Supplemental Online Content

Mintoff D, Pace NP, Borg I. *NCSTN* in-frame deletions in Maltese patients with hidradenitis suppurativa. *JAMA Dermatol*. Published online July 26, 2023. doi:10.1001/jamadermatol.2023.2227

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This supplemental material has been provided by the authors to give readers additional information about their work.

eMethods

DNA extraction and PCR

Genomic DNA was isolated from peripheral blood leukocytes using a QIAmp® DNA Blood Mini Kit (Qiagen Inc, CA, USA) according to the manufacturer's instructions. DNA concentrations were measured using a NanoDrop® 2000 Spectrophotometer (Thermo Scientific, USA) and concentration adjusted to 40 ng/µL.

A 290 bp region flanking the variant of interest within exon 6 of *NCSTN* was amplified using the following primers designed using Primer3[1] (forward: CAG CAC CAA CCT TCC CAC TAT, reverse: CCC TCT GCC CCT TAT TCC AT). PCR reactions were set up in 50µL volumes, containing 40ng of genomic DNA, 10µM of each forward and reverse primer, 25µL of PCRBIO TaqMix® Red (PCR Biosystems, USA) and 22µL of molecular biology grade water. Amplification was performed using the following cycling conditions: 95°C for 5 minutes, followed by 30 cycles of 30 seconds at 95°C, 40 seconds at 64°C, 40 seconds at 72 °C then at 72°C for 10 minutes. Following PCR amplification and size verification by 2% agarose gel electrophoresis, amplicons underwent spin column purification with a MinElute Reaction Cleanup Kit (Qiagen) and bi-directional Sanger sequencing using a dye terminator cycle sequencing kit (ABI PRISM Big Dye Terminator v2.0) and an ABI 3100 sequencer (Applied Biosystems).

Targeted variant analysis RFLP and Sanger Sequencing

We screened the HS population for the *NCSTN* in frame deletion of interest by using the restriction enzyme BtsI v2 (New England BioLabs® Inc). The wild-type genotype is indicated by the presence of two fragments at 187 and 103bp. The deletion abolishes the restriction site for BtsI-v2, creating an undigested 290bp band.

The RFLP reaction was set up according to the manufacturer's recommendations using 5 Units of restriction enzyme and incubated at 37 degrees Celsius for 30 minutes. RFLP products were subsequently subjected to electrophoretic separation on 2% agarose till the restriction fragments could be adequately resolved to enable genotype calls to be made.

Variant genotypes detected by RFLP were confirmed by Sanger sequencing. Internal positive and negative controls were included with each assay. Representative results are shown in **eFigure 1 & 2** in the **Supplement.**

Controls

Briefly, exon enrichment for next generation sequencing was performed using the SureSelectXT All Exon V6 capture design (Agilent Technologies). Each individual exomic library was prepared from 300ng of DNA according to the manufacturer's protocols. The resulting libraries were sequenced in 150 bp paired-end reads on a NovaSeq®6000 sequencer (Illumina®, Inc.).

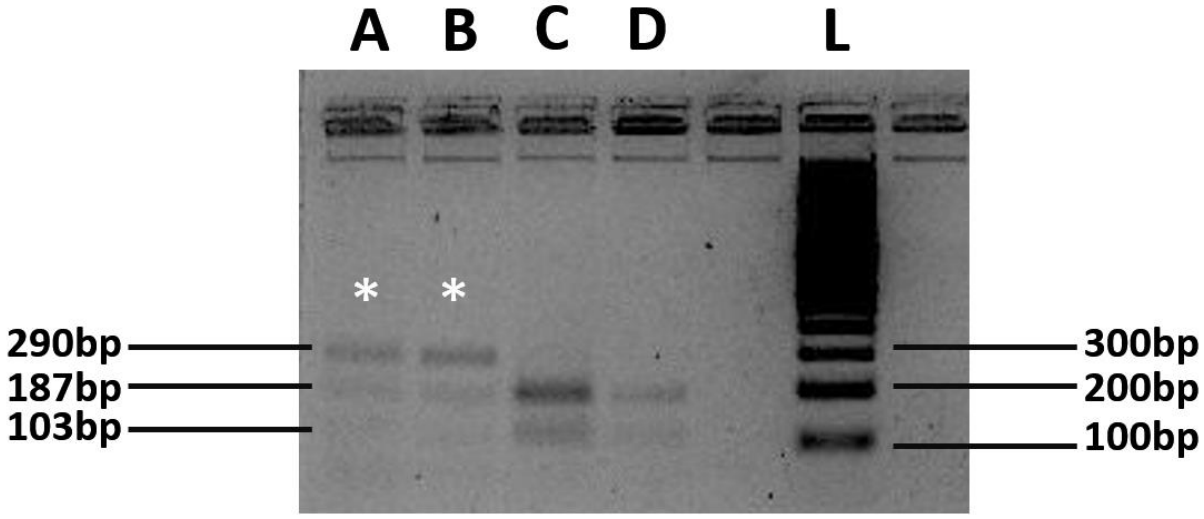
We used VarAft to explore on-target *NCSTN* sequencing depth and *NCSTN* exon 6 depth in the control reference population. Summary sequencing metrics are outlined in the table below.

	Mean depth	SD Depth	Coverage 20X	Coverage 30X
***NCSTN* NM_015331**	71.11	13.29	100	98.34
NCSTN exon 6	68.33	10.3	100	97.63

eTable 1: *Summary Sequencing metrics for NCSTN NM_015331 and NCSTN exon 6 in the control population*

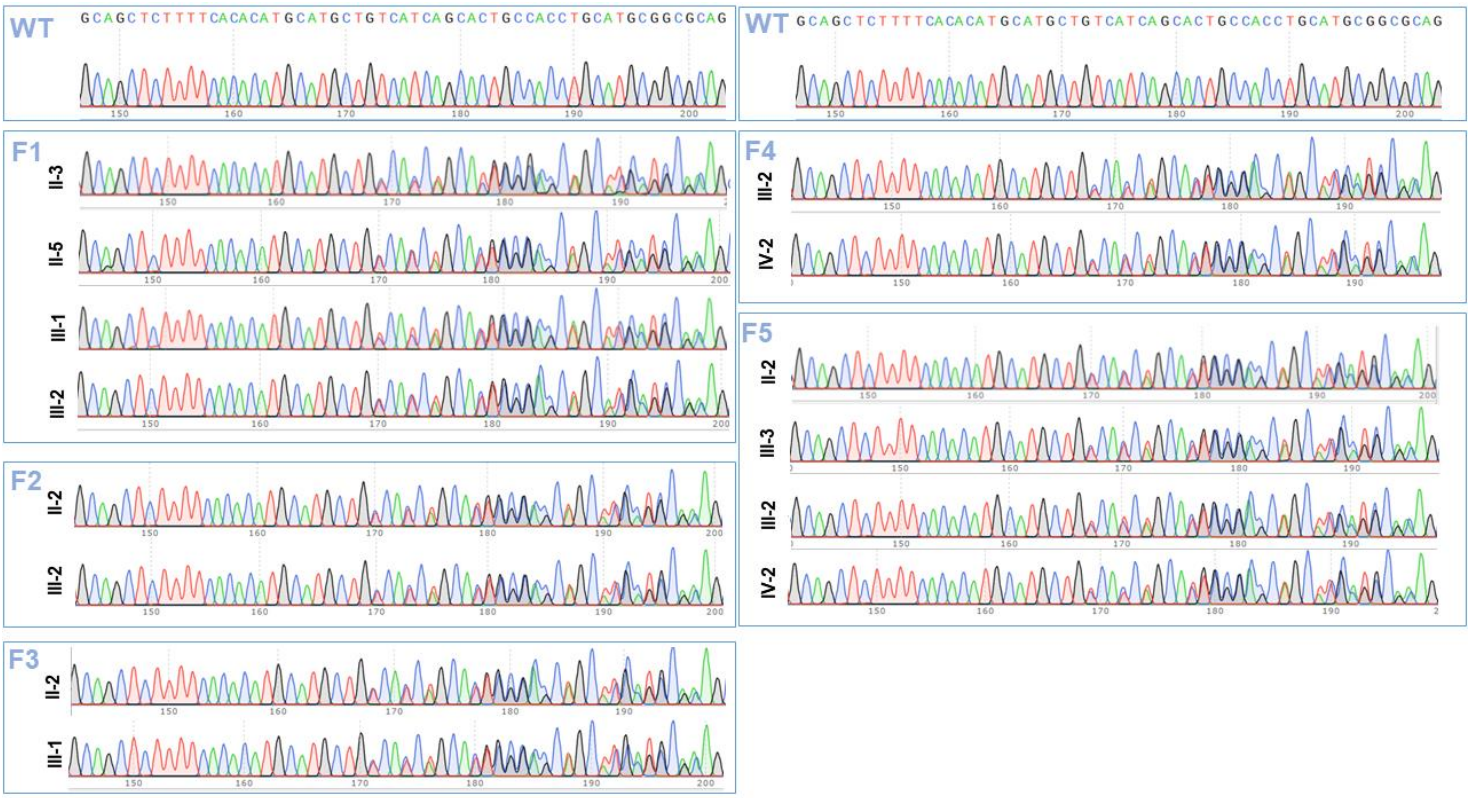
Assessing evolutionary conservation analysis

Multiple species amino acid sequence alignment of the NCSTN protein was performed using Clustal Omega (European Bioinformatics Institute, EMBL-EBI; http://www.ebi.ac.uk/Tools/msa/clustalo/). The following Uniprot identifiers were used: Q92542 (Human – *Homo sapiens*), A0A8D1l1Z1 (Pig – *Sus scrofa),* A0A2R9ATE9 (Pygmy chimpanzee – *Pan paniscus*) M1VPL1 (Dog – *Canis familiaris*), P57716 (Mouse – *Mus musculus*), Q5ZJH2 (Chicken - *Gallus gallus*). Sequence alignment quality was interpreted according to Blocks Substitution Matrix (BLOSUM62) score[2] and the results were visualized using Jalview 2.0.[3] The MetaDome webserver (https://stuart.radboudumc.nl/metadome/) was used to evaluate the regional mutation tolerance at each position in NCSTN protein.[4]



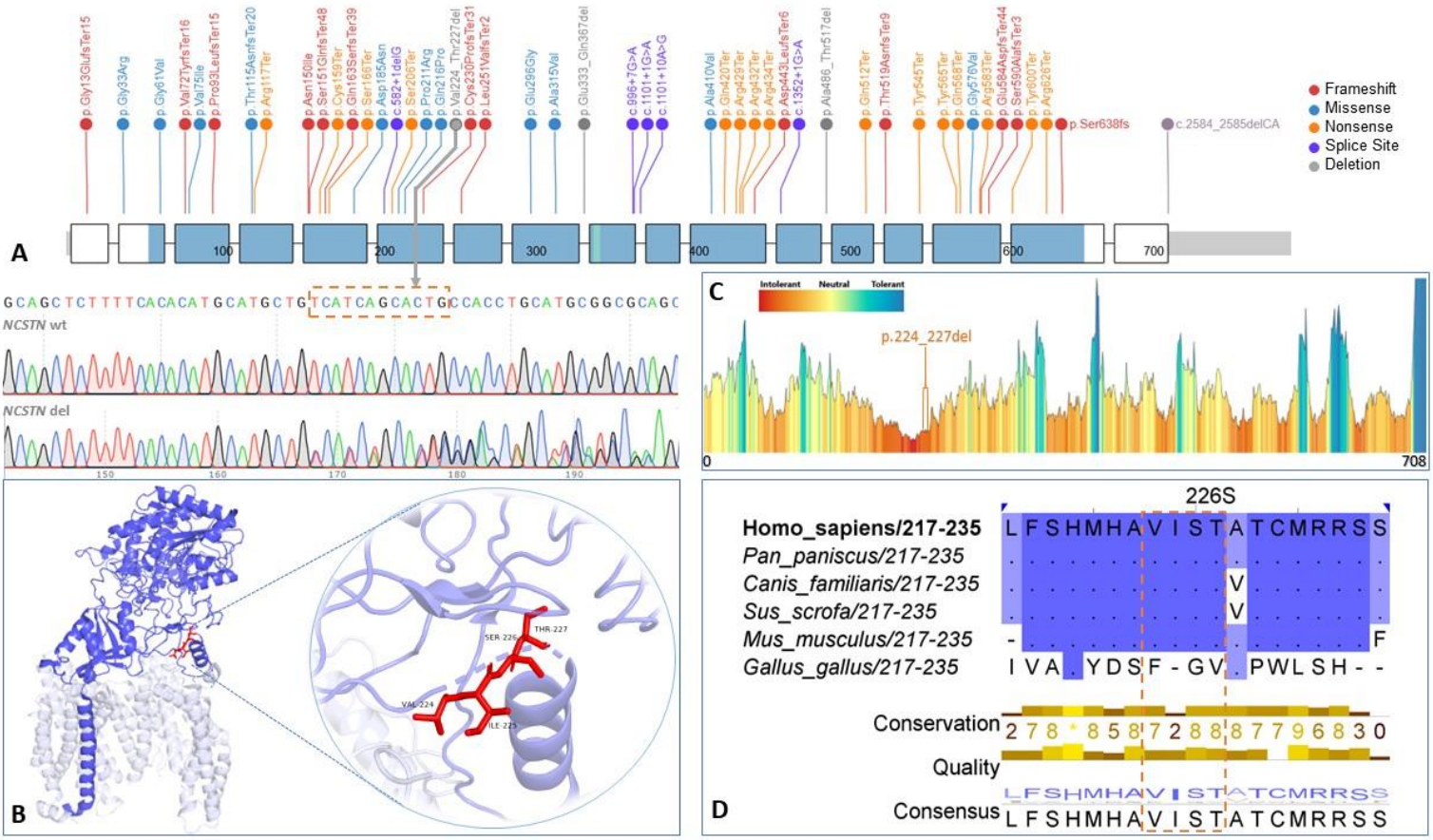
eFigure1: Representative RFLP Results.

Lanes ‘A’ to ‘D’ represent individual HS patients, Lane ‘L’ represents a 100bp ladder DNA marker. The arrows indicate the three restriction fragment bands at 290, 187 and 103bp. Lanes ‘A’ and ‘B’ (*) signifying loss of the restriction site secondary to deletion in the heterozygous state. Lanes ‘C’ and ‘D’ show the two bands (at 187bp and 103bp). The deletion was not identified in these two patients.



eFigure 2: Sanger Traces

Sanger traces of patients heterozygous for the *NCSTN*:c.671_682del in Families (F) 1-5. Trace for wild type (WT) *NCSTN* are shown for reference.



eFigure 3: The *NCSTN* (NM_015331.3):c.671_682delTCATCAGCACTG variant.

(A) The spectrum of HS-associated *NCSTN* variants reported to date. The deletion described in this report is shown in the grey arrow. Sanger traces showing the control sequence (top trace) and the deletion (bottom trace). The superimposed traces represent chromatogram shift due to the heterozygous deletion displayed in the bottom trace but not in the control sequence. (B) The deletion results in loss of the four amino acids Val224, Ile225, Ser226, Thr227 in the extracellular domain of *NCSTN*. This variant lies in a region which is intolerant to variation (C) and which is highly conserved (D).

Cohort	% of unrelated probands with pathogenic/LP/VUS *NCSTN* variation	*NCSTN* variant(s)	Ethnic background	Reference
Syndromic HS	20% (2/10)	c.1140_1141del (p.Asp381SerfsTer7) c.482delA (p.Ile162TyrfsTer7)	Italian	Marzano et al [5]
Stand alone HS	0.03% (1/31)	c.38delG (p.Glu13GlufsTer15)	German	Vural et al[6]
Stand alone HS	0.03% (2/59)	c.436+129A>G c.582+1G>A	Singaporean Chinese	Shen et al[7]
Stand alone HS	0 (0/11)		Danish	Theut Riis et al[8]
Stand alone HS, syndromic (8%) and Collision disease (3%)	0.01% (2/169)	c.182G>T (p.Gly61Val) c.1727G>T (p.Gly576Val)	European (63%), African (14%), Asian (2%)	Duchatelet et al[9]
Stand alone HS	0.02% (2/95)	c.349C>T (p.Arg117Ter)	European	Liu et al[10]
Stand alone HS	0/20		South Wales	Ingram et al[11]
Stand alone HS	0.04% (2/48)	c.553G>A (p.Asp185Asn) c.996+7G>A	Mixed ethnicity	Pink et al[12]

eTable2: A summary of studies investigating the prevalence of GSC variation in cohorts.

eReferences ;

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