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ORIGINAL ARTICLE

Immunolocalization and Expression of JAK/STAT Signaling Molecules in the Skin of NCSTN Knockout Mouse

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SUMMARY

Background: **Mutations in the NCSTN gene are believed to be involved in the pathogenesis of acne inversa (AI). Further, certain cytokines are overexpressed in the inflammatory milieu of AI skin. Considering its central role in cytokine regulation, the JAK/STAT signaling pathway should be examined as a pathogenic factor and potential therapeutic target in AI.**
Methods: **$NCSTN^{flox/+}$, CAGGCre-ERTM mice were given 10 mg/kg/day tamoxifen for 6 days. Knockout of the NCSTN gene was confirmed by PCR. The levels of JAK1, JAK2, JAK3, and TYK2 in the skin tissue of NCSTN conditional knockout mice and WT mice were measured by IHC.**
Results: **By objective scoring using ImageJ software, JAK2 ($p = 0.013$) expression was increased in the dermis of KO mice, and JAK1 ($p = 0.032$), JAK3 ($p = 0.028$), and TYK2 ($p = 0.029$) were upregulated in the epidermis of KO mice versus WT mice.**
Conclusions: **This study suggests that the JAK/STAT pathway is important in AI, rendering it a potential therapeutic target. Targeting the JAK1/2 may be more prominent in the treatment of HS.**
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KEYWORDS

NCSTN, Notch, gene knockout mouse, hidradenitis suppurativa, acne inversa, JAK/STAT

INTRODUCTION

Acne inversa (AI), also known as Hidradenitis suppurativa, is a chronic, recurrent inflammatory disease that affects approximately 1% of the global population [1]. Typically, its lesions present as deep-seated painful nodules, abscesses, suppurative sinus tracts or tunnels, bridged scars, and double-ended and multi-ended comedones [2]. AI can repeatedly cause skin abscesses, ultimately leading to disfiguring scars and disfigurement or disability in patients, seriously affecting their physical and mental health [3]. There is no ideal treatment for this disease, necessitating the study of its etiology and pathogenesis.

Regarding the pathogenesis of AI, NCSTN is a pathogenic gene in this disease [4-6]. To this end, we established an NCSTN knockout mouse model to further examine its function in the pathogenesis of AI [7]. NCSTN gene mutations may lead to AI by affecting the Notch signaling pathway [8,9]. Proinflammatory cytokines, such as IL-1, IL-17, IL-23, and TNF-a, are elevated in the inflammatory skin manifestations of AI, but inhibitors of JAK-mediated cytokine signaling are believed to constitute a more effective treatment for AI than targeting individual cytokines [10]. Thus, several JAK inhibitors, including tofacitinib, povidontinib, and rosoliztinib, are currently examined in clinical trials [11]. However, the underlying molecular mechanisms by which JAK/STAT signaling is involved in the pathogenesis of AI remain to be determined.

There is no data regarding the localization or expression of the JAK/STAT pathway in the skin of AI in mouse models. This study compared the localization and expression of JAK1, JAK2, JAK3, and TYK2 in mouse skin before and after knockout of NCSTN, testing the hypothesis that knockout of NCSTN would alter these components of the JAK/STAT pathway, providing a basis for further research on the specific mechanisms of the JAK/STAT pathway in the pathogenesis of AI.

MATERIALS AND METHODS

Animals

NCSTN $^{flox/+}$ CAGGCre ERTM mice were housed in a warm animal room without specific pathogen conditions, given normal Co60-irradiated feed and purified water, and exposed to light for 12 hours per day. All animal experiments were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals and approved by our institutional review committee.

We selected C57BL/6 male mice and collaborated with Cyagen Biosciences Inc., using CRISPR-Cas9 gene editing technology to obtain the NCSTN $^{flox/+}$ CAGGCre ERTM mice. At the age of 3 weeks, CAGGCre ERTM mice were treated with tamoxifen to knockout the NCSTN gene. After 21 days (calculated from the second day after the end of gavage), ear tissue samples were harvested for genotyping by PCR to confirm knockout of the NCSTN gene. Four KO mice were generated.

Cutaneous lesions were harvested from NCSTN knockout mice (KO), with age- and lesion-matched wild-type (WT) mice serving as controls. This experimental design ensured direct comparability of all pathological and molecular parameters between genotypes. Skin tissues were fixed with 4% paraformaldehyde for HE staining and immunohistochemical experiments.

Immunohistochemistry (IHC)

Skin tissue was embedded in paraffin, and sections (4 μm) were prepared for staining. Antigen repair solution (pH 6.1 or 9, 97°C) was used to unmask the antigen for 10 minutes, and the sections were blocked with peroxidase inhibitor for 10 minutes and regular bovine serum at room temperature for 10 minutes. Next, the primary antibody was incubated at room temperature for 1 hour, and signals were detected using a DAB kit. The sections were counterstained with hematoxylin and mounted with neutral gum, and images were taken using a Zeiss microscope.

Evaluation of immunohistochemical staining

Immunohistochemical staining was assessed using a combined scoring system incorporating both subjective evaluation and objective quantification.

Subjective evaluation: five representative regions of each section were analyzed with 400 x magnification, and subjective scores were obtained based on the intensity and degree of staining in the epidermis and dermis. This rating was evaluated by 4 well-trained researchers (pathological doctors). In the epidermis, the intensity and degree of staining were scored, and the 2 scores were summed up for comparison (degree 0: negative, 1: 1/2 of epidermal thickness, 2: epidermal thickness greater than 1/2; intensity 0 - 3: 0 = missing, 3 = severe). In the dermis, the staining intensity and degree for each image were scored on a scale of 0 - 3 (0 = missing, 3 = severe) and 0 - 4 (0 = baseline, 4 = severe), respectively. The scores were then added and divided by the number of hair follicles for comparison. This facilitates the quantification of subjective evaluations, and subsequent statistical analysis reduces inter-observer variability.

Objective quantification: ImageJ was used to perform semiquantitative objective calculations of the amount of immunostaining in each slice.

Statistical analysis

In the statistical analysis, continuous data were expressed as mean ± standard deviation. Student's *t*-test was employed for comparing continuous variables between two groups. A two-tailed p-value of < 0.05 was deemed statistically significant. Data analysis was performed using the Statistical Package for the Social Sciences (IBM Corp., Armonk, NY, USA), version 27.

RESULTS

Subjective evaluation

JAK1 was distributed primarily in the internal root sheath, external root sheath, and the upper layer of the epidermis. JAK2 was expressed throughout the epidermis, upper dermis, cortex, and medulla of the hair shaft. JAK3 was present in the epidermis, dermis, cortex, and medulla of the hair shaft. It should be noted that JAK3 exhibited stratum corneum-predominant expression in the KO mice, while it showed pan-epidermal and dermal distribution in the WT mice. TYK2 was expressed weakly in the epidermis but was distributed throughout

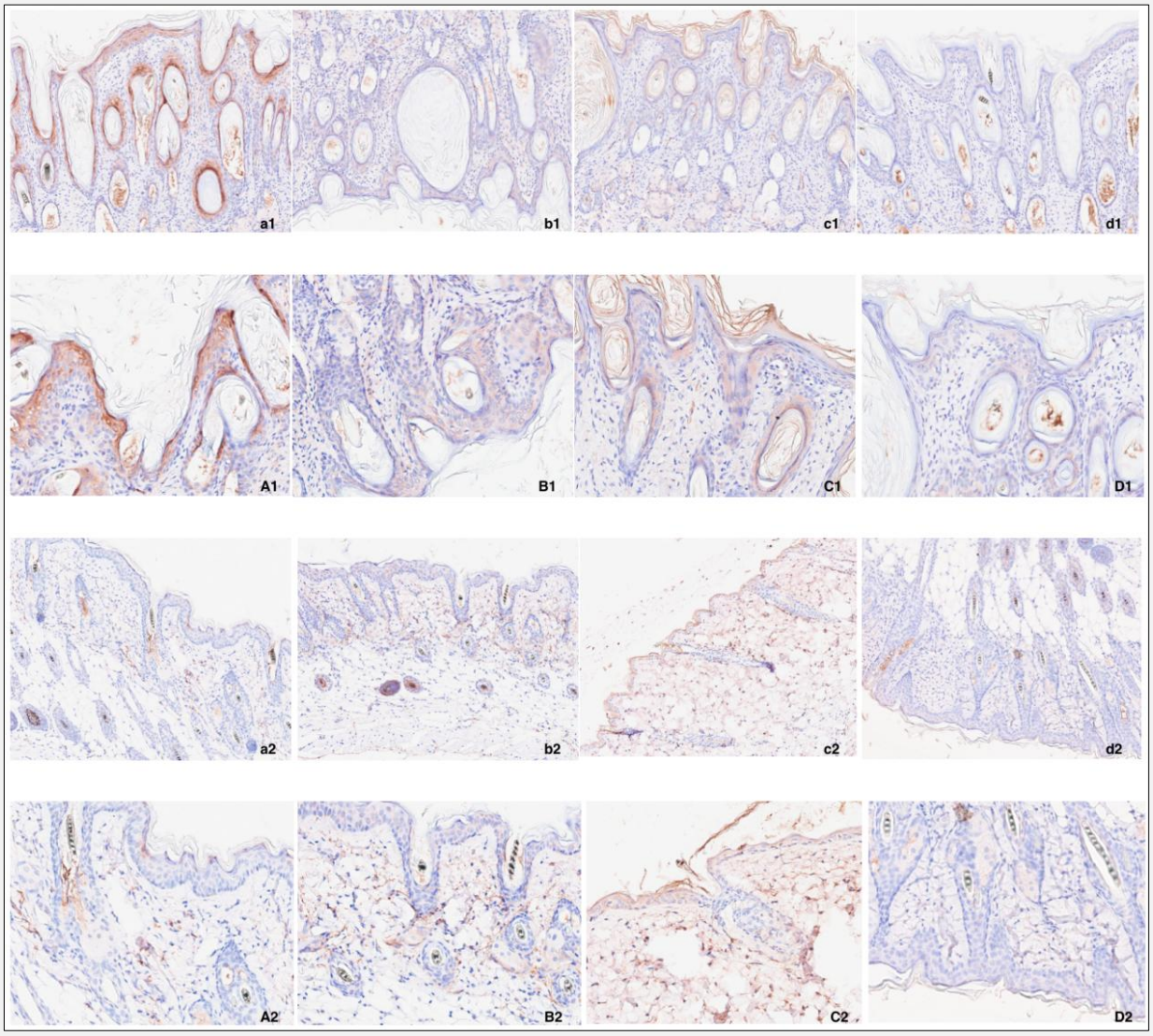

Figure 1. Knockout type (a1 - d1 × 100, A1 - D1 × 200) and wild-type mice (a2 - d2 × 100, immunohistochemistry of A2 - D2 × 200 skin specimens).

JAK1 (A1 and A2 and a1 and a2) was distributed primarily in the internal root sheath, external root sheath, and the upper layer of the epidermis. JAK2 (B1 and B2 and b1 and b2) was expressed throughout the epidermis, upper dermis, cortex, and medulla of the hair shaft. JAK3 (C1 and C2 and c1 and c2) was present in the epidermis, dermis, cortex, and medulla of the hair shaft. It should be noted that JAK3 exhibited stratum corneum-predominant expression in the KO mice, while it showed pan-epidermal and dermal distribution in the WT mice. TYK2 (D1 and D2 and d1 and d2) was expressed weakly in the epidermis but was distributed throughout the cortex and medulla of the hair shaft. Compared with WT mice (A1 - D1; a1 - d1), the expression of all four proteins was elevated in NCSTN KO mice (A2 - D2; a2 - a2).

the cortex and medulla of the hair shaft (Figures 1 and 2).

Semiquantitative assessment (ImageJ)

We tracked the epidermis and dermis and calculated the total number of immune pixels per unit area. Compared with WT mice, the expression of JAK1 ($p = 0.032$), JAK3 ($p = 0.028$), and TYK2 ($p = 0.029$) were higher in the epidermis. In the dermis, the expression of JAK2 ($p = 0.013$) increased (Figures 1 and 3).

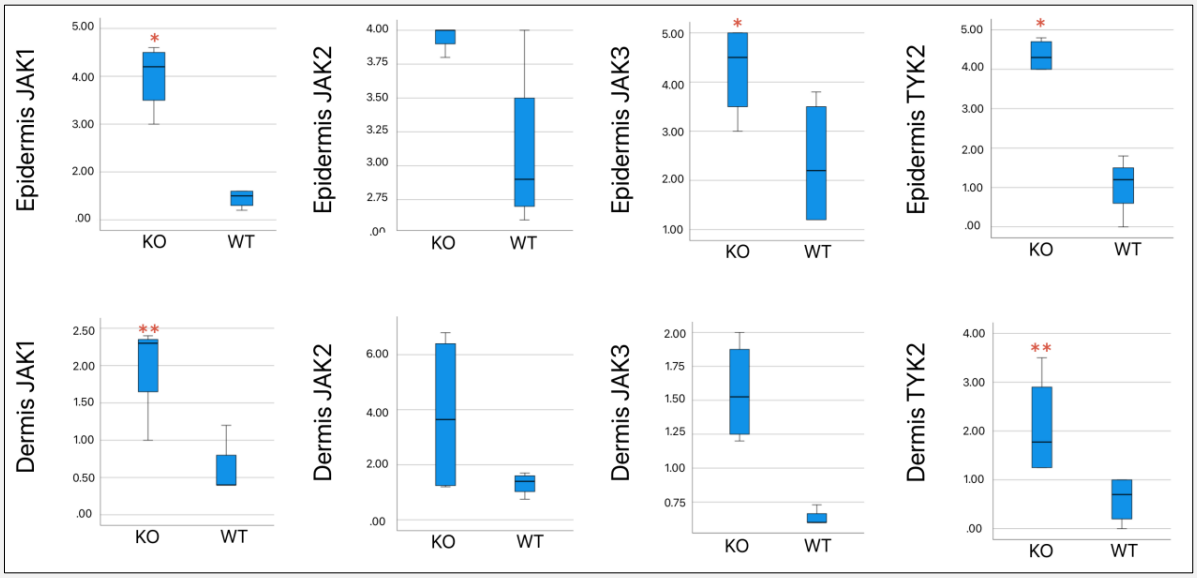

Figure 2. SPSS statistical analysis of the NCSTN KO mice' subjective score compared with WT mice (for JAK1, JAK2, JAK3, and TYK2).

*** $p \leq 0.05$, ** $p \leq 0.001$.**

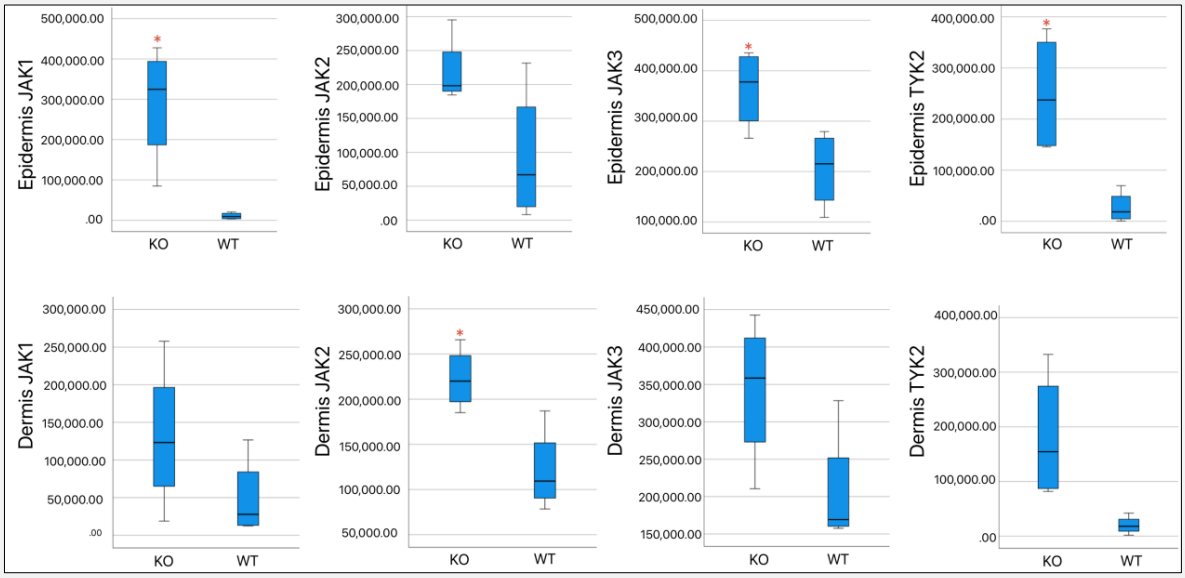

Figure 3. SPSS statistical analysis of the total number of immune pixels per unit area (ImageJ) of NCSTN KO mice compared with WT mice (for JAK1, JAK2, JAK3, and TYK2).

*** $p \leq 0.05$, ** $p \leq 0.001$.**
Compared with WT mice, the expressions of JAK1 ($p = 0.032$), JAK3 ($p = 0.028$), and TYK2 ($p = 0.029$) were higher in the epidermis. In the dermis, the expression of JAK2 ($p = 0.013$) increased.

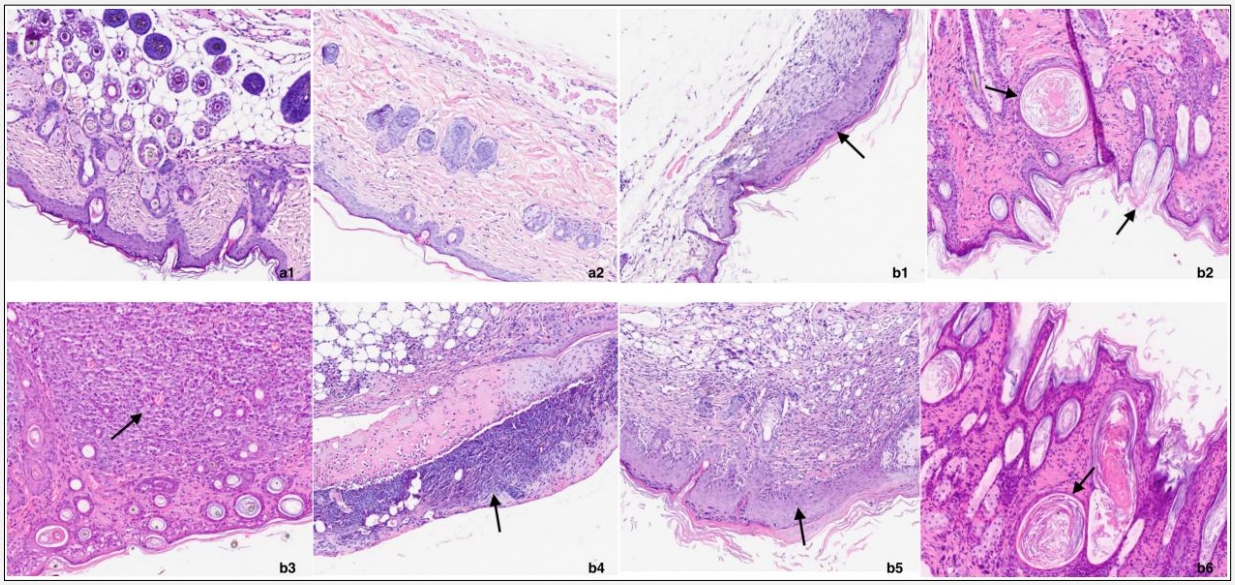

Figure 4. Microscopic characteristics of skin in NCSTN KO mice and WT mice.

A1 and a2 are pathological manifestations of the skin in WT mice (× 100). In the lesions of NCSTN KO mice, pathology showed the formation of hair follicle horn plugs and horn cysts similar to AI as well as infiltration of inflammatory cells in the skin tissue around the hair follicles. Psoriatic-like hyperplasia and hypertrophy of the spinous layer were observed in the epidermis, and the number of inflammatory cells infiltrating the lower hair follicles in the dermis decreased, even leading to loss of epidermal structure.

DISCUSSION

AI is a recurrent inflammatory skin disease with complex pathogenesis, and there is currently no ideal treatment. AI can attack any skin that contains a follicular portion of folliculopilosebaceous units. AI usually appears in the axillary, groin, perianal, perineal, and inframammary regions. The exact pathogenesis of AI is unknown. However, it is believed to be attributed to the combined actions of genetics, the environmental, and the immune system. Approximately one-third of AI patients have a family history, inherited through autosomal dominance [9].

In 2010, Wang et al. discovered mutations in the gamma secretase (GS) gene in familial AI patients. Gamma secretase [12] is an intramembrane protease complex with 20 transmembrane domains, responsible for the intramembrane cleavage of various type 1 transmembrane proteins, including amyloid precursor protein and Notch receptor. It contains 4 subunits, comprising presenilin (PS), nicastrin (NCSTN), presenilineenhancer-2 (PSENEN), and anterior pharyngeal defect protein-1 (APH-1), encoded by the PSEN1/PSEN2, NCSTN, PSENEN, and APH1A/APH1B genes, respectively [12].

Some groups believe that the NCSTN gene is involved in the pathogenesis of AI by affecting the transmission of Notch signaling [13,14]. In the Notch pathway, receptor proteins are cleaved by GS to release the intracellular domain NICD, which enters the nucleus and binds to DNA-binding proteins to activate downstream target genes, including Hes and Hey [8]. The Notch pathway is believed to be associated with abnormal hair follicle growth and development. Damage to this pathway can lead to abnormal keratinization of hair follicles, a classic histological feature of AI [9].

Early lesions of AI also show pronounced perifolliculitis and rupture of HF and epidermal cysts [15]. Meanwhile, inflammatory nodules form around disrupted hair follicles. Tertiary lymphoid structures containing B cells, plasma cells, and T cells develop within these areas [16]. Subsequent massive neutrophil infiltration induces tissue liquefaction, culminating in pus-filled abscesses. During this stage, macrophages and dendritic cells begin to release pro-inflammatory cytokines, including TNF and IL-1 β. Moreover, IL-1 β induces specific cytokines such as IL-6, IL-36, and granulocyte colony-stimulating factor (G-CSF) [17]. Notch signaling can also regulate immune responses [18].

In our previous work [7], we generated and tested an AI mouse model. We found that knockout of the NCSTN genes results in impaired GS activity with deficient Notch signaling. And in the lesions of NCSTN KO

mice, pathology showed the formation of hair follicle horn plugs and horn cysts similar to AI, as well as infiltration of inflammatory cells in the skin tissue around the hair follicles. Psoriatic-like hyperplasia and hypertrophy of the spinous layer were observed in the epidermis, and the number of inflammatory cells infiltrating the lower hair follicles in the dermis decreased, even leading to loss of epidermal structure (Figure 4). We conclude that NCSTN $^{flox/+}$ CAGGCre ERTM mice can serve as an animal model for AI.

Interplay between the Notch pathway and JAK-STAT signaling has been shown to mediate cell fate determination during development. Genetic interactions between these two signaling pathways appear to be highly diverse. Depending on the developmental context, these two pathways interact as upstream or downstream regulators of each other [19,20]. One study found that activation of the JAK-STAT pathway was significantly upregulated with Notch-low status [21]. Interplay between Notch and JAK-STAT signaling pathway may play a pivotal role in AI. In our experiments, in AI mouse model skin, Notch signaling was downregulated [7], whereas the expression of JAK signaling components increased. And it is worth noting that most of inflammatory factors (IL-6, G-CSF) exert their effects through the JAK/STAT pathway [22]. So, AI may be related to the JAK/STAT signaling pathway. This study used NCSTN knockout mice as an animal model of AI to examine the relationship between the disease and the JAK/STAT pathway.

We obtained skin tissues from NCSTN knockout and WT mice and measured JAK1, JAK2, JAK3, and TYK2 - components in the JAK/STAT pathway - by immunohistochemistry. The results showed that JAK1 was distributed primarily in the internal root sheath, external root sheath, and the upper layer of the epidermis. JAK2 was expressed throughout the epidermis, upper dermis, cortex, and medulla of the hair shaft. JAK3 was present in the epidermis, dermis, cortex, and medulla of the hair shaft. It should be noted that JAK3 exhibited stratum corneum-predominant expression in the KO mice, while it showed pan-epidermal and dermal distribution in the WT mice. TYK2 was expressed weakly in the epidermis but was distributed throughout the cortex and medulla of the hair shaft. In the epidermis of NCSTN knockout mice, JAK1, JAK3, and TYK2 levels were higher than in WT mice, based on objective scores. In the dermis of NCSTN knockout mice, JAK2 had higher objective scores (i.e. expression) than in WT mice.

Based on our results, we speculate which JAK inhibitor is more effective in treating AI. Current research on the expression of the JAK/STAT pathway in inflammatory skin diseases is based on AI patient skin [23]. This research found that the expression of JAK2, JAK3, and TYK2 was higher in the skin of AI patients than in healthy people, and that JAK1 was not intensely expressed in the dermis. This study [23] suggested that targeting TYK2 and JAK3 may have more potential in the treatment of AI. Our results are generally consistent with the current research [23]. However, we further focused on the AI with NCSTN gene mutations. Compared with the study of skin samples from the patient, which comes from multiple AI types, this provides a certain basis for the treatment of NCSTN mutations AI. In addition, our study added observation and description of location in hair follicles, providing additional ideas for the selection of JAK inhibitors.

There are many clinical trials on JAK inhibitors for the treatment of AI. Ruxolitinib is a JAK1/2-specific inhibitor that can be used topically or orally [11]. Currently, there are two phase 2 trials that are examining the efficacy of local rosoliztinib use in the treatment of AI [24]: one is an open-label trial that evaluates HiSCR in week 16, and the other is a double-blind trial that is comparing 1.5% rosoliztinib cream with excipient twice daily, with the main outcome being changes in abscess and inflammatory nodule (AN) counts at week 16. In our experiment, the significant differential expression of JAK1 and JAK2 in the skin between WT and KO mice may provide an explanation for these clinical trials. And according to the immunohistochemical staining results of JAK1 and JAK2 in the skin of NCSTN knockout mice, JAK1 is almost not expressed in the epidermis, while JAK2 is expressed in both the epidermis and dermis. This can also provide some explanation for the better combined inhibition of JAK1 and JAK2.

In another study, these components were examined with regard to specific cell type and localization. TYK2 was found to be expressed in not only the cytoplasm but also the nucleus, whereas JAK2 and JAK3 were observed to have perinuclear localization. In our study, JAK1, JAK2, JAK3, and TYK2 were expressed in the cytoplasm of KO mouse skin tissue. JAK3 was expressed in the nucleus, as was JAK1 in a small number of slides (n = 3). TYK2 was not found to be expressed in the nucleus.

The limitations of our study include the small number of mice. Another potential limitation is the subjective nature of our immunohistochemical analysis. Therefore, we evaluated the intensity and degree of staining in the epidermis and dermis using subjective scores. This facilitates the quantification of subjective evaluations, and subsequent statistical analysis reduces inter-observer variability. Immunohistochemical staining was assessed using a combined scoring system incorporating both subjective evaluation and objective quantification. Overall, this study contributes to the research on the JAK/STAT pathway in AI and provides some ideas for the development of AI therapeutic strategies. Further research is needed to better determine the expression of the JAK/STAT pathway and its role in the pathogenesis of AI.

Ethical Approval and Consent to Participate:

All animal experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals and approved by the Institutional Review Board of

the Fifth Clinical Medical College of Henan University of Chinese Medicine.

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Declaration of Interest:
The authors declare no competing interests.

References:

1. Sabat R, Alavi A, Wolk K, et al. Hidradenitis suppurativa. Lancet 2025;405(10476):420-38. (PMID: 39862870)

2. Saunte DML, Jemec GBE. Hidradenitis Suppurativa: Advances in Diagnosis and Treatment. JAMA 2017;318(20):2019-32. (PMID: 29183082)

3. Sampogna F, Fania L, Mastroeni S, Fusari R, Panebianco A, Abeni D. Quality of life in patients with early- and late-onset hidradenitis suppurativa. Arch Dermatol Res 2023;315(3):473-9. (PMID: 35997825)

4. Gao M, Wang P-G, Cui Y, et al. Inversa acne (hidradenitis suppurativa): a case report and identification of the locus at chromosome 1p21.1-1q25.3. J Invest Dermatol 2006;126(6):1302-6. (PMID: 16543891)

5. Al-Ali FMS, Ratnamala U, Mehta TY, et al. Hidradenitis suppurativa (or Acne inversa) with autosomal dominant inheritance is not linked to chromosome 1p21.1-1q25.3 region. Exp Dermatol 2010;19(9):851-3. (PMID: 20698881)

6. Wang B, Yang W, Wen W, et al. Gamma-secretase gene mutations in familial acne inversa. Science 2010;330(6007):1065. (PMID: 20929727)

7. Yan L, Song Y-S, Zhou J, et al. Expression of nicastrin, NICD1, and Hes1 in NCSTN knockout mice: implications for hidradenitis suppurativa, Alzheimer's, and liver cancer. Eur J Med Res 2024; 29(1):622. (PMID: 39719647)

8. Melnik BC, Plewig G. Impaired Notch-MKP-1 signalling in hidradenitis suppurativa: an approach to pathogenesis by evidence from translational biology. Exp Dermatol 2013;22(3):172-7. (PMID: 23489419)

9. Nomura T. Hidradenitis Suppurativa as a Potential Subtype of Autoinflammatory Keratinization Disease. Front Immunol 2020; 11:847. (PMID: 32508815)

10. Frew JW, Marzano AV, Wolk K, et al. A Systematic Review of Promising Therapeutic Targets in Hidradenitis Suppurativa: A Critical Evaluation of Mechanistic and Clinical Relevance. J Invest Dermatol 2021;141(2):316-24.e2. (PMID: 32919760)

11. Jaguan D, Nguyen KT, Goldfarb N. Selective small molecule inhibitors for hidradenitis suppurativa: Today and tomorrow. J Am Acad Dermatol 2024;91(6S):S31-6. (PMID: 39626997)

12. Wang Z, Yan Y, Wang B. γ-Secretase Genetics of Hidradenitis Suppurativa: A Systematic Literature Review. Dermatology 2021;237(5):698-704. (PMID: 33333507)

13. Ingram JR. The Genetics of Hidradenitis Suppurativa. Dermatol Clin 2016;34(1):23-8. (PMID: 26617354)

14. Pace NP, Mintoff D, Borg I. The Genomic Architecture of Hidradenitis Suppurativa-A Systematic Review. Front Genet 2022;13: 861241. (PMID: 35401657)

15. Wolk K, Join-Lambert O, Sabat R. Aetiology and pathogenesis of hidradenitis suppurativa. Br J Dermatol 2020;183(6):999-1010. (PMID: 33048349)

16. Sabat R, Šimaitė D, Gudjonsson JE, et al. Neutrophilic granulocyte-derived B-cell activating factor supports B cells in skin lesions in hidradenitis suppurativa. J Allergy Clin Immunol 2023; 151(4):1015-26. (PMID: 36481267)

17. Moran B, Smith CM, Zaborowski A, et al. Targeting the NLRP3 inflammasome reduces inflammation in hidradenitis suppurativa skin. Br J Dermatol 2023;189(4):447-58. (PMID: 37243544)

18. Zhang Q, Wang C, Liu Z, et al. Notch signal suppresses Toll-like receptor-triggered inflammatory responses in macrophages by inhibiting extracellular signal-regulated kinase 1/2-mediated nuclear factor κB activation. J Biol Chem 2012;287(9):6208-17. (PMID: 22205705)

19. Gratton R, Tricarico PM, Moltrasio C, et al. Pleiotropic Role of Notch Signaling in Human Skin Diseases. Int J Mol Sci 2020; 21(12):4214. (PMID: 32545758)

20. Okuyama R, Tagami H, Aiba S. Notch signaling: its role in epidermal homeostasis and in the pathogenesis of skin diseases. J Dermatol Sci 2008;49(3):187-94. (PMID: 17624739)

21. Ghosh S, Mitra P, Saha U, et al. NOTCH pathway inactivation reprograms stem-like oral cancer cells to JAK-STAT dependent state and provides the opportunity of synthetic lethality. Transl Oncol 2023;32:101669. (PMID: 37054548)

22. Luo Y, Alexander M, Gadina M, O'Shea JJ, Meylan F, Schwartz DM. JAK-STAT signaling in human disease: From genetic syndromes to clinical inhibition. J Allergy Clin Immunol 2021; 148(4):911-25. (PMID: 34625141)

23. Demirci Yildirim T, Kahraman A, Köken Avşar A, Onen F, Akar S, Sari İ. Quantitative analysis of JAK/STAT signaling pathway in patients of inflammatory skin disorders. Rheumatol Int 2024; 44(12):3009-15. (PMID: 37558928)

24. Papp K, Szepietowski JC, Kircik L, et al. Efficacy and safety of ruxolitinibcream for the treatment of atopic dermatitis: Results from 2 phase 3, randomized, double-blind studies. J Am Acad Dermatol 2021;85(4):863-72. (PMID: 33957195)