# Molecular link between itch and atopic dermatitis

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Atopic dermatitis (AD) is a relapsing inflammatory skin disease often associated with intractable chronic itch. The sensation of itch depends on the activity of pruriceptive sensory neurons whose nerve fibers innervate the dermis and epidermis. These fibers can respond to factors secreted by keratinocytes (e.g., thymic stromal lymphopoietin) and immune cells (e.g., IL-31, histamine, and proteases) (1). However, the pathogenesis of chronic itch and inflammation in AD is not well understood, and therapeutic options are limited. In PNAS, Emrick et al. (2) perform an elegant study in which they determine the cellular and molecular mechanisms by which the AD-associated gene *Tmem79* drives skin inflammation and itch. Emrick et al. find that TMEM79 acts as a putative glutathione transferase to decrease oxidative stress in keratinocytes to prevent mast cell activation and histamine-driven neuronal activation and itch (Fig. 1). This study is an important advance in our understanding of the molecular and cellular mechanisms of AD.

AD affects ∼20% of children in the developed world and 2 to 8% of adults, with 87 to 100% of these patients experiencing chronic itch (3, 4). It is generally thought that AD is caused by a combination of skin barrier dysfunction and immune dysregulation. Several genetic predispositions have been linked to AD. The most common mutations in AD patients are loss-of-function mutations in the filaggrin (FLG) gene (5). FLG encodes for profilaggrin, a protein at the skin barrier necessary to maintain epidermal hydration and low pH. *Tmem79* is another gene recently linked to AD in animals and humans (5–7). Mutations in the *Tmem79* gene are responsible for the matted hair and AD-like phenotypes observed in flaky tail (*ma/ma* $Flg^{ft/ft}$) mice, characterized by skin lesions, dermal thickening, and inflammation (5–7). In humans, a missense mutation in the *Tmem79* gene was recently discovered and associated with AD patients (6). *Tmem79* encodes a protein with five transmembrane domains, found to be expressed in the outermost layers of the stratum granulosum in the epidermis (7). However, the molecular functions of TMEM79 and how its deficiency drives AD are not clear.

Emrick et al. (2) used transgenic and knockout mouse studies to determine the role of *Tmem79* in skin inflammation and itch. They generated $Tmem79^{-/-}$ mice, which developed spontaneous itch, dermal thickening, and immune cell accumulation in the skin. $Tmem79^{-/-}$ mice showed significant expansion of mast cells and IL-17–expressing γδ-T cells. Next, to determine the cell types in which *Tmem79* was specifically expressed and that mediated its function, Emrick et al. generated a *Tmem79*-knockin GFP reporter mouse with an inverted floxed cassette, allowing the analysis of *Tmem79* promoter-driven gene expression and subsequent targeted deletion of this gene from specific cell types via Cre recombinase. The authors found that *Tmem79* was expressed both by keratinocytes and dorsal root ganglion sensory neurons. A majority of $Tmem79^{+}$ neurons also expressed histamine receptor 4 (H4R) (80%) and peripherin (74%), which marks small-diameter C-fiber sensory neurons. Using keratinocyte-specific (K14-Cre) and sensory neuron-specific (Prph-Cre) deletion of *Tmem79*, Emrick et al. show that both cell types contribute to itch. While keratinocyte ablation of *Tmem79* induced significant itch, *Tmem79* deletion from both keratinocytes and neurons induced greater itch and skin inflammation to levels that phenocopied $Tmem79^{-/-}$ mice.

To investigate the molecular function of TMEM79, Emrick et al. (2) performed homology analysis and found that TMEM79 shared 30% amino acid sequence homology with microsomal glutathione transferases [i.e., membrane-associated proteins in eicosanoid and glutathione metabolism (MAPEGs)], a family of detoxification enzymes that protect cellular macromolecules from attack by reactive species (RS) (8). This led Emrick et al. (2) to hypothesize that TMEM79 could act as a glutathione transferase to decrease oxidative stress in cells. The authors found that $Tmem79^{-/-}$ keratinocytes had greater accumulation of RS when challenged with the oxidant 3-morpholinosydnonimine (SIN-1), and that HEK293T cells overexpressing TMEM79 better reduced RS in response to SIN-1. These data demonstrate

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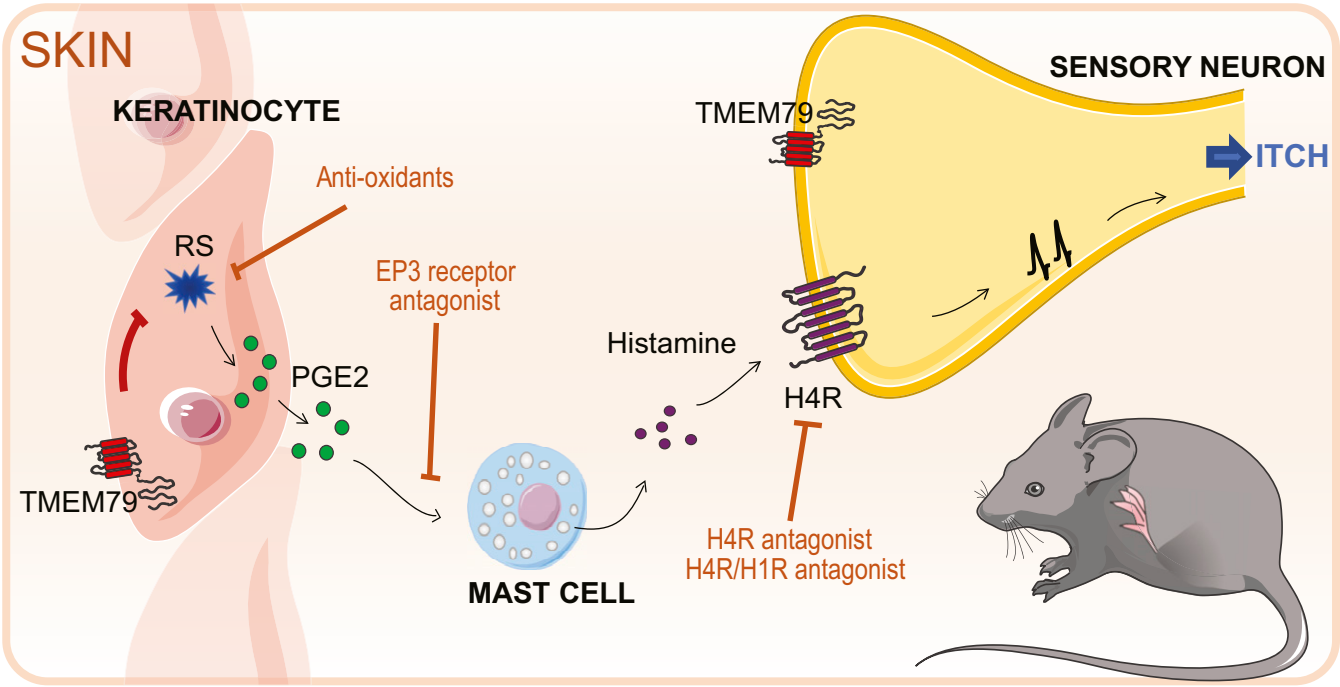


**Fig. 1.** ***Tmem79*** **reduces oxidative stress to protect against itch and AD.** ***Tmem79*****, a gene linked to AD, is expressed in keratinocytes and sensory neurons. Emrick et al. (2) find that loss of** ***Tmem79*** **causes an increase in RS in keratinocytes and the induction of $PGE_2$ levels. $PGE_2$ acts through EP3 receptors to recruit dermal mast cells. Mast cell degranulation results in the release of histamine, which acts on H1R and H4R expressed by C-fiber sensory neurons to drive itch. Potential strategies to inhibit itch in AD suggested by this study include the use of antioxidants, EP3 receptor antagonists, and histamine receptor (H4R/H1R) antagonists.**

that TMEM79 is necessary and sufficient to regulate RS. The protective effect against RS was lost when two putative active sites were mutated in the *Tmem79* region homologous with MAPEG family members.

Keratinocytes respond to oxidative stress by producing eicosanoids, including prostaglandin $E_2$ ($PGE_2$) (9). Emrick et al. (2) hypothesized that elevated oxidative stress in *Tmem79*$^{-/-}$ mice could result in increased $PGE_2$ and downstream inflammation. $PGE_2$ levels were indeed increased in keratinocytes from *Tmem79*$^{-/-}$ mice, which may relate to increased levels of $PGE_2$ observed in skin plaques from AD patients (10). Blocking prostaglandin production using a cyclooxygenase inhibitor or blocking the $PGE_2$ receptor EP3 reduced scratching and accumulation of dermal mast cells in *Tmem79*$^{-/-}$ mice (2). These findings nicely complement a study showing that $PGE_2$ triggers mast cell activation through EP3 receptors (11). Given the key role of mast cells in allergic inflammation, Emrick et al. (2) next investigated whether they mediated itch in *Tmem79*$^{-/-}$ mice. They found that blocking mast cell degranulation reduced scratching in *Tmem79*$^{-/-}$ mice. Mast cells release histamine, a potent inducer of pruriception and itch. Emrick et al. found that blocking H4R reduced scratching in *Tmem79*$^{-/-}$ mice, while blocking only the histamine receptor 1 (H1R) had no effect; however, blocking both receptors (H4R and H1R) completely abolished scratching behaviors. They next found that H4R agonists triggered calcium influx into sensory neurons independent of the transient receptor potential (TRP) ion channels TRPV1 and TRPA1. A TRPA1 antagonist partially reduced scratching in *Tmem79*$^{-/-}$ mice, indicating a role for TRPA1 in itch-induction mechanisms that remains to be clarified. Together, these data indicate that loss of *Tmem79* in keratinocytes leads to elevation of $PGE_2$ levels, which activates EP3 signaling in mast cells. Mast cells then release histamine, which acts on sensory neurons via H4R/H1R receptors to drive itch (Fig. 1).

In AD, the molecular mechanisms involved in linking itch, inflammation, and barrier dysfunction remain poorly understood. Current hypotheses concerning these mechanisms include increased transepidermal water loss (TEWL), with subsequent epidermal pH rise and protease activation, which could explain how TEWL is correlated with itch intensity in patients (4). Dysfunction of the barrier could also allow irritants and allergens to enter more easily, including bacterial products and proteases that directly or indirectly activate sensory neurons to mediate itch. Another promising hypothesis is an underlying role for oxidative stress in AD. For the last 15 y, studies have shown that AD patients showed evidence of oxidative stress (e.g., nitrite/nitrate and lipid peroxidation) and decreased antioxidant capability (e.g., lower levels of vitamins A, C, and E) (12). A polymorphism in GST was recently associated with increased AD susceptibility (13). With this study, Emrick et al. (2) contribute to the emerging concept that oxidative stress could mediate AD, by showing that TMEM79 belongs to a protective system against oxidative stress in keratinocytes and potentially in sensory neurons, and that loss of this protection leads to mast cell activation, dermatitis, and chronic itch.

Therapeutic options are limited to effectively treat AD, especially for the chronic itch that remains a huge burden on the quality of life for many patients. In recent years, there has been a push to translate several therapies specifically targeting immune cell signaling and cytokines to treat AD (e.g., dupilumab, an IL-4/IL-13 signaling blocker; and nemolizumab, an IL-31 receptor inhibitor) (14). The findings by Emrick et al. (2) provide arguments for several targeted strategies to block itch in AD (Fig. 1). While it remains to be determined whether these mechanisms apply more broadly to AD or mainly to patients bearing a *Tmem79* mutation, potential strategies include reducing oxidative stress, blocking the $PGE_2$/EP3 pathway, or blocking histamine signaling in neurons



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using an H4R antagonist or a combination of H4R/H1R antagonists. A recent trial showed promising, although not conclusive, results using an H4R inhibitor in alleviating pruritus in AD patients (15). While the trial was terminated because of off-target effects of the inhibitor, H4R antagonists without side effects are currently under investigation (16). As well as being a relevant model of human disease, the *Tmem79*$^{-/-}$ mouse may be a useful preclinical tool for development of novel therapeutics to treat inflammation and itch in AD.

In addition to therapeutic implications, the study by Emrick et al. (2) provides insights into the function of TMEM79 that merit future investigation. Biochemical studies are needed to define the exact protein domains and molecular mechanisms by which TMEM79 mediates RS reduction. Previous studies have shown that loss-of-function mutations in *Tmem79* induced abnormal function of lamellar granules, including reduced protein secretion and impaired stratum corneum formation (7). It would be interesting to determine whether the role of TMEM79 in oxidative stress also impacts lamellar granule secretion. It would also be informative to determine whether TMEM79 reduces oxidative stress in sensory neurons. Emrick et al. (2) make the interesting observation that γδ-T cells and IL-17 production were significantly elevated in *Tmem79*$^{-/-}$ mice. AD has potential links with IL-17–driven pathology (17). It would be interesting in future work to determine whether γδ-T cells or IL-17 signaling also drives skin thickening or itch induction in *Tmem79*$^{-/-}$ mice.

In summary, the Emrick et al. (2) study is a tour de force, establishing a molecular link between chronic itch and AD. It shows that loss of the AD-associated gene *Tmem79* in keratinocytes and neurons results in skin inflammation and itch. The study demonstrates a protective role of TMEM79 against oxidative stress, which prevents keratinocyte-mediated $PGE_2$ induction, mast cell accumulation, and histaminergic itch. It also introduces exciting research questions about the TMEM79 protein and highlights potential therapeutic avenues for AD.

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