

## Expression of Histidine Decarboxylase in the Epidermis of Primates with Chronic Itch

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Chronic itch is a burdensome clinical issue that has a significant negative impact on quality of life. In the past two decades, the volume of research on itch has increased immensely. However, there is still a significant lack of translation from scientific findings into medical practice. We previously identified a colony of *Cynomolgus* macaques (*Macaca fascicularis*) suffering from idiopathic chronic itch (1). This non-human primate chronic itch model could provide a translational bridge for studying chronic itch.

Histidine decarboxylase (HDC) is the enzyme that catalyzes the synthesis of histamine. Recently, an increase in epidermal HDC was shown in acute and chronic itch-related behaviors induced by topical application of anionic surfactants in mice (2, 3). Additionally, epidermal HDC is elevated in mice with  $\alpha$ -melanocyte-stimulating hormone-induced itch (4). Thus, these findings suggest that increased HDC in the epidermis might play a role in itch. In the present study, we investigated whether epidermal HDC is associated with itch severity in the primate model.

### MATERIALS AND METHODS

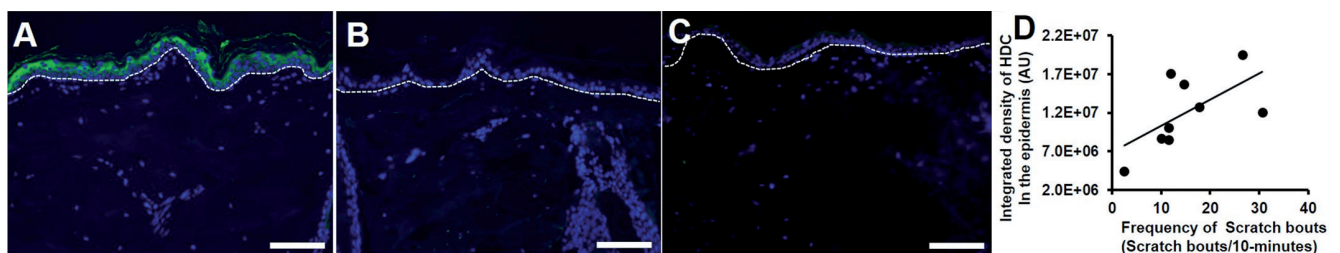
The skin of adult female *Cynomolgus* macaques ( $n=8$ ; *Macaca fascicularis*) suffering from varying degrees of idiopathic chronic itch was used. These primates had no inflammatory skin lesions or infections (e.g. mites), nor received any antipruritic therapy. Behavior was observed in 10-min sessions, at similar times during the day (early afternoon), twice weekly for a 4-year period by two trained observers. The frequency of scratch bouts (number of scratching episodes per focal observation) was documented using a validated focal animal observation technique (5; described in detail in 1). A single bout of scratching was defined as moving the fingertips repeatedly across the same skin area for duration longer than one second and is distinct from grooming behaviors.

The frequencies recorded over the 4 years were then averaged for each primate, thus representing a total of 68 h of observation per animal. Skin tissue was then collected and fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS; pH 7.4) over night at 4°C, and embedded in paraffin.

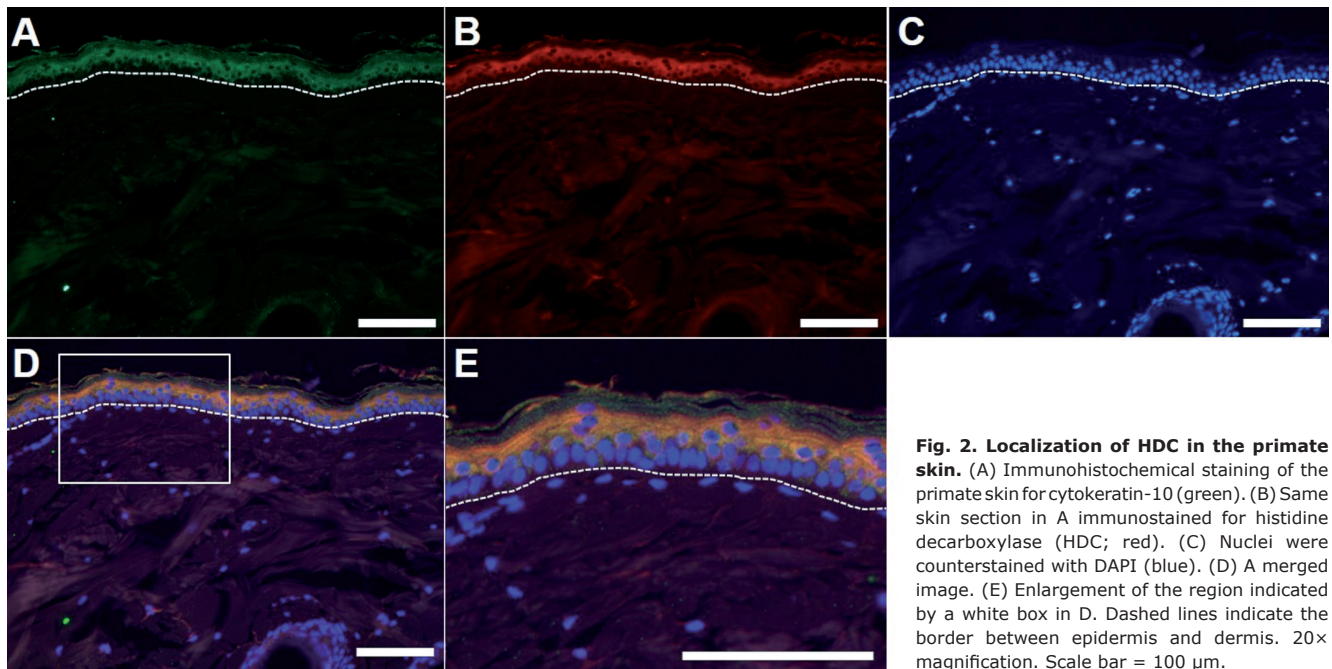
For immunohistochemical analysis, the paraffin-embedded skin was sectioned at a 5- $\mu$ m thickness. After dewaxing and antigen retrieval using DAKO Target Retrieval Solution (S1699; DAKO Corporation, Carpinteria, CA) overnight at 60°C, sections were blocked in PBS containing 0.2% Triton X-100 and 5% donkey serum (Jackson Immunoresearch Laboratories, Inc., West Grove, PA) at room temperature for 2 h. The following primary antibodies were then incubated over night at 4°C: rabbit polyclonal anti-HDC antibody (1:100; HPA038891, Atlas antibodies Co., Stockholm, Sweden) or mouse polyclonal anti-cytokeratin 10 antibody (1:100; ab9026, Abcam Inc., Cambridge, MA). After washing with PBS, the sections were incubated for 2 h at room temperature with the corresponding secondary antibodies conjugated to either Alexa Fluor 488 or Alexa Fluor 594 (1:300; Invitrogen Co., Carlsbad, CA). Nuclei were counterstained with DAPI (4,6-diamidino-2-phenylindole). Specificity of the anti-HDC antibody was confirmed by preabsorption of antibody with the immunogenic peptide (1:100; APrEST70111, Atlas Antibodies, Stockholm, Sweden). Immunofluorescence was visualized using a fluorescence microscope (E1000; Nikon Eclipse, Tokyo, Japan) and 7 images (field size per image: 526.7  $\times$  703.5  $\mu$ m) were randomly selected from each primate to be captured under 20 $\times$  magnification. For immunoreactivity quantitation, signal intensity in the epidermis was determined for each image after background subtraction using Image J software (NIH, Bethesda, MD). Correlation between primate scratching behavior and immunoreactivity was performed using Spearman correlation analysis with a probability of  $p < 0.05$  considered to be significant (SPSS Statistics).

### RESULTS

HDC immunoreactivity was exclusively detected in the primate epidermis (Fig. 1A). This immunoreactivity was completely abolished by preabsorption of the antibody



**Fig. 1. Positive correlation between epidermal HDC expression and itch severity in primates.** (A) A typical example of expression of histidine decarboxylase (HDC; green) in the skin of primates with chronic itch. Nuclei were counterstained with DAPI (blue). A white dashed line indicates the border between epidermis and dermis. 20 $\times$  magnification. Scale bar = 100  $\mu$ m. (B) As in A for preabsorption test. HDC expression was abolished by preabsorption of the blocking peptide. (C) As in A for the skin of a primate showing the lowest scratch number in the colony. HDC expression was barely detected. (D) The expression level of HDC in the epidermis correlated with the frequency of scratch bouts in 10-min session (Spearman correlation,  $r=0.750$ ,  $p=0.02$ ).



**Fig. 2. Localization of HDC in the primate skin.** (A) Immunohistochemical staining of the primate skin for cyokeratin-10 (green). (B) Same skin section in A immunostained for histidine decarboxylase (HDC; red). (C) Nuclei were counterstained with DAPI (blue). (D) A merged image. (E) Enlargement of the region indicated by a white box in D. Dashed lines indicate the border between epidermis and dermis. 20× magnification. Scale bar = 100 µm.

with recombinant fragment of HDC (Fig. 1B). HDC was barely detectable in the epidermis of a primate with the lowest frequency of scratch bouts in the colony (Fig. 1C). This primate did not show any sign of chronic itch (e.g. lichenification). The expression level of epidermal HDC was significantly correlated with the frequency of scratch bouts ( $r=0.750$ ,  $p=0.02$ ; Fig. 1D). The localization of HDC was determined by double staining for cyokeratin-10, a marker of suprabasal layers of the epidermis. HDC was colocalized with cyokeratin-10 in the epidermis, indicating that HDC is localized in suprabasal layers of the epidermis (Fig. 2).

## DISCUSSION

We found that the expression level of epidermal HDC was positively correlated with the frequency of scratch bouts in non-human primates with chronic itch, implying that HDC may play a role in itch in primates. This finding is supported from previous studies showing that epidermal HDC was increased in itchy mice treated with a surfactant (2, 3). Furthermore, a recent study showed that a significant increase in epidermal HDC was observed in atopic dermatitis patients, but not in psoriasis patients (6). However, this is the first study to correlate HDC levels to itch severity. Our study also extends these previous findings by showing that HDC was colocalized with cyokeratin-10, a marker of suprabasal layers of the epidermis. Further studies are required to investigate

whether epidermal HDC is involved in chronic itch in human skin diseases and whether the expression of HDC correlates to itch intensity.

## ACKNOWLEDGMENTS

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