General discussion

Parts of the discussion are based on:

Hidradenitis suppurativa: a systematic review integrating inflammatory pathways into a
cohesive pathogenic model

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CONCLUSIONS AND GENERAL DISCUSSION

Hidradenitis suppurativa (HS) is a chronic, recurrent skin disease harbouring a complex pathogenesis that is not yet fully understood. By identifying the latest publications on the pathophysiology of HS, our systematic review has collated substantial evidence that HS is an immune-mediated inflammatory disease (IMID) with both endogenous and exogenous factors contributing to the onset and progression of the disease.

Firstly, genetic factors play a key role in causing HS. Mutations in a range of genes, including *NCSTN* mutations in the γ-secretase complex and *PSTPIP1* mutations, are directly associated with auto-inflammatory disease.[1-4] However, the majority of HS cases appear to be non-familial, suggesting the existence of separate subsets and the need for stratification within patients diagnosed with HS.[5] Secondly, the simultaneous activation of multiple inflammatory pathways (inflammasome, Th1/Th17, toll-like receptor signalling) result in the upregulation of cytokines and chemokines, including TNF-α and a range of interleukins, which are connected to auto-inflammatory mechanisms in the pathogenesis of HS.[6-8] Thirdly, there is an alteration in the local microbiome of normal-appearing versus lesional skin.[9-12] Data also suggest that bacterial aggregates are associated with inflammation of chronic HS lesions, and it is proposed that they most likely occur as a secondary event, possibly due to predisposing local anatomical changes such as sinus tracts (tunnels), keratinous detritus and dilated hair follicles.[9] Finally, enhancement of HS risk and severity occurs via a range of physiological and environmental factors like smoking, obesity and mechanical friction.[13-16]

As there are multiple gaps in HS knowledge, the aim of this thesis was not restricted to one single facet of the disease. Instead, we took a broader approach and focused on both clinical and translational aspects of HS. Four key themes have emerged from this thesis. First, pruritus or itch is a frequent and bothersome symptom in patients with HS. We detected several pathophysiological substrates that could explain the occurrence of HS-related pruritus. Second, the overexpression of chemokines and cytokines in HS lesional skin reflects a chronic, activated local inflammatory milieu, indicating the need for effective anti-inflammatory HS therapies. Third, the potency and efficacy of novel anti-inflammatory agents for HS were demonstrated in respectively laboratory and clinical trial settings. Fourth, two treatment strategies (primarily) targeting the hair follicle were evaluated. This chapter closes with future perspectives on HS research.

Importance of the symptom itch including possible pathophysiological substrates

Key symptoms of HS are acute and chronic pain, discomfort, and a purulent, foul-smelling discharge, which overall contribute to a decreased quality of life.[17,18] Previous clinical studies have mainly focused on these well-known symptoms, while less is known about pruritus.[17,19,20] Therefore, we determined the prevalence and explored the characteristics of pruritus in a cohort of HS patients. In **Chapter 2** we found a high prevalence rate of 57% in 211 HS patients, who reported a mean (± SD) NRS score (range 0-10) of 6.1 ± 2.0 in the past 7 days. The majority (81%) of patients ranked the severity of pruritus as moderate-to-severe on a five-point Likert scale. Moreover, sleep and ADL were negatively impacted by pruritus in more than half of the patients. The mean modified 5-D itch score of 13.7 ± 3.6 in 52 HS patients is comparable with the 5-D score in 51 patients with an inflammatory skin condition like burn wounds (13.5 ± 3.2).[21]

The prevalence rate we found in HS was similar in a Spanish cohort of 191 HS patients (59%), and higher than a Polish cohort of 103 HS patients (42%).[22,23] However, Matusiak *et al*. did not report the VAS/NRS cut-off point for determination of the prevalence rate in their HS patients (N = 103).[22] In comparison with other dermatologic conditions, the rate in our cohort was similar to that in patients with psoriasis (49-90%),[24,25] although in the past psoriasis was considered a non-pruritic disease. The rate of pruritus in HS patients is lower than patients suffering burn injuries (67-93%)[26,27] or chronic idiopathic urticaria (79%).[28]

In our study, the degree of pruritus was positively correlated with pain intensity, number of HS-affected areas, and active smoking. In addition, we found a significant correlation between the intensity of pruritus and DLQI scores (N = 422, r = 0.47, p = 2.0 e-24, unpublished data) in a subpopulation of the HiScreen Registry, consisting of HS patients from the department of Dermatology in the Erasmus MC and DermaHaven. A similar correlation (r = 0.48) was found in the study of Matusiak *et al* (N = 103).[22] Furthermore, pruritus scores were responsive to anti-inflammatory treatment, as shown by the significant different trends of NRS scores for patients receiving apremilast versus patients receiving placebo (**Chapter 5.1**).

The occurrence of pruritus from HS lesions could be explained by inflammatory cell infiltration, especially the influx of eosinophils and the presence of neurogenic inflammation (**Chapter 2**). Moreover, protein levels of β-nerve growth factor were significantly elevated in lesional skin versus non-lesional skin in the same anatomical area of 20 HS patients (**Chapter 5.2**). Nerve growth factor is known to increase neuropeptide levels. These upregulated neuropeptides, such as substance P and calcitonin gene-related peptide, are associated with neurogenic inflammation and the hypersensitive perception of pruritus.[29,30]

Another explanation for the report of pruritus by HS patients is the presence of tryptase-positive mast cells, which were found to be increased in all stages of the disease including perilesional skin.[31] In **Chapter 3** we found overexpression of CCL-26 (eotaxin-3) in the circulation and lesional skin of HS patients. Additionally, increased serum levels of IgE have been reported in patients with HS.[32] The latter two findings in combination with a dense infiltration of mast cells in HS could trigger degranulation of these cells, releasing histamine and other mediators, such as proteases, which causes pruritus. Levels of IL-2, CCL-11, and CCL-17 (TARC), all inflammatory markers that are associated with pruritus, were not elevated in HS plasma and lesional skin (**Chapter 3**). However, the cause of pruritus in HS is probably multifactorial. Other possible mechanisms leading to itch are a small fibre neuropathy as a result of scar formation,[33] irritant contact dermatitis due to maceration or purulent discharge of HS lesions, and alteration in the signalling of mammalian target of rapamycin (mTOR).[34,35]

Overexpression of chemokines and cytokines reflects chronic inflammation

An aberrant immune response, characterised by the overexpression of several markers of inflammation, is an important element of the HS pathophysiology. Unravelling the role of cytokines and chemokines in disease initiation and progression is essential for the clinical and therapeutic stratification of HS. In **Chapter 3** we showed that the *in vivo* protein levels of IL-12/23p40, IL-16, IL-17A, CXCL-8, CXCL-10, CCL-4, and CCL-26 were significantly higher in HS patients compared with healthy controls. A limitation was the small sample size, which did not allow for subgroup analysis by Hurley stage disease severity. Interestingly, there was no significant correlation between protein levels in patient plasma and lesional skin. The question is whether pathway and drug target discovery in plasma or serum is useful in HS. In another study (**Chapter 5.2**), *ex vivo* protein levels of multiple inflammatory markers including IL-12/23p40, IL-17A and S100A12 (calgranulin C) were significantly elevated in lesional skin versus non-lesional skin in 20 patients suffering moderate HS.

Our results obtained in lesional skin confirm previous findings demonstrating overexpression of IL-17 pathway-associated cytokines and chemokines such as IL-17A, IL-12/23 and CXCL-8 in HS.[36-38] In the context of a strong upregulation of S100A12, CXCL-8, IL-17A and IL-23, we hypothesise that IL-16, CCL-4 and CXCL-10 may participate in the recruitment of leucocyte subsets, especially neutrophils, eosinophils, monocytes and dendritic cells, into the inflamed HS skin.[39,40] The significance of neutrophils in the HS pathogenesis is highlighted by the (very) high levels of CXCL-8 that can be cleaved by neutrophil elastase to activate Th17 cells to produce bioactive IL-17.[41] Furthermore, it has been demonstrated that activated neutrophils induce che-

motaxis of Th17 cells by a reciprocal cross-talk.[42] Some previously published results that showed upregulation of TNF-α, IL-1ß and IL-10 in (peri)lesional HS skin could not be reproduced.[38,43] This can be explained by the different methodologies used. In our study (**Chapter 3**), biopsies were homogenised for *in situ* analysis, while van der Zee *et al.* and Kelly *et al.* cultured the skin specimens for 24 and 3 hours respectively, and measured cytokines in the supernatant.[38,43]

The evidence presented above supports HS as a chronic inflammatory skin disorder associated with alterations in predominantly the innate immune system. An informal literature review was additionally conducted to consider our (**Chapter 3** and **Chapter 5.2**) and previous findings in relation to the immunopathogenesis of other established IMIDs such as Crohn's disease, ulcerative colitis, ankylosing spondylitis, psoriasis and psoriatic arthritis, pyoderma gangrenosum, and Behçet's disease. Although these IMIDs are characterised by different pathogeneses, they also share common immunological mechanisms and activated inflammatory pathways (Table 1). The most striking similarity among these diseases is that of aberrations in the innate immune response. Several cytokines are systemically-raised in many of these IMIDs, particularly those implicated in the Th1 and Th17 responses, including TNF-α, IFN-γ, IL-12/23, IL-17, IL-1β and other cytokines of the IL-1 family such as IL-36.[44-48] Several of the pro-inflammatory cytokines have also been shown to be upregulated in HS (e.g. IFN-γ[6], IL-2, TNF-α[7,46] and TNF-β[6]), and are produced by Th1 cells, implicating the Th1 pathway in the pathogenesis of HS. Understanding the distinct and shared immunologic characteristics of IMIDs will aid the development of effective treatments to target the pathogenic mechanisms involved and to modify the disease course.

Novel anti-inflammatory treatments in laboratory and clinical settings

Most evidence to guide management decisions for HS is based on small cohort studies, case reports, and expert opinion.[49] This is illustrated by the low number (only eight) of randomised controlled trials that have investigated the efficacy of anti-inflammatory agents in HS. First-line treatments encompass oral antibiotics with anti-inflammatory properties, mainly tetracyclines and the combination of clindamycin and rifampicin.[19] Second-line therapies include anti-TNF-α biologics such as adalimumab, which is the only registered drug for moderate-to-severe HS with clinically relevant improvements.[50] Other biologic therapies such as secukinumab (anti-IL-17A) and ustekinumab (anti-IL-12/23p40) have not widely been studied in patients with HS or are in the early stages of clinical development.[51] Moreover, very little is known about small molecule drugs that modulate the production of pro- and anti-inflammatory cytokines in HS. Taken together, there is limited high-quality evidence on HS treatment indicating a significant need for novel efficacious anti-inflammatory therapies.

Table 1. Key cytokines of established immune mediated inflammatory diseases in relation to hidradenitis suppurativa.

Disease	Disease overview	Key^ cytokine profile	References
HS	Inflammatory skin disease with genetic, immunological, and environmental background	Th1, Th17 IL-1β, 6, CXCL/IL-8,12, 17, 23, IFN-γ, TNF-α	Banerjee *et al.*, 2017; Boer, 2017; Calderon-Castrat *et al.*, 2016; Di Caprio *et al.*, 2017; Hessam *et al.*, 2015; Jimenez-Gallo *et al.*, 2017; Kromann *et al.*, 2014; Marzano *et al.*, 2017; Marzano *et al.*, 2013; Nomura *et al.*, 2013; Thomi *et al.*, 2017; Xiao *et al.*, 2016; Zhang *et al.*, 2013
CD	Imbalance between gut microbiome and host immune system with genetic background	Th1, Th17 IL-1β, 6, 12, 17, 23, IFN-γ, TNF-α	Hugot *et al.*, 2001; Ogura *et al.*, 2001; Park *et al.*, 2017
UC	Imbalance between gut microbiome and host immune system with genetic background	Th2, Th17 IL-1β, 6, 12, 13, 17, 23 TNF-α	Park *et al.*, 2017
AS	Imbalance between gut microbiome and host immune system with genetic background	Th17 IL-6, 17, 22, 23, 26, IFN-γ, TNF-α	Brown, 2017; Gooren *et al.*, 2000; Jethwa and Bowness, 2016; Li and Brown, 2017; O'Rielly *et al.*, 2016; Sparks and Costenbader, 2014; Videm *et al.*, 2014
Psoriasis	Inflammatory skin disease with genetic and immunological background	Th1, Th17 IL-2, 17, 22, 23, 26, TNF-α, IFN-γ	El-Boghdady *et al.*, 2017; Ho *et al.*, 2005; Love *et al.*, 2012; Nguyen *et al.*, 2018; Ogawa *et al.*, 2017; Strange *et al.*, 2010
PsA	Inflammatory arthritis associated with psoriasis with genetic, immunological, and environmental background	Th1, Th17 IL-17, 23, TNF-α	
PG	Inflammatory, ulcerating, neutrophilic skin disease with genetic, immunological, and environmental background	IL-1β, 17, TNF-α	Al Ghazal *et al.*, 2012; Marzano *et al.*, 2017; Shavit *et al.*, 2017; Thomsen and Sorensen, 2010
Behçet's disease	Multi-systemic, inflammatory, vasculitis with genetic, immunological, and environmental background	Th1, Th17 IL-6, 11, 17, 21, 22, 26, TNF-α, Chitinase3-like1, gp130/sIL -6Rb, sTNF-R1, sTNF-R2	Hemminki *et al.*, 2012; Lopalco *et al.*, 2017; Scherrer *et al.*, 2017; Thomsen and Sorensen, 2010

^ Data summarise key cytokines of these diseases but many other genes, cells types and mediators are involved in the pathogenesis. AS: ankylosing spondylitis. CD: Crohn's disease. HS: hidradenitis suppurativa. PG: pyoderma gangrenosum; PsA, psoriasis and psoriatic arthritis; UC, ulcerative colitis.

In an *ex vivo* disease model, we evaluated the anti-inflammatory effects of currently available biologics targeting TNF-α, IL-17A, IL-12/23p40, and CD20. Adalimumab, infliximab, secukinumab, ustekinumab and rituximab in addition to prednisolone significantly inhibited a selection of pro-inflammatory cytokines and antimicrobial peptides in HS lesional skin (**Chapter 4**). Furthermore, adalimumab, infliximab and prednisolone reduced the levels of a broader mix of individual cytokines than secukinumab, ustekinumab, and rituximab. These findings correspond with the observed efficacy of both TNF-α inhibitors, and systemic and intralesional corticosteroids in the treatment of HS patients in daily practice.[50,52,53] Moreover, the inter-patient variability in the response to the biologics could explain why some HS patients are successfully treated while others show a lesser response. Interestingly, in our *ex vivo* assay, secukinumab did not inhibit protein concentration of respectively IL-17 in the same way as adalimumab and infliximab reduced that of TNF-α. Unfortunately, IL-12p40 protein, an important indicator of the IL-17 pathway, fell below the level of detection in the multiplex assay. However, the lower mRNA expression of the AMPs, IL-6 and CXCL-8 can be considered as the indirect result of blocking the bioactivity of IL-17A and IL-12/23p40 by secukinumab and ustekinumab, respectively. Rituximab was the only biologic without a significant inhibitory effect on individual inflammatory mRNA and protein levels. This is not surprising as B cell blockade in inflammatory diseases acts via inhibition of antibody production, antigen presentation and indirectly via cytokine reduction.[54]

In conclusion, our *ex vivo* skin culture system represents an adequate model for studies in search of novel candidate drugs for the treatment of HS, and to personalise the treatment in specific patients. Future *ex vivo* studies could focus on dose-response relationships, combinations of monoclonal antibodies or bi/trifunctional antibodies, and biologics in combination with low dose prednisolone.

In a clinical setting, we studied the efficacy and tolerability of apremilast at a dosage of 30 mg twice daily in patients with moderate HS. A clinical response as measured by the HiSCR50 was met in 8 of 15 (53.3%) patients in the apremilast group and none of 5 patients (0%) in the placebo group at week 16 (**Chapter 5.1**). This response rate is within the range of the proportion of HiSCR50 achievers reported in four studies which have investigated biologics in patients suffering moderate-to-severe HS; 41.8% to 60.0%.[50,55,56] In addition, patients treated with apremilast showed a significantly lower abscess and nodule count, and levels of pain and pruritus over 16 weeks compared with placebo-treated patients. Although the DLQI improved in the apremilast-treated patients, its trend over time was not significantly different between the treatment arms, possibly because of the large variability observed. Furthermore, treatment with apremilast was generally well tolerated, similar to the safety data from larger trials in patients with psoriasis and psoriatic arthritis.[57,58]

We believe that apremilast is a valuable option after failure of conventional treatments such as the combination of clindamycin and rifampicin. Moreover, apremilast may have the following potential advantages over both antibiotics and biologics. A drawback of recurrent or long-term antibiotic treatment is the risk of inducing bacterial resistance. A problem with the use of TNF-α antagonists such as adalimumab and infliximab is the risk of anti-drug antibody formation with neutralisation of the therapeutic effect over time.[59]

In **Chapter 5.2** we aimed to detect changes in the expression of important inflammatory markers in lesional skin between the two treatment arms. Although we did not observe significant differences, related S100A12 and IL-17A were significantly elevated in lesional skin and showed a decline only in the apremilast group. Moreover, in psoriasis, the significant reduction of IL-17A and IL-17F plasma protein levels after apremilast treatment highlights its impact on the Th17 pathway.[60] Our nonsignificant results could be explained by several determinants. First, the regression to the mean phenomenon should be considered as a possible cause as the highly inflammatory index lesions could have spontaneously improved over time in the context of the fluctuating nature of the disease, especially in the placebo group. Second, the inflammatory infiltrate and substrates for the foreign body inflammation were gradually reduced by successively taking biopsies from the same nodule. Third, the small and possibly underpowered study population could have resulted in the nonsignificant findings. Previously, two open label studies investigating respectively infliximab and ustekinumab in HS patients neither found a change in inflammatory protein serum levels after treatment, nor could link translational data to the clinical response.[61,62] In conclusion, assessing pharmacodynamics (in the skin) in a highly fluctuating inflammatory disease remains challenging. A better translational model in clinical trials involving HS has yet to be developed.

Modifying the disease course by targeting the hair follicle yielded ambiguous results

Current treatment strategies primarily focus on treating the consequences rather than preventing flare-ups or sustaining remission. Because follicular occlusion is considered to be the primary event in the pathogenesis of HS, we hypothesised that targeting the hair follicle would ameliorate the disease. Therefore, two non-invasive techniques that could potentially improve the symptoms and clinical course of HS were separately studied: hair reduction using the 1064-nm neodymium-doped yttrium aluminium garnet (Nd:YAG) laser and microwave ablation (miraDry).

Three previous studies that used the Nd:YAG laser in HS primarily focused on targeting the inflammatory lesions rather than destroying the hair follicles.[63-65] We retrospectively evaluated the effect of Nd:YAG depilation of a whole anatomic area

in 15 patients with Hurley stage I and HS-PGA 2. Because of the different approach in using the Nd:YAG laser, our HS patients were less severely affected than those in previous studies (Hurley II and III disease severity). Treatment with Nd:YAG resulted in a significant decrease in the number of patient-reported monthly flares after a follow-up period of on average more than one year (**Chapter 6.1**). In addition, mean HS disease severity after depilation as measured by a NRS was significantly lower in comparison with before therapy. Two-thirds of the patients would recommend Nd:YAG depilation to other HS patients. These results suggest that laser hair removal could be a novel therapeutic approach to prevent disease progression or ameliorate the disease, especially in HS patients with the follicular sub-phenotype.[66] However, our findings could be biased due to natural fluctuation of the disease course as there was no control group. Other study limitations are a possible recall bias and the absence of physician-reported outcomes such as an abscess and nodule count. Prospective randomised controlled trials are warranted to confirm the mechanism of action and long-term efficacy of laser hair removal in mild HS.

Recently, non-invasive microwave ablation (MWA) using the miraDry device have demonstrated promising results for permanent reduction of axillary hairs.[67] There may be advantages of MWA over light- and laser-based methods. MWA requires only 1 or 2 sessions to achieve 70% reduction of hair growth,[67] while light- and laser-based methods often require 6 to 10 sessions to realise such results. Moreover, outcomes of MWA are independent of skin type and hair colour.[67]

We hypothesised that MWA could potentially improve the symptoms of HS by reducing the number of hair follicles (primary action) and the destruction of the inflammatory cell infiltrate (secondary action) in HS lesions. Therefore, in **Chapter 6.2**, we evaluated the efficacy and safety of MWA for mild axillary HS in a randomised intrapatient-controlled trial. Only 9 of 20 planned HS patients were included because the study was prematurely terminated due to negative clinical outcomes. In total, 8 patients completed the three month follow-up, of which 5 showed worsening of their disease after microwave ablative therapy. Commercial miraDry clinics in The Netherlands also observed a few cases of flaring of the disease in HS patients (personal communication).

As the miraDry device targets a zone rather than a particular structure, its non-selectivity might have resulted in the poor study outcomes. Accordingly, we argue that the microwave energy is able to rupture pre-existing and subclinical or microscopic HS precursor lesions (cysts), subsequently resulting in an intense inflammatory response beyond the initially visible lesions. In addition, the development of HS-like lesions such as abscesses and nodules are a relatively frequent complication of miraDry treatment in otherwise healthy subjects with axillary hyperhidrosis.[68] We used miraDry energy level 5 (manufacturer's recommended setting) corresponding

with an energy delivery time of 3.0 seconds. Although the delivery time in level 1 (lowest setting) is only 0.6 seconds shorter than level 5 (highest setting), the effect on hair reduction using the lowest setting has never been reported. In conclusion, our findings indicate that MWA using the miraDry device has no apparent clinical benefit and may even be harmful in patients with mild axillary HS. Therefore, we question the utility of microwave ablative therapy in patients with HS in clinical practice.

FUTURE PERSPECTIVES

Pathomechanisms

Despite the rising number of publications on HS in the recent years, there are still many questions to be addressed. Therefore, further research in various arenas is warranted to ultimately improve the management and treatment of patients with HS and related syndromic conditions. Large gaps remain in the understanding of the pathogenesis of HS. Genetic research should aim to add more detail to the proposed mechanism by which loss of function of NCSTN or of other γ-secretase proteins causes familial HS, and to better stratify patients with HS. Immunologic studies should focus on molecular drivers of tissue inflammation and injury in HS and the relationship between the HS cytokine profile and disease activity. Next generation sequencing methods will help unravelling the genome, transcriptome, and proteome of HS patients. Furthermore, microbiome research is needed to better characterise the disruption to the microbial ecosystem and to elucidate whether the disruption causes the disease or whether the disease causes the dysbiosis. High-throughput metagenomic methods can make this work possible. Finally, it will be important to focus research on the interaction of environmental factors and immunogenetic factors.

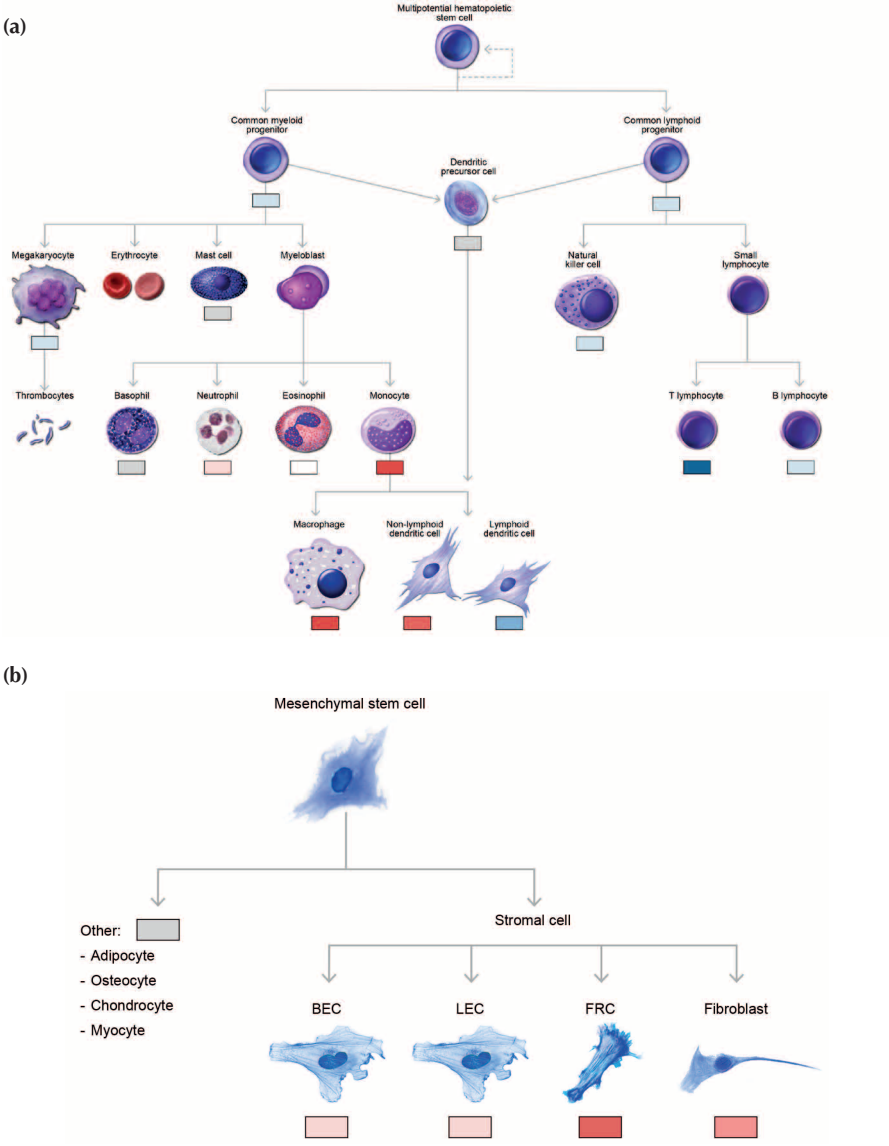
Immunogenetic research in progress

In 2017, we reported a novel *NCSTN* mutation in a three-generation Dutch family with HS.[69] In HS, 83% (30/36) of the previously reported sequence variants in the γ-secretase complex are scattered throughout the extracellular domain of the *NCSTN* gene without particular hotspots,[70] indicating a critical role for *NCSTN* in the stability of the γ-secretase complex.[71] Because the nicastrin protein exhibits multiple conserved residues and is for 88% (63/72 amino acids) homologous to the murine counterpart, we used the microarray dataset of the Immunological Genome Project (ImmGen) to perform a thorough dissection of the expression and function of *NCSTN* in the immune system. In short, the expression data of the *NCSTN* gene were normalised as part of the ImmGen pipeline by Robust Multichip Average as described by Jojic *et al*.[72]

Gene Expression Omnibus data were subsequently log^2-transformed and signatures for 16 cell lineages (both hematopoietic and mesenchymal) were calculated.

Wildtype *NCSTN* appeared to be upregulated in myeloid cells like monocytes and macrophages, and mesenchymal cells such as fibroblastic reticular cells and fibroblasts (Figure 1). We hypothesise that mutated *NCSTN* variants could affect the function of these cell lineages, ultimately leading to an aberrant immune response, especially in the skin. In addition, *ARNT*, *PPARδ* and *CAPNS1* were identified in the 25 highest co-expressed genes with *NCSTN* (Figure 2). The *ARNT* gene encodes the aryl hydrocarbon receptor nuclear translocator protein. The aryl hydrocarbon receptor (AhR) is involved in the induction of several enzymes that participate in xenobiotic metabolism, including dioxin and polycyclic aromatic hydrocarbons which are present in cigarette smoke.[73] *PPARδ* facilitates AhR signalling, enhances fatty acid catabolism, and induces keratinocyte differentiation.[74,75] Calpain-like proteases process the precursor form of IL-1α into the biologically active mature form, an important pro-inflammatory cytokine in epithelial and myeloid cells.[76,77]

In summary, the associated immunobiological functions of *NCSTN*, *ARNT* and *PPARδ* link genetics to the environment, which are smoking, the metabolic syndrome, and the skin microbiome.[78] Because we observed a positive association between pack years of smoking and disease severity in the three affected family members, we are investigating the role of AhR ligands and its relation to bacterial products in patients with both familial and common HS in a laboratory setting.



Legend

Count per cell line	Z-score ≥ 0	Z-score ≤ 0
No activity		
0[a]		
1 to 3[b]		
4 to 6		
7 to 9		
10 to 12		
13 to 15		
16+		
No ImmGen reference		

[a] 0 corresponds with ^{2}Log ≤0.5 or ≥-0.5
[b] ≥1 corresponds with ^{2}Log ≥0.5 or ≤-0.5

Figure 1. Wildtype *NCSTN* expression in hematopoietic and mesenchymal cell lineages. The colour intensity correlates with the degree of change. **(a)** Myeloid signature with upregulation in monocytes, macrophages, non-lymphoid dendritic cells, and neutrophils. **(b)** Upregulation of stromal cells and typically fibroblastic reticular cells and fibroblasts. BEC: blood endothelial cell. FRC: fibroblastic reticular cell. LEC: lymphatic endothelial cell.

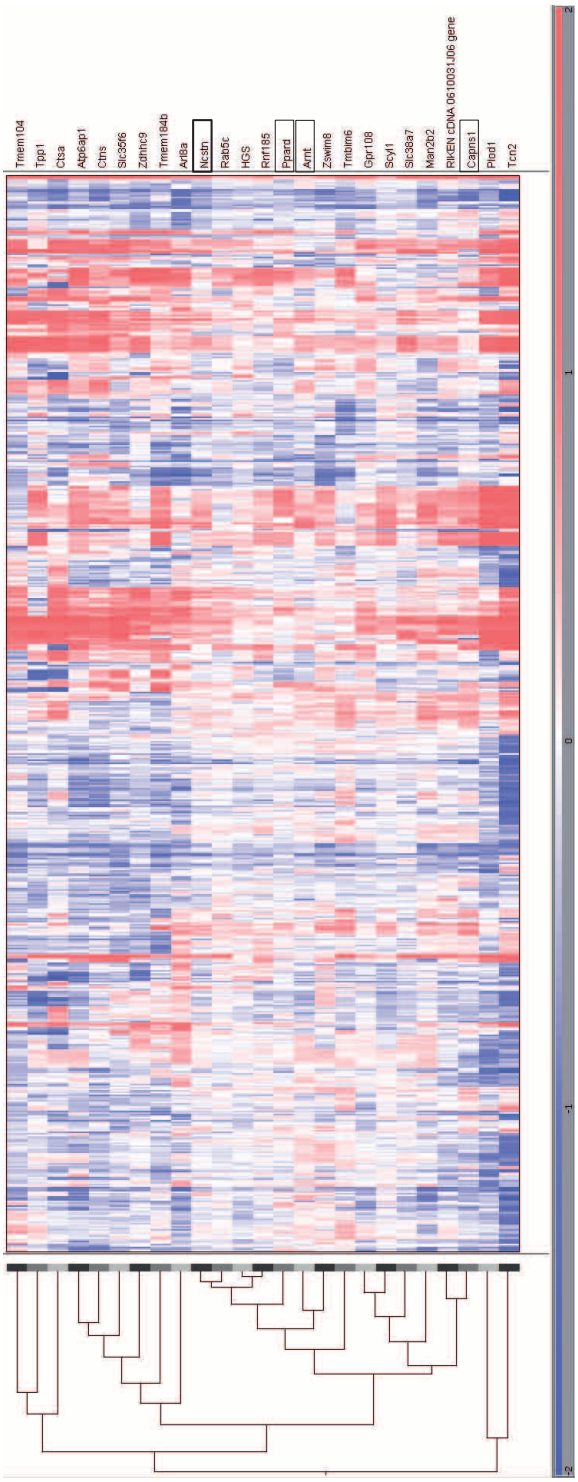


Figure 2. OmniViz Treescape showing the top 25 co-expressed genes related to *NCSTN*. Gene expression levels: red colour, up-regulated genes compared with the geometric mean; blue colour, down-regulated genes compared with the geometric mean. The colour intensity positively correlates with the degree of change. *ARNT*: aryl hydrocarbon receptor nuclear transporter. CAPNS1: calpain small subunit 1. *PPARD*: peroxisome proliferator-activated receptor delta.

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