NEUROSCIENCE

Piezo2 channel–Merkel cell signaling modulates the conversion of touch to itch

Jing Feng,[1] Jialie Luo,[1] Pu Yang,[1] Junhui Du,[2] Brian S. Kim,[1,3,4] Hongzhen Hu[1]*

The somatosensory system relays many signals ranging from light touch to pain and itch. Touch is critical to spatial awareness and communication. However, in disease states, innocuous mechanical stimuli can provoke pathologic sensations such as mechanical itch (alloknesis). The molecular and cellular mechanisms that govern this conversion remain unknown. We found that in mice, alloknesis in aging and dry skin is associated with a loss of Merkel cells, the touch receptors in the skin. Targeted genetic deletion of Merkel cells and associated mechanosensitive Piezo2 channels in the skin was sufficient to produce alloknesis. Chemogenetic activation of Merkel cells protected against alloknesis in dry skin. This study reveals a previously unknown function of the cutaneous touch receptors and may provide insight into the development of alloknesis.

Chronic pruritus or itch is an increasingly common and debilitating problem in the elderly. Age-related dry skin is strongly associated with alloknesis, whereby the sensation of itch is evoked by innocuous mechanical stimulation such as light touch. We adapted the well-established von Frey technique to mechanically irritate the skin of young and aged mice (2 months and >24 months of age, respectively) at varying levels of mechanical force. Mechanical stimulation ranging from 0.02 to 0.16 g evoked scratching behavior in aged mice in a manner not observed in young mice (Fig. 1A and movies S1 and S2). In contrast, acute itch in response to classical pruritogens such as histamine (His) or chloroquine (CQ) was unaffected by aging (Fig. 1, B and C). Mechanical pain (2.0 g von Frey hair force) and thermal pain responses did not reveal any differences in aged mice relative to young mice (fig. S1).

Recent studies have identified a subpopulation of neuropeptide Y (NPY)–positive interneurons that are innervated by hairy-skin low-threshold mechanoreceptors (LTMRs) and critically regulate mechanical itch in the spinal cord (*1*). To investigate whether aging also affects the firing properties of LTMRs, we analyzed the firing patterns of different types of cutaneous fibers by means of ex vivo skin-nerve recordings (*2*–*6*). In marked contrast to the sustained firing of type I slowly adapting (SAI) afferents in young mice, skin-nerve preparations from the aged mice displayed truncated static firing, which was classified as intermediately adapting (IA) responses (Fig. 1, E and F) (*4*). The firing rates of SAI afferents elicited by a range of mechanical force from 1 to 150 mN were significantly reduced starting from 50 mN (Fig. 1, D and E), and the firing threshold of SAI afferents was markedly increased in aged mice versus young mice (Fig. 1G). Spike counts in both dynamic and static phases were also markedly reduced in aged mice (Fig. 1, H and I). However, comparable firing properties of rapidly adapting (RA), A delta (Aδ), and C LTMRs were evident between aged and young mice (figs. S2 to S4). The overall proportions of Aβ fiber subtypes and conduction velocities were also similar in the two groups (fig. S5). Although the spinal NPY-expressing interneurons were reported to selectively receive mechanosensory inputs only from hairy skin (*1*), we found no significant difference in firing properties between the SAI fibers innervating hairy and glabrous skin in either young or aged mice (fig. S6), which suggests that both NPY^+ and unidentified NPY^- inhibitory interneurons likely receive inputs from SAI afferents.

Because Merkel cells in the epidermis and SAI afferents make "synapse-like" contacts and encode unique SAI firing patterns (*3*, *4*, *7*–*9*), we speculated that Merkel cells may act upstream and thus may be implicated in the selective attenuation of the SAI responses in the setting of aging-associated alloknesis. We therefore used immunofluorescent staining to determine the numbers and morphology of Merkel cells in aged skin. There were significantly fewer Merkel cells in the epidermal mechanosensory touch domes of aged mice (Fig. 1, J and K). In contrast, the afferent ending structures in the touch domes were intact and comparable between the aged and young mice (Fig. 1, J and L). Given that Merkel cells are specifically required for sustained action potential firing of touch-dome afferents (*8*), the loss of Merkel cells in aging skin may promote alloknesis.

We thus used the well-established acetone-ether-water (AEW) model, which recapitulates dry skin itch as observed in elderly patients and manifests alloknesis (*10*–*13*). AEW-treated mice displayed enhanced alloknesis relative to water-treated control mice starting from day 3, and the alloknesis score reached a plateau at day 5 (Fig. 2A). The number of Merkel cells was markedly reduced in the AEW-treated mice relative to control mice, whereas the total length of the nerve fibers was comparable between the two groups (Fig. 2, B to D). We next investigated whether the loss of Merkel cells affects the SAI responses by targeted recording from touch-dome afferents. The touch-dome afferents from the AEW-treated mice also displayed markedly different firing pattern of IA responses versus the SAI responses in water-treated mice (Fig. 2, E and H). Although the conduction velocities of SAI afferents were comparable in both groups (fig. S7), touch-dome afferents from the AEW-treated mice displayed significantly higher firing thresholds (Fig. 2, F and G) and fewer firing counts in both dynamic and static phases (Fig. 2, I and J).

To directly test the contribution of Merkel cells in regulating alloknesis, we measured mechanical itch in Merkel cell–deficient $K14^{Cre}$; $Atoh1^{f/f}$ mice (*4*, *14*). These mice showed significantly increased scratching in response to mild von Frey filament stimulations when compared with Cre^- littermate control mice (Fig. 3A). Neither acute itch responses induced by intradermal injections of His and CQ nor evoked mechanical and thermal pain responses were altered in the $K14^{Cre}$; $Atoh1^{f/f}$ mice (fig. S8).

We then tested whether activation of Merkel cells could act on the same pathways that would suppress alloknesis. By crossing the $Atoh1^{CreERT}$; *Ai9* reporter mice with *Gq*-coupled *DREADD* (designer receptors exclusively activated by designer drugs) mice, we generated mice in which Merkel cells could be selectively activated by chemogenetic stimulation (*15*). After tamoxifen induction, the $Atoh1^{CreERT}$; *Ai9* reporter mice showed strong expression of tdTomato in the Merkel cells but not other structures such as sensory nerve endings (fig. S9). The DREADD ligand clozapine N-oxide (CNO) elicited a rapid intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) response in the tdTomato-positive Merkel cells in the enlargement segment of freshly isolated whisker hair follicles from Cre^+ mice but not Cre^- *Atoh1-Gq-DREADD* mice (Fig. 3B). Treatment with CNO significantly increased the firing frequency (Fig. 3, C and D), especially the static phase of SAI firing, in response to mechanical stimulation forces in the skin-nerve preparations from the AEW-treated Cre^+ mice but not Cre^- *Atoh1-Gq-DREADD* mice (Fig. 3G). This finding suggests that activation of *Gq-DREADDs* in spared Merkel cells promotes sustained firing of the SAI afferents in the AEW-treated mice. We therefore tested whether broad chemogenetic activation of Merkel cells could suppress alloknesis in the *Atoh1-Gq-DREADD* mice by measuring mechanical itch at day 5 after AEW treatment. Indeed, the administration of CNO significantly suppressed alloknesis

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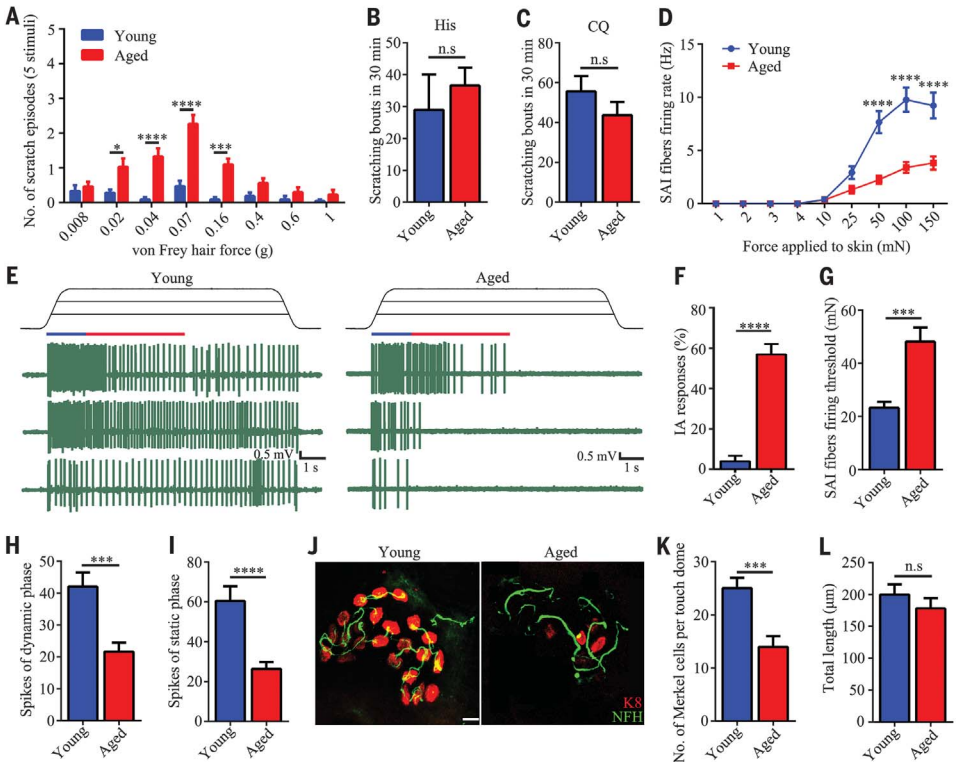

Fig. 1. Alloknesis in aged mice is correlated with a decline in SAI responses and reduced numbers of touch-dome Merkel cells. (**A**) Alloknesis scores in young (n = 7) and aged (n = 10) mice. (**B** and **C**) Scratching responses to intradermal injections of His (B) and CQ (C) in young (n = 8) and aged (n = 8) mice. (**D**) Firing rates of SAI fibers from young (23 units) and aged (38 units) mice. (**E**) Touch-dome afferents firing in young and aged mice. Top, ramp-and-hold displacements (50, 100, and 150 mN); bottom, corresponding spike trains. Blue and red lines indicate dynamic and static phases. (**F**) Proportion of IA responses from young (4.0 ± 2.6%) and aged (56.9 ± 5.1%) mice; n = 7 mice for each group. (**G**) Firing threshold of SAI fibers from young (25.8 ± 2.5 mN, n = 23) and aged (47.4 ± 6.8 mN, n = 38) mice. (**H**) Maximum number of spikes in the dynamic phases; n = 23 from young mice (42.0 ± 4.4 counts), n = 38 from aged mice (21.9 ± 2.9 counts). (**I**) Maximum number of spikes in the static phases; n = 23 from young mice (60.5 ± 7.3 counts), n = 38 from aged mice (26.4 ± 3.5 counts). (**J**) Confocal images of keratin-8 (K8, red) and neurofilament heavy polypeptide (NFH, green) costaining in a single touch dome. Scale bar, 10 μm. (**K**) Merkel cell numbers in each touch dome of young (25.1 ± 1.9, n = 30) and aged (14.0 ± 2.0, n = 30) mice. (**L**) Length of NFH$^+$ fiber innervating touch domes of young (200.2 ± 15.8 μm, n = 30) and aged (178.2.0 ± 16.2 μm, n = 30) mice. Data are presented as mean ± SEM. *P < 0.05, ***P < 0.001, ****P < 0.0001 [Student t test in (A) to (C), (H), (I), (K), and (L); two-way analysis of variance (ANOVA) with Bonferroni post hoc analysis in (D); Fisher's exact test in (F); Mann-Whitney U test in (G)]; n.s, not significant.

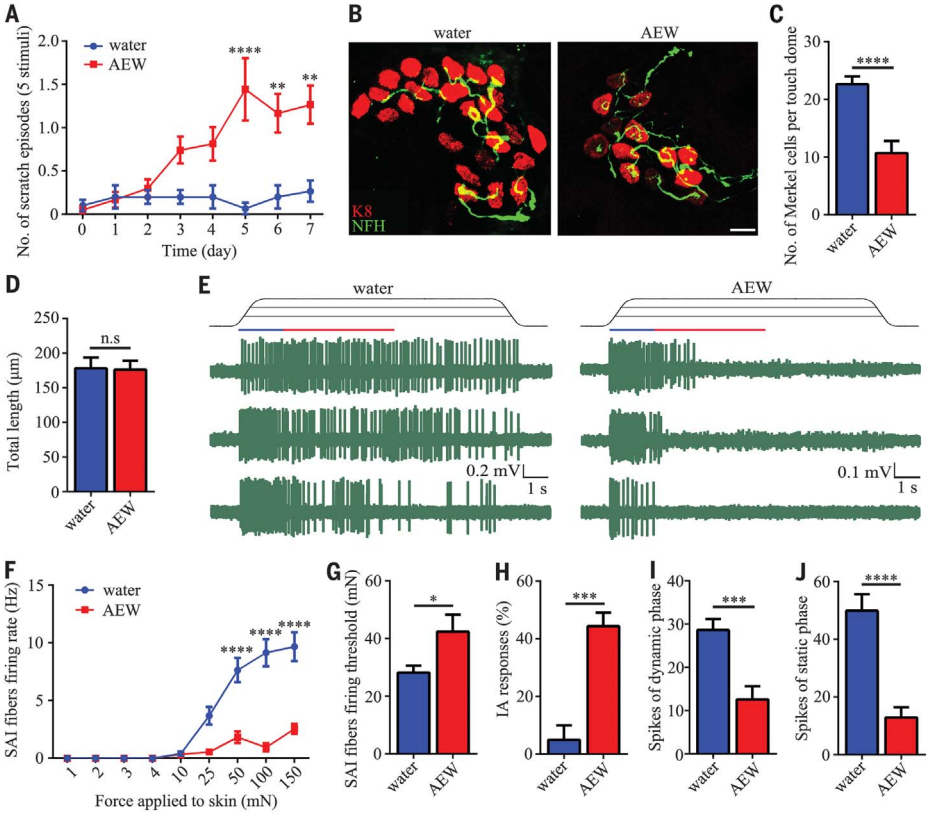

Fig. 2. Alloknesis in a mouse model of dry skin is correlated with a decline in SAI responses and reduced numbers of touch-dome Merkel cells. (**A**) Time course of alloknesis in mice treated with AEW (n = 10) or water (n = 10). (**B**) Confocal images of K8 (red) and NFH (green) costaining in a single touch dome. Scale bar, 10 μm. (**C**) Merkel cell numbers in touch domes of water-treated (22.6 ± 1.3, n = 26) and AEW-treated (9.6 ± 1.9, n = 26) mice. (**D**) Length of NFH$^+$ fiber innervating touch domes of water-treated (178.3 ± 15.4 μm, n = 26) and AEW-treated (176.4 ± 13.0 μm, n = 26) mice. (**E**) Firing traces of SAI afferents in response to mechanical stimulations (50, 100, and 150 mN) in water-treated and AEW-treated mice. (**F**) Firing rates of SAI fibers from water-treated (n = 17 units) and AEW-treated (n = 19 units) mice. (**G**) Firing threshold of SAI afferents of water-treated (29.3 ± 2.5 mN, n = 20) and AEW-treated (44.4 ± 8.1 mN, n = 19) mice. (**H**) Proportion of IA responses of water-treated (5.0 ± 5.0%, n = 5) and AEW-treated (41.7 ± 4.8%, n = 5) groups. (**I**) Maximum number of spikes in the dynamic phases (water-treated, 28.6 ± 2.5 counts, n = 20; AEW-treated, 13.6 ± 4.3 counts, n = 19). (**J**) Maximum number of spikes in the static phases (water-treated, 50.0 ± 5.8 counts, n = 20; AEW-treated, 9.5 ± 2.3 counts, n = 19). Data are presented as mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 [two-way ANOVA with Bonferroni post hoc analysis in (A) and (F); Student t test in (C), (D), (I), and (J); Mann-Whitney U test in (G); Fisher's exact test in (H)].

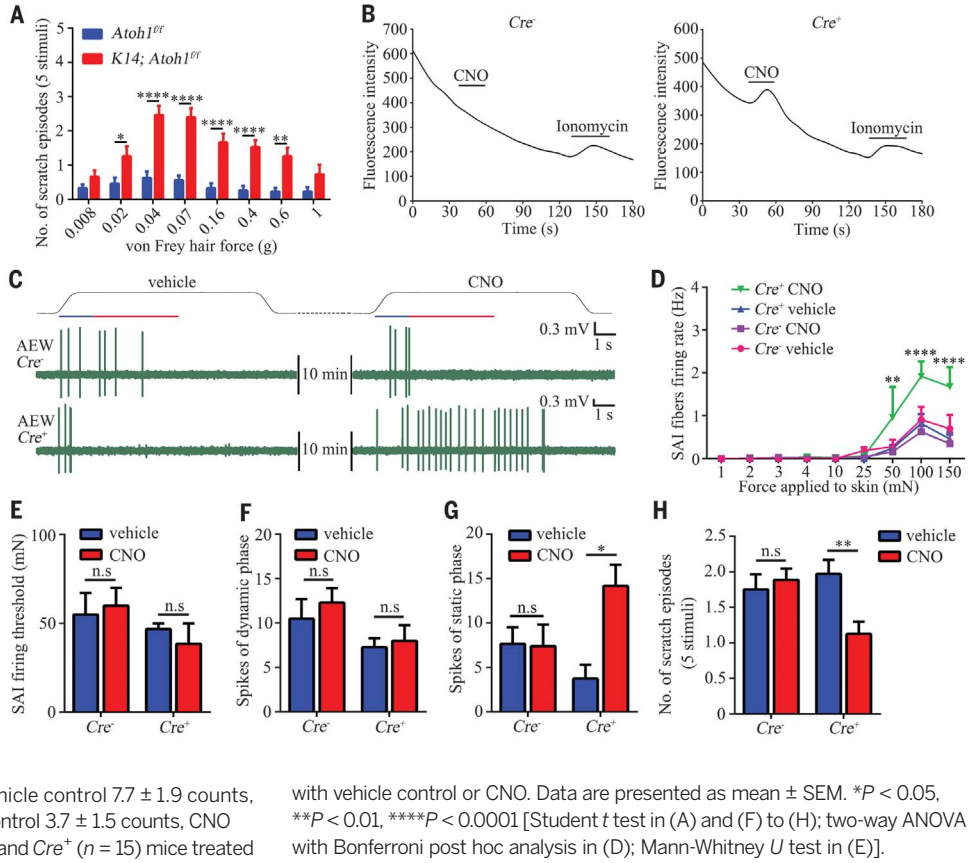

Fig. 3. Chemogenetic activation of Merkel cells is sufficient to modulate alloknesis. (**A**) Alloknesis score in Merkel cell–deficient *K14^{Cre}; Atoh1$^{f/f}$* mice (n = 10) and wild-type littermates (n = 10). (**B**) Traces showing that 300 μM CNO elicited $[Ca^{2+}]_i$ responses in tdTomato-positive Merkel cells in hair follicles isolated from *Cre*$^+$ but not *Cre*$^-$ mice. Ionomycin (10 μM) was used as positive control; n = 25 hair follicles from 5 mice. (**C**) Firings of SAI afferents of AEW-treated *Cre*$^-$ and *Cre*$^+$ mice in response to 100-mN mechanical stimulation in the absence and presence of 300 μM CNO. (**D**) Firing rates of SAI fibers of AEW-treated *Cre*$^-$ (n = 10 units) and *Cre*$^+$ (n = 5 units) mice in response to increased forces of mechanical stimulation in the absence and presence of 300 μM CNO. (**E**) Firing threshold of SAI afferents of AEW-treated *Cre*$^-$ and *Cre*$^+$ mice in the absence and presence of 300 μM CNO (*Cre*$^-$ n = 10, vehicle control 55.0 ± 12.2 mN, CNO 60.0 ± 10.0 mN; *Cre*$^+$ n = 5, vehicle control 46.9 ± 3.1 mN, CNO 38.5 ± 11.5 mN). (**F**) Maximum number of spikes in the dynamic phases (n = 5 *Cre*$^-$ mice, vehicle control 10.5 ± 2.2 counts, CNO 12.3 ± 1.6 counts; n = 5 *Cre*$^+$ mice, vehicle control 7.3 ± 1.0 counts, CNO 8.0 ± 1.8 counts). (**G**) Maximum number of spikes in the static phases (n = 5 *Cre*$^-$ mice, vehicle control 7.7 ± 1.9 counts, CNO 7.4 ± 2.4 counts; n = 5 *Cre*$^+$ mice, vehicle control 3.7 ± 1.5 counts, CNO 14.2 ± 2.4 counts). (**H**) Alloknesis of *Cre*$^-$ (n = 15) and *Cre*$^+$ (n = 15) mice treated with vehicle control or CNO. Data are presented as mean ± SEM. *P < 0.05, **P < 0.01, ****P < 0.0001 [Student t test in (A) and (F) to (H); two-way ANOVA with Bonferroni post hoc analysis in (D); Mann-Whitney U test in (E)].

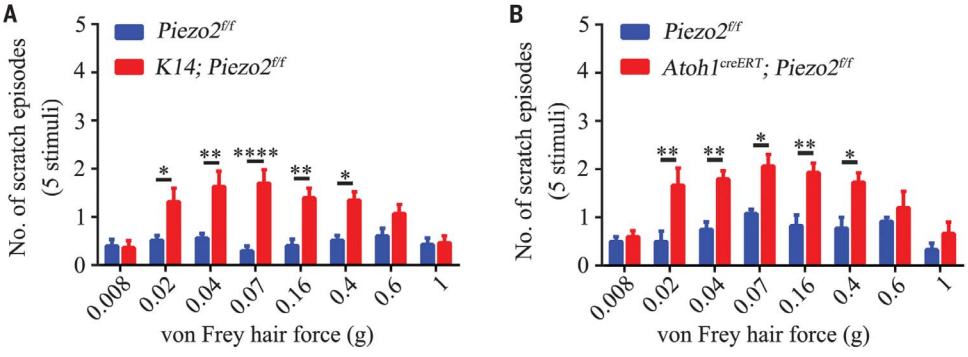

Fig. 4. Genetic ablation of Piezo2 function in Merkel cells produces alloknesis. (**A**) Alloknesis score in *K14^{Cre}; Piezo2$^{f/f}$* mice (n = 10) and wild-type *Piezo2$^{f/f}$* littermates (n = 10). (**B**) Alloknesis score in *Atoh1^{CreERT}; Piezo2$^{f/f}$* mice (n = 5) and wild-type *Piezo2$^{f/f}$* littermates (n = 4). Data are presented as mean ± SEM. *P < 0.05, **P < 0.01, ****P < 0.0001 (Student t test).

in *Cre*$^+$ *Atoh1-Gq-DREADD* mice when compared with vehicle. The alloknesis score was comparable between the vehicle-injected and CNO-injected *Cre*$^-$ littermates (Fig. 3H).

The mechanosensitive ion channel Piezo2 is a canonical light-touch receptor expressed by cutaneous Merkel cells (*16*) and is required for normal SAI responses (*4*, *8*). We directly tested whether Piezo2 was involved in the modulation of mechanical itch in the skin. *Cre*$^+$ *K14^{Cre}; Piezo2$^{f/f}$* mice displayed markedly increased alloknesis to low mechanical stimulation relative to *Cre*$^-$ mice (Fig. 4A). We then specifically ablated Piezo2 function in Merkel cells by crossing *Atoh1^{CreERT}* with *Piezo2$^{f/f}$* mice. *Cre*$^+$ but not *Cre*$^-$ mice showed significantly increased mechanical itch behavior after tamoxifen induction (Fig. 4B). In contrast, itch responses induced by His or CQ were comparable between *Cre*$^-$ and *Cre*$^+$ *K14^{Cre}; Piezo2$^{f/f}$* mice and *Atoh1^{CreERT}; Piezo2$^{f/f}$* mice (fig. S10, A, B, E, and F). Neither mechanical nor thermal pain responses were altered in the *K14^{Cre}; Piezo2$^{f/f}$* and *Atoh1^{CreERT}; Piezo2$^{f/f}$* mice (fig. S10, C, D, G, and H).

Our results show that cutaneous Piezo2 channel–Merkel cell signaling is critical in modulating the conversion of touch to itch, and is evidently required for the normal function of peripheral mechanoreceptors that convert mechanical stimuli from the skin to the spinal cord; in turn, these stimuli may control the activity of both NPY$^+$ and NPY$^-$ inhibitory interneurons (*1*) (fig. S11). Our findings may also offer an explanation for the loss of mechanical itch gating under aging and chronic itch conditions.

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SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/360/6388/530/suppl/DC1
Materials and Methods
Figs. S1 to S11
Movies S1 and S2

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Piezo2 channel–Merkel cell signaling modulates the conversion of touch to itch

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Loss of touch receptors leads to itch

Itch in response to light touch of the skin is an aging-associated problem. This phenomenon is called alloknesis and can become a major medical condition associated with dry skin. Feng *et al.* discovered that loss or dysfunction of Merkel cells causes scratching in mice (see the Perspective by Lewis and Grandl). Reduction of Merkel cell numbers results in reduced firing patterns and frequencies and changes the activation thresholds of slowly adapting afferent nerve fibers. Like hair cells, Merkel cells are lost with age. A painful scratch will temporarily alleviate itch because it induces enough activity through the remaining Merkel cells.

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