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

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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-fl exural 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.
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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,
mechanical friction and hormones, whereas endogenous factors comprise female gender, an aberrant immunity and genetic predisposition. 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. However, a familial presentation 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 $\gamma$-secretase subunits, which consists of presenilin ($PSEN1$ [MIM: 142690] 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]). To date, HS-related mutations of the $\gamma$-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. 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. 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). 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.

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 leukocytes.

**Whole-genome sequencing and validation analysis**

Whole-genome sequencing was performed using the BGI’s Complete Genomics platform as previously published. 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. 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 leukocytes 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) 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. GEO sample (GSM) data were log2 transformed and signatures for 16 lineages, both hematopoietic and mesenchymal, were subsequently calculated. Cut-off values of $\leq -0.5$ and $\geq 0.5$ were applied on the Log2 scores in order to...
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 < 5). 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.

**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 (EV),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], POTE [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

**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           | past [36]      | positive [26] | negative |
| Acne (history of)        | yes            | no   | no   |
| Diabetes mellitus (history of) | no           | yes  | no   |
| Hypertension (history of) | no             | yes  | no   |
| Abnormal lipid profile (history of) | no        | no   | no   |
| Body Mass Index (kg/m²)  | 24.6           | 24.1 | 27.5 |
| HS-PGA (0–5)             | 5              | 3    | 2    |
| Modified Sartorius Score | 191            | 19   | 10   |
| Refined Hurley stage (°) | III            | IIIB | 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.

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**Notes:**

1. Alzheimer’s disease within the pedigree was only reported in the unaffected father of the proband.
2. The variant NCSTN c.1912_1915delCAGT had the highest CADD score (35).
3. None of the other six gene mutations could be directly linked to HS pathophysiology.
4. The HGMD database licensed through Qiagen/Biobase confirmed the previously identified mutations at the NCSTN locus.
mutation in NCSTN to be causative in and associated with familial HS. 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).

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.

| # | 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 | 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_002582:c.233T>A | p.I78K | Missense 27.8 | Deleterious |

* CADD v1.3: Combined Annotation Dependent Depletion
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 \( \beta \)-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 \( \beta \)-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 \( \beta \)-secretase complex (Table S1A,B).28 Most mutations in NCSTN cause a frameshift and premature translation stop, potentially leading to impaired activity of the \( \gamma \)-secretase complex. NCSTN acts to sterically block substrates with large ectodomain, providing the mechanism by which \( \beta \)-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 \( \gamma \)-secretase complex, and
the amyloid precursor protein (APP) and the Notch receptor are two of the most widely known and studied substrates. 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. However, proteomic and functional studies investigating the impact of NCSTN mutations on Notch signalling in HS are limited and have shown contradictory results. 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.

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). 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. 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
catabolism through beta-oxidation, facilitates AhR signalling and induces keratinocyte differentiation.\textsuperscript{42–44} Lastly, we identified \textit{CAPNS1}, also known as \textit{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-1\(\alpha\) into the biologically active mature form, an important pro-inflammatory cytokine in epithelial and myeloid cells.\textsuperscript{45,46} The importance of IL-1\(\alpha\) was illustrated in one study, investigating a human antibody targeting IL-1\(\alpha\), which showed promising results in moderate to severe patients with HS after 12 weeks of treatment.\textsuperscript{47}

In addition to \textit{NCSTN}, we found six other gene mutations, none of which could be directly linked to HS pathophysiology. However, the proteins encoded by \textit{PCNA}, \textit{CDH19}, and \textit{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.\textsuperscript{48–50}

A combination of careful delineation of the familial phenotype, together with functional analysis of \textit{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 \(\gamma\)-secretase mutations.\textsuperscript{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 \((\gamma\)-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 \textit{NCSTN} gene splice site mutation, exon 16: c.1912\_1915\textit{delCAGT}, in a three-generation Caucasian family. Our explorative analysis demonstrates that the \(\gamma\)-secretase component \textit{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 \textit{NCSTN}, and it is co-expressed genes \textit{ARNT} and \textit{PPARD} link genetics to well-known environmental and metabolic triggers (smoking and obesity), both associated with HS and disease severity.

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**Declaration of Interests**

The authors declare no competing interests.

**Accession numbers**

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

refseq Gene ID: NG_027935
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Supporting information

Additional Supporting Information may be found in the online version of this article:
Table S1. A) alpha-secretase mutations found in familial HS, updated on the 15th of August 2019. (B) alpha-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_1915del-CAGT) 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.