TO THE EDITOR

The cutaneous sensation of itch is common to human and nonhuman primates. Developments in human itch research have revealed molecular underpinnings that span immune and neural networks within the skin and the nervous system. This work presents the differentially expressed genes (DEGs) and immunohistochemistry profile of primates with itch and provides an interesting comparison with the known itch pathways in humans as well as insight into the mediators that represent promising therapeutic targets.

Animal experiments were approved by the Wake Forest University Animal Care and Use Committee. Adult female cynomolgus macaques (Macaca fascicularis) (n = 35) with itch were observed over a 4-year period for frequency and time spent scratching. Veterinary evaluation revealed no inflammatory, systemic, or infectious etiology, and their itch was considered idiopathic in nature. Primates were classified as severe (n = 14) or mild and/or moderate (n = 21) scratchers, and skin sections were collected from lichenified skin (LS) of severe scratchers and non-LS of mild and/or moderate scratchers (Supplementary Figures S1 and S2). RNA sequencing through Illumina HiSeq2000 (Illumina, San Diego, CA) was performed at a depth of 200 million paired-end reads of 100 base pairs and aligned to the reference genome (GCA_000364345.1) to identify DEGs. Paraffin-embedded skin sections were double stained for antibodies to the following: substance P, NK1R, tryptase, PAR2, histamine, TRPV1, and TRPA1 and analyzed for epidermal fluorescence and positive mast cell and nerve counts. Detailed methods for data collection, RNA sequencing, and immunohistochemistry are available in the Supplementary Materials and Methods.

Sequencing identified 2,264 DEGs, with approximately 400 genes implicated in known itch pathways. Genes of interest that code for receptor channels, proteases, neuropeptides, cytokines, and chemokines are shown in Figure 1. Complete DEG results are available in the Supplementary Results. Immunohistochemistry results show significantly increased epidermal fluorescence intensity of PAR2 and tryptase-positive mast cells in the LS compared with those in the non-LS (Figure 2). Fluorescence of TRPV1, TRPA1, NK1R, and substance P-positive nerve cells was also significantly increased in the epidermis of LS (Supplementary Figure S3). There was no significant increase in the number of TRPV1-positive and TRPA1-positive nerves in the LS (Supplementary Figure S3). Histamine-positive mast cells were not overexpressed in the LS compared with those in the non-LS (data not shown).

Human chronic itch is mediated through nonhistaminergic pathways, and primate LS exhibited DEGs consistent with the activation of these pathways (Ikoma et al., 2006). Although histamine decarboxylase was upregulated, the histamine receptor 1 was not, and immunohistochemistry showed no histamine overexpression.

G-protein coupled receptors (GPCRs) play a key role in nonhistaminergic itch signaling. Notable GPCR DEGs were PAR2 (F2RL1), MRGPRX2, and NK1R (TACR1) along...
with their ligands, tryptase (TPSAB1), kallikrein 5, CTSS, and substance P (TAC1). PAR2 and its ligands have been observed in the skin of humans with atopic dermatitis and scabies (Nattkemper et al., 2018; Sanders et al., 2019; Steinhoff et al., 2003). MRGPRX2 is overexpressed in human atopic dermatitis and psoriatic skin as well as in allergic contact dermatitis, and its ligand CTSS is overexpressed in human and murine pruritic disease models (Kim et al., 2012; Meixiong et al., 2019; Nattkemper et al., 2018; Schönefuss et al., 2010). Substance P is overexpressed in pruritic conditions in humans, and drugs targeting this pathway have been reported in phase II trials to demonstrate antipruritic effect for chronic itch of multiple etiologies (Yosipovitch et al., 2018). Common overexpression of these GPCRs in the itch of primates and humans suggests that these pathways are promising targets for itch therapy generally.

Activation of pruritogenic GPCRs leads to the opening of transient receptor potential receptors and sodium voltage-gated (NaV) ion channels that propagate the action potential along the itch-selective unmyelinated C fibers. DEGs for several transient receptor potential and NaV channels were evident in the primates. TRPV1 and TRPV3, implicated in nonhistaminergic itch, are overexpressed in human pruritic diseases (Li et al., 2014; Nattkemper et al., 2018; Sanders et al., 2019; Valdes-Rodriguez et al., 2013). NaV1.7 is overexpressed in human itch and gain-of-function mutations in its precursor gene SCN9A cause familial paroxysmal itch (Devigili et al., 2014; Nattkemper et al., 2018). NaV1.7-inhibiting compounds reduce mice scratching behavior after itch stimulation (Lee et al., 2014). The common finding of transient receptor potential and NaV channel upregulation among primates and humans with various itch etiologies is consistent with upstream GPCR activation by itch mediators. Given their role in the final step in signaling before the propagation of the action potential, the transient receptor potential and NaV channels represent a promising target for itch therapy.

A phospholipase A2 transcript, PLA2G4D, had a fold change of 9.31, the highest of any DEG in this study. Phospholipase A2 is upregulated in the nonhistaminergic itch of atopic dermatitis and psoriasis (Nattkemper et al., 2018). It is also implicated in the pain and itch reaction associated with bee venom, which is proposed to be through the modifications of the cell membrane and activation of proinflammatory pathways in keratinocytes (Nakashima et al., 2020). Given its role in a number of itch etiologies, phospholipase A2 represents a target for antipruritic therapy.

An imbalance of endogenous opioids is proposed to lead to neuronal sensitization as a mechanism of chronic itch (Hashimoto and Yosipovitch, 2019). Primate DEGs were consistent with this hypothesis, with downregulation of the OPRK1 and dynorphin A precursor gene PDYN as well as upregulation of the β-endorphin precursor POMC and the µ-opioid receptor OPRM1. Human gene expression in atopic dermatitis and psoriatic skin are consistent with the primate findings, showing upregulation of POMC and downregulation of OPRK1 (Nattkemper et al., 2018).

This study demonstrates the cutaneous gene expression profile of primates with nonhistaminergic itch. Receptors and their ligands paralleling human nonhistaminergic itch were upregulated as were downstream ion channels for neuronal itch signal propagation. Known mediators of pruritic diseases were represented, although the primates were not suspected to suffer from these conditions, suggesting that these mediators underly itch in different etiologies. The involvement of other pathways such as those involved in endogenous opioid regulation was consistent with human forms of chronic itch. The application of these findings is limited owing to a lack of comparison with primate healthy skin and the uncertain etiology of the primate itch. Further research in human itch pathways and primates with known itch etiologies would provide supportive data to these findings.
TO THE EDITOR

Atopic dermatitis (AD) represents a complex intersection between skin barrier dysfunction, host immunologic response, and external factors such as allergenic triggers and the skin microbiome. However, the relative contributions of these factors are not clear. There is a surge in *Staphylococcus aureus* on the skin during flares of AD (Kong et al., 2012). *S. aureus* strains seen in AD flares are unique to the host and capable of eliciting varied immune responses (Byrd et al., 2017). No direct relationship between severity and in vitro toxin genotype has been established (Kim et al., 2009). *S. epidermidis* may similarly contribute to the propagation of AD through the elucidation of toxins (Martinez-Garcia et al., 2018). Further information on strains of staphylococci found on patients with AD may aid in developing therapeutic targets.

Abbreviation: AD, atopic dermatitis

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