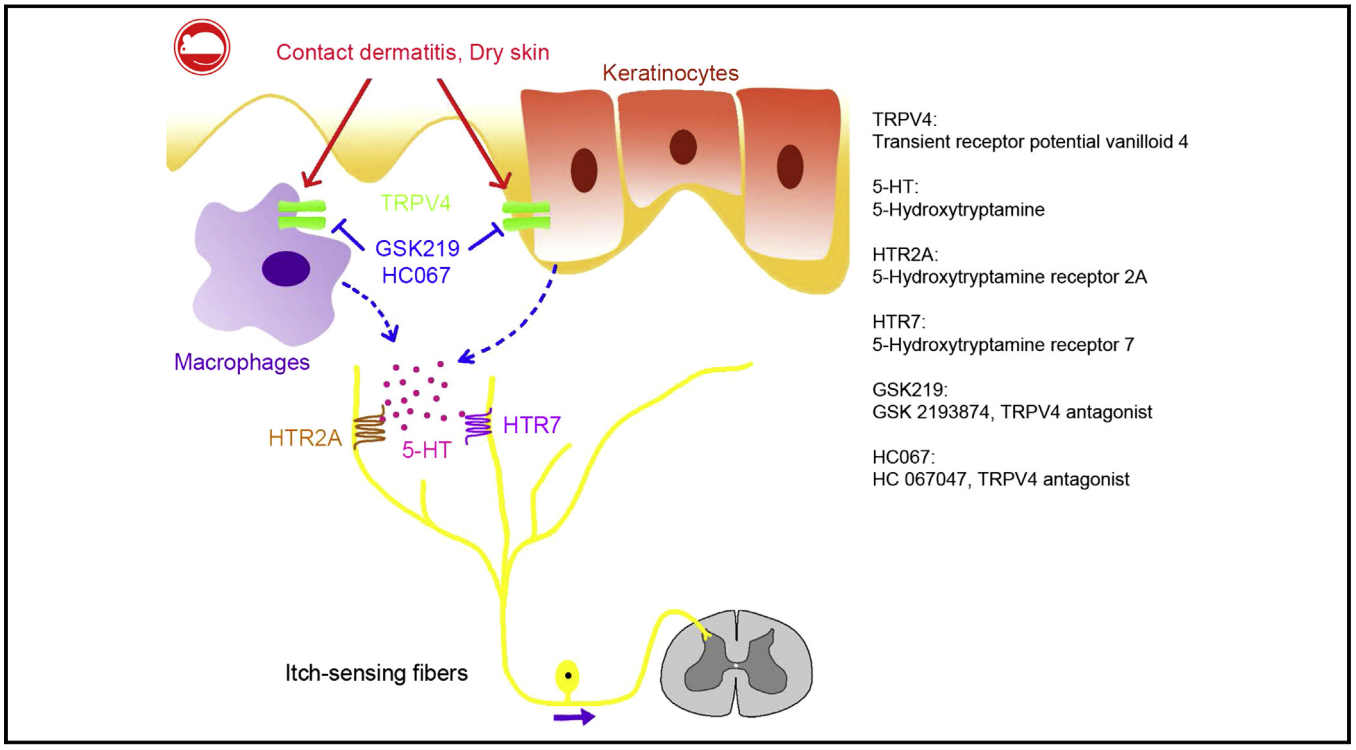
Transient receptor potential vanilloid 4–expressing macrophages and keratinocytes contribute differentially to allergic and nonallergic chronic itch

Jialie Luo, PhD,[a]* Jing Feng, PhD,[a]* Guang Yu, PhD,[a,b] Pu Yang, PhD,[a] Madison R. Mack, BA,[a] Junhui Du, PhD,[c] Weihua Yu, PhD,[d] Aihua Qian, PhD,[e] Yujin Zhang, PhD,[f] Shenbin Liu, PhD,[a] Shijin Yin, PhD,[g] Amy Xu, BS,[a] Jizhong Cheng, PhD,[h] Qingyun Liu, PhD,[i] Roger G. O'Neil, PhD,[j] Yang Xia, PhD,[f] Liang Ma, PhD,[k] Susan M. Carlton, PhD,[c] Brian S. Kim, MD,[a,k] Kenneth Renner, PhD,[l] Qin Liu, PhD,[a] and Hongzhen Hu, PhD[a]

St Louis, Mo; Nanjing, Chongqing, Shanghai, and Wuhan, China; Galveston and Houston, Tex; and Vermillion, SD

GRAPHICAL ABSTRACT



From [a]the Center for the Study of Itch, Department of Anesthesiology, and [k]the Division of Dermatology, Department of Medicine, Washington University School of Medicine, St Louis; [b]the School of Medicine and Life Sciences, Nanjing University of Chinese Medicine; [c]the Department of Neuroscience and Cell Biology, University of Texas Medical Branch, Galveston; [d]the Department of Anatomy, Chongqing Medical University; [e]the Department of Gastroenterology, Ruijin Hospital, Shanghai Jiaotong University, Shanghai; [f]the Department of Biochemistry and Molecular Biology and [j]the Department of Integrative Biology and Pharmacology, the University of Texas Medical School at Houston; [g]the College of Pharmacy, South-Central University for Nationalities, Wuhan; [h]the Department of Medicine, Baylor College of Medicine, Houston; [i]the Brown Foundation Institute of Molecular Medicine and Texas Therapeutics Institute, University of Texas Health Science Center at Houston; and [l]the Center for Brain and Behavior Research, Biology Department, University of South Dakota, Vermillion.

*These authors contributed equally to this work.

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Corresponding author: Hongzhen Hu, PhD, Or Qin Liu, PhD, Department of Anesthesiology, Center for the Study of Itch, Washington University School of Medicine in St Louis, Campus Box 8054, 660 South Euclid Ave, St Louis, MO 63110-1093. E-mail: hongzhen.hu@wustl.edu. Or: qinliu@wustl.edu.

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Background: Chronic itch is a highly debilitating symptom that underlies many medical disorders with no universally effective treatments. Although unique neuronal signaling cascades in the sensory ganglia and spinal cord have been shown to critically promote the pathogenesis of chronic itch, the role of skin-associated cells remains poorly understood.

Objective: We sought to examine the cutaneous mechanisms underlying transient receptor potential vanilloid 4 (TRPV4)–mediated allergic and nonallergic chronic itch.

Methods: Expression of TRPV4 in chronic itch and healthy control skin preparations was examined by using real-time RT-PCR. *Trpv4*^{eGFP} mice were used to study the expression and function of TRPV4 in the skin by means of immunofluorescence staining, flow cytometry, calcium imaging, and patch-clamp recordings. Genetic and pharmacologic approaches were used to examine the role and underlying mechanisms of TRPV4 in mouse models of dry skin–associated chronic itch and spontaneous scratching associated with squaric acid dibutylester–induced allergic contact dermatitis.

Results: TRPV4 is selectively expressed by dermal macrophages and epidermal keratinocytes in mice. Lineage-specific deletion of TRPV4 in macrophages and keratinocytes reduces allergic and nonallergic chronic itch in mice, respectively. Importantly, TRPV4 expression is significantly increased in skin biopsy specimens from patients with chronic idiopathic pruritus in comparison with skin from healthy control subjects. Moreover, TRPV4-dependent chronic itch requires 5-hydroxytryptamine (5-HT) signaling secondary to activation of distinct 5-HT receptors in both patients with allergic and those with nonallergic chronic itch conditions.

Conclusion: Our study reveals previously unrecognized mechanisms by which TRPV4-expressing epithelial and immune cells in the skin critically and dynamically mediate chronic itch and unravels novel targets for therapeutics in the setting of chronic itch. (J Allergy Clin Immunol 2017;■■■:■■■-■■■.)

Key words: *Transient receptor potential vanilloid 4, chronic itch, macrophage, keratinocyte*

Abbreviations used

ACD:	Allergic contact dermatitis
AEW:	Acetone/ether mixture followed by distilled water
$[Ca^{2+}]_i$:	Intracellular Ca^{2+}
CIP:	Chronic idiopathic pruritus
C_t:	Cycle threshold
DRG:	Dorsal root ganglion
DTX:	Diphtheria toxin
eGFP:	Enhanced green fluorescent protein
GAPDH:	Glyceraldehyde-3-phosphate dehydrogenase
GFP:	Green fluorescent protein
5-HT:	5-Hydroxytryptamine
Htr2a:	5-HT receptor 2a
Htr7:	5-HT receptor 7
IRB:	Institutional review board
PBS+TX:	PBS with 0.1% Triton X-100
pCPA:	p-Chlorophenylalanine
SADBE:	Squaric acid dibutylester
TPH:	Tryptophan hydroxylase
TRPV4:	Transient receptor potential vanilloid 4
WT:	Wild-type

Chronic itch, a symptom of many primary skin disorders and systemic diseases, is a major medical issue affecting 10% to 20% of the general population and has deleterious effects on both quality of life and productivity.[1,2] Despite decades of research, how chronic itch is generated at the molecular and cellular levels is poorly understood. Recent studies have identified multiple itch-related G protein–coupled receptors and ion channels in the primary sensory neurons, which enable sensory neurons to detect a variety of pruritogens.[3-5] However, upstream pathways, such as the identity of putative receptors that trigger epithelial and immune cells to elicit itch, remain unknown. Lack of this critical information has severely limited the development of effective therapies for most types of chronic itch.

Transient receptor potential vanilloid 4 (TRPV4) is a Ca^{2+}-permeable cation channel in the TRPV family and is abundantly expressed in the skin, renal, and urinary bladder epithelia (www.biogps.org).[6,7] TRPV4 is a polymodal sensory transducer that integrates a variety of thermal, mechanical, and chemical stimuli, including warmth (27°C to 35°C), hypo-osmotic stimulation, and many inflammatory metabolites.[8] As a result, TRPV4 channels are involved in many physiologic and pathologic processes. Although it was recently reported that TRPV4 is involved in acute itch elicited by exogenously applied histamine and 5-hydroxytryptamine (5-HT), its precise mechanism in itch induction remains controversial. Indeed, whether TRPV4 predominantly mediates itch indirectly through skin-associated cells or by directly stimulating dorsal root ganglion (DRG) neurons is an active area of investigation.[9,10] More importantly, the role of TRPV4 in the development of chronic itch remains unexplored.

In the current study we show that these osmosensitive TRPV4 channels are selectively expressed by skin keratinocytes and dermal macrophages and that their activation promotes downstream 5-HT signaling, resulting in itch-specific behavioral responses in mouse models of chronic itch. Importantly, TRPV4-expressing macrophages and keratinocytes are differentially involved in the generation of spontaneous itch behaviors in mouse models of allergic and nonallergic chronic itch, respectively. Furthermore, we identified increased expression of TRPV4 in the skin of patients with chronic idiopathic pruritus (CIP). Collectively, our data demonstrate that TRPV4-expressing cells in the skin are a critical component in the pathogenesis of chronic itch.

METHODS

Animals

Adult male and female C57BL/6J (Jackson Laboratories, Bar Harbor, Me), *Trpv4*^{eGFP} (Mutant Mouse Regional Resource Centers), *Trpv4*$^{-/-}$,[11] *Kit*$^{W\text{-}sh/W\text{-}sh}$ (Jackson Laboratories), 5-HT receptor 7 (*Htr7*$^{-/-}$) (Jackson Laboratories), and 5-HT receptor 2a (*Htr2a*)$^{-/-}$ (a kind gift from Dr Jay Gingrich at Columbia University) mice were used for the study. *Cre*$^{+}$ and *Cre*$^{-}$ *Pf4*^{Cre};*iDTR* (Pf4-Cre^{+} and Pf4-Cre^{-}) mice were obtained by crossing *Rosa26*^{iDTR} mice (Jackson Laboratories) with *Pf4-Cre* mice (Jackson Laboratories). To generate *Trpv4*$^{f/f}$ mice, 3 of the properly targeted embryonic stem cell clones were obtained from the Knockout Mouse Project Repository and used for blastocyst injections, and 1 clone led to high-contribution chimeras that produced germline-transmitted offspring, as assayed by using black coat color. This chimera line was then mated to FLPo mice (Jackson Laboratories) to remove the neomycin cassette and generate heterozygous *Trpv4*$^{f/+}$ mice,