DOI: 10.1111/jdv.16310 JEADV

ORIGINAL ARTICLE

A novel nicastrin mutation in a three-generation Dutch family with hidradenitis suppurativa: a search for functional significance

A.R.J.V. Vossen,[1,*] K.R. van Straalen,[1] S.M.A. Swagemakers,[2] J.E.M.M. de Klein,[3] A.P. Stubbs,[2] D.J. Venter,[4] H.H. van der Zee,[1] P.J. van der Spek,[2,†], E.P. Prens[1,†]

[1]Department of Dermatology, Erasmus University Medical Center, Rotterdam, The Netherlands
[2]Department of Pathology and Clinical Bioinformatics, Erasmus University Medical Center, Rotterdam, The Netherlands
[3]Department of Clinical Genetics, Erasmus University Medical Center, Rotterdam, The Netherlands
[4]Department of Pathology, Mater Health Services, South Brisbane, Queensland, Australia
*Correspondence: A.R.J.V. Vossen. E-mail: a.vossen@erasmusmc.nl

Abstract

Background Mutations in the γ-secretase enzyme subunits have been described in multiple kindreds with familial hidradenitis suppurativa (HS).

Objective In this study, we report a novel nicastrin (*NCSTN)* mutation causing HS in a Dutch family. We sought to explore the immunobiological function of *NCSTN* mutations using data of the Immunological Genome Project.

Methods Blood samples of three affected and two unaffected family members were collected. Whole-genome sequencing was performed using genomic DNA isolated from peripheral blood leucocytes. Sanger sequencing was done to confirm the causative *NCSTN* variant and the familial segregation. The microarray data set of the Immunological Genome Project was used for thorough dissection of the expression and function of wildtype *NCSTN* in the immune system.

Results In a family consisting of 23 members, we found an autosomal dominant inheritance pattern of HS and detected a novel splice site mutation (c.1912_1915delCAGT) in the *NCSTN* gene resulting in a frameshift and subsequent premature stop. All affected individuals had HS lesions on non-flexural and atypical locations. Wildtype *NCSTN* appears to be upregulated in myeloid cells like monocytes and macrophages, and in mesenchymal cells such as fibroblastic reticular cells and fibroblasts. In addition, within the 25 highest co-expressed genes with *NCSTN* we identified *CAPNS1*, *ARNT* and *PPARD*.

Conclusion This study reports the identification a novel *NCSTN* gene splice site mutation which causes familial HS. The associated immunobiological functions of *NCSTN* and its co-expressed genes *ARNT* and *PPARD* link genetics to the most common environmental and metabolic HS risk factors which are smoking and obesity.

Received: 6 September 2019; Accepted: 30 January 2020

Conflicts of interest

ARJVV, KRvS, SMAS, JEMMdK, APS, DJV, HHvdZ, PJvdS, EPP; none to declare.

Funding sources

None.

Introduction

Hidradenitis suppurativa (HS), also known as acne inversa, is a chronic, inflammatory and debilitating skin disease characterized by painful, deep-seated, inflammatory nodules and abscesses, and in later stages sinus tract formation and scarring. The characteristic lesions are mainly located in the inverse body areas such as the axillary, inguinal and anogenital regions.[1]

The complex pathogenesis of HS starts with hyperplasia of the follicular epithelium with infundibular hyperkeratosis and subsequent follicular occlusion.[2,3] The consequential dilatation of the hair follicle results in a rupture with a foreign body-type inflammatory immune response.[4,5] In addition, multiple factors are associated with the development and maintenance of the disease. Known environmental factors include smoking, obesity,

Ethical review board approval: IRB Erasmus University Medical Center Rotterdam (NL45264.078.13).
†These authors contributed equally to this work and share last authorship.
Prior presentation: 6th EHSF Conference, February 2017, Copenhagen

© 2020 The Authors. Journal of the European Academy of Dermatology and Venereology published by John Wiley & Sons Ltd on behalf of European Academy of Dermatology and Venereology
This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

mechanical friction and hormones, whereas endogenous factors comprise female gender, an aberrant immunity and genetic predisposition.[6]

A family history of HS is reported in up to 40% of patients. A hereditary form of HS was first described in three English families in 1984.[7] However, a familial presentation [MIM: 142690] displaying an autosomal dominant pattern of inheritance with high penetrance is rare. The genetic basis of this hereditary form of HS was first described in a Chinese family in 2010 and involves heterozygous gene mutations in the γ-secretase subunits, which consists of presenilin (*PSEN1* [MIM: 104311] and *PSEN2* [MIM: 600759]), presenilin enhancer 2 (*PSENEN* [MIM: 607632]), nicastrin (*NCSTN* [MIM: 605254]) and anterior pharynx defective 1 (*APH1A* [MIM: 607629] and *APH1B* [MIM: 607630]).[8] To date, HS-related mutations of the γ-secretase genes have been identified in 35 multiplex kindreds: 21 Chinese,4 French, 2 Japanese, 2 British, 2 Indian, 1 African American, 1 Iranian and 1 Jewish Askenazi.[9] Moreover, other genes have been associated with syndromic HS. Most recently, a *FGFR2* [MIM: 176943] missense mutation was found in one case displaying generalized comedones, acne and HS.[10] In addition, the *PSTPIP1* gene [MIM: 606347] has been implicated in patients with a syndromic form of HS comprising HS, pyoderma gangrenosum, acne and pyogenic arthritis (PASH/PAPASH).[11] However, the functional implications of the above-mentioned sequence variants remain unknown, and functional and proteomic studies investigating the pathogenic mechanisms in familial HS are currently scarce.[11]

In this study, we report a novel *NCSTN* mutation causing HS in at least three generations of a Caucasian Dutch family, with an autosomal dominant inheritance. We sought to explore the immunobiological function of *NCSTN* mutations using the Immunological Genome Project (ImmGen) data.

Material and Methods

Ethical statement

The research protocol was approved by the Institutional Review Board of the Erasmus University Medical Center, Rotterdam (reference NL45264.078.13). Written informed consent for the diagnostic procedures including whole-genome sequencing was obtained from all participants in accordance with the Declaration of Helsinki.

The pedigree

A three-generation Caucasian family with HS was ascertained via identification of the proband at the department of Dermatology of the Erasmus University Medical Center in the Netherlands. The pedigree displayed an autosomal dominant inheritance. The family consisted of three generations including 23 individuals, of which 10 were affected (60% male) and 13 were unaffected (Fig. 1). Blood samples of three affected (HS2A, HS2C and HS3B) and two unaffected (HS1A, HS2B) individuals were collected and genomic DNA was isolated from peripheral blood leucocytes.

Whole-genome sequencing and validation analysis

Whole-genome sequencing was performed using the BGI's Complete Genomics platform as previously published.[12] Sequence reads were mapped to the reference genome (GRCh38), and variants were called by local de novo assembly according to the methods previously described by Carnevali *et al.*[13] Analysis of the whole-genome sequencing data from all sequenced family members was performed using Complete Genomics Analysis tools, version 1.3.0, build 9 and TIBCO Spotfire software version 3.3.1. Mapped sequences of the five samples varied between 132 and 146 Gb, resulting in an average coverage between, respectively, 42- and 47-fold per genome. Subsequently, the pathogenic status of the identified sequence variants was interpreted using Alamut®. DNA flank primers were designed to study DNA expression of the mutant allele on peripheral blood leucocytes from all analysed individuals. Confirmation of the causative *NCSTN* variant and the familial segregation were performed using Sanger sequencing.

Dissection of expression and function

The protein sequence of *NCSTN* exhibits multiple conserved residues and is for 89% homologous to the murine counterpart. Therefore, the microarray data set of the Immunological Genome Project (GSE15907)[14–16] was used to perform a thorough search for the expression and function of murine *NCSTN* in the immune system. The expression data of the *NCSTN* gene were normalized as part of the ImmGen pipeline by RMA as described by Jojic *et al.*[17] GEO sample (GSM) data were log2 transformed and signatures for 16 lineages, both hematopoietic and mesenchymal, were subsequently calculated. Cut-off values of ≤ -0.5 and ≥ 0.5 were applied on the Log2 scores in order to

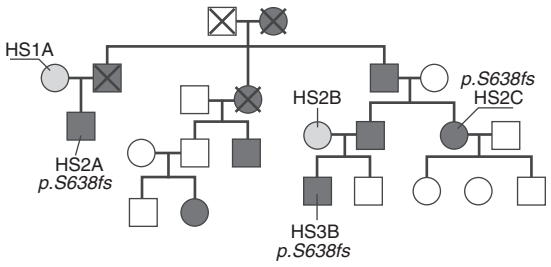

Figure 1 The pedigree is consistent with autosomal dominant inheritance. Five individuals were investigated: HS2A (proband), HS2C and HS3B were affected by a frameshift mutation (p.S638 fs), while HS1A and HS2B were unaffected.

© 2020 The Authors. Journal of the European Academy of Dermatology and Venereology published by John Wiley & Sons Ltd on behalf of European Academy of Dermatology and Venereology

select the most significant lineages. Stimulated cells were excluded from the analysis as infectious mediators are not considered to be involved in the primary pathophysiology of HS. Mast cells, basophilic granulocytes, dendritic precursor cells (haematopoietic lineages), and adipocytes, osteocytes, chondrocytes, tenocytes and myocytes (mesenchymal lineages) are not included in the GSE 15907 data set.

NCSTN-related transcripts

Using above-mentioned data set, we identified the 25 highest co-expressed transcripts compared with wildtype *NCSTN*. Raw intensity values of all samples were normalized by background correction and quantile normalization using the V.6.4 Robust Multichip Analysis (Partek Genomics Suite software, V.6.6; 2014 Partek Inc., St. Louis, Missouri, USA). The normalized data file was transposed and imported into OmniViz V.6.1 (Instem) for further analysis. Correlations with the expression of *NCSTN* were calculated for all probes.

Results

Phenotype of affected individuals

The three-generation family comprised 23 individuals, of which 10 were affected (60% male) and 13 were unaffected (Fig. 1). Phenotyping of the individuals HS2A (proband), HS2C and HS3B revealed a disease severity ranging from mild to severe (Table 1). The proband displayed multiple, interconnected, inflammatory lesions in the axillary, inguinal and gluteal regions, resulting in a HS-PGA of 5 (very severe) and significant impact on quality of life (DLQI = 17). The perianal and perineal areas were affected for > 1% of the total body surface area (BSA) consistent with a Hurley III disease stage. In addition, multiple papules and cysts were found in non-flexural and atypical locations including the back, nape of the neck and the retro-auricular regions (Fig. 2). In contrast, individuals HS2C and HS3B displayed mild to moderate inflammatory activity (HS-PGA 2 to 3) and low impact on quality of life (DLQI < 5) (Fig. S1 and S2). HS onset before the age of twenty, the presence of follicular lesions, and involvement of atypical locations such as the head and neck region were found in all affected patients (Table 1). Interestingly, HS2A (past smoker) and HS2C (current smoker) suffered more severe disease than HS3B (non-smoker). None of the affected patients was obese. Late-onset Alzheimer's disease within the pedigree was only reported in the unaffected father of the proband.

Table 1 Patient characteristics of the affected individuals (HS2A, HS2C and HS3B).

	HS2A (proband)	HS2C	HS3B
Age (years)	52	58	26
Gender	Male	Female	Male
Age of HS onset (years)	18	16	16
Smoking status [pack years]	past [36]	positive [26]	negative
Acne (history of)	yes	no	no
Diabetes mellitus (history of)	no	no	no
Hypertension (history of)	no	yes	no
Abnormal lipid profile (history of)	no	no	no
Body Mass Index (kg/m^2)	24.6	24.1	27.5
HS-PGA (0–5)	5	3	2
Modified Sartorius Score	191	19	10
Refined Hurley stage[52]	III	IIB	IA
History of HS surgery	yes	yes	no
Locations†			
Flexural	+	+	–
Non-flexural	+	+	+
Atypical	+	+	+
Lesion types‡			
Comedones	–	+	–
Papules and folliculitis	+	+	+
Cysts [location]	+ [neck/ auricular]	+ [neck/face]	+ [auricular]
NRS pain/itch (0–10)	4/5	0/0	0/0
DLQI (0–30)	17	4	0

†Flexural: armpit, groin, perianal/perineal, sub-mammary folds. Non-flexural: nape, back, buttocks. Atypical: face, limbs, abdomen, other.
‡Not including typical HS lesions (abscesses, nodules, sinuses).
DLQI, dermatology life quality index; HS-PGA, hidradenitis suppurativa – physician global assessment scale; NRS, numerical rating scale.

NCSTN p.S638 fs mutation

The affected individuals were heterozygous, whereas the healthy individuals demonstrated wildtype genotype. In total 8,479,202 variants were detected in one or more family members, including single-nucleotide variants and small insertions, deletions and substitutions (up to about 50 bp). After filtering for heterozygous non-synonymous variants and variants present in splice sites, seven variants were selected (Table 2). None of these were present in the Wellderly database (490 control samples sequenced by Complete Genomics). The variant *NCSTN* c.1912_1915delCAGT had the highest CADD score (35) and was not detected in any control data set (Exome Aggregation Consortium (ExAC),[18] 1000 Genomes Project,[19] Wellderly Database, and Exome Variant Server (EVS),[20] NHLBI GO Exome Sequencing Project (ESP), Seattle, WA (URL: http://evs.gs.washington.edu/EVS/)). None of the other six gene mutations (*MKRN1* [MIM: 607754], *POTEA* [MIM: 608915], *EPS8* [MIM: 600206], *RBBP6* [MIM: 600938], *CDH19* [MIM: 603016], *PCNA* [MIM: 176740]) could be directly linked to HS pathophysiology. Moreover, the HGMD database licensed through Qiagen/Biobase confirmed the previously identified

© 2020 The Authors. Journal of the European Academy of Dermatology and Venereology published by John Wiley & Sons Ltd on behalf of European Academy of Dermatology and Venereology

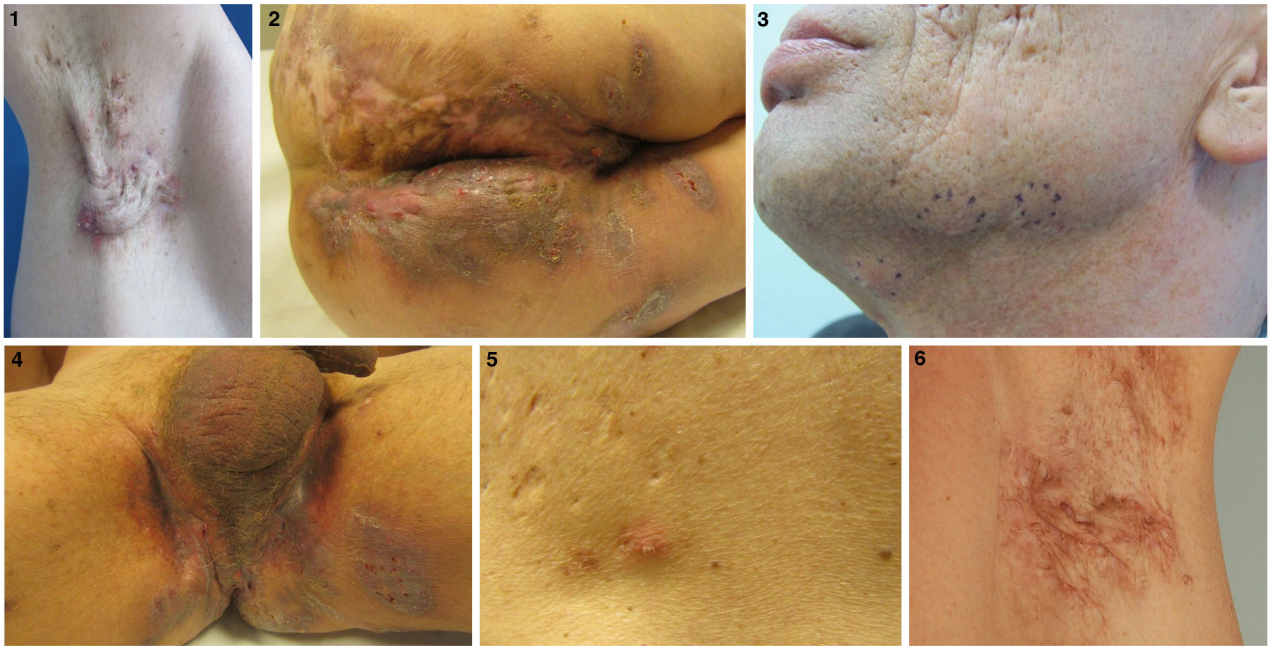

Figure 2 Phenotype of the proband with (1) overview of the right axilla with sinuses and scar contraction displaying Hurley stage II (2) patient on left lateral recumbent with gluteal involvement displaying Hurley stage III, wound contractions after surgical excision, and HS plaques in the upper leg (3) nodules/cysts (pencil marked) and atrophic scarring of the face and nape region, (4) overview of the scrotal, inguinal area including HS plaque in the medial thigh, (5) detail of scarring and folliculitis on the back, (6) overview of the left axilla with superficial lesions displaying Hurley stage II.

Table 2 Summary of results of whole-genome sequencing after filtering for heterozygous non-synonymous variants and variants present in splice sites resulted in seven causal candidate sequence variants. The HGVS-nomenclature (versions 15.11) was used to describe sequence variants[53]

#	Gene	Chr	genomic DNA change (GRCh37)	coding DNA change	protein change	mutation type	CADD*	SIFT
1	**NCSTN**	1	g.160326951_160326954del	NM_001290186:c.1498_1501del, NM_015331:c.1912_1915del, NM_001290184:c.1852_1855del	p.S500 fs p.S638 fs p.S618 fs	Frameshift	35	Unknown
2	**MKRN1**	7	g.140154896C>G	NM_013446:c.1235G>C	p.R412P	Missense	23.5	Tolerated
3	**POTEA**	8	g.43211968del	NM_001002920:c.1149delG, NM_001005365:c.1287delG	p.E383 fs p.E429 fs	Frameshift	7.7	Unknown
4	**EPS8**	12	g.15811097_15811098insAGAAAAC	NM_004447:c.1027insGTTTTCT	Unknown	Unknown	3.7	Unknown
5	**RBBP6**	16	g.24581613C>T	NM_018703:c.3500C>T, NM_006910:c.3602C>T	p.S1167F p.S1201F	Missense	19.4	Deleterious
6	**CDH19**	18	g.64211217G>T	NM_021153:c.1205C>A	p.S402Y	Missense	25.9	Deleterious
7	**PCNA**	20	g.5099501A>T	NM_002592:c.233T>A	p.I78K	Missense	27.8	Deleterious

*CADD v1.3: Combined Annotation Dependent Depletion

mutation in *NCSTN* to be causative in and associated with familial HS.[21] Results of the Sanger sequencing subsequently confirmed the c.1912_1915delCAGT variant and the familial segregation (Fig. S3). The CAGT deletion (p.S638) results in a frameshift and premature termination codon. Protein impact was visualized on structural data derived from NCBI Homologene database and Protein Data Bank database and compared with the amino acid sequence of other species (Fig. 3). The C-terminal deletion present in the extracellular (luminal) domain of the multiple alignment affects the tail of the protein, resulting in loss of adherence with the membrane structures (Fig. 4).

© 2020 The Authors. Journal of the European Academy of Dermatology and Venereology published by John Wiley & Sons Ltd on behalf of European Academy of Dermatology and Venereology

Figure 3 Sequence alignment of NCSTN in human and other species. The amino acid sequence is given in the one-letter code. The deleted serine (S; yellow) located in the C-terminus results in a frameshift. The altered sequence of mutated NCSTN (red) causes a premature stop (*). Conserved residues situated in the C-terminus are indicated in bold (S; N; F, Y and W). Identical amino acids in human and murine are presented in grey boxes. WT: wildtype.

Expression and function of NCSTN and immune-related transcripts

The dissection of the expression and function of *NCSTN* in the immune system resulted in 681 unique lineages. The application of the cut-off values resulted in 123 unique GSM samples, of which 71 upregulated and 62 down-regulated. Nineteen of these samples, n = 16 simulated cells and n = 3 microglia, were excluded. A semi-quantitative analysis was performed on the remaining 104 most significant samples to segregate both hematopoietic and mesenchymal lineages. Each sample was categorized to one immunological cell class leading to a lineage-specific signature (Figs S4 and S5). Wildtype *NCSTN* appeared to be upregulated in the myeloid cells: monocytes, macrophages, non-lymphoid dendritic cells and to a lesser extent neutrophilic granulocytes (Fig. S4. *NCSTN* also affected the mesenchymal lineages by upregulation of fibroblastic reticular cells and fibroblasts (Fig. S5). In addition, three genes of interest with similar immunobiological function were identified among the 25 highest co-expressed genes: *CAPNS1* [MIM: 114170], *ARNT* [MIM: 126110] and *PPARD* [MIM: 600409] of which the latter two are most closely related to *NCSTN*, suggesting a common role in the same pathway (Fig. 5).

Discussion

In this study, we report the identification of a novel *NCSTN* gene splice site mutation, exon 16: c.1912_1915delCAGT, which causes HS in a three-generation Dutch family. The affected individuals of the pedigree were predominately men (male-to-female ratio of 1.5:1), which is in accordance with literature on familial HS describing a male-to-female ratio of 1.7:1.[22–24] In contrast, in common HS, women are generally more affected than men with an associated male-to-female ratio of 1:3.[25] In addition, a history of acne with involvement of the back, the presence of papules and cysts in non-flexural and atypical locations including the back, nape and auricular regions represent the follicular phenotype, which characterize HS patients with γ-secretase mutations.[24,26,27] Similarly, for a number of families the genetic causes of HS are not yet identified as known gene mutations were already excluded, suggesting that HS is most likely a heterogeneous disease and additional genes may contribute to the phenotype.

Compared to mutations in the other genes of the γ-secretase complex, sequence variants of the subunit *NCSTN* have been most frequently reported in HS: 80% (28/35) in hereditary HS and 90% (9/10) in individual cases of HS, indicating a critical role for *NCSTN* in the stability of the γ-secretase complex (Table S1A,B).[28] Most mutations in *NCSTN* cause a frameshift and premature translation stop, potentially leading to impaired activity of the γ-secretase complex.

NCSTN acts to sterically block substrates with large ectodomains, providing the mechanism by which γ-secretase selectively recruits ectodomain-shed substrates while also preventing cleavage of non-substrates.[29] Over 90 transmembrane proteins have been reported to be substrates of the γ-secretase complex, and

© 2020 The Authors. Journal of the European Academy of Dermatology and Venereology published by John Wiley & Sons Ltd on behalf of European Academy of Dermatology and Venereology

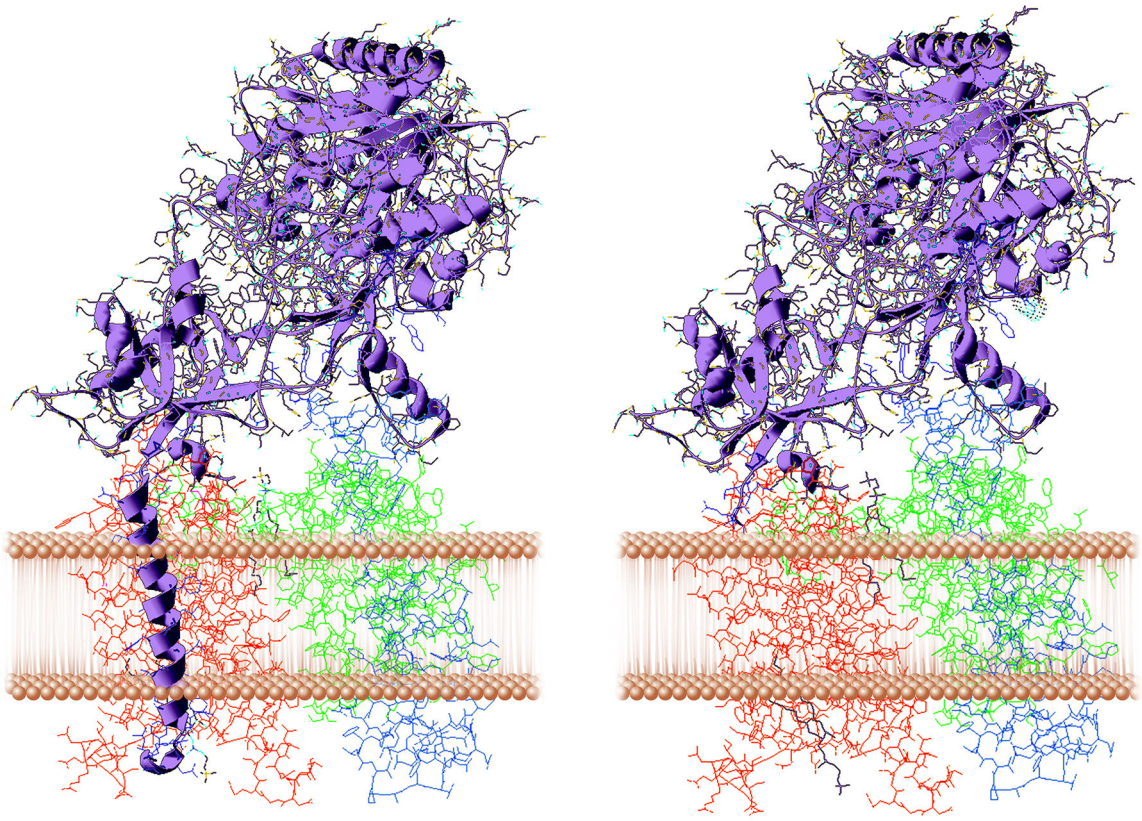

Figure 4 The gamma-secretase complex with wildtype nicastrin (purple), presinilin-1 (green), PEN-2 (blue) and APH-1 (orange). Mutated NCSTN with the C-terminal deletion affects the tail of the protein resulting in a loss of interaction with the luminal and cytoplasmic membrane. The left panel displays wildtype NCSTN. The right panel displays mutated NCSTN.

the amyloid precursor protein (APP) and the Notch receptor are two of the most widely known and studied substrates.[30] APP plays a central role in the development of Alzheimer's disease, while the Notch receptor regulates a variety of developmental processes by controlling cell fate. Members of the Notch transmembrane protein family share multiple epidermal growth-like factor (EGF) repeats in the ectodomain. In HS, it is hypothesized that decreased Notch signalling causes a blockade of epidermal cell differentiation resulting in follicular keratinization, ultimately leading to cyst formation and a subsequent rupture followed by an inflammatory response.[31–33] However, proteomic and functional studies investigating the impact of *NCSTN* mutations on Notch signalling in HS are limited and have shown contradictory results.[32,34–36] A recent study demonstrated that defective expression of *NCSTN* promotes keratinocytes proliferation via decreased miR-100-5p expression. More studies are needed to investigate the role of miRNAs in HS patients with *NCSTN* mutations.[37]

Our explorative analysis investigating the immunobiological function of wildtype *NCSTN* revealed a myeloid and stromal cell signature with upregulation in, respectively, monocytes, macrophages, non-lymphoid dendritic cells, and fibroblasts and fibroblastic reticular cells. We hypothesize that mutated *NCSTN* could disturb the function of these cell lineages and ultimately result in an aberrant immune response, especially in the skin. In addition, three transcripts with similar immunobiological function were identified within the 25 highest co-expressed genes related to *NCSTN* in the ImmGen data. One of the genes was *ARNT*. The *ARNT* gene encodes the aryl hydrocarbon receptor nuclear translocator protein and forms a complex with the ligand-bound aryl hydrocarbon receptor (AhR).[38] AhR is involved in the induction of several enzymes that participate in xenobiotic metabolism including dioxin and polycyclic aromatic hydrocarbons (PAH) which are present in tobacco smoke. Hereby, AhR is able to regulate immunological responses via B lymphocytes, which are important in HS pathophysiology.[39–41] The second upregulated gene *PPARD* is a member of the nuclear-hormone-receptor superfamily and governs a variety of biological processes in peroxisomes. This gene enhances fatty acid

© 2020 The Authors. Journal of the European Academy of Dermatology and Venereology published by John Wiley & Sons Ltd on behalf of European Academy of Dermatology and Venereology

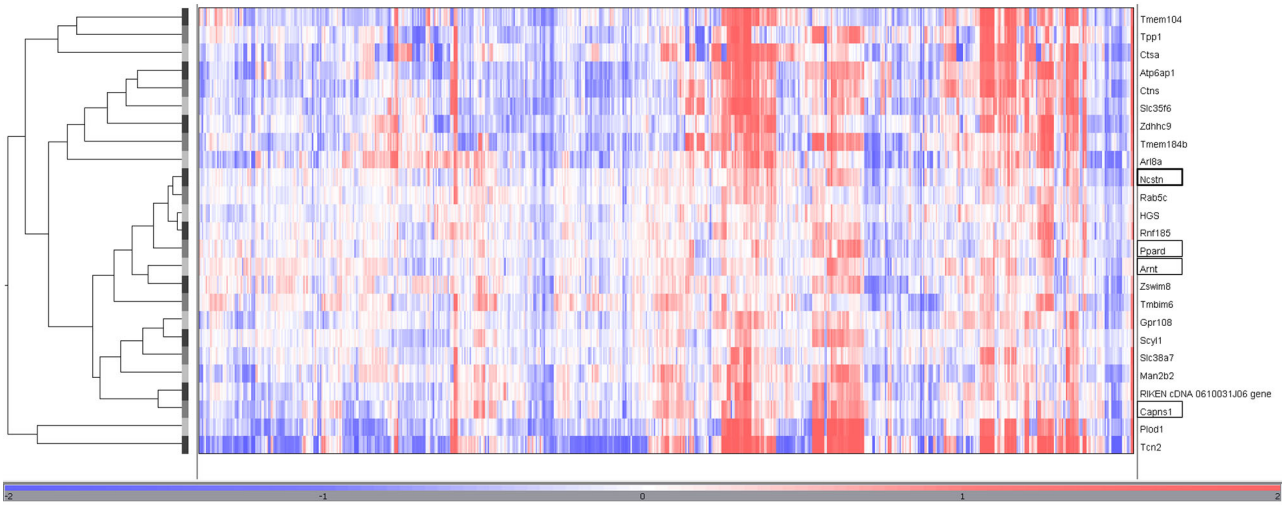

Figure 5 OmniViz heatmap showing the top 25 co-expressed genes related to NCSTN. Gene expression levels: red, upregulated genes compared to the geometric mean; blue, down-regulated genes compared to the geometric mean. The colour intensity correlates with the degree of change. Abbreviations for co-expressed genes of interest: ARNT: aryl hydrocarbon receptor nuclear transporter. PPARD: Peroxisome proliferator-activated receptor delta. CAPNS1: Calpain Small Subunit 1.

catabolism through beta-oxidation, facilitates AhR signalling and induces keratinocyte differentiation.[42–44] Lastly, we identified *CAPNS1*, also known as *CAPN4*, which is a part of the well-conserved family of calcium-dependent cysteine proteases (Fig. 5). 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.[45,46] The importance of IL-1α was illustrated in one study, investigating a human antibody targeting IL-1α, which showed promising results in moderate to severe patients with HS after 12 weeks of treatment.[47]

In addition to *NCSTN,* we found six other gene mutations, none of which could be directly linked to HS pathophysiology. However, the proteins encoded by *PCNA*, *CDH19*, *ESP8* are known to play a role in cell cycle, cytoskeletal signalling and immunological responses. Mutations in these genes could modify cell proliferation and alter the innate immune response possibly contributing to HS.[48–50]

A combination of careful delineation of the familial phenotype, together with functional analysis of *NCSTN* sequence variants and highly co-expressed genes are needed to gain a better understanding of the underlying mechanisms in the pathophysiology of both common and familial HS. Furthermore, larger samples sizes are needed and international collaborations have the potential to identify multiple familial cases with γ-secretase mutations.[51] Ongoing efforts to elicit the functional consequences of these mutations on gamma-secretase activity and downstream signalling pathways have the potential to identify novel therapeutic targets in this debilitating condition. We suggest to search for (γ-secretase) mutations when HS occurs in both three consecutive generations and patients display follicular lesions including involvement of atypical body sites such as the head and neck.

This study reports a novel *NCSTN* gene splice site mutation, exon 16: c.1912_1915delCAGT, in a three-generation Caucasian family. Our explorative analysis demonstrates that the γ-secretase component *NCSTN*, which is the most frequently reported sequence variant in familial HS, has a function in myeloid cells and fibroblasts in the skin. In addition, the associated immunobiological functions of *NCSTN,* and it is co-expressed genes *ARNT* and *PPARD* link genetics to well-known environmental and metabolic triggers (smoking and obesity), both associated with HS and disease severity.

Acknowledgements

The authors would like to express their gratitude to the patients and family members for participating in this study. The patients in this manuscript have given written informed consent to publication of their case details. We thank Mirjam van der Burg (head Laboratory Medical Immunology, Department of Immunology Erasmus University Medical Center) and her team for the Sanger validation of the mutation.

Declaration of Interests

The authors declare no competing interests.

Accession numbers

The accession number(s) for the NCSTN sequence(s) reported in this paper are:

refseq Gene ID: NG_027935

© 2020 The Authors. Journal of the European Academy of Dermatology and Venereology published by John Wiley & Sons Ltd on behalf of European Academy of Dermatology and Venereology

NCSTN:NM_001290186:exon13:c.1498_1501del:p.S500 fs
NCSTN:NM_015331:exon16:c.1912_1915del:p.S638 fs
NCSTN:NM_001290184:exon17:c.1852_1855del:p.S618 fs

References

1 Jemec GB. Clinical practice Hidradenitis suppurativa. *N Engl J Med* 2012; **366**: 158–164.
2 von Laffert M, Helmbold P, Wohlrab J *et al.* Hidradenitis suppurativa (acne inversa): early inflammatory events at terminal follicles and at interfollicular epidermis. *Exp Dermatol* 2010; **19**: 533–537.
3 Yu CC, Cook MG. Hidradenitis suppurativa: a disease of follicular epithelium, rather than apocrine glands. *Br J Dermatol* 1990; **122**: 763–769.
4 Prens E, Deckers I. Pathophysiology of Hidradenitis suppurativa: an update. *J Am Acad Dermatol* 2015; **73**(5 Suppl 1): S8–S11.
5 Vossen A, van der Zee H, Tsoi LC *et al.* Novel cytokine and chemokine markers of hidradenitis suppurativa reflect chronic inflammation and itch. *Allergy* 2018; **74**: 631–634.
6 Vossen ARJV, van der Zee HH, Prens EP. Hidradenitis suppurativa: a systematic review integrating inflammatory pathways into a cohesive pathogenic model. *Front Immunol* 2018; **9**: 2965.
7 Fitzsimmons JS, Fitzsimmons EM, Gilbert G. Familial hidradenitis suppurativa: evidence in favour of single gene transmission. *J Med Genet* 1984; **21**: 281–285.
8 Wang B, Yang W, Wen W *et al.* Gamma-secretase gene mutations in familial acne inversa. *Science* 2010; **330**: 1065.
9 Li A, Peng Y, Taiclet LM, Tanzi RE. Analysis of hidradenitis suppurativa–linked mutations in four genes and the effects of PSEN1-P242LfsX11 on cytokine and chemokine expression in macrophages. *Hum Mol Genet* 2018; **28**: 1173–1182.
10 Higgins R, Pink A, Hunger R, Yawalkar N, Navarini AA. Generalized comedones, acne, and hidradenitis suppurativa in a patient with an FGFR2 missense mutation. *Front Med* 2017; **4**: 16.
11 Frew JW, Vekic DA, Woods J, Cains GD. A systematic review and critical evaluation of reported pathogenic sequence variants in hidradenitis suppurativa. *Br J Dermatol* 2017; **177**: 987–998.
12 Drmanac R, Sparks AB, Callow MJ *et al.* Human genome sequencing using unchained base reads on self-assembling DNA nanoarrays. *Science* 2010; **327**: 78–81.
13 Carnevali P, Baccash J, Halpern AL *et al.* Computational techniques for human genome resequencing using mated gapped reads. *J Comput Biol* 2012; **19**: 279–292.
14 Clancy T, Hovig E. Differential protein network analysis of the immune cell lineage. *Biomed Res Int* 2014; **2014**: 363408.
15 Robinette ML, Fuchs A, Cortez VS *et al.* Transcriptional programs define molecular characteristics of innate lymphoid cell classes and subsets. *Nat Immunol* 2015; **16**: 306–317.
16 Mostafavi S, Ortiz-Lopez A, Bogue MA *et al.* Variation and genetic control of gene expression in primary immunocytes across inbred mouse strains. *J Immunol* 2014; **193**: 4485–4496.
17 Jojic V, Shay T, Sylvia K *et al.* Identification of transcriptional regulators in the mouse immune system. *Nat Immunol* 2013; **14**: 633–643.
18 Lek M, Karczewski KJ, Minikel EV *et al.* Analysis of protein-coding genetic variation in 60,706 humans. *Nature* 2016; **536**: 285–291.
19 Birney E, Soranzo N. Human genomics: the end of the start for population sequencing. *Nature* 2015; **526**: 52–53.
20 Erikson GA, Bodian DL, Rueda M *et al.* Whole-genome sequencing of a healthy aging cohort. *Cell* 2016; **165**: 1002–1011.
21 Stenson PD, Ball EV, Mort M *et al.* The Human Gene Mutation Database (HGMD) and its exploitation in the fields of personalized genomics and molecular evolution. *Curr Protoc Bioinformatics* 2012; Chapter 1: **Unit1**: 13.
22 Canoui-Poitrine F, Revuz JE, Wolkenstein P *et al.* Clinical characteristics of a series of 302 French patients with hidradenitis suppurativa, with an analysis of factors associated with disease severity. *J Am Acad Dermatol* 2009; **61**: 51–57.
23 Schrader AM, Deckers IE, van der Zee HH, Boer J, Prens EP. Hidradenitis suppurativa: a retrospective study of 846 Dutch patients to identify factors associated with disease severity. *J Am Acad Dermatol* 2014; **71**: 460–467.
24 Xu H, Xiao X, Hui Y *et al.* Phenotype of 53 Chinese individuals with nicastrin gene mutations in association with familial hidradenitis suppurativa (acne inversa). *Br J Dermatol* 2016; **174**: 927–929.
25 Zouboulis CC, Desai N, Emtestam L *et al.* European S1 guideline for the treatment of hidradenitis suppurativa/acne inversa. *J Eur Acad Dermatol Venereol* 2015; **29**: 619–644.
26 van der Zee HH, Jemec GB. New insights into the diagnosis of hidradenitis suppurativa: clinical presentations and phenotypes. *J Am Acad Dermatol* 2015; **73**(5 Suppl 1): S23–S26.
27 Canoui-Poitrine F, Le Thuaut A, Revuz JE *et al.* Identification of three hidradenitis suppurativa phenotypes: latent class analysis of a cross-sectional study. *J Invest Dermatol* 2013; **133**: 1506–1511.
28 Zhang YW, Luo WJ, Wang H *et al.* Nicastrin is critical for stability and trafficking but not association of other presenilin/gamma-secretase components. *J Biol Chem* 2005; **280**: 17020–17026.
29 Bolduc DM, Montagna DR, Gu Y, Selkoe DJ, Wolfe MS. Nicastrin functions to sterically hinder gamma-secretase-substrate interactions driven by substrate transmembrane domain. *Proc Natl Acad Sci USA* 2016; **113**: E509–E518.
30 Haapasalo A, Kovacs DM. The many substrates of presenilin/gamma-secretase. *J Alzheimers Dis* 2011; **25**: 3–28.
31 Ratnamala U, Jhala D, Jain NK *et al.* Expanding the spectrum of gamma-secretase gene mutation-associated phenotypes: two novel mutations segregating with familial hidradenitis suppurativa (acne inversa) and acne conglobata. *Exp Dermatol* 2016; **25**: 314–316.
32 Pink AE, Simpson MA, Desai N, Trembath RC, Barker JN. gamma-Secretase mutations in hidradenitis suppurativa: new insights into disease pathogenesis. *J Invest Dermatol* 2013; **133**: 601–607.
33 Lu P, Bai XC, Ma D *et al.* Three-dimensional structure of human gamma-secretase. *Nature* 2014; **512**: 166–170.
34 Zhang X, Sisodia SS. Acne inversa caused by missense mutations in NCSTN is not fully compatible with impairments in Notch signaling. *J Invest Dermatol* 2015; **135**: 618–620.
35 Melnik BC, Plewig G. Impaired Notch signalling: the unifying mechanism explaining the pathogenesis of hidradenitis suppurativa (acne inversa). *Br J Dermatol* 2013; **168**: 876–878.
36 Takeichi T, Matsumoto T, Nomura T *et al.* A novel NCSTN missense mutation in the signal peptide domain causes hidradenitis suppurativa, which has features characteristic of an autoinflammatory keratinization disease. *Br J Dermatol* 2019; **182**: 491–493.
37 He Y, Li C, Xu H *et al.* AKT-dependent hyperproliferation of keratinocytes in familial hidradenitis suppurativa with NCSTN mutation: a potential role of defective miR-100-5p. *Br J Dermatol* 2019; **182**: 500–502.
38 Labrecque MP, Prefontaine GG, Beischlag TV. The aryl hydrocarbon receptor nuclear translocator (ARNT) family of proteins: transcriptional modifiers with multi-functional protein interfaces. *Curr Mol Med* 2013; **13**: 1047–1065.
39 Ikuta T, Ohba M, Zouboulis CC, Fujii-Kuriyama Y, Kawajiri K. B lymphocyte-induced maturation protein 1 is a novel target gene of aryl hydrocarbon receptor. *J Dermatol Sci* 2010; **58**: 211–216.
40 Blevins LK, Zhou J, Crawford R, Kaminski NE. TCDD-mediated suppression of naïve human B cell IgM secretion involves aryl hydrocarbon receptor-mediated reduction in STAT3 serine 727 phosphorylation and is restored by interferon-γ. *Cell Signal* 2020; **65**: 109447.
41 Byrd AS, Carmona-Rivera C, O'Neil LJ *et al.* Neutrophil extracellular traps, B cells, and type I interferons contribute to immune

© 2020 The Authors. Journal of the European Academy of Dermatology and Venereology published by John Wiley & Sons Ltd on behalf of European Academy of Dermatology and Venereology

dysregulation in hidradenitis suppurativa. *Sci Transl Med* 2019; **11**: eaav5908.
42 Borland MG, Krishnan P, Lee C *et al.* Modulation of aryl hydrocarbon receptor (AHR)-dependent signaling by peroxisome proliferator-activated receptor beta/delta (PPARbeta/delta) in keratinocytes. *Carcinogenesis* 2014; **35**: 1602–1612.
43 Mao-Qiang M, Fowler AJ, Schmuth M *et al.* Peroxisome-proliferator-activated receptor (PPAR)-gamma activation stimulates keratinocyte differentiation. *J Invest Dermatol* 2004; **123**: 305–312.
44 Kleiner S, Nguyen-Tran V, Bare O *et al.* PPAR{delta} agonism activates fatty acid oxidation via PGC-1{alpha} but does not increase mitochondrial gene expression and function. *J Biol Chem* 2009; **284**: 18624–18633.
45 Kavita U, Mizel SB. Differential sensitivity of interleukin-1 alpha and -beta precursor proteins to cleavage by calpain, a calcium-dependent protease. *J Biol Chem* 1995; **270**: 27758–27765.
46 Gross O, Yazdi AS, Thomas CJ *et al.* Inflammasome activators induce interleukin-1alpha secretion via distinct pathways with differential requirement for the protease function of caspase-1. *Immunity* 2012; **36**: 388–400.
47 Kanni T, Argyropoulou M, Spyridopoulos T *et al.* MABp1 targeting IL-1α for moderate to severe hidradenitis suppurativa not eligible for adalimumab: a randomized study. *J Invest Dermatol* 2018; **138**: 795–801.
48 Bowen AR, Hanks AN, Murphy KJ, Florell SR, Grossman D. Proliferation, apoptosis, and survivin expression in keratinocytic neoplasms and hyperplasias. *Am J Dermatopathol* 2004; **26**: 177.
49 Niu J, Azfer A, Zhelyabovska O, Fatma S, Kolattukudy PE. Monocyte chemotactic protein (MCP)-1 promotes angiogenesis via a novel transcription factor, MCP-1-induced protein (MCPIP). *J Biol Chem* 2008; **283**: 14542–14551.
50 Chen Y-J, Hsieh M-Y, Chang MY *et al.* Eps8 protein facilitates phagocytosis by increasing TLR4-MyD88 protein interaction in lipopolysaccharide-stimulated macrophages. *J Biol Chem* 2012; **287**: 18806–18819.
51 Philippakis AA, Azzariti DR, Beltran S *et al.* The Matchmaker Exchange: a platform for rare disease gene discovery. *Hum Mutat* 2015; **36**: 915–921.
52 Horváth B, Janse IC, Blok JL *et al.* Hurley staging refined: a proposal by the Dutch Hidradenitis Suppurativa Expert Group. *Acta Derm Venereol* 2017; **97**: 412–413.
53 den Dunnen JT, Dalgleish R, Maglott DR *et al.* HGVS recommendations for the description of sequence variants: 2016 update. *Hum Mutat* 2016; **37**: 564–569.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1. A) γ-secretase mutations found in familial HS, updated on the 15th of August 2019. (B) γ-secretase mutations described in individual (sporadic) cases of HS.

Figure S1. Phenotype of HS2C with (1) right axilla including scars and an inflammatory nodule (2) surgical scar after excision of cyst (3) groins and pubic area displaying Hurley IIB with moderate inflammation.

Figure S2. Phenotype of HS3B with (1) papules in the nape, arrow indicating an auricular cyst (2) no disease activity in the axillae (3) diffuse pattern of folliculitis in the gluteal area.

Figure S3. DNA sequence of the mutant allele (c.1912_1915delCAGT) and wildtype NCSTN. The CAGT bases are indicated in the black box.

Figure S4. Wildtype NCSTN expression in hematopoietic cell lineages display a myeloid signature with upregulation (red color) in monocytes and macrophages. The colour intensity correlates with the degree of change.

Figure S5. Mesenchymal cell lineages with wildtype NCSTN RNA expression show upregulation of stromal cells and typically FRC's and fibroblasts. BEC: blood endothelial cell. LEC: lymphatic endothelial cell. FRC: fibroblastic reticular cell. The colour intensity correlates with the degree of RNA change.

© 2020 The Authors. Journal of the European Academy of Dermatology and Venereology published by John Wiley & Sons Ltd on behalf of European Academy of Dermatology and Venereology