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CASE REPORT

Novel Mutation of the *NCSTN* Gene Identified in a Chinese Acne Inversa Family

Jing Wu[1,2,*], Huiyao Ge[1,2,*], Yiming Fan[4,*], Qi Zhen[1,2], Lili Tang[1,2], Liangdan Sun[1,2,3]

[1]*Department of Dermatology of First Affiliated Hospital, Anhui Medical University,* [2]*Key Laboratory of Dermatology, Ministry of Education, Anhui Medical University,* [3]*Anhui Province Key Laboratory of Major Autoimmune Disease, Hefei,* [4]*Department of Dermatology, Affiliated Hospital of Guangdong Medical University, Zhanjiang, China*

Acne inversa is a chronic inflammatory follicular disease with autosomal dominant inheritance. In recent years, many functional mutations in the *NCSTN* genes have been identified as the cause of familial acne inversa. Herein, we recruited four patients and seven unaffected individuals from a Chinese family and performed Sanger sequencing of the *NCSTN* gene. One novel frameshift mutation, c.450_459del (p.Ser 151GlnfsX48), was identified in exon 5 of the *NCSTN* gene. Three normal-looking children carrying the mutation were proven to be patients. We also presented a literature review from previous studies of acne inversa, suggesting that *NCSTN* is a hotspot gene for acne inversa. Most affected individuals experienced onset in adolescence. We confirmed the diagnosis in this family based on the mutation. This finding will help expound the relationship between the *NCSTN* gene and the pathogenesis of acne inversa and emphasize the value of genetic diagnosis in monogenic disorder. **(Ann Dermatol 32(3) 237～242, 2020)**

-Keywords-
Chinese patients, Hidradenitis suppurativa, Mutation analysis, *NCSTN* gene

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*These authors have equally contributed to the article.

Corresponding author: Liangdan Sun, Department of Dermatology of First Affiliated Hospital, Anhui Medical University, No. 81 Meishan Road, Shushan District, Hefei 230032, China. Tel: 86-551-62923102, Fax: 86-551-65138576, E-mail: ahmusld@163.com
ORCID: https://orcid.org/0000-0002-8499-5353

INTRODUCTION

Acne inversa (AI; Online Mendelian Inheritance in Man [OMIM] #142690), also called hidradenitis suppurativa (HS), remains a challenging disease for both patients and clinicians. AI is an autosomal dominant monogenic disorder with genetic heterogeneity, usually presenting after puberty on the apocrine gland-bearing areas of the body[1]. The average onset of AI is in the early 20s, and substantial data suggest a female-predominance with a 3:1 sex ratio[2]. AI commonly occurs in the armpit and inguinal folds as well as near the genitalia, the inframammary skin and buttocks[3]. Early skin lesions take the form of acne, papules, and nodules, followed by the formation of cysts, abscesses, and sinuses, causing abnormal pus to flow out and scars to form. The pathogenesis of AI is not fully understood, although it is a multifaceted disease triggered by the follicular occlusions, hereditary components, immune dysregulation and other factors[3,4]. AI patients are at significantly increased risk for various autoimmune diseases and metabolic syndromes, consisting of rheumatoid arthritis, inflammatory bowel disease, psoriasis, systemic lupus erythematous, diabetes, hypertension, and pilonidal cysts[5,6]. Depending upon the severity of the disease, AI patients are treated with different methods, such as antibiotics, immunosuppressives, biological agents, antidiabetics, glucocorticoids, retinoids, laser therapy and surgery.

So far, three different genes have been identified in AI patients, including *NCSTN*, *PSENEN*, and *PSEN1*[7]. Genetic inactivation of these genes in mouse skin produces epidermal and follicular abnormalities that are histopathologically similar to those observed in human AI[8]. After this dis-

covery, loss-of-function mutations in these genes were identified in AI families and AI sporadic individuals, and the most common of these is mutation of the *NCSTN* gene[9]. The diagnosis of AI is mainly based on the clinical features. However, with the development of the sequencing technology, sequencing has been used as a high-efficiency tool to make an early diagnosis due to the particular advantage in finding mutations of rare diseases. To confirm the value of genetic diagnosis and further investigate the genetic mechanisms underlying AI, we conducted a mutation analysis of *NCSTN* in a Chinese family with AI.

CASE REPORT

Patients and controls

A family from Guangdong Province of China were recruited (Fig. 1A). The proband was a 27-year-old male who presented inflammatory papules, painful nodules, sinus tracts and atrophic scars especially on his armpit, back and buttocks over 11 years (Fig. 1B, C). Histopathologic results of the proband showed hyperkeratinization of hair follicles and structural destruction of hair follicles, extensive infiltration of neutrophils and lymphoid cells, which further confirmed the reliability of the diagnosis (Fig. 2A, B). His father was a 64-year-old male who had exhibited similar skin lesions on his back and buttocks for more than 50 years, showing widespread skin abscesses, disfiguring scars and post-inflammatory hyperpigmentation (Fig. 1D, E). The age of onset of 4 patients was 14 years old (I1), 15 years old (II6), 12 years old (II8), and 16 years old (II10). Thus far, the third generation (III1, III2, III3, III4, III5) in this family has no clinical phenotype. There was no autoimmune or metabolic disease associated with this AI family. Written informed consents were obtained from 11 members of this family. This study was approved by the ethics committee of Anhui Medical University (IRB No. 20150051) and was managed in accordance with Declaration of Helsinki principles. We received the patient's consent form about publishing all photographic materials.

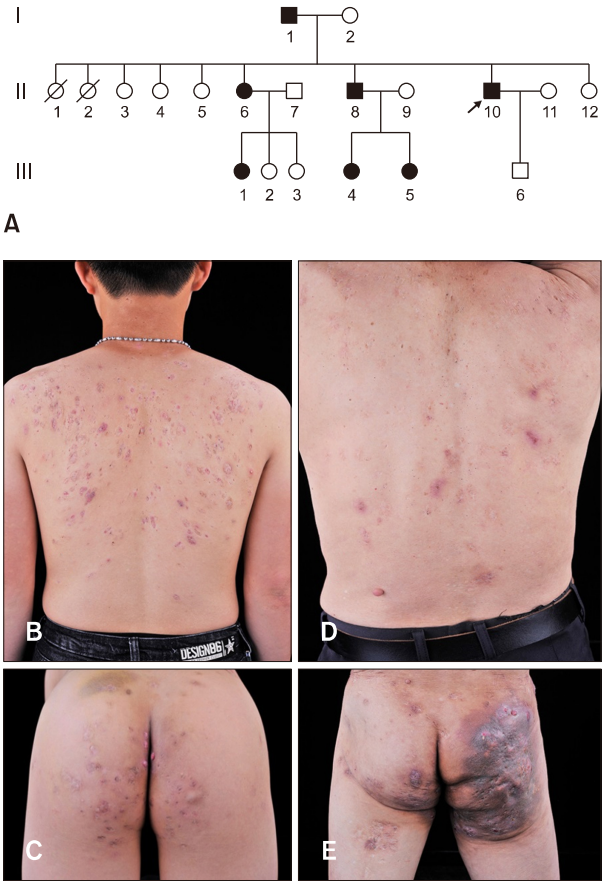

Fig. 1. (A) Genealogical tree of the acne inversa family. The arrow in the pedigree refers to the proband. (B, C) Inflammatory papules, painful nodules, sinus tracts and atrophic scarring are distributed on the back and buttocks of the proband. (D, E) The father of the proband showed widespread sinus tracts, inflamed cysts, skin abscesses, disfiguring scars and post-inflammatory hyperpigmentation on his back and buttocks.

Mutation sequencing and analysis

DNA was extracted from 2 ml venous blood using the QIAamp DNA blood mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. DNA samples were amplified using a polymerase chain reaction (PCR) with specially designed primer pairs covering all exons and exon-introns of *NCSTN* (Table 1). The DNA was amplified by cycling at 95°C for 15 minutes; 11 cycles of 94°C for 15 seconds, 62°C to 0.5°C per cycle for 40 seconds, 72°C for 1 minute; 24 cycles of 94°C for 15 seconds, 57°C for 30 seconds, 72°C for 1 minute; 72°C for 2 minutes. Primers were designed using primer3 (http://bioinfo.ut.ee/primer3-0.4.0/; HHMI, San Francisco, CA, USA). Direct DNA sequencing of *NCSTN* was performed using an ABI3730XL genetic analyzer (Thermo Fisher Scientific, Waltham, MA, USA) and the results were analyzed by the Polyphred software (UW, Seattle, WA, USA) and revised manually.

NCSTN mutation identification and analysis

Four patients (I1, II6, II8, II10), two controls (I2, II9), five children without clinical phenotype (III1, III2, III3, III4, III5) were sequenced using Sanger sequencing (Applied Biosystems, Foster City, CA, USA) (Fig. 2C, D). A novel frameshift mutation, c.450_459del (p.Ser151GlnfsX48), in exon 5 of *NCSTN* (NM_015331.2) existed in all the affected individuals (I1, II6, II8, II10) and three children with normal phenotype (III1, III4, III5). Notably, this mutation was absent in other two unaffected adults (I2, II9) and two chil-

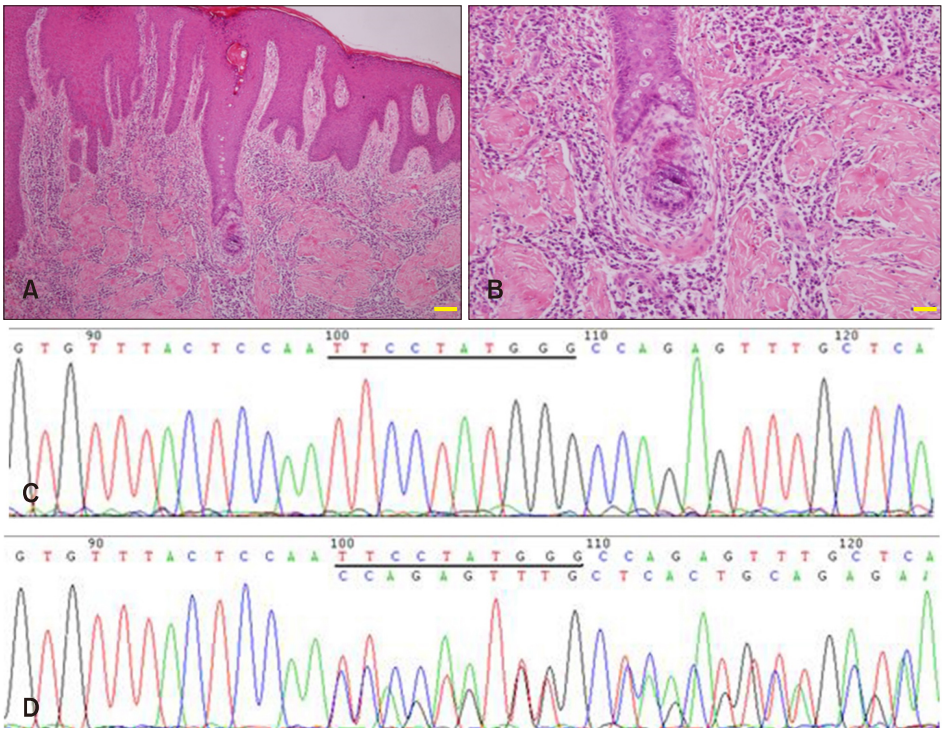

Fig. 2. (A, B) Skin biopsy from the proband showed hyperkeratinization and structural destruction of hair follicles, extensive infiltration of neutrophils and lymphoid cells (A, H&E, ×40; B, H&E, ×100). (C, D) Sanger sequencing confirmed a novel frameshift mutation in the *NCSTN* gene (C, Control; D, Patient, c.450_459del and p.Ser151GlnfsX48).

Table 1. *NCSTN* gene exon polymerase chain reaction amplification primer sequence information

Exon	Forward primer	Reverse primer	Product length
1	GCGCTCTTGGTCTCGAATTT	CTTTGCCTGGACCTGAAGGT	516
2-3	TGATCACCTGTGCAACCA	GTCCTGCTTAGGAGATTTGACA	520
4	TTGTGACATCTTTAGGGGATAAAA	CTGGTCTGGTCAGGGGAGAG	340
5	CCTCCCTGGGGTCCTTACTC	TTCTTTGAGTCACCACCTCCT	653
6	TCACCACCTCCTTTTGAACTCTT	CTTTCCCCACATTTTGCTCC	611
7	AGTCTGCAACCCTTTGTAACTC	TTTCCAATGTTGCCTTTATAGC	636
8-9	GGCAGCTTGTTCCTAAAGTGG	TGACCTAAGTTGTTAACCAGCACA	795
10-11	AGCTGATGTTCATCTTAGACCTTT	GGTGTTGGCCAAAGGATGA	752
12	TAGGGTAGCTCCCCAAGCAG	ACAAGCATGGGAAGGATGGT	620
13-14	CACCCCTTTCTTCTGATGCT	CTCATGCCCCAGAGAGCTCT	564
15-16	TCTATCTGGCCAGTCTGGTTC	GAAAGTTGGAGGTTCTTCTAGACCT	678
17	GGAAAGTTGGAGGTTCTTCTAGACC	TAGCCCTTTCCATCTCCCAT	540
18_1	CAGCTGGGATGAGTCAGTCT	CCTGTTTAACTCCCTAGTTACCCA	749
18_2	CCTGGGCCTGTCTCAGATT	GAGGACCCAAGGAGTAAGGC	703

dren without a clinical phenotype (III2, III3). According to the result of the function annotation, the mutation found in this family is properly disease-causing.

DISCUSSION

AI is a chronic, debilitating, painful inflammatory disease for which inherent unpredictability poses a major challenge for patients in terms of disease progression and response to treatment.

NCSTN exerts anti-proliferation and differentiation-promoting effects in human keratinocytes, and functional deletion mutation of *NCSTN* in familial AI promotes proliferation and inhibits differentiation of keratinocytes mainly through Notch and PI3K-AKT signaling pathways[10]. Moreover, epidermal and follicular hyperkeratosis and epidermal cyst formation are associated with disruption of Notch signaling pathways, leading to subsequent blockage, rupture and infection, a process strikingly similar to the pathogenesis of familial AI[11]. In addition, the NCSTN/miR-30a-3p/RAB31 contributed to the impaired activation of epidermal growth factor receptor signaling pathway which was followed by dysregulated keratinocyte differentiation in AI progression with the *NCSTN* mutation[12]. These find-

ings confirmed the association of *NCSTN* with proliferation, cell cycle control and differentiation of keratinocytes, which is closely related to the pathogenetic mechanism of AI.

We searched the literature of AI or HS cases in PubMed. A total of 37 unique mutations have been identified in familial or sporadic AI patients with a diversity of mutation types in Chinese, Caucasian, Japanese, British, Germany, French, Indian, or African ethnic origin (Table 2). Twenty-seven located in *NCSTN* (73.0%, 27/37), six located in *PSENEN*, two located in *PSEN1* and two located in *POFUT1*. Of which eight resulted in frameshifts and nine resulted in splice sites, seven were missense mutations and thirteen were nonsense mutations (Table 2). Obesity, smoking, hormones, bacterial infections, mechanical friction, and diabetes can aggravate AI symptoms. Four AI patients with *NCSTN* mutation were combined with cutaneous squamous cell carcinoma, three AI patients with *PSENEN* mutation and two AI patients with *POFUT1* mutation were combined with Dowling-Degos disease. These observations have enriched our understanding of AI and highlighted that *NCSTN* may play a pivotal role in AI. Moreover, most affected individuals who have been reported experience onset in adolescence with lesions often occurring in the neck, back, armpits, breast, buttocks and groin areas. These previous studies have helped us to ra-

Table 2. All mutations identified in *NCSTN*, *PSEN1*, *PSENEN*, and *POGLUT1* for acne inversa patients to date

No.	Familial/sporadic	Gene	Exon	Mutation type	Nucleotide mutation	Protein alteration	Origin
1	Familial	*NCSTN*	Ex3	Frameshift	c.210-211delAG	p.Thr70fs18X	Chinese
2	Familial	*NCSTN*	Ex3	Missense	c.223G>A	p.Val75Ile	Chinese
3	Familial	*NCSTN*	Ex4	Nonsense	c.218delC	p.P73Lfs*15	Chinese
4	Familial	*NCSTN*	Ex4	Nonsense	c.349C>T	p.Arg117X	Chinese African
5	Case	*NCSTN*	Ex5	Missense	c.553G>A	p.Asp185Asn	British
6	Familial	*NCSTN*	Int5	Splice site	c.582+1delG	p. F145fs_X54	Japanese
7	Familial	*NCSTN*	Ex5	Frameshift	c.487delC	p.Gln163SerfsX39	French
8	Familial	*NCSTN*	Ex5	Nonsense	c.477C>A	p.C159X	Chinese
9	Familial	*NCSTN*	Ex6	Nonsense	c.617C>A	p.S206X	Chinese
10	Case	*NCSTN*	Ex6	Missense	c.632C>G	p.P211R	Chinese
11	Familial	*NCSTN*	Ex6	Missense	c.647A>C	p.Q216P	Chinese
12	Familial	*NCSTN*	Ex6	Frameshift	c.687insCC	p.Cys230ProfsX31	Indian
13	Case	*NCSTN*	Int8	Splice site	c.996+7G>A	p.L282_G332del	British
14	Familial	*NCSTN*	Ex8	Missense	c.944C>T	p.Ala315Val	Chinese
15	Familial	*NCSTN*	Int9	Splice site	c.1101+1G>A	p.E333_Q367del	British
16	Case	*NCSTN*	Int9	Splice site	c.1101+10A>G	p.E333_Q367del	African
17	Familial	*NCSTN*	Int11	Splice site	c.1352+1G>A	p.Q393fs_X9	Chinese
18	Familial	*NCSTN*	Ex11	Nonsense	c.1300C>T	p.Arg434X	French
19	Familial	*NCSTN*	Ex11	Nonsense	c.1258C>T	p.Q420X	Chinese
20	Familial	*NCSTN*	Int11	Splice site	c.1180-5C>G		British
21	Familial	*NCSTN*	Int13	Splice site	c.1551+1G>A	p.A486-T517del	Chinese
22	Familial	*NCSTN*	Ex15	Frameshift	c.1752delG	p.E584DfsX44	Chinese
23	Familial	*NCSTN*	Ex15	Nonsense	c.1635C>G	p.Tyr545	Iranian
24	Familial	*NCSTN*	Ex15	Nonsense	c.1695T>G	p.Y565X	Chinese
25	Familial	*NCSTN*	Ex15	Missense	c.1768A>G	p.Ser590AlafsX3	French
26	Familial	*NCSTN*	Ex15	Nonsense	c.1702C>T	p.Gln568Term	Japanese
27	Familial	*NCSTN*	Ex16	Nonsense	c.1799delTG	p.Leu600X	Indian
1	Familial	*PSENEN*	Ex3	Frameshift	c.66delG	p.F23LfsX46	Chinese
2	Familial	*PSENEN*	Ex3	Frameshift	c.279delC	p.F94SfsX51	Chinese
3	Familial	*PSENEN*	Ex3	Frameshift	c.66-67insG	p.F23VfsX98	British
4	Familial	*PSENEN*	Ex3	Splice site	c.167-2A>G	p.G55-101Pdel	Chinese
5	Familial	*PSENEN*	Ex3	Missense	c.194T>G	p.L65R	Chinese
6	Case	*PSENEN*	Ex3	Nonsense	c.168T>G	p.Y56X	Germany
1	Familial	*PSEN1*	Ex7	Frameshift	c.725delC	p.P242LfsX11	Chinese
2	Familial	*PSEN1*	Ex9	Nonsense	c.953A> G	p.Glu318Gly	British
1	Case	*POFUT1*	Ex9	Nonsense	c.814C>T	p.R272*	Caucasian
2	Case	*POFUT1*	Ex4	Splice site	c.430-1G>A	p.K246_392Ldel	Caucasian

Int: intron, Ex: exon.

tionally select hotspot mutation genes for sequencing when encounter patients with AI.

Furthermore, three mutations located in exon 5 of *NCSTN* causing AI have been reported so far. A nonsense mutation, c.477C>A (p.C159X), in exon 5 of the *NCSTN* gene was detected that causes messenger RNA and protein expression evident reduction in the lesion[10]. A frameshift mutation, c.487delC, resulted in early termination of the codon (p.Gln163SerfsX39) and caused haploinsufficiency and severe reduction of *NCSTN* transcript levels in AI patients[13]. Finally, the missense variant in exon 5 of *NCSTN* (c.553G>A, p.Asp185Asn) was identified in a 45-year old female who presented inflammatory nodules, abscesses, and scarring in the breast area. It was predicted that this mutation caused the evolutionary conserved aspartic acid residue to be replaced by an asparagine residue[14]. In addition, we identified another novel frameshift mutation, c.450_459del (p.Ser151GlnfsX48) in exon 5 of *NCSTN* in this study, which has not been reported in the National Center for Biotechnology Information (NCBI) or OMIM database.

From the current physical examination, 7 individuals had no lesions. However, the results of the genetic test proven that 3 children of them were actual patients. According to the age in the onset of AI lesions in this family, these three children were too young to develop the skin rash. As summarized above in the literature, AI is not a disease with clinical manifestations at birth. From this point, the mutation analysis is a reliable supplement, suggesting the value of genetic diagnosis. Considering that all four patients in this family have a clinical phenotype presenting after the age of 12, these 3 children are likely to develop the disease in future, suggesting the significance of a timely and regular follow-up.

In conclusion, patients with AI experience chronic pain and have substantial physical, emotional and psychological effects. Our research reveals the cause of this Chinese family and extends the gene database of AI in China, which helps us to conduct clinical diagnosis and early intervention. Moreover, the ongoing recognition of unique mutations may allow people to understand the mechanisms that are still unknown in the development of AI and provide valuable information for further studies of treatment programs.

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CONFLICTS OF INTEREST

The authors have nothing to disclose.

ORCID

Jing Wu, https://orcid.org/0000-0001-6971-4640
Huiyao Ge, https://orcid.org/0000-0002-4928-1777
Yiming Fan, https://orcid.org/0000-0001-5001-0651
Qi Zhen, https://orcid.org/0000-0002-8344-4461
Lili Tang, https://orcid.org/0000-0002-5269-3297
Liangdan Sun, https://orcid.org/0000-0002-8499-5353

REFERENCES

1. Kiss N, Plázár D, Lőrincz K, Bánvölgyi A, Valent S, Wikonkál N. [Gynecological aspects of hidradenitis suppurativa]. Orv Hetil 2019;160:291-299. Hungarian.
2. Vinkel C, Thomsen SF. Hidradenitis suppurativa: causes, features, and current treatments. J Clin Aesthet Dermatol 2018; 11:17-23.
3. Negus D, Ahn C, Huang W. An update on the pathogenesis of hidradenitis suppurativa: implications for therapy. Expert Rev Clin Immunol 2018;14:275-283.
4. Theut Riis P, Thorlacius LR, Jemec GB. Investigational drugs in clinical trials for hidradenitis suppurativa. Expert Opin Investig Drugs 2018;27:43-53.
5. Lee JH, Kwon HS, Jung HM, Kim GM, Bae JM. Prevalence and comorbidities associated with hidradenitis suppurativa in Korea: a nationwide population-based study. J Eur Acad Dermatol Venereol 2018;32:1784-1790.
6. Ergun T. Hidradenitis suppurativa and the metabolic syndrome. Clin Dermatol 2018;36:41-47.
7. Wang B, Yang W, Wen W, Sun J, Su B, Liu B, et al. Gamma-secretase gene mutations in familial acne inversa. Science 2010;330:1065.
8. Danby FW, Margesson LJ. Hidradenitis suppurativa. Dermatol Clin 2010;28:779-793.
9. Ratnamala U, Jhala D, Jain NK, Saiyed NM, Raveendrababu M, Rao MV, et al. Expanding the spectrum of γ-secretase gene mutation-associated phenotypes: two novel mutations segregating with familial hidradenitis suppurativa (acne inversa) and acne conglobata. Exp Dermatol 2016;25:314-316.
10. Xiao X, He Y, Li C, Zhang X, Xu H, Wang B. Nicastrin mutations in familial acne inversa impact keratinocyte proliferation and differentiation through the Notch and phosphoinositide 3-kinase/AKT signalling pathways. Br J Dermatol 2016;174:522-532.
11. Yamamoto N, Tanigaki K, Han H, Hiai H, Honjo T. Notch/RBP-J signaling regulates epidermis/hair fate determination of hair follicular stem cells. Curr Biol 2003;13:333-338.
12. He Y, Xu H, Li C, Zhang X, Zhou P, Xiao X, et al. Nicastrin/miR-30a-3p/RAB31 axis regulates keratinocyte differentiation by impairing EGFR signaling in familial acne inversa. J

Invest Dermatol 2019;139:124-134.
13. Miskinyte S, Nassif A, Merabtene F, Ungeheuer MN, Join-Lambert O, Jais JP, et al. Nicastrin mutations in French families with hidradenitis suppurativa. J Invest Dermatol 2012;132: 1728-1730.
14. Pink AE, Simpson MA, Desai N, Dafou D, Hills A, Mortimer P, et al. Mutations in the γ-secretase genes NCSTN, PSENEN, and PSEN1 underlie rare forms of hidradenitis suppurativa (acne inversa). J Invest Dermatol 2012;132:2459-2461.