



The Genetics of Chronic Itch: Gene Expression in the Skin of Patients with Atopic Dermatitis and Psoriasis with Severe Itch

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To identify itch-related mediators and receptors that are differentially expressed in pruritic skin, we used RNA sequencing to analyze the complete transcriptome in skin from paired itchy, lesional and nonitchy, nonlesional skin biopsies from 25 patients with atopic dermatitis and 25 patients with psoriasis and site-matched biopsies from 30 healthy controls. This analysis identified 18,000 differentially expressed genes common between itchy atopic and psoriatic skin compared with healthy skin. Of those, almost 2,000 genes were differentially expressed between itchy and nonitchy skin in atopic and psoriatic subjects. Overexpression of several genes, such as phospholipase A2 IVD, substance P, voltage-gated sodium channel 1.7, and transient receptor potential (TRP) vanilloid 1, in itchy skin was positively correlated with itch intensity ratings in both atopic dermatitis and psoriasis. Cytokines such as IL-17A, IL-23A, and IL-31 had elevated gene transcript levels in both itchy atopic and psoriatic skin. However, expression of genes for TRP vanilloid 2, TRP ankyrin 1, protease-activated receptor 2, protease-activated receptor 4, and IL-10 was found to be increased only in pruritic atopic skin, whereas expression of genes for TRP melastatin 8, TRP vanilloid 3, phospholipase C, and IL-36 α/γ was elevated only in pruritic psoriatic skin. This “itchscriptome” analysis will lead to an increased understanding of the molecular mechanisms of chronic pruritus and provide targets for itch treatment irrespective of disease state.

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INTRODUCTION

The majority of patients with chronic itch have a diagnosis of atopic dermatitis (AD) or psoriasis (PS) (Feramisico et al., 2010). Between 87% and 100% of patients with AD report chronic itch, making it a defining feature of the disease (Dawn et al., 2009; Yosipovitch et al., 2002). In patients with PS, pruritus is the second most (79%) reported symptom (Yosipovitch et al., 2000). Although pruritus research has advanced in the last decade, the exact mechanisms and genetics involved in AD and PS itch are still unknown.

Because itch involves a complex interaction among skin cells, immune cells, secreted factors, and cutaneous neural networks, there is no one specific cause of chronic itch. Furthermore, these components may interact differently in various pruritic disease states and ethnicities (Noda et al., 2015). Identifying the elements that are unique to each disease state and those shared across multiple pruritic diseases is crucial for the development of itch-specific drug therapies.

RNA sequencing (RNA-seq) allows for precise gene identification and quantification of gene expression, even when genes are expressed at low levels (Wang et al., 2009). Although a few studies have used genotyping by RNA-seq to examine the genetics involved in AD and PS (Jabbari et al., 2012; Li et al., 2014; Suárez-Fariñas et al., 2015), no study has yet examined gene expression specifically linked to chronic itch. The previous genotypic studies conducted in AD and PS did not include any assessment of itch in their subjects. To better understand itch on the peripheral level, we aimed to assess the genetic expression profiles in the skin of patients with chronic itch from AD and PS, comparing genetic expression with itch severity scores and across ethnicities. After identifying differentially expressed genes (DEGs) of interest, we used immunohistochemistry to examine the level of corresponding protein expression in itchy and nonitchy skin. This “itchscriptome” analysis elucidates specific genotypic features that define chronic itch in AD and PS.

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Abbreviations: AD, atopic dermatitis; CCL, chemokine (C-C motif) ligand; CXCL, chemokine (C-X-C motif) ligand; CXCR, chemokine (C-X-C motif) receptor; DEG, differentially expressed gene; FC, fold changes; KLK, kallikrein; MRGPR, Mas-related G-protein coupled receptor; Nav, voltage-gated sodium channels; NK-1R, neurokinin-1; PAR, protease-activated receptor; PLA2, phospholipase A2; PS, psoriasis; RNA-seq, RNA sequencing; TRP, transient receptor potential; TRPA1, TRP ankyrin 1; TRPV1, TRP vanilloid 1

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RESULTS

Analysis of DEGs

This analysis identified 18,000 DEGs common between itchy, lesional atopic, and psoriatic skin compared with healthy skin (Supplementary Table S1 online; Tab 1). Of those, almost 2,000 genes were differently expressed between itchy and nonitchy skin in atopic and psoriatic subjects. A heat map summarizing key, established itch-associated DEGs is shown in Figure 1. For these genes, fold changes (FC) and correlations to itch intensity ratings are listed in Supplementary Table S1 (Tab 2).

DEGs in pruritic skin (compared with nonpruritic skin) in both atopic and psoriatic subjects included those encoding several inflammatory mediators, such as chemokine (C-C motif) ligand (*CCL* 2, *CCL3*, *CCL17*, *CCL18*), chemokine (C-X-C motif) ligand (*CXCL* 1, *CXCL10*), chemokine (C-X-C motif) receptor (*CXCR* 1, *CXCR3*, *IL6*, *IL8*, *IL17A*, *IL17F*, *IL22*, *IL23*, and *IL31*). *TAC1* (substance P [SP]), *TACR1* (its receptor neurokinin-1 [NK-1R]), transient receptor potential vanilloid 1 (*TRPV1*), Mas-related G-protein coupled receptor (*MRGPR*) X2, and *SCN3A*, *SCN9A*, *SCN11A* (voltage-gated sodium channels [Nav] 1.3, 1.7, and 1.9) were some of the neuronal components that were overexpressed with the highest FCs. Phospholipase A2 (PLA2) group IV (*PLA2G4*) B, *PLA2G4D*, and *PLA2G4E* were also prominent DEGs with high FCs that were significantly correlated with the itch severity scores. Of note, *TPSAB1* (tryptase), histamine receptor 2 (*HRH2*), kallikrein (*KLK*) 6 and *KLK14*, and *S100A9* and *A15* were also increased in itchy skin.

Several DEGs were unique to pruritic atopic skin or pruritic psoriatic skin. *CCL1*, *CCL26*, *CCL27*, *CXCL3*, *CXCL11*, *IL4*, *IL7*, *IL9*, *IL10*, and *IFNG* (IFN- γ) were DEGs only in pruritic atopic skin. In contrast, *CCL4*, *CCL7*, *CCL8*, *CCL14*, *CCL20*, *IL19*, *IL20*, *IL26*, *IL36A*, *IL36G*, and tumor necrosis factor- α were DEGs only in pruritic psoriatic skin. Interestingly, *TRPA1* (TRP ankyrin 1) and *TRPV2* had high FCs in the itchy atopic skin, whereas *TRPV3* and *TRP melastatin 8* were elevated only in itchy psoriatic skin. Other notable genes only overexpressed in itchy AD skin are *EDN1* (endothelin-1), *EDNRA* (its receptor endothelin receptor A), *F2RL1* and 3 (protease-activated receptor [PAR] 2 and PAR4), *HRH4*, serotonin receptor (*HTR*) 3B, *HTR3C*, *HTR7*, *KLK5*, *KLK13*, and *S100A2* and *G*. In itchy PS skin, *PLCG1* (phospholipase C γ), *MRGPRX3*, *F2RL2* (PAR3), *HRH3*, *KLK8*, *S100A7*, and *S100P* were overexpressed.

Analysis of DEGs in ethnic groups

Only a few itch-related gene transcripts were significantly different among ethnic groups. *TRPA1* was found to be elevated in Caucasians, whereas African Americans exhibited higher expression of *TRP melastatin 8* and decreased expression of *TRPV1*. In the Asian population, *S100A2*, *IL19*, and a potassium-dependent sodium/calcium exchanger protein *SLC24A5* were overexpressed in itchy skin. The Hispanic population was too limited to reach significant statistical power in this study.

Immunohistochemistry of selected DEG products

Immunohistochemistry was performed on itchy AD/PS, nonitchy AD/PS, and healthy control skin to validate selected findings from the RNA-seq. *TRPV1* and *TRPA1* mRNA levels

were shown to be increased, but the numbers of *TRPV1*⁺ and *TRPA1*⁺ cutaneous nerve fibers were not significantly different between groups. However, the fluorescence intensity for *TRPV1* was increased ($P < 0.0001$) throughout the epidermis in itchy AD skin (18.5% increase from nonitchy skin) and itchy PS skin (14.4% increase from nonitchy skin; Figure 2a). *TRPA1* fluorescence was also elevated ($P < 0.0001$) in the epidermis of itchy AD skin (34.9% increase from nonitchy skin) but not in itchy PS skin (13.9% increase from nonitchy skin; Figure 2b).

Tryptase and its receptor PAR2 were found to be elevated ($P < 0.0001$) in itchy AD and PS skin. Tryptase was detected in numerous mast cells scattered within the dermis of all skin types. However, most Tryptase⁺ mast cells were located in the papillary dermis at the dermal-epidermal junction in AD and PS skin. Tryptase⁺ mast cells were significantly increased in itchy AD (40.5.25 \pm 6) and itchy PS (42.75 \pm 4.5) skin when compared with healthy (6.75 \pm 2.25) and nonitchy AD (28.25 \pm 9.5) and PS (32.35 \pm 4.5) skin (Figure 3a). The largest increases of PAR2 expression were found throughout in the epidermis of itchy AD skin (48.2% increase from nonitchy AD skin). PAR2 was predominantly found in keratinocytes of the granular layer in healthy and PS skin, but there was no significant expression of PAR2 in itchy PS skin (18.4% increase from nonitchy PS skin; Figure 3b).

Immunohistochemical analysis showed that the number of SP⁺ nerve fibers was increased ($P < 0.0001$) in the itchy skin of subjects with AD (7.8 \pm 1.8) and PS (8.6 \pm 2.7) (Figure 4a). This increase of SP was usually seen in nerve fibers that were in close proximity to the dermal-epidermal junction. Moreover, the receptor NK-1R was overexpressed ($P < 0.0001$) within the epidermis of itchy AD skin (88% increase from nonitchy AD skin) and itchy PS skin (30.1% increase from nonitchy PS skin; Figure 4b).

DISCUSSION

We have defined an RNA-seq profile for chronic pruritus in AD and PS by comparing DEGs in pruritic, lesional and nonpruritic, nonlesional skin. Inflammatory mediators, including many chemokines and cytokines, were commonly overexpressed in itchy atopic and psoriatic skin. With the emergence of new biologic drugs indicated for PS and AD, our data suggest that these treatments should also be tested in other chronic itch conditions. Psoriatic drugs secukinumab and ustekinumab, which target IL-17A and IL-12/23 respectively, may also be effective in AD, where these cytokines are similarly overexpressed (Kavanaugh et al., 2016; Strober et al., 2016). Likewise, the IL-31 receptor-targeting drug nemolizumab tested in AD should also be examined in other chronic itch diseases (Ruzicka et al., 2017).

The gene transcripts of both the neuropeptide SP and its receptor NK-1R were elevated in itchy atopic and psoriatic skin. SP has been shown to be involved in the mechanism of chronic itch in several disease states, including AD, prurigo nodularis, and PS (Amatya et al., 2011; Järvikallio et al., 2003; Pincelli et al., 1990; Tobin et al., 1992). Accordingly, the NK-1R antagonist aprepitant has been shown to be an effective antipruritic in patients with refractory chronic pruritus from several underlying diseases, such as AD, uremic pruritus, and prurigo nodularis (Ständer et al., 2010). New

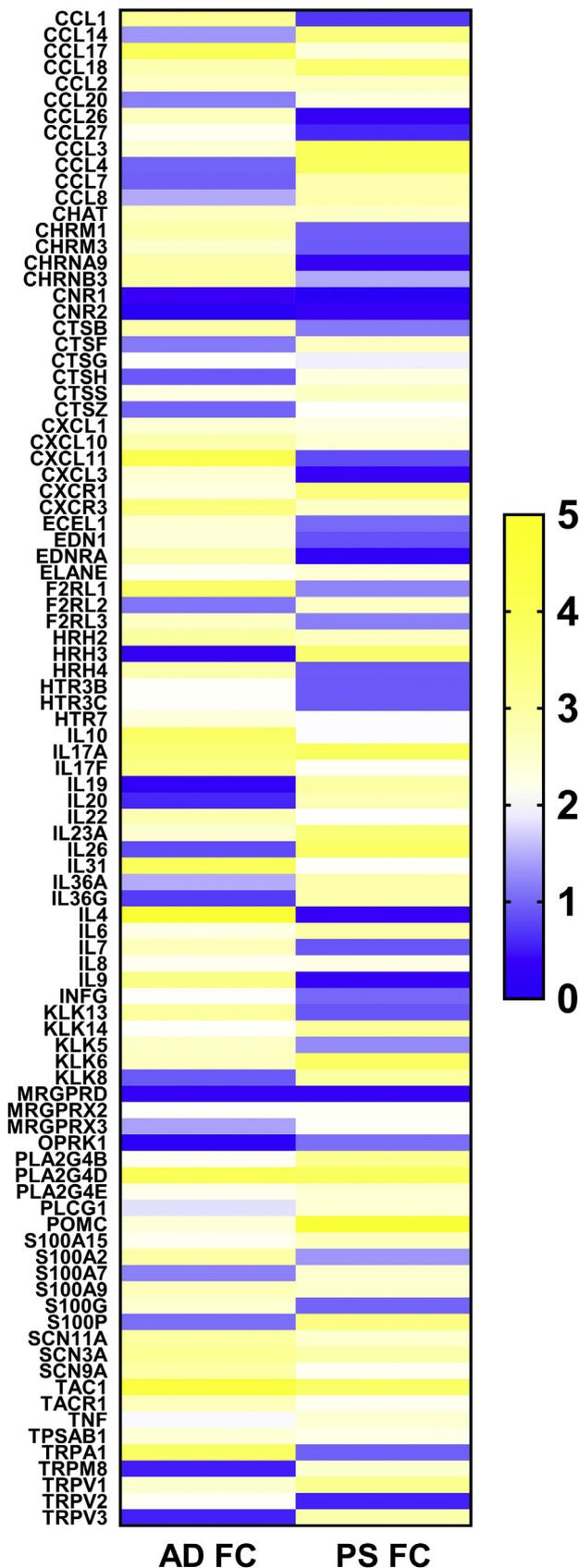


Figure 1. Heat map of itch-associated gene fold changes (FC) between pruritic, lesional and nonpruritic, nonlesional skin in patients with atopic dermatitis (AD) and psoriasis (PS). Scale bar represents FC, where blue is underexpressed (FC < 2) and yellow is overexpressed (FC > 2) in pruritic skin.

NK-1R antagonists, serlopitant and tradipitant, are currently being tested in AD and prurigo nodularis. The success of these drugs across disease states may be due to the common overexpression of SP and NK-1R across multiple chronic itch conditions.

Several itch-associated signaling genes were revealed to have increased expression in itchy skin. Mrgprs, orphan GPCRs, have recently emerged as novel nonhistaminergic receptors. MrgprX2 has recently been shown to be activated by SP to induce inflammation (McNeil et al., 2015). However, this receptor was not antagonized by aprepitant, but by a tripeptide NK-1R antagonist (Azimi et al., 2016), suggesting that antagonists of MrgprX2 may be of benefit in combination with drugs such as aprepitant and tradipitant. Recently, Nav1.7 was shown to play a role in itch (Lee et al., 2014). Although selective Nav1.7 antagonists are still in clinical development, an antibody that inhibits Nav1.7 did reduce itch in mice (Lee et al., 2014). Also of note, the precursor gene for β -endorphin (*POMC*) was significantly upregulated in both pruritic atopic and psoriatic skin, whereas the κ -opioid receptor gene (*OPRK1*) was downregulated. This imbalance of μ -opioids may play a significant role in the propagation of chronic itch (Cowan et al., 2015).

Cytosolic members of the group IV PLA2 family of enzymes had significantly higher FCs in itchy atopic and psoriatic skin. These enzymes are involved in cell signaling and the inflammation response via production of arachidonic acid, which is a precursor for eicosanoids. The eicosanoid subfamily of prostaglandins and leukotrienes is known to be involved in itch of AD and PS (Brain et al., 1984; Neisius et al., 2002; Ruzicka et al., 1986). Furthermore, TRPV1, which was also significantly elevated in itchy skin, mediates histamine-induced itching via activation of PLA2 (Shim et al., 2007). Therefore, inhibition of PLA2 may provide a promising antipruritic target.

Of note, *HRH1*, the gene for histamine receptor 1, was not found to be overexpressed in pruritic, lesional skin. H1 antihistamines are frequently given as antipruritic agents; however, these treatments are usually ineffective for chronic pruritus, with the exception of urticaria (Pereira and Ständer, 2017). This supports the theory that most chronic itch conditions are mediated by nonhistaminergic pathways (Lavery et al., 2016).

Our study also identified gene transcripts unique to each pruritic disease state. These genes are mainly involved in the inflammatory process or in cell signaling. Our results are consistent with previous RNA-seq studies when comparing lesional with nonlesional atopic or psoriatic skin. Multiple RNA-seq studies in psoriatic skin revealed enriched DEGs commonly involved in inflammatory response, cytokine-receptor interaction, cell division, and keratinization pathways (Jabbari et al., 2012; Li et al., 2014; Sarkar et al., 2017). These findings include increased expression of several cytokines (*IL6*, *IL12B*, *IL17A*, *IL17F*, *IL21*, *IL22*, *IL24*, *IL26*, *IFNG*, and *IFNE*), cytokine receptors (*IL21R* and *IL23R*), and transcription regulators (signal transducer and activator of transcription [*STAT1*], *STAT3*, CCAAT/enhancer binding protein β [*CERB*], and nuclear factor kappa-light-chain-enhancer of B cells [*NFKB1*]). A study in atopic skin found DEGs that consisted of inflammatory mediators (*S100A7*, *S100A8*,

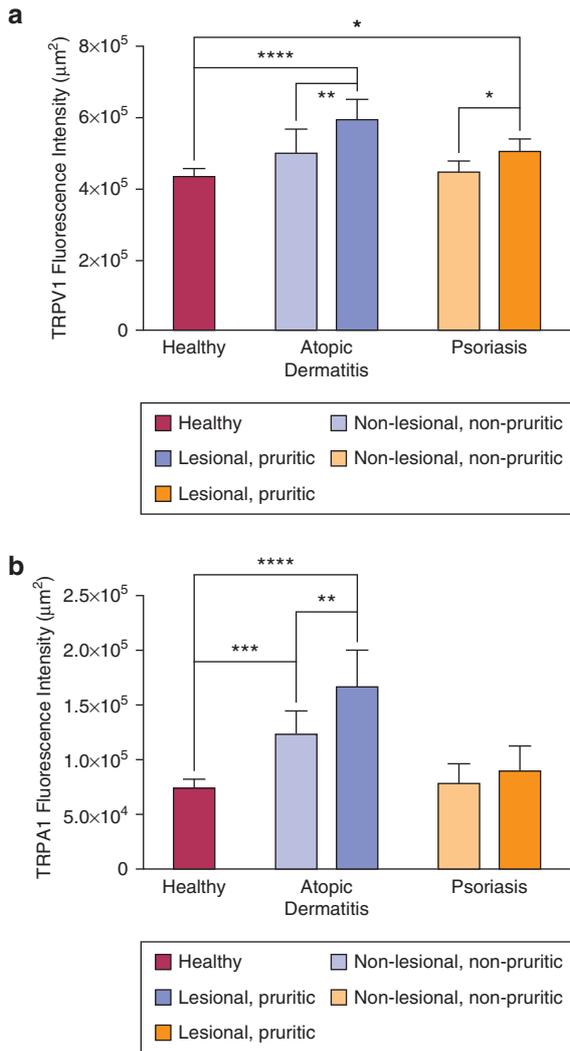


Figure 2. The expression of TRPV1 and TRPA1 in atopic, psoriatic, and healthy skin. (a) TRPV1 was overexpressed throughout the epidermis of pruritic atopic and psoriatic skin. (b) TRPA1 was overexpressed in both nonpruritic and pruritic atopic skin. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; **** $P < 0.0001$. TRPA1, transient receptor potential ankyrin 1; TRPV1, transient receptor potential vanilloid 1.

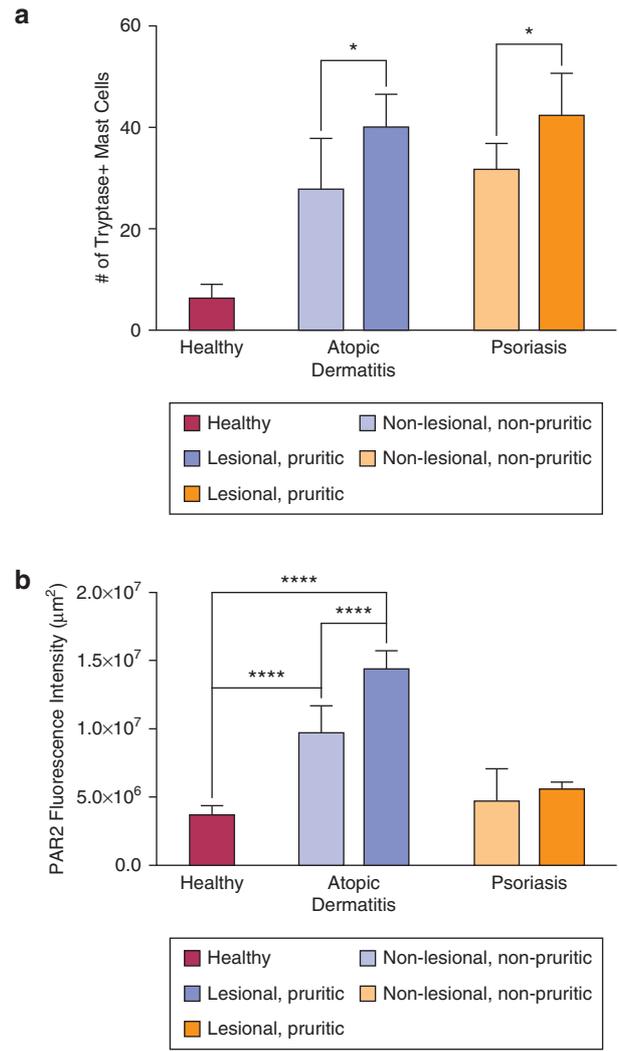


Figure 3. The expression of Tryptase⁺ mast cells and PAR2 in atopic, psoriatic, and healthy skin. (a) A significantly high number of Tryptase⁺ mast cells were located in the papillary dermis at the dermal-epidermal junction in pruritic atopic and psoriatic skin. (b) PAR2 was overexpressed throughout the epidermis in both nonpruritic and pruritic atopic skin. PAR2 was predominantly found in keratinocytes of the granular layer in psoriatic and healthy skin. * $P < 0.05$; **** $P < 0.0001$. PAR2, protease-activated receptor 2.

S100A9, *CCL2*, *CCL3*, *IL36A*, *IL36G*, and *IL36RN*, triggering receptor expressed on myeloid cells [*TREM* 1] and skin barrier proteins (marker of proliferation Ki67 [*MKI67*] and keratin 16 [*KRT16*]) (Suárez-Fariñas et al., 2015).

Although our genotypic study explored the itch component of AD and PS, we were unable to include the analysis of nonitchy, lesional or itchy, nonlesional skin as other controls. Therefore, the observed changes in our itchy, lesional skin might also be a result of scratching. Of note, we only analyzed DEGs in atopic and psoriatic skin that had significant FCs compared with healthy skin. Therefore, although some genes were downregulated in pruritic skin compared with nonpruritic skin, they remained upregulated in non-pruritic AD and PS skin compared with healthy controls. These upregulations in nonpruritic skin may point to underlying differences because of disease state. In addition, some genes, such as *MrgprX1*, that have been shown to play a role in itch signaling were not over- or underexpressed in pruritic

skin. It is possible that, despite normal mRNA expression, these itch mediators could be dysfunctional in other ways under chronic itch conditions. Our findings among different ethnic groups were limited to genes mainly involved in structural and anatomical differences. Future studies should focus on phenotyping and genotyping the ethnic differences of chronic itch with larger subject numbers. We were also unable to localize these changes to specific cell types because whole skin biopsies were used in this analysis. However, immunohistochemistry of the selected gene products helped to visualize some of these changes.

This study allowed for the identification of an “itch-scriptome” or fingerprint of itch-associated mediators and receptors in AD and PS (Figure 5). Although we found distinct gene expression patterns associated with each group, a common core of components was revealed. The products of

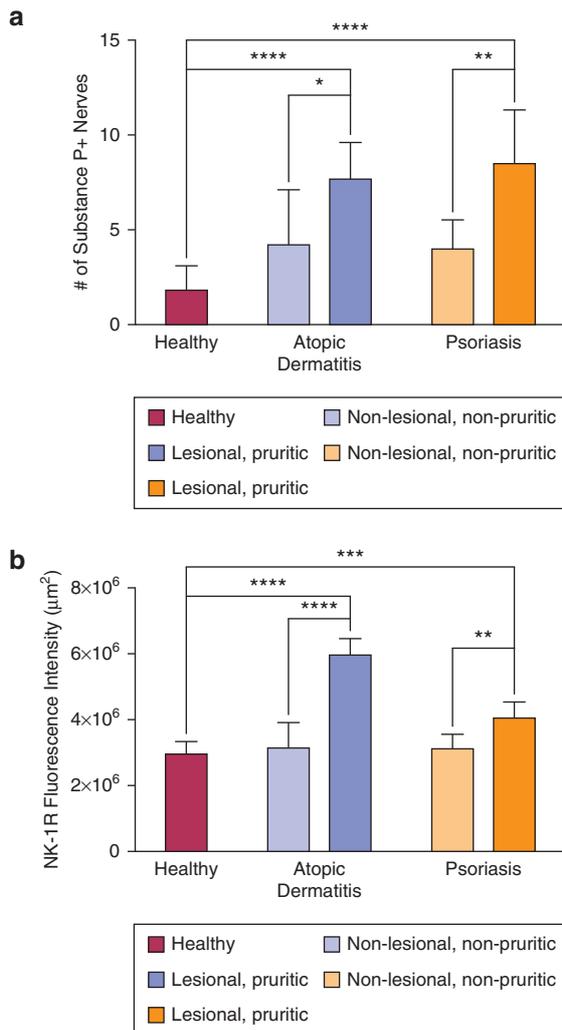


Figure 4. The expression of SP⁺ nerve fibers and NK-1R in atopic, psoriatic, and healthy skin. (a) SP⁺ nerves at the dermal epidermal junction were overall increased in atopic and psoriatic skin, with the largest increases in the pruritic skin. (b) NK-1R was overexpressed throughout the epidermis in both nonpruritic and pruritic atopic skin, with the highest expression seen in pruritic skin. **P* < 0.05, ***P* < 0.01, ****P* < 0.001; *****P* < 0.0001. NK-1R, neurokinin-1; SP, substance P.

these genes are connected to all aspects of itch transmission at the peripheral level and are expressed by skin cells, immune cells, and nerves. Although these data do not confirm pruritic mechanisms or drug potential, we believe that treatments targeting these common elements could provide itch relief that is effective across multiple disease states.

MATERIALS AND METHODS

Patients

Healthy controls and patients with AD and PS with severe pruritus (numeric rating scale ≥ 7) were enrolled from Wake Forest University and Temple University in the USA and the National Skin Centre in Singapore (Table 1) in accordance with the Declaration of Helsinki and with approval by each institutional review board. After giving written, informed consent, all subjects provided demographic information, including age, gender, ethnicity, medical history, and current medications. Subjects with chronic itch completed the itch questionnaire and rated their itch in the lesional site with a 0–10

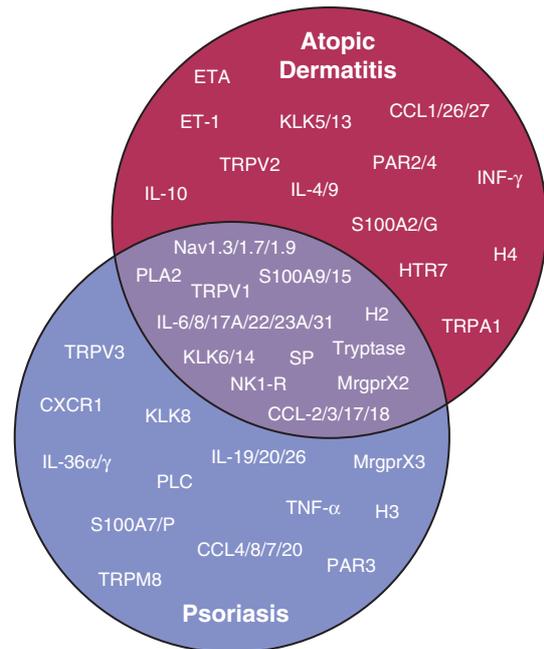


Figure 5. The “itchscriptome” or a fingerprint of itch-associated mediators and receptors in atopic dermatitis and psoriasis. This Venn diagram shows genes differently expressed in both conditions (middle) or unique to atopic dermatitis or psoriasis. CCL, chemokine (C-C motif) ligand; CXCR, chemokine (C-X-C motif) receptor; KLK, kallikrein; MRGPR, Mas-related G-protein coupled receptor; Nav, voltage-gated sodium channels; PAR, protease-activated receptor; PLA2, phospholipase A2; PLC, phospholipase C; SP, substance P; TNF- α , tumor necrosis factor- α ; TRPA1, transient receptor potential ankyrin 1; TRPV1, transient receptor potential vanilloid 1.

numeric rating scale. All subjects with chronic itch underwent two 4-mm skin punch biopsies, with one biopsy at an itchy, lesional site and the other biopsy at a nonitchy, nonlesional site. One 4-mm biopsy, corresponding to the location of a lesional biopsy, was performed on age- and sex-matched healthy subjects. Half of each biopsy was used for RNA-seq and the other half for immunohistochemistry.

RNA isolation and sequencing

Skin biopsies were fixed and stabilized using the PAXgene tissue container. RNA was isolated and purified with the PAXgene tissue RNA kit (PreAnalytiX, Feldbachstrasse, Switzerland). Purified RNA was quantified with a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA) and a Nanodrop ND 1000 spectrophotometer (Fisher Scientific, Pittsburg, PA).

Polyadenylated mRNA libraries were prepared using TruSeq Stranded mRNA with Ribo-Zero (Illumina, San Diego, CA). RNA-seq was performed with the Illumina NextSeq (Illumina) at a depth of 25 million single-end reads of 75 bp. Quality control was performed on all raw sequence data using FastQC, and reads were aligned Using TopHat. Read counts were normalized to the number of reads per kilobase per million mapped reads. A Wilcoxon rank-sum test with a Bonferroni corrected *P*-value (*P* < 0.000001) was used to identify DEGs (based on a false discovery rate less than 0.05 and an FC greater than 2.0) in AD and PS pruritic skin versus healthy skin. Reads per kilobase per million mapped reads data from these DEGs were then analyzed between pruritic and non-pruritic AD and PS skin. These reads per kilobase per million

Table 1. Subject demographics and itch characteristics

	Atopic dermatitis	Psoriasis	Healthy
N	25	25	30
Age	40 ± 13	41 ± 16	38 ± 14
Male:female	11:14	12:13	13:17
NRS itch intensity	8.4 ± 1.5	8.6 ± 1.8	0
EASI/PASI score	42.6 ± 30.1	21 ± 16.7	0
Chronic itch duration	5–57 y	2–28 y	N/A
Ethnicity			
Caucasian	7	10	14
African American	5	4	3
Asian	11	10	10
Hispanic	2	1	3

Abbreviations: EASI, Eczema Area and Severity Index; NRS, numerical rating scale; PASI, psoriasis area and severity index.

mapped reads data (log2 transformed) were also correlated with the itch intensity ratings using Spearman’s correlation analysis. Ethnic influence was analyzed using multivariate logistic regression analysis.

Immunohistochemistry

Researchers were blinded to the identity of the biopsies, and the results were only decoded after the immunohistochemical analysis was fully performed. Twenty-micrometer-thick sections of paraffin-embedded skin tissue were double stained from each biopsy. Sections were deparaffinized, underwent antigen retrieval using Target Retrieval Solutions (DAKO, Glostrup, Denmark) heated in a humidified oven overnight at 60°C, and then washed in phosphate buffered saline. Sections were blocked with 5% normal donkey serum and 0.2% Triton X-100 in phosphate buffered saline for 2 hours at room temperature and then incubated with primary antibodies overnight at 4°C.

Primary antibodies were anti-SP (1:1,000; ab106291; Abcam, Cambridge, MA), anti-NK-1R (1:750; PA3-301; Pierce Thermo Scientific, Rockford, IL), antitryptase (1:100; ab2378; Abcam), anti-PAR2 (1:100; sc-5597; Santa Cruz, Dallas, TX), anti-TRPV1 (1:200; ab3487; Abcam), anti-TRPA1 (1:500; ab62053; Abcam), and anti-β-tubulin III (1:300; M015013; Neuromics, Edina, MN).

Alexa Fluor (488 & 594, 1:300; Molecular Probes, Eugene, OR) secondary antibodies were used for detection. All slides were mounted with Vectashield with DAPI (Vector Laboratories, Burlingame, CA) and imaged under a fluorescence microscope. Sections treated without any primary antibodies were used as negative controls. Furthermore, specificity of each primary antibody was confirmed by preabsorption with its respective peptide in blocking solution overnight at 4°C with gentle agitation. Solutions were centrifuged, and the supernatant was used for immunohistochemistry as described above. In each case, this process resulted in blocking of the primary antibody’s immunoreactivity.

Three fields (×20 objective magnification) were measured for every section. The total field and selected field (epidermis) fluorescence areas (in μm²) were measured and normalized to background staining using ImageJ Software (National Institutes of Health, Bethesda, MD). Data are presented as mean epidermal fluorescence normalized to mean total field fluorescence. Mast cell (Tryptase⁺ cells) and nerve (β-tubulin III⁺) counts were also performed using ImageJ Software and normalized to epidermal length as previously

described (McArther et al., 1998). All data are reported as mean ± standard deviation or as percentage change. One-way analyses of variance with Bonferroni post hoc tests were used to compare the differences between groups. Statistical significance was set at P < 0.05 (GraphPad Prism, La Jolla, CA).

CONFLICT OF INTEREST

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at <https://doi.org/10.1016/j.jid.2017.12.029>.

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