dermal fibroblasts. Notably, down-regulation of anti-fibrotic miR-7 and miR-196a have been demonstrated in the skin and serum of patients with morphoea, potentially contributing to overproduction of collagen type I.

TRIGGERING EVENTS
An array of environmental triggers are widely reported in the aetiology of morphoea, seemingly linking susceptibility mechanisms and eventual, but not inevitable, disease onset (see Figure 1 and 2). Trauma, in the form of insect bites, injection/vaccination, repeated friction, surgery, penetrating trauma, radiotherapy and extreme exercise, may trigger morphoea in up to 16% of adults and 9% of children. Trauma related morphoea may occur at the affected site, or a more systemic response may be triggered, with site unrelated skin sclerosis also seen. Of relevance is isomorphic disseminated plaque morphoea, which occurs symmetrically and at sites of repeated minor friction induced trauma along the waistline, bra strap and inguinal creases (see Figure 1). The mechanisms of trauma related morphoea remain somewhat elusive; enhanced innate signalling via toll-like receptor (TLR)-ligands inducing fibroblast activation and an abnormal wound healing response has been proposed. Several vaccinations are implicated temporally and anatomically with morphoea, including hepatitis B, tetanus, vitamin B12 and more. Whether this is purely trauma related (due to vessel injury, tissue hypoxia and subsequent immune activation) or potentially due to common adjuvant vaccine constituents, remains uncertain. Somewhat similarly, post-irradiation morphoea predominantly occurs at the site of radiation and within 12 months of completing treatment (most commonly in the setting of breast cancer). However up to one-quarter of cases may extend beyond the radiation field and the immunopathology of this is not clear, but increased IL-4, IL-5 and TGF-β is suggested. Infection also triggers morphoea and perhaps most controversially, Borrelia species are associated in some studies. Finally, various drugs can seemingly initiate the development of skin sclerosis, and drug cessation does not necessarily result in resolution. Specific drug related lymphocyte responses and autoantibody production, with consequent vascular damage, reactive oxygen species, IL-1, tumour necrosis factor (TNF)-α and TGF-β production may be involved.

PATHOGENESIS
A plausible model of morphoea pathogenesis would involve a triggering event in a susceptible individual that results in a cascade of innate and adaptive immunoinflammatory and profibrotic responses, involving potential epidermal signalling and mesenchymal drivers (see Figure 2). Notwithstanding morphologic variation and distinct patterning observed in different morphoea subsets, many of these fundamental immunopathogenic mechanisms are likely to be shared.

The epidermis
Keratinocytes
A number of factors known to be involved in dermal fibrosis can be produced by keratinocytes, including TGF-β, IL-1, IL-6, TNF-α, PDGF, fibroblast growth factor (FGF), CCL2, endothelin (ET)-1, fibrillin-1, friend leukaemia integrated transcription factor (Fli)-1, S100A9,
alpha-melanocyte-stimulating hormone (α-MSH) and others\textsuperscript{83} (see Figure 2). Accordingly, fibroblasts are more contractile when cultured with epithelial cells\textsuperscript{84} and keratinocytes can alter the expression of extracellular matrix (ECM) gene modulators such as connective tissue growth factor (CTGF), fibronectin and type 1 collagen\textsuperscript{85, 86} in experimental models. Furthermore, although little is documented in morphea specifically, in the context of SSc skin, activated highly proliferative keratinocytes have been demonstrated in the epidermis\textsuperscript{23, 87, 88} and importantly, unstimulated keratinocytes promote myofibroblast activation independent of TGF-β\textsuperscript{89}.

S100A9 is induced in keratinocytes during epidermal stress and, via TLR4, stimulates fibroblast proliferation and over expression of CTGF by SSc fibroblasts\textsuperscript{88, 90}. Over expression of TLR-4 occurs in fibrosis and its profibrotic effects are related to potentiating TGF-β activity and suppression of antifibrotic miRNAs (eg. miR-29)\textsuperscript{46}. CTGF mRNA and protein have been identified in dermal fibroblasts in morphea\textsuperscript{91, 92}. Keratinocyte FGF-receptor 1 and 2 are also linked to S100A9 activity and fibrosis. FGF-receptor knockout mice demonstrate loss of claudins and occludin which causes transepidermal water loss, severe xerosis and resultant activation of γδ-T-cells and keratinocytes to produce IL-1, S100A8 and S100A9, promoting a profibrotic response\textsuperscript{90}.

α-MSH mediates melanocyte pigment production via melanocortin-1 receptors (MC1R), is up regulated in the epidermis and fibroblasts of human burn wounds and hypertrophic scars\textsuperscript{93}, and modulates pro and anti-inflammatory cytokines produced by keratinocytes, monocytes and fibroblasts\textsuperscript{94}. Conversely, and signifying its physiological homeostatic role, α-MSH (via MC1R) antagonizes cutaneous fibrosis induced by repeated TGF-β exposure and bleomycin\textsuperscript{95-97}. As such, MC1R knockout mice have demonstrated a susceptibility to fibrosis\textsuperscript{98}.

Fibrillin-1 is a major component of the microfibrillar ECM network. Its expression has been associated with diffuse cutaneous SSc of the ‘fibroproliferative’ signature\textsuperscript{23} and antibodies against fibrillin-1 have been documented in patients with morphea and SSc\textsuperscript{99}. Stiff skin syndrome is due to mutations in the domain of fibrillin-1 that mediates integrin binding, and is characterized by sclerotic skin usually over the entire body; somewhat reminiscent of pansclerotic morphea\textsuperscript{100, 101}. Further evidence connecting fibrillin-1 to sclerotic skin disease is the suggested link between variation at the fibrillin-1 gene locus and SSc in Choctaw Indians\textsuperscript{102}.

Finally, keratinocyte derived transcription factor Fli-1 has a demonstrated role in dermal fibrosis mouse models and SSc. Down-regulation of Fli-1 induces an SSc-like phenotype in fibroblasts, vascular endothelium and macrophages. Additionally, keratinocyte Fli-1 knockout mice exhibit enhanced skin fibrosis with increased IL-1, IL-6 and IL-8 expression\textsuperscript{103}.

**Epidermal-dermal signaling and developmental pathways**

Epidermal-dermal morphogenic signaling pathways (including Wingless and int homolog (Wnt), Sonic hedgehog (Shh) and Jagged-Notch) involved in embryonic development, tissue patterning, morphogenesis and wound repair, are of increasing interest in skin fibrosis\textsuperscript{90, 104-108} (see Figure 2). These pathways have been implicated in SSc\textsuperscript{109-113} and are also of
potential relevance to patterning and morphologic variation seen clinically in morphoea; although little is confirmed in the present literature in this regard.

Jagged-Notch pathways, which control formation of boundaries between groups of cells during embryogenesis and mediate epithelial-mesenchymal transition, are over activated in fibroblasts in SSc.\textsuperscript{46, 114} Similarly, Shh is elevated in fibroblasts, endothelial cells and keratinocytes of SSc patients, allowing degradation of Gli proteins, thus promoting fibrosis\textsuperscript{46} \textsuperscript{111, 115}.

Finally, and perhaps especially significant, is Wnt signaling, which originates in the epidermis. Normal stimulation of fibroblasts with Wnt ligands results in β-catenin-mediated expression of collagen, other matrix proteins, CTGF, enhanced fibroblast proliferation, myofibroblast differentiation and increased cell migration\textsuperscript{116-120}. Genome expression profiling has demonstrated elevated expression of several Wnt ligands, receptors and decreased expression of Wnt antagonists in SSc\textsuperscript{115, 116, 118-127}. Of further relevance to skin sclerosis, Wnt-β-catenin signaling and TGF-β pathways are known to interact, and peroxisome proliferator-activated receptor (PPAR)-γ (a recognized antifibrotic receptor which inhibits Wnt-β-catenin signaling) is decreased in SSc, thus promoting fibrosis\textsuperscript{120}. Additionally, Wnt signaling inhibits epidermal responsiveness to FGF-receptors, providing another link between potentially important epidermal inciting pathways and dermal fibrosis. Finally, β-catenin independent keratinocyte derived Wnt5a is highly expressed in SSc fibroblasts, and Wnt5a knockout mice appear resistant to Bleomycin induced skin sclerosis\textsuperscript{128}.

Immunoinflammation

\textbf{Innate responses}

The potential role of the innate immune system in triggering the complex profibrotic cascade is of increasing interest. Type 1 IFNs are key innate mediators and are intimately linked with TLR-signaling (see Figure 2). TLR-3 is involved in downstream increases in inflammatory and profibrotic cytokines and chemokines such as IL-6 and IP10 (CXCL10), as well as increased responsiveness of fibroblasts to TGF-β\textsuperscript{46}. Additionally, synthetic TLR3-ligand induces the expression of several ECM genes in fibroblasts and dermal fibrosis in mouse models\textsuperscript{129}.

Interferon-regulatory factor 5 (IRF5) is a transcription factor involved in TLR-signaling and activation of target IFN genes\textsuperscript{130}. IRF5 has been coined a susceptibility factor to SSc and its potential role in pulmonary fibrosis has been identified\textsuperscript{130}.

Further implicating IFN in fibrosis is signal transducer and activator of transcription 4 (STAT4), which induces expression of type 1 IFNs, and STAT4 null mice exposed to bleomycin develop reduced fibrosis compared to controls\textsuperscript{130}. STAT4 is also pivotal in pro-inflammatory cytokine production including TNF-α, IL-2 and IL-6; hence its role in an inflammatory model of fibrosis is potentially relevant to morphoea, where an inflammatory signature has been demonstrated in some\textsuperscript{23}.

NF-kappaB, the master regulator of innate immune signaling, is important in skin homeostasis and more recently a profibrotic role of the c-Rel subunit expression within keratinocytes has been suggested, with abnormal epidermal expression patterns in SSc skin compared to healthy controls\textsuperscript{131}.

Inflammasome activation and IL-1β production is also implicated in many animal models of fibrosis, and has a potential role in SSc\textsuperscript{132, 133} (see Figure 1 and 2). Indeed, NACHT, LRR and PYD domains-containing protein 3 (NLRP3) inflammasome and IL-1β levels correlate with
mRSS and immunohistochemistry staining shows strong NLRP3 and IL-1β staining in the epidermis of SSC skin. Similarly, ET-1 levels correlate with NLRP3 and mRSS, ET-1 receptors are increased in SSC, and external stimuli can induce ET-1 and concurrent TGF-β production by oral mucosal keratinocytes in oral submucous fibrosis.

Adaptive responses

A cytokine profile based conceptual model of the immunopathology of morphoea was recently proposed and has been supported by further cytokine expression profiling. An early active disease phase characterized by inflammation with a Th1 cytokine response (mediated by IL-2, TNF-α and IL-6) appears to be followed by ongoing inflammation and initiation of fibrosis driven by Th17 related IL-1, IL-17, IL-22 and TGF-β production. Subsequent progression to a final fibrotic and atrophic phase then occurs, predominated by Th2 cytokines (IL-4 and IL-13) (see Figure 1 and 2).

Correspondingly, raised IL-2, IL-2 receptor and TNF-α levels are seen in early phase morphoea and correlate with IL-4 and IL-6 levels, objective skin scores, anti-histone and anti-ssDNA antibodies. Serum IL-1 and IL-6 levels (Th-17 inducers) are also elevated during early disease (<24 months), whilst levels of Th17 effectors (IL-17F and IL-22) become elevated later (24-48 months). IL-6, stimulated by IL-1, is required for wound healing and fibroblast activity, and is intimately involved in morphoea. In response to injury, IL-1, via IL-6 and PDGF, promotes fibroblast proliferation, resultant transcription of type I, III and IV collagens and CTGF expression. IL-1 pathways can also activate fibroblasts in SSC. Further, low IL-17C with high IL-17E and IL-22 increases fibroblast profibrotic responses which is further enhanced in the presence of IL-22 and TNF activated keratinocytes in both SSC and morphoea. IL-17A also appears important, with levels decreasing in correlation with TGF-β and IL-22 in the setting of polymerised collagen treatment and associated normalisation of dermal architecture in morphoea skin.

TGF-β is recognized as a potential master regulator of wound healing and a driver of pathological fibrosis. Increased levels of TGF-β and TGF-β receptor-I and II have been demonstrated in skin biopsies and sera of morphoea and SSC patients. In addition, and perhaps highly relevant, is the key role of TGF-β in regulating normal embryonic and fetal growth and development, as well as postnatal growth of connective tissue. A number of MMPs and tissue inhibitor of metalloproteinases (TIMPs) are also deregulated by TGF-β expression; promoting ECM production and limiting degradation. MMP-12 is over expressed in SSC and associated with extent of skin disease, disease duration and severity of pulmonary fibrosis. Similarly, MMP-12 may be implicated in the antifibrotic affects of UVA1 phototherapy.

Finally in this cytokine profile based model of morphoea, Th2 related IL-4 and IL-13 appear to be are linked to the late fibrotic, as well as final atrophic and/or hyperpigmented phases of morphoea morphology. Regarding fibrosis, IL-4 and IL-13 up-regulate collagen synthesis, inhibit collagenase activity, with elevated IL-13 levels have been demonstrated in some morphoea patients in the late fibrotic stage. In addition, IL-13 pathways may be interlinked with TGF-β and CCL2, and recent gene expression profiling of morphoea patients confirmed increased IL-13 and CCL2 activity.

The role of B-cells in morphoea is also well supported. As mentioned, a personal or family history of an associated autoimmune or rheumatologic diagnosis can occur in 9 to 46% of those with morphoea. Accordingly, the systemic autoimmune nature of morphoea is
further recognised by a variety of autoantibodies\(^2,7,10,11\), and an increased level of B-cell activating factor in the serum of affected patients further infers systemic autoimmunity\(^{162}\). Antinuclear antibodies (ANA) can occur in 18 to 68%\(^2,9,12-20\). Furthermore, B-cells are known to produce profibrotic cytokines such as IL-6 and TGF-β, promote a profibrotic Th2 response and regulatory B-cells (which inhibit Th1 and Th17) are decreased in SSc\(^{163}\).

**Mesenchyme and Fibroblasts**

Myofibroblasts are the key effector cell in fibrosis. Upon injury, fibroblasts migrate towards a wound and, under the influence of growth factors, differentiate into secretory myofibroblasts; central to repair during wound healing\(^{115}\). In wounds, the process of myofibroblast activation and population expansion are appropriately switched off via apoptosis or de-activation. In contrast, this physiological balance is deregulated in pathological fibrosis, including morphea, and a pro-fibrotic environment rich in activating signals such as TGF-β, FGFs and CTGF inappropriately persists\(^{115}\) (see Figure 1 and 2).

Studies support the role of fibroblast transdifferentiation in fibrotic skin disease, which expands resident fibroblast populations from many other differentiated cell lineages. Bone morphogenetic protein-2 (BMP-2) induced TNF-α, TGF-β Smad signaling, CTGF, FGF as well as developmental pathways (Wnt, Shh and Jagged-Notch) all potentially induce epithelial to myofibroblast transformation in keratinocytes, and are separately implicated in skin fibrosis\(^{164-169}\). Interestingly, and of potential relevance to Blaschkoid morphea, is the increased keratinocyte expression of α-smooth muscle actin in patients with stiff skin syndrome (fibrillin-1 gene mutation) and the related lack of normal basal layer keratinocyte columnar organization\(^{100,101}\). In addition to transdifferentiation, the aberrant differentiation of circulating bone marrow-derived mesenchymal progenitors (fibrocytes) into fibroblasts and myofibroblasts is also described in SSc\(^{170,171}\).

**Mesenchymal patterning and location-specific gene expression**

Fibroblasts show location specific gene expression, formally referred to as ‘positional identity’. The master regulating genes of positional identity during development are the homeodomain genes of the HOX sub-family, along with their co-factors, TALE. HOX-genes encode transcriptional factors that determine the positional identity of fibroblasts along anterior-posterior and secondary axes\(^{109,172-175}\). Hence fibroblasts from different sources, although immunophenotypically very similar, display distinct and site-specific HOX and TALE signatures; for example, HOXA13 is expressed in distal fibroblasts and is required for normal toe, finger and foreskin development\(^{172,173}\). Importantly, site-specific HOX and TALE transcriptional patterns remain stable throughout life and hence epigenetic factors are at play\(^{172,173}\).

As one may elucidate, such site specific gene signatures are not only responsible for determining position and patterning during development, but also play a pivotal role in subsequent downstream orchestration of site-specific mesenchymal cell differentiation and related pathway signaling; acting as micromanagers of adult cell differentiation\(^{109,176}\). Many patterning and developmental pathways, including Shh and Wnt are involved\(^{177}\), and the resultant regional diversification, which can be traced back to specific axial positions, may suggest that fibroblasts at different sites should actually be considered as groups of distinctly differentiated cells\(^{109,177-180}\). Importantly, these many pathways which are
instrumental in developmental patterning, positional identity, regional-specific mesenchymal differentiation and overlying epidermal fate, are intricately linked to pathogenesis of fibrosis via site specific expression of factors including TGF-β, Wnt, FGFs, BMP, receptor kinase, phosphatase families and G-protein signaling\(^{109\ 173}\) (see Figure 1). Hence somewhat unsurprisingly their pathogenic role in SSc specific skin and internal organ disease is documented\(^{46\ 181}\), and although likely of relevance, currently there are no specific investigations into the role of these mechanisms in morphoea (see Figure 2).

**POTENTIAL MOLECULAR MECHANISM OF MORPHEOA CLINICAL SUBSETS**

Although there are clearly several shared aetiopathogenic mechanisms across the spectrum of morphoea, there must be underlying determinants of anatomical patterning and the variably sclerotic, atrophic and pigmented morphology observed clinically (see Figure 1). In some, this will likely reflect local susceptibility factors including regional genetic and/or epigenetic perturbation, as described above. Whilst in others it may suggest distinct pathogenic mechanisms. As alluded to, morphoea classically evolves through three clinical stages; active inflammation, followed by sclerosis and then dyspigmentation and/or atrophy. However, there is marked variation within this phenotypic spectrum, both between and within individual patients; for example, atrophoderma may present without clinically apparent preceding inflammation or sclerosis in some or all lesions. Epigenetics, perturbation of homeostatic signaling (eg. α-MSH/MRC1, positional identify or Wnt-signaling) and/or aberrant recruitment of antifibrotic pathways may be at play. Of relevance, FDH (an X-linked genodermatosis; porcupine homolog gene (PORCN) mutation) results in Blaschko distributed dermal atrophy and pigmentation. PORCN gene expression causes epidermally-derived palmitoleic acid induced Wnt-signaling and protein release. It has been suggested that this subsequently regulates underlying dermal development and could account for the Blaschko distribution of FDH\(^{40}\). Thus, beyond providing an example of a dermal Blaschko process, the pathogenesis of FDH may in fact be more closely related to morphoea than immediately appreciated.

**IMPLICATIONS FOR THERAPY**

With improved understanding of the molecular pathogenesis of morphoea, it is likely that more precisely targeted biologic therapies directed towards specific immunoinflammatory and connective tissue repair pathways will become increasingly available (see Table 1). As discussed, the role of activated T-cells is described in the pathogenesis of morphoea and well understood in SSc and animal models of skin fibrosis\(^{138\ 182\ 183}\). Abatacept (recombinant IgG1 fusion protein to cytotoxic T-lymphocyte antigen 4 (CTLA4)), inhibits activated T-cells by binding to CD80 and CD86, thereby blocking interactions with CD28. Thus, abatacept has resulted in significant improvement in two cases of deep and extensive morphoea\(^{184}\) and further cases of SSc\(^{185\ 186}\).

Infliximab (chimeric monoclonal antibody to TNF-α) has induced remission in one case of generalised morphoea\(^{187}\) and three cases of eosinophilic fasciitis\(^{188-190}\). As a Th1 cytokine linked to the early inflammatory phase of morphoea, one can elucidate how TNF-α inhibition could be a useful therapy. However, etanercept (TNF-α receptor fusion protein) has been linked to the subsequent development of disseminated plaque morphoea (at sites related and completely separate to injections) in a patient with psoriasis receiving therapy for 18 months\(^{191}\). Whether this was due to injection site related trauma and a subsequent
systemic response, or a trauma unrelated paradoxical immunological drug response, remains unclear.

Imatinib (tyrosine kinase inhibitor) via anti-TGF-β and PDGF effects, showed promising results in three cases of morphea \(^{192-194}\) (see Table 1). Whilst not yet used in morphea, tocilizumab (humanized IL-6 receptor monoclonal antibody) has been used with some success in SSC \(^{195-197}\). In experimental SSC mouse models, PPAR (γ and α) agonist IVA337 can decrease ECM deposition and TGF-β related Smad-signaling \(^{198}\). And finally, PDE4 inhibition (Apremalast\(^{®}\)) reduces and inhibits fibrosis in a mouse model of sclerotic skin disease, with amelioration of skin fibrosis in sclerodermoid GVHD \(^{103}\).

Based on present understanding of pathogenesis, future targets for morphea could include IL-1 (Anakinra; anti-IL1), IL-13, IL17E, TGF-β or Wnt-signaling pathway mediators, among others (see Table 1). Importantly, the potential for non-systemic routes of administration, such as topical preparations in optimized vehicles, long acting patches, or local intradermal or subcutaneous injection, may improve tolerability and increase therapeutic benefit to risk ratios.

**CONCLUSIONS**

The complexity of morphea aetiopathogenesis is clear. Whilst some broadly involved immunoinflammatory and profibrotic signaling cascades are elucidated, an overlay of clinical heterogeneity means regional susceptibility mechanisms and distinct pathogenic determinants are likely implicated. In the age of genetic profiling and advanced molecular science, more precise characterisation of systemic and local genetic and immunopathological factors underpinning the anatomical and morphologic variability in morphea, may open the door to novel targeted therapeutic approaches.

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Table 1. Selected potential key molecular pathogenic mechanisms in morphea and related future treatment targets.

<table>
<thead>
<tr>
<th>Mechanism / evidence of profibrotic action(s)</th>
<th>Target Therapies (experimental / available)</th>
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</thead>
<tbody>
<tr>
<td><strong>EPIDERMAL / KERATINOCYTE SIGNALLING</strong></td>
<td></td>
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<tr>
<td>Key examples; IL1, IL6, TNF-α, PDGF, FGF-receptors, CCL2, ET-1, Fibrillin-1, Fli-1, morphogenic signalling pathways</td>
<td></td>
</tr>
<tr>
<td>αMSH / MCR1</td>
<td>Homeostatic pathway which modulates pro and anti-inflammatory cytokines to antagonize TGF-β induced skin fibrosis</td>
</tr>
<tr>
<td>S100A9</td>
<td>Stimulates fibroblast proliferation and CTGF via TLR4 signalling</td>
</tr>
<tr>
<td>Keratinocyte FGF-receptors</td>
<td>Receptor loss leads to transepidermal water loss with IL-1, S100A8 and S100A9 production and resultant profibrotic response</td>
</tr>
<tr>
<td>Fibrillin-1</td>
<td>ECM microfibrillar component overexpressed in some SSc populations, antibodies demonstrated in morphea and mutation responsible for stiff skin syndrome</td>
</tr>
<tr>
<td>Fli-1</td>
<td>Transcription factor; down-regulation induces SSc-like phenotype and enhances skin fibrosis via IL-1, IL-6 and IL-8 over-expression</td>
</tr>
<tr>
<td>Wnt-signalling</td>
<td>Stimulates enhanced fibroblast proliferation, myofibroblast differentiation, collagen and ECM expression and CTGF. Inhibited by PPAR-γ receptor agonists. Inhibits epidermal responsiveness to FGF-receptors.</td>
</tr>
<tr>
<td><strong>INNATE IMMUNITY</strong></td>
<td></td>
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<tr>
<td>TLR3</td>
<td>IL-6, IP10</td>
</tr>
<tr>
<td></td>
<td>Heightened fibroblast responsiveness to TGF-β</td>
</tr>
<tr>
<td>STAT4</td>
<td>proinflammatory / profibrotic cytokines; TNF-α, IL-2, IL-6</td>
</tr>
<tr>
<td></td>
<td>Type-1 IFNs, leading to increased fibrosis</td>
</tr>
<tr>
<td>NF-κB</td>
<td>Skin homeostatic pathway, profibrotic c-Rel subunit abnormally expressed in SSc keratinocytes</td>
</tr>
<tr>
<td>IL-1 / NLRP3 inflammasome</td>
<td>Levels correlate with mRSS</td>
</tr>
<tr>
<td><strong>ADAPTIVE RESPONSES</strong></td>
<td></td>
</tr>
<tr>
<td>Early active inflammatory phase</td>
<td></td>
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<td>Th2 cytokines: IL-2, TNF-α, IL-6</td>
<td>Increased levels in early (&lt;24 months) morphea, correlate with skin scores</td>
</tr>
<tr>
<td>Amplified inflammation and initiation of fibrosis</td>
<td></td>
</tr>
<tr>
<td>Th17 cytokines: IL-1, IL-17, IL-22</td>
<td>Increased levels later (24-48 months)</td>
</tr>
<tr>
<td></td>
<td>IL-1 promotes fibroblast proliferation, collagen production and CTGF expression</td>
</tr>
<tr>
<td></td>
<td>TGF-β</td>
</tr>
<tr>
<td></td>
<td>Increased levels in skin and serum</td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>
Up-regulates MMPs eg. MMP12 over-expressed in SSc

**Final fibrotic and atrophic phase**

**Th2 cytokines:**

- **IL-4, IL-13**
  - Up-regulate collagen synthesis and inhibit collagenase activity
  - ↑IL-13 levels and activity in morphoea
  - IL-4 levels correlate with early inflammatory phase cytokines and skin scores

**MESENCHYME AND FIBROBLASTS**

<table>
<thead>
<tr>
<th><strong>Fibrocyte to myofibroblast trans-differentiation</strong></th>
<th>Aberrant differentiation of mesenchymal progenitors described in SSc</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Epithelial to myofibroblast trans-differentiation</strong></td>
<td>Induced by cytokines and signalling involved in morphoea related fibrosis; TNF-α, TGF-β, CTGF, FGF2, Wnt, Shh and Jagged-notched.</td>
</tr>
<tr>
<td><strong>Location specific mesenchymal gene expression and patterning</strong></td>
<td>Demonstrated in stiff skin syndrome</td>
</tr>
<tr>
<td></td>
<td>Determined by pathways and molecular mechanisms intricately linked to pathological fibrosis; TGF-β, Wnt, FGFs and others.</td>
</tr>
<tr>
<td>ANATOMICAL DISTRIBUTION</td>
<td>MORPHOLOGY</td>
</tr>
<tr>
<td>-------------------------</td>
<td>------------</td>
</tr>
<tr>
<td><strong>LIMITED</strong></td>
<td><strong>INFLAMMATORY</strong></td>
</tr>
<tr>
<td>Plaques affecting ≥ 2 anatomical sites</td>
<td>- erythematous</td>
</tr>
<tr>
<td><strong>GENERALIZED</strong></td>
<td><strong>SCLEROTIC</strong></td>
</tr>
<tr>
<td>Plaques affecting ≥ 3 anatomical sites</td>
<td>- blue or non-pigmented</td>
</tr>
<tr>
<td><strong>BLASCHKOID</strong></td>
<td><strong>DYSPIGMENTED</strong></td>
</tr>
<tr>
<td>Follows Blaschko's lines = linear morpheces</td>
<td>- hyperpigmented</td>
</tr>
<tr>
<td><strong>MIXED</strong></td>
<td><strong>ATROPHIC</strong></td>
</tr>
<tr>
<td>≥ 2 distributions: eg. limited plaque and BlaschkoID</td>
<td>- atrophy</td>
</tr>
</tbody>
</table>

**REGIONAL DIVERSIFICATION**

- Regional genetic / epigenetic susceptibility mechanisms
  - BlaschkoID mutations
  - Epidermal signaling (eg. VHL, PORCN gene)
  - Keratinocytes
  - eg Smad4, fibroblast, R1, innate pathways
  - Mesenchymal patterning and location specific gene expression
  - Postional identity
  - Site specific expression (eg. TGFβ, FGF)

**LOCATION SPECIFIC TRIGGERS**

- eg Retinol (isomorphic disseminated plaque morpheces), radiation field morpheces

**IMMUNOINFLAMMATORY AND PROFIBROTIC MECHANISMS**

- Innate
  - IFN, IL6, IL8, STAT4, NFκB, IL1
- Adaptive
  - IL2, TNFα, IL4, IL6, IL1, TGFβ, IL17, IL13, CCL2
- Connective tissue
  - FGF, CTGF, MMPs, TIMPs
- Developmental and homeostatic
  - VHL, Jagged-Kloth, Shh
  - αSMA
- Aberrant antifibrotic mechanisms
- Inflammatory versus profibrotic genetic signatures

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Figure 1. Linking clinical morphoea variability (anatomical, morphology and depth of tissue involvement), to possible underlying genetic and molecular mechanisms. Specifically, anatomical distributions may be explained by certain factors of regional diversification, whilst variations in morphology and depth of tissue involvement may be determined by differing immunoinflammatory and pro / anti-fibrotic pathways.

Figure 2. Proposed morphoea pathogenesis; underlying susceptibility mechanisms plus epigenetic and environmental factors combine to trigger a cascade of epidermal (keratinocyte) and dermal (fibroblast) innate, adaptive, fibrotic molecular mechanisms and signaling pathways, culminating in fibrosis.

α-MSH = α-melanocyte stimulating hormone, ET1 = endothelin 1; Fli1 = , Shh = Sonic hedgehog, Wnt = , PPAR = , Kϕ FGFR = keratinocyte fibroblast growth factor receptors, CTGF = connective tissue growth factor, TGF-β = transforming growth factor, TLR = toll-like receptor, IRF5 = , STAT4 = , NLRP3 = , IL = interleukin, TNFα = tumour necrosis factor, PDFG = platelet derived growth factor, MMPs = matrix metalloproteins, TIMPs = tissue inhibitors of metalloproteinases, FGF = fibroblast growth factor, COMP = cartilage oligomeric matrix protein.