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Novel nicastrin mutation in hidradenitis suppurativa-Dowling Degos disease clinical phenotype: more than just clinical overlap?

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In familial hidradenitis suppurativa (HS), mutations in the genes encoding three subunits of the γ-secretase complex, *PSEN1*, *PSENEN* and *NCSTN*, have pointed to an impaired NOTCH signalling as a pathogenic disease mechanism.[1]

Dowling Degos Disease (DDD; MIM 179850, 615327, and 615696)—a rare reticulated pigmentary disorder— has also been associated with a deficient NOTCH signalling and patients with mutations in *PSENEN* suffering from both disorders seem to confirm a potential link between two apparently different conditions.[2-3] However, why mutations in *PSENEN* cause DDD, HS or the combined HS-DDD phenotype, and why mutations in *NCSTN* have never been associated to DDD is not yet understood.

In this study, we describe a patient with familial HS and concomitant DDD harbouring—a novel nonsense mutation in *NCSTN* associated with a reduced quantity of critical subunits of the γ-secretase.

A 54-year-old male patient, with a diagnosis of HS and Hurley II stage disease, was examined for recurrent inflammatory lesions affecting the inguinal and genital regions. He reported a HS family history (father and daughter). In addition, physical examination showed a typical reticulated flexural pigmentation, localized on the scrotum and inguinal crease. Histopathological examination of lesional skin confirmed classic DDD diagnosis (Figure 1a). Onset of HS lesions was reported at the age of 25 years, while first signs of flexural hyperpigmentation were reported at the age of 40 years. The patient never smoked, had a normal Body Mass Index (BMI) and no other comorbidities.

Patient's DNA was extracted from saliva. We sequenced, with the Sanger method, using exon flanking intronic primers, all coding regions of genes associated to DDD: *KRT5* (9 exons), *POGLUT1* (11 exons), *POFUT1* (7 exons) and *PSENEN* (4 exons). We identified only two missense single–nucleotide variation in *KRT5*, both reported as benign in ClinVar.

Since the proband suffered from HS, we sequenced the coding region of *NCSTN* (17 exons): a single–nucleotide variation in *NCSTN* exon 15 (NM_015331.2:c.1747C>T), coding for a premature stop codon (NP_056146.1:p.(R583*)), was detected (Figure 1c). This genetic variant was not present in the Genome Aggregation Database (gnomAD) v.2.1.1. Subsequently, we sequenced *NCSTN* from his 30-year old HS-affected daughter (Figure 1b) and his clinically healthy son, observing only in the daughter a co-segregation of the *NCSTN* heterozygous single–nucleotide variation (Figure 1c).

Interestingly, physical examination of the patient's daughter did not reveal any signs of DDD disease.

As the substitution encoded for a premature stop codon, we wondered whether the mutation could cause haploinsufficiency following nonsense-mediated RNA decay (NMD) of *NCSTN*. To confirm this, we compared NCSTN expression by outer root sheath (ORS) cells isolated from the patient's plucked hairs and those of the healthy son.

It has been reported that NCSTN is dispensable for NOTCH receptors processing by γ-secretase, so we questioned whether NCSTN haploinsufficiency could lead to γ-secretase complex instability and degradation of its subunits. To verify this, we studied the PEN2, PSEN1 (N-ter and C-ter fragments), and PSEN2 expression in ORS cells extracts. After normalization with Beta-Actin, we observed that the quantity of these subunits was significantly diminished in ORS cells from our patient compared with the healthy son ($p<0.05$; unpaired t-test; Figure 1d).

The combined HS-DDD phenotype has been reported in different families and case-series. Their *PSENEN* mutations have been associated with HS, HS/DDD or isolated DDD.[2-4] Most of the pathogenic variants in *PSENEN* are nonsense or frameshift mutations causing an haploinsufficiency of PEN2 either by its proteasomal degradation or by NMD.[2]

We further expanded the spectrum of the combined HS/DDD phenotype, reporting the novel role of *NCSTN* and suggesting a putative link between auto-inflammatory and pigmentary disorders. Interestingly, the *NCSTN* mutation of our patient affected PEN2 quantity, mimicking its haploinsufficiency. Preliminary experimental evidence suggests a role of NCSTN deficiency in pigmentary disorders, by modulating melanosome degradation.[5]

In autoinflammatory conditions, such as HS, NCSTN, haploinsufficiency seems to stimulate the proliferation, type I-interferon gene expression and TNF-alpha induced inflammatory response of keratinocytes.[6] The fact that our patient has managed to control skin inflammation avoiding well-known risk-factors could have helped to detect the DD phenotype, which could arise late in life.[7] The affected daughter did not present any clinical signs of DDD yet, which might develop at a later age, as observed in her father. The clinical and genetic overlap of HS-DDD may also have a clinical relevance, translating in a personalized therapeutic management, such as the combination of retinoids and sulphones.[8]

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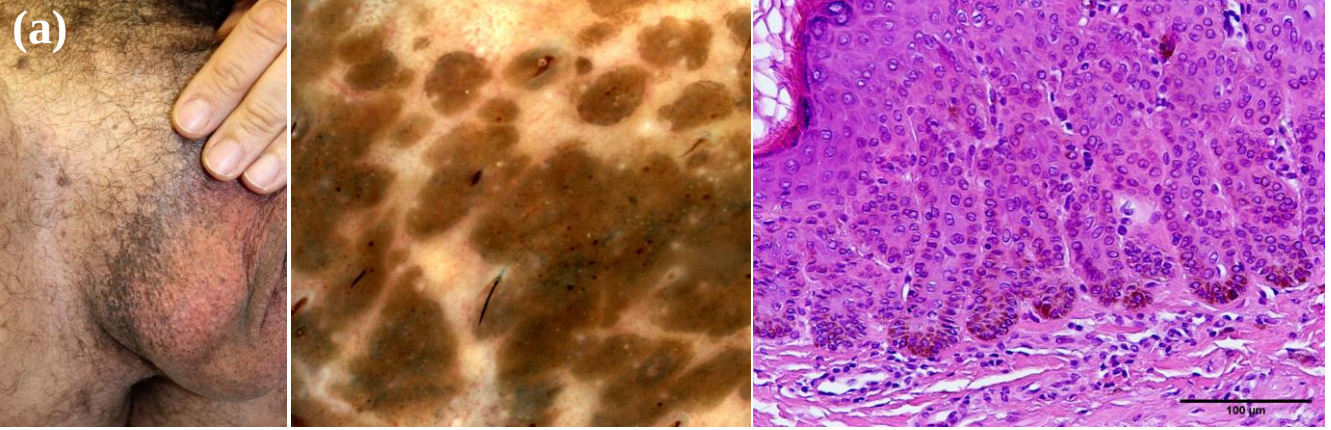
Figure legends:

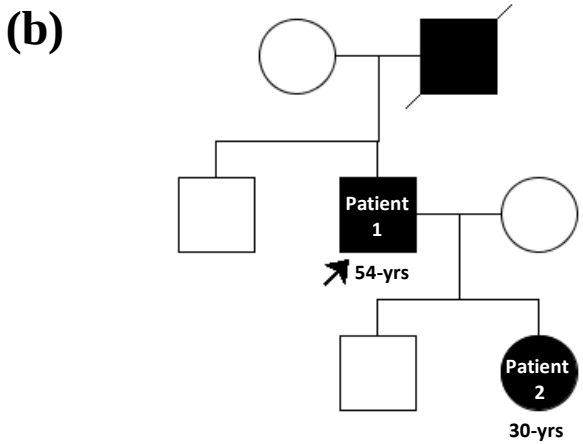
1a. Dowling Degos Disease: flexural reticulate hyperpigmentation with representative dermoscopy and histology

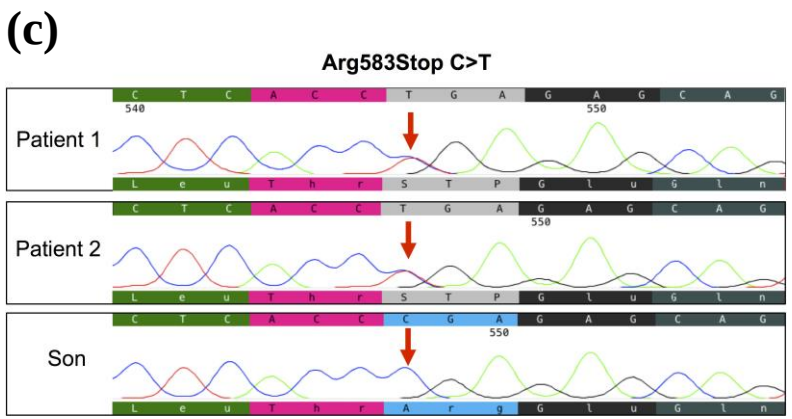
1b. Family pedigree of the proband, HS individuals are labelled in bold.

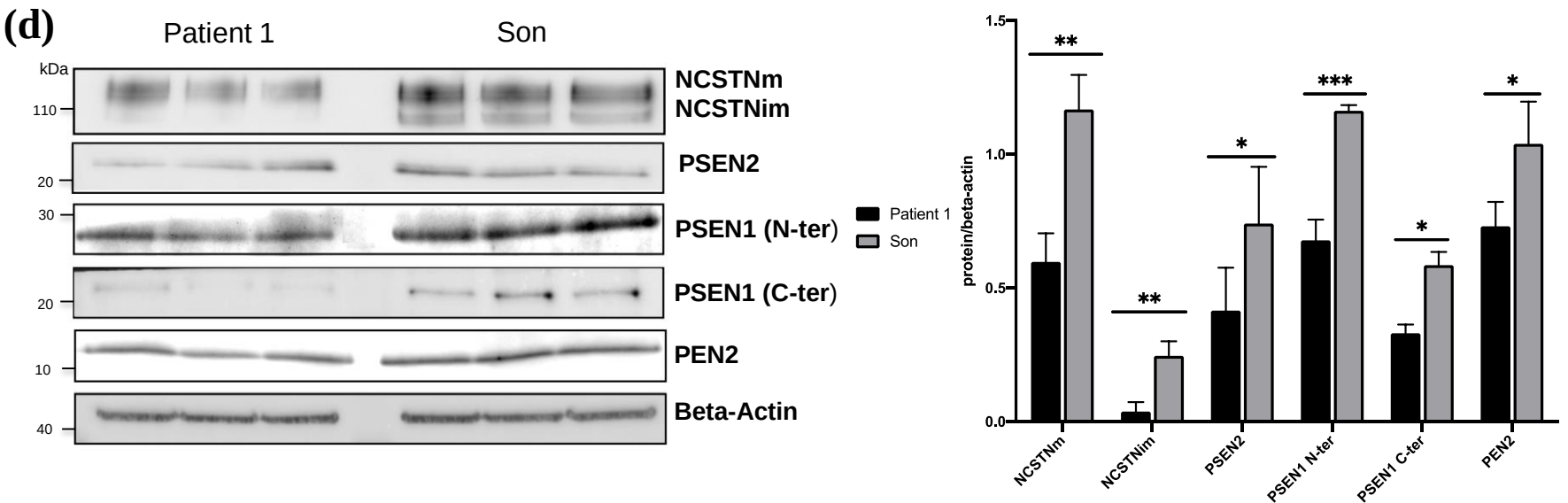
1c. Chromatograms of the heterozygous single–nucleotide variation in *NCSTN* exon 15 coding for a premature stop codon identified in the proband (Patient 1) and HS-affected daughter (Patient 2), absent in the clinically healthy son.

1d. WB analysis of 3 independent cultures of ORS cells isolated from the proband suffering from HS and DD and his healthy son. Passage 1 ORS cells were seeded in a 6-well plate, grown to confluency and proteins extracted in RIPA buffer. γ-secretase proteins expression was studied with antibodies against NCSTN (clone 716910), PEN2 (D6G8), PSEN2 (D30G3) and PSEN1 N-ter (E3L9X) and C-ter (D39D1) . Beta-actin was used as loading control. Results from the three experiences were pulled and their distribution compared with the unpaired t-test (* : $p<0.05$; ** : $p<0.01$; *** $p<0.001$)


(a)
100 µm


(b)
Patient 1
54-yrs
Patient 2
30-yrs


(c)
Arg583Stop C>T
Patient 1
Patient 2
Son
540
550


(d)
Patient 1
Son
kDa
110
20
30
20
10
40
NCSTNm
NCSTNim
PSEN2
PSEN1 (N-ter)
PSEN1 (C-ter)
PEN2
Beta-Actin
Patient 1
Son
protein/beta-actin
1.5
1.0
0.5
0.0
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*
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NCSTNm
NCSTNim
PSEN2
PSEN1 N-ter
PSEN1 C-ter
PEN2